[図13]図１３は、ヒト化ｈＧ０１９－薬物コンジュゲートＨ０１Ｌ０２－ＤＸｄ又はＮＯＶ０７１２－ＤＭ４又はＮＯＶ０７１２－ＤＸｄのｉｎ　ｖｉｖｏ抗腫瘍効果を示す。ＣＤＨ６陽性ヒト卵巣腫瘍細胞株ＰＡ－１を免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

[図14]図１４は、ヒト化ｈＧ０１９－薬物コンジュゲートＨ０１Ｌ０２－ＤＸｄ又はＮＯＶ０７１２－ＤＭ４のｉｎ　ｖｉｖｏ抗腫瘍効果を示す。ＣＤＨ６陽性ヒト卵巣腫瘍細胞株ＮＩＨ：ＯＶＣＡＲ－３を免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

[図15]図１５は、ヒト化ｈＧ０１９－薬物コンジュゲートＨ０１Ｌ０２－ＤＸｄ又はＮＯＶ０７１２－ＤＭ４のｉｎ　ｖｉｖｏ抗腫瘍効果を示す。ＣＤＨ６陽性ヒト腎細胞腫瘍細胞株７８６－Ｏを免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

[図16]図１６は、ヒト化ｈＧ０１９－薬物コンジュゲートＨ０１Ｌ０２－ＤＸｄ又はＮＯＶ０７１２－ＤＭ４のｉｎ　ｖｉｖｏ抗腫瘍効果を示す。ＣＤＨ６陰性ヒト卵巣腫瘍細胞株ＥＳ－２を免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

発明を実施するための形態

[0025] 以下、本発明を実施するための好適な形態について図面を参照しながら説明する。なお、以下に説明する実施形態は、本発明の代表的な実施形態の一例を示したものであり、これにより本発明の範囲が狭く解釈されることはない。

[0026] 本明細書中、「がん」と「腫瘍」は同じ意味に用いられる。

[0027] 本明細書中、「遺伝子」という語には、ＤＮＡのみならずそのｍＲＮＡ、ｃＤＮＡ及びそのｃＲＮＡも含まれる。

[0028] 本明細書中、「ポリヌクレオチド」又は「ヌクレオチド」は、核酸と同じ意味で用いており、ＤＮＡ、ＲＮＡ、プローブ、オリゴヌクレオチド、及びプライマーも含まれる。本明細書中、「ポリヌクレオチド」と「ヌクレオチド」は、特に指定されない限り、交換可能に使用され得る。

[0029] 本明細書中、「ポリペプチド」と「タンパク質」は交換可能に使用され得る。

[0030] 本明細書中、「細胞」には、動物個体内の細胞、培養細胞も含む。

[0031] 本明細書中、「ＣＤＨ６」は、ＣＤＨ６タンパク質と同じ意味で使用され得る。本明細書中、ヒトＣＤＨ６を「ｈＣＤＨ６」と記載する場合がある。

[0032] 本明細書中、「細胞傷害活性」とは、何らかの形で、細胞に病理的な変化を引き起こすことをいい、直接的な外傷にとどまらず、ＤＮＡの切断や塩基の二量体の形成、染色体の切断、細胞分裂装置の損傷、各種酵素活性の低下などあらゆる細胞の構造や機能上の損傷を引き起こすことをいう。

[0033] 本明細書中、「細胞内で毒性を発揮する」とは、何らかの形で、細胞内で毒性を示すことをいい、直接的な外傷にとどまらず、ＤＮＡの切断や塩基の二量体の形成、染色体の切断、細胞分裂装置の損傷、各種酵素活性の低下、細胞増殖因子の作用の抑制などあらゆる細胞の構造や機能、代謝に影響をもたらすことをいう。

[0034] 本明細書中、「抗体の機能性断片」とは、「抗体の抗原結合断片」とも呼ばれ、抗原との結合活性を有する抗体の部分断片を意味しており、Ｆａｂ、Ｆ（ａｂ’）２、Ｆｖ、ｓｃＦｖ、ｄｉａｂｏｄｙ、線状抗体及び抗体断片より形成された多特異性抗体等を含む。また、Ｆ（ａｂ’）２を還元条件下で処理した抗体の可変領域の一価の断片であるＦａｂ’も抗体の抗原結合断片に含まれる。但し、抗原との結合能を有している限りこれらの分子に限定されない。また、これらの抗原結合断片には、抗体蛋白質の全長分子を適当な酵素で処理したもののみならず、遺伝子工学的に改変された抗体遺伝子を用いて適当な宿主細胞において産生された蛋白質も含まれる。

[0035] 本明細書中、「エピトープ」とは、特定の抗ＣＤＨ６抗体の結合するＣＤ

Ｈ６の部分ペプチド又は部分立体構造を意味する。前記のＣＤＨ６の部分ペプチドであるエピトープは免疫アッセイ法等当業者にはよく知られている方法によって、決定することができる。まず、抗原の様々な部分構造を作製する。部分構造の作製にあたっては、公知のオリゴヌクレオチド合成技術を用いることができる。例えば、ＣＤＨ６のＣ末端又はＮ末端から適当な長さで順次短くした一連のポリペプチドを当業者に周知の遺伝子組み換え技術を用いて作製した後、それらに対する抗体の反応性を検討し、大まかな認識部位を決定した後に、更に短いペプチドを合成してそれらのペプチドとの反応性を検討することによって、エピトープを決定することができる。また、複数の細胞外ドメインからなる膜タンパク質に結合する抗体が、複数のドメインからなる立体構造をエピトープとしている場合は、特定の細胞外ドメインのアミノ酸配列を改変することによって、立体構造を改変することによってどのドメインと結合するかを決定することができる。特定の抗体の結合する抗原の部分立体構造であるエピトープは、Ｘ線構造解析によって抗体と隣接する抗原のアミノ酸残基を特定することによっても決定することができる。

[0036] 本明細書中、「同一のエピトープに結合する」とは、共通のエピトープに結合する抗体を意味している。第一の抗体の結合する部分ペプチド又は部分立体構造に第二の抗体が結合すれば、第一の抗体と第二の抗体が同一のエピトープに結合すると判定することができる。あるいは、第一の抗体の抗原に対する結合に第二の抗体が競合する（即ち、第二の抗体が第一の抗体と抗原の結合を妨げる）ことを確認することによって、具体的なエピトープの配列又は構造が決定されていなくても、第一の抗体と第二の抗体の同一のエピトープに結合すると判定することができる。本明細書中、「同一のエピトープに結合する」とは、第一の抗体と第二の抗体が、当該判定方法のいずれか一方又は両方により、共通のエピトープに結合すると判定された場合をいう。第一の抗体と第二の抗体が同一のエピトープに結合し、かつ第一の抗体が抗腫瘍活性や内在化活性等の特殊な効果を有する場合、第二の抗体も同様の活性を有することが期待できる。

[0037] 本明細書中、「ＣＤＲ」とは、相補性決定領域（ＣＤＲ；Ｃｏｍｐｌｅｍｅｎｔａｒｉｔｙ　Ｄｅｔｅｒｍｉｎｉｎｇ　Ｒｅｇｉｏｎ）を意味する。抗体分子の重鎖及び軽鎖にはそれぞれ３箇所のＣＤＲがあることが知られている。ＣＤＲは、超可変領域（ｈｙｐｅｒｖａｒｉａｂｌｅ　ｒｅｇｉｏｎ）とも呼ばれ、抗体の重鎖及び軽鎖の可変領域内にあって、一次構造の変異性が特に高い部位であり、重鎖及び軽鎖のポリペプチド鎖の一次構造上において、それぞれ３ヶ所に分離している。本明細書中においては、抗体のＣＤＲについて、重鎖のＣＤＲを重鎖アミノ酸配列のアミノ末端側からＣＤＲＨ１、ＣＤＲＨ２、ＣＤＲＨ３と表記し、軽鎖のＣＤＲを軽鎖アミノ酸配列のアミノ末端側からＣＤＲＬ１、ＣＤＲＬ２、ＣＤＲＬ３と表記する。これらの部位は立体構造の上で相互に近接し、結合する抗原に対する特異性を決定している。

[0038] 本明細書中、「ストリンジェントな条件下でハイブリダイズする」とは、市販のハイブリダイゼーション溶液ＥｘｐｒｅｓｓＨｙｂ　Ｈｙｂｒｉｄｉｚａｔｉｏｎ　Ｓｏｌｕｔｉｏｎ（クロンテック社）中、６８℃でハイブリダイズすること、又はＤＮＡを固定したフィルターを用いて０．７～１．０ＭのＮａＣｌ存在下６８℃でハイブリダイゼーションを行った後、０．１～２倍濃度のＳＳＣ溶液（１倍濃度ＳＳＣとは１５０ｍＭ　ＮａＣｌ、１５ｍＭクエン酸ナトリウムからなる）を用い、６８℃で洗浄することによって同定することができる条件又はそれと同等の条件でハイブリダイズすることをいう。

[0039] 本明細書中、「１～数個」とは、１～１０個、１～９個、１～８個、１～７個、１～６個、１～５個、１～４個、１～３個又は１～２個を意味する。

[0040] １．ＣＤＨ６

カドヘリンは、細胞膜表面に存在する糖タンパク質で、カルシウムイオン依存的にＮ末端側の細胞外ドメイン同士が結合することで、細胞間接着分子として、また細胞間相互作用を担うシグナル分子として機能する。カドヘリンスーパーファミリーの内、クラシックカドヘリンに分類される分子群は、

細胞外に５個の細胞外ドメイン（ＥＣドメイン）と１個の膜貫通領域、及び細胞内ドメインから構成される１回膜貫通タンパク質である。

[0041] ＣＤＨ６（Ｃａｄｈｅｒｉｎ－６） は、タイプIIカドヘリンファミリーに分類される、７９０アミノ酸からなる１回膜貫通タンパク質であり、Ｎ末端側を細胞外、Ｃ末端側を細胞内に持つ。ヒトＣＤＨ６遺伝子は１９９５年に初めてクローニングされ（非特許文献１）、ＮＭ＿００４９３２、ＮＰ＿００４９２３（ＮＣＢＩ）等のアクセッション番号により参照可能である。

[0042] 本発明で用いるＣＤＨ６タンパク質は、ヒト、非ヒト哺乳動物（ラット、マウス、サル等）のＣＤＨ６発現細胞から直接精製して使用するか、あるいは当該細胞の細胞膜画分を調製して使用することができ、また、ＣＤＨ６をｉｎ ｖｉｔｒｏにて合成する、あるいは遺伝子操作によって宿主細胞に産生させることによって得ることができる。遺伝子操作では、具体的には、ＣＤＨ６ ｃＤＮＡを発現可能なベクターに組み込んだ後、転写と翻訳に必要な酵素、基質及びエネルギー物質を含む溶液中で合成する、あるいは他の原核生物、又は真核生物の宿主細胞を形質転換させることによってＣＤＨ６を発現させることによって、該タンパク質を得ることが出来る。また、前記の遺伝子操作によるＣＤＨ６発現細胞、あるいはＣＤＨ６を発現している細胞株をＣＤＨ６タンパク質として使用することも可能である。また、ＣＤＨ６のｃＤＮＡを組み込んだ発現ベクターを直接被免疫動物に投与し被免疫動物の体内でＣＤＨ６を発現させることもできる。

[0043] また、上記ＣＤＨ６のアミノ酸配列において、１又は数個のアミノ酸が置換、欠失及び／又は付加されたアミノ酸配列からなり、当該タンパク質と同等の生物活性を有するタンパク質もＣＤＨ６に含まれる。

[0044] ヒトＣＤＨ６タンパク質は、配列番号１に記載のアミノ酸配列を有する。ヒトＣＤＨ６タンパク質の細胞外領域は、配列番号１に記載のアミノ酸配列の５４～１５９番目のアミノ酸配列を有する細胞外ドメイン１（本明細書中、ＥＣ１とも称する）、配列番号１に記載のアミノ酸配列の１６０～２６８番目のアミノ酸配列を有する細胞外ドメイン２（本明細書中、ＥＣ２とも称

する。）、配列番号１に記載のアミノ酸配列の２６９～３８３番目のアミノ酸配列を有する細胞外ドメイン３（本明細書中、ＥＣ３とも称する。）、配列番号１に記載のアミノ酸配列の３８４～４８６番目のアミノ酸配列を有する細胞外ドメイン４（本明細書中、ＥＣ４とも称する。）、及び配列番号１に記載のアミノ酸配列の４８７～６０８番目のアミノ酸配列を有する細胞外ドメイン５（本明細書中、ＥＣ５とも称する。）から構成される。ＥＣ１～ＥＣ５のアミノ酸配列を、それぞれ、配列番号２～６とする（表１）。

[0045] ２．抗ＣＤＨ６抗体の製造

本発明の抗ＣＤＨ６抗体の一例として、配列番号４に示すアミノ酸配列を含むアミノ酸配列を認識し、かつ内在化活性を有する抗ＣＤＨ６抗体を挙げることができる。本発明の抗ＣＤＨ６抗体の一例として、配列番号４に示すアミノ酸配列を含むアミノ酸配列を特異的に認識し、かつ内在化活性を有する抗ＣＤＨ６抗体を挙げることができる。本発明の抗ＣＤＨ６抗体の一例として、配列番号４に示すアミノ酸配列からなるアミノ酸配列を認識し、かつ内在化活性を有する抗ＣＤＨ６抗体を挙げることができる。本発明の抗ＣＤＨ６抗体の一例として、配列番号４に示すアミノ酸配列からなるアミノ酸配列を特異的に認識し、かつ内在化活性を有する抗ＣＤＨ６抗体を挙げることができる。抗体が「配列番号４に示すアミノ酸配列を含むアミノ酸配列を特異的に認識」する又は「ＥＣ３ドメインを特異的に認識」するとは、抗体が、ＣＤＨ６のＥＣ３ドメインをＣＤＨ６の他の細胞外ドメインと比較して強く認識する又は強く結合することをいう。

[0046] 本発明の抗ＣＤＨ６抗体は、いずれの種に由来してもよいが、好ましくは、ヒト、サル、ラット、マウス又はウサギを例示できる。ヒト以外の種に由来する場合は、周知の技術を用いて、キメラ化又はヒト化することが好ましい。本発明の抗体は、ポリクローナル抗体であっても、モノクローナル抗体であってもよいが、モノクローナル抗体が好ましい。

[0047] 本発明の抗ＣＤＨ６抗体は腫瘍細胞を標的にできる抗体であり、すなわち腫瘍細胞を認識できる特性、腫瘍細胞に結合できる特性、及び／又は腫瘍細

胞内に取り込まれて内在化する特性等を備えている。したがって、本発明の抗ＣＤＨ６抗体と抗腫瘍活性を有する化合物を、リンカーを介して結合させて抗体－薬物コンジュゲートとすることができる。

[0048] 抗体の腫瘍細胞への結合性は、フローサイトメトリーを用いて確認できる。腫瘍細胞内への抗体の取り込みは、（１）治療抗体に結合する二次抗体（蛍光標識）を用いて細胞内に取り込まれた抗体を蛍光顕微鏡で可視化するアッセイ（Ｃｅｌｌ Ｄｅａｔｈ ａｎｄ Ｄｉｆｆｅｒｅｎｔｉａｔｉｏｎ，２００８，１５，７５１－７６１）、（２）治療抗体に結合する二次抗体（蛍光標識）を用いて細胞内に取り込まれた蛍光量を測定するアッセイ（Ｍｏｌｅｃｕｌａｒ Ｂｉｏｌｏｇｙ ｏｆ ｔｈｅ Ｃｅｌｌ Ｖｏｌ．１５，５２６８－５２８２，Ｄｅｃｅｍｂｅｒ ２００４）又は（３）治療抗体に結合するイムノトキシンを用いて、細胞内に取り込まれると毒素が放出されて細胞増殖が抑制されるというＭａｂ－ＺＡＰアッセイ（Ｂｉｏ Ｔｅｃｈｎｉｑｕｅｓ ２８：１６２－１６５，Ｊａｎｕａｒｙ ２０００）を用いて確認できる。イムノトキシンとしては、ジフテテリア毒素の触媒領域とプロテインＧとのリコンビナント複合タンパク質タンパク質も使用可能である。

[0049] 本明細書で言う「高い内在化能」とは、当該抗体とサポリン標識抗ラットＩｇＧ抗体を投与したＣＤＨ６発現細胞の生存率（抗体未添加時の細胞生存率を１００％とした相対率で表したもの）が、好ましくは７０％以下、より好ましくは６０％以下であることを意味する。

[0050] 抗体－薬物コンジュゲートは抗腫瘍効果を発揮する化合物を結合させてあるので、抗体自体が抗腫瘍効果を有することは、好ましいが、必須ではない。抗腫瘍性化合物の細胞傷害性を腫瘍細胞において特異的及び／又は選択的に発揮させる目的からは、抗体が内在化して腫瘍細胞内に移行する性質のあることが重要であり、好ましい。

[0051] 抗ＣＤＨ６抗体は、この分野で通常実施される方法を用いて、抗原となるポリペプチドを動物に免疫し、生体内に産生される抗体を採取、精製するこ

とによって得ることができるが、好ましくは、立体構造を保持したＣＤＨ６を抗原として用いることが好ましい。そのような方法として、ＤＮＡ免疫法を挙げることができる。

[0052] 抗原の由来はヒトに限定されず、マウス、ラット等のヒト以外の動物に由来する抗原を動物に免疫することもできる。この場合には、取得された異種抗原に結合する抗体とヒト抗原との交差性を試験することによって、ヒトの疾患に適用可能な抗体を選別できる。

[0053] また、公知の方法（例えば、Ｋｏｈｌｅｒ ａｎｄ Ｍｉｌｓｔｅｉｎ，Ｎａｔｕｒｅ（１９７５）２５６，４９５－４９７、Ｋｅｎｎｅｔ，Ｒ．ｅｄ．，Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｉｅｓ，３６５－３６７，Ｐｌｅｎｕｍ Ｐｒｅｓｓ，Ｎ．Ｙ．（１９８０））に従って、抗原に対する抗体を産生する抗体産生細胞とミエローマ細胞とを融合させることによってハイブリドーマを樹立し、モノクローナル抗体を得ることもできる。

[0054] 以下、具体的にＣＤＨ６に対する抗体の取得方法を説明する。

[0055] （１）抗原の調製

抗原は抗原タンパク質をコードする遺伝子を遺伝子操作によって宿主細胞に産生させることによって得ることができる。具体的には、抗原遺伝子を発現可能なベクターを作製し、これを宿主細胞に導入して該遺伝子を発現させ、発現した抗原を精製すればよい。上記の遺伝子操作による抗原発現細胞、あるいは抗原を発現している細胞株を動物に免疫する方法を用いることによっても抗体を取得できる。

[0056] また、抗原タンパク質を用いずに、抗原タンパク質のｃＤＮＡを発現ベクターに組み込んで被免疫動物に投与し、被免疫動物の体内で抗原タンパク質を発現させ、抗原タンパク質に対する抗体を産生させることによっても抗体を取得することができる。

[0057] （２）抗ＣＤＨ６モノクローナル抗体の製造

本発明で使用される抗ＣＤＨ６抗体は、特に制限はないが、例えば、本願の配列表で示されたアミノ酸配列で特定される抗体を好適に使用することが

できる。本発明において使用される抗ＣＤＨ６抗体としては、以下の特性を有するものが望ましい。

（１）以下の特性を有することを特徴とする抗体；

（ａ）ＣＤＨ６に特異的に結合する

（ｂ）ＣＤＨ６と結合することによってＣＤＨ６発現細胞に内在化する活性を有する

（２）ＣＤＨ６がヒトＣＤＨ６である上記（１）に記載の抗体又は当該抗体。

（３）ヒトＣＤＨ６のＥＣ３を特異的に認識し、かつ内在化活性を有する

本発明のＣＤＨ６に対する抗体の取得方法は、抗ＣＤＨ６抗体を取得できる限りにおいて、特に制限されないが、高次構造を保持したＣＤＨ６を抗原として用いることが好ましい。

[0058] 好ましい抗体の取得方法の一例としてＤＮＡ免疫法を挙げることができる。ＤＮＡ免疫法とは、抗原発現プラスミドをマウスやラットなどの動物個体に遺伝子導入し、抗原を個体内で発現させることによって、抗原に対する免疫を誘導する手法である。遺伝子導入の手法には、直接プラスミドを筋肉に注射する方法や、リポソームやポリエチレンイミンなどの導入試薬を静脈注射する方法、ウイルスベクターを用いる手法、プラスミドを付着させた金粒子をＧｅｎｅ　Ｇｕｎにより射ち込む手法、急速に大量のプラスミド溶液を静脈注射するＨｙｄｒｏｄｙｎａｍｉｃ法などが存在する。発現プラスミドの筋注による遺伝子導入法に関して、発現量を向上させる手法として、プラスミドを筋注後、同部位にエレクトロポレーションを加えるｉｎ　ｖｉｖｏ　ｅｌｅｃｔｒｏｐｏｒａｔｉｏｎという技術が知られている（Ａｉｈａｒａ Ｈ，Ｍｉｙａｚａｋｉ Ｊ．Ｎａｔ Ｂｉｏｔｅｃｈｎｏｌ．１９９８ Ｓｅｐ；１６（９）：８６７－７０又はＭｉｒ ＬＭ，Ｂｕｒｅａｕ Ｍ Ｆ，Ｇｅｈｌ Ｊ，Ｒａｎｇａｒａ Ｒ，Ｒｏｕｙ Ｄ，Ｃａｉｌｌａｕｄ ＪＭ，Ｄｅｌａｅｒｅ Ｐ，Ｂｒａｎｅｌｌｅｃ Ｄ，Ｓｃｈｗａｒｔｚ Ｂ，Ｓｃｈｅｒｍａｎ Ｄ．Ｐｒｏｃ Ｎａｔｌ Ａｃａｄ Ｓｃｉ Ｕ

ＳＡ．１９９９　Ａｐｒ　１３；９６（８）：４２６２－７．）。本手法はプラスミドの筋注前にヒアルロニダーゼで筋肉を処理することにより、さらに発現量が向上する（ＭｃＭａｈｏｎ　ＪＭ１，　Ｓｉｇｎｏｒｉ　Ｅ，Ｗｅｌｌｓ　ＫＥ，Ｆａｚｉｏ　ＶＭ，Ｗｅｌｌｓ　ＤＪ．Ｇｅｎｅ　Ｔｈｅｒ．２００１　Ａｕｇ；８（１６）：１２６４－７０）。また、ハイブリドーマの作製は公知の方法によって行うことができ、例えば、Ｈｙｂｒｉｍｕｎｅ　Ｈｙｂｒｉｄｏｍａ　Ｐｒｏｄｕｃｔｉｏｎ　Ｓｙｓｔｅｍ（Ｃｙｔｏ　Ｐｕｌｓｅ　Ｓｃｉｅｎｃｅｓ社）を用いて行うこともできる。

[0059]　具体的なモノクローナル抗体取得の例としては、以下を挙げることができる。

（ａ）ＣＤＨ６のｃＤＮＡを発現ベクター（例えば、ｐｃＤＮＡ３．１：Ｔｈｅｒｍｏ　Ｆｉｓｈｅｒ　Ｓｃｉｅｎｔｉｆｉｃ）に組み込み、エレクトロポレーションや遺伝子銃等の方法によって、そのベクターを直接被免疫動物（例えば、ラットやマウス）に投与することによって、動物体内においてＣＤＨ６を発現させることによって、免疫反応を誘起させることができる。エレクトロポレーション等によるベクターの投与は抗体価を上げるために必要であれば、１回でも複数回でもよく、好ましくは複数回である；

（ｂ）免疫反応を誘起された上述の動物より、抗体産生細胞を含む組織（例えばリンパ節）を採取する；

（ｃ）骨髄腫細胞（以下「ミエローマ」という）（例えば、マウスミエローマＳＰ２／０－ａｇ１４細胞）の調製；

（ｄ）抗体産生細胞とミエローマとの細胞融合；

（ｅ）目的とする抗体を産生するハイブリドーマ群の選別；

（ｆ）単一細胞クローンへの分割（クローニング）；

（ｇ）場合によっては、モノクローナル抗体を大量に製造するためのハイブリドーマの培養、又はハイブリドーマを移植した動物の飼育；及び／又は

（ｈ）このようにして製造されたモノクローナル抗体の生理活性（内在化活性）、及びその結合特異性の検討、あるいは標識試薬としての特性の検定。

[0060] ここで用いられる抗体価の測定法としては、例えば、フローサイトメトリー又はＣｅｌｌ－ＥＬＩＳＡ法を挙げることができるがこれらの方法に制限されない。

[0061] このようにして樹立されたハイブリドーマ株の例としては、抗ＣＤＨ６抗体産生ハイブリドーマｒＧ０１９、ｒＧ０５５、ｒＧ０５６及びｒＧ０６１を挙げることができる。なお、本明細書中においては、抗ＣＤＨ６抗体産生ハイブリドーマｒＧ０１９が産生する抗体を、「ｒＧ０１９抗体」又は単に「ｒＧ０１９」と記載し、ハイブリドーマｒＧ０５５が産生する抗体を、「ｒＧ０５５抗体」又は単に「ｒＧ０５５」と記載し、ハイブリドーマｒＧ０５６が産生する抗体を、「ｒＧ０５６抗体」又は単に「ｒＧ０５６」と記載し、ハイブリドーマｒＧ０６１が産生する抗体を、「ｒＧ０６１抗体」又は単に「ｒＧ０６１」とする。

[0062] ｒＧ０１９抗体の軽鎖可変領域は、配列番号１０に示されるアミノ酸配列からなる。該ｒＧ０１９抗体の軽鎖可変領域のアミノ酸配列は、配列番号１１に示されるヌクレオチド配列でコードされる。ｒＧ０１９抗体の軽鎖可変領域は、配列番号１２に示されるアミノ酸配列からなるＣＤＲＬ１、配列番号１３に示されるアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に示されるアミノ酸配列からなるＣＤＲＬ３を有する。ｒＧ０１９抗体の重鎖可変領域は、配列番号１５に示されるアミノ酸配列からなる。該ｒＧ０１９抗体の重鎖可変領域のアミノ酸配列は、配列番号１６に示されるヌクレオチド配列でコードされる。ｒＧ０１９抗体の重鎖可変領域は、配列番号１７に示されるアミノ酸配列からなるＣＤＲＨ１、配列番号１８に示されるアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に示されるアミノ酸配列からなるＣＤＲＨ３を有する。ｒＧ０１９抗体の配列を表１に示す。

[0063] ｒＧ０５５抗体の軽鎖可変領域は、配列番号２０に示されるアミノ酸配列からなる。該ｒＧ０５５抗体の軽鎖可変領域のアミノ酸配列は、配列番号２１に示されるヌクレオチド配列でコードされる。ｒＧ０５５抗体の軽鎖可変領域は、配列番号２２に示されるアミノ酸配列からなるＣＤＲＬ１、配列番

号２３に示されるアミノ酸配列からなるＣＤＲＬ２及び配列番号２４に示されるアミノ酸配列からなるＣＤＲＬ３を有する。ｒＧ０５５抗体の重鎖可変領域は、配列番号２５に示されるアミノ酸配列からなる。該ｒＧ０５５抗体の重鎖可変領域のアミノ酸配列は、配列番号２６に示されるヌクレオチド配列でコードされる。ｒＧ０５５抗体の重鎖可変領域は、配列番号２７に示されるアミノ酸配列からなるＣＤＲＨ１、配列番号２８に示されるアミノ酸配列からなるＣＤＲＨ２及び配列番号２９に示されるアミノ酸配列からなるＣＤＲＨ３を有する。ｒＧ０５５抗体の配列を表１に示す。

[0064] ｒＧ０５６抗体の軽鎖可変領域は、配列番号３０に示されるアミノ酸配列からなる。該ｒＧ０５６抗体の軽鎖可変領域のアミノ酸配列は、配列番号３１に示されるヌクレオチド配列でコードされる。ｒＧ０５６抗体の軽鎖可変領域は、配列番号３２に示されるアミノ酸配列からなるＣＤＲＬ１、配列番号３３に示されるアミノ酸配列からなるＣＤＲＬ２及び配列番号３４に示されるアミノ酸配列からなるＣＤＲＬ３を有する。ｒＧ０５６抗体の重鎖可変領域は、配列番号３５に示されるアミノ酸配列からなる。該ｒＧ０５６抗体の重鎖可変領域のアミノ酸配列は、配列番号３６に示されるヌクレオチド配列でコードされる。ｒＧ０５６抗体の重鎖可変領域は、配列番号３７に示されるアミノ酸配列からなるＣＤＲＨ１、配列番号３８に示されるアミノ酸配列からなるＣＤＲＨ２及び配列番号３９に示されるアミノ酸配列からなるＣＤＲＨ３を有する。ｒＧ０５６抗体の配列を表１に示す。

[0065] ｒＧ０６１抗体の軽鎖可変領域は、配列番号４０に示されるアミノ酸配列からなる。該ｒＧ０６１抗体の軽鎖可変領域のアミノ酸配列は、配列番号４１に示されるヌクレオチド配列でコードされる。ｒＧ０６１抗体の軽鎖可変領域は、配列番号４２に示されるアミノ酸配列からなるＣＤＲＬ１、配列番号４３に示されるアミノ酸配列からなるＣＤＲＬ２及び配列番号４４に示されるアミノ酸配列からなるＣＤＲＬ３を有する。ｒＧ０６１抗体の重鎖可変領域は、配列番号４５に示されるアミノ酸配列からなる。該ｒＧ０６１抗体の重鎖可変領域のアミノ酸配列は、配列番号４６に示されるヌクレオチド配

列でコードされる。ｒＧ０６１抗体の重鎖可変領域は、配列番号４７に示されるアミノ酸配列からなるＣＤＲＨ１、配列番号４８に示されるアミノ酸配列からなるＣＤＲＨ２及び配列番号４９に示されるアミノ酸配列からなるＣＤＲＨ３を有する。ｒＧ０６１抗体の配列を表１に示す。

[0066] さらに、「２．抗ＣＤＨ６抗体の製造」（ａ）乃至（ｈ）の工程を再度実施して別途に独立してモノクローナル抗体を取得した場合や他の方法によって別途にモノクローナル抗体を取得した場合においても、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体と同等の内在化活性を有する抗体を取得することが可能である。このような抗体の一例として、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体と同一のエピトープに結合する抗体を挙げることができる。新たに作製されたモノクローナル抗体が、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体の結合する部分ペプチド又は部分立体構造に結合すれば、該モノクローナル抗体がｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体と同一のエピトープに結合すると判定することができる。また、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体のＣＤＨ６に対する結合に対して該モノクローナル抗体が競合する（即ち、該モノクローナル抗体が、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体とＣＤＨ６の結合を妨げる）ことを確認することによって、具体的なエピトープの配列又は構造が決定されていなくても、該モノクローナル抗体が抗ＣＤＨ６抗体と同一のエピトープに結合すると判定することができる。エピトープが同一であることが確認された場合、該モノクローナル抗体がｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体と同等の抗原結合能、生物活性及び／又は内在化活性を有していることが強く期待される。

[0067] （３）その他の抗体

本発明の抗体には、上記ＣＤＨ６に対するモノクローナル抗体に加え、ヒトに対する異種抗原性を低下させること等を目的として人為的に改変した遺

伝子組換え型抗体、例えば、キメラ（Ｃｈｉｍｅｒｉｃ）抗体、ヒト化（Ｈｕｍａｎｉｚｅｄ）抗体又はヒト抗体等も含まれる。これらの抗体は、既知の方法を用いて製造することができる。

[0068] キメラ抗体としては、抗体の可変領域と定常領域が互いに異種である抗体、例えばマウス又はラット由来抗体の可変領域をヒト由来の定常領域に接合したキメラ抗体を挙げることができる（Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．Ｕ．Ｓ．Ａ．，８１，６８５１－６８５５，（１９８４）参照）。

[0069] ラット抗ヒトＣＤＨ６抗体由来のキメラ抗体の例としては、例えば、本明細書に記載のラット抗ヒトＣＤＨ６抗体（例えば、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体）の各軽鎖可変領域とヒト由来の定常領域を含む軽鎖、及び、各重鎖可変領域とヒト由来の定常領域を含む重鎖からなる抗体が挙げられる。

[0070] ラット抗ヒトＣＤＨ６抗体由来のキメラ抗体の別の例としては、例えば、本明細書に記載のラット抗ヒトＣＤＨ６抗体（例えば、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体）の各軽鎖可変領域中の１～数残基、１～３残基、１～２残基、好ましくは１残基のアミノ酸を別のアミノ酸残基に置換した軽鎖可変領域を含む軽鎖、及び、各重鎖可変領域中の１～数残基、１～３残基、１～２残基、好ましくは１残基のアミノ酸を別のアミノ酸残基に置換した重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。

[0071] ラット抗ヒトＣＤＨ６抗体由来のキメラ抗体の別の例としては、例えば、本明細書に記載のラット抗ヒトＣＤＨ６抗体（例えば、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体）の各軽鎖可変領域中のいずれか１～３個のＣＤＲ中の１～２残基、好ましくは１残基のアミノ酸を別のアミノ酸残基に置換した軽鎖可変領域を含む軽鎖、及び、各重鎖可変領域中のいずれか１～３個のＣＤＲ中の１～２残基、好ましくは１残基のアミノ酸を別のアミノ酸残基に置換した重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。

[0072] ｒＧ０１９抗体由来のキメラ抗体としては、例えば、配列番号１０に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号１５に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。

[0073] ｒＧ０１９抗体由来のキメラ抗体の別の例としては、例えば、配列番号１０に示されるアミノ酸配列からなる軽鎖可変領域中の１～数残基、１～３残基、１～２残基、好ましくは１残基のアミノ酸を別のアミノ酸残基に置換した軽鎖可変領域を含む軽鎖、及び、配列番号１５に示されるアミノ酸配列からなる重鎖可変領域中の１～数残基、１～３残基、１～２残基、好ましくは１残基のアミノ酸を別のアミノ酸残基に置換した重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。

[0074] ｒＧ０１９抗体由来のキメラ抗体の別の例としては、例えば、配列番号１０に示されるアミノ酸配列からなる軽鎖可変領域中のいずれか１～３個のＣＤＲ中の１又は２残基（好ましくは１残基）のアミノ酸を別のアミノ酸残基に置換した軽鎖可変領域を含む軽鎖、及び、配列番号１５に示されるアミノ酸配列からなる重鎖可変領域中のいずれか１～３個のＣＤＲ中の１又は２残基（好ましくは１残基）のアミノ酸を別のアミノ酸残基に置換した重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。

[0075] ｒＧ０１９抗体由来のキメラ抗体の別の例としては、例えば、配列番号１０に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号５８に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。配列番号５８に示されるアミノ酸配列は、配列番号１５に示されるアミノ酸配列中のＣＤＲＨ２中のシステイン残基をプロリン残基に置換した配列である。

[0076] ｒＧ０１９抗体由来のキメラ抗体の具体例としては、配列番号５３に示さ

れる軽鎖全長アミノ酸配列からなる軽鎖、及び、配列番号５６に示される重鎖全長アミノ酸配列からなる重鎖からなる抗体が挙げられ、当該キメラ抗ヒトＣＤＨ６抗体を本明細書中、「キメラＧ０１９抗体」、「ｃｈＧ０１９抗体」又は「ｃｈＧ０１９」と称する。ｃｈＧ０１９抗体の軽鎖全長アミノ酸配列は配列番号５４に示されるヌクレオチド配列でコードされ、ｃｈＧ０１９抗体の重鎖全長アミノ酸配列は配列番号５７に示されるヌクレオチド配列でコードされる。

[0077] ｃｈＧ０１９抗体の軽鎖可変領域アミノ酸配列は、ｒＧ０１９抗体の軽鎖可変領域アミノ酸配列と同一であり、配列番号１０に示されるアミノ酸配列からなる。ｃｈＧ０１９抗体の軽鎖は、配列番号１２に示されるアミノ酸配列からなるＣＤＲＬ１、配列番号１３に示されるアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に示されるアミノ酸配列からなるＣＤＲＬ３を有し、それぞれ、ｒＧ０１９の軽鎖のＣＤＲＬ１、ＣＤＲＬ２及びＣＤＲＬ３と同一である。ｃｈＧ０１９抗体の軽鎖可変領域アミノ酸は、配列番号５５に示されるヌクレオチド配列でコードされる。

[0078] ｃｈＧ０１９抗体の重鎖可変領域アミノ酸配列は、配列番号５８に示されるアミノ酸配列からなる。ｃｈＧ０１９抗体の重鎖は、配列番号１７に示されるアミノ酸配列からなるＣＤＲＨ１、配列番号６０に示されるアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に示されるアミノ酸配列からなるＣＤＲＨ３を有する。配列番号５８に示されるアミノ酸配列は、配列番号１５に示されるアミノ酸配列中のＣＤＲＨ２中のシステイン残基をプロリン残基に置換した配列である。配列番号６０に示されるアミノ酸配列からなるＣＤＲＨ２は、配列番号１８に示されるｒＧ０１９　ＣＤＲＨ２中のシステイン残基をプロリン残基に置換した配列である。ｃｈＧ０１９抗体の重鎖可変領域アミノ酸配列は、配列番号５９に示されるヌクレオチド配列でコードされる。

[0079] ｃｈＧ０１９抗体の配列を表１に示す。

[0080] ラット抗ヒトＣＤＨ６抗体ｒＧ０５５抗体由来のキメラ抗体としては、例

えば、配列番号２０に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号２５に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなるキメラ抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。

[0081] ラット抗ヒトＣＤＨ６抗体ｒＧ０５６抗体由来のキメラ抗体としては、例えば、配列番号３０に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号３５に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなるキメラ抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。

[0082] ラット抗ヒトＣＤＨ６抗体ｒＧ０６１抗体由来のキメラ抗体としては、例えば、配列番号４０に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号４５に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなるキメラ抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてよい。

[0083] ヒト化抗体としては、相補性決定領域（ＣＤＲ；Ｃｏｍｐｌｅｍｅｎｔａｒｉｔｙ Ｄｅｔｅｒｍｉｎｉｎｇ Ｒｅｇｉｏｎ）のみをヒト由来の抗体に組み込んだ抗体（Ｎａｔｕｒｅ（１９８６）３２１，ｐ．５２２－５２５参照）、ＣＤＲ移植法によって、ＣＤＲの配列に加え一部のフレームワークのアミノ酸残基もヒト抗体に移植した抗体（国際公開第９０／０７８６１号）、更に、抗原に対する結合能を維持しつつ、一部のＣＤＲのアミノ酸配列を改変した抗体を挙げることができる。

[0084] 本明細書において、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体又はｃｈＧ０１９抗体由来のヒト化抗体とは、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体又はｃｈＧ０１９抗体のいずれかについて、各々に固有の６つのＣＤＲ配列を全て保持し、かつ、内在化活性を有するヒト化抗体であればよく、特定のヒト化抗体に限定されない。当該ヒト化抗体は、内在化活性を有する限り、更に一部のＣＤＲの一部のアミノ酸配列が改変されていてもよい。

[0085] ｃｈＧ０１９抗体のヒト化抗体の実例としては、（１）配列番号６３又は６７に記載のアミノ酸配列、（２）上記（１）のアミノ酸配列に対して少なくとも９５％以上の同一性を有するアミノ酸配列（好ましくは、各ＣＤＲ配列以外のフレームワーク領域の配列に対して少なくとも９５％以上の配列同一性を有するアミノ酸配列）、及び（３）上記（１）のアミノ酸配列において１又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか１つに記載の軽鎖可変領域を含む軽鎖、並びに（４）配列番号７１、７５又は７９に記載のアミノ酸配列、（５）上記（４）のアミノ酸配列に対して少なくとも９５％以上の同一性を有するアミノ酸配列（好ましくは、各ＣＤＲ配列以外のフレームワーク領域の配列に対して少なくとも９５％以上の配列同一性を有するアミノ酸配列）、及び（６）上記（４）のアミノ酸配列において１又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか１つに記載の重鎖可変領域を含む重鎖の任意の組合せを挙げることができる。

[0086] また、重鎖又は軽鎖の一方をヒト化し、他方をラット抗体やキメラ抗体の軽鎖又は重鎖とした抗体も用いることができる。そのような抗体の例としては、（１）配列番号６３又は６７に記載のアミノ酸配列、（２）上記（１）のアミノ酸配列に対して少なくとも９５％以上の同一性を有するアミノ酸配列（好ましくは、各ＣＤＲ配列以外のフレームワーク領域の配列に対して少なくとも９５％以上の配列同一性を有するアミノ酸配列）、及び（３）上記（１）のアミノ酸配列において１又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか１つに記載の軽鎖可変領域を含む軽鎖、並びに（４）配列番号１５、２５、３５、４５又は５８に記載のアミノ酸配列、（５）上記（４）のアミノ酸配列に対して少なくとも９５％以上の同一性を有するアミノ酸配列（好ましくは、各ＣＤＲ配列以外のフレームワーク領域の配列に対して少なくとも９５％以上の配列同一性を有するアミノ酸配列）、及び（６）上記（４）のアミノ酸配列において１又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群か

ら選択されるいずれか１つに記載の重鎖可変領域を含む重鎖の任意の組合せを挙げることができる。他の例としては、（１）配列番号１０、２０、３０又は４０に記載のアミノ酸配列、（２）上記（１）のアミノ酸配列に対して少なくとも９５％以上の同一性を有するアミノ酸配列（好ましくは、各ＣＤＲ配列以外のフレームワーク領域の配列に対して少なくとも９５％以上の配列同一性を有するアミノ酸配列）、及び（３）上記（１）のアミノ酸配列において１又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか１つに記載の軽鎖可変領域を含む軽鎖、並びに（４）配列番号７１、７５又は７９に記載のアミノ酸配列、（５）上記（４）のアミノ酸配列に対して少なくとも９５％以上の同一性を有するアミノ酸配列（好ましくは、各ＣＤＲ配列以外のフレームワーク領域の配列に対して少なくとも９５％以上の配列同一性を有するアミノ酸配列）、及び（６）上記（４）のアミノ酸配列において１又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか１つに記載の重鎖可変領域を含む重鎖の任意の組合せを挙げることができる。

[0087] また、本明細書中におけるアミノ酸の置換としては保存的アミノ酸置換が好ましい。保存的アミノ酸置換とは、アミノ酸側鎖に関連のあるアミノ酸グループ内で生じる置換である。好適なアミノ酸グループは、以下のとおりである：酸性グループ＝アスパギン酸、グルタミン酸；塩基性グループ＝リシン、アルギニン、ヒスチジン；非極性グループ＝アラニン、バリン、ロイシン、イソロイシン、プロリン、フェニルアラニン、メチオニン、トリプトファン；及び非帯電極性ファミリー＝グリシン、アスパラギン、グルタミン、システイン、セリン、スレオニン、チロシン。他の好適なアミノ酸グループは次のとおりである：脂肪族ヒドロキシグループ＝セリン及びスレオニン；アミド含有グループ＝アスパラギン及びグルタミン；脂肪族グループ＝アラニン、バリン、ロイシン及びイソロイシン；並びに芳香族グループ＝フェニルアラニン、トリプトファン及びチロシン。かかるアミノ酸置換は元のアミノ酸配列を有する物質の特性を低下させない範囲で行うのが好ましい。

[0088] 上記軽鎖及び重鎖の好適な組合せの抗体としては、配列番号６３に示される軽鎖可変領域アミノ酸配列（本明細書中、ｈＬ０２軽鎖可変領域アミノ酸配列とも称する）を有する軽鎖又は配列番号６７に示される軽鎖可変領域アミノ酸配列（本明細書中、ｈＬ０３軽鎖可変領域アミノ酸配列とも称する）を有する軽鎖、及び、配列番号７１に示される重鎖可変領域アミノ酸配列（本明細書中、ｈＨ０１重鎖可変領域アミノ酸配列とも称する）を有する重鎖、配列番号７５に示される重鎖可変領域アミノ酸配列（本明細書中、ｈＨ０２重鎖可変領域アミノ酸配列とも称する）を有する重鎖又は配列番号７９に示される重鎖可変領域アミノ酸配列（本明細書中、ｈＨ０４重鎖可変領域アミノ酸配列とも称する）を有する重鎖からなる抗体が挙げられる。好ましい例としては、配列番号６３に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７１に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号６３に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７５に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号６３に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７９に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号６７に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７１に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号６７に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７５に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；又は、配列番号６７に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７９に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体が挙げられる。より好ましい例としては、配列番号６３に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７１に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号６３に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７５に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号６３に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７９に示される重鎖可変領域ア

ミノ酸配列を有する重鎖からなる抗体；又は、配列番号６７に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号７５に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体が挙げられる。

[0089] 上記軽鎖及び重鎖の好適な組合せの抗体の別の例としては、配列番号６１に示される軽鎖全長アミノ酸配列（本明細書中、ｈＬ０２軽鎖全長アミノ酸配列とも称する）の２１～２３３番目のアミノ酸配列からなる軽鎖又は配列番号６５に示される軽鎖全長アミノ酸配列（本明細書中、ｈＬ０３軽鎖全長アミノ酸配列とも称する）の２１～２３３番目のアミノ酸配列からなる軽鎖、及び、配列番号６９に示される重鎖全長アミノ酸配列（本明細書中、ｈＨ０１重鎖全長アミノ酸配列とも称する）の２０～４７１番目のアミノ酸配列からなる重鎖、配列番号７３に示される重鎖全長アミノ酸配列（本明細書中、ｈＨ０２重鎖全長アミノ酸配列とも称する）の２０～４７１番目のアミノ酸配列からなる重鎖又は配列番号７７に示される重鎖全長アミノ酸配列（本明細書中、ｈＨ０４重鎖全長アミノ酸配列とも称する）の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体が挙げられる。好ましい例としては、配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；配列番号６５に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；配列番号６５に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３に示される重鎖全長

アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；又は、配列番号６５に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体が挙げられる。より好ましい例としては、配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体（本明細書中、「Ｈ０１Ｌ０２抗体」又は「Ｈ０１Ｌ０２」とも称する）；配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体（本明細書中、「Ｈ０２Ｌ０２抗体」又は「Ｈ０２Ｌ０２」とも称する）；配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体（本明細書中、「Ｈ０４Ｌ０２抗体」又は「Ｈ０４Ｌ０２」とも称する）；又は、配列番号６５に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体（本明細書中、「Ｈ０２Ｌ０３抗体」又は「Ｈ０２Ｌ０３」とも称する）が挙げられる。Ｈ０１Ｌ０２抗体、Ｈ０２Ｌ０２抗体、Ｈ０２Ｌ０３抗体又はＨ０４Ｌ０２抗体の配列を表１に示す。

[0090] 上記の重鎖アミノ酸配列及び軽鎖アミノ酸配列と高い同一性を示す配列を組み合わせることによって、上記の各抗体と同等の生物活性を有する抗体を選択することが可能である。このような同一性は、一般的には８０％以上の同一性であり、好ましくは９０％以上の同一性であり、より好ましくは９５％以上の同一性であり、最も好ましくは９９％以上の同一性である。また、重鎖又は軽鎖のアミノ酸配列に１乃至数個のアミノ酸残基が置換、欠失又は

付加されたアミノ酸配列を組み合わせることによっても、上記の各抗体と同等の生物活性を有する抗体を選択することが可能である。

[0091] 二種類のアミノ酸配列間の同一性は、ＣｌｕｓｔａｌＷ ｖｅｒｓｉｏｎ ２ （Ｌａｒｋｉｎ ＭＡ, Ｂｌａｃｋｓｈｉｅｌｄｓ Ｇ, Ｂｒｏｗｎ ＮＰ, Ｃｈｅｎｎａ Ｒ, ＭｃＧｅｔｔｉｇａｎ ＰＡ, ＭｃＷｉｌｌｉａｍ Ｈ, Ｖａｌｅｎｔｉｎ Ｆ, Ｗａｌｌａｃｅ ＩＭ, Ｗｉｌｍ Ａ, Ｌｏｐｅｚ Ｒ, Ｔｈｏｍｐｓｏｎ ＪＤ, Ｇｉｂｓｏｎ ＴＪ ａｎｄ Ｈｉｇｇｉｎｓ ＤＧ（２００７），「Ｃｌｕｓｔａｌ Ｗ ａｎｄ Ｃｌｕｓｔａｌ Ｘ ｖｅｒｓｉｏｎ ２．０」, Ｂｉｏｉｎｆｏｒｍａｔｉｃｓ．２３（２１）：２９４７－２９４８）のデフォルトパラメーターを使用して配列を整列させることによって決定することができる。

[0092] なお、配列番号６１に示されるｈＬ０２軽鎖全長アミノ酸配列中、１～２０番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、２１～１２８番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、１２９～２３３番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号６２に示されるｈＬ０２軽鎖全長ヌクレオチド配列中、１～６０番目のヌクレオチドからなるヌクレオチド配列はシグナル配列をコードし、６１～３８４番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードし、３８５～６９９番目のヌクレオチドからなるヌクレオチド配列は定常領域をコードしている。

[0093] 配列番号６５に示されるｈＬ０３軽鎖全長アミノ酸配列中、１～２０番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、２１～１２８番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、１２９～２３３番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号６６に示されるｈＬ０３軽鎖全長ヌクレオチド配列中、１～６０番目のヌクレオチドからなるヌクレオチド配列はシグナル配列をコードし、６１～３８４番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードし、３８

５～６９９番目のヌクレオチドからなるヌクレオチド配列は定常領域をコードしている。

[0094] 配列番号６９に示されるｈＨ０１重鎖全長アミノ酸配列中、１～１９番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、２０～１４１番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、１４２～４７１番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号７０に示されるｈＨ０１重鎖全長ヌクレオチド配列中、１～５７番目のヌクレオチドからなるヌクレオチド配列はシグナル配列をコードし、５８～４２３番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードし、４２４～１４１３番目のヌクレオチドからなるヌクレオチド配列は定常領域をコードしている。

[0095] 配列番号７３に示されるｈＨ０２重鎖全長アミノ酸配列中、１～１９番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、２０～１４１番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、１４２～４７１番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号７４に示されるｈＨ０２重鎖全長ヌクレオチド配列中、１～５７番目のヌクレオチドからなるヌクレオチド配列はシグナル配列をコードし、５８～４２３番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードし、４２４～１４１３番目のヌクレオチドからなるヌクレオチド配列は定常領域をコードしている。

[0096] 配列番号７７に示されるｈＨ０４重鎖全長アミノ酸配列中、１～１９番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、２０～１４１番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、１４２～４７１番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号７８に示されるｈＬ０４重鎖全長ヌクレオチド配列で、１～５７番目のヌクレオチドからなるヌクレオチド配列はシグナル配列であり、５８～４２３番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードしており、４２４～１４１３番目のヌクレオチドからなるヌクレオチド配列は定常領域を

コードしている。

[0097] [表1-1]

配列番号		配列
1	ヒトCDH6 ORFのアミノ酸配列	MRTYRYFLLLFWVGQPYPTLSTPLSKRTSGFPAKKRALELSGNSKNELNRSKRSWMW NQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIQATK RLDREEKPVYILRAQAINRRTGRPVEPESEFIIKIHDINDNEPIFTKEVYTATVPEM SDVGTFVVQVTATDADDPTYGNSAKVVYSILQGQPYFSVESETGIIKTALLNMDREN REQYQVVIQAKDMGGQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPPGT PIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQETQEGIITVKKLLDFEKKKV YTLKVEASNPYVEPRFLYLGPFKDSATVRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLDRETLLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQTLHAVD KDDPYSGHQFSFSLAPEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHPTGLSTGALVAILLCIVILL VTVVLFAALRRQRKKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIE DNKLRRDIVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPYDSLATYAYE GTGSVADSLSSLESVTTDADQDYDYLSDWGPRFKKLADMYGGVDSDKDS
2	ヒトCDH6 EC1	SWMWNQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDI QATKRLDREEKPVYILRAQAINRRTGRPVEPESEFIIKIHDINDNEPIF
3	ヒトCDH6 EC2	TKEVYTATVPEMSDVGTFVVQVTATDADDPTYGNSAKVVYSILQGQPYFSVESETGI IKTALLNMDRENREQYQVVIQAKDMGGQMGGLSGTTTVNITLTDVNDNPPRF
4	ヒトCDH6 EC3	PQSTYQFKTPESSPPGTPIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQETQ EGIITVKKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFKDSATVRIVVEDVDEPPV F
5	ヒトCDH6 EC4	SKLAYILQIREDAQINTTIGSVTAQDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSI FTSKLLDRETLLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAP
6	ヒトCDH6 EC5	EFAEFYETFVCEKAKADQLIQTLHAVDKDDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPVVISDNDYPVQSSTGTVTVRVCACDHHGNMQSC HAEALIHP
7	マウスCDH6 ORFのアミノ酸配列	MRTYRYFLLLFWVGQPYPTFSNPLSKRTSGFPAKRKALELSANSRNELSRSKRSWMW NQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIQATK RLDREEKPVYILRAQAVNRRTGRPVEPESEFIIKIHDINDNEPIFTKDVYTATVPEM ADVGTFVVQVTATDADDPTYGNSAKVVYSILQGQPYFSVESETGIIKTALLNMDREN REQYQVVIQAKDMGGQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPPGT PIGRIKASDADVGENAEIEYSITDGEGHEMFDVITDQETQEGIITVKKLLDFEKKKV YTLKVEASNPHVEPRFLYLGPFKDSATVRIVVDDVDEPPVFSKLAYILQIREDARIN TTIGSVAAQDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLDRETLLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQTLRAVD KDDPYSGHQFSFSLAPEAASSSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHPTGLSTGALVAILLCIVILL VTVVLFAALRRQRKKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAME DSKSRRDIVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPYDSLATYAYE GTGSVADSLSSLESVTTDGDQDYDYLSDWGPRFKKLADMYGGMDSDKDS

[0098]

[表1-2]

8	ラットCDH6 ORFのアミノ酸配列	MRTYRYFLLLFWVGQPYPTFSNPLSKRTSGFPAKRRALELSANSRNELSRSKRSWMW NQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIQATK RLDREEKPVYILRAQAINRRTGRPVEPESEFIIKIHDINDNEPIFTKDVYTATVPEM ADVGTFVVQVTATDADDPTYGNSAKVVYSILQGQPYFSVESETGIIKTALLNMDREN REQYQVVIQAKDMGGQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPPGT PIGRIKASDADVGENAEIEYSITDGEGHDMFDVITDQETQEGIITVKKLLDFEKKRV YTLKVEASNPHIEPRFLYLGPFKDSATVRIVVDDVDEPPVFSKPAYILQIREDAQIN TTIGSVAAQDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLDRETLLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQTLHAVD KDDPYSGHQFSFSLAPEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHPTGLSTGALVAILLCIVILL VTVVLFAALRRQRKKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDIGTLRNPKPWR QQSRRDMVPEALFLPRRTPTARDNTDVRDFISQRLRKMNTDPTAPPYDSLATYAYEG TGSVADSLSSLESVTTDGDQDYGYLSDWGPRFKKLADMYGGMDSDKDS
9	カニクイザル CDH6 ORFのアミノ酸配列	MRTYRYFLLLFWVGQPYPTLSTPLSKRTSGFPAKKRALELSGNSKNELNRSKRSWMW NQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIQATK RLDREEKPVYILRAQAINRRTGRPVEPESEFIIKIHDINDNEPIFTKEVYTATVPEM SDVGTFVVQVTATDADDPTYGNSAKVVYSILQGQPYFSVESETGIIKTALLNMDREN REQYQVVIQAKDMGGQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPPGT PIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQETQEGIITVKKLLDFEKKKV YTLKVEASNPHVEPRFLYLGPFKDSATVRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLDRETLLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQTLRAVD KDDPYSGHQFSFSLAPEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHPTGLSTGALVAILLCIVILL VTVVLFAALRRQRKKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIE DNKLRRDIVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPYDSLATYAYE GTGSVADSLSSLESVTTDGDQDYDYLSDWGPRFKKLADMYGGVDSDKDS
10	rGO19軽鎖可変領域アミノ酸配列	DIQMTQSPSLLSASVGDRVTLNCKASQNIYKNLAWYQQKLGEGPKLLIYDANTLQTG IPSRFSGSGSGSDFTLTISSLQPEDVATYFCQQYYSGWAFGGVTNLELKRA
11	rGO19軽鎖可変領域ヌクレオチド配列	GACATCCAGATGACCCAGTCTCCTTCACTCCTGTCTGCATCTGTGGGAGACAGAGTC ACTCTCAACTGCAAAGCAAGTCAGAATATTTATAAGAACTTAGCCTGGTATCAGCAA AAGCTTGGAGAAGGTCCCAAACTCCTGATTTATGATGCAAACACTTTGCAAACGGGC ATCCCATCAAGGTTCAGTGGCAGTGGATCTGGTTCAGATTTCACACTCACCATCAGC AGCCTGCAGCCTGAAGATGTTGCCACATATTTCTGCCAGCAGTACTATAGCGGGTGG GCGTTCGGTGGAGTCACCAACCTGGAATTGAAACGGGCT
12	rGO19 CDRL1	KASQNIYKNLA
13	rGO19 CDRL2	DANTLQT
14	rGO19 CDRL3	QQYYSGWA

[0099]

[表1-3]

15	rGO19重鎖可変領域アミノ酸配列	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFMHWIKQQPGNGLEWIGWIYCGDGETEYNQKFNGKATLTADRSSSTAYMELSRLTSEDSAVYFCARGVYGGFAGGYFDFWGQGVMVTVSS
16	rGO19重鎖可変領域ヌクレオチド配列	CAGGTACAGCTGCAGCAATCTGGGGCTGAACTGGTGAAGCCTGGGTCCTCAGTGAAAATTTCCTGCAAGGCTTCTGGCTACACCTTCACCAGGAACTTTATGCACTGGATAAAACAGCAGCCTGGAAATGGCCTTGAGTGGATTGGGTGGATTTATTGTGGAGATGGTGAGACAGAGTACAATCAAAAGTTCAATGGGAAGGCAACACTCACTGCGGACAGATCCTCCAGCACAGCCTATATGGAGCTCAGCAGACTGACATCTGAGGACTCTGCAGTCTATTTCTGTGCAAGAGGGGTTTACGGAGGGTTTGCCGGGGGCTACTTTGATTTCTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
17	rGO19 CDRH1	GYTFTRNFMH
18	rGO19 CDRH2	WIYCGDGETE
19	rGO19 CDRH3	GVYGGFAGGYFDF
20	rGO55軽鎖可変領域アミノ酸配列	DVQMTHSPSYLAASPGESVSISCKTSKNISNYLVWYQQKPGEAYKLLIYSGSTLQSGTPSRFSGSGSGTDFTLTIRSLEPEDFGLYFCQQYYEKPFTFGSGTKLEIKRA
21	rGO55軽鎖可変領域ヌクレオチド配列	GATGTCCAGATGACCCACTCTCCGTCTTATCTTGCTGCGTCTCCTGGAGAAAGTGTTTCCATCAGTTGCAAGACAAGTAAGAACATTAGTAATTATTTAGTCTGGTATCAACAGAAACCTGGGGAAGCATATAAGCTTCTTATCTATTCTGGGTCAACTTTGCAATCTGGAACTCCATCAAGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACTCTCACTATCAGAAGCCTGGAGCCTGAAGATTTTGGACTCTATTTCTGTCAACAGTATTATGAAAAACCATTCACGTTCGGCTCAGGGACGAAGTTGGAAATAAAACGGGCT
22	rGO55 CDRL1	KTSKNISNYLV
23	rGO55 CDRL2	SGSTLQS
24	rGO55 CDRL3	QQYYEKPFT
25	rGO55重鎖可変領域アミノ酸配列	EVQLQESGPGLVRPSQSLSLSCSVTDYSITSNYWGWIRRFPGNKMEWMGYITYSGYTSYNPSLQSRISITRDTSKNQFFLQLNSVTAEDTATYYCARSINHGGYSYVVDAWGPGASVTVSS
26	rGO55重鎖可変領域ヌクレオチド配列	GAGGTGCAACTTCAGGAGTCAGGACCTGGCCTTGTGAGACCCTCACAGTCACTCTCCCTCTCCTGTTCTGTCACTGATTACTCCATCACTAGTAATTACTGGGGCTGGATCCGGAGGTTCCCAGGAAATAAAATGGAGTGGATGGGATACATAACCTATAGTGGTTACACTAGCTACAACCCATCTCTCCAAAGTCGAATCTCCATTACTAGAGACACATCGAAGAATCAGTTCTTCCTGCAGTTGAACTCTGTAACTGCTGAGGACACAGCCACATATTACTGTGCAAGATCGATTAACCACGGAGGATATAGTTATGTTGTGGATGCCTGGGGTCCGGGAGCTTCAGTCACTGTCTCCTCA
27	rGO55 CDRH1	DYSITSNYWG
28	rGO55 CDRH2	YITYSGYTS
29	rGO55 CDRH3	SINHGGYSYVVDA

[0100]

[表1-4]

30	rGO56軽鎖可変領域アミノ酸配列	DVQMTQSPSSLAASPGESVSISCRATKSIGIYLAWYQQKPGKTFKLLIYSGSTLQSG TPSRFSGSGSGTDFTLTIRSLEPEDFGLYFCQQFYENPFTFGSGTKLEIRRA
31	rGO56軽鎖可変領域ヌクレオチド配列	GATGTCCAGATGACCCAGTCTCCGTCTTCTCTTGCTGCGTCTCCTGGAGAAAGTGTT TCCATCAGTTGCAGGGCAACTAAGAGCATTGGTATTTATTTAGCCTGGTATCAACAG AAACCTGGGAAAACATTTAAGCTTCTTATCTACTCTGGGTCAACTTTGCAATCTGGA ACTCCATCAAGGTTCAGTGGCAGTGGGTCTGGTACAGATTTCACTCTCACCATCAGA AGCCTGGAGCCTGAAGATTTTGGACTCTATTTCTGTCAACAGTTTTATGAAAACCCA TTCACGTTCGGCTCAGGGACGAAGTTGGAAATAAGACGGGCT
32	rGO56 CDRL1	RATKSIGIYLA
33	rGO56 CDRL2	SGSTLQS
34	rGO56 CDRL3	QQFYENPFT
35	rGO56重鎖可変領域アミノ酸配列	EVQLQESGPGLVKPSQSLSLTCSVTDYSITTYFWGWIRKFPGNKMEWMGYMSYRGGT SYNPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCARCPNYGGHSLVFDYWGQG VMVTVSS
36	rGO56重鎖可変領域ヌクレオチド配列	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTGAAACCCTCACAGTCACTCTCC CTCACCTGTTCTGTCACTGATTACTCCATCACTACTTATTTCTGGGGCTGGATCCGG AAGTTCCCAGGAAATAAAATGGAGTGGATGGGATACATGAGCTACCGTGGTGGCACT TCCTACAACCCATCTCTCAAGAGTCGAATCTCCATTACTAGAGACACATCGAAGAAT CAGTTCTTCCTGCAGTTGAACTCTGTAACTACTGAGGACACAGCCACATATTACTGT GCAAGATGCCCTAACTACGGAGGGCATTCCCTTGTTTTTGATTACTGGGGCCAAGGA GTCATGGTCACAGTGTCCTCA
37	rGO56 CDRH1	DYSITTYFWG
38	rGO56 CDRH2	YMSYRGGTS
39	rGO56 CDRH3	CPNYGGHSLVFDY
40	rGO61軽鎖可変領域アミノ酸配列	DVQMTQSPSYLAASPGESVSISCKATKSISNYLAWYQQKPGEAYKVLIYSGSTLQSG TPSRFSGSGSGTDFTLTIRSLEPEDFGLYSCQQYYEKPLTFGSGTKLEIKRA
41	rGO61軽鎖可変領域ヌクレオチド配列	GATGTCCAGATGACCCAGTCTCCGTCTTATCTTGCTGCGTCTCCTGGAGAAAGTGTT TCCATCAGTTGCAAGGCAACTAAGAGCATTAGTAATTATTTAGCCTGGTATCAACAG AAACCTGGGGAAGCATATAAGGTTCTTATCTATTCTGGGTCAACTTTGCAATCTGGA ACTCCATCAAGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACTCTCACCATCAGA AGCCTGGAGCCTGAAGATTTTGGACTCTATTCCTGTCAACAGTATTATGAAAAACCG CTCACGTTCGGTTCTGGGACCAAGCTGGAGATCAAACGGGCT
42	rGO61 CDRL1	KATKSISNYLA
43	rGO61 CDRL2	SGSTLQS
44	rGO61 CDRL3	QQYYEKPLT

[0101]

[表1-5]

45	rGO61重鎖可変領域アミノ酸配列	EVQLQESGPGLVKPSQSLSLTCSVTGYSITTYYWGWIRKFPGNKMEWMGYISYSGRTSYNPSLKSRMSITRDASKNQFFLQLNSVTTDDTATYYCARSPINHGGYWYFDFWGPGTMVTVSS
46	rGO61重鎖可変領域ヌクレオチド配列	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTGAAACCCTCACAGTCACTCTCCCTCACCTGTTCTGTCACTGGTTACTCCATCACTACTTATTACTGGGGCTGGATCCGGAAGTTCCCAGGAAATAAAATGGAGTGGATGGGGTACATAAGCTACAGTGGTCGCACTAGTTATAACCCATCTCTCAAAAGTCGAATGTCCATTACTAGAGACGCATCGAAGAATCAGTTCTTCCTACAGTTGAACTCTGTAACTACTGACGACACAGCCACATATTACTGTGCAAGATCCCCAATTAACCACGGAGGGTACTGGTACTTTGACTTCTGGGGCCCAGGAACCATGGTCACCGTGTCCTCA
47	rGO61 CDRH1	GYSITTYYWG
48	rGO61 CDRH2	YISYSGRTS
49	rGO61 CDRH3	SPINHGGYWYFDF
50	ヒト軽鎖シグナル配列及びヒトκ鎖定常領域をコードするDNA配列を含むDNA断片	gcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGATATCGTGATGATTAAACGTACGGTGGCCGCCCCCTCCGTGTTCATCTTCCCCCCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCCAGAGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGCCTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGTtaggggcccgtttaaacgggggaggcta

[0102]

[表1-6]

51	ヒト重鎖シグナル配列及びヒトIgG1定常領域をコードするDNA配列を含むDNA断片	gcctccggactctagagccaccATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGC AGCTCCCAGATGGGTGCTGAGCCAGGTGCAATTGTGCAGGCGGTTAGCTCAGCCTCC ACCAAGGGCCCAAGCGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGCGGC ACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCCGTGACCGTGAGC TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCCGCTGTCCTGCAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGA GTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCCTGCCCAGCACCTGAA CTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATG ATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCT GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG CCCCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTG CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCCAGCCCCGGGAACCACAG GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGC CAGCCCGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACTCCGACGGCTCCTTC TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGCAACGTCTTC TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGAGCCTCTCC CTGTCTCCCGGCAAAtgagatatcgggcccgtttaaacgggggaggcta
52	chGO19軽鎖をコードするDNA配列を含むDNA断片	ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTCATCAGCCT GCTGCTGTGGATCAGCGGCGCCTACGGCGACATCCAGATGACCCAGAGCCCTAGCCT GCTGAGCGCCAGCGTGGGCGATAGAGTGACCCTGAACTGCAAGGCCAGCCAGAACAT CTACAAGAACCTGGCCTGGTATCAGCAGAAGCTGGGCGAGGGCCCCAAGCTGCTGAT CTACGACGCCAACACCCTGCAGACCGGCATCCCCAGCAGATTTTCTGGCAGCGGCAG CGGCTCCGACTTCACCCTGACAATCAGCAGCCTGCAGCCCGAGGACGTGGCCACCTA CTTTTGCCAGCAGTACTACAGCGGCTGGGCCTTCGGCGGCGTGACCAACCTGGAACT GAAGAGAGCCGTGGCCGCTCCCTCCGTGTTCATCTTCCCACCTAGCGACGAGCAGCT GAAGTCCGGCACAGCCTCTGTCGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGC CAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGTCTGGCAACAGCCAGGAAAGCGT GACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGACCCTGAG CAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCT GTCTAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGTtgagtttaaacgggggaggctaact
53	chGO19軽鎖全長アミノ酸配列	MVLQTQVFISLLLWISGAYGDIQMTQSPSLLSASVGDRVTLNCKASQNIYKNLAWYQ QKLGEGPKLLIYDANTLQTGIPSRFSGSGSGSDFTLTISSLQPEDVATYFCQQYYSG WAFGGVTNLELKRAVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC

[0103]

[表1-7]

54	chGO19軽鎖全長ヌクレオチド配列	ATGGTGCTGCAGACCCAGGTGTTCATCAGCCTGCTGCTGTGGATCAGCGGCGCCTAC GGCGACATCCAGATGACCCAGAGCCCTAGCCTGCTGAGCGCCAGCGTGGGCGATAGA GTGACCCTGAACTGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAG CAGAAGCTGGGCGAGGGCCCCAAGCTGCTGATCTACGACGCCAACACCCTGCAGACC GGCATCCCCAGCAGATTTTCTGGCAGCGGCAGCGGCTCCGACTTCACCCTGACAATC AGCAGCCTGCAGCCCGAGGACGTGGCCACCTACTTTTGCCAGCAGTACTACAGCGGC TGGGGCTTCGGCGGCGTGACCAACCTGGAACTGAAGAGAGCCGTGGCCGCTCCCTCC GTGTTCATCTTCCCACCTAGCGACGAGCAGCTGAAGTCCGGCACAGCCTCTGTCGTG TGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAAT GCCCTGCAGTCTGGCAACAGCCAGGAAAGCGTGACCGAGCAGGACAGCAAGGACTCC ACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAG GTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTC AACCGGGGGCGAGTGT
10	chGO19軽鎖可変領域アミノ酸配列	DIQMTQSPSLLSASVGDRVTLNCKASQNIYKNLAWYQQKLGEGPKLLIYDANTLQTG IPSRFSGSGSGSDFTLTISSLQPEDVATYFCQQYYSGWAFGGVTNLELKRA
55	chGO19軽鎖可変領域ヌクレオチド配列	GACATCCAGATGACCCAGAGCCCTAGCCTGCTGAGCGCCAGCGTGGGCGATAGAGTG ACCCTGAACTGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAGCAG AAGCTGGGCGAGGGCCCCAAGCTGCTGATCTACGACGCCAACACCCTGCAGACCGGC ATCCCCAGCAGATTTTCTGGCAGCGGCAGCGGCTCCGACTTCACCCTGACAATCAGC AGCCTGCAGCCCGAGGACGTGGCCACCTACTTTTGCCAGCAGTACTACAGCGGCTGG GCCTTCGGCGGCGTGACCAACCTGGAACTGAAGAGAGCC
12	chGO19 CDRL1	KASQNIYKNLA
13	chGO19 CDRL2	DANTLQT
14	chGO19 CDRL3	QQYYSGWA
56	chGO19重鎖全長アミノ酸配列	MKHLWFFLLLVAAPRWVLSQVQLQQSGAELVKPGSSVKISCKASGYTFTRNFMHWIK QQPGNGLEWIGWIYPGDGETEYNQKFNGKATLTADRSSSTAYMELSRLTSEDSAVYF CARGVYGGFAGGYFDFWGQGVMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK

[0104]

[表1-8]

57	chGO19重鎖全長ヌクレオチド配列	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGC CAGGTGCAGCTGCAGCAGTCTGGCGCCGAGCTCGTGAAGCCTGGCAGCAGCGTGAAG ATCAGCTGCAAGGCCAGCGGCTACACCTTCACCCGGAACTTCATGCACTGGATCAAG CAGCAGCCCGGCAACGGCCTGGAATGGATCGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACAACCAGAAGTTCAACGGCAAGGCCACCCTGACCGCCGACAGAAGCAGC TCCACCGCCTACATGGAACTGAGCCGGCTGACCAGCGAGGACAGCGCCGTGTACTTT TGCGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTTGGGGCCAG GGCGTGATGGTCACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTCCCCCTG GCACCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACTCAGGCGCCCTGACCAGCGGC GTGCACACCTTCCCCGCTGTCCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACT CACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTC TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCCGGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACC CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACCCAGAAGAGCCTCTCCCTGTCTCCCGGCAAA
58	chGO19重鎖可変領域アミノ酸配列	QVQLQQSGAELVKPGSSVKISCKASGYTFTRNFMHWIKQQPGNGLEWIGWIYPGDGE TEYNQKFNGKATLTADRSSSTAYMELSRLTSEDSAVYFCARGVYGGFAGGYFDFWGQ GVMVTVSS
59	chGO19重鎖可変領域ヌクレオチド配列	CAGGTGCAGCTGCAGCAGTCTGGCGCCGAGCTCGTGAAGCCTGGCAGCAGCGTGAAG ATCAGCTGCAAGGCCAGCGGCTACACCTTCACCCGGAACTTCATGCACTGGATCAAG CAGCAGCCCGGCAACGGCCTGGAATGGATCGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACAACCAGAAGTTCAACGGCAAGGCCACCCTGACCGCCGACAGAAGCAGC TCCACCGCCTACATGGAACTGAGCCGGCTGACCAGCGAGGACAGCGCCGTGTACTTT TGCGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTTGGGGCCAG GGCGTGATGGTCACCGTCAGCTCA
17	chGO19 CDRH1	GYTFTRNFMH
60	chGO19 CDRH2	WIYPGDGETE
19	chGO19 CDRH3	GVYGGFAGGYFDF

[0105]

[表1-9]

61	hLO2軽鎖全長アミノ酸配列	MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTITCKASQNIYKNLAWYQQKPGKAPKLLIYDANTLQTGVPSRFSGSGSGSDFTLTISSLQPEDFATYFCQQYYSGWAFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
62	hLO2軽鎖全長ヌクレオチド配列	ATGGTGCTGCAGACCCAGGTGTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACGACGCCAACACCCTGCAGACCGGCGTGCCCAGCAGATTTTCTGGCAGCGGCAGCGGCTCCGACTTCACCCTGACAATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTTTTGCCAGCAGTACTACAGCGGCTGGGCCTTCGGCCAGGGCACCAAGGTGGAAATCAAGCGTACGGTGGCCGCCCCCTCCGTGTTCATCTTCCCCCCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCCAGAGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGCCTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGT
63	hLO2軽鎖可変領域アミノ酸配列	DIQMTQSPSSLSASVGDRVTITCKASQNIYKNLAWYQQKPGKAPKLLIYDANTLQTGVPSRFSGSGSGSDFTLTISSLQPEDFATYFCQQYYSGWAFGQGTKVEIKRT
64	hLO2軽鎖可変領域ヌクレオチド配列	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACGACGCCAACACCCTGCAGACCGGCGTGCCCAGCAGATTTTCTGGCAGCGGCAGCGGCTCCGACTTCACCCTGACAATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTTTTGCCAGCAGTACTACAGCGGCTGGGCCTTCGGCCAGGGCACCAAGGTGGAAATCAAGCGTACG
65	hLO3軽鎖全長アミノ酸配列	MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTITCKASQNIYKNLAWYQQKLGEGPKLLIYDANTLQTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSGWAFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0106]

[表1-10]

66	hLO3軽鎖全長ヌクレオチド配列	ATGGTGCTGCAGACCCAGGTGTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTAC GGCGACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGA GTGACCATCACATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAG CAGAAGCTGGGCGAGGGCCCCAAGCTGCTGATCTACGACGCCAACACCCTGCAGACC GGCGTGCCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCCTGACAATC AGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACTACAGCGGC TGGGCCTTTGGCCAGGGCACCAAGGTGGAAATCAAGCGTACGGTGGCCGCCCCCTCC GTGTTCATCTTCCCCCCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTG TGCCTGCTGAATAACTTCTACCCCAGAGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGC ACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGAGCTCCCCCGTCACCAAGAGCTTC AACAGGGGGGAGTGT
67	hLO3軽鎖可変領域アミノ酸配列	DIQMTQSPSSLSASVGDRVTITCKASQNIYKNLAWYQQKLGEGPKLLIYDANTLQTG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSGWAFGQGTKVEIKRT
68	hLO3軽鎖可変領域ヌクレオチド配列	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTG ACCATCACATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAGCAG AAGCTGGGCGAGGGCCCCAAGCTGCTGATCTACGACGCCAACACCCTGCAGACCGGC GTGCCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCCTGACAATCAGC AGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACTACAGCGGCTGG GCCTTTGGCCAGGGCACCAAGGTGGAAATCAAGCGTACG
69	hHO1重鎖全長アミノ酸配列	MKHLWFFLLLVAAPRWVLSEVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFMHWVR QAPGQGLEWMGWIYPGDGETEYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYY CARGVYGGFAGGYFDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK

[0107]

[表1-11]

70	hH01重鎖全長ヌクレオチド配列	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGC GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCCTGCAAGGCCAGCGGCTACACCTTTACCCGGAACTTCATGCACTGGGTGCGC CAGGCTCCAGGCCAGGGACTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACGCCCAGAAATTCCAGGGCAGAGTGACCATCACCGCCGACACCAGCACC TCCACCGCCTACATGGAACTGAGCAGCCTGCGGAGCGAGGACACCGCCGTGTACTAT TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTTGGGGCCAG GGCACCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTCCCCCTG GCACCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACTCAGGCGCCCTGACCAGCGGC GTGCACACCTTCCCCGCTGTCCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACT CACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTC TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCCGGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACC CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACCCAGAAGAGCCTCTCCCTGTCTCCCGGCAAA
71	hH01重鎖可変領域アミノ酸配列	EVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFMHWVRQAPGQGLEWMGWIYPGDGE TEYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCARGVYGGFAGGYFDFWGQ GTLVTVSS
72	hH01重鎖可変領域ヌクレオチド配列	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCCTGCAAGGCCAGCGGCTACACCTTTACCCGGAACTTCATGCACTGGGTGCGC CAGGCTCCAGGCCAGGGACTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACGCCCAGAAATTCCAGGGCAGAGTGACCATCACCGCCGACACCAGCACC TCCACCGCCTACATGGAACTGAGCAGCCTGCGGAGCGAGGACACCGCCGTGTACTAT TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTTGGGGCCAG GGCACCCTCGTGACCGTCAGCTCA

[0108]

[表1-12]

73	hH02重鎖全長アミノ酸配列	MKHLWFFLLLVAAPRWVLSEVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFMHWVR QAPGQGLEWMGWIYPGDGETEYNQKFQGRVTITADRSTSTAYMELSSLRSEDTAVYF CARGVYGGFAGGYFDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
74	hH02重鎖全長ヌクレオチド配列	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGC GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCCTGCAAGGCCAGCGGCTACACCTTTACCCGGAACTTCATGCACTGGGTGCGC CAGGCTCCAGGCCAGGGACTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACAACCAGAAATTCCAGGGCAGAGTGACCATCACCGCCGACAGAAGCACC AGCACCGCCTACATGGAACTGAGCAGCCTGCGGAGCGAGGATACCGCCGTGTACTTC TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTTGGGGCCAG GGCACCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTCCCCCTG GCACCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACTCAGGCGCCCTGACCAGCGGC GTGCACACCTTCCCCGCTGTCCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACT CACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTC TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCCGGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACC CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACCCAGAAGAGCCTCTCCCTGTCTCCCGGCAAA
75	hH02重鎖可変領域アミノ酸配列	EVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFMHWVRQAPGQGLEWMGWIYPGDGE TEYNQKFQGRVTITADRSTSTAYMELSSLRSEDTAVYFCARGVYGGFAGGYFDFWGQ GTLVTVSS

[0109]

[表1-13]

76	hH02重鎖可変領域ヌクレオチド配列	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCCTGCAAGGCCAGCGGCTACACCTTTACCCGGAACTTCATGCACTGGGTGCGC CAGGCTCCAGGCCAGGGACTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACAACCAGAAATTCCAGGGCAGAGTGACCATCACCGCCGACAGAAGCACC AGCACCGCCTACATGGAACTGAGCAGCCTGCGGAGCGAGGATACCGCCGTGTACTTC TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTTGGGGCCAG GGCACCCTCGTGACCGTCAGCTCA
77	hH04重鎖全長アミノ酸配列	MKHLWFFLLLVAAPRWVLSQVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFMHWIR QAPGQGLEWMGWIYPGDGETEYAQKFQGRVTLTADRSTSTAYMELSSLRSEDTAVYY CARGVYGGFAGGYFDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
78	hH04重鎖全長ヌクレオチド配列	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGC CAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCCTGCAAGGCCAGCGGCTACACCTTTACCCGGAACTTCATGCACTGGATCCGG CAGGCCCCTGGACAGGGCCTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACGCCCAGAAATTCCAGGGCAGAGTGACCCTGACCGCCGACAGAAGCACC AGCACCGCCTACATGGAACTGAGCAGCCTGCGGAGCGAGGACACCGCCGTGTACTAT TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTTGGGGCCAG GGCACCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTCCCCCTG GCACCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACTCAGGCGCCCTGACCAGCGGC GTGCACACCTTCCCCGCTGTCCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACT CACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTC TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCCGGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACC CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACCCAGAAGAGCCTCTCCCTGTCTCCCGGCAAA

[0110]

[表1-14]

79	hH04重鎖可変領域アミノ酸配列	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFMHWIRQAPGQGLEWMGWIYPGDGETEYAQKFQGRVTLTADRSTSTAYMELSSLRSEDTAVYYCARGVYGGFAGGYFDFWGQGTLVTVSS
80	hH04重鎖可変領域ヌクレオチド配列	CAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAGGTGTCCTGCAAGGCCAGCGGCTACACCTTTACCCGGAACTTCATGCACTGGATCCGGCAGGCCCCTGGACAGGGCCTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAGACAGAGTACGCCCAGAAATTCCAGGGCAGAGTGACCCTGACCGCCGACAGAAGCACCAGCACCGCCTACATGGAACTGAGCAGCCTGCGGAGCGAGGACACCGCCGTGTACTATTGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTTGGGGCCAGGGCACCCTCGTGACCGTCAGCTCA
81	NOV0712軽鎖全長アミノ酸配列	MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAVSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSGTFPPTTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
82	NOV0712軽鎖全長ヌクレオチド配列	ATGGTGCTGCAGACCCAGGTGTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAACTGCTGATCTACGCCGTGTCCACACTGCAGAGCGGCGTGCCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCCTGACAATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGTCAGCAGTCCGGCACCTTCCCCCCCACCACATTTGGCCAGGGCACCAAGGTGGAAATCAAGCGTACGGTGGCCGCCCCCTCCGTGTTCATCTTCCCCCCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCCAGAGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGCCTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGT
83	NOV0712重鎖全長アミノ酸配列	MKHLWFFLLLVAAPRWVLSQVQLLESGGGLVQPGGSLRLSCAASGFTFSSHGMHWVRQAPGKGLEWVSVISGSGSNTGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARQWGSYAFDSWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0111]

[表1-15]

84	NOV0712重鎖全長ヌクレオチド配列	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGC CAGGTGCAGCTGCTGGAATCTGGCGGAGGACTGGTGCAGCCTGGCGGCTCTCTGAGA CTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGCCACGGAATGCACTGGGTGCGC CAGGCCCCTGGAAAGGGACTGGAATGGGTGTCCGTGATCAGCGGCAGCGGCTCCAAT ACCGGCTACGCCGATAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGCGGGGCCGAGGACACCGCCGTGTACTAT TGTGCCAGACAGTGGGGCAGCTACGCCTTCGATTCTTGGGGCCAGGGCACCCTCGTG ACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTCCCCCTGGCACCCTCCTCC AAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCC GAACCCGTGACCGTGAGCTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTC CCCGCTGTCCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC ACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCA CCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCCCAAAA CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG CATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTC AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGC CAGCCCCGGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAG AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG GAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACCCCTCCCGTGCTG GACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG CAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACCCAGAAGAGCCTCTCCCTGTCTCCCGGCAAA

[0112] （本明細書中、表１－１～表１－１５を表１と総称する場合がある。）

本発明の抗体としては、さらに、ＣＤＨ６に結合する、ヒト抗体を挙げることができる。抗ＣＤＨ６ヒト抗体とは、ヒト染色体由来の抗体の遺伝子配列のみを有するヒト抗体を意味する。抗ＣＤＨ６ヒト抗体は、ヒト抗体の重鎖と軽鎖の遺伝子を含むヒト染色体断片を有するヒト抗体産生マウスを用いた方法（Ｔｏｍｉｚｕｋａ，Ｋ．ｅｔ　ａｌ．，Ｎａｔｕｒｅ　Ｇｅｎｅｔｉｃｓ（１９９７）１６，ｐ．１３３－１４３，；Ｋｕｒｏｉｗａ，Ｙ．ｅｔ．ａｌ．，Ｎｕｃｌ．Ａｃｉｄｓ　Ｒｅｓ．（１９９８）２６，ｐ．３４４７－３４４８；Ｙｏｓｈｉｄａ，Ｈ．ｅｔ．ａｌ．，Ａｎｉｍａｌ　Ｃｅｌｌ　Ｔｅｃｈｎｏｌｏｇｙ：Ｂａｓｉｃ　ａｎｄ　Ａｐｐｌｉｅｄ　Ａｓｐｅｃｔｓ　ｖｏｌ．１０，ｐ．６９－７３（Ｋｉｔａｇａｗａ，Ｙ．，Ｍａｔｓｕｄａ，Ｔ．ａｎｄ　Ｉｉｊｉｍａ，Ｓ．ｅｄｓ．），Ｋｌｕｗｅｒ　Ａｃａｄｅｍｉｃ　Ｐｕｂｌｉｓｈｅｒｓ，１９９９．；Ｔｏｍｉｚｕｋａ，Ｋ．ｅｔ．ａｌ．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ（２０００）９７，ｐ．７２２－７２７等を参照。）によって取得することができる。

[0113] このようなヒト抗体産生マウスは、具体的には、内在性免疫グロブリン重鎖及び軽鎖の遺伝子座が破壊され、代わりに酵母人工染色体（Ｙｅａｓｔ ａｒｔｉｆｉｃｉａｌ ｃｈｒｏｍｏｓｏｍｅ，ＹＡＣ）ベクター等を介してヒト免疫グロブリン重鎖及び軽鎖の遺伝子座が導入された遺伝子組み換え動物として、ノックアウト動物及びトランスジェニック動物の作製及びこれらの動物同士を掛け合わせることによって作り出すことができる。

[0114] また、遺伝子組換え技術によって、そのようなヒト抗体の重鎖及び軽鎖の各々をコードするｃＤＮＡ、好ましくは該ｃＤＮＡを含むベクターによって真核細胞を形質転換し、遺伝子組換えヒトモノクローナル抗体を産生する形質転換細胞を培養することによって、この抗体を培養上清中から得ることもできる。

[0115] ここで、宿主としては例えば真核細胞、好ましくはＣＨＯ細胞、リンパ球やミエローマ等の哺乳動物細胞を用いることができる。

[0116] また、ヒト抗体ライブラリーより選別したファージディスプレイ由来のヒト抗体を取得する方法（Ｗｏｒｍｓｔｏｎｅ，Ｉ．Ｍ．ｅｔ．ａｌ，Ｉｎｖｅｓｔｉｇａｔｉｖｅ Ｏｐｈｔｈａｌｍｏｌｏｇｙ ＆ Ｖｉｓｕａｌ Ｓｃｉｅｎｃｅ．（２００２）４３（７），ｐ．２３０１－２３０８；Ｃａｒｍｅｎ，Ｓ．ｅｔ．ａｌ．，Ｂｒｉｅｆｉｎｇｓ ｉｎ Ｆｕｎｃｔｉｏｎａｌ Ｇｅｎｏｍｉｃｓ ａｎｄ Ｐｒｏｔｅｏｍｉｃｓ（２００２），１（２），ｐ．１８９－２０３；Ｓｉｒｉｗａｒｄｅｎａ，Ｄ．ｅｔ．ａｌ．，Ｏｐｈｔｈａｌｍｏｌｏｇｙ（２００２）１０９（３），ｐ．４２７－４３１等参照。）も知られている。

[0117] 例えば、ヒト抗体の可変領域を一本鎖抗体（ｓｃＦｖ）としてファージ表面に発現させて、抗原に結合するファージを選択するファージディスプレイ法（Ｎａｔｕｒｅ Ｂｉｏｔｅｃｈｎｏｌｏｇｙ（２００５），２３，（９），ｐ．１１０５－１１１６）を用いることができる。

[0118] 抗原に結合することで選択されたファージの遺伝子を解析することによって、抗原に結合するヒト抗体の可変領域をコードするＤＮＡ配列を決定する

ことができる。

[0119] 抗原に結合するｓｃＦｖのＤＮＡ配列が明らかになれば、当該配列を有する発現ベクターを作製し、適当な宿主に導入して発現させることによってヒト抗体を取得することができる（国際公開第９２／０１０４７号、同９２／２０７９１号、同９３／０６２１３号、同９３／１１２３６号、同９３／１９１７２号、同９５／０１４３８号、同９５／１５３８８号、Ａｎｎｕ．Ｒｅｖ．Ｉｍｍｕｎｏｌ（１９９４）１２，ｐ．４３３－４５５、Ｎａｔｕｒｅ Ｂｉｏｔｅｃｈｎｏｌｏｇｙ（２００５）２３（９），ｐ．１１０５－１１１６）。

[0120] 新たに作製されたヒト抗体が、本明細書に記載のラット抗ヒトＣＤＨ６抗体、キメラ抗ヒトＣＤＨ６抗体又はヒト化抗ヒトＣＤＨ６抗体（例えば、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体、ｃｈＧ０１９抗体、Ｈ０１Ｌ０２抗体、Ｈ０２Ｌ０２抗体、Ｈ０２Ｌ０３抗体又はＨ０４Ｌ０２抗体）のいずれか１つが結合する部分ペプチド又は部分立体構造に結合すれば、該ヒト抗体が当該ラット抗ヒトＣＤＨ６抗体、キメラ抗ヒトＣＤＨ６抗体又はヒト化抗ヒトＣＤＨ６抗体と同一のエピトープに結合すると判定することができる。あるいは、本明細書に記載のラット抗ヒトＣＤＨ６抗体、キメラ抗ヒトＣＤＨ６抗体又はヒト化抗ヒトＣＤＨ６抗体（例えば、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体、ｃｈＧ０１９抗体、Ｈ０１Ｌ０２抗体、Ｈ０２Ｌ０２抗体、Ｈ０２Ｌ０３抗体又はＨ０４Ｌ０２抗体）のＣＤＨ６に対する結合に対して該ヒト抗体が競合する（例えば、該ヒト抗体が、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体、ｃｈＧ０１９抗体、Ｈ０１Ｌ０２抗体、Ｈ０２Ｌ０２抗体、Ｈ０２Ｌ０３抗体又はＨ０４Ｌ０２抗体とＣＤＨ６、好ましくは、ＣＤＨ６のＥＣ３への結合を妨げる）ことを確認することによって、具体的なエピトープの配列又は構造が決定されていなくても、該ヒト抗体が本明細書に記載のラット抗ヒトＣＤＨ６抗体、キメラ抗ヒトＣＤＨ６抗体又はヒト化抗ヒトＣＤＨ６抗体と同一のエピトープに結合すると判定することが

できる。本明細書において、当該判定方法の少なくともいずれか一方の方法により「同一のエピトープに結合する」と判定された場合、新たに作製されたヒト抗体が、本明細書に記載のラット抗ヒトＣＤＨ６抗体、キメラ抗ヒトＣＤＨ６抗体又はヒト化抗ヒトＣＤＨ６抗体と「同一のエピトープに結合する」といえる。エピトープが同一であることが確認された場合、該ヒト抗体がラット抗ヒトＣＤＨ６抗体、キメラ抗ヒトＣＤＨ６抗体又はヒト化抗ヒトＣＤＨ６抗体（例えば、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体、ｃｈＧ０１９抗体、Ｈ０１Ｌ０２抗体、Ｈ０２Ｌ０２抗体、Ｈ０２Ｌ０３抗体又はＨ０４Ｌ０２抗体）と同等の生物活性を有していることが期待される。

[0121] 以上の方法によって得られたキメラ抗体、ヒト化抗体、又はヒト抗体は、公知の方法等によって抗原に対する結合性を評価し、好適な抗体を選抜することができる。

[0122] 抗体の性質を比較する際の別の指標の一例としては、抗体の安定性を挙げることができる。示差走査カロリメトリー（ＤＳＣ）は、蛋白の相対的構造安定性のよい指標となる熱変性中点（Ｔｍ）を素早く、また正確に測定することができる装置である。ＤＳＣを用いてＴｍ値を測定し、その値を比較することによって、熱安定性の違いを比較することができる。抗体の保存安定性は、抗体の熱安定性とある程度の相関を示すことが知られており（Ｌｏｒｉ Ｂｕｒｔｏｎ，ｅｔ．ａｌ．，Ｐｈａｒｍａｃｅｕｔｉｃａｌ Ｄｅｖｅｌｏｐｍｅｎｔ ａｎｄ Ｔｅｃｈｎｏｌｏｇｙ（２００７）１２，ｐ．２６５－２７３）、熱安定性を指標に、好適な抗体を選抜することができる。抗体を選抜するための他の指標としては、適切な宿主細胞における収量が高いこと、及び水溶液中での凝集性が低いことを挙げることができる。例えば収量の最も高い抗体が最も高い熱安定性を示すとは限らないので、以上に述べた指標に基づいて総合的に判断して、ヒトへの投与に最も適した抗体を選抜する必要がある。

[0123] 本発明の抗体には抗体の修飾体も含まれる。当該修飾体とは、本発明の抗

体に化学的又は生物学的な修飾が施されてなるものを意味する。化学的な修飾体には、アミノ酸骨格への化学部分の結合、Ｎ－結合又はＯ－結合炭水化物鎖の化学修飾体等が含まれる。生物学的な修飾体には、翻訳後修飾（例えば、Ｎ－結合又はＯ－結合への糖鎖付加、Ｎ末又はＣ末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、又はＮ末グルタミンもしくはＮ末グルタミン酸のピログルタミン酸化）されたもの、原核生物宿主細胞を用いて発現させることによってＮ末にメチオニン残基が付加したもの等が含まれる。また、本発明の抗体又は抗原の検出又は単離を可能にするために標識されたもの、例えば、酵素標識体、蛍光標識体、アフィニティ標識体もかかる修飾物の意味に含まれる。このような本発明の抗体の修飾物は、抗体の安定性及び血中滞留性の改善、抗原性の低減、抗体又は抗原の検出又は単離等に有用である。

[0124] また、本発明の抗体に結合している糖鎖修飾を調節すること（グリコシル化、脱フコース化等）によって、抗体依存性細胞傷害活性を増強することが可能である。抗体の糖鎖修飾の調節技術としては、国際公開第１９９９／５４３４２号、同２０００／６１７３９号、同２００２／３１１４０号等が知られているが、これらに限定されるものではない。本発明の抗体には当該糖鎖修飾を調節された抗体も含まれる。

[0125] 抗体遺伝子を一旦単離した後、適当な宿主に導入して抗体を作製する場合には、適当な宿主と発現ベクターの組み合わせを使用することができる。抗体遺伝子の具体例としては、本明細書に記載された抗体の重鎖配列をコードする遺伝子、及び軽鎖配列をコードする遺伝子を組み合わせたものを挙げることができる。宿主細胞を形質転換する際には、重鎖配列遺伝子と軽鎖配列遺伝子は、同一の発現ベクターに挿入されていることが可能であり、又別々の発現ベクターに挿入されていることも可能である。

[0126] 真核細胞を宿主として使用する場合、動物細胞、植物細胞、真核微生物を用いることができる。特に動物細胞としては、哺乳類細胞、例えば、サルの細胞であるＣＯＳ細胞（Ｇｌｕｚｍａｎ，Ｙ．Ｃｅｌｌ（１９８１）２３，

ｐ．１７５－１８２、ＡＴＣＣ　ＣＲＬ－１６５０）、マウス線維芽細胞ＮＩＨ３Ｔ３（ＡＴＣＣ　Ｎｏ．ＣＲＬ－１６５８）やチャイニーズ・ハムスター卵巣細胞（ＣＨＯ細胞、ＡＴＣＣ　ＣＣＬ－６１）のジヒドロ葉酸還元酵素欠損株（Ｕｒｌａｕｂ，Ｇ．ａｎｄ　Ｃｈａｓｉｎ，Ｌ．Ａ．Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．Ｕ．Ｓ．Ａ．（１９８０）７７，ｐ．４１２６－４２２０）、ＦｒｅｅＳｔｙｌｅ　２９３Ｆ細胞（Ｉｎｖｉｔｒｏｇｅｎ社）を挙げることができる。

[0127] 原核細胞を使用する場合は、例えば、大腸菌、枯草菌を挙げることができる。

[0128] これらの細胞に目的とする抗体遺伝子を形質転換によって導入し、形質転換された細胞をｉｎ　ｖｉｔｒｏで培養することによって抗体が得られる。当該培養においては抗体の配列によって収量が異なる場合があり、同等な結合活性を持つ抗体の中から収量を指標に医薬としての生産が容易なものを選別することが可能である。よって、本発明の抗体には、上記形質転換された宿主細胞を培養する工程、及び当該工程で得られた培養物から目的の抗体又は当該抗体の機能性断片を採取する工程を含むことを特徴とする当該抗体の製造方法によって得られる抗体も含まれる。

[0129] なお、哺乳類培養細胞で生産される抗体の重鎖のカルボキシル末端のリシン残基が欠失することが知られており（Ｊｏｕｒｎａｌ　ｏｆ　Ｃｈｒｏｍａｔｏｇｒａｐｈｙ　Ａ，７０５：１２９－１３４（１９９５））、また、同じく重鎖カルボキシル末端のグリシン、リシンの２アミノ酸残基が欠失し、新たにカルボキシル末端に位置するプロリン残基がアミド化されることが知られている（Ａｎａｌｙｔｉｃａｌ　Ｂｉｏｃｈｅｍｉｓｔｒｙ，３６０：７５－８３（２００７））。しかし、これらの重鎖配列の欠失及び修飾は、抗体の抗原結合能及びエフェクター機能（補体の活性化や抗体依存性細胞傷害作用等）には影響を及ぼさない。従って、本発明に係る抗体には、当該修飾を受けた抗体及び当該抗体の機能性断片も含まれ、重鎖カルボキシル末端において１又は２つのアミノ酸が欠失した欠失体、及びアミド化された当

該欠失体（例えば、カルボキシル末端部位のプロリン残基がアミド化された重鎖）等も包含される。但し、抗原結合能及びエフェクター機能が保たれている限り、本発明に係る抗体の重鎖のカルボキシル末端の欠失体は上記の種類に限定されない。本発明に係る抗体を構成する２本の重鎖は、完全長及び上記の欠失体からなる群から選択される重鎖のいずれか一種であってもよいし、いずれか二種を組み合わせたものであってもよい。各欠失体の量比は本発明に係る抗体を産生する哺乳類培養細胞の種類及び培養条件に影響を受け得るが、本発明に係る抗体の主成分としては２本の重鎖の双方でカルボキシル末端の１つのアミノ酸残基が欠失している場合を挙げることができる。

[0130] 本発明の抗体のアイソタイプとしては、例えばＩｇＧ（ＩｇＧ１、ＩｇＧ２、ＩｇＧ３、ＩｇＧ４）等を挙げることができるが、好ましくはＩｇＧ１又はＩｇＧ４を挙げることができる。

[0131] 抗体の生物活性としては、一般的には抗原結合活性、抗原と結合することによって該抗原を発現する細胞に内在化する活性、抗原の活性を中和する活性、抗原の活性を増強する活性、抗体依存性細胞傷害（ＡＤＣＣ）活性、補体依存性細胞傷害（ＣＤＣ）活性及び抗体依存性細胞媒介食作用（ＡＤＣＰ）を挙げることができるが、本発明に係る抗体が有する機能は、ＣＤＨ６に対する結合活性であり、好ましくはＣＤＨ６と結合することによってＣＤＨ６発現細胞に内在化する活性である。さらに、本発明の抗体は、細胞内在化活性に加えて、ＡＤＣＣ活性、ＣＤＣ活性及び／又はＡＤＣＰ活性を併せ持っていても良い。

[0132] 得られた抗体は、均一にまで精製することができる。抗体の分離、精製は通常のタンパク質で使用されている分離、精製方法を使用すればよい。例えばカラムクロマトグラフィー、フィルター濾過、限外濾過、塩析、透析、調製用ポリアクリルアミドゲル電気泳動、等電点電気泳動等を適宜選択、組み合わせれば、抗体を分離、精製することができる（Ｓｔｒａｔｅｇｉｅｓ ｆｏｒ Ｐｒｏｔｅｉｎ Ｐｕｒｉｆｉｃａｔｉｏｎ ａｎｄ Ｃｈａｒａｃｔｅｒｉｚａｔｉｏｎ：Ａ Ｌａｂｏｒａｔｏｒｙ Ｃｏｕｒｓｅ Ｍａ

nual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988))が、これらに限定されるものではない。

[0133] クロマトグラフィーとしては、アフィニティークロマトグラフィー、イオン交換クロマトグラフィー、疎水性クロマトグラフィー、ゲル濾過クロマトグラフィー、逆相クロマトグラフィー、吸着クロマトグラフィー等を挙げることができる。

[0134] これらのクロマトグラフィーは、ＨＰＬＣやＦＰＬＣ等の液体クロマトグラフィーを用いて行うことができる。

[0135] アフィニティークロマトグラフィーに用いるカラムとしては、プロテインＡカラム、プロテインＧカラムを挙げることができる。例えばプロテインＡカラムを用いたカラムとして、Ｈｙｐｅｒ Ｄ，ＰＯＲＯＳ，Ｓｅｐｈａｒｏｓｅ Ｆ．Ｆ．（ファルマシア）等を挙げることができる。

[0136] また抗原を固定化した担体を用いて、抗原への結合性を利用して抗体を精製することも可能である。

[0137] ３．抗ＣＤＨ６抗体－薬物コンジュゲート

（１）薬物

上記「２．抗ＣＤＨ６抗体の製造」にて取得された抗ＣＤＨ６抗体はリンカー構造部分を介して薬物を結合させることによって、抗ＣＤＨ６抗体－薬物コンジュゲートとすることができる。薬物としては、リンカー構造に結合できる置換基、部分構造を有するものであれば特に制限はない。抗ＣＤＨ６抗体－薬物コンジュゲートは結合する薬物に応じて種々の用途に用いることができる。そのような薬物の例としては、抗腫瘍活性を有する物質、血液疾患に対する効果を有する物質、自己免疫疾患に対する効果を有する物質、抗炎症物質、抗菌物質、抗真菌物質、抗寄生虫物質、抗ウイルス物質、抗麻酔

物質等を挙げることができる。

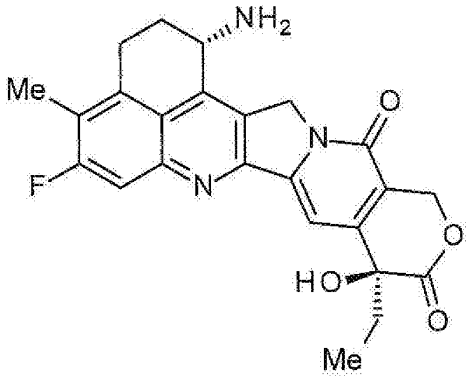
[0138] （１）－１　抗腫瘍化合物

本発明の抗ＣＤＨ６抗体－薬物コンジュゲートに結合される化合物として抗腫瘍性化合物を用いる例について以下に述べる。抗腫瘍性化合物としては、抗腫瘍効果を有する化合物であって、リンカー構造に結合できる置換基、部分構造を有するものであれば特に制限はない。抗腫瘍性化合物は、リンカーの一部又は全部が腫瘍細胞内で切断されて抗腫瘍性化合物部分が遊離されて抗腫瘍効果が発現される。リンカーが薬物との結合部分で切断されれば抗腫瘍性化合物が本来の構造で遊離され、その本来の抗腫瘍効果が発揮される。

[0139] 上記「２．抗ＣＤＨ６抗体の製造」にて取得された抗ＣＤＨ６抗体はリンカー構造部分を介して抗腫瘍性化合物を結合させることによって、抗ＣＤＨ６抗体－薬物コンジュゲートとすることができる。

[0140] 本発明で使用される抗腫瘍性化合物の一つの例として、カンプトテシン誘導体であるエキサテカン（（１Ｓ，９Ｓ）－１－アミノ－９－エチル－５－フルオロ－２，３－ジヒドロ－９－ヒドロキシ－４－メチル－１Ｈ，１２Ｈ－ベンゾ［ｄｅ］ピラノ［３’，４’：６，７］インドリジノ［１，２－ｂ］キノリン－１０，１３（９Ｈ，１５Ｈ）－ジオン；次式：）

[0141] [化5]



[0142] を好適に使用することができる。同化合物は、例えば、米国特許公開公報Ｕ

Ｓ２０１６／０２９７８９０号に記載の方法やその他の公知の方法で容易に取得でき、１位のアミノ基をリンカー構造への結合部位として好適に使用することができる。また、エキサテカンはリンカーの一部が結合した状態で腫瘍細胞内で遊離される場合もあるが、この様な状態でも優れた抗腫瘍効果が発揮される化合物である。

[0143] エキサテカンはカンプトテシン構造を有するので、酸性水性媒体中（例えばｐＨ３程度）ではラクトン環が形成された構造（閉環体）に平衡が偏り、一方、塩基性水性媒体中（例えばｐＨ１０程度）ではラクトン環が開環した構造（開環体）に平衡が偏ることが知られている。このような閉環構造及び開環構造に対応するエキサテカン残基を導入した薬物コンジュゲートであっても同等の抗腫瘍効果が期待され、いずれのものも本発明の範囲に包含されることはいうまでもない。

[0144] 他の抗腫瘍性化合物として例えば、文献（Ｐｈａｒｍａｃｏｌｏｇｉｃａｌ Ｒｅｖｉｅｗｓ， ６８，ｐ３－１９，２０１６）記載の抗腫瘍化合物を挙げることができ、その一例として、ドキソルビシン（Ｄｏｘｏｒｕｂｉｃｉｎ）、カルケマイシン（Ｃａｌｉｃｈｅａｍｉｃｉｎｅ）、ドラスタチン（Ｄｏｒａｓｔａｔｉｎ）１０、モノメチルアウリスタチンＥ（ＭＭＡＥ）、モノメチルアウリスタチンＦ（ＭＭＡＦ）等のアウリスタチン類（Ａｕｒｉｓｔａｔｉｎｓ）、ＤＭ1、ＤＭ4等のメイタンシノイド類（Ｍａｙｔａｎｓｉｎｏｉｄｓ）、ピロロベンゾジアゼピン（Ｐｙｒｒｏｌｏｂｅｎｚｏｄｉａｚｅｐｉｎｅ）二量体のＳＧ２０００（ＳＪＧ－１３６）、カンプトテシンの誘導体であるＳＮ－３８、ＣＣ－１０６５等のデュオカルマイシン類（Ｄｕｏｃａｒｍｙｃｉｎｓ）、アマニチン（Ａｍａｎｉｔｉｎ）、ダウノルビシン、マイトマイシンＣ、ブレオマイシン、シクロシチジン、ビンクリスチン、ビンブラスチン、メトトレキセート、白金系抗腫瘍剤（シスプラチン若しくはその誘導体）、タキソール若しくはその誘導体等を挙げることができる。

[0145] 抗体－薬物コンジュゲートにおいて、抗体１分子への薬物の結合数は、そ

の有効性、安全性に影響する重要因子である。抗体－薬物コンジュゲートの製造は、薬物の結合数が一定の数となるよう、反応させる原料・試薬の使用量等の反応条件を規定して実施されるが、低分子化合物の化学反応とは異なり、異なる数の薬物が結合した混合物として得られるのが通常である。抗体１分子への薬物の結合数は平均値、すなわち、平均薬物結合数として特定され、表記される。本発明でも原則として断りのない限り、すなわち、異なる薬物結合数をもつ抗体－薬物コンジュゲート混合物に含まれる特定の薬物結合数をもつ抗体－薬物コンジュゲートを示す場合を除き、薬物の結合数は平均値を意味する。抗体分子へのエキサテカンの結合数はコントロール可能であり、１抗体あたりの薬物平均結合数として、１～１０個程度のエキサテカンを結合させることができるが、好ましくは２～８個、３～８個、４～８個、５～８個、６～８個、７～８個であり、より好ましくは５～８個であり、更に好ましくは７～８個であり、更により好ましくは８個である。なお、当業者であれば本願の実施例の記載から抗体に必要な数の薬物を結合させる反応を設計することができ、エキサテカンの結合数をコントロールした抗体－薬物コンジュゲートを取得することができる。

[0146] （２）リンカー構造

本発明の抗ＣＤＨ６抗体－薬物コンジュゲートにおいて薬物を抗ＣＤＨ６抗体に結合させるリンカー構造について述べる。

[0147] 本願の抗体－薬物コンジュゲートにおいて、抗ＣＤＨ６抗体と薬物とを結合するリンカー構造は、抗体－薬物コンジュゲートとして用いることができる限りにおいて特に制限されず、使用目的に応じて適宜選択して用いることもできる。リンカー構造の一例としては、公知文献（Ｐｈａｒｍａｃｏｌ Ｒｅｖ ６８：３－１９，Ｊａｎｕａｒｙ ２０１６、Ｐｒｏｔｅｉｎ Ｃｅｌｌ ＤＯＩ １０．１００７／ｓ１３２３８－０１６－０３２３－０等）に記載のリンカーを挙げることができ、更に具体的な例としては、ＶＣ（バリン－シトルリン：ｖａｌｉｎｅ－ｃｉｔｒｕｌｌｉｎｅ）、ＭＣ（マレインアミドカプロイル：ｍａｌｅｉｍｉｄｏｃａｐｒｏｙｌ）、ＳＭＣＣ（

スクシニミジル　４－（Ｎ－マレイミドメチル）シクロヘキサン－１－カルボキシレート：ｓｕｃｃｉｎｉｍｉｄｙｌ　４－（Ｎ－ｍａｌｅｉｍｉｄｏｍｅｔｈｙｌ）　ｃｙｃｌｏｈｅｘａｎｅ－１－ｃａｒｂｏｘｙｌａｔｅ）、ＳＰＰ（Ｎ－スクシニミジル　４－（２－ピリジルジチオ）ペンタン酸：Ｎ－ｓｕｃｃｉｎｉｍｉｄｙｌ　４－（２－ｐｙｒｉｄｙｌｄｉｔｈｉｏ）ｐｅｎｔａｎｏａｔｅ、ＳＳ（ジスルフィド：ｄｉｓｕｌｆｉｄｅ）、ＳＰＤＢ（Ｎ－スクシミジル　４－（２－ピリジルジチオ）ブチラート）Ｎ－ｓｕｃｃｉｎｉｍｉｄｙｌ　４－（２－ｐｙｒｉｄｙｌｄｉｔｈｉｏ）ｂｕｔｙｒａｔｅ、ＳＳ／ヒドラゾン、ヒドラゾン、カルボネートを挙げることができる。

[0148]　他の一例としては、例えば、米国特許公開公報ＵＳ２０１６／０２９７８９０に記載のリンカー構造（一例として、段落［０２６０］～［０２８９］に記載のもの）を挙げることができ、以下の構造のものを好適に使用することができる。なお以下に示す構造の左端が抗体との結合部位であり、右端が薬物との結合部位である。また、下記のリンカー構造中のＧＧＦＧは、グリシン－グリシン－フェニルアラニン－グリシン（ＧＧＦＧ）からなるペプチド結合でつながっているアミノ酸配列を示す。

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＮＨ－CH_2CH_2O－CH_2CH_2O－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－。

[0149] より好ましくは、次のものを挙げることができる。

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－。

さらにより好ましくは、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－、

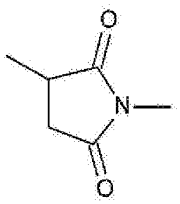
－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－。

を挙げることができる。

[0150] 抗体は－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）の末端、（例えば、『－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－』においては、（－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－）が結合するのとは反対側の末端（左末端））で結合し、抗腫瘍性化合物は－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）とは反対側の末端（上記例では右末端の、ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－のカルボニル基で結合する。『－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－』は、次式：

[0151]

[化6]



[0152] で示される構造を有する。この部分構造における３位が抗ＣＤＨ６抗体への結合部位である。この３位での該抗体との結合は、チオエーテルを形成して結合することが特徴である。この構造部分の１位の窒素原子は、この構造が含まれるリンカー内に存在するメチレンの炭素原子と結合する。

[0153] 薬物をエキサテカンとする本発明の抗体－薬物コンジュゲートにおいては、下記の構造の薬物－リンカー構造部分を抗体に結合させたものが好ましい。これらの薬物－リンカー構造部分は、１抗体あたりの平均結合数として、１から１０を結合させればよいが、好ましくは２から８であり、より好ましくは５から８であり、更に好ましくは７から８であり、更により好ましくは８である。

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＮＨ－CH_2CH_2O－CH_2CH_2O－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－Ｎ

Ｈ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）。

[0154] より、好ましくは、次のものである。

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）。

[0155] さらに、より好ましくは、次のものを挙げることができる。

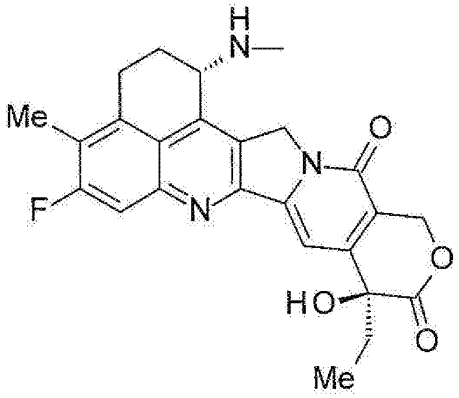
－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－（ＮＨ－ＤＸ）。

[0156] また、－（ＮＨ－ＤＸ）は次式：

[0157]

[化7]



[0158] で示される構造であり、エキサテカンの１位のアミノ基の水素原子１個が除かれて生成する基を示す。

[0159] （３）抗体－薬物コンジュゲートの製造方法

本発明の抗体ー薬物コンジュゲートに用いることができる抗体は、上記「２．抗ＣＤＨ６抗体の製造」の項及び実施例に記載の内在化活性を有する抗ＣＤＨ６抗体及び該抗体の機能性断片であれば特に制限がない。

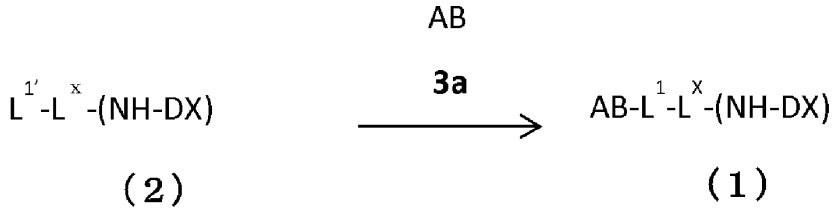
[0160] 次に、本発明の抗体－薬物コンジュゲートの代表的な製造方法について説明する。なお、以下において、化合物を示すために、各反応式中に示される化合物の番号を用いる。すなわち、『式（１）の化合物』、『化合物（１）』等と称する。また、これ以外の番号の化合物についても同様に記載する。

[0161] （３）－１　製造方法１

下記式（１）で示される抗体－薬物コンジュゲートのうち、チオエーテルを介して抗ＣＤＨ６抗体とリンカー構造が結合しているものは抗ＣＤＨ６抗体を還元してジスルフィド結合をスルヒドリル基に変換した抗体に対して、既知の方法によって入手しうる化合物（２）（例えば、ＵＳ２０１６／２９７８９０号公開特許公報に記載の方法（例えば段落［０３３６］～［０３７４］に記載の方法）で入手可能）を反応させることによって製造することができる。例えば下記の方法によって製造することができる。

[0162]

[数1]



(2) (1)

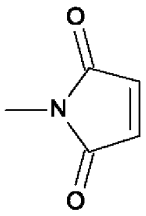
[0163] ［式中、ＡＢは、スルフヒドリル基を有する抗体を示す。

ここで、Ｌ1は、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－の構造で示される。

Ｌ1’は次式で示される、マレイミジル基を示す。］

[0164] [化8]



[0165] －Ｌ1－Ｌxは、以下の式で示されるいずれかの構造を有する。

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ2ＣＨ2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ2ＣＨ2ＣＨ2－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ2ＣＨ2ＣＨ2ＣＨ2ＣＨ2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ2ＣＨ2ＣＨ2－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ2ＣＨ2ＣＨ2ＣＨ2ＣＨ2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ2－Ｏ－ＣＨ2－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ2ＣＨ2ＣＨ2ＣＨ2ＣＨ2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ2ＣＨ2－Ｏ－ＣＨ2－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ2ＣＨ2－Ｃ（＝Ｏ）－ＮＨ－ＣＨ2ＣＨ2Ｏ－ＣＨ2ＣＨ2Ｏ－ＣＨ2ＣＨ2－Ｃ（＝Ｏ）－ＧＧＦＧ－Ｎ

Ｈ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－。

[0166] これらのうちでより好ましくは、次のものである。

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－。

[0167] さらに、好ましくは、次のものを挙げることができる。

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－、

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－。

[0168] また、上記の反応式において抗体－薬物コンジュゲート（１）では、薬物からリンカー末端までの構造部分１個が１個の抗体に対して結合した構造として記載されているが、これは説明のための便宜的な記載であって、実際には当該構造部分が１個の抗体分子に対して複数個が結合している場合が多い。この状況は以下の製造方法の説明においても同様である。

[0169] すなわち、既知の方法によって入手しうる化合物（２）（例えば、ＵＳ２０１６／２９７８９０号公開特許公報に記載の方法（例えば段落［０３３６］～［０３７４］に記載の方法）で入手可能）で入手可能）と、スルフヒドリル基を有する抗体（３ａ）を反応させることによって、抗体－薬物コンジュゲート（１）を製造することができる。

[0170] スルフヒドリル基を有する抗体（３ａ）は、当業者周知の方法で得ることができる（Ｈｅｒｍａｎｓｏｎ，Ｇ．Ｔ、Ｂｉｏｃｏｎｊｕｇａｔｅ Ｔｅｃｈｎｉｑｕｅｓ、ｐｐ．５６－１３６、ｐｐ．４５６－４９３、Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ（１９９６））。例えば、Ｔｒａｕｔ’ｓ試薬を抗体のアミノ基に作用させる；Ｎ－サクシンイミジルＳ－アセチルチオアルカノエート類を抗体のアミノ基に作用させた後、ヒドロキシルアミンを作用させる；Ｎ－サクシンイミジル３－（ピリジルジチオ）プロピオネートを作用させた後、還元剤を作用させる；ジチオトレイトール、２－メルカプトエタノール、トリス（２－カルボキシエチル）ホスフィン塩酸塩（ＴＣＥＰ）等の還元剤を抗体に作用させて抗体内鎖間部のジスルフィド結合を還元しスルフヒドリル基を生成させる；等の方法を挙げることができるがこれらに限定されることはない。

[0171] 具体的には、還元剤としてＴＣＥＰを、抗体内鎖間部ジスルフィド一個当たりに対して０．３乃至３モル当量用い、キレート剤を含む緩衝液中で、抗体と反応させることで、抗体内鎖間部ジスルフィドが部分的もしくは完全に還元された抗体を得ることができる。キレート剤としては、例えばエチレンジアミン四酢酸（ＥＤＴＡ）やジエチレントリアミン５酢酸（ＤＴＰＡ）等を挙げることができる。これ等を１ｍＭ乃至２０ｍＭの濃度で用いればよい。緩衝液としては、リン酸ナトリウムやホウ酸ナトリウム、酢酸ナトリウム溶液等を用いることができる。具体的な例において、抗体は４℃乃至３７℃にて１乃至４時間ＴＣＥＰと反応させることで部分的もしくは完全に還元されたスルフヒドリル基を有する抗体（３ａ）を得ることが出来る。

[0172] なお、ここでスルフヒドリル基を薬物－リンカー部分に付加させる反応を実施させることでチオエーテル結合によって薬物－リンカー部分を結合させることができる。

[0173] 次に、スルフヒドリル基を有する抗体（３ａ）一個あたり、２乃至２０モル当量の化合物（２）を使用して、抗体１個当たり２個乃至８個の薬物が結合した抗体一薬物コンジュゲート（１）を製造することができる。具体的に

は、スルフヒドリル基を有する抗体（３ａ）を含む緩衝液に、化合物（２）を溶解させた溶液を加えて反応させればよい。ここで、緩衝液としては、酢酸ナトリウム溶液、リン酸ナトリウムやホウ酸ナトリウム、等を用いればよい。反応時のｐＨは５乃至９であり、より好適にはｐＨ７付近で反応させればよい。化合物（２）を溶解させる溶媒としては、ジメチルスルホキシド（ＤＭＳＯ）、ジメチルホルムアミド（ＤＭＦ）、ジメチルアセトアミド（ＤＭＡ）、Ｎ－メチル－２－ピリドン（ＮＭＰ）等の有機溶媒を用いることができる。化合物（２）を溶解させた有機溶媒溶液を、スルフヒドリル基を有する抗体（３ａ）を含む緩衝液に１乃至２０％ｖ／ｖを加えて反応させればよい。反応温度は、０乃至３７℃、より好適には１０乃至２５℃であり、反応時間は、０．５乃至２時間である。反応は、未反応の化合物（２）の反応性をチオール含有試薬によって失活させることによって終了できる。チオール含有試薬は例えば、システイン又はＮ－アセチル－Ｌ－システイン（ＮＡＣ）である。より具体的には、ＮＡＣを、用いた化合物（２）に対して、１乃至２モル当量加え、室温で１０乃至３０分インキュベートすることにより反応を終了できる。

[0174] （４）抗体ー薬物コンジュゲートの同定

製造した抗体－薬物コンジュゲート（１）は、以下の共通操作によって濃縮、バッファー交換、精製、抗体濃度及び抗体一分子あたりの薬物平均結合数の測定を行い、抗体－薬物コンジュゲート（１）の同定を行うことができる。

[0175] （４）－１　共通操作Ａ：抗体もしくは抗体－薬物コンジュゲート水溶液の濃縮

Ａｍｉｃｏｎ　Ｕｌｔｒａ（５０，０００　ＭＷＣＯ，Ｍｉｌｌｉｐｏｒｅ　Ｃｏｒｐｏｒａｔｉｏｎ）の容器内に抗体もしくは抗体－薬物コンジュゲート溶液を入れ、遠心機（Ａｌｌｅｇｒａ　Ｘ－１５Ｒ，Ｂｅｃｋｍａｎ　Ｃｏｕｌｔｅｒ，Ｉｎｃ．）を用いた遠心操作（２０００Ｇ乃至３８００Ｇにて５乃至２０分間遠心）にて、抗体もしくは抗体－薬物コンジュゲート

溶液を濃縮した。

[0176] （４）－２　共通操作Ｂ：抗体の濃度測定

ＵＶ測定器（Ｎａｎｏｄｒｏｐ　１０００，Ｔｈｅｒｍｏ　Ｆｉｓｈｅｒ　Ｓｃｉｅｎｔｉｆｉｃ　Ｉｎｃ．）を用いて、メーカー規定の方法に従い、抗体濃度の測定を行った。その際に、抗体ごとに異なる２８０ｎｍ吸光係数（１．３ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$乃至１．８ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$）を用いた。

[0177] （４）－３　共通操作Ｃ：抗体のバッファー交換

Ｓｅｐｈａｄｅｘ　Ｇ－２５担体を使用したＮＡＰ－２５カラム（Ｃａｔ．Ｎｏ．１７－０８５２－０２，ＧＥ　Ｈｅａｌｔｈｃａｒｅ　Ｊａｐａｎ　Ｃｏｒｐｏｒａｔｉｏｎ）を、メーカー規定の方法に従い、塩化ナトリウム（５０ｍＭ）及びＥＤＴＡ（２ｍＭ）を含むリン酸緩衝液（５０ｍＭ，ｐＨ６．０）（本明細書でＰＢＳ６．０／ＥＤＴＡと称する。）にて平衡化させた。このＮＡＰ－２５カラム一本につき、抗体水溶液２．５ｍＬをのせたのち、ＰＢＳ６．０／ＥＤＴＡ３．５ｍＬで溶出させた画分（３．５ｍＬ）を分取した。この画分を共通操作Ａによって濃縮し、共通操作Ｂを用いて抗体濃度の測定を行ったのちに、ＰＢＳ６．０／ＥＤＴＡを用いて２０ｍｇ／ｍＬに抗体濃度を調整した。

[0178] （４）－４　共通操作Ｄ：抗体－薬物コンジュゲートの精製

市販のＳｏｒｂｉｔｏｌ（５％）を含む酢酸緩衝液（１０ｍＭ，ｐＨ５．５；本明細書でＡＢＳと称する。）のいずれかの緩衝液でＮＡＰ－２５カラムを平衡化させた。このＮＡＰ－２５カラムに、抗体－薬物コンジュゲート反応水溶液（約２．５ｍＬ）をのせ、メーカー規定の量の緩衝液で溶出させることで、抗体画分を分取した。この分取画分を再びＮＡＰ－２５カラムにのせ緩衝液で溶出させるゲルろ過精製操作を計２乃至３回繰り返すことで、未結合の薬物リンカーや低分子化合物（トリス（２－カルボキシエチル）ホスフィン塩酸塩（ＴＣＥＰ），Ｎ－アセチル－Ｌ－システイン（ＮＡＣ），ジメチルスルホキシド）を除いた抗体－薬物コンジュゲートを得た。

[0179] （４）－５　共通操作Ｅ：抗体－薬物コンジュゲートにおける抗体濃度及

び抗体一分子あたりの薬物平均結合数の測定

抗体－薬物コンジュゲートにおける結合薬物濃度は、抗体－薬物コンジュゲート水溶液の２８０ｎｍ及び３７０ｎｍの二波長におけるＵＶ吸光度を測定したのちに下記の計算を行うことで、算出することができる。

[0180] ある波長における全吸光度は系内に存在する全ての吸収化学種の吸光度の和に等しい［吸光度の加成性］ことから、抗体と薬物のコンジュゲーション前後において、抗体及び薬物のモル吸光係数に変化がないと仮定すると、抗体－薬物コンジュゲートにおける抗体濃度及び薬物濃度は、下記の関係式で示される。

$$A_{280}=A_{D,280}+A_{A,280}=\varepsilon_{D,280}C_D+\varepsilon_{A,280}C_A \quad \text{式（１）}$$

$$A_{370}=A_{D,370}+A_{A,370}=\varepsilon_{D,370}C_D+\varepsilon_{A,370}C_A \quad \text{式（２）}$$

ここで、A_{280}は２８０ｎｍにおける抗体－薬物コンジュゲート水溶液の吸光度を示し、A_{370}は３７０ｎｍにおける抗体－薬物コンジュゲート水溶液の吸光度を示し、$A_{A,280}$は２８０ｎｍにおける抗体の吸光度を示し、$A_{A,370}$は３７０ｎｍにおける抗体の吸光度を示し、$A_{D,280}$は２８０ｎｍにおけるコンジュゲート前駆体の吸光度を示し、$A_{D,370}$は３７０ｎｍにおけるコンジュゲート前駆体の吸光度を示し、$\varepsilon_{A,280}$は２８０ｎｍにおける抗体のモル吸光係数を示し、$\varepsilon_{A,370}$は３７０ｎｍにおける抗体のモル吸光係数を示し、$\varepsilon_{D,280}$は２８０ｎｍにおけるコンジュゲート前駆体のモル吸光係数を示し、$\varepsilon_{D,370}$は３７０ｎｍにおけるコンジュゲート前駆体のモル吸光係数を示し、C_Aは抗体－薬物コンジュゲートにおける抗体濃度を示し、C_Dは抗体－薬物コンジュゲートにおける薬物濃度を示す。

[0181] ここで、$\varepsilon_{A,280}$、$\varepsilon_{A,370}$、$\varepsilon_{D,280}$、$\varepsilon_{D,370}$は、事前に用意した値（計算推定値もしくは化合物のＵＶ測定から得られた実測値）が用いられる。例えば、$\varepsilon_{A,280}$は、抗体のアミノ酸配列から、既知の計算方法（Ｐｒｏｔｅｉｎ Ｓｃｉｅｎｃｅ，１９９５，ｖｏｌ．４，２４１１－２４２３）によって推定することが出来る。$\varepsilon_{A,370}$は、通常、ゼロである。$\varepsilon_{D,280}$及び$\varepsilon_{D,370}$は、用いるコンジュゲート前駆体をあるモル濃度に溶解させた溶液の吸

光度を測定することで、ランベルト・ベールの法則（吸光度＝モル濃度×モル吸光係数×セル光路長）によって、得ることができる。抗体－薬物コンジュゲート水溶液のA_{280}及びA_{370}を測定し、これらの値を式（１）及び（２）に代入して連立方程式を解くことによって、C_A及びC_Dを求めることができる。さらにC_DをC_Aで除することで１抗体あたりの薬物平均結合数が求めることができる。

[0182] （４）－６　共通操作Ｆ：抗体－薬物コンジュゲートにおける抗体一分子あたりの薬物平均結合数の測定（２）

抗体－薬物コンジュゲートにおける抗体一分子あたりの薬物平均結合数は、前述の「（４）－５　共通操作Ｅ」に加え、以下の方法を用いる高速液体クロマトグラフィー（ＨＰＬＣ）分析によっても求めることができる。以下に、抗体と薬物リンカーがジスルフィド結合している場合のＨＰＬＣによる薬物平均結合数の測定方法を記載する。当業者は、この方法を参照して、抗体と薬物リンカーとの結合様式に依存して適宜、ＨＰＬＣにより薬物平均結合数を測定し得る。

[0183] Ｆ－１．ＨＰＬＣ分析用サンプルの調製（抗体－薬物コンジュゲートの還元）

抗体－薬物コンジュゲート溶液（約１ｍｇ／ｍＬ、６０μＬ）をジチオトレイトール（ＤＴＴ）水溶液（１００ｍＭ、１５μＬ）と混合する。混合物を３７℃で３０分インキュベートすることで、抗体－薬物コンジュゲートの軽鎖及び重鎖間のジスルフィド結合を切断したサンプルを、ＨＰＬＣ分析に用いる。

[0184] Ｆ－２．ＨＰＬＣ分析

ＨＰＬＣ分析を、下記の測定条件にて行う。

[0185] ＨＰＬＣシステム：Ａｇｉｌｅｎｔ　１２９０　ＨＰＬＣシステム（Ａｇｉｌｅｎｔ　Ｔｅｃｈｎｏｌｏｇｉｅｓ）

検出器：紫外吸光度計（測定波長：２８０ｎｍ）

カラム：ＡＣＱＵＩＴＹ　ＵＰＬＣ　ＢＥＨ　Ｐｈｅｎｙｌ（２．１×５

０ｍｍ、１．７μｍ、１３０Å；Ｗａｔｅｒｓ、Ｐ／Ｎ　１８６００２８８４）

カラム温度：８０℃

移動相Ａ：０．１０％トリフルオロ酢酸（ＴＦＡ）、１５％２－プロパノールを含む水溶液

移動相Ｂ：０．０７５％ＴＦＡ、１５％２－プロパノールを含むアセトニトリル溶液

グラジエントプログラム：１４％－３６％（０分－１５分）、３６％－８０％（１５分－１７分）、８０％－１４％（１７分―１７．０１分）、１４％（１７．０１分―２５分）

サンプル注入量：１０μＬ

Ｆ－３．データ解析

Ｆ－３－１　薬物の結合していない抗体の軽鎖（Ｌ０）及び重鎖（Ｈ０）に対して、薬物の結合した軽鎖（薬物がi個結合した軽鎖：$Ｌ_{i}$）及び重鎖（薬物がi個結合した重鎖：$Ｈ_{i}$）は、結合した薬物の数に比例して疎水性が増し保持時間が大きくなることから、例えば、Ｌ０、Ｌ１、Ｈ０、Ｈ１、Ｈ２、Ｈ３の順に溶出される。Ｌ０及びＨ０との保持時間比較により検出ピークをＬ０、Ｌ１、Ｈ０、Ｈ１、Ｈ２、Ｈ３のいずれかに割り当てることができる。薬物結合数は、当業者によって定義され得るが、好ましくは、Ｌ０、Ｌ１、Ｈ０、Ｈ１、Ｈ２、Ｈ３である。

[0186] Ｆ－３－２　薬物リンカーにＵＶ吸収があるため、薬物リンカーの結合数に応じて、軽鎖、重鎖及び薬物リンカーのモル吸光係数を用いて下式に従ってピーク面積値の補正を行う。

[0187] [数2]

$$\text{薬物がｉ個結合した軽鎖ピーク面積補正値}(A_{Li})=\text{ピーク面積}\times\frac{\text{軽鎖のモル吸光係数}}{\text{軽鎖のモル吸光係数}+\text{結合薬物数（i）}\times\text{薬物リンカーのモル吸光係数}}$$

[0188] [数3]

$$\text{薬物がｉ個結合した重鎖ピーク面積補正値}（A_{Hi}）＝\text{ピーク面積}\times\frac{\text{重鎖のモル吸光係数}}{\text{重鎖のモル吸光係数}+\text{結合薬物数}（\mathrm{i}）\times\text{薬物リンカーのモル吸光係数}}$$

[0189] ここで、各抗体における軽鎖及び重鎖のモル吸光係数（２８０ｎｍ）は、既知の計算方法（Ｐｒｏｔｅｉｎ Ｓｃｉｅｎｃｅ，１９９５，ｖｏｌ．４，２４１１－２４２３）によって、各抗体の軽鎖及び重鎖のアミノ酸配列から推定される値を用いることができる。Ｈ０１Ｌ０２の場合、そのアミノ酸配列に従って、軽鎖のモル吸光係数として３１７１０を、重鎖のモル吸光係数として７９９９０を推定値として用いた。また、薬物リンカーのモル吸光係数（２８０ｎｍ）は、各薬物リンカーをメルカプトエタノール又はＮ－アセチルシステインで反応させ、マレイミド基をサクシニイミドチオエーテルに変換した化合物の実測のモル吸光係数（２８０ｎｍ）を用いた。吸光度を測定する波長は、当業者によって適宜設定され得るが、好ましくは、抗体のピークが測定され得る波長であり、より好ましくは２８０ｎｍである。

[0190] Ｆ－３－３ ピーク面積補正値合計に対する各鎖ピーク面積比（％）を下式に従って計算する。

[0191] [数4]

$$\text{薬物がｉ個結合した軽鎖ピーク面積比}=\frac{A_{Li}}{A_{L0}+A_{L1}}\times 100$$

$$\text{薬物がｉ個結合した重鎖ピーク面積比}=\frac{A_{Hi}}{A_{H0}+A_{H1}+A_{H2}+A_{H3}}\times 100$$

A_{Li}，A_{Hi}：Ｌｉ，Ｈｉ各々のピーク面積補正値

[0192] Ｆ－３－４ 抗体－薬物コンジュゲートにおける抗体一分子あたりの薬物平均結合数を、下式に従って計算する。

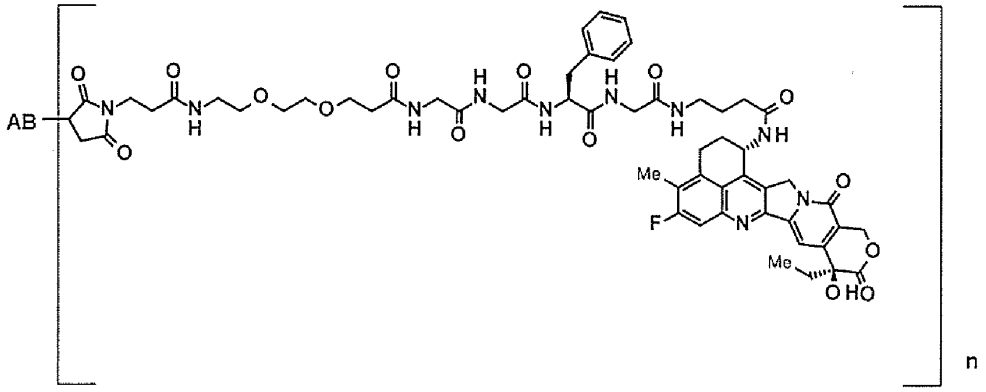
[0193] 薬物平均結合数＝（$Ｌ_0$ピーク面積比ｘ０＋$Ｌ_1$ピーク面積比ｘ１＋$Ｈ_0$ピー

ク面積比ｘ０＋H_1ピーク面積比ｘ１＋H_2ピーク面積比ｘ２＋H_3ピーク面積比ｘ３）／１００ｘ２

なお、抗体－薬物コンジュゲートの量を確保するために、同様な条件で作製して得られた平均薬物数が同程度の複数の抗体－薬物コンジュゲート（例えば±１程度）を混合して新たなロットにすることができる。その場合、平均薬物数は混合前の平均薬物数の間に収まる。

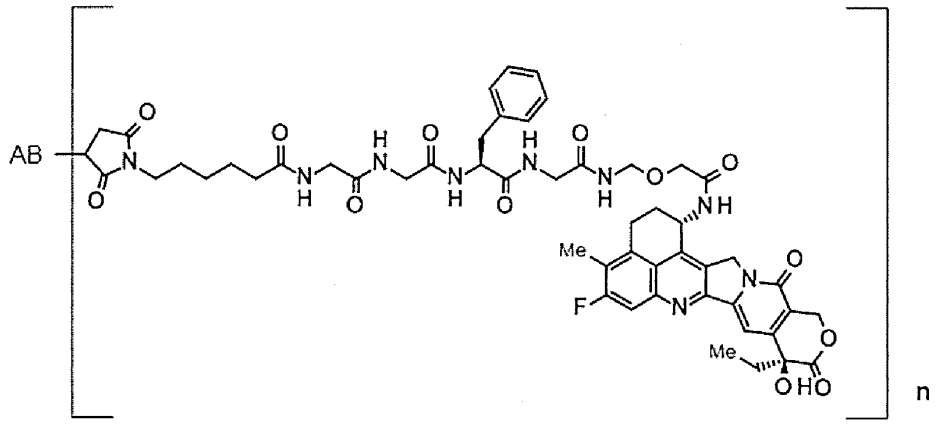
[0194] 本発明の抗体－薬物コンジュゲートの一つの具体例としては次式：

[0195] [化9]



[0196] 又は次式：

[0197] [化10]



[0198] で示される構造を有するものを挙げることができる。

[0199] ここで、ＡＢは本明細書で開示される抗ＣＤＨ６抗体を示し、抗体由来の

スルフヒドリル基を介して結合リンカーと結合している。ここで、ｎはいわゆるＤＡＲ（Ｄｒｕｇ－ｔｏ－Ａｎｔｉｂｏｄｙ　Ｒａｔｉｏ）と同義であり、１抗体あたりの薬物抗体比を示す。すなわち、抗体１分子への薬物の結合数を示すが、これは平均値、すなわち、平均薬物結合数として特定され、表記される数値である。本発明の[化９]及び[化１０]で示される抗体－薬物コンジュゲートの場合、共通操作Ｆによる測定で、ｎは、２から８であればよく、好ましくは５から８であり、更に好ましくは７から８であり、更により好ましく８である。

[0200] 本発明の抗体薬物－コンジュゲートの一例として、上記式［化９］又は［化１０］に示される構造において、ＡＢで示される抗体が、以下の（ａ）乃至（ｇ）からなる群から選択されるいずれか１項に記載の重鎖及び軽鎖の抗体又はその機能性断片を含む、抗体－薬物コンジュゲート又はその薬理学上許容される塩を挙げることができる：

（ａ）配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；

（ｂ）配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；

（ｃ）配列番号６１に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；

（ｄ）配列番号６５に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；

（ｅ）配列番号６５に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；

（ｆ）配列番号６５に示される軽鎖全長アミノ酸配列の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７に示される重鎖全長アミノ酸配列の２０～４７１番目のアミノ酸配列からなる重鎖からなる抗体；又は

（ｇ）重鎖又は軽鎖がＮ－結合への糖鎖付加、Ｏ－結合への糖鎖付加、Ｎ末のプロセッシング、Ｃ末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、Ｎ末にメチオニン残基の付加、プロリン残基のアミド化、Ｎ末グルタミンやＮ末グルタミン酸のピログルタミン酸化などに代表される翻訳後修飾、及びカルボキシル末端における１つ又は２つのアミノ酸欠失からなる群より選択される１又は２以上の修飾を含む、（ａ）～（ｆ）からなる群から選択されるいずれか１つに記載の抗体。

[0201] ４．医薬

上記、「２．抗ＣＤＨ６抗体の製造」の項及び実施例に記載された本発明の抗ＣＤＨ６抗体及び当該抗体の機能性断片は、腫瘍細胞表面のＣＤＨ６に結合し、内在化活性を有することから、単独であるいは他の薬剤と組み合わせて、医薬として、腎細胞腫瘍及び卵巣腫瘍等のがん、例えば、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巣癌、卵巣漿液性腺癌、甲状腺癌、胆管癌、肺癌（例えば、小細胞肺癌又は非小細胞肺癌）、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫の治療剤として用いることができる。

[0202] また、ＣＤＨ６を発現している細胞の検出に用いることができる。

[0203] 更に、本発明の抗ＣＤＨ６抗体及び当該抗体の機能性断片は、内在化活性を有することから、抗体－薬物コンジュゲートに用いる抗体として供することができる。

[0204] 上記「３．抗ＣＤＨ６抗体－薬物コンジュゲート」の項及び実施例に記載の本発明の抗ＣＤＨ６抗体－薬物コンジュゲートのうちで薬物として細胞傷害活性等の抗腫瘍活性を有する薬物が用いられているものは、内在化活性を有する抗ＣＤＨ６抗体及び／又は当該抗体の機能性断片と細胞傷害活性等の抗腫瘍活性を有する薬物のコンジュゲートであり、ＣＤＨ６を発現している

がん細胞に対して抗腫瘍活性を示すことから、医薬として、特にがんに対する治療剤及び／又は予防剤として使用することができる。

[0205] 本発明の抗ＣＤＨ６抗体－薬物コンジュゲートは、大気中に放置したり、又は再結晶や精製操作をしたりすることにより、水分を吸収し、あるいは吸着水が付着するなどして、水和物になる場合があり、そのような水を含む化合物又は薬理学的に許容され得る塩も本発明に包含される。

[0206] 本発明の抗ＣＤＨ６抗体－薬物コンジュゲートが、アミノ基などの塩基性基を有する場合、所望により薬理学的に許容され得る酸付加塩を形成することができる。そのような酸付加塩としては、例えばフッ化水素酸塩、塩酸塩、臭化水素酸塩、ヨウ化水素酸塩などのハロゲン化水素酸塩；硝酸塩、過塩素酸塩、硫酸塩、燐酸塩などの無機酸塩；メタンスルホン酸塩、トリフルオロメタンスルホン酸塩、エタンスルホン酸塩などの低級アルカンスルホン酸塩；ベンゼンスルホン酸塩、ｐ－トルエンスルホン酸塩などのアリールスルホン酸塩；蟻酸塩、酢酸塩、トリフルオロ酢酸塩、りんご酸塩、フマル酸塩、コハク酸塩、クエン酸塩、酒石酸塩、シュウ酸塩、マレイン酸塩などの有機酸塩；又はオルニチン酸塩、グルタミン酸塩、アスパラギン酸塩などのアミノ酸塩などを挙げることができる。

[0207] 本発明の抗ＣＤＨ６抗体－薬物コンジュゲートが、カルボキシ基などの酸性基を有する場合、所望により薬理学的に許容され得る塩基付加塩を形成することができる。そのような塩基付加塩としては、例えばナトリウム塩、カリウム塩、リチウム塩などのアルカリ金属塩；カルシウム塩、マグネシウム塩などのアルカリ土類金属塩；アンモニウム塩などの無機塩；ジベンジルアミン塩、モルホリン塩、フェニルグリシンアルキルエステル塩、エチレンジアミン塩、Ｎ－メチルグルカミン塩、ジエチルアミン塩、トリエチルアミン塩、シクロヘキシルアミン塩、ジシクロヘキシルアミン塩、Ｎ，Ｎ’－ジベンジルエチレンジアミン塩、ジエタノールアミン塩、Ｎ－ベンジル－Ｎ－（２－フェニルエトキシ）アミン塩、ピペラジン塩、テトラメチルアンモニウム塩、トリス（ヒドロキシメチル）アミノメタン塩などの有機アミン塩など

を挙げることができる。

[0208] 本発明はまた、抗体－薬物コンジュゲートを構成する原子の１以上が、その原子の同位体で置換された抗ＣＤＨ６抗体－薬物コンジュゲートを包含し得る。同位体には放射性同位体及び安定同位体の２種類が存在し、同位体の例としては、例えば、水素の同位体（２Ｈ及び３Ｈ）、炭素の同位体（１１Ｃ、１３Ｃ及び１４Ｃ）、窒素の同位体（１３Ｎ及び１５Ｎ）、酸素の同位体（１５Ｏ、１７Ｏ及び１８Ｏ）、フッ素の同位体（１８Ｆ）などを挙げることができる。同位体で標識された抗体－薬物コンジュゲートを含む組成物は、例えば、治療剤、予防剤、研究試薬、アッセイ試薬、診断剤、インビボ画像診断剤などとして有用である。同位体で標識された抗体－薬物コンジュゲート、及び、同位体で標識された抗体－薬物コンジュゲートの任意の割合の混合物もすべて本発明に包含される。同位体で標識された抗体－薬物コンジュゲートは、当該分野で公知の方法により、例えば、後述する本発明の製造方法における原料の代わりに同位体で標識された原料を用いることにより、製造することができる。

[0209] ｉｎ ｖｉｔｒｏでの殺細胞活性は例えば、細胞の増殖抑制活性で測定することができる。例えば、ＣＤＨ６を過剰発現している癌細胞株を培養し、培養系に種々の濃度で抗ＣＤＨ６抗体－薬物コンジュゲートを添加し、フォーカス活性、コロニー形成及びスフェロイド増殖に対する抑制活性を測定することができる。ここで、例えば、腎細胞腫瘍及び卵巣腫瘍由来がん細胞株等を用いることで、腎細胞腫瘍及び卵巣腫瘍に対する細胞増殖抑制活性を調べることができる。

[0210] ｉｎ ｖｉｖｏでの実験動物を用いたがんに対する治療効果は、例えば、ＣＤＨ６を高発現している腫瘍細胞株を移植したヌードマウスに抗ＣＤＨ６抗体－薬物コンジュゲートを投与し、がん細胞の変化を測定することができる。ここで、例えば、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巣癌、卵巣漿液性腺癌又は甲状腺癌由来の細胞を免疫不全マウスに移植した動物モデルを用いることで、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巣癌、

卵巣漿液性腺癌又は甲状腺癌に対する治療効果を測定することができる。

[0211] 本発明の抗ＣＤＨ６抗体－薬物コンジュゲートが適用されるがんの種類としては、治療対象となるがん細胞においてＣＤＨ６を発現しているがんであれば特に制限されないが、例えば、腎細胞癌（例えば、腎淡明細胞癌又は乳頭状腎細胞癌）、卵巣癌、卵巣漿液性腺癌、甲状腺癌、胆管癌、肺癌（例えば、小細胞肺癌又は非小細胞肺癌）、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫を挙げることができるが、ＣＤＨ６を発現している限りこれらに制限されない。より好ましいがんの例としては、腎細胞癌（例えば、腎淡明細胞癌、乳頭状腎細胞癌）又は卵巣癌を挙げることができる。

[0212] 本発明の抗ＣＤＨ６抗体－薬物コンジュゲートは、哺乳動物に対して好適に投与することができるが、より好ましくはヒトである。

[0213] 本発明の抗ＣＤＨ６抗体－薬物コンジュゲートが含まれる医薬組成物において使用される物質としては、投与量や投与濃度において、この分野において通常使用される製剤添加物その他から適宜選択して適用することができる。

[0214] 本発明の抗ＣＤＨ６抗体－薬物コンジュゲートは、１種以上の薬学的に適合性の成分を含む薬学的組成物として投与され得る。例えば、上記薬学的組成物は、代表的には、１種以上の薬学的キャリア（例えば、滅菌した液体（例えば、水及び油（石油、動物、植物、又は合成起源の油（例えば、ラッカセイ油、大豆油、鉱油、ごま油など）を含む））を含む。水は、上記薬学的組成物が静脈内投与される場合に、より代表的なキャリアである。食塩水溶液、ならびにデキストロース水溶液及びグリセロール水溶液もまた、液体キャリアとして、特に、注射用溶液のために使用され得る。適切な薬学的賦形剤は、当該分野で公知である。上記組成物はまた、所望であれば、微量の湿潤剤もしくは乳化剤、又はｐＨ緩衝化剤を含み得る。適切な薬学的キャリアの例は、Ｅ．Ｗ．Ｍａｒｔｉｎによる「Ｒｅｍｉｎｇｔｏｎ’ｓ Ｐｈａｒｍａｃｅｕｔｉｃａｌ Ｓｃｉｅｎｃｅｓ」に記載される。その処方は、投

与の態様に対応する。

[0215] 種々の送達システムが公知であり、本発明の抗ＣＤＨ６抗体－薬物コンジュゲートを投与するために使用され得る。導入方法としては、皮内、筋肉内、腹腔内、静脈内、及び皮下の経路が挙げられるが、これらに限定されない。投与は、例えば、注入又はボーラス注射によるものであり得る。特定の好ましい実施形態において、上記抗体－薬物コンジュゲートの投与は、注入によるものである。非経口的投与は、好ましい投与経路である。

[0216] 代表的実施形態において、上記薬学的組成物は、ヒトへの静脈内投与に適合した薬学的組成物として、常習的手順に従って処方される。代表的には、静脈内投与のための組成物は、滅菌の等張性の水性緩衝液中の溶液である。必要である場合、上記医薬はまた、可溶化剤及び注射部位での疼痛を和らげるための局所麻酔剤（例えば、リグノカイン）を含み得る。一般に、上記成分は、（例えば、活性剤の量を示すアンプル又はサシェなどに密封してシールされた容器中の乾燥凍結乾燥粉末又は無水の濃縮物として、別個に、又は単位剤形中で一緒に混合して、のいずれかで供給される。上記医薬が注入によって投与される予定である場合、それは、例えば、滅菌の製薬グレードの水又は食塩水を含む注入ボトルで投薬され得る。上記医薬が注射によって投与される場合、注射用滅菌水又は食塩水のアンプルは、例えば、上記成分が投与前に混合され得るように、提供され得る。

[0217] 本発明の医薬組成物には本願の抗ＣＤＨ６抗体－薬物コンジュゲートのみを含む医薬組成物であってもよいし、抗ＣＤＨ６抗体－薬物コンジュゲート及び少なくとも一つのこれ以外のがん治療剤を含む医薬組成物であってもよい。本発明の抗ＣＤＨ６抗体－薬物コンジュゲートは、他のがん治療剤と共に投与することもでき、これによって抗がん効果を増強させることができる。このような目的で使用される他の抗がん剤は、抗体－薬物コンジュゲートと同時に、別々に、あるいは連続して個体に投与されてもよいし、それぞれの投与間隔を変えて投与してもよい。このようながん治療剤として、Ｉｍａｔｉｎｉｂ、Ｓｕｎｉｔｉｎｉｂ、Ｒｅｇｏｒａｆｅｎｉｂなどを含むチロ

シンキナーゼ阻害剤、Ｐａｌｂｏｃｉｃｌｉｂなどを含むＣＤＫ４／６阻害剤、ＴＡＳ－１１６などを含むＨＳＰ９０阻害剤、ＭＥＫ１６２などを含むＭＥＫ阻害剤、ニボルマブ、ペンブロリズマブ、イピリムマブなどを含む免疫チェックポイント阻害剤等を挙げることができるが、抗腫瘍活性を有する薬剤であれば限定されることはない。

[0218] このような医薬組成物は、選択された組成と必要な純度を持つ製剤として、凍結乾燥製剤あるいは液状製剤として製剤化すればよい。凍結乾燥製剤として製剤化する際には、この分野において使用される適当な製剤添加物が含まれる製剤であってもよい。また液剤においても同様にして、この分野において使用される各種の製剤添加物を含む液状製剤として製剤化することができる。

[0219] 医薬組成物の組成及び濃度は投与方法によっても変化するが、本発明の医薬組成物に含まれる抗ＣＤＨ６抗体－薬物コンジュゲートは、抗体－薬物コンジュゲートの抗原に対する親和性、すなわち、抗原に対する解離定数（Ｋｄ値）の点において、親和性が高い（Ｋｄ値が低い）ほど、少量の投与量であっても薬効を発揮させることができる。したがって、抗体－薬物コンジュゲートの投与量の決定に当たっては、抗体－薬物コンジュゲートと抗原との親和性の状況に基づいて投与量を設定することもできる。本発明の抗体－薬物コンジュゲートをヒトに対して投与する際には、例えば、約０．００１～１００ｍｇ／ｋｇを１回あるいは１～１８０日間に１回の間隔で複数回投与すればよい。好適には、０．１～５０ｍｇ／ｋｇを、さらに好適には、１～５０ｍｇ／ｋｇ、１～３０ｍｇ／ｋｇ、１～２０ｍｇ／ｋｇ、１～１５ｍｇ／ｋｇ、２～５０ｍｇ／ｋｇ、２～３０ｍｇ／ｋｇ、２～２０ｍｇ／ｋｇ又は２～１５ｍｇ／ｋｇを１～４週間に１回、好ましくは２～３週間に１回の間隔で複数回投与すればよい。

実施例

[0220] 以下に示す実施例によって本発明を具体的に説明するが、本発明はこれらに限定されるものではない。また、これらはいかなる意味においても限定的

に解釈されるものではない。なお、下記実施例において遺伝子操作に関する各操作は特に明示がない限り、「モレキュラークローニング（Ｍｏｌｅｃｕｌａｒ　Ｃｌｏｎｉｎｇ）」（Ｓａｍｂｒｏｏｋ，Ｊ．，Ｆｒｉｔｓｃｈ，Ｅ．Ｆ．及びＭａｎｉａｔｉｓ，Ｔ．著，Ｃｏｌｄ　ＳｐｒｉｎｇＨａｒｂｏｒ　Ｌａｂｏｒａｔｏｒｙ　Ｐｒｅｓｓより１９８９年発刊）に記載の方法及びその他の当業者が使用する実験書に記載の方法により行うか、又は、市販の試薬やキットを用いる場合には市販品の指示書に従って行った。また、本明細書において、特に記載のない試薬、溶媒及び出発材料は、市販の供給源から容易に入手可能である。

[0221] ［実施例１：内在化活性を有するラット抗ヒトＣＤＨ６抗体の取得］

１）－１　ヒト、マウス、ラットおよびカニクイザルＣＤＨ６発現ベクターの構築

ヒトＣＤＨ６タンパク質（ＮＰ＿００４９２３）をコードするｃＤＮＡ発現ベクター（ＯｒｉＧｅｎｅ社、ＲＣ２１７８８９）を用いて、当業者に公知な方法に従い哺乳動物発現用ベクターに組み込むことによってヒトＣＤＨ６発現ベクターｐｃＤＮＡ３．１－ｈＣＤＨ６が作製された。ヒトＣＤＨ６　ＯＲＦ（Ｏｐｅｎ　Ｒｅａｄｉｎｇ　Ｆｒａｍｅ）のアミノ酸配列を配列番号１に示す。

[0222] マウスＣＤＨ６タンパク質（ＮＰ＿０３１６９２）をコードするｃＤＮＡ発現ベクター（ＯｒｉＧｅｎｅ社、ＭＣ２２１６１９）を用いて、当業者に公知な方法に従い哺乳動物発現用ベクターに組み込むことによってマウスＣＤＨ６発現ベクターｐｃＤＮＡ３．１－ｍＣＤＨ６、及びｐ３ｘＦＬＡＧ－ＣＭＶ－９－ｍＣＤＨ６が作製された。マウスＣＤＨ６　ＯＲＦのアミノ酸配列を配列番号７に示す。

[0223] ラットＣＤＨ６タンパク質（ＮＰ＿０３７０５９）をコードするｃＤＮＡ発現ベクター（ＯｒｉＧｅｎｅ社、ＲＮ２１１８５０）の各ｃＤＮＡ部分を用いて、当業者に公知な方法に従い哺乳動物発現用ベクターに組み込むことによってヒトＣＤＨ６発現ベクターｐｃＤＮＡ３．１－ｒＣＤＨ６、及びｐ

３ｘＦＬＡＧ－ＣＭＶ－９－ｒＣＤＨ６が作製された。ラットＣＤＨ６　ＯＲＦのアミノ酸配列を配列番号８に示す。

[0224] カニクイザルＣＤＨ６タンパク質をコードするｃＤＮＡは、カニクイザル腎臓のｔｏｔａｌ　ＲＮＡより合成したｃＤＮＡを鋳型にｐｒｉｍｅｒ　１（５’－ＣＡＣＣＡＴＧＡＧＡＡＣＴＴＡＣＣＧＣＴＡＣＴＴＣＴＴＧＣＴＧＣＴＣ－３’）（配列番号８５）及びｐｒｉｍｅｒ　２（５’－ＴＴＡＧＧＡＧＴＣＴＴＴＧＴＣＡＣＴＧＴＣＣＡＣＴＣＣＴＣＣ－３’）（配列番号８６）を用いてクローニングを行った。得られた配列が、カニクイザルＣＤＨ６（ＮＣＢＩ，ＸＰ＿００５５５６６９１．１）の細胞外領域と一致することを確認した。また、ＥＭＢＬで登録されたカニクイザルＣＤＨ６（ＥＨＨ５４１８０．１）と全長配列が一致することを確認した。当業者に公知な方法に従い哺乳動物発現用ベクターに組み込むことによってカニクイザルＣＤＨ６発現ベクターｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６が作製された。カニクイザルＣＤＨ６　ＯＲＦのアミノ酸配列を配列番号９に示す。

[0225] 作製したプラスミドＤＮＡの大量調製は、ＥｎｄｏＦｒｅｅ　Ｐｌａｓｍｉｄ　Ｇｉｇａ　Ｋｉｔ（ＱＩＡＧＥＮ）を用いて行った。

[0226] １）－２　免疫

免疫にはＷＫＹ／ｌｚｍラットの雌（日本エスエルシー社）を使用した。まずラット両足下腿部をＨｙａｌｕｒｏｎｉｄａｓｅ（ＳＩＧＭＡ－ＡＬＤＲＩＣＨ社）にて前処理後、同部位に実施例１）－　１で作製したヒトＣＤＨ６発現ベクターｐｃＤＮＡ３．１－ｈＣＤＨ６を筋肉内注射した。続けて、ＥＣＭ８３０（ＢＴＸ社）を使用し、２ニードル電極を用いて、同部位にインビボエレクトロポレーションを実施した。約二週間に一度、同様のインビボエレクトロポーレーションを繰り返した後、ラットのリンパ節又は脾臓を採取しハイブリドーマ作製に用いた。

[0227] １）－３　ハイブリドーマ作製

リンパ節細胞あるいは脾臓細胞とマウスミエローマＳＰ２／０－ａｇ１４細胞（ＡＴＣＣ，　Ｎｏ．ＣＲＬ－１　５８１）とをＬＦ３０１　Ｃｅｌｌ

Ｆｕｓｉｏｎ Ｕｎｉｔ（ＢＥＸ社）を用いて電気細胞融合した後、ＣｌｏｎａＣｅｌｌ－ＨＹ Ｓｅｌｅｃｔｉｏｎ Ｍｅｄｉｕｍ Ｄ（ＳｔｅｍＣｅｌｌ Ｔｅｃｈｎｏｌｏｇｉｅｓ社）に懸濁し、希釈して３７℃、５％ CO_2の条件下で培養した。出現した各々のハイブリドーマコロニーは、モノクローンとして回収され、ＣｌｏｎａＣｅｌｌ－ＨＹ Ｓｅｌｅｃｔｉｏｎ Ｍｅｄｉｕｍ Ｅ（ＳｔｅｍＣｅｌｌ Ｔｅｃｈｎｏｌｏｇｉｅｓ社）に懸濁して３７℃、５％ CO_2の条件下で培養した。適度に細胞が増殖した後、各々のハイブリドーマ細胞の凍結ストックを作製すると共に、得られたハイブリドーマ培養上清を抗ヒトＣＤＨ６抗体産生ハイブリドーマのスクリーニングに用いた。

[0228] １）－４ Ｃｅｌｌ－ＥＬＩＳＡ法による抗体産生ハイブリドーマのスクリーニング

１）－４－１ Ｃｅｌｌ－ＥＬＩＳＡ用抗原遺伝子発現細胞の調製

２９３α細胞（インテグリンαｖ及びインテグリンβ３を発現するＨＥＫ２９３由来の安定発現細胞株）を１０％ ＦＢＳ含有ＤＭＥＭ培地中５ｘ１０^{5}細胞／ｍＬになるよう調製した。Ｌｉｐｏｆｅｃｔａｍｉｎｅ ２０００（Ｔｈｅｒｍｏ Ｆｉｓｈｅｒ Ｓｃｉｅｎｆｔｉｆｉｃ社）を用いた形質移入手順に従って、この２９３α細胞に対して、ｐｃＤＮＡ３．１－ｈＣＤＨ６もしくはｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６または、陰性コントロールとしてｐｃＤＮＡ３．１のＤＮＡを導入し、９６－ｗｅｌｌ ｐｌａｔｅ（Ｃｏｒｎｉｎｇ社）に１００μＬずつ分注後、１０％ ＦＢＳ含有ＤＭＥＭ培地中で３７℃、５％ CO_2の条件下で２４から２７時間培養した。得られた形質移入細胞を接着状態のまま、Ｃｅｌｌ－ＥＬＩＳＡに使用した。

[0229] １）－４－２ Ｃｅｌｌ－ＥＬＩＳＡ

実施例１）－４－１で調製した発現ベクター導入２９３α細胞の培養上清を除去後、ｐｃＤＮＡ３．１－ｈＣＤＨ６もしくはｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６またはｐｃＤＮＡ３．１導入２９３α細胞の各々に対しハイブリドーマ培養上清を添加し、４℃で１時間静置した。ｗｅｌｌ中の細胞を５

％　ＦＢＳ含有ＰＢＳ（＋）で１回洗浄後、５％　ＦＢＳ含有ＰＢＳ（＋）で５００倍に希釈したＡｎｔｉ－Ｒａｔ　ＩｇＧ－Ｐｅｒｏｘｉｄａｓｅ　ａｎｔｉｂｏｄｙ　ｐｒｏｄｕｃｅｄ　ｉｎ　ｒａｂｂｉｔ（ＳＩＧＭＡ社）を加えて、４℃で１時間静置した。ｗｅｌｌ中の細胞を５％　ＦＢＳ含有ＰＢＳ（＋）で３回洗浄した後、ＯＰＤ発色液（ＯＰＤ溶解液（０．０５Ｍ　クエン酸３ナトリウム、０．１Ｍ　リン酸水素２ナトリウム・１２水　ｐＨ４．５）にｏ－フェニレンジアミン二塩酸塩（和光純薬社）、H_2O_2をそれぞれ０．４ｍｇ／ｍＬ、０．６％（ｖ／ｖ）になるように溶解）を１００μＬ／ｗｅｌｌで添加した。時々攪拌しながら発色反応を行い、１Ｍ　ＨＣｌを１００μＬ／ｗｅｌｌで添加して発色反応を停止させた後、プレートリーダー（ＥＮＶＩＳＩＯＮ：ＰｅｒｋｉｎＥｌｍｅｒ社）で４９０ｎｍの吸光度を測定した。コントロールのｐｃＤＮＡ３．１導入２９３α細胞と比較し、ｐｃＤＮＡ３．１－ｈＣＤＨ６ならびにｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６発現ベクター導入２９３α細胞の方でより高い吸光度を示す培養上清を産生するハイブリドーマをヒト、かつ、カニクイサルＣＤＨ６に結合する抗体産生ハイブリドーマとして選択した。

[0230]　１）－５　フローサイトメトリーによるカニクイサルＣＤＨ６への選択的結合抗体のスクリーニング

１）－５－１　フローサイトメトリー解析用抗原遺伝子発現細胞の調製

２９３Ｔ細胞を５×１０^{4}細胞／ｃｍ^{2}になるよう２２５ｃｍ^{2}フラスコ（住友ベークライト社）に播種し、１０％　ＦＢＳ含有ＤＭＥＭ培地中で３７℃、５％　CO_2の条件下で一晩培養した。この２９３Ｔ細胞に、ｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６または、陰性コントロールとしてｐｃＤＮＡ３．１をＬｉｐｏｆｅｃｔａｍｉｎｅ　２０００を用いて導入し、３７℃、５％　CO_2の条件下でさらに一晩培養した。各ベクターを導入した２９３Ｔ細胞をＴｒｙｐＬＥ　Ｅｘｐｒｅｓｓ（Ｔｈｅｒｍｏ　Ｆｉｓｈｅｒ　Ｓｃｉｅｎｔｉｆｉｃ社）で処理し、１０％　ＦＢＳ含有ＤＭＥＭで細胞を洗浄した後、５％　ＦＢＳ含有ＰＢＳに懸濁した。得られた細胞懸濁液をフローサイ

トメトリー解析に使用した。

[0231] １）－５－２　フローサイトメトリー解析

実施例１）－４のＣｅｌｌ－ＥＬＩＳＡで選択されたヒト、かつ、カニクイサルＣＤＨ６に結合する抗体産生ハイブリドーマが産生する抗体のカニクイサルＣＤＨ６に対する結合特異性をフローサイトメトリー法によりさらに確認した。実施例１）－５－１で調製した一過性発現２９３Ｔ細胞の懸濁液を遠心し、上清を除去した後、各々に対しハイブリドーマ培養上清を加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、５％　ＦＢＳ含有ＰＢＳで５００倍に希釈したＡｎｔｉ－Ｒａｔ　ＩｇＧ　ＦＩＴＣ　ｃｏｎｊｕｇａｔｅ（ＳＩＧＭＡ社）を加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、２μｇ／ｍＬ　７－ａｍｉｎｏａｃｔｉｎｏｍｙｃｉｎ　Ｄ（Ｍｏｌｅｃｕｌａｒ　Ｐｒｏｂｅｓ社）を含む５％　ＦＢＳ含有ＰＢＳに再懸濁し、フローサイトメーター（ＦＣ５００：ＢｅｃｋｍａｎＣｏｕｌｔｅｒ社）で検出した。データ解析はＦｌｏｗＪｏ（ＴｒｅｅＳｔａｒ社）で行った。７－ａｍｉｎｏａｃｔｉｎｏｍｙｃｉｎ　Ｄ陽性の死細胞をゲートで除外した後、生細胞のＦＩＴＣ蛍光強度のヒストグラムを作成した。コントロールであるｐｃＤＮＡ３．１導入２９３Ｔ細胞の蛍光強度ヒストグラムに対しｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６導入２９３Ｔ細胞のヒストグラムが強蛍光強度側にシフトしている抗体を産生するハイブリドーマを細胞膜表面上に発現するカニクイサルＣＤＨ６に特異的に結合する抗体産生ハイブリドーマとして選択した。

[0232] １）－６　ラットモノクローナル抗体のアイソタイプの決定

実施例１）－　５で選択したラット抗ＣＤＨ６抗体産生ハイブリドーマの中から、ヒトならびにサルＣＤＨ６に強く特異的に結合することが示唆されたクローンｒＧ０１９、ｒＧ０５５、ｒＧ０５６、ｒＧ０６１を選抜し、各抗体のアイソタイプを同定した。抗体の重鎖のサブクラス、軽鎖のタイプは、ＲＡＴ　ＭＯＮＯＣＬＯＮＡＬ　ＡＮＴＩＢＯＤＹ　ＩＳＯＴＹＰＩＮＧ　ＴＥＳＴ　ＫＩＴ（ＤＳファーマバイオメディカル社）により決定された

。その結果、ｒＧ０１９、ｒＧ０５５、ｒＧ０５６、ｒＧ０６１の４クローンともサブクラスは、ＩｇＧ２ｂ、タイプはκ鎖であることが確認された。

[0233] １）－７　ラット抗ＣＤＨ６抗体の調製

１）－７－１　培養上清作製

ラット抗ヒトＣＤＨ６モノクローナル抗体は、ハイブリドーマ培養上清から精製した。まず、ラット抗ＣＤＨ６モノクローナル抗体産生ハイブリドーマをＣｌｏｎａＣｅｌｌ－ＨＹ　Ｓｅｌｅｃｔｉｏｎ　Ｍｅｄｉｕｍ　Ｅ（ＳｔｅｍＣｅｌｌ　Ｔｅｃｈｎｏｌｏｇｉｅｓ社）で充分量まで増殖させた後、Ｕｌｔｒａ　Ｌｏｗ　ＩｇＧ　ＦＢＳ（Ｔｈｅｒｍｏ　Ｆｉｓｈｅｒ　Ｓｃｉｅｎｆｔｉｆｉｃ社）を２０％添加したＨｙｂｒｉｄｏｍａ　ＳＦＭ（Ｔｈｅｒｍｏ　Ｆｉｓｈｅｒ　Ｓｃｉｅｎｆｔｉｆｉｃ社）に培地交換し、４－５日間培養した。本培養上清を回収し、０．８μｍのフィルターに通した後、さらに０．２μｍのフィルターに通して不溶物を除去した。

[0234] １）－７－２　ラット抗ＣＤＨ６抗体の精製

実施例１）－７－１で作製したハイブリドーマの培養上清から抗体（ラット抗ＣＤＨ６抗体（ｒＧ０１９，ｒＧ０５５，ｒＧ０５６，ｒＧ０６１））をＰｒｏｔｅｉｎ　Ｇアフィニティークロマトグラフィーで精製した。Ｐｒｏｔｅｉｎ　Ｇカラム（ＧＥ　Ｈｅａｌｔｈｃａｒｅ　Ｂｉｏｓｃｉｅｎｃｅ社）に抗体を吸着させ、ＰＢＳでカラムを洗浄後に０．１Ｍ　グリシン／塩酸水溶液（ｐＨ２．７）で溶出した。溶出液に１Ｍ　Ｔｒｉｓ－ＨＣｌ（ｐＨ９．０）を加えてｐＨ７．０～７．５に調整した後に、Ｃｅｎｔｒｉｆｕｇａｌ　ＵＦ　Ｆｉｌｔｅｒ　Ｄｅｖｉｃｅ　ＶＩＶＡＳＰＩＮ２０（分画分子量ＵＦ３０Ｋ、Ｓａｒｔｏｒｉｕｓ社）にてＨＢＳｏｒ（２５ｍＭヒスチジン／５％　ソルビトール、ｐＨ６．０）へのバッファー置換を行うとともに抗体の濃縮を行い、抗体濃度を１ｍｇ／ｍＬに調製した。最後にＭｉｎｉｓａｒｔ－Ｐｌｕｓ　ｆｉｌｔｅｒ（Ｓａｒｔｏｒｉｕｓ社）でろ過し、精製サンプルとした。

[0235]

［実施例２：ラット抗ＣＤＨ６抗体のｉｎ　ｖｉｔｒｏ評価］

２）－１　フローサイトメトリーによるラット抗ＣＤＨ６抗体の結合能評価

実施例１）－７で作製したラット抗ＣＤＨ６抗体のヒトＣＤＨ６結合性をフローサイトメトリー法により評価した。実施例１）－１で作製したｐｃＤＮＡ３．１－ｈＣＤＨ６を２９３Ｔ細胞（ＡＴＣＣ）にＬｉｐｏｆｅｃｔａｍｉｎｅ　２０００（Ｔｈｅｒｍｏ　Ｆｉｓｈｅｒ　Ｓｃｉｅｎｔｉｆｉｃ）を用いて一過性に導入し、３７℃、５％　CO_2の条件下で一晩培養した後、細胞懸濁液を調製した。遺伝子導入した２９３Ｔ細胞懸濁液を遠心し、上清を除去した後、実施例１）－７で調製したラット抗ＣＤＨ６モノクローナル抗体４種（クローン番号は、ｒＧ０１９、ｒＧ０５５、ｒＧ０５６及びｒＧ０６１）又はラットＩｇＧコントロール（Ｒ＆Ｄ　Ｓｙｓｔｅｍｓ）を終濃度１０ｎｇ／ｍＬ加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、５％　ＦＢＳ含有ＰＢＳで５０倍に希釈したＡｎｔｉ－Ｒａｔ　ＩｇＧ（ｗｈｏｌｅ　ｍｏｌｅｃｕｌｅ）－ＦＩＴＣ　ａｎｔｉｂｏｄｙ　ｐｒｏｄｕｃｅｄ　ｉｎ　ｒａｂｂｉｔ（ＳＩＧＭＡ）を加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、フローサイトメーター（ＦＣ５００：Ｂｅｃｋｍａｎ　Ｃｏｕｌｔｅｒ）で検出を行った。データ解析はＦｌｏｗＪｏ（ＴｒｅｅＳｔａｒ）で行った。その結果を図１に示す。図１のヒストグラムにおいて、横軸は抗体結合量を表すＦＩＴＣの蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムはｈＣＤＨ６を導入していない陰性コントロール２９３Ｔ細胞を用いた場合を示し、白抜き実線で示すヒストグラムは、ｈＣＤＨ６導入２９３Ｔ細胞を用いた場合を示す。細胞表面のｈＣＤＨ６に抗体が結合したことによって蛍光強度が増強したことを示す。ラットＩｇＧコントロールはいずれの細胞にも結合しない。この結果、作製したラット抗ＣＤＨ６モノクローナル抗体４種はｐｃＤＮＡ３．１－ｈＣＤＨ６導入２９３Ｔ細胞に結合することを確認した。

[0236] ２）－２　フローサイトメトリーによるラット抗ＣＤＨ６抗体のＣＤＨ６結合部位の解析

２）－２－１　ヒトＣＤＨ６各ドメイン欠失体発現ベクターの構築

ヒトＣＤＨ６の細胞外全長には５つの細胞外ドメイン、ＥＣ１（配列番号２）、ＥＣ２（配列番号３）、ＥＣ３（配列番号４）、ＥＣ４（配列番号５）、ＥＣ５（配列番号６）が存在する。ヒトＣＤＨ６全長から、５つのＥＣドメインを各一箇所ずつ欠失発現した遺伝子をＧｅｎｅＡｒｔ社で合成し、当業者に公知な方法に従い哺乳動物発現用ベクターｐ３ｘＦＬＡＧ－ＣＭＶ－９ベクター（ＳＩＧＭＡ－ＡＬＤＲＩＣＨ社）へ組み込み、ＥＣ１からＥＣ５をそれぞれ欠失した、各ドメイン欠失体発現ベクターを作製した。

[0237] ２）－２－２　フローサイトメトリーによるドメイン欠失体を用いたラット抗ＣＤＨ６抗体のエピトープ解析

各ＥＣドメイン欠失ベクターを導入した２９３α細胞株を用いたフローサイトメトリー解析により、ラット抗ヒトＣＤＨ６抗体の結合エピトープを同定した。インテグリンαｖおよびインテグリンβ３発現ベクターをＨＥＫ２９３細胞内に安定形質移入した細胞株２９３α細胞株に対して、実施例２）－２－１で作成した各ドメイン欠失体発現ベクター、及び全長ヒトＣＤＨ６を発現するｐｃＤＮＡ３．１－ｈＣＤＨ６をＬｉｐｏｆｅｃｔａｍｉｎｅ２０００（Ｔｈｅｒｍｏ　Ｆｉｓｈｅｒ　Ｓｃｉｅｎｔｉｆｉｃ）を用いて一過性に導入し、３７℃、５％　CO_2の条件下で一晩培養した後、細胞懸濁液を調製した。導入２９３α細胞懸濁液を遠心し、上清を除去した後、実施例１）－７で調製したラット抗ＣＤＨ６モノクローナル抗体４種（クローン番号は、ｒＧ０１９、ｒＧ０５５、ｒＧ０５６及びｒＧ０６１）又はラットＩｇＧコントロール（Ｒ＆Ｄ　Ｓｙｓｔｅｍｓ）を終濃度２０ｎＭで加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、５％　ＦＢＳ含有ＰＢＳで５０倍に希釈したＡｎｔｉ－Ｒａｔ　ＩｇＧ（ｗｈｏｌｅ　ｍｏｌｅｃｕｌｅ）－ＦＩＴＣ　ａｎｔｉｂｏｄｙ　ｐｒｏｄｕｃｅｄ　ｉｎ　ｒａｂｂｉｔ（ＳＩＧＭＡ）を加えて懸濁し、４℃で１時

間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、フローサイトメーター（ＣａｎｔｏII：ＢＤ　Ｂｉｏｓｃｉｅｎｃｅｓ）で検出を行った。データ解析はＦｌｏｗＪｏ（ＴｒｅｅＳｔａｒ）で行った。その結果を図２－１～図２－６に示す。図２－１～図２－６のヒストグラムにおいて、横軸は抗体結合量を表すＦＩＴＣの蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムは遺伝子を導入していない陰性コントロール２９３α細胞を用いた場合を示し、白抜き実線で示すヒストグラムは、全長ｈＣＤＨ６又は各ＥＣドメイン欠失２９３細胞を用いた場合を示す。細胞表面の全長ｈＣＤＨ６又は各ＥＣドメイン欠失体に抗体が結合した場合は、蛍光強度が増強する。ラットＩｇＧコントロールはいずれの導入細胞にも結合しない。作製したラット抗ＣＤＨ６モノクローナル抗体４種は、全長ｈＣＤＨ６、ＥＣ１欠失体、ＥＣ２欠失体、ＥＣ４欠失体、及びＥＣ５欠失体には結合するが、ＥＣ３欠失体には結合しない。この結果から、ラット抗ＣＤＨ６モノクローナル抗体４種はｈＣＤＨ６のＥＣ３をエピトープとして特異的に結合することが示された。

[0238]　２）－３　ラット抗ＣＤＨ６抗体の内在化活性

２）－３－１　ヒト腫瘍細胞株におけるＣＤＨ６の発現確認

取得抗体の評価に用いるＣＤＨ６陽性ヒト腫瘍細胞株を選抜するため、公知データベースからＣＤＨ６発現情報を検索し、フローサイトメトリー法により細胞膜表面でのＣＤＨ６の発現を評価した。ヒト卵巣腫瘍細胞株ＮＩＨ：ＯＶＣＡＲ－３，ＰＡ－１，ＥＳ－２及びヒト腎細胞腫瘍細胞株７８６－Ｏ（全てＡＴＣＣから入手）を３７℃、５％　CO_2の条件下で培養した後、細胞懸濁液を調製した。細胞を遠心し、上清を除去した後、市販抗ヒトＣＤＨ６抗体（ＭＡＢＵ２７１５，Ｒ＆Ｄ　Ｓｙｓｔｅｍｓ）又は陰性コントロールとしてマウスＩｇＧ１（ＢＤ　Ｐｈａｒｍｉｎｇｅｎ）を終濃度５０ｕｇ／ｍＬ加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、５％　ＦＢＳ含有ＰＢＳで５０倍に希釈したＦ（ａｂ’）２　Ｆｒａｇｍｅｎｔ　ｏｆ　ＦＩＴＣ－ｃｏｎｊｕｇａｔｅｄ　Ｇｏａｔ

Ａｎｔｉ－ｍｏｕｓｅ ｉｍｍｕｎｏｇｌｏｂｕｌｉｎｓ（Ｄａｋｏ）を加えて懸濁し、４℃で１時間静置した。５％ ＦＢＳ含有ＰＢＳで２回洗浄した後、フローサイトメーター（ＣａｎｔｏII：ＢＤ Ｂｉｏｓｃｉｅｎｃｅｓ）で検出を行った。データ解析はＦｌｏｗＪｏ（ＴｒｅｅＳｔａｒ）で行った。その結果を図３に示す。図３のヒストグラムにおいて、横軸は抗体結合量を表すＦＩＴＣの蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムは陰性コントロールｍＩｇＧ１で染色した場合を示し、白抜き実線で示すヒストグラムは抗ヒトＣＤＨ６抗体で染色した場合を示す。細胞表面のｈＣＤＨ６に抗体が結合したことによって蛍光強度が増強したことを示す。ｍＩｇＧ１コントロールはいずれの細胞にも結合しない。この結果、ＮＩＨ：ＯＶＣＡＲ－３，ＰＡ－１及び７８６－Ｏ細胞株は、内在的にＣＤＨ６を細胞表面上に発現することが示された。一方、ＥＳ－２細胞株はＣＤＨ６を全く発現しないことが示された。

[0239] ２）－３－２ ラット抗ＣＤＨ６抗体の内在化活性評価

ラット抗ＣＤＨ６抗体の内在化活性は、タンパク質合成を阻害する毒素（サポリン）を結合させた抗ラットＩｇＧ試薬Ｒａｔ－ＺＡＰ（ＡＤＶＡＮＣＥＤ ＴＡＲＧＥＴＩＮＧ ＳＹＳＴＥＭＳ）を用いて評価した。すなわち、ヒトＣＤＨ６陽性卵巣腫瘍細胞株ＮＩＨ：ＯＶＣＡＲ－３（ＡＴＣＣ）を４ｘ１０^{3} ｃｅｌｌｓ／ｗｅｌｌで９６ｗｅｌｌプレートに播種して３７℃、５％ ＣＯ２の条件下で一晩培養した。ヒトＣＤＨ６陽性腎細胞腫瘍細胞株７８６－Ｏ（ＡＴＣＣ）は１ｘ１０^{3} ｃｅｌｌｓ／ｗｅｌｌで９６ｗｅｌｌプレートに播種して一晩培養した。翌日、ラット抗ＣＤＨ６抗体（終濃度：１ｎＭ）又は、陰性コントロール抗体としてラットＩｇＧ２ｂ抗体（Ｒ＆Ｄ Ｓｙｓｔｅｍｓ）を添加した。さらに、Ｒａｔ－ＺＡＰ（終濃度：０．５ｎＭ）または、陰性コントロールとして毒素を結合していないＧｏａｔ Ａｎｔｉ－Ｒａｔ ＩｇＧ，Ｆｃ（ｇａｍｍａ）Ｆｒａｇｍｅｎｔ Ｓｐｅｃｉｆｉｃ（ＪＡＣＫＳＯＮ ＩＭＭＵＮＯＲＥＳＥＡＲＣＨ）（終濃度：０．５ｎＭ）を添加し、３日間３７℃、５％ＣＯ２条件下で培養した。生

存細胞数は、ＣｅｌｌＴｉｔｅｒ－Ｇｌｏ™ Ｌｕｍｉｎｅｓｃｅｎｔ Ｃｅｌｌ Ｖｉａｂｉｌｉｔｙ Ａｓｓａｙ（Ｐｒｏｍｅｇａ）によるＡＴＰ活性（ＲＬＵ）の定量で測定した。この評価では、ラット抗ＣＤＨ６抗体の内在化活性に依存してＲａｔ－ＺＡＰが細胞内に取り込まれ、タンパク合成を阻害するサポリンが細胞内に放出されることで、細胞増殖が抑制される。抗ＣＤＨ６抗体添加による細胞増殖抑制作用は、Ｒａｔ－ＺＡＰの代わりに陰性コントロールを添加したウェルの生存細胞数を１００％とした相対生存率で表記した。図４にグラフおよび細胞生存率の表を示す。この結果、ラット抗ＣＤＨ６抗体はＣＤＨ６に結合して内在化を引き起こすことが示された。

[0240] ［実施例３：ラット抗ＣＤＨ６抗体の可変領域をコードするｃＤＮＡのヌクレオチド配列の決定］

３）－１　ｒＧ０１９重鎖可変領域及び軽鎖可変領域の遺伝子断片の増幅および配列決定

３）－１－１　Ｇ０１９からのｔｏｔａｌ　ＲＮＡの調製

ｒＧ０１９の可変領域を含むｃＤＮＡを増幅するためＧ０１９よりＴＲＩｚｏｌ　Ｒｅａｇｅｎｔ（Ａｍｂｉｏｎ社）を用いてｔｏｔａｌ　ＲＮＡを調製した。

[0241] ３）－１－２　５’－ＲＡＣＥ　ＰＣＲによるｒＧ０１９の重鎖可変領域を含むｃＤＮＡの増幅とヌクレオチド配列の決定

重鎖可変領域を含むｃＤＮＡの増幅は、実施例３）－１－１で調製したｔｏｔａｌ　ＲＮＡの約１μｇとＳＭＡＲＴｅｒ　ＲＡＣＥ　ｃＤＮＡ　Ａｍｐｌｉｆｉｃａｔｉｏｎ　Ｋｉｔ（Ｃｌｏｎｔｅｃｈ社）を用いて実施した。ｒＧ０１９の重鎖遺伝子の可変領域のｃＤＮＡをＰＣＲで増幅するためのプライマーとして、ＵＰＭ　（Ｕｎｉｖｅｒｓａｌ　Ｐｒｉｍｅｒ　Ａ　Ｍｉｘ：ＳＭＡＲＴｅｒ　ＲＡＣＥ　ｃＤＮＡ　Ａｍｐｌｉｆｉｃａｔｉｏｎ　Ｋｉｔに付属）、及び公知のラット重鎖の定常領域の配列から設計したプ

ライマーを用いた。

[0242] ５’－ＲＡＣＥ　ＰＣＲで増幅した重鎖の可変領域を含むｃＤＮＡをプラスミドにクローニングし、次に重鎖の可変領域のｃＤＮＡのヌクレオチド配列のシークエンス解析を実施した。

[0243] 決定されたｒＧ０１９の重鎖の可変領域をコードするｃＤＮＡのヌクレオチド配列を配列番号１６に示し、アミノ酸配列を配列番号１５に示した。

[0244] ３）－１－３　５’－ＲＡＣＥ　ＰＣＲによるｒＧ０１９の軽鎖可変領域を含むｃＤＮＡの増幅とヌクレオチド配列の決定

実施例３）－１－２と同様の方法で実施した。ただし、ｒＧ０１９の軽鎖遺伝子の可変領域のｃＤＮＡをＰＣＲで増幅するためのプライマーとして、ＵＰＭ（Ｕｎｉｖｅｒｓａｌ　Ｐｒｉｍｅｒ　Ａ　Ｍｉｘ：ＳＭＡＲＴｅｒ　ＲＡＣＥ　ｃＤＮＡ　Ａｍｐｌｉｆｉｃａｔｉｏｎ　Ｋｉｔに付属）、及び公知のラット軽鎖の定常領域の配列から設計したプライマーを用いた。

[0245] 決定されたｒＧ０１９の軽鎖の可変領域をコードするｃＤＮＡのヌクレオチド配列を配列番号１１に示し、アミノ酸配列を配列番号１０に示した。

[0246] ３）－２　ｒＧ０５５重鎖可変領域及び軽鎖可変領域の遺伝子断片の増幅および配列決定

実施例３）－１と同様の方法で配列を決定した。

[0247] 決定されたｒＧ０５５の重鎖の可変領域をコードするｃＤＮＡのヌクレオチド配列を配列番号２６に示し、アミノ酸配列を配列番号２５に示した。軽鎖の可変領域をコードするｃＤＮＡのヌクレオチド配列を配列番号２１に示し、アミノ酸配列を配列番号２０に示した。

[0248] ３）－３　ｒＧ０５６重鎖可変領域及び軽鎖可変領域の遺伝子断片の増幅および配列決定

実施例３）－１と同様の方法で配列を決定した。

[0249] 決定されたｒＧ０５６の重鎖の可変領域をコードするｃＤＮＡのヌクレオチド配列を配列番号３６に示し、アミノ酸配列を配列番号３５に示した。軽鎖の可変領域をコードするｃＤＮＡのヌクレオチド配列を配列番号３１に示

し、アミノ酸配列を配列番号３０に示した。

[0250] ３）－４　ｒＧ０６１重鎖可変領域及び軽鎖可変領域の遺伝子断片の増幅および配列決定

実施例３）－１と同様の方法で配列を決定した。

[0251] 決定されたｒＧ０６１の重鎖の可変領域をコードするｃＤＮＡのヌクレオチド配列を配列番号４６に示し、アミノ酸配列を配列番号４５に示した。軽鎖の可変領域をコードするｃＤＮＡのヌクレオチド配列を配列番号４１に示し、アミノ酸配列を配列番号４０に示した。

[0252]

［実施例４：ヒトキメラ化抗ＣＤＨ６抗体ｃｈＧ０１９の作製］

４）－１　ヒトキメラ化抗ＣＤＨ６抗体ｃｈＧ０１９の発現ベクターの構築

４）－１－１　キメラ及びヒト化軽鎖発現ベクターｐＣＭＡ－ＬＫの構築

プラスミドｐｃＤＮＡ３．３－ＴＯＰＯ／ＬａｃＺ（Ｉｎｖｉｔｒｏｇｅｎ社）を制限酵素ＸｂａＩ及びＰｍｅＩで消化して得られる約５．４ｋｂのフラグメントと、配列番号５０に示すヒト軽鎖シグナル配列及びヒトκ鎖定常領域をコードするＤＮＡ配列を含むＤＮＡ断片をＩｎ－Ｆｕｓｉｏｎ　Ａｄｖａｎｔａｇｅ　ＰＣＲクローニングキット（Ｃｌｏｎｔｅｃｈ社）を用いて結合して、ｐｃＤＮＡ３．３／ＬＫを作製した。

[0253] ｐｃＤＮＡ３．３／ＬＫからネオマイシン発現ユニットを除去することによりｐＣＭＡ－ＬＫを構築した。

[0254] ４）－１－２　キメラ及びヒト化ＩｇＧ１タイプ重鎖発現ベクターｐＣＭＡ－Ｇ１の構築

ｐＣＭＡ－ＬＫをＸｂａＩ及びＰｍｅＩで消化して軽鎖シグナル配列及びヒトκ鎖定常領域を取り除いたＤＮＡ断片と、配列番号５１で示されるヒト重鎖シグナル配列及びヒトＩｇＧ１定常領域をコードするＤＮＡ配列を含むＤＮＡ断片をＩｎ－Ｆｕｓｉｏｎ　Ａｄｖａｎｔａｇｅ　ＰＣＲクローニングキット（Ｃｌｏｎｔｅｃｈ社）を用いて結合して、ｐＣＭＡ－Ｇ１を構築

した。

[0255] ４）－１－３　ｃｈＧ０１９重鎖発現ベクターの構築

配列番号５７に示すｃｈＧ０１９重鎖のヌクレオチド配列のヌクレオチド番号３６乃至４４０に示されるＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。Ｉｎ－Ｆｕｓｉｏｎ　ＨＤ　ＰＣＲクローニングキット（Ｃｌｏｎｔｅｃｈ社）を用いて、ｐＣＭＡ－Ｇ１を制限酵素ＢｌｐＩで切断した箇所に合成したＤＮＡ断片を挿入することによりｃｈＧ０１９重鎖発現ベクターを構築した。なお、ｃｈＧ０１９重鎖は予期せぬジスルフィド結合を防ぐため、ＣＤＲ中のシステインをプロリンに置換した配列を用いた。

[0256] ４）－１－４　ｃｈＧ０１９軽鎖発現ベクターの構築

配列番号５２に示すｃｈＧ０１９軽鎖をコードするＤＮＡ配列を含むＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。Ｉｎ－Ｆｕｓｉｏｎ　ＨＤ　ＰＣＲクローニングキット（Ｃｌｏｎｔｅｃｈ社）を用いて、合成したＤＮＡ断片とｐＣＭＡ－ＬＫをＸｂａＩ及びＰｍｅＩで消化して軽鎖シグナル配列及びヒトκ鎖定常領域を取り除いたＤＮＡ断片を結合することにより、ｃｈＧ０１９軽鎖発現ベクターを構築した。

[0257] ４）－２　ヒトキメラ化抗ＣＤＨ６抗体ｃｈＧ０１９の生産及び精製

４）－２－１　ｃｈＧ０１９の生産

ＦｒｅｅＳｔｙｌｅ　２９３Ｆ細胞（Ｉｎｖｉｔｒｏｇｅｎ社）はマニュアルに従い、継代、培養をおこなった。対数増殖期の１．２×10^9個のＦｒｅｅＳｔｙｌｅ　２９３Ｆ細胞（Ｉｎｖｉｔｒｏｇｅｎ社）を３Ｌ　Ｆｅｒｎｂａｃｈ　Ｅｒｌｅｎｍｅｙｅｒ　Ｆｌａｓｋ（ＣＯＲＮＩＮＧ社）に播種し、ＦｒｅｅＳｔｙｌｅ２９３　ｅｘｐｒｅｓｓｉｏｎ　ｍｅｄｉｕｍ（Ｉｎｖｉｔｒｏｇｅｎ社）で希釈して２．０×10^6細胞／ｍｌに調製した。４０ｍｌのＯｐｔｉ－Ｐｒｏ　ＳＦＭ培地（Ｉｎｖｉｔｒｏｇｅｎ社）に０．２４ｍｇの重鎖発現ベクターと０．３６ｍｇの軽鎖発現ベクターと１．８ｍｇのＰｏｌｙｅｔｈｙｌｅｎｅｉｍｉｎｅ（Ｐｏｌｙｓｃｉｅｎｃｅ　＃２４７６５）を加えて穏やかに攪拌し、さらに５分間放置した後にＦｒｅｅ

Ｓｔｙｌｅ ２９３Ｆ細胞に添加した。３７℃、８％CO_2インキュベーターで４時間、９０ｒｐｍで振とう培養後に６００ｍｌのＥＸ－ＣＥＬＬ ＶＰＲＯ培地（ＳＡＦＣ Ｂｉｏｓｃｉｅｎｃｅｓ社）、１８ｍｌのＧｌｕｔａＭＡＸ Ｉ（ＧＩＢＣＯ社）、及び３０ｍｌのＹｅａｓｔｏｌａｔｅ Ｕｌｔｒａｆｉｌｔｒａｔｅ（ＧＩＢＣＯ社）を添加し、３７℃、８％CO_2インキュベーターで７日間、９０ｒｐｍで振とう培養して得られた培養上清をＤｉｓｐｏｓａｂｌｅ Ｃａｐｓｕｌｅ Ｆｉｌｔｅｒ （Ａｄｖａｎｔｅｃ ＃ＣＣＳ－０４５－Ｅ１Ｈ）でろ過した。

[0258] ４）－２－２ ｃｈＧ０１９の精製

実施例４）－２－１で得られた培養上清をｒＰｒｏｔｅｉｎ Ａアフィニティークロマトグラフィーの１段階工程で精製した。培養上清をＰＢＳで平衡化したＭａｂＳｅｌｅｃｔＳｕＲｅが充填されたカラム（ＧＥ Ｈｅａｌｔｈｃａｒｅ Ｂｉｏｓｃｉｅｎｃｅ社製）にアプライしたのちに、カラム容量の２倍以上のＰＢＳでカラムを洗浄した。次に２Ｍアルギニン塩酸塩溶液（ｐＨ４．０）で溶出し、抗体の含まれる画分を集めた。その画分を透析（Ｔｈｅｒｍｏ Ｓｃｉｅｎｔｉｆｉｃ社、Ｓｌｉｄｅ－Ａ－Ｌｙｚｅｒ Ｄｉａｌｙｓｉｓ Ｃａｓｓｅｔｔｅ）によりＨＢＳｏｒ（２５ｍＭ ヒスチジン／５％ ソルビトール、ｐＨ６．０）へのバッファー置換を行った。Ｃｅｎｔｒｉｆｕｇａｌ ＵＦ Ｆｉｌｔｅｒ Ｄｅｖｉｃｅ ＶＩＶＡＳＰＩＮ２０（分画分子量ＵＦ１０Ｋ，Ｓａｒｔｏｒｉｕｓ社）で抗体を濃縮し、ＩｇＧ濃度を５ｍｇ／ｍｌ以上に調製した。最後にＭｉｎｉｓａｒｔ－Ｐｌｕｓ ｆｉｌｔｅｒ（Ｓａｒｔｏｒｉｕｓ社）でろ過し、精製サンプルとした。

[0259] ４）－３ ヒトキメラ化抗ＣＤＨ６抗体ｃｈＧ０１９の結合性評価

４）－２で精製したヒトキメラ化抗ＣＤＨ６抗体ｃｈＧ０１９のＣＤＨ６結合性をフローサイトメトリー法により確認した。実施例１）－１で作製したｐｃＤＮＡ３．１－ｈＣＤＨ６、又はｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６、又はｐｃＤＮＡ３．１をそれぞれ２９３α細胞にＬｉｐｏｆｅｃｔａｍ

ｉｎｅ ２０００を用いて一過性に導入し、３７℃、５％ CO_2の条件下で一晩培養した後、細胞懸濁液を調製した。これらの細胞懸濁液にｃｈＧ０１９を加えて４℃で１時間静置した後、５％ ＦＢＳ含有ＰＢＳで２回洗浄し、５％ ＦＢＳ含有ＰＢＳで５００倍に希釈したＰＥ標識Ｆ（ａｂ’）２ Ｆｒａｇｍｅｎｔ抗ヒトＩｇＧ，Ｆｃγ抗体（ＪＡＣＫＳＯＮ ＩＭＭＵＮＯＲＥＳＥＡＲＣＨ）を加えて懸濁し、４℃で１時間静置した。５％ ＦＢＳ含有ＰＢＳで２回洗浄した後、５％ ＦＢＳ含有ＰＢＳに再懸濁し、フローサイトメーター（ＣａｎｔｏII：ＢＤ Ｂｉｏｓｃｉｅｎｃｅｓ）で検出を行った。データ解析はＦｌｏｗＪｏ（ＴｒｅｅＳｔａｒ）で行った。図５に示すとおり、ｃｈＧ０１９は陰性コントロールであるｐｃＤＮＡ３．１導入２９３Ｔ細胞には結合せず、ｐｃＤＮＡ３．１－ｈＣＤＨ６及びｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６導入２９３Ｔ細胞に抗体濃度依存的に結合した。図５において横軸は抗体濃度を示し、縦軸は結合量をＭｅａｎＦｌｕｏｒｅｓｃｅｎｔ Ｉｎｔｅｎｓｉｔｙ（平均蛍光強度）で示す。この結果から、ｃｈＧ０１９が、ヒトおよびカニクイザルＣＤＨ６に対して特異的に結合し、結合活性はほぼ同等であることがわかる。

[0260]

［実施例５：ヒト化抗ＣＤＨ６抗体の作製］

５）－１ 抗ＣＤＨ６抗体のヒト化体デザイン

５）－１－１ ｃｈＧ０１９の可変領域の分子モデリング

ｃｈＧ０１９の可変領域の分子モデリングは、ホモロジーモデリングとして公知の方法（Ｍｅｔｈｏｄｓ ｉｎ Ｅｎｚｙｍｏｌｏｇｙ，２０３，１２１－１５３，（１９９１））を利用した。ｃｈＧ０１９の重鎖と軽鎖の可変領域に対して高い配列同一性を有するＰｒｏｔｅｉｎ Ｄａｔａ Ｂａｎｋ（Ｎｕｃ．Ａｃｉｄ Ｒｅｓ．３５，Ｄ３０１－Ｄ３０３（２００７））に登録されている構造（ＰＤＢ ＩＤ：２Ｉ９Ｌ）を鋳型に、市販のタンパク質立体構造解析プログラムＢｉｏＬｕｍｉｎａｔｅ（Ｓｃｈｒｏｄｉｎｇｅｒ社製）を用いて行った。

[0261] ５）－１－２　ヒト化ｈＧ０１９に対するアミノ酸配列の設計

ｃｈＧ０１９は、ＣＤＲグラフティング（Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ　８６，１００２９－１００３３（１９８９））によりヒト化した。ＫＡＢＡＴ　ｅｔ　ａｌ．（Ｓｅｑｕｅｎｃｅｓ　ｏｆ　Ｐｒｏｔｅｉｎｓ　ｏｆ　Ｉｍｍｕｎｏｌｏｇｉｃａｌ　Ｉｎｔｅｒｅｓｔ，５ｔｈ　Ｅｄ．Ｐｕｂｌｉｃ　Ｈｅａｌｔｈ　Ｓｅｒｖｉｃｅ　Ｎａｔｉｏｎａｌ　Ｉｎｓｔｉｔｕｔｅｓ　ｏｆ　Ｈｅａｌｔｈ，Ｂｅｔｈｅｓｄａ，ＭＤ．（１９９１））において既定されるヒトのｇａｍｍａ鎖サブグループ１およびｋａｐｐａ鎖サブグループ１のコンセンサス配列が、ｃｈＧ０１９のフレームワーク領域に対して高い同一性を有することから、それぞれ、重鎖と軽鎖のアクセプターとして選択された。アクセプター上に移入すべきドナー残基は、Ｑｕｅｅｎ　ｅｔ　ａｌ．（Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ　８６，１００２９－１００３３（１９８９））によって与えられる基準などを参考に三次元モデルを分析することで選択された。

[0262] ５）－２　ｃｈＧ０１９重鎖のヒト化

設計された３種の重鎖をｈＨ０１、ｈＨ０２及びｈＨ０４と命名した。ｈＨ０１の重鎖全長アミノ酸配列を、配列番号６９に記載する。配列番号６９のアミノ酸配列をコードするヌクレオチド配列を、配列番号７０に記載する。ｈＨ０２の重鎖全長アミノ酸配列を、配列番号７３に記載する。配列番号７３のアミノ酸配列をコードするヌクレオチド配列を、配列番号７４に記載する。ｈＨ０４の重鎖全長アミノ酸配列を、配列番号７７に記載する。配列番号７７のアミノ酸配列をコードするヌクレオチド配列を、配列番号７８に記載する。

[0263] ５）－３　ｃｈＧ０１９軽鎖のヒト化

設計された２種の軽鎖をｈＬ０２およびｈＬ０３と命名した。ｈＬ０２の軽鎖全長アミノ酸配列を、配列番号６１に記載する。配列番号６１のアミノ酸配列をコードするヌクレオチド配列を、配列番号６２に記載する。ｈＬ０３の軽鎖全長アミノ酸配列を、配列番号６５に記載する。配列番号６５のア

ミノ酸配列をコードするヌクレオチド配列は、配列番号６６に記載する。

[0264] ５）－４　重鎖及び軽鎖の組み合わせによるヒト化ｈＧ０１９の設計

ｈＨ０１及びｈＬ０２からなる抗体を「Ｈ０１Ｌ０２抗体」又は「Ｈ０１Ｌ０２」と称する。ｈＨ０２及びｈＬ０２からなる抗体を「Ｈ０２Ｌ０２抗体」又は「Ｈ０２Ｌ０２」と称する。ｈＨ０２及びｈＬ０３からなる抗体を「Ｈ０２Ｌ０３抗体」又は「Ｈ０２Ｌ０３」と称する。ｈＨ０４及びｈＬ０２からなる抗体を「Ｈ０４Ｌ０２抗体」又は「Ｈ０４Ｌ０２」と称する。

[0265] ５）－５　ヒト化抗ＣＤＨ６抗体の発現

５）－５－１　ヒト化ｈＧ０１９の重鎖発現ベクターの構築

５）－５－１－１　ヒト化ｈＧ０１９－Ｈ０１タイプ重鎖発現ベクターの構築

配列番号７０に示すヒト化ｈＧ０１９－Ｈ０１タイプ重鎖のヌクレオチド配列のヌクレオチド番号３６乃至４４０に示されるＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。実施例４）－１－３と同様の方法でヒト化ｈＧ０１９－Ｈ０１タイプ重鎖発現ベクターを構築した。

[0266] ５）－５－１－２　ヒト化ｈＧ０１９－Ｈ０２タイプ重鎖発現ベクターの構築

配列番号７４に示すヒト化ｈＧ０１９－Ｈ０２タイプ重鎖のヌクレオチド配列のヌクレオチド番号３６乃至４４０に示されるＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。実施例４）－１－３と同様の方法でヒト化ｈＧ０１９－Ｈ０２タイプ重鎖発現ベクターを構築した。

[0267] ５）－５－１－３　ヒト化ｈＧ０１９－Ｈ０４タイプ重鎖発現ベクターの構築

配列番号７８に示すヒト化ｈＧ０１９－Ｈ０４タイプ重鎖のヌクレオチド配列のヌクレオチド番号３６乃至４４０に示されるＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。実施例４）－１－３と同様の方法でヒト化ｈＧ０１９－Ｈ０４タイプ重鎖発現ベクターを構築した。

[0268] ５）－５－２　ヒト化ｈＧ０１９の軽鎖発現ベクターの構築

５）－５－２－１　ヒト化ｈＧ０１９－Ｌ０２タイプ軽鎖発現ベクターの構築

配列番号６２に示すヒト化ｈＧ０１９－Ｌ０２タイプ軽鎖のヌクレオチド配列のヌクレオチド番号３７乃至３９９に示されるヒト化ｈＧ０１９－Ｌ０２タイプ軽鎖の可変領域をコードするＤＮＡ配列を含むＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。Ｉｎ－Ｆｕｓｉｏｎ　ＨＤ　ＰＣＲクローニングキット（Ｃｌｏｎｔｅｃｈ社）を用いて、ｐＣＭＡ－ＬＫを制限酵素ＢｓｉＷＩで切断した箇所に合成したＤＮＡ断片を挿入することによりヒト化ｈＧ０１９－Ｌ０２タイプ軽鎖発現ベクターを構築した。

[0269] ５）－５－２－２　ヒト化ｈＧ０１９－Ｌ０３タイプ軽鎖発現ベクターの構築

配列番号６６に示すヒト化ｈＧ０１９－Ｌ０３タイプ軽鎖のヌクレオチド配列のヌクレオチド番号３７乃至３９９に示されるヒト化ｈＧ０１９－Ｌ０３タイプ軽鎖の可変領域をコードするＤＮＡ配列を含むＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。実施例５）－５－２－１と同様の方法でヒト化ｈＧ０１９－Ｌ０３タイプ軽鎖発現ベクターを構築した。

[0270] ５）－５－３　ヒト化ｈＧ０１９の調製

５）－５－３－１　Ｈ０１Ｌ０２、Ｈ０２Ｌ０２、Ｈ０２Ｌ０３、Ｈ０４Ｌ０２の生産

実施例４）－２－１と同様の方法で生産した。実施例５）－４に示した重鎖と軽鎖の組み合わせにより、Ｈ０１Ｌ０２、Ｈ０２Ｌ０２、Ｈ０２Ｌ０３、Ｈ０４Ｌ０２を生産した。

[0271] ５）－５－３－２　Ｈ０１Ｌ０２、Ｈ０２Ｌ０２、Ｈ０２Ｌ０３、Ｈ０４Ｌ０２の２ｓｔｅｐ精製

実施例５）－５－３－１で得られた培養上清をｒＰｒｏｔｅｉｎ　Ａアフィニティークロマトグラフィーとセラミックハイドロキシアパタイトの２段階工程で精製した。培養上清をＰＢＳで平衡化したＭａｂＳｅｌｅｃｔＳｕＲｅが充填されたカラム（ＧＥ　Ｈｅａｌｔｈｃａｒｅ　Ｂｉｏｓｃｉｅｎ

ｃｅ社製）にアプライした後に、カラム容量の２倍以上のＰＢＳでカラムを洗浄した。次に２Ｍアルギニン塩酸塩溶液（ｐＨ４．０）で抗体を溶出した。抗体の含まれる画分を透析（Ｔｈｅｒｍｏ　Ｓｃｉｅｎｔｉｆｉｃ社、Ｓｌｉｄｅ－Ａ－Ｌｙｚｅｒ　Ｄｉａｌｙｓｉｓ　Ｃａｓｓｅｔｔｅ）によりＰＢＳへのバッファー置換を行い、５ｍＭリン酸ナトリウム／５０ｍＭ　ＭＥＳ／ｐＨ７．０のバッファーで５倍希釈した後に、５ｍＭ　ＮａＰｉ／５０ｍＭ　ＭＥＳ／３０ｍＭ　ＮａＣｌ／ｐＨ７．０のバッファーで平衡化したセラミックハイドロキシアパタイトカラム（日本バイオラッド、Ｂｉｏ－Ｓｃａｌｅ　ＣＨＴ　Ｔｙｐｅ－１　Ｈｙｄｒｏｘｙａｐａｔｉｔｅ　Ｃｏｌｕｍｎ）にアプライした。塩化ナトリウムによる直線的濃度勾配溶出を実施し、抗体の含まれる画分を集めた。その画分を透析（Ｔｈｅｒｍｏ　Ｓｃｉｅｎｔｉｆｉｃ社、Ｓｌｉｄｅ－Ａ－Ｌｙｚｅｒ　Ｄｉａｌｙｓｉｓ　Ｃａｓｓｅｔｔｅ）によりＨＢＳｏｒ（２５ｍＭ　ヒスチジン／５％　ソルビトール、ｐＨ６．０）へのバッファー置換を行った。Ｃｅｎｔｒｉｆｕｇａｌ　ＵＦ　Ｆｉｌｔｅｒ　Ｄｅｖｉｃｅ　ＶＩＶＡＳＰＩＮ２０（分画分子量ＵＦ１０Ｋ，Ｓａｒｔｏｒｉｕｓ社）にて抗体を濃縮し、ＩｇＧ濃度を２０ｍｇ／ｍｌに調製した。最後にＭｉｎｉｓａｒｔ－Ｐｌｕｓ　ｆｉｌｔｅｒ（Ｓａｒｔｏｒｉｕｓ社）でろ過し、精製サンプルとした。

[0272]

［参考例１：抗ＣＤＨ６抗体ＮＯＶ０７１２の作製］

実施例で用いた抗ＣＤＨ６抗体ＮＯＶ０７１２は、国際公開第２０１６／０２４１９５号に記載のＮＯＶ０７１２の軽鎖全長、及び重鎖全長のアミノ酸配列（それぞれ、国際公開第２０１６／０２４１９５の配列番号２３５及び配列番号２３４）を参照して作製した。

[0273] 参考例１）－１抗ＣＤＨ６抗体ＮＯＶ０７１２

参考例１）－１－１　抗ＣＤＨ６抗体ＮＯＶ０７１２の重鎖発現ベクターの構築

配列番号８４に示すＮＯＶ０７１２の重鎖のヌクレオチド配列のヌクレオ

チド番号３６乃至４２８に示されるＮＯＶ０７１２の重鎖の可変領域を含むＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。実施例４）－１－３と同様の方法でＮＯＶ０７１２の重鎖発現ベクターを構築した。当該ＮＯＶ０７１２の重鎖発現ベクターにより発現するＮＯＶ０７１２の重鎖のアミノ酸配列を、配列番号８３に示す。配列番号８３に示されるアミノ酸配列中、１～１９番目のアミノ酸残基からなるアミノ酸配列はシグナル配列である。

[0274] 参考例１）－１－２　抗ＣＤＨ６抗体ＮＯＶ０７１２の軽鎖発現ベクターの構築

配列番号８２に示すＮＯＶ０７１２の軽鎖のヌクレオチド配列のヌクレオチド番号３７乃至４０５に示されるＮＯＶ０７１２の軽鎖の可変領域をコードするＤＮＡ配列を含むＤＮＡ断片を合成した（ＧＥＮＥＡＲＴ社）。実施例５）－５－２－１と同様の方法でＮＯＶ０７１２の軽鎖発現ベクターを構築した。当該ＮＯＶ０７１２の軽鎖発現ベクターにより発現するＮＯＶ０７１２の軽鎖のアミノ酸配列を、配列番号８１に示す。配列番号８１に示されるアミノ酸配列中、１～２０番目のアミノ酸残基からなるアミノ酸配列はシグナル配列である。

[0275] 参考例１）－２　抗ＣＤＨ６抗体ＮＯＶ０７１２の調製

参考例１）－２－１　抗ＣＤＨ６抗体ＮＯＶ０７１２の生産

実施例４）－２－１と同様の方法でＮＯＶ０７１２を生産した。

[0276] 参考例１）－２－２　抗ＣＤＨ６抗体ＮＯＶ０７１２の１ｓｔｅｐ精製

参考例１）－２－１で得られた培養上清から実施例４）－２－２と同様の方法で抗ＣＤＨ６抗体ＮＯＶ０７１２を精製した（抗体濃度ＨＢＳｏｒで５ｍｇ／ｌ）。

[0277]

［実施例６：ヒト化ｈＧ０１９およびＮＯＶ０７１２のｉｎ　ｖｉｔｒｏ評価］

６）－１　ヒト化ｈＧ０１９の結合性評価

６）－１－１　ヒト化ｈＧ０１９のヒトＣＤＨ６抗原結合能

抗体と抗原（Ｒｅｃｏｍｂｉｎａｎｔ　Ｈｕｍａｎ　ＣＤＨ６　Ｆｃ　Ｈｉｓ　ｃｈｉｍｅｒａ，　Ｒ＆Ｄ　Ｓｙｓｔｅｍｓ）との解離定数測定は、Ｂｉａｃｏｒｅ　Ｔ２００（ＧＥヘルスケアバイオサイエンス）を使用し、固定化した抗Ｈｉｓ抗体に抗原をリガンドとして捕捉（キャプチャー）し、抗体をアナライトとして測定するキャプチャー法にて行った。抗ヒスチジン抗体（Ｈｉｓ　ｃａｐｔｕｒｅ　ｋｉｔ、ＧＥヘルスケアバイオサイエンス）は、センサーチップＣＭ５（ＧＥヘルスケアバイオサイエンス）へ、アミンカップリング法にて約１０００ＲＵ共有結合させた。リファレンスセルにも同様に固定化した。ランニングバッファーとして１ｍＭ　$CaCl_2$を添加したＨＢＳ－Ｐ＋（１０ｍＭ　ＨＥＰＥＳ　ｐＨ７．４、０．１５Ｍ　ＮａＣｌ、０．０５％　Ｓｕｒｆａｃｔａｎｔ　Ｐ２０）を用いた。抗ヒスチジン抗体を固定化したチップ上に、抗原を６０秒間添加した後、抗体の希釈系列溶液（０．３９１－１００ｎＭ）を流速３０μｌ／分で３００秒間添加し、引き続き６００秒間の解離相をモニターした。再生溶液として、５Ｍ　ＭｇＣｌ２を添加したＧｌｙｃｉｎｅ溶液ｐＨ１．５を流速１０μｌ／分で３０秒間、２回添加した。データの解析は、分析ソフトウェア（ＢｉＡｅｖａｌｕａｔｉｏｎ　ｓｏｆｔｗａｒｅ，　ｖｅｒｓｉｏｎ　４．１）のＳｔｅａｄｙ　Ｓｔａｔｅ　Ａｆｆｉｎｉｔｙモデルを用いて、解離定数（ＫＤ）を算出した。結果を表２に示す。

[0278] [表2]

	抗体	ＫＤ（Ｍ）
1	Ｈ０２Ｌ０２	１．５Ｅ－０９
2	Ｈ０２Ｌ０２	１．１Ｅ－０９
3	Ｈ０２Ｌ０３	１．４Ｅ－０９
4	Ｈ０４Ｌ０２	１．１Ｅ－０９

[0279]　６）－１－２　ヒト、サル、マウス、ラットＣＤＨ６に対する結合性

実施例１）－１で作製したｐｃＤＮＡ３．１－ｈＣＤＨ６、ｐｃＤＮＡ３．１－ｃｙｎｏＣＤＨ６、ｐ３ｘＦＬＡＧ－ＣＭＶ－９－ｍＣＤＨ６、ｐ３ｘＦＬＡＧ－ＣＭＶ－９－ｒＣＤＨ６を、２９３α細胞にＬｉｐｏｆｅｃｔａｍｉｎｅ　２０００を用いて一過性に導入し、３７℃、５％　CO_2の条件下で一晩培養した後、細胞懸濁液を調製した。陰性コントロールとして、遺伝子導入していない２９３α細胞を用いた。上記にて作製した２９３α細胞懸濁液を遠心し、上清を除去した後、実施例５）－５－３で調製したヒト化ｈＧ０１９抗体４種（クローン番号は、Ｈ０１Ｌ０２、Ｈ０２Ｌ０２、Ｈ０２Ｌ０３およびＨ０４Ｌ０２）、又は、ヒトＩｇＧ１コントロール（Ｃａｌｂｉｏｃｈｅｍ）を加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、５％　ＦＢＳ含有ＰＢＳで５００倍に希釈したａｎｔｉ－ｈｕｍａｎ　Ｆｃｇ　ＰＥ　ｇｏａｔ　Ｆ（ａｂ’）（Ｊａｃｋｓｏｎ　ｌａｂｏｒａｔｏｒｙ）を加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、フローサイトメーター（ＣａｎｔｏII：ＢＤ　Ｂｉｏｓｃｉｅｎｃｅｓ）で検出を行った。データ解析はＦｌｏｗＪｏ（ＴｒｅｅＳｔａｒ）で行った。図６－１及び図６－２において横軸は抗体濃度を示し、縦軸は結合量をＭｅａｎＦｌｕｏｒｅｓｃｅｎｔ　Ｉｎｔｅｎｓｉｔｙ（平均蛍光強度）で示す。図６－１及び図６－２に示すとおり、陰性コントロールであるヒトＩｇＧ１コントロールは、いずれのＣＤＨ６遺伝子導入細胞に対しても結合しない。ヒト化ｈＧ０１９抗体４種（クローン番号は、Ｈ０１Ｌ０２、Ｈ０２Ｌ０２、Ｈ０２Ｌ０３およびＨ０４Ｌ０２）はヒト及びカニクイザルＣＤＨ６に結合するが、マウス及びラットＣＤＨ６には結合しない。いずれの抗体も、陰性コントロールである空ベクターｐｃＤＮＡ３．１導入細胞には結合しない。一方、ＮＯＶ０７１２抗体はヒト、カニクイザル、マウス、及びラットＣＤＨ６全てに結合活性を示すことが国際公開第２０１６／０２４１９５号に示されている。この結果、本明細書で取得したヒト化ｈＧ０１９抗体４種は、ＮＯＶ０７１２抗体と異なる結合性質を示す抗ＣＤＨ６抗体であることが示された。

[0280] ６）－２　ヒト化ｈＧ０１９およびＮＯＶ０７１２のＣＤＨ６結合部位の解析

６）－２－１　ドメイン欠失体を用いたエピトープ解析

実施例２）－２－１で作成した各ドメイン欠失体発現ベクター、及び全長ｈＣＤＨ６を発現するｐｃＤＮＡ３．１－ｈＣＤＨ６をＬｉｐｏｆｅｃｔａｍｉｎｅ　２０００（Ｔｈｅｒｍｏ　Ｆｉｓｈｅｒ　Ｓｃｉｅｎｔｉｆｉｃ）を用いて一過性に導入し、３７℃、５％　CO_2の条件下で一晩培養した後、細胞懸濁液を調製した。遺伝子導入した２９３α細胞懸濁液を遠心し、上清を除去した後、実施例５）－５－３で調製したヒト化ｈＧ０１９抗体４種（クローン番号は、Ｈ０１Ｌ０２、Ｈ０２Ｌ０２、Ｈ０２Ｌ０３およびＨ０４Ｌ０２）、又は、参考例１で調製した抗ＣＤＨ６抗体ＮＯＶ０７１２、又は、陰性コントロールとしてヒトＩｇＧ１（Ｃａｌｂｉｏｃｈｅｍ）を加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、５％　ＦＢＳ含有ＰＢＳで５００倍に希釈したＡＰＣ－ａｎｔｉ－ｈｕｍａｎ　ＩｇＧ　ｇｏａｔ　Ｆ（ａｂ’）２（Ｊａｃｋｓｏｎ　ｌａｂｏｒａｔｏｒｙ）を加えて懸濁し、４℃で１時間静置した。５％　ＦＢＳ含有ＰＢＳで２回洗浄した後、フローサイトメーター（ＣａｎｔｏII：ＢＤ　Ｂｉｏｓｃｉｅｎｃｅｓ）で検出を行った。データ解析はＦｌｏｗＪｏ（ＴｒｅｅＳｔａｒ）で行った。その結果を図７－１～図７－６に示す。図７－１～図７－６のヒストグラムにおいて、横軸は抗体結合量を表すＡＰＣの蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムは遺伝子を導入していない陰性コントロール２９３α細胞を用いた場合を示し、白抜き実線で示すヒストグラムは、全長ｈＣＤＨ６又は各ＥＣドメイン欠失２９３α細胞を用いた場合を示す。細胞表面の全長ｈＣＤＨ６又は各ＥＣドメイン欠失体に抗体が結合した場合は、蛍光強度が増強する。ヒトＩｇＧ１コントロールはいずれの導入細胞にも結合しない。ヒト化ｈＧ０１９抗体４種（クローン番号は、Ｈ０１Ｌ０２、Ｈ０２Ｌ０２、Ｈ０２Ｌ０３およびＨ０４Ｌ０２）は、全長ｈＣＤＨ６、ＥＣ１欠失体、ＥＣ２欠失体、ＥＣ４欠失体、及びＥＣ５

欠失体には結合するが、ＥＣ３欠失体には結合しない。すなわち、ヒト化ｈＧ０１９抗体４種はｈＣＤＨ６のＥＣ３をエピトープとして特異的に結合することが示された。一方、抗ＣＤＨ６抗体ＮＯＶ０７１２は、全長ｈＣＤＨ６、ＥＣ１欠失体、ＥＣ２欠失体、ＥＣ３欠失体、及びＥＣ４欠失体には結合するが、ＥＣ５欠失体には結合しない。すなわち、抗ＣＤＨ６抗体ＮＯＶ０７１２抗体はｈＣＤＨ６のＥＣ５をエピトープとして特異的に結合することが示され、国際公開第２０１６／０２４１９５号に記載されているＮＯＶ０７１２のエピトープ情報と一致する。この結果から、ＮＯＶ０７１２と本明細書で取得したヒト化ｈＧ０１９抗体４種は、異なる性質を示す抗ＣＤＨ６抗体であることが示された。

[0281] ６）－２－２　抗体の結合競合アッセイ

６）－２－２－１　７８６－Ｏ／ｈＣＤＨ６安定発現細胞株の作製

７８６－Ｏ／ｈＣＤＨ６安定発現細胞株は、７８６－Ｏ細胞（ＡＴＣＣ）にヒトＣＤＨ６全長発現用組換えレトロウイルスを感染させることにより作製した。ヒトＣＤＨ６発現レトロウイルスベクター（ｐＱＣＸＩＮ－ｈＣＤＨ６）は、ヒトＣＤＨ６タンパク質（ＮＰ＿００４９２３）をコードするｃＤＮＡ発現ベクター（ＯｒｉＧｅｎｅ社ＲＣ２１７８８９）を用いて、当業者に公知な方法に従いレトロウイルスベクターｐＱＣＸＩＮ（ＣＬＯＮＴＥＣＨ）に組み込むことにより作製された。ＦｕＧｅｎｅ　ＨＤ（Ｐｒｏｍｅｇａ）を用いてレトロウイルスパッケージング細胞ＲｅｔｒｏＰａｃｋ　ＰＴ６７（ＣＬＯＮＴＥＣＨ）にｐＱＣＸＩＮ－ｈＣＤＨ６を一過性に導入し、４８時間後に組換えレトロウイルスを含む培養上清を回収し、７８６－Ｏ細胞培養系に添加することで同細胞に感染させた。感染３日後から、Ｇ４１８（Ｇｉｂｃｏ）を終濃度５０ｍｇ／ｍＬで添加した培地で３７℃、５％ＣＯ２の条件下で培養を行い感染細胞の薬剤選抜を行うことで、ヒトＣＤＨ６を安定的に発現する細胞株７８６－Ｏ／ｈＣＤＨ６を樹立した。実施例２）－３－１と同様にフローサイトメトリーにて安定発現株でのヒトＣＤＨ６の高発現を確認した（図８）。検出用抗体には５％　ＦＢＳ含有ＰＢＳで５

００倍に希釈したＧｏａｔ ａｎｔｉ－Ｍｏｕｓｅ ＩｇＧ１ Ｓｅｃｏｎｄａｒｙ Ａｎｔｉｂｏｄｙ Ａｌｅｘａ Ｆｌｕｏｒ ６４７（Ｔｈｅｒｍｏ Ｆｉｓｈｅｒ Ｓｃｉｅｎｔｉｆｉｃ）を使用した。その結果を図８に示す。図８のヒストグラムにおいて、横軸は抗体結合量を表すＡｌｅｘａ Ｆｌｕｏｒ ６４７の蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムは陰性コントロールｍＩｇＧ１で染色した場合を示し、白抜き実線で示すヒストグラムは抗ヒトＣＤＨ６抗体で染色した場合を示す。細胞表面のｈＣＤＨ６に抗体が結合したことによって蛍光強度が増強したことを示す。ｍＩｇＧ１コントロールはいずれの細胞にも結合しない。この結果、７８６－Ｏ／ｈＣＤＨ６安定発現細胞株では親株７８６－Ｏ細胞と比較して、ヒトＣＤＨ６を高発現することが示された。

[0282] ６）－２－２－２ ラベル化Ｈ０１Ｌ０２及びラベル化ＮＯＶ０７１２を用いた結合競合アッセイ

Ａｌｅｘａ Ｆｌｕｏｒ ４８８ Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｙ Ｌａｂｅｌｉｎｇ Ｋｉｔ（Ｔｈｅｒｍｏ Ｆｉｓｈｅｒ）を用いて、ラベル化Ｈ０１Ｌ０２及びラベル化ＮＯＶ０７１２を作製した。７）－２－２－１で作製した７８６－Ｏ／ｈＣＤＨ６安定発現細胞株の細胞懸濁液を遠心し、上清を除去した後、ラベル化ＮＯＶ０７１２又はラベル化Ｈ０１Ｌ０２を終濃度５ｎＭで添加し、さらに実施例５）－５－３で調製したヒト化ｈＧ０１９抗体４種（クローン番号は、Ｈ０１Ｌ０２、Ｈ０２Ｌ０２、Ｈ０２Ｌ０３およびＨ０４Ｌ０２）、又は、参考例１で調製した抗ＣＤＨ６抗体ＮＯＶ０７１２、又は、陰性コントロールとしてヒトＩｇＧ１（Ｃａｌｂｉｏｃｈｅｍ）を図９の横軸で示す終濃度で添加して懸濁し、４℃で１時間静置した。５％ ＦＢＳ含有ＰＢＳで２回洗浄した後、フローサイトメーター（Ｃａｎｔｏ ＩＩ：ＢＤ Ｂｉｏｓｃｉｅｎｃｅｓ）で検出を行った。データ解析はＦｌｏｗＪｏ（ＴｒｅｅＳｔａｒ）で行った。その結果を図９に示す。横軸はラベル化していない抗体の添加時の終濃度を示し、縦軸は結合量をＭｅａｎ Ｆｌｕｏｒｅｓｃｅｎｔ Ｉｎｔｅｎｓｉｔｙ（平均蛍光強度）

で示す。ラベル化ＮＯＶ０７１２を添加した細胞にラベル化していないＮＯＶ０７１２を添加した場合は、同じエピトープを持ち結合が競合するため、添加濃度依存的にラベル化していない抗体に置き換わりラベル化抗体の結合量が減少する。一方、ラベル化ＮＯＶ０７１２を添加した細胞にヒト化ｈＧ０１９抗体４種、又は陰性コントロールとしてヒトＩｇＧ１を添加しても、ラベル化抗体の結合量に変化は無いことから、これらの抗体はエピトープが異なり結合が競合しないことが示される。同様に、ラベル化Ｈ０１Ｌ０２を添加した細胞にラベル化していないヒト化ｈＧ０１９抗体４種を添加した場合は、同じエピトープを持ち結合が競合するため、添加濃度依存的にラベル化していない抗体に置き換わりラベル化抗体の結合量が減少する。一方、ラベル化Ｈ０１Ｌ０２を添加した細胞にＮＯＶ０７１２、又は陰性コントロールとしてヒトＩｇＧ１を添加しても、ラベル化抗体の結合量に変化は無いことから、これらの抗体はエピトープが異なり結合が競合しないことが示される。

[0283] ６）－３　ヒト化ｈＧ０１９およびＮＯＶ０７１２の内在化活性評価

ヒト化ｈＧ０１９およびＮＯＶ０７１２の内在化活性は、タンパク質合成を阻害する毒素（サポリン）を結合させた抗ヒトＩｇＧ試薬Ｈｕｍ－ＺＡＰ（ＡＤＶＡＮＣＥＤ　ＴＡＲＧＥＴＩＮＧ　ＳＹＳＴＥＭＳ）を用いて評価した。すなわち、ヒトＣＤＨ６陽性卵巣腫瘍細胞株ＮＩＨ：ＯＶＣＡＲ－３（ＡＴＣＣ）を４ｘ１０^{3}　ｃｅｌｌｓ／ｗｅｌｌで９６ｗｅｌｌプレートに播種して３７℃、５％　ＣＯ２の条件下で一晩培養した。ヒトＣＤＨ６陽性腎細胞腫瘍細胞株７８６－Ｏ（ＡＴＣＣ）は１ｘ１０^{3}　ｃｅｌｌｓ／ｗｅｌｌで９６ｗｅｌｌプレートに播種して一晩培養した。ヒトＣＤＨ６陽性卵巣腫瘍細胞株ＰＡ－１（ＡＴＣＣ）を１ｘ１０^{3}　ｃｅｌｌｓ／ｗｅｌｌで９６ｗｅｌｌプレートに播種して３７℃、５％　ＣＯ２の条件下で一晩培養した。翌日、抗ＣＤＨ６抗体（終濃度：１ｎＭ）、又は、陰性コントロール抗体としてヒトＩｇＧ１抗体（Ｃａｌｂｉｏｃｈｅｍ）を添加した。さらにＨｕｍ－ＺＡＰ（終濃度：０．５ｎＭ）または、陰性コントロールとして毒素を

結合していないＦ（ａｂ’）２ Ｆｒａｇｍｅｎｔ Ｇｏａｔ Ａｎｔｉ－ｈｕｍａｎ ＩｇＧ，Ｆｃ（ｇａｍｍａ） Ｆｒａｇｍｅｎｔ Ｓｐｅｃｉｆｉｃ（ＪＡＣＫＳＯＮ ＩＭＭＵＮＯＲＥＳＥＡＲＣＨ）（終濃度：０．５ｎＭ）を添加し、３日間３７℃、５％ＣＯ２条件下で培養した。生存細胞数は、ＣｅｌｌＴｉｔｅｒ－Ｇｌｏ™ Ｌｕｍｉｎｅｓｃｅｎｔ Ｃｅｌｌ Ｖｉａｂｉｌｉｔｙ ＡｓｓａｙによるＡＴＰ活性（ＲＬＵ）の定量で測定した。この評価では、ヒト化抗ＣＤＨ６抗体の内在化活性に依存してＨｕｍ－ＺＡＰが細胞内に取り込まれ、タンパク合成を阻害するサポリンが細胞内に放出されることで、細胞増殖が抑制される。抗ＣＤＨ６抗体添加による細胞増殖抑制作用は、Ｈｕｍ－ＺＡＰの代わりに陰性コントロールを添加したウェルの生存細胞数を１００％とした相対生存率で表記した。図１０－1～図１０－３にグラフおよび細胞生存率の表を示す。本実験において内在化活性が強い抗体は低い細胞生存率を示すと考えられる。この結果、ＮＯＶ０７１２はヒト化ｈＧ０１９抗体４種は３種の細胞株いずれにおいても細胞生存率から予測される内在化率は約５０～７５％であり、非常に高い内在化活性を示し、ＮＯＶ０７１２と比較して、さらに高い内在活性を示す。ＡＤＣの薬効メカニズムから、高い内在化活性を有する抗体は、ＡＤＣ化抗体をして、より適していると考えられる。

[0284]

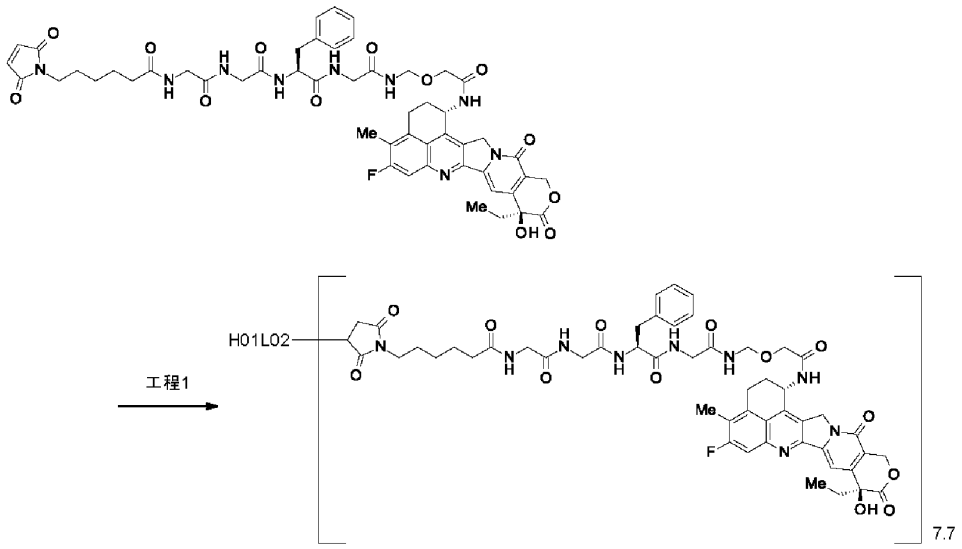
［実施例７：ヒト化ｈＧ０１９－薬物コンジュゲートの作製］

７）－１ 抗体－薬物コンジュゲートの作製 Ｈ０１Ｌ０２－ＤＸｄ

工程１：抗体－薬物コンジュゲート（１）

[0285]

[化11]



[0286] 抗体の還元：実施例５にて作製したＨ０１Ｌ０２を、製造方法１に記載した共通操作Ｂ（２８０ｎｍ吸光係数として１．５３ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$を使用）及びＣを用いて、ＰＢＳ６．０／ＥＤＴＡにて９．８５ｍｇ／ｍＬに調製した。本溶液（５．７ｍＬ）に１０ｍＭ　ＴＣＥＰ（東京化成工業株式会社）水溶液（０．２３１ｍＬ；抗体一分子に対して６．０当量）及び１Ｍリン酸水素二カリウム水溶液（Ｎａｃａｌａｉ　Ｔｅｓｑｕｅ，Ｉｎｃ．；０．０８５５ｍＬ）を加えた。本溶液のｐＨが７．０±０．１内であることを確認した後に、３７℃で２時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

[0287] 抗体と薬物リンカーのコンジュゲーション：上記溶液を１５℃で１０分間インキュベートした。次いでＮ－［６－（２，５－ジオキソ－２，５－ジヒドロ－１Ｈ－ピロール－１－イル）ヘキサノイル］グリシルグリシル－Ｌ－フェニルアラニル－Ｎ－［（２－｛［（１Ｓ，９Ｓ）－９－エチル－５－フルオロ－９－ヒドロキシ－４－メチル－１０，１３－ジオキソ－２，３，９，１０，１３，１５－ヘキサヒドロ－１Ｈ，１２Ｈ－ベンゾ［ｄｅ］ピラノ［３’，４’：６，７］インドリジノ［１，２－ｂ］キノリン－１－イル］

アミノ｝－２－オキソエトキシ）メチル］グリシンアミドの１０ｍＭ　ジメチルスルホキシド溶液（０．３８６ｍＬ；抗体一分子に対して１０当量）を加え、１５℃で１時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、１００ｍＭ　ＮＡＣ（Ｓｉｇｍａ－Ａｌｄｒｉｃｈ　Ｃｏ．ＬＬＣ）水溶液（０．０３４７ｍＬ；抗体一分子に対して９当量）を加え、さらに室温にて２０分間撹拌し、薬物リンカーの反応を停止させた。

[0288]　精製：上記溶液を、製造方法１に記載した共通操作Ｄによる精製を行い、標記抗体－薬物コンジュゲート「Ｈ０１Ｌ０２－ＡＤＣ」を含有する溶液を１９ｍＬ得た。

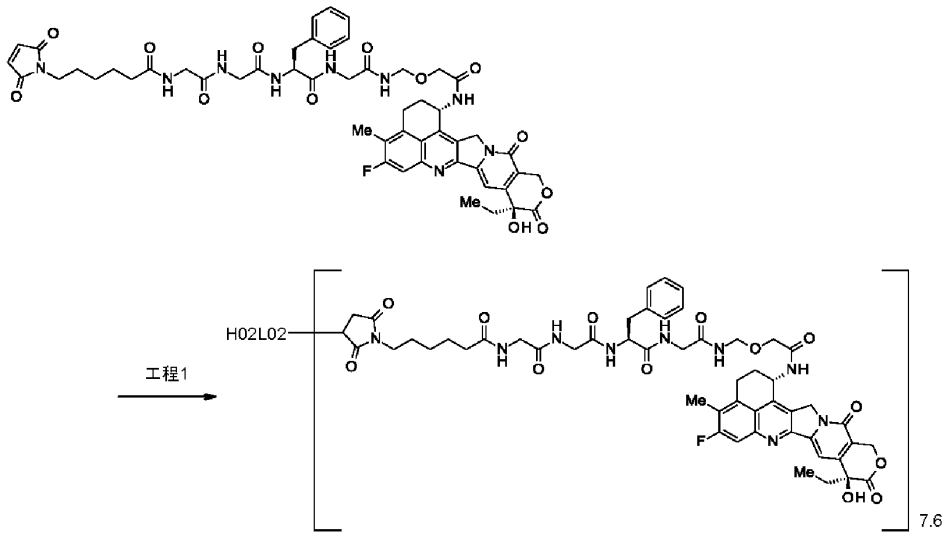
[0289]　特性評価：製造方法１に記載した共通操作Ｅ（$\varepsilon_{D,280}$＝５４４０、$\varepsilon_{D,370}$＝２１２４０を使用）を使用して、下記の特性値を得た。

抗体濃度：２．２６ｍｇ／ｍＬ，抗体収量：４２．９ｍｇ（７６％），共通操作Ｅにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：５．９；共通操作Ｆにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：７．７。

[0290]　７）－２　抗体－薬物コンジュゲートの作製　Ｈ０２Ｌ０２－ＤＸｄ

工程１：抗体－薬物コンジュゲート（２）

[0291] [化12]



[0292] 抗体の還元：実施例５にて作製したＨ０２Ｌ０２を、製造方法１に記載した共通操作Ｂ（２８０ｎｍ吸光係数として１．５１ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$を使用）及びＣを用いて、ＰＢＳ６．０／ＥＤＴＡにて９．９５ｍｇ／ｍＬに調製した。本溶液（５．７ｍＬ）に１０ｍＭ　ＴＣＥＰ（東京化成工業株式会社）水溶液（０．２３４ｍＬ；抗体一分子に対して６．０当量）及び１Ｍリン酸水素二カリウム水溶液（Ｎａｃａｌａｉ　Ｔｅｓｑｕｅ，Ｉｎｃ．；０．０８５５ｍＬ）を加えた。本溶液のｐＨが７．０±０．１内であることを確認した後に、３７℃で２時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

[0293] 抗体と薬物リンカーのコンジュゲーション：上記溶液を１５℃で１０分間インキュベートした。次いでＮ－［６－（２，５－ジオキソ－２，５－ジヒドロ－１Ｈ－ピロール－１－イル）ヘキサノイル］グリシルグリシル－Ｌ－フェニルアラニル－Ｎ－［（２－｛［（１Ｓ，９Ｓ）－９－エチル－５－フルオロ－９－ヒドロキシ－４－メチル－１０，１３－ジオキソ－２，３，９，１０，１３，１５－ヘキサヒドロ－１Ｈ，１２Ｈ－ベンゾ［ｄｅ］ピラノ［3’，4’：6，7］インドリジノ［1，2－ｂ］キノリン－１－イル］アミノ｝－２－オキソエトキシ）メチル］グリシンアミドの１０ｍＭ　ジメチルスルホキシド溶液（０．３８９ｍＬ；抗体一分子に対して１０当量）を加え、１５℃で１時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、１００ｍＭ　ＮＡＣ（Ｓｉｇｍａ－Ａｌｄｒｉｃｈ　Ｃｏ．ＬＬＣ）水溶液（０．０３５０ｍＬ；抗体一分子に対して９当量）を加え、さらに室温にて２０分間撹拌し、薬物リンカーの反応を停止させた。

[0294] 精製：上記溶液を、製造方法１に記載した共通操作Ｄによる精製を行い、標記抗体－薬物コンジュゲート「Ｈ０２Ｌ０２－ＡＤＣ」を含有する溶液を１９ｍＬ得た。

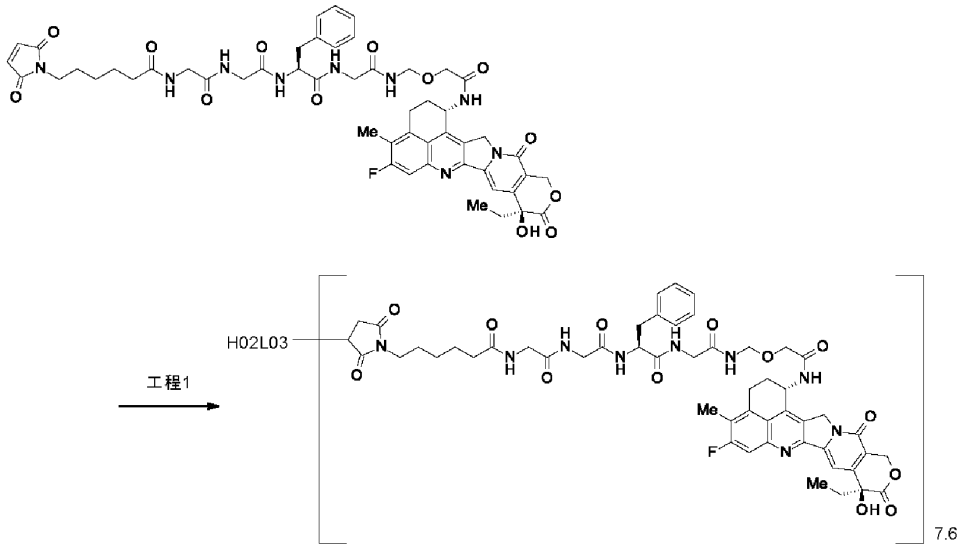
[0295] 特性評価：製造方法１に記載した共通操作Ｅ（$\varepsilon_{D,280}$＝５４４０、$\varepsilon_{D,370}$＝２１２４０を使用）を使用して、下記の特性値を得た。

抗体濃度：２．６１ｍｇ／ｍＬ，抗体収量：４９．６ｍｇ（８７％），共通操作Ｅにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：５．９；共通操作Ｆにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：７．６。

[0296] ７）－３　抗体－薬物コンジュゲートの作製　Ｈ０２Ｌ０３－ＤＸｄ

工程１：抗体－薬物コンジュゲート（３）

[0297] [化13]



[0298] 抗体の還元：実施例５にて作製したＨ０２Ｌ０３を、製造方法１に記載した共通操作Ｂ（２８０ｎｍ吸光係数として１．５３ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$を使用）及びＣを用いて、ＰＢＳ６．０／ＥＤＴＡにて９．８６ｍｇ／ｍＬに調製した。本溶液（５．７ｍＬ）に１０ｍＭ　ＴＣＥＰ（東京化成工業株式会社）水溶液（０．２７０ｍＬ；抗体一分子に対して７．０当量）及び１Ｍリン酸水素二カリウム水溶液（Ｎａｃａｌａｉ　Ｔｅｓｑｕｅ，Ｉｎｃ．；０．０８５５ｍＬ）を加えた。本溶液のｐＨが７．０±０．１内であることを確認した後に、３７℃で２時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

[0299] 抗体と薬物リンカーのコンジュゲーション：上記溶液を１５℃で１０分間

インキュベートした。次いでＮ－［６－（２，５－ジオキソ－２，５－ジヒドロ－１Ｈ－ピロール－１－イル）ヘキサノイル］グリシルグリシル－Ｌ－フェニルアラニル－Ｎ－［（２－｛［（１Ｓ，９Ｓ）－９－エチル－５－フルオロ－９－ヒドロキシ－４－メチル－１０，１３－ジオキソ－２，３，９，１０，１３，１５－ヘキサヒドロ－１Ｈ，１２Ｈ－ベンゾ［ｄｅ］ピラノ［３’，４’：６，７］インドリジノ［１，２－ｂ］キノリン－１－イル］アミノ｝－２－オキソエトキシ）メチル］グリシンアミドの１０ｍＭ　ジメチルスルホキシド溶液（０．３８６ｍＬ；抗体一分子に対して１０当量）を加え、１５℃で１時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、１００ｍＭ　ＮＡＣ（Ｓｉｇｍａ－Ａｌｄｒｉｃｈ　Ｃｏ．ＬＬＣ）水溶液（０．０３４７ｍＬ；抗体一分子に対して９当量）を加え、さらに室温にて２０分間撹拌し、薬物リンカーの反応を停止させた。

[0300]　精製：上記溶液を、製造方法１に記載した共通操作Ｄによる精製を行い、標記抗体－薬物コンジュゲート「Ｈ０１Ｌ０２－ＡＤＣ」を含有する溶液を１９ｍＬ得た。

[0301]　特性評価：製造方法１に記載した共通操作Ｅ（$\varepsilon_{D,280}$＝５４４０、$\varepsilon_{D,370}$＝２１２４０を使用）を使用して、下記の特性値を得た。

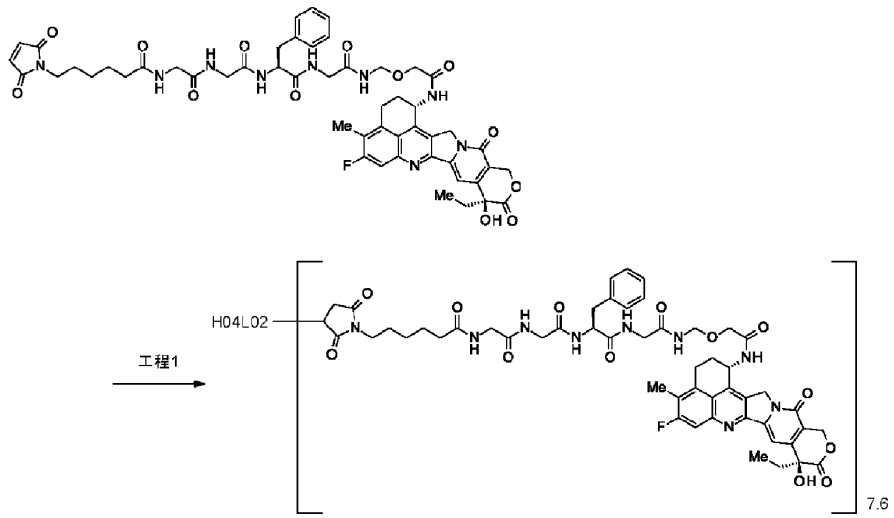
抗体濃度：２．７１ｍｇ／ｍＬ，抗体収量：５１．４ｍｇ（９１％），共通操作Ｅにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：５．７；共通操作Ｆにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：７．６。

[0302]　７）－４　抗体－薬物コンジュゲートの作製　Ｈ０４Ｌ０２－ＤＸｄ

工程１：抗体－薬物コンジュゲート（４）

[0303]

[化14]



[0304] 抗体の還元：実施例５にて作製したＨ０４Ｌ０２を、製造方法１に記載した共通操作Ｂ（２８０ｎｍ吸光係数として１．５３ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$を使用）及びＣを用いて、ＰＢＳ６．０／ＥＤＴＡにて９．８６ｍｇ／ｍＬに調製した。本溶液（５．７ｍＬ）に１０ｍＭ　ＴＣＥＰ（東京化成工業株式会社）水溶液（０．２３２ｍＬ；抗体一分子に対して６．０当量）及び１Ｍリン酸水素二カリウム水溶液（Ｎａｃａｌａｉ　Ｔｅｓｑｕｅ，Ｉｎｃ．；０．０８５５ｍＬ）を加えた。本溶液のｐＨが７．０±０．１内であることを確認した後に、３７℃で２時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

[0305] 抗体と薬物リンカーのコンジュゲーション：上記溶液を１５℃で１０分間インキュベートした。次いでＮ－［６－（２，５－ジオキソ－２，５－ジヒドロ－１Ｈ－ピロール－１－イル）ヘキサノイル］グリシルグリシル－Ｌ－フェニルアラニル－Ｎ－［（２－｛［（１Ｓ，９Ｓ）－９－エチル－５－フルオロ－９－ヒドロキシ－４－メチル－１０，１３－ジオキソ－２，３，９，１０，１３，１５－ヘキサヒドロ－１Ｈ，１２Ｈ－ベンゾ［ｄｅ］ピラノ［３’，４’：６，７］インドリジノ［１，２－ｂ］キノリン－１－イル］

アミノ｝－２－オキソエトキシ）メチル］グリシンアミドの１０ｍＭ　ジメチルスルホキシド溶液（０．３８６ｍＬ；抗体一分子に対して１０当量）を加え、１５℃で１時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、１００ｍＭ　ＮＡＣ（Ｓｉｇｍａ－Ａｌｄｒｉｃｈ　Ｃｏ．ＬＬＣ）水溶液（０．０３４７ｍＬ；抗体一分子に対して９当量）を加え、さらに室温にて２０分間撹拌し、薬物リンカーの反応を停止させた。

[0306] 精製：上記溶液を、製造方法１に記載した共通操作Ｄによる精製を行い、標記抗体－薬物コンジュゲート「Ｈ０４Ｌ０２－ＡＤＣ」を含有する溶液を１９ｍＬ得た。

[0307] 特性評価：製造方法１に記載した共通操作Ｅ（$\varepsilon_{D,280}$＝５４４０、$\varepsilon_{D,370}$＝２１２４０を使用）を使用して、下記の特性値を得た。

抗体濃度：２．５６ｍｇ／ｍＬ，抗体収量：４８．７ｍｇ（８７％），共通操作Ｅにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：５．８；共通操作Ｆにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：７．６。

[0308]

［参考例２：ＮＯＶ０７１２－薬物コンジュゲートの作製］

参考例２）－１　抗体－薬物コンジュゲートの作製　ＮＯＶ０７１２－ＤＭ４

抗体－薬物コンジュゲート（５）

抗体と薬物リンカーのコンジュゲーション：参考例1にて作製したＮＯＶ０７１２を、製造方法１に記載した共通操作Ｂ（２８０ｎｍ吸光係数として１．５１ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$を使用）及びＣを用いて、２０ｍＭ　ＨＥＰＥＳ８．１（ＬＩＦＥ　ＴＥＣＨＮＯＬＯＧＩＥＳ社製　ＨＥＰＥＳ，　１Ｍ　Ｂｕｆｆｅｒ　Ｓｏｌｕｔｉｏｎ（２０ｍＬ）を１M水酸化ナトリウムでｐＨ８．１とした後、蒸留水にて１Ｌとした）にて９．７ｍｇ／ｍＬに調製し、２０℃で１０分間インキュベートした。次いでＷＯ２０１６／０２４１９５号公開特許公報に記載の１０ｍＭ　１－（２，５－ジオキソピロリジン－１

－イルオキシ）－１－オキソ－４－（ピリジン－２－イルジスルファニル）ブタン－２－スルフォン酸のＤＭＡ溶液（０．３６６ｍＬ；抗体一分子に対して５．２当量）、１０ｍＭ　Ｎ２－デアセチル－デアセチル－Ｎ２－（４－メチル－４－メルカプト－１－オキソペンチル）－メイタンシン（ＤＭ４）のＤＭＡ溶液（０．３６６ｍＬ；抗体一分子に対して６．８当量）、及び０．２４３ｍＬのＤＭＡを加え、２０℃で１６時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、１Ｍ　酢酸水溶液を加えｐＨ５．０とし、さらに室温にて２０分間撹拌し、薬物リンカーの反応を停止させた。

[0309] 精製：上記溶液を、製造方法１に記載した共通操作Ｄによる精製を行い、標記抗体－薬物コンジュゲート「ＮＯＶ０７１２－ＤＭ４」を含有する溶液を２８ｍＬ得た。

[0310] 特性評価：製造方法１に記載した共通操作Ｅ（$\varepsilon_{A,280}$＝２００５００、$\varepsilon_{A,252}$＝７６２９５、$\varepsilon_{D,280}$＝４３１７０、及び$\varepsilon_{D,252}$＝２３２２４を使用）を使用して、下記の特性値を得た。

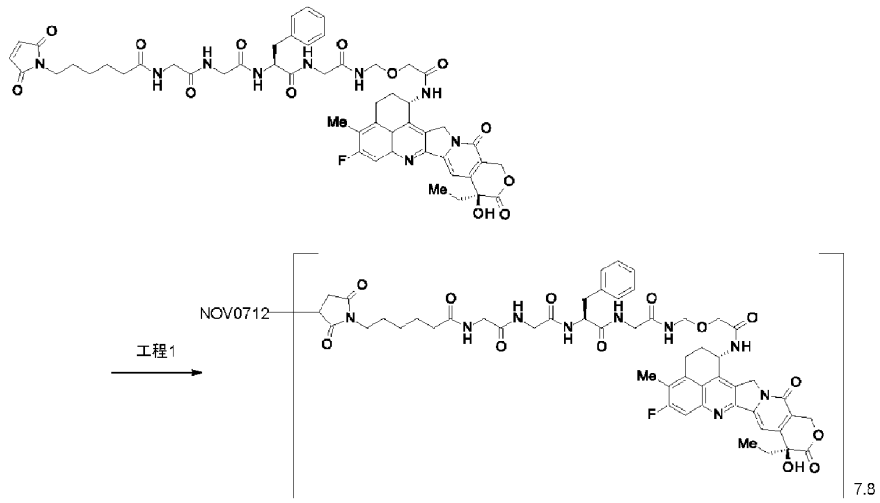
抗体濃度：２．５８ｍｇ／ｍＬ，抗体収量：７２．２ｍｇ（９３％），共通操作Ｅにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：３．０。

[0311] 参考例２）－２　抗体－薬物コンジュゲートの作製　ＮＯＶ０７１２－ＤＸｄ

工程１：抗体－薬物コンジュゲート（６）

[0312]

[化15]



[0313] 抗体の還元：参考例１にて作製したＮＯＶ０７１２を、製造方法１に記載した共通操作Ｂ（２８０ｎｍ吸光係数として１．５１ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$を使用）及びＣを用いて、ＰＢＳ６．０／ＥＤＴＡにて９．２６ｍｇ／ｍＬに調製した。本溶液（６．６ｍＬ）に１０ｍＭ　ＴＣＥＰ（東京化成工業株式会社）水溶液（０．２５４ｍＬ；抗体一分子に対して６．０当量）及び１Ｍリン酸水素二カリウム水溶液（Ｎａｃａｌａｉ　Ｔｅｓｑｕｅ，Ｉｎｃ．；０．０９９０ｍＬ）を加えた。本溶液のｐＨが７．０±０．１内であることを確認した後に、３７℃で２時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

[0314] 抗体と薬物リンカーのコンジュゲーション：上記溶液を１５℃で１０分間インキュベートした。次いでＮ－［６－（２，５－ジオキソ－２，５－ジヒドロ－１Ｈ－ピロール－１－イル）ヘキサノイル］グリシルグリシル－Ｌ－フェニルアラニル－Ｎ－［（２－｛［（１Ｓ，９Ｓ）－９－エチル－５－フルオロ－９－ヒドロキシ－４－メチル－１０，１３－ジオキソ－２，３，９，１０，１３，１５－ヘキサヒドロ－１Ｈ，１２Ｈ－ベンゾ［ｄｅ］ピラノ［３’，４’：６，７］インドリジノ［１，２－ｂ］キノリン－１－イル］アミノ｝－２－オキソエトキシ）メチル］グリシンアミドの１０ｍＭ　ジメ

チルスルホキシド溶液（０．３８１ｍＬ；抗体一分子に対して９当量）を加え、１５℃で１時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、１００ｍＭ　ＮＡＣ（Ｓｉｇｍａ－Ａｌｄｒｉｃｈ　Ｃｏ．ＬＬＣ）水溶液（０．０３８１ｍＬ；抗体一分子に対して９当量）を加え、さらに室温にて２０分間撹拌し、薬物リンカーの反応を停止させた。

[0315] 精製：上記溶液を、製造方法１に記載した共通操作Ｄによる精製を行い、標記抗体－薬物コンジュゲート「ＮＯＶ０７１２－ＡＤＣ」を含有する溶液を２３．５ｍＬ得た。

[0316] 特性評価：製造方法１に記載した共通操作Ｅ（$\varepsilon_{D,280}$＝５４４０、$\varepsilon_{D,370}$＝２１２４０を使用）を使用して、下記の特性値を得た。

抗体濃度：２．２６ｍｇ／ｍＬ，抗体収量：５６．４ｍｇ（９２％），共通操作Ｅにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：６．４；共通操作Ｆにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：７．８。

[0317]

［参考例３：Ｈ０１Ｌ０２－ＤＭ４の作製］

参考例３）－１　抗体－薬物コンジュゲートの作製　Ｈ０１Ｌ０２－ＤＭ４

抗体－薬物コンジュゲート（７）

抗体と薬物リンカーのコンジュゲーション：実施例５にて作製したＨ０１Ｌ０２を、製造方法１に記載した共通操作Ｂ（２８０ｎｍ吸光係数として１．５３ｍＬｍｇ$^{-1}$ｃｍ$^{-1}$を使用）及びＣを用いて、２０ｍＭ　ＨＥＰＥＳ８．１（ＬＩＦＥ　ＴＥＣＨＮＯＬＯＧＩＥＳ社製　ＨＥＰＥＳ，　１Ｍ　Ｂｕｆｆｅｒ　Ｓｏｌｕｔｉｏｎ（２０ｍＬ）を１Ｍ水酸化ナトリウムでｐＨ８．１とした後、蒸留水にて１Ｌとした）にて９．８ｍｇ／ｍＬに調製し、２０℃で１０分間インキュベートした。次いでＷＯ２０１６／０２４１９５号公開特許公報に記載の１０ｍＭ　１－（２，５－ジオキソピロリジン－１－イルオキシ）－１－オキソ－４－（ピリジン－２－イルジスルファニル）

ブタン－２－スルフォン酸のＤＭＡ溶液（０．０６２ｍＬ；抗体一分子に対して１１．５当量）、１０ｍＭ　Ｎ２－デアセチル－Ｎ２－（４－メチル－４－メルカプト－１－オキソペンチル）－メイタンシン（ＤＭ４）のＤＭＡ溶液（０．０８２ｍＬ；抗体一分子に対して１５．１当量）を加え、２０℃で１８時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、１Ｍ　酢酸水溶液を加えｐＨ５．０とし、さらに室温にて２０分間撹拌し、薬物リンカーの反応を停止させた。

[0318] 精製：上記溶液を、製造方法１に記載した共通操作Ｄによる精製を行い、標記抗体－薬物コンジュゲート「Ｈ０１Ｌ０２－ＤＭ４」を含有する溶液を３．５ｍＬ得た。

[0319] 特性評価：製造方法１に記載した共通操作Ｅ（εＡ，２８０＝２２３４００、εＡ，２５２＝８５６４６、εＤ，２８０＝４３１７、及びεＤ，２５２＝２３２２４を使用）を使用して、下記の特性値を得た。

抗体濃度：１．９７ｍｇ／ｍＬ，抗体収量：６．９０ｍｇ（８８％），共通操作Ｅにて測定された抗体一分子あたりの薬物平均結合数（ｎ）：３．６。

[0320]

［実施例８：抗体－薬物コンジュゲートのｉｎ　ｖｉｔｒｏ活性評価］

８）－１　ＣＤＨ６陽性ヒト腫瘍細胞株に対する抗体－薬物コンジュゲートのｉｎ　ｖｉｔｒｏ細胞増殖抑制活性評価

ＣＤＨ６陽性ヒト卵巣腫瘍細胞株ＰＡ－１を１０％　ＦＢＳ含有ＭＥＭ培地中２ｘ１０^{3}細胞／１００μＬ／ｗｅｌｌになるよう９６ウェルプレートに播種し、３７℃、５％　CO_2の条件下で一晩培養した。翌日、実施例７で作製したヒト化ｈＧ０１９－薬物コンジュゲート４種（クローン名は、Ｈ０１Ｌ０２－ＤＸｄ、Ｈ０２Ｌ０２－ＤＸｄ、Ｈ０２Ｌ０３－ＤＸｄ及びＨ０４Ｌ０２－ＤＸｄ）、又は参考例２で作製したＮＯＶ０７１２－薬物コンジュゲート（ＮＯＶ０７１２－ＤＭ４）を終濃度０．０００１（ｎＭ）から１００（ｎＭ）となるように添加した。４日間培養後に生存細胞数をＣｅｌｌＴｉｔｅｒ－Ｇｌｏ™ Ｌｕｍｉｎｅｓｃｅｎｔ Ｃｅｌｌ Ｖｉａｂｉｌｉ

ｔｙ　Ａｓｓａｙ（Ｐｒｏｍｅｇａ）によるＡＴＰの定量で測定した。図１１に抗体－薬物コンジュゲートを各々添加した際の濃度依存的な細胞増殖抑制活性を示す。この結果、ヒト化ｈＧ０１９－薬物コンジュゲート４種は、ＮＯＶ０７１２－薬物コンジュゲートと比較して、より低い添加濃度から腫瘍細胞の増殖抑制活性を示し、抗腫瘍活性が高いことが示される。

[0321]

［実施例９：抗体－薬物コンジュゲートのｉｎ　ｖｉｖｏ抗腫瘍効果］

抗体－薬物コンジュゲートの抗腫瘍効果は、ＣＤＨ６陽性ヒト腫瘍細胞株の細胞を免疫不全マウスに移植した動物モデルを用いて評価した。４－５週齢のＢＡＬＢ／ｃ　ヌードマウス（ＣＡｎＮ．Ｃｇ－Ｆｏｘｎｌ［ｎｕ］／ＣｒｌＣｒｌｊ［Ｆｏｘｎｌｎｕ／Ｆｏｘｎｌｎｕ］、日本チャールス・リバー）及び、ＳＣＩＤマウス（ＣＢ１７／Ｉｃｒ－Ｐｒｋｄｃ［ｓｃｉｄ］／ＣｒｌＣｒｌｊ、日本チャールス・リバー）を実験使用前にＳＰＦ条件化で３日間以上馴化した。マウスには滅菌した固形飼料（ＦＲ－２，Ｆｕｎａｂａｓｈｉ　Ｆａｒｍｓ　Ｃｏ．，Ｌｔｄ）を給餌し、滅菌した水道水（５－１５ｐｐｍ次亜塩素酸ナトリウム溶液を添加して調製）を与えた。移植した腫瘍の長径及び短径を電子式デジタルノギス（ＣＤ－１５ＣＸ，Ｍｉｔｕｔｏｙｏ　Ｃｏｒｐ．）で１週間に２回測定し、以下に示す計算式により腫瘍体積を算出した。

腫瘍体積（mm^3）＝１／２×長径（ｍｍ）×［短径（ｍｍ）］^{2}

抗体－薬物コンジュゲートは全てＡＢＳ緩衝液（１０ｍＭ－Ａｃｅｔａｔｅ　Ｂｕｆｆｅｒ，　５％　Ｓｏｒｂｉｔｏｌ，　ｐＨ５．５）（ＮＡＣＡＬＡＩ）で希釈し、各実施例に示す用量にて尾静脈内投与した。また、コントロール群（Ｖｅｈｉｃｌｅ群）としてＡＢＳ緩衝液を同様に投与した。１群あたり６匹のマウスを実験に用いた。

[0322]　９）－１　抗腫瘍効果（１）

実施例２）－３－１にてＣＤＨ６発現を確認した、ＣＤＨ６陽性ヒト腎細胞腫瘍細胞株７８６－Ｏ（ＡＴＣＣ）をマトリゲル（コーニング社）に懸濁

し、５×１０^{6}ｃｅｌｌｓを雄ＳＣＩＤマウスの右側腹部に皮下移植し（Ｄａｙ０）、Ｄａｙ１８に無作為に群分けを実施した。群分け実施日に、実施例７で作製した抗体－薬物コンジュゲート４種（クローン名は、Ｈ０１Ｌ０２－ＤＸｄ、Ｈ０２Ｌ０２－ＤＸｄ、Ｈ０２Ｌ０３－ＤＸｄ及びＨ０４Ｌ０２－ＤＸｄ）、又は参考例２で作製したＮＯＶ０７１２－ＤＭ４を３ｍｇ／ｋｇの用量で尾静脈内投与した。結果を図１２に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

[0323] ＮＯＶ０７１２－ＤＭ４は本腫瘍モデルにおいて顕著な抗腫瘍効果を示さなかった。実施例７で作成した抗体－薬物コンジュゲート４種はいずれも投与後腫瘍体積が減少し、顕著な腫瘍の退縮が認められ、投与後２４日の間、腫瘍退縮効果が持続した（図１２）。

[0324] ９）－２　抗腫瘍効果（２）

実施例２）－３－１にてＣＤＨ６発現を確認した、ＣＤＨ６陽性ヒト卵巣腫瘍細胞株ＰＡ－１（ＡＴＣＣ）をマトリゲル（コーニング社）に懸濁し、８．５×１０^{6}ｃｅｌｌｓを雌ヌードマウスの右側腹部に皮下移植し（Ｄａｙ０）、Ｄａｙ１１に無作為に群分けを実施した。群分け実施日に、実施例７で作製した抗体－薬物コンジュゲートＨ０１Ｌ０２－ＤＸｄ又は参考例２で作製したＮＯＶ０７１２－ＤＭ４もしくはＮＯＶ０７１２－ＤＸｄを、１及び３ｍｇ／ｋｇの用量で尾静脈内投与した。結果を図１３に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

[0325] ＮＯＶ０７１２－ＤＭ４は本腫瘍モデルにおいて１及び３ｍｇ／ｋｇいずれの用量でも抗腫瘍効果を示さなかった。一方、Ｈ０１Ｌ０２－ＤＸｄは１及び３ｍｇ／ｋｇいずれの用量でも投与後腫瘍体積が著しく減少し、腫瘍を縮退させる効果を発揮した（図１３）。また、本明細書で取得したＨ０１Ｌ０２抗体もしくはＮＯＶ０７１２抗体に同じ薬物ＤＸｄをコンジュゲートした検体の薬効を比較したところ、Ｈ０１Ｌ０２－ＤＸｄは、１及び３ｍｇ／ｋｇいずれの用量でもＮＯＶ０７１２－ＤＸｄに比べて強い抗腫瘍効果を発揮した。すなわち、本発明のＨ０１Ｌ０２抗体は、ＮＯＶ０７１２抗体に比

べて、抗腫瘍剤としての抗体－薬物コンジュゲートの抗体としてより優れた抗体であることが示された（図１３）。

[0326] ９）－３　抗腫瘍効果（３）

実施例２）－３－１にてＣＤＨ６発現を確認した、ＣＤＨ６陽性ヒト卵巣腫瘍細胞株ＮＩＨ：ＯＶＣＡＲ－３（ＡＴＣＣ）をマトリゲル（コーニング社）に懸濁し、１×１０^{7}ｃｅｌｌｓを雌ヌードマウスの右側腹部に皮下移植し（Ｄａｙ０）、Ｄａｙ２２に無作為に群分けを実施した。群分け実施日に、実施例７で作製した抗体－薬物コンジュゲートＨ０１Ｌ０２－ＤＸｄ又は参考例２で作製したＮＯＶ０７１２－ＤＭ４を、１及び３ｍｇ／ｋｇの用量で尾静脈内投与した。結果を図１４に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

[0327] ＮＯＶ０７１２－ＤＭ４は、１ｍｇ／ｋｇでは全く抗腫瘍効果を示さず、３ｍｇ／ｋｇでは抗腫瘍効果を示すものの、投与２週間後から腫瘍の再増殖が認められた。一方、Ｈ０１Ｌ０２－ＤＸｄは１及び３ｍｇ／ｋｇいずれの用量でも、投与後腫瘍体積の増加を著しく抑制し、特に３ｍｇ／ｋｇでは投与後３１日間の長期にわたって腫瘍増殖抑制効果が持続した。（図１４）。

[0328] また、ＰＡ－１細胞を用いて同様に、参考例２で作製したＮＯＶ０７１２－ＤＭ４もしくは参考例３で作成したＨ０１Ｌ０２－ＤＭ４の腫瘍増殖抑制効果を評価した。Ｈ０１Ｌ０２－ＤＭ４はＮＯＶ０７１２－ＤＭ４よりも腫瘍体積を縮退させ、本発明のＨ０１Ｌ０２抗体は、ＮＯＶ０７１２抗体に比べて、抗腫瘍剤としての抗体－薬物コンジュゲートの抗体としてより優れた抗体であった。

[0329] ９）－４　抗腫瘍効果（４）

実施例２）－３－１にてＣＤＨ６発現を確認した、ＣＤＨ６陽性ヒト腎細胞腫瘍細胞株７８６－Ｏ（ＡＴＣＣ）をマトリゲル（コーニング社）に懸濁し、５×１０^{6}ｃｅｌｌｓを雄ＳＣＩＤマウスの右側腹部に皮下移植し（Ｄａｙ０）、Ｄａｙ２０に無作為に群分けを実施した。群分け実施日に、実施例７で作製した抗体－薬物コンジュゲートＨ０１Ｌ０２－ＤＸｄ又は参考例２

で作製したＮＯＶ０７１２－ＤＭ４を、１及び３ｍｇ／ｋｇの用量で尾静脈内投与した。結果を図１５に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

[0330] ＮＯＶ０７１２－ＤＭ４は本腫瘍モデルにおいて１及び３ｍｇ／ｋｇいずれの用量でも顕著な抗腫瘍効果を示さなかった。一方、Ｈ０１Ｌ０２－ＤＸｄは１及び３ｍｇ／ｋｇいずれの用量でも投与後腫瘍体積が減少し、特に３ｍｇ／ｋｇでは顕著な腫瘍の退縮が認められ、投与後２０日の間、腫瘍退縮効果が持続した（図１５）。

[0331] ９）－５　抗腫瘍効果（５）

実施例２）－３－１にてＣＤＨ６を発現しないことを確認した、ＣＤＨ６陰性ヒト卵巣腫瘍細胞株ＥＳ－２（ＡＴＣＣ）を生理食塩水に懸濁し、１×10^6ｃｅｌｌｓを雌ヌードマウスの右側腹部に皮下移植し（Ｄａｙ０）、Ｄａｙ７に無作為に群分けを実施した。群分け実施日に、実施例７で作成した抗体－薬物コンジュゲートＨ０１Ｌ０２－ＤＸｄ又は参考例２で作製したＮＯＶ０７１２－ＤＭ４を、１及び３ｍｇ／ｋｇの用量で尾静脈内投与した。結果を図１６に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲はＳＥ値を示す。

[0332] ＣＤＨ６を発現しない本腫瘍モデルでは、Ｈ０１Ｌ０２－ＤＸｄ及びＮＯＶ０７１２－ＤＭ４はいずれの用量でも抗腫瘍効果を全く示さなかった。この結果から、実施例９）－１、９）－２、９）－３、９）－４で示されたＣＤＨ６陽性腫瘍モデルでの抗体－薬物コンジュゲートの抗腫瘍効果は、腫瘍細胞のＣＤＨ６発現に依存して起こる効果であり、ＣＤＨ６陽性の腫瘍に特異的に抗腫瘍効果を示し、ＣＤＨ６陰性の正常組織には細胞障害を起こさない選択的で安全な抗腫瘍薬剤と考えられる（図１６）。

産業上の利用可能性

[0333] 本発明により、内在化活性を有する抗ＣＤＨ６抗体及び該抗体を含む抗体－薬物コンジュゲートが提供された。該抗体－薬物コンジュゲートは癌に対する治療薬等として用いることができる。

請求の範囲

[請求項1] 配列番号４に記載のアミノ酸配列に特異的に結合し、細胞内に取り込まれる内在化能を有する、抗体又は当該抗体の機能性断片。

[請求項2] 配列番号４に記載のアミノ酸配列への結合に対して、以下の（１）～（５）：

（１）配列番号５３の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号５６の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、

（２）配列番号６１の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、

（３）配列番号６１の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、

（４）配列番号６５の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、及び

（５）配列番号６１の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、

からなる群から選択される抗体のうち少なくともいずれか１つと競合阻害活性を有する請求項１に記載の抗体又は当該抗体の機能性断片。

[請求項3] 以下の（１）～（４）：

（１）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に記載のアミノ酸配列からなるＣＤＲＬ３、

（２）配列番号２２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号２３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号２４

に記載のアミノ酸配列からなるＣＤＲＬ３、

（３）配列番号３２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号３３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号３４に記載のアミノ酸配列からなるＣＤＲＬ３、及び

（４）配列番号４２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号４３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号４４に記載のアミノ酸配列からなるＣＤＲＬ３、

からなる群から選択されるいずれか１つに記載のＣＤＲＬ１、ＣＤＲＬ２及びＣＤＲＬ３、並びに、

以下の（５）～（９）：

（５）配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号１８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３、

（６）配列番号２７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号２８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号２９に記載のアミノ酸配列からなるＣＤＲＨ３、

（７）配列番号３７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号３８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号３９に記載のアミノ酸配列からなるＣＤＲＨ３、

（８）配列番号４７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号４８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号４９に記載のアミノ酸配列からなるＣＤＲＨ３、及び

（９）配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号６０に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３、

からなる群から選択されるいずれか１つに記載のＣＤＲＨ１、ＣＤＲＨ２及びＣＤＲＨ３を含む、

請求項１又２のいずれか１項に記載の抗体又は当該抗体の機能性断片

。

[請求項4] 以下の（１）～（５）：

（１）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号１８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３、

（２）配列番号２２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号２３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号２４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号２７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号２８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号２９に記載のアミノ酸配列からなるＣＤＲＨ３、

（３）配列番号３２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号３３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号３４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号３７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号３８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号３９に記載のアミノ酸配列からなるＣＤＲＨ３、

（４）配列番号４２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号４３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号４４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号４７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号４８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号４９に記載のアミノ酸配列からなるＣＤＲＨ３、及び

（５）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４

に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号６０に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３、

からなる群から選択されるＣＤＲＬ１、ＣＤＲＬ２及びＣＤＲＬ３、並びに、ＣＤＲＨ１、ＣＤＲＨ２及びＣＤＲＨ３を含む、

請求項１～３のいずれか１項に記載の抗体又は当該抗体の機能性断片。

[請求項5] ヒト化されている請求項１～４のいずれか１項に記載の抗体又は当該抗体の機能性断片。

[請求項6] 以下の（１）～（４）：

（１）配列番号６３に記載のアミノ酸配列からなる軽鎖可変領域、

（２）配列番号６７に記載のアミノ酸配列からなる軽鎖可変領域、

（３）（１）～（２）のアミノ酸配列において各ＣＤＲ配列以外のフレームワーク領域の配列に対して少なくとも９５％以上の配列同一性を有するアミノ酸配列、及び

（４）（１）～（３）のアミノ酸配列において各ＣＤＲ配列以外のフレームワーク領域の配列において１又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列

からなる群から選択されるいずれか１つに記載の軽鎖可変領域、並びに、

以下の（５）～（９）：

（５）配列番号７１に記載のアミノ酸配列からなる重鎖可変領域、

（６）配列番号７５に記載のアミノ酸配列からなる重鎖可変領域、

（７）配列番号７９に記載のアミノ酸配列からなる重鎖可変領域、

（８）（５）～（７）のアミノ酸配列において各ＣＤＲ配列以外のフレームワーク領域の配列に対して少なくとも９５％以上の配列相同性を有するアミノ酸配列、及び

（９）（５）～（８）のアミノ酸配列において各ＣＤＲ配列以外のフレームワーク領域の配列において１又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列
からなる群から選択されるいずれか１つに記載の重鎖可変領域、
を有する請求項１～５のいずれか１項に記載の抗体又は当該抗体の機能性断片。

[請求項7] 以下の（１）～（４）：
（１）配列番号６３に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号７１に記載のアミノ酸配列からなる重鎖可変領域、
（２）配列番号６３に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号７５に記載のアミノ酸配列からなる重鎖可変領域、
（３）配列番号６７に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号７５に記載のアミノ酸配列からなる重鎖可変領域、又は
（４）配列番号６３に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号７９に記載のアミノ酸配列からなる重鎖可変領域、
のいずれかの軽鎖可変領域及び重鎖可変領域、
を含む請求項１～６のいずれか１項に記載の抗体又は当該抗体の機能性断片。

[請求項8] 以下の（１）～（４）：
（１）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖、
（２）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖、
（３）配列番号６５の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖、
又は
（４）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖、

のいずれかを有する請求項１～７のいずれか１項に記載の抗体又は当該抗体の機能性断片。

[請求項9] 配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖を有する請求項８に記載の抗体又は当該抗体の機能性断片。

[請求項10] 配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖を有する請求項８に記載の抗体又は当該抗体の機能性断片。

[請求項11] 配列番号６５の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖を有する請求項８に記載の抗体又は当該抗体の機能性断片。

[請求項12] 配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖を有する請求項８に記載の抗体又は当該抗体の機能性断片。

[請求項13] 機能性断片がＦａｂ、Ｆ（ａｂ’）２、Ｆａｂ’及びＦｖからなる群から選択される請求項１～１２のいずれか１項に記載の抗体の機能性断片。

[請求項14] 請求項１～１３のいずれか１項に記載の抗体又は当該抗体の機能性断片をコードするポリヌクレオチド。

[請求項15] 以下の（１）～（５）：

（１）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号１８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、

（２）配列番号２２に記載のアミノ酸配列からなるＣＤＲＬ１、配列

番号２３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号２４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号２７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号２８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号２９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、

（３）配列番号３２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号３３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号３４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号３７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号３８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号３９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、

（４）配列番号４２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号４３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号４４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号４７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号４８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号４９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、並びに

（５）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号６０に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、

からなる群から選択されるいずれか１つに記載のポリヌクレオチドを

含む請求項１４に記載のポリヌクレオチド。

[請求項16] 配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項１４又は１５に記載のポリヌクレオチド。

[請求項17] 配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項１４又は１５に記載のポリヌクレオチド。

[請求項18] 配列番号６５の２１～２３３番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項１４又は１５に記載のポリヌクレオチド。

[請求項19] 配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項１４又は１５に記載のポリヌクレオチド。

[請求項20] 請求項１４～１９のいずれか１項に記載のポリヌクレオチドを含有する発現ベクター。

[請求項21] 請求項２０に記載の発現ベクターにより形質転換された宿主細胞。

[請求項22] 宿主細胞が真核細胞である請求項２１に記載の宿主細胞。

[請求項23] 請求項２１又は２２に記載の宿主細胞を培養する工程、及び当該工程で得られた培養物から目的の抗体又は当該抗体の機能性断片を採取する工程を含むことを特徴とする当該抗体又は当該抗体の機能性断片の製造方法。

[請求項24] 重鎖又は軽鎖が、Ｎ－結合への糖鎖付加、Ｏ－結合への糖鎖付加、Ｎ末のプロセッシング、Ｃ末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、Ｎ末にメチオニン残基の付加、

プロリン残基のアミド化、Ｎ末グルタミンもしくはＮ末グルタミン酸のピログルタミン酸化、及びカルボキシル末端における１つ又は２つのアミノ酸欠失からなる群より選択される１又は２以上の修飾をうけた、請求項１～１３のいずれか１項に記載の抗体又は当該抗体の機能性断片。

[請求項25] 重鎖のカルボキシル末端において１つ又は２つのアミノ酸が欠失している請求項２４に記載の抗体。

[請求項26] ２本の重鎖の双方でカルボキシル末端において１つのアミノ酸が欠失している請求項２５に記載の抗体。

[請求項27] 重鎖のカルボキシル末端のプロリン残基が更にアミド化されている請求項２４～２６のいずれか１項に記載の抗体。

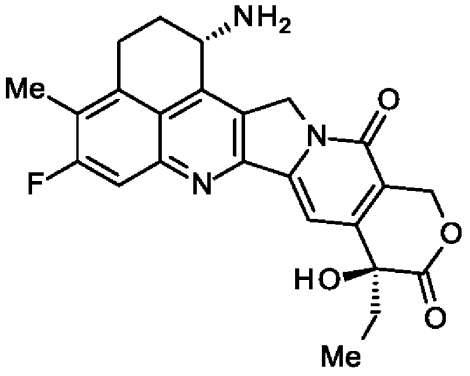
[請求項28] 抗体依存性細胞傷害活性を増強させるために糖鎖修飾が調節されている請求項１～１３及び請求項２４～２７からなる群から選択されるいずれか１項に記載の抗体又は当該抗体の機能性断片。

[請求項29] 請求項１～１３及び請求項２４～２８からなる群から選択されるいずれか１項に記載の抗体又は当該抗体の機能性断片に薬物が結合している抗体－薬物コンジュゲート。

[請求項30] 薬物が抗腫瘍性化合物である、請求項２９に記載の抗体－薬物コンジュゲート。

[請求項31] 抗腫瘍性化合物が次式：

[化1]



で示される抗腫瘍性化合物である請求項３０に記載の抗体－薬物コンジュゲート。

[請求項32] 抗体と薬物が、次式（ａ）～（ｆ）：

（ａ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

（ｂ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

（ｃ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、

（ｄ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、

（ｅ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＮＨ－CH_2CH_2O－CH_2CH_2O－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、及び

（ｆ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＮＨ－CH_2CH_2O－CH_2CH_2O－CH_2CH_2O－CH_2CH_2O－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

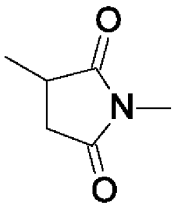
からなる群から選択されるいずれかの構造のリンカーを介して結合している、請求項２９～３１のいずれか１項に記載の抗体－薬物コンジュゲート。

（ここで、抗体は、－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）の末端において結合する。抗腫瘍性化合物は、１位のアミノ基の窒素原子を結合部位として、（ａ）、（ｂ）、（ｅ）又は（ｆ）の－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－部分、（ｃ）のCH_2－Ｏ－CH_2－Ｃ（＝

Ｏ）－部分又は（ｄ）のＣＨ$_2$ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－部分のカルボニル基に結合する。上記式中ＧＧＦＧは、グリシン－グリシン－フェニルアラニン－グリシンからなるペプチド結合でつながっているアミノ酸配列を示す。

－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－は次式：

[化2]



で示される構造であり、このものの３位で抗体と結合し、１位の窒素原子上でこれを含むリンカー構造内のメチレン基と結合する。）。

[請求項33] リンカーが以下の（ｃ）、（ｄ）及び（ｅ）からなる群から選択されるいずれかの式で示される請求項２９～３２のいずれか１項に記載の抗体－薬物コンジュゲート：

（ｃ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－、

（ｄ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$－Ｏ－ＣＨ$_2$－Ｃ（＝Ｏ）－、

（ｅ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＮＨ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$Ｏ－ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－ＣＨ$_2$ＣＨ$_2$ＣＨ$_2$－Ｃ（＝Ｏ）－。

[請求項34] リンカーが以下の（ｃ）又は（ｅ）の式で示される請求項２９～３３のいずれか１項に記載の抗体－薬物コンジュゲート：

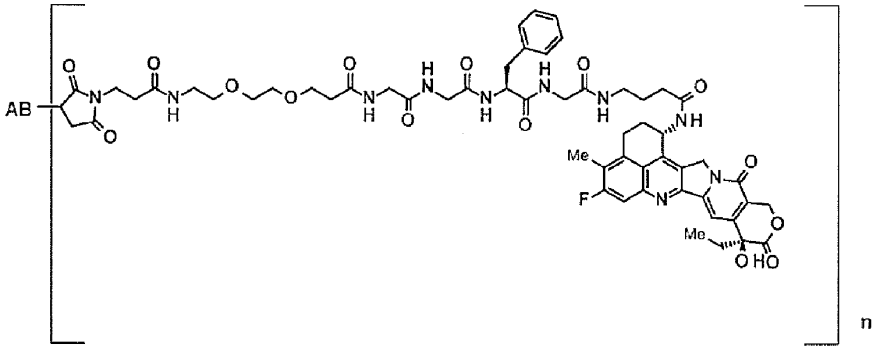
（ｃ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－ＣＨ$_2$ＣＨ$_2$Ｃ

$H_2CH_2CH_2-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-$、

(e)－(Succinimid－3－yl－N)－$CH_2CH_2-C(=O)-NH-CH_2CH_2O-CH_2CH_2O-CH_2CH_2-C(=O)-GGFG-NH-CH_2CH_2CH_2-C(=O)-$。

[請求項35] 以下の式：

[化3]

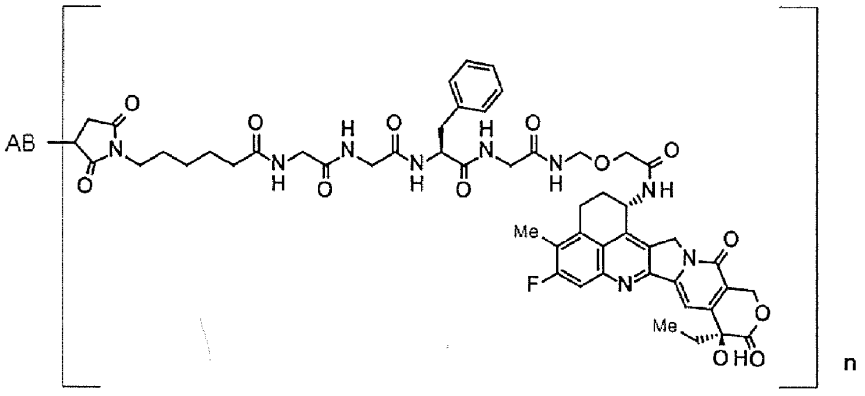


で示される構造を有する、請求項２９～３４のいずれか１項に記載の抗体－薬物コンジュゲート：

ここで、ＡＢは抗体又は該抗体の機能性断片を示す。ｎは抗体と結合している薬物－リンカー構造の１抗体あたりの平均結合数を示す。抗体とリンカーは抗体由来のスルフヒドリル基を介して結合している。

[請求項36] 以下の式：

[化4]



で示される構造を有する、請求項２９～３４のいずれか１項に記載の抗体－薬物コンジュゲート：

ここで、ＡＢは抗体又は該抗体の機能性断片を示す。ｎは抗体と結合している薬物－リンカー構造の１抗体あたりの平均結合数を示す。抗体とリンカーは抗体由来のスルフヒドリル基を介して結合している。

[請求項37] 抗体が以下の（１）～（４）：

（１）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖、

（２）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖、

（３）配列番号６５の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖、又は

（４）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖、

からなる群から選択されるいずれか１つに記載の軽鎖及び重鎖を含む抗体又は当該抗体の機能性断片である、請求項２９～３６のいずれか１項に記載の抗体－薬物コンジュゲート。

[請求項38] 抗体が、配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖を含む抗体又は当該抗体の機能性断片である、請求項３７に記載の抗体－薬物コンジュゲート。

[請求項39] 抗体が、配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖を含む抗体又は当該抗体の機能性断片である、請求項３７に記載の抗体－薬物コンジュゲート。

[請求項40] 重鎖又は軽鎖が、Ｎ－結合への糖鎖付加、Ｏ－結合への糖鎖付加、Ｎ末のプロセッシング、Ｃ末のプロセッシング、脱アミド化、アスパラ

ギン酸の異性化、メチオニンの酸化、Ｎ末にメチオニン残基の付加、プロリン残基のアミド化、Ｎ末グルタミンもしくはＮ末グルタミン酸のピログルタミン酸化及びカルボキシル末端における１つ又は２つのアミノ酸欠失からなる群から選択される１又は２以上の修飾をうけた、請求項２９～３９のいずれか１項に記載の抗体－薬物コンジュゲート。

[請求項41] 選択された１種の薬物－リンカー構造の１抗体あたりの平均結合数が１～１０個の範囲である請求項２９～４０のいずれか１項に記載の抗体－薬物コンジュゲート。

[請求項42] 選択された１種の薬物－リンカー構造の１抗体あたりの平均結合数が２～８個の範囲である請求項４１に記載の抗体－薬物コンジュゲート。

[請求項43] 選択された１種の薬物－リンカー構造の１抗体あたりの平均結合数が５～８個の範囲である請求項４２に記載の抗体－薬物コンジュゲート。

[請求項44] 選択された１種の薬物－リンカー構造の１抗体あたりの平均結合数が７～８個である請求項４３に記載の抗体－薬物コンジュゲート。

[請求項45] 請求項２９～４４のいずれか１項に記載の抗体－薬物コンジュゲート、その塩、又はそれらの水和物を含むことを特徴とする医薬組成物。

[請求項46] 抗腫瘍薬であることを特徴とする、請求項４５に記載の医薬組成物。

[請求項47] 腫瘍がＣＤＨ６を発現する腫瘍であることを特徴とする、請求項４６に記載の医薬組成物。

[請求項48] 腫瘍が、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巣癌、卵巣漿液性腺癌、甲状腺癌、胆管癌、肺癌、小細胞肺癌、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫であることを特徴とする、請求項４６又は４７に記載の医薬組成物。

[請求項49] 請求項２９～４４のいずれか１項に記載の抗体－薬物コンジュゲート、その塩、又はそれらの水和物から選択されるいずれかを個体に投与

することを特徴とする腫瘍の治療方法。

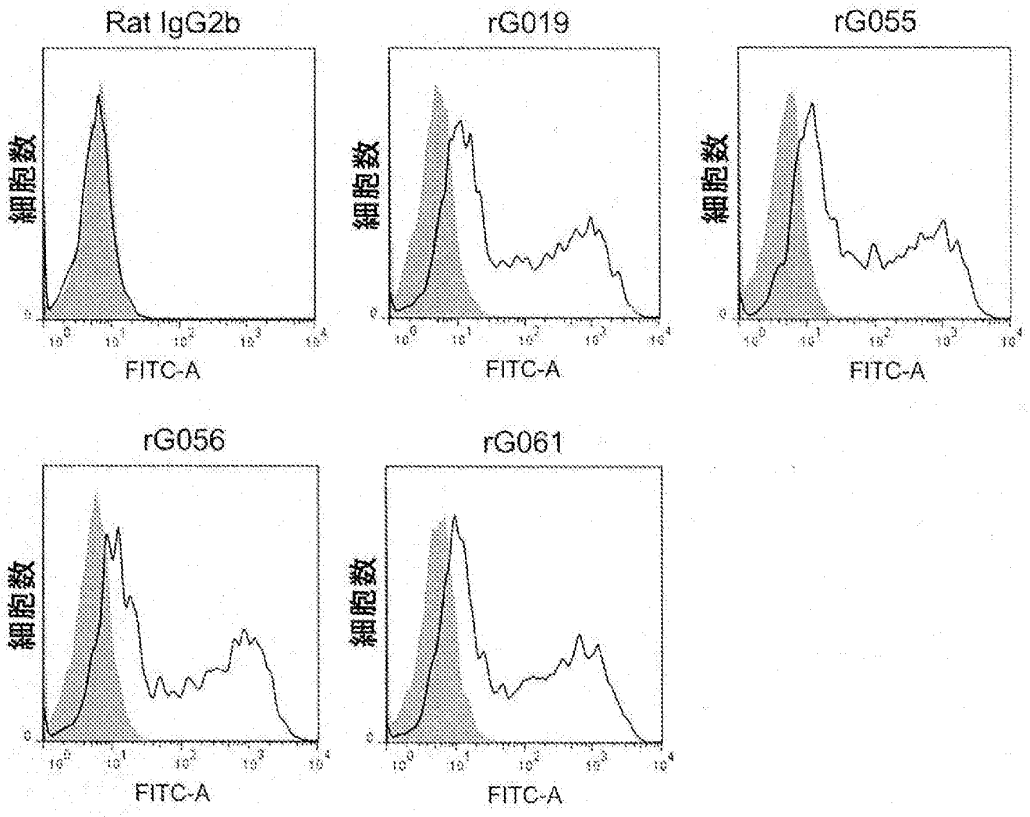
[請求項50] 腫瘍がＣＤＨ６が発現している腫瘍であることを特徴とする、請求項４９に記載の治療方法。

[請求項51] 腫瘍が、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巣癌、卵巣漿液性腺癌、甲状腺癌、胆管癌、肺癌、小細胞肺癌、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫であることを特徴とする、請求項４９又は５０に記載の治療方法。

[請求項52] 請求項２９～４４のいずれか１項に記載の抗体－薬物コンジュゲート、その塩、又はそれらの水和物から選択される少なくとも一つを含む医薬組成物及び少なくとも一つの抗腫瘍薬を、同時に、別々に又は連続して個体に投与することを特徴とする腫瘍の治療方法。

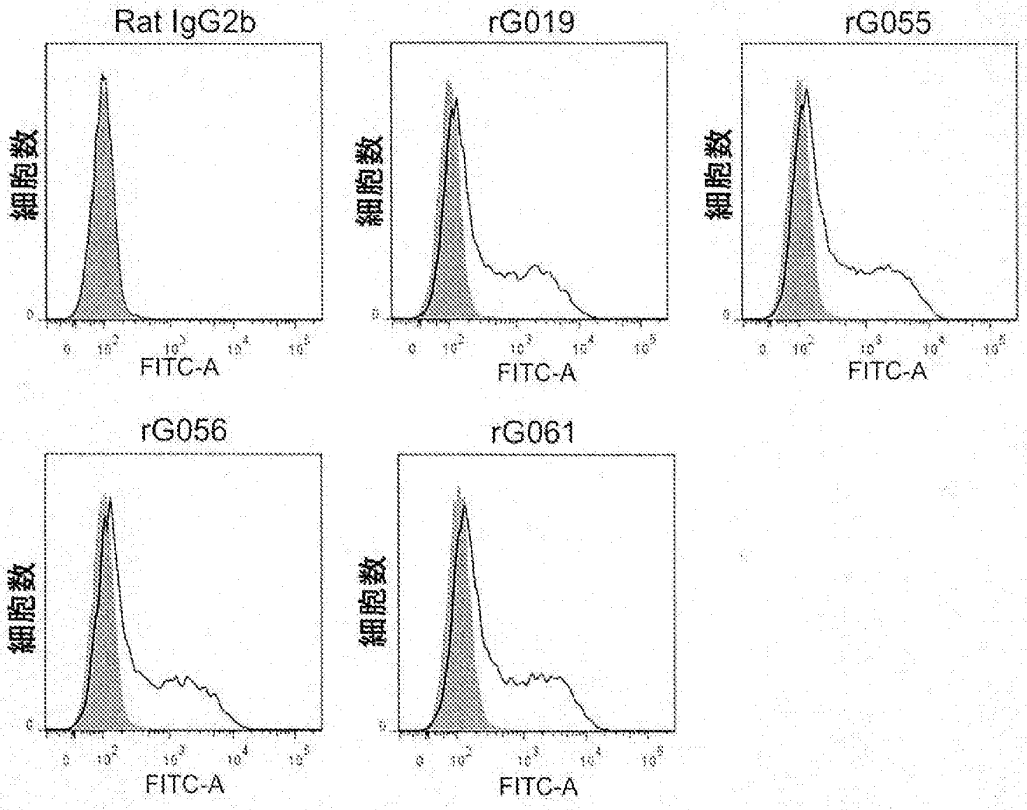
[請求項53] 請求項１～１３及び請求項２４～２８からなる群から選択されるいずれか１項に記載の抗体又は当該抗体の機能性断片或いは請求項２３に記載の製造方法で得られる抗体又は当該抗体の機能性断片と、薬物－リンカー中間化合物を反応させる工程を含むことを特徴とする、抗体－薬物コンジュゲートの製造方法。

[図1]



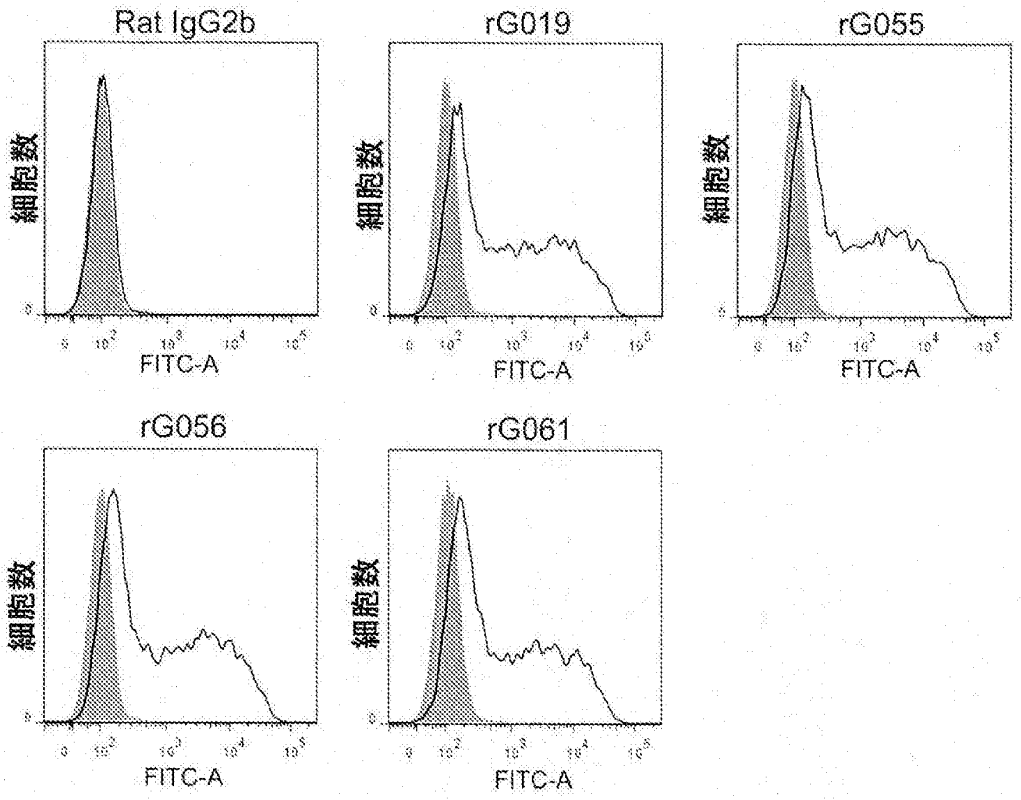
[図2-1]

(1) コントロール細胞又は全長ｈＣＤＨ６導入細胞



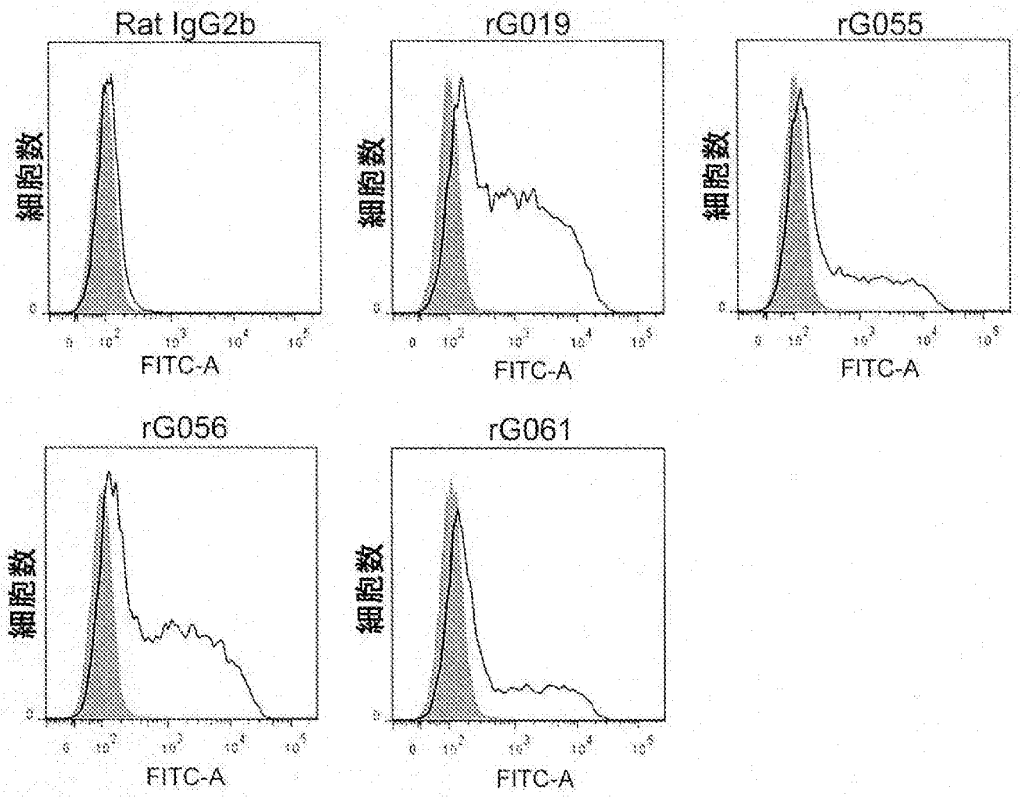
[図2-2]

(2) コントロール細胞又はＥＣ１欠失ｈＣＤＨ６導入細胞



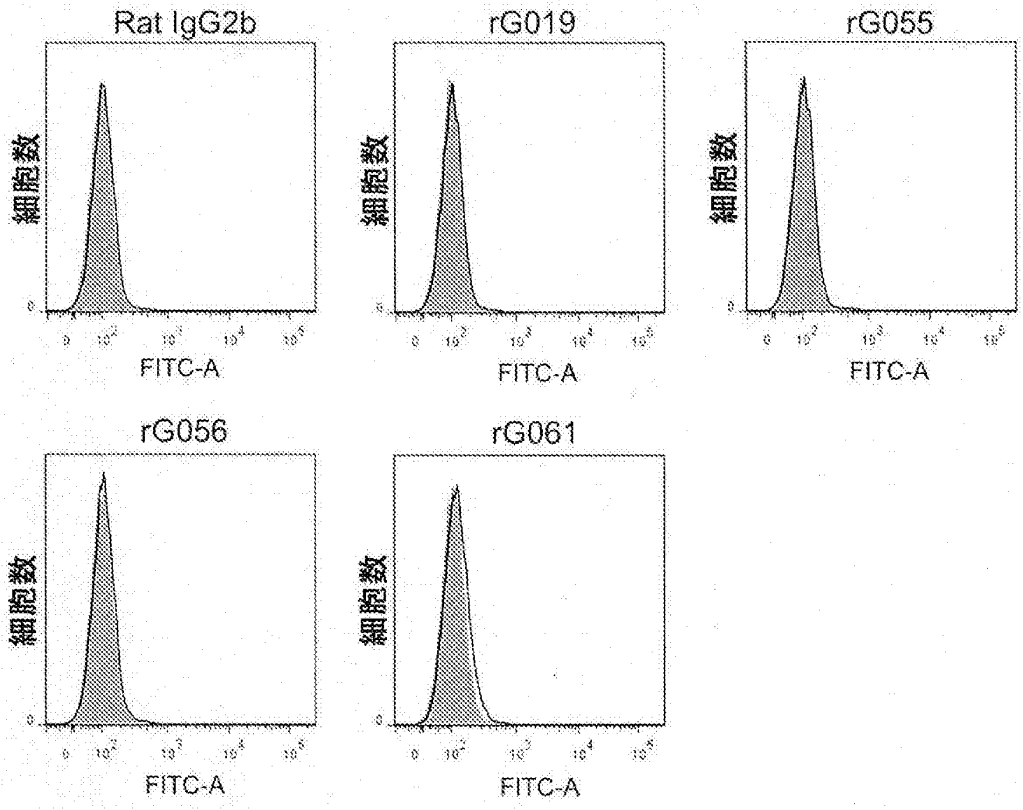
[図2-3]

(3) コントロール細胞又はＥＣ２欠失ｈＣＤＨ６導入細胞



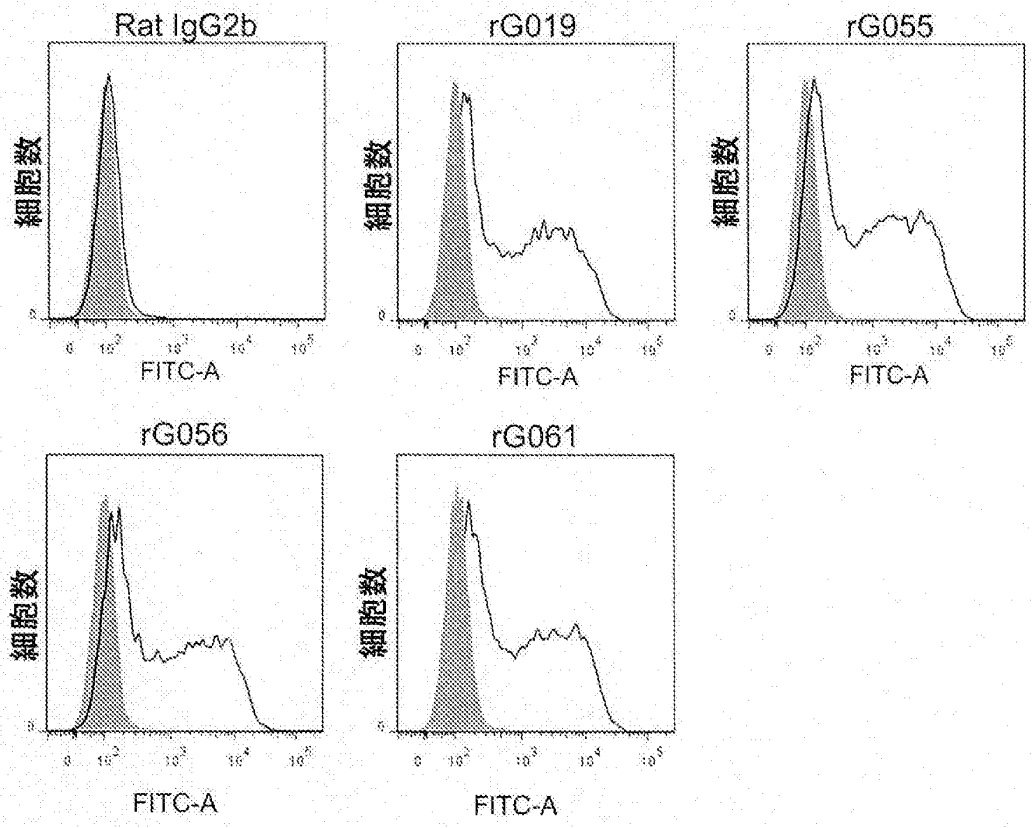
[図2-4]

(4) コントロール細胞又はＥＣ３欠失ｈＣＤＨ６導入細胞



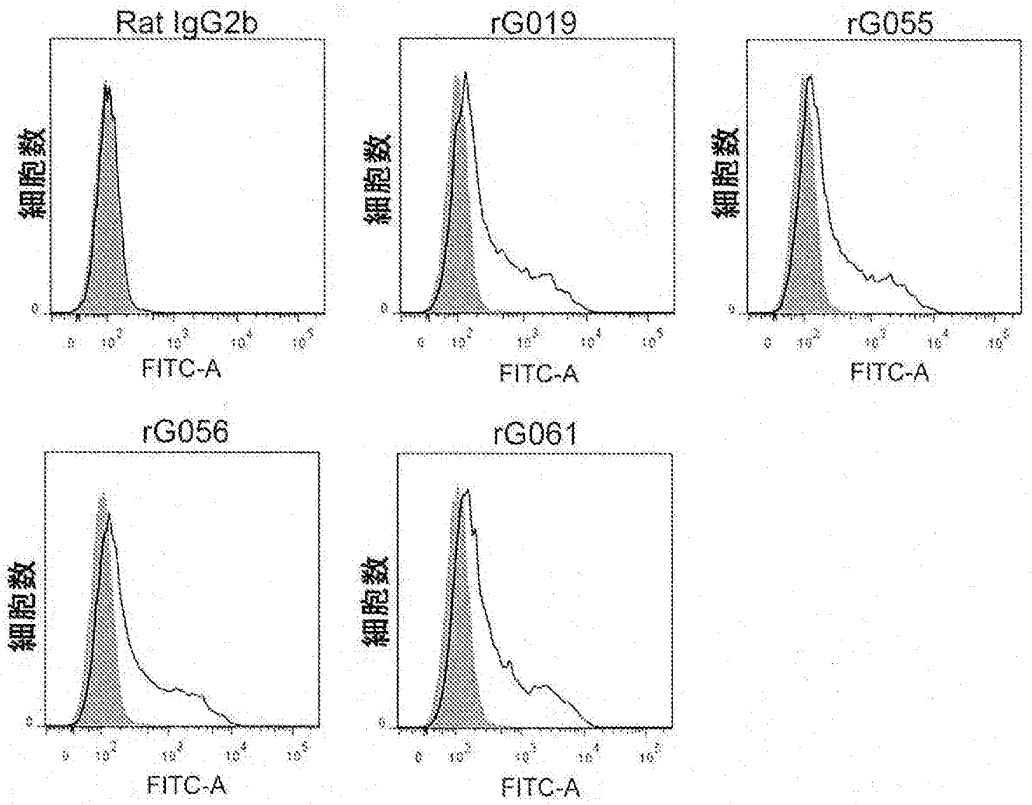
[図2-5]

(5) コントロール細胞又はＥＣ４欠失ｈＣＤＨ６導入細胞

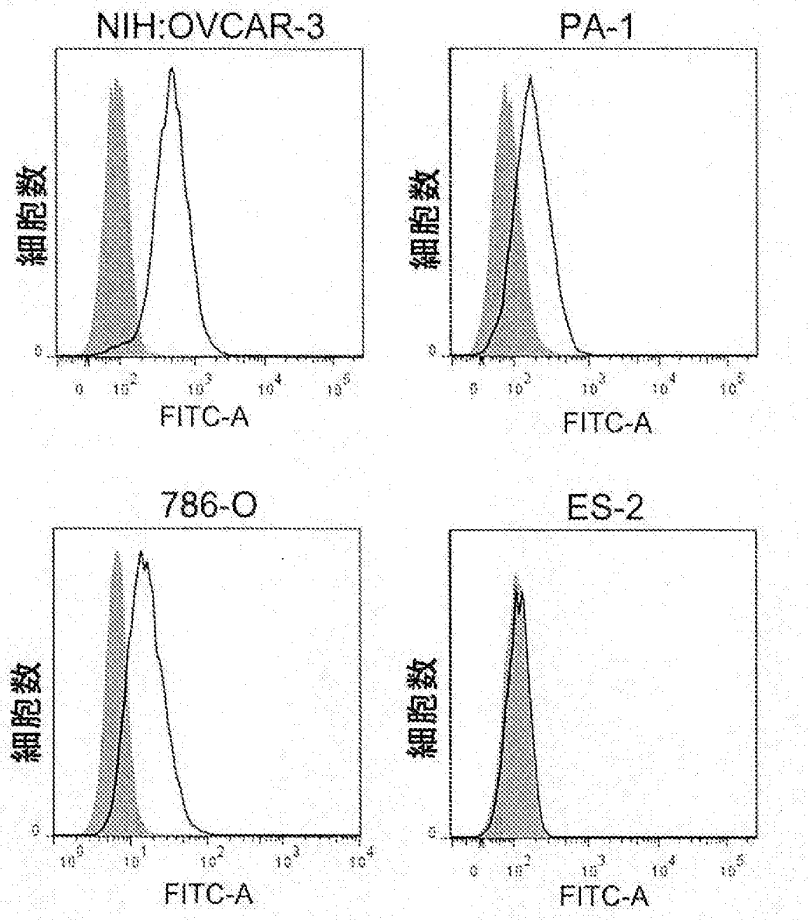


[図2-6]

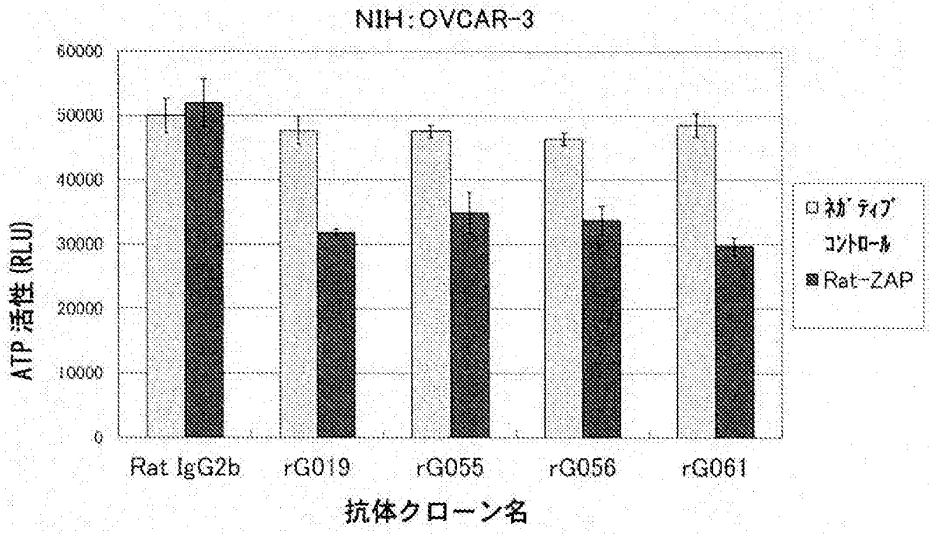
(6) コントロール細胞又はＥＣ５欠失ｈＣＤＨ６導入細胞



[図3]

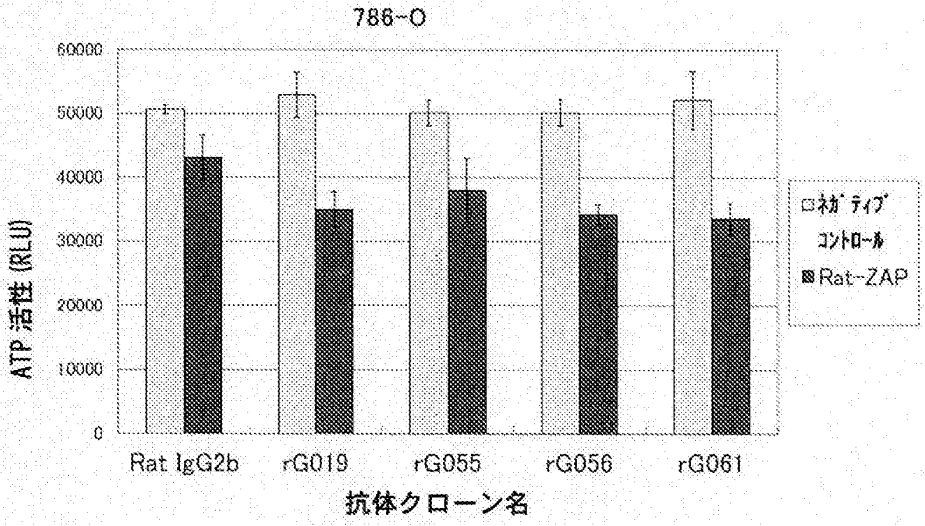


[図4]



Ｒａｔ－ＺＡＰを用いたＮＩＨ：ＯＶＣＡＲ-3細胞生存率（%）

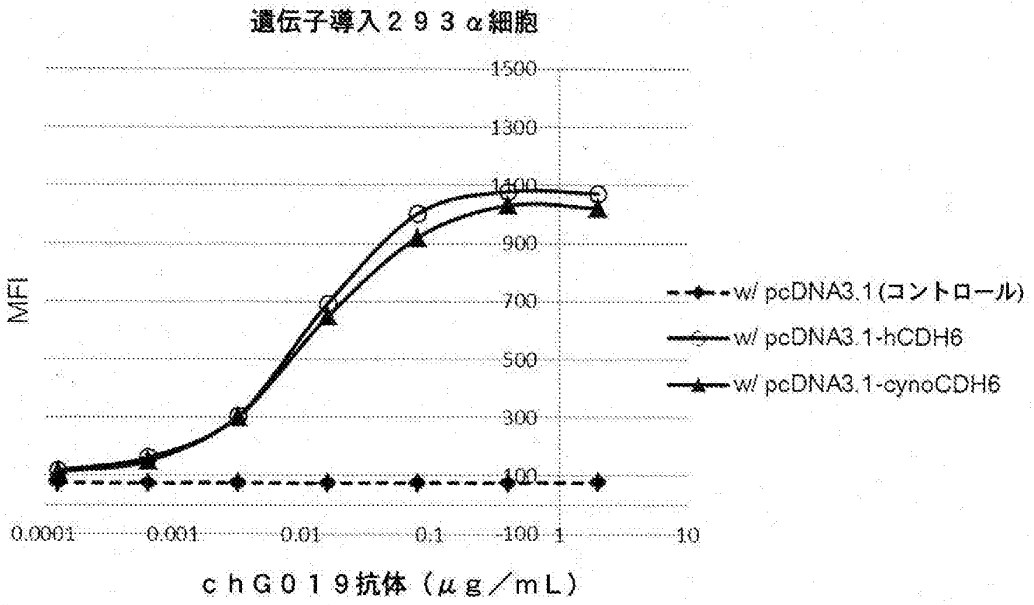
Rat IgG2b	rG019	rG055	rG056	rG061
104.1	66.8	73.3	72.8	61.2



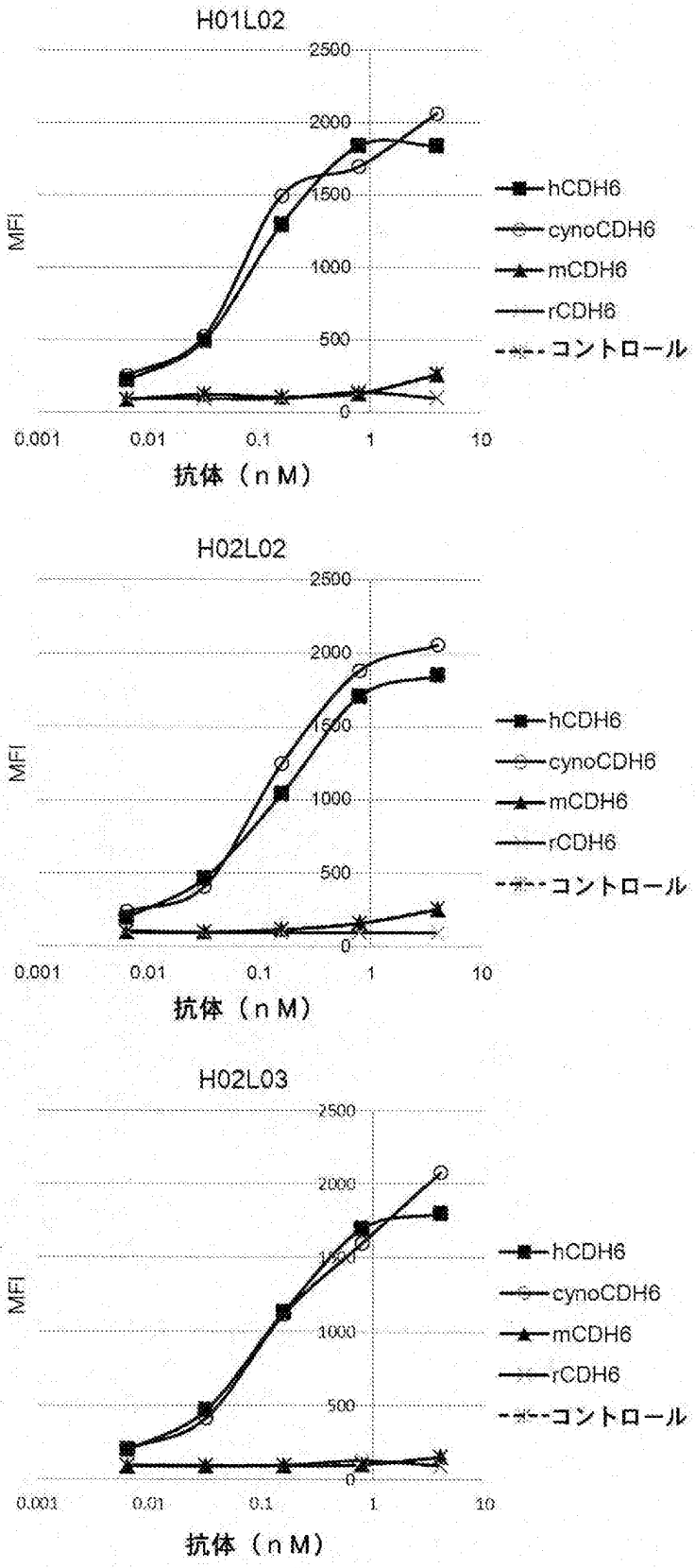
Ｒａｔ－ＺＡＰを用いた７８６－Ｏ細胞生存率（%）

Rat IgG2b	rG019	rG055	rG056	rG061
85.2	66.1	75.7	68.1	64.2

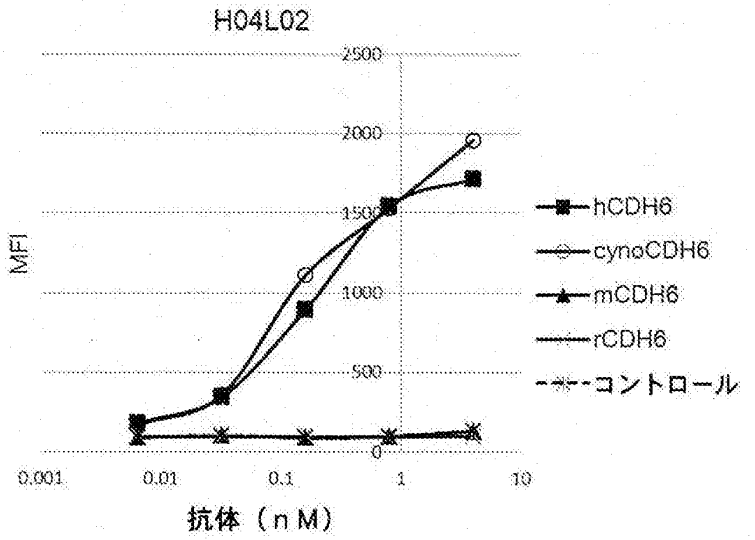
[図5]

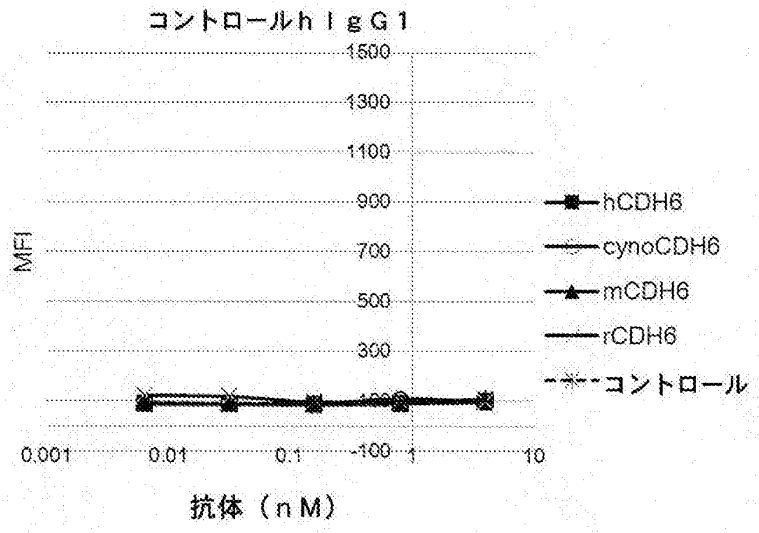

遺伝子導入２９３αα細胞
MFI
1500
1300
1100
900
700
500
300
100
0.0001
0.001
0.01
0.1
100
1
10
w/ pcDNA3.1(コントロール)
w/ pcDNA3.1-hCDH6
w/ pcDNA3.1-cynoCDH6
ｃｈＧ０１９抗体（μｇ／ｍＬ）

[図6-1]


H01L02
MFI
2500
2000
1500
1000
500
0
0.001
0.01
0.1
1
10
抗体（n M）
hCDH6
cynoCDH6
mCDH6
rCDH6
コントロール
H02L02
MFI
抗体（n M）
hCDH6
cynoCDH6
mCDH6
rCDH6
コントロール
H02L03
MFI
抗体（n M）
hCDH6
cynoCDH6
mCDH6
rCDH6
コントロール

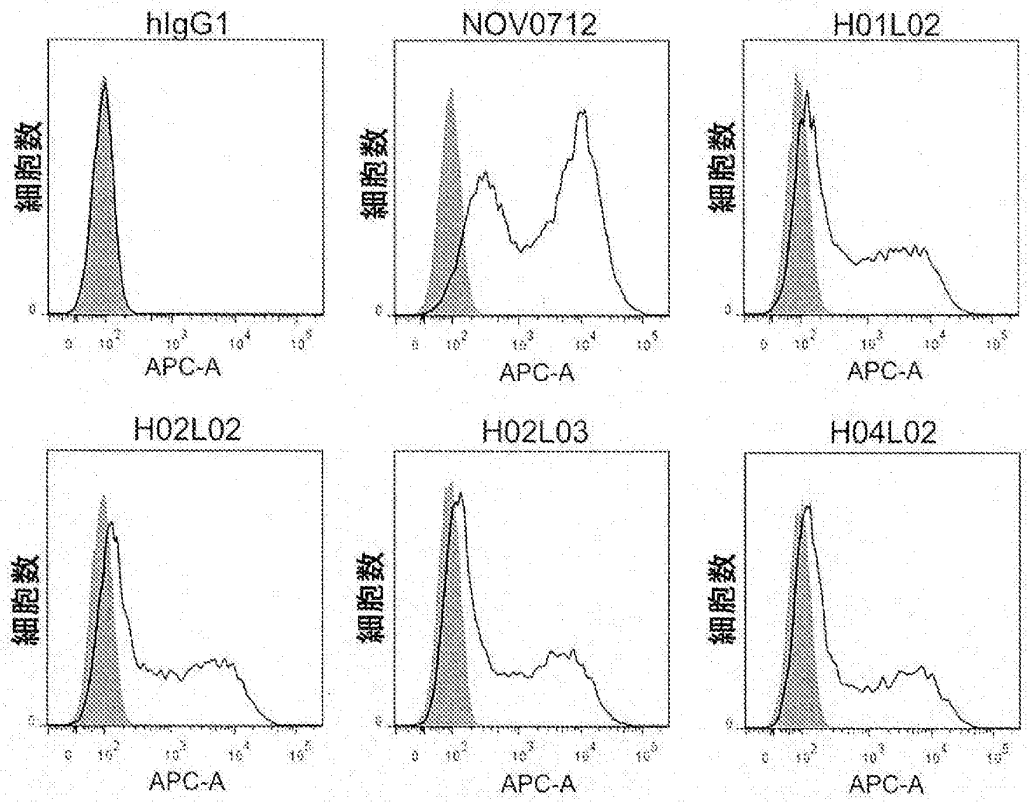
[図6-2]


H04L02
MFI
2500
2000
1500
1000
500
0
0.001
0.01
0.1
1
10
抗体（ｎＭ）
hCDH6
cynoCDH6
mCDH6
rCDH6
コントロール


コントロールｈＩｇＧ１
MFI
1500
1300
1100
900
700
500
300
100
-100
0.001
0.01
0.1
1
10
抗体（ｎＭ）
hCDH6
cynoCDH6
mCDH6
rCDH6
コントロール

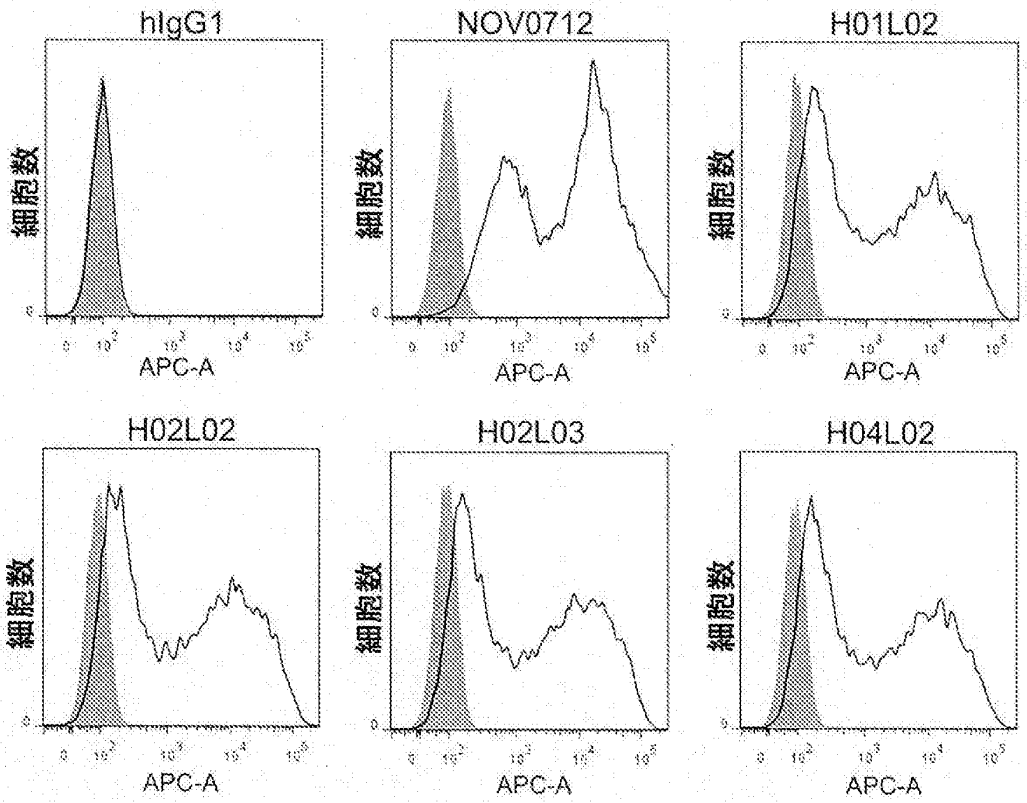
[図7-1]

(1) コントロール細胞又は全長ｈＣＤＨ６導入細胞



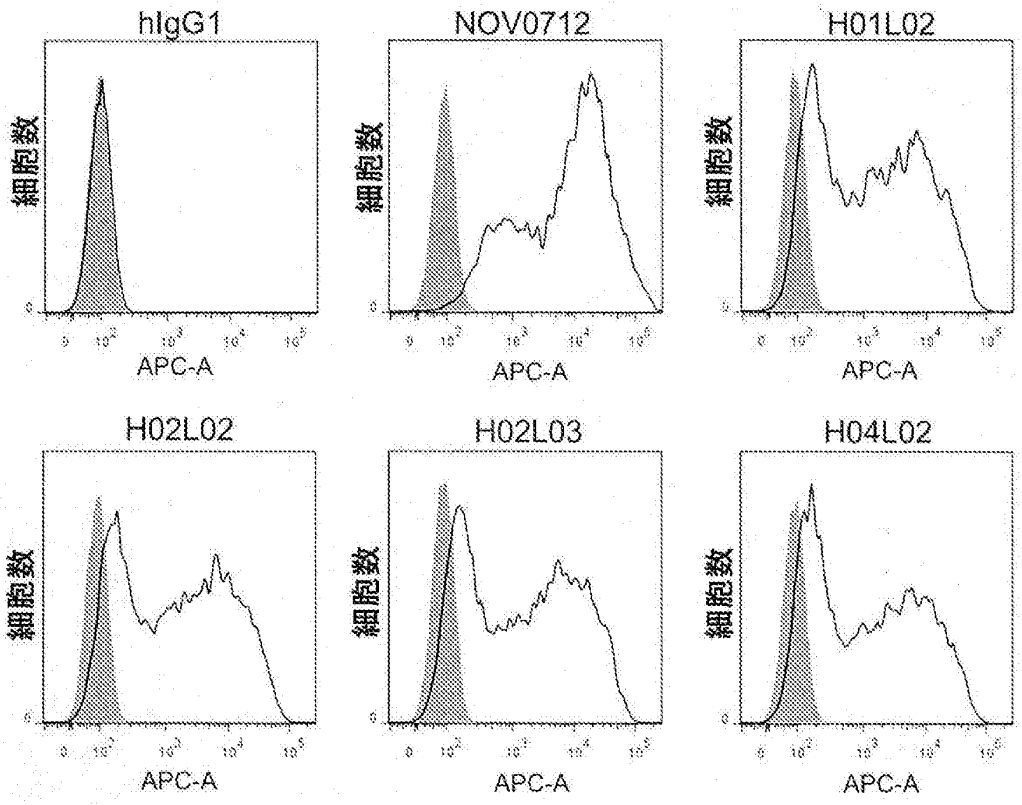
[図7-2]

(2) コントロール細胞又はＥＣ１欠失ｈＣＤＨ６導入細胞



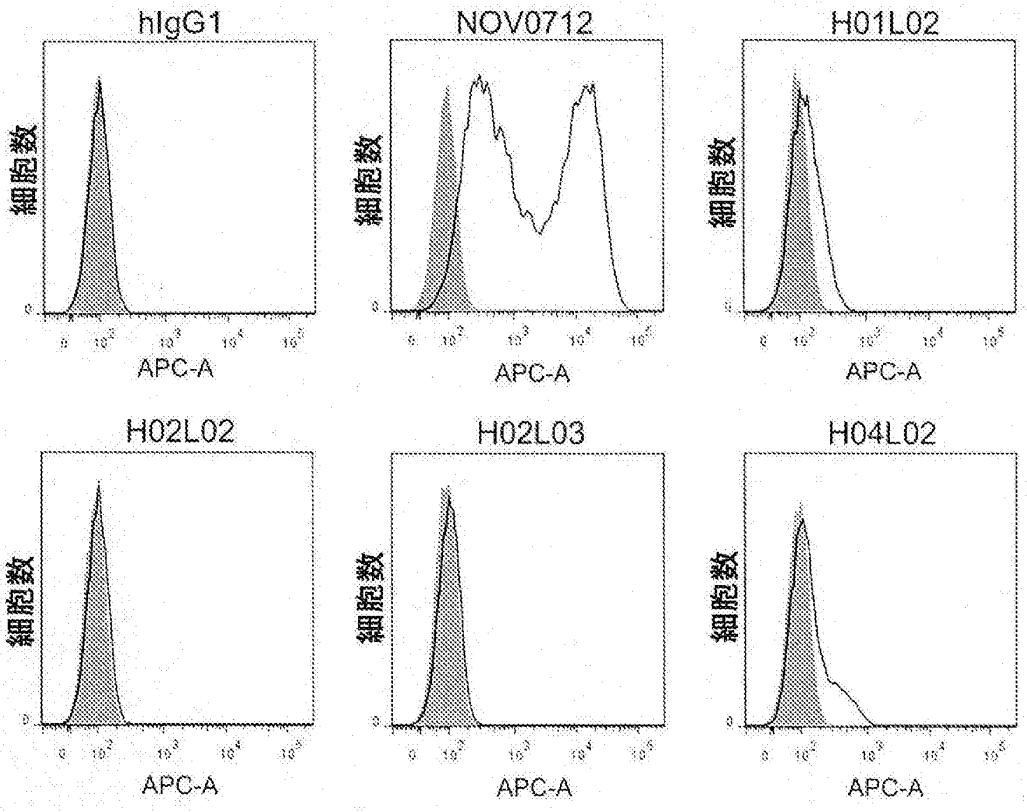
[図7-3]

(3) コントロール細胞又はＥＣ２欠失ｈＣＤＨ６導入細胞



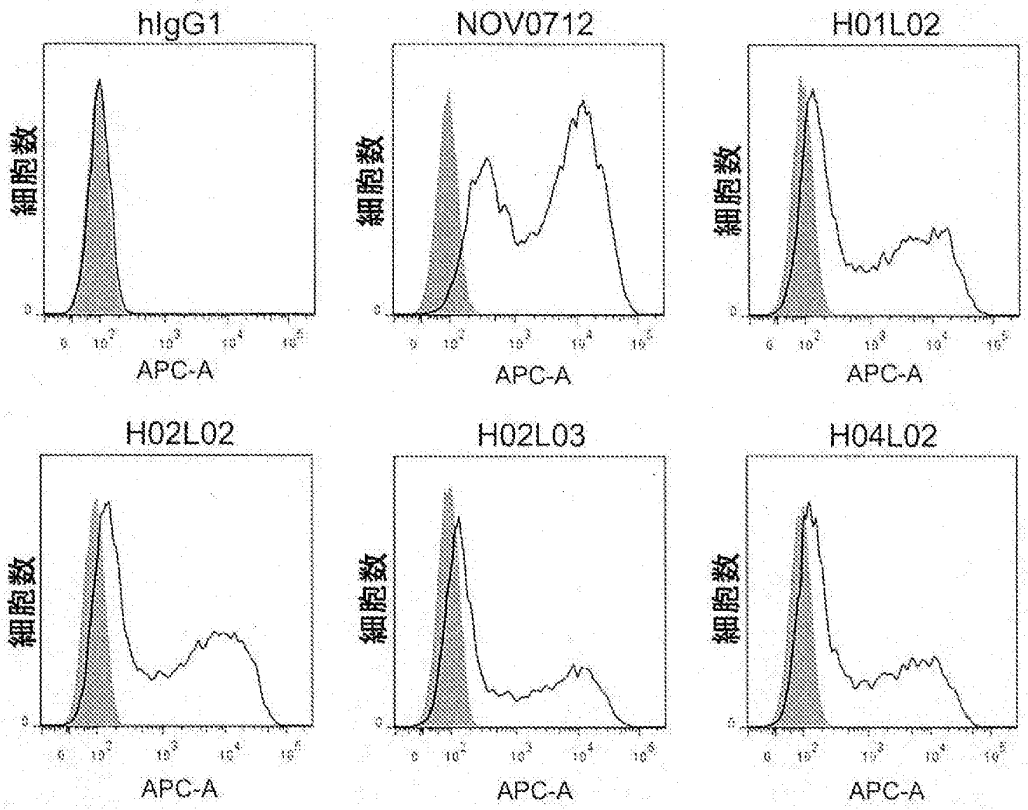
[図7-4]

(4)コントロール細胞又はＥＣ３欠失ｈＣＤＨ６導入細胞



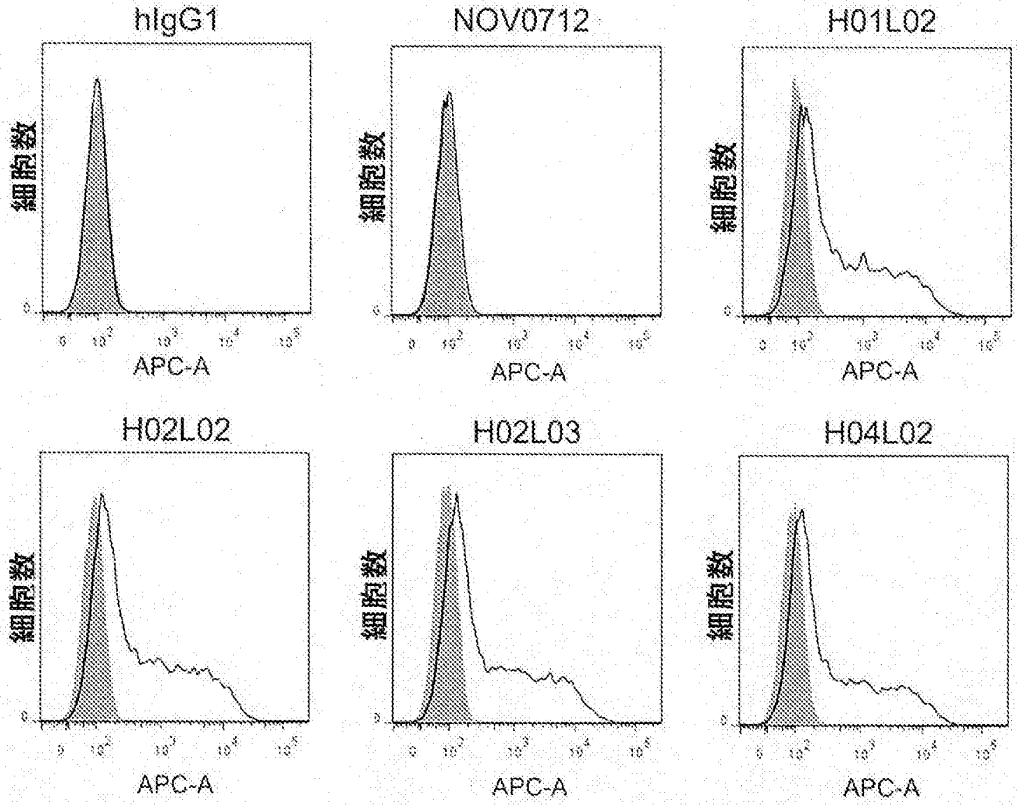
[図7-5]

(5)コントロール細胞又はＥＣ４欠失ｈＣＤＨ６導入細胞

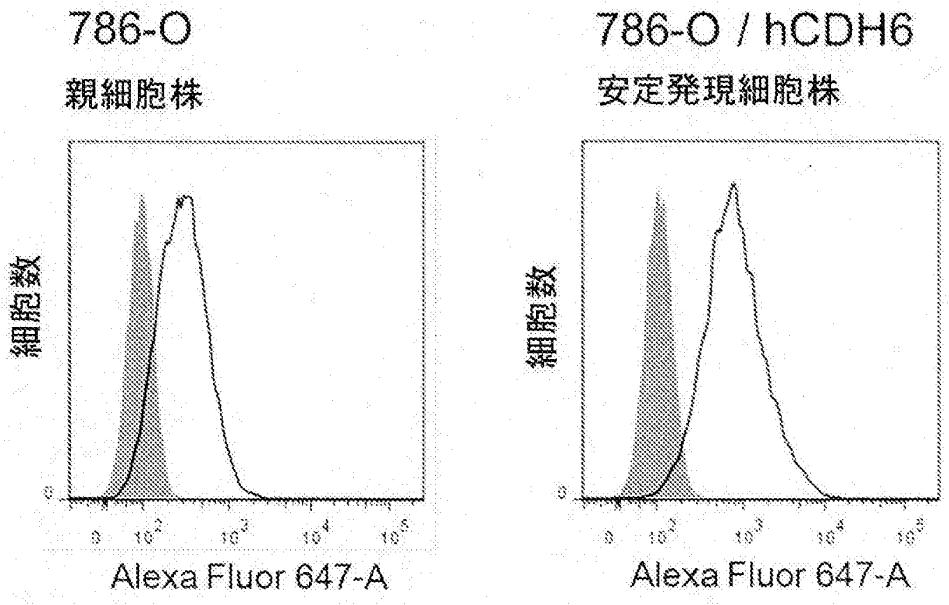


[図7-6]

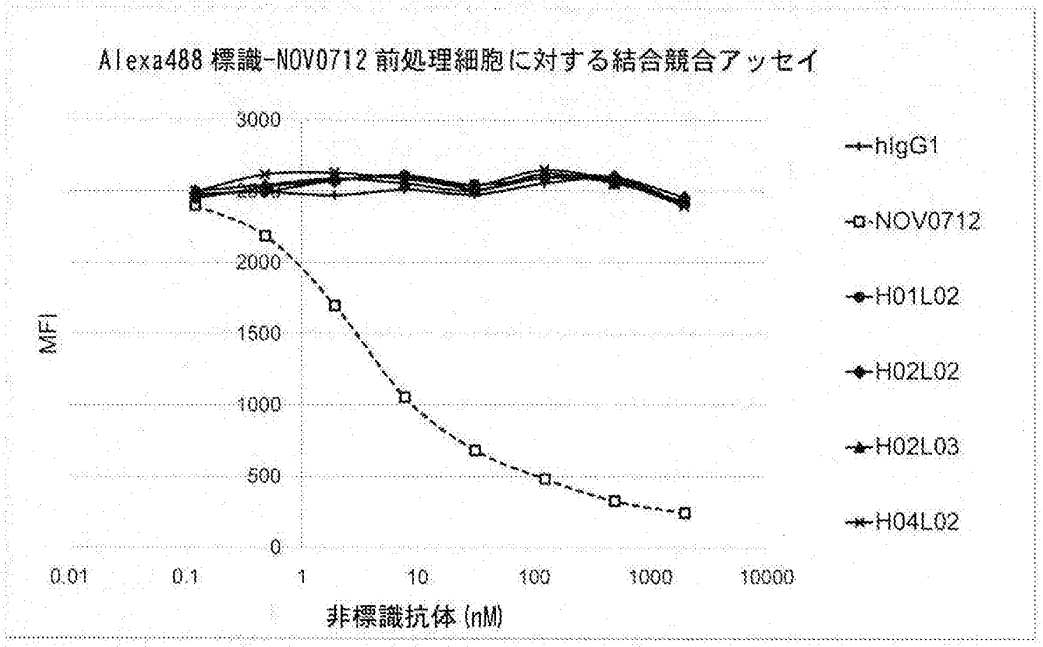
(6) コントロール細胞又はＥＣ５欠失ｈＣＤＨ６導入細胞

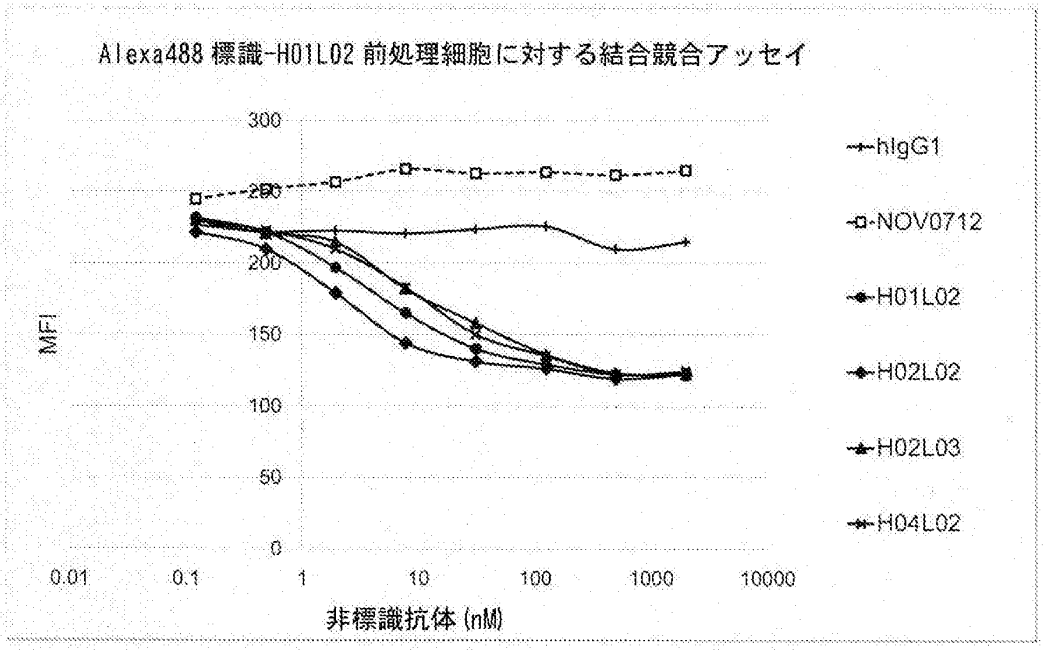


[図8]

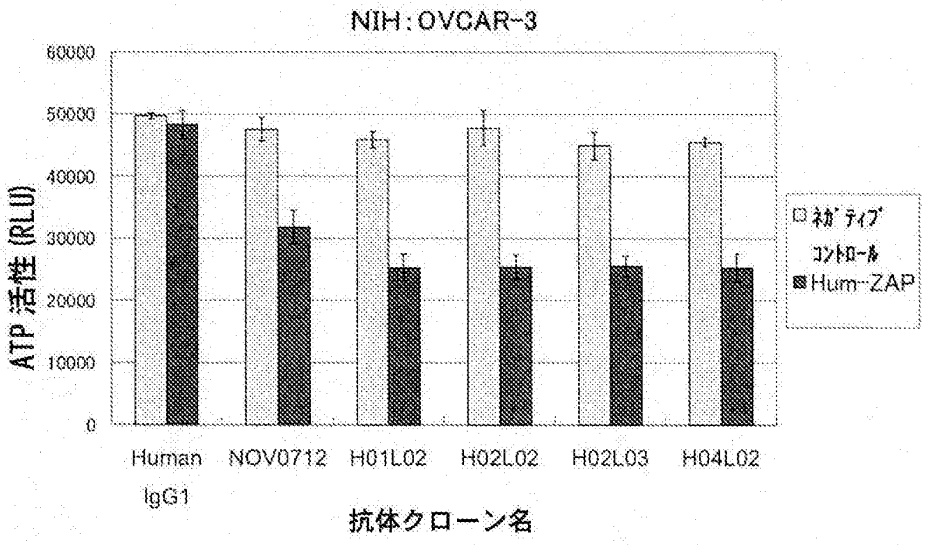


[図9]


Alexa488 標識-NOV0712 前処理細胞に対する結合競合アッセイ
MFI
3000
2000
1500
1000
500
0
0.01
0.1
1
10
100
1000
10000
非標識抗体 (nM)
hIgG1
NOV0712
H01L02
H02L02
H02L03
H04L02


Alexa488 標識-H01L02 前処理細胞に対する結合競合アッセイ
MFI
300
200
150
100
50
0
0.01
0.1
1
10
100
1000
10000
非標識抗体 (nM)
hIgG1
NOV0712
H01L02
H02L02
H02L03
H04L02

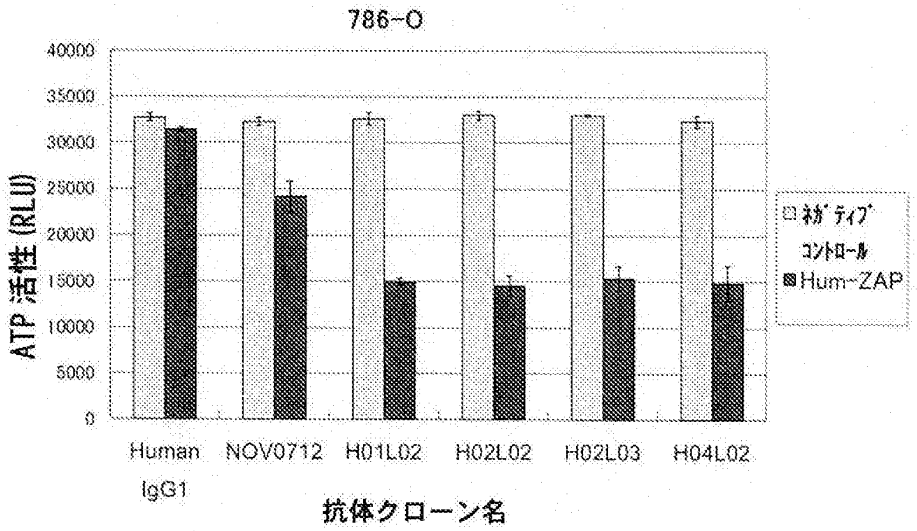
[図10-1]



Ｈｕｍ－ＺＡＰを用いたＮＩＨ：ＯＶＣＡＲ－３細胞生存率（％）

Human IgG1	NOV0712	H01L02	H02L02	H02L03	H04L02
97.3	67.0	55.1	53.1	56.7	55.6

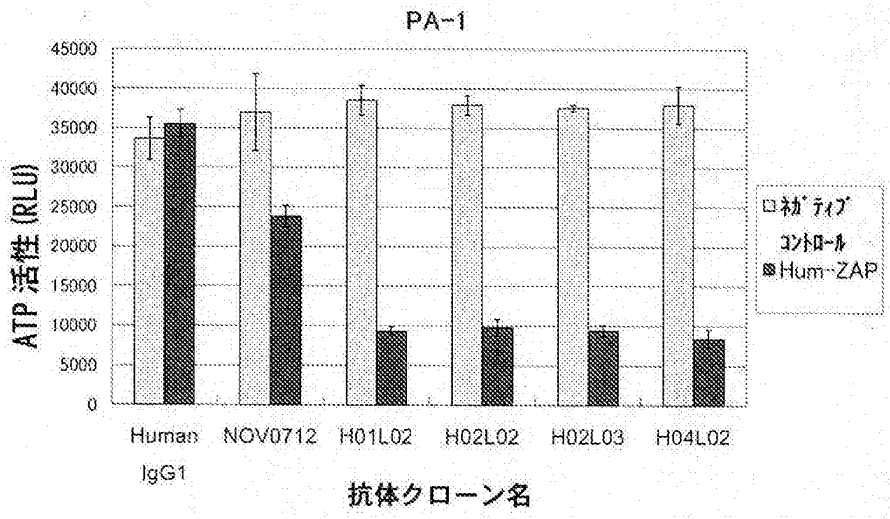
[図10-2]



Ｈｕｍ－ＺＡＰを用いた７８６－Ｏ細胞生存率（％）

Human IgG1	NOV0712	H01L02	H02L02	H02L03	H04L02
95.9	74.8	46.0	44.1	46.5	45.9

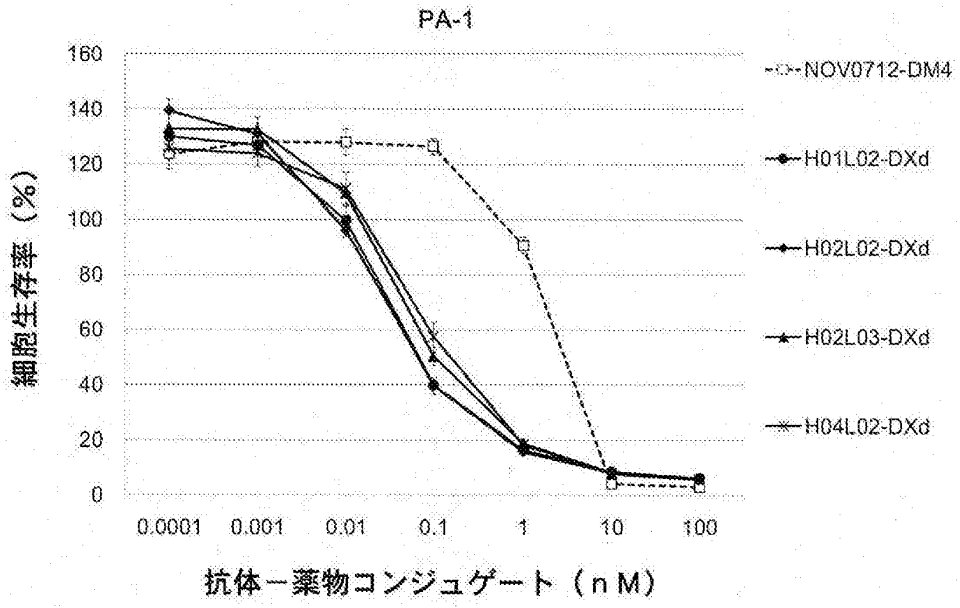
[図10-3]



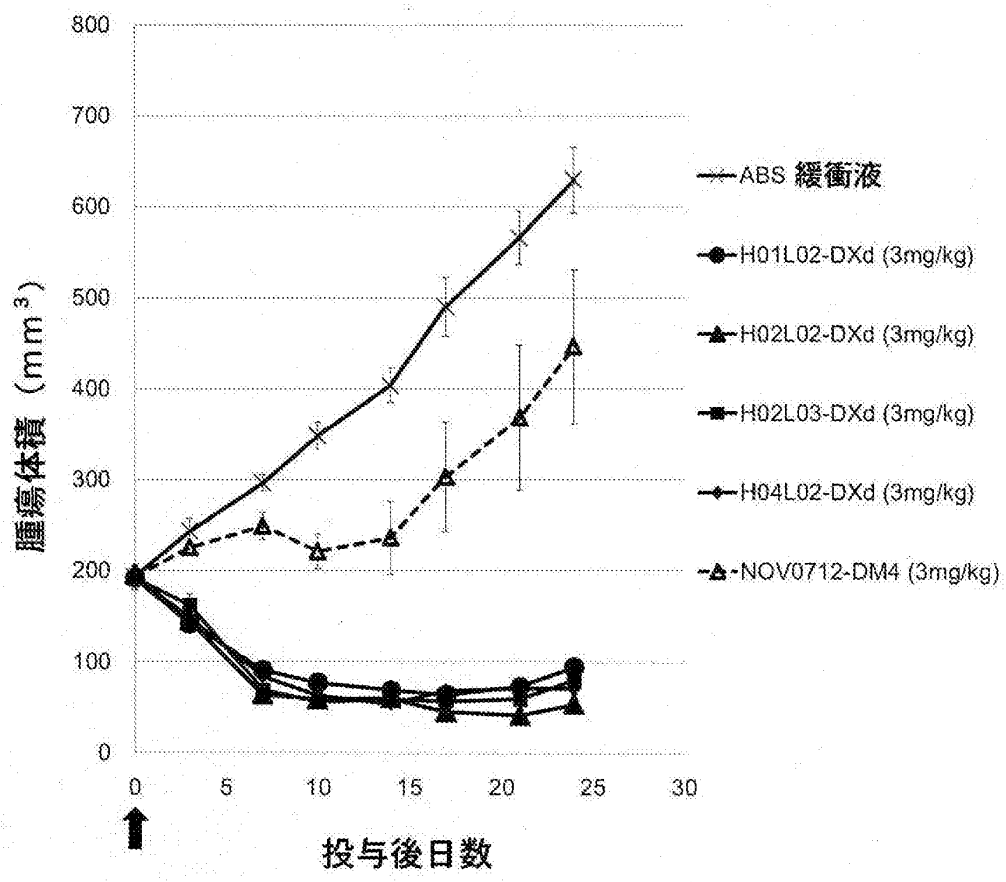
Ｈｕｍ－ＺＡＰを用いたＰＡ－１細胞生存率（％）

Human IgG1	NOV0712	H01L02	H02L02	H02L03	H04L02
105.6	64.5	24.3	25.8	25.1	22.2

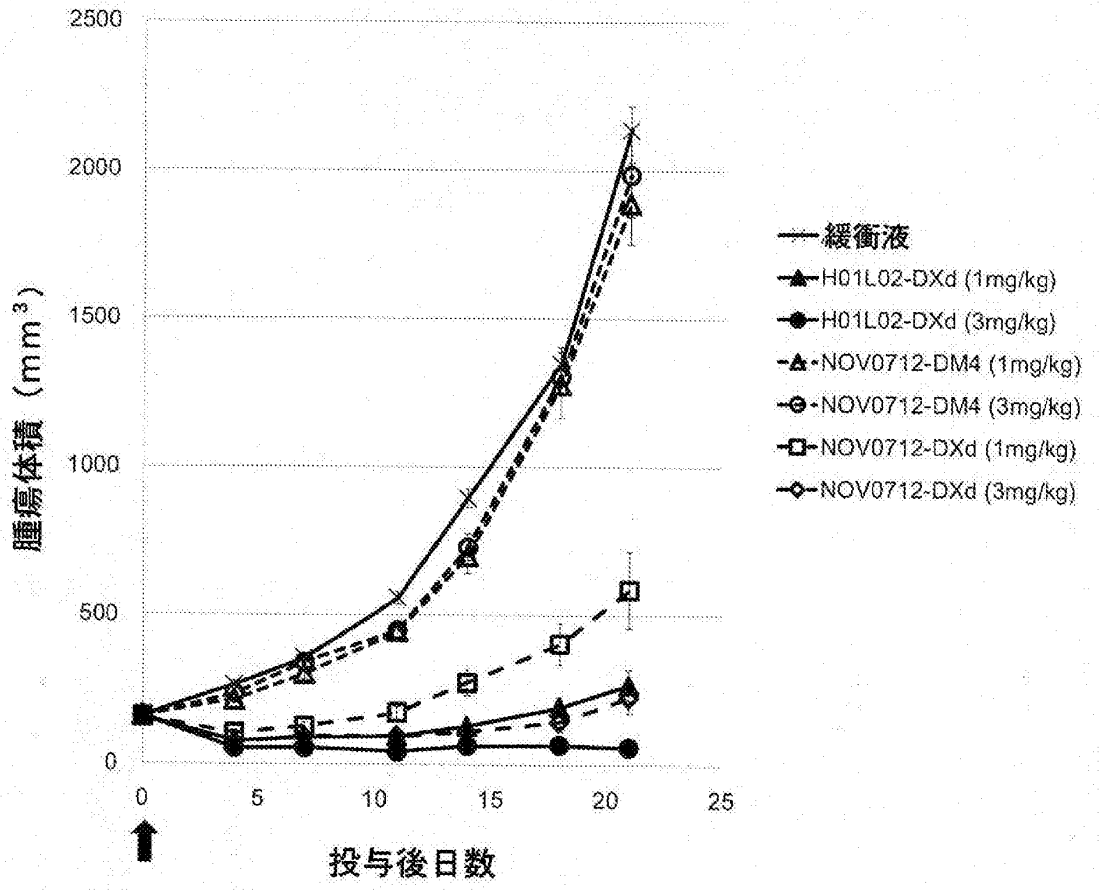
[図11]



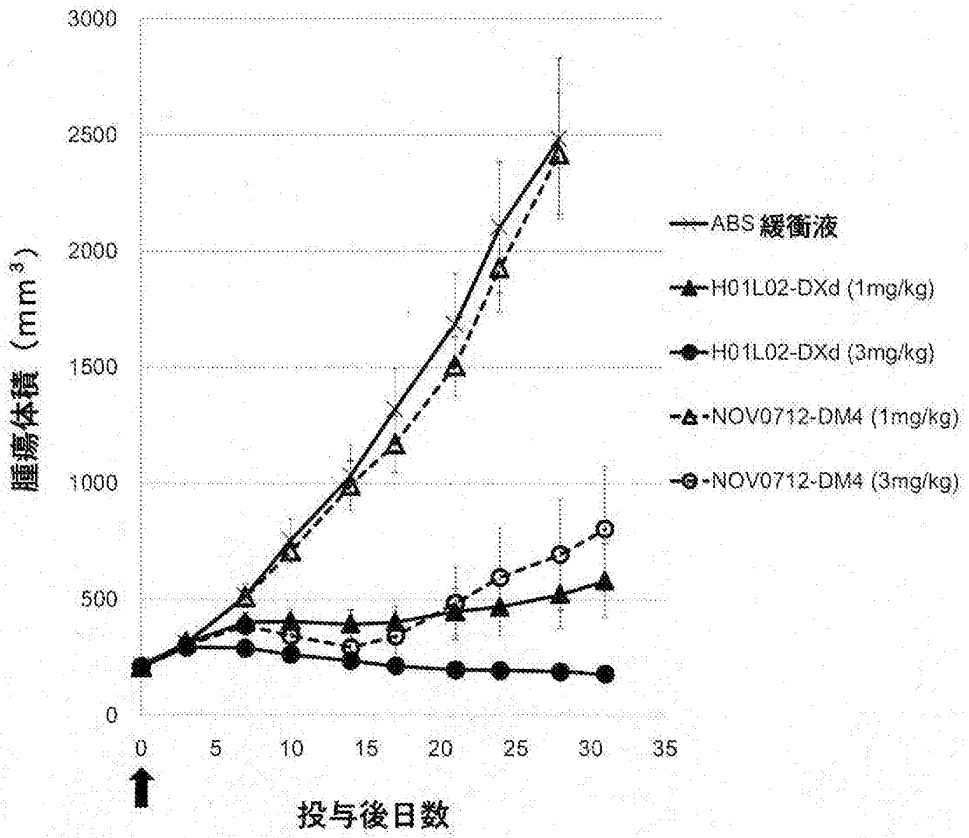
[図12]


800
700
600
500
400
300
200
100
0
腫瘍体積（mm³）
0
5
10
15
20
25
30
投与後日数
ABS 緩衝液
H01L02-DXd (3mg/kg)
H02L02-DXd (3mg/kg)
H02L03-DXd (3mg/kg)
H04L02-DXd (3mg/kg)
NOV0712-DM4 (3mg/kg)

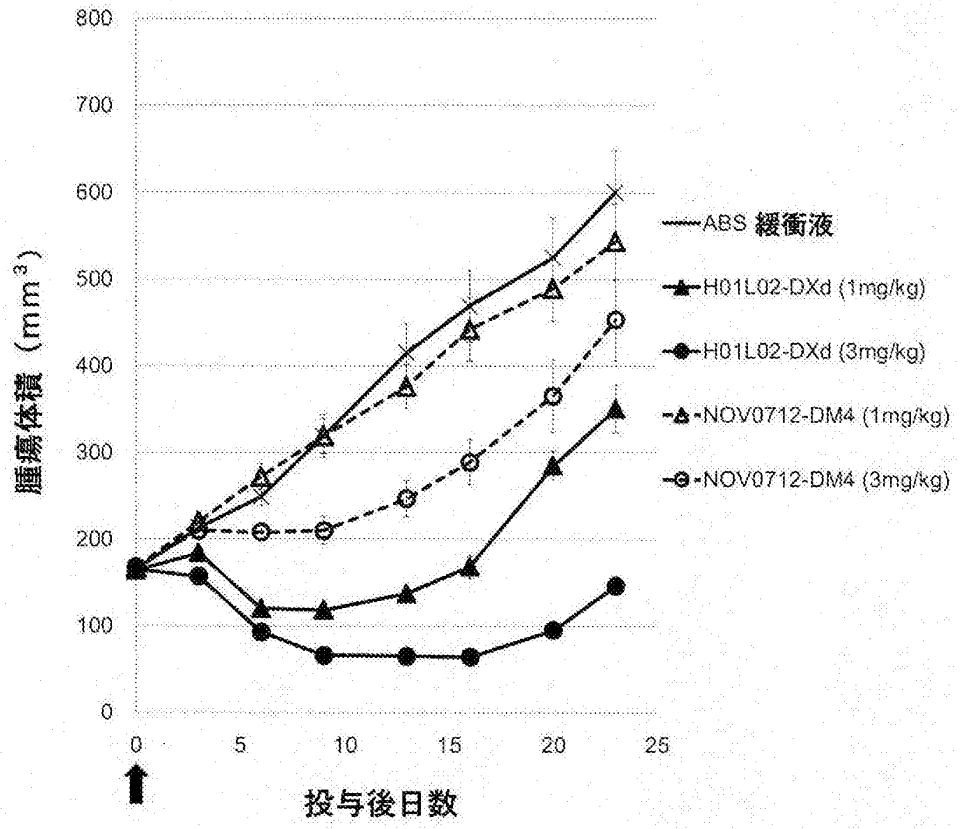
[図13]


2500
2000
1500
1000
500
0
腫瘍体積（mm3）
0
5
10
15
20
25
投与後日数
緩衝液
H01L02-DXd (1mg/kg)
H01L02-DXd (3mg/kg)
NOV0712-DM4 (1mg/kg)
NOV0712-DM4 (3mg/kg)
NOV0712-DXd (1mg/kg)
NOV0712-DXd (3mg/kg)

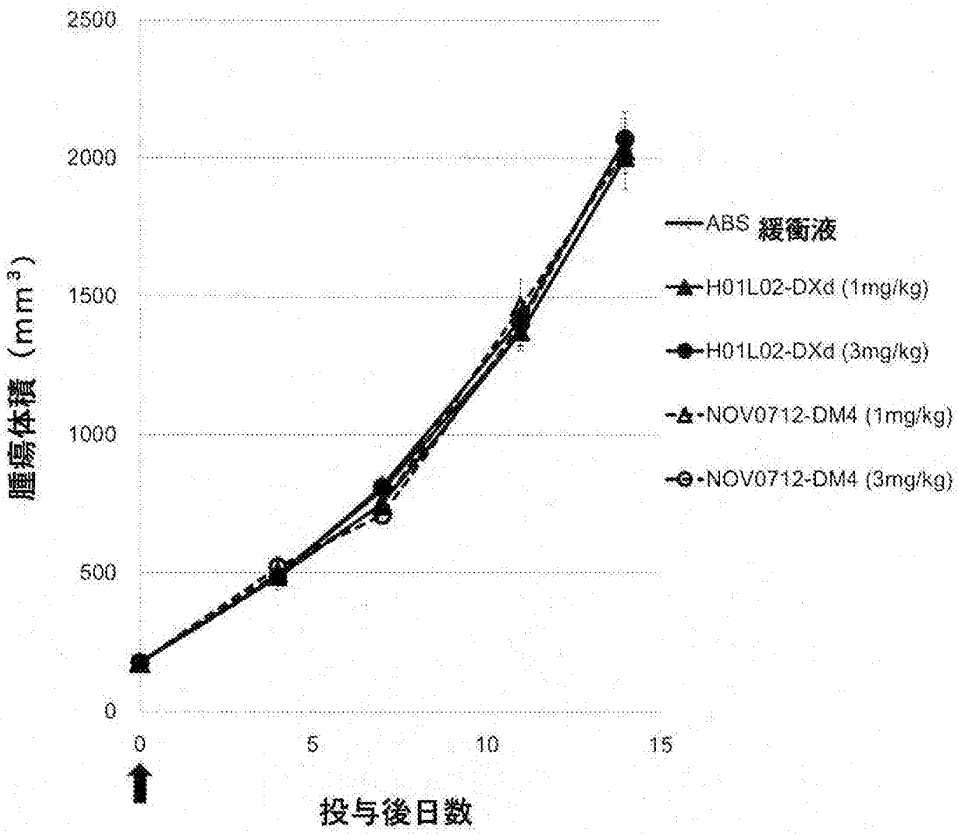
[図14]


3000
2500
2000
1500
1000
500
0
腫瘍体積（mm³）
0
5
10
15
20
25
30
35
投与後日数
ABS 緩衝液
H01L02-DXd (1mg/kg)
H01L02-DXd (3mg/kg)
NOV0712-DM4 (1mg/kg)
NOV0712-DM4 (3mg/kg)

[図15]


800
700
600
500
400
300
200
100
0
腫瘍体積（mm³）
0
5
10
15
20
25
投与後日数
ABS 緩衝液
H01L02-DXd (1mg/kg)
H01L02-DXd (3mg/kg)
NOV0712-DM4 (1mg/kg)
NOV0712-DM4 (3mg/kg)

[図16]


2500
2000
1500
1000
500
0
腫瘍体積（mm3）
0
5
10
15
投与後日数
ABS 緩衝液
H01L02-DXd (1mg/kg)
H01L02-DXd (3mg/kg)
NOV0712-DM4 (1mg/kg)
NOV0712-DM4 (3mg/kg)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2018/018572

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl. See extra sheet
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl. C12N15/13, A61K9/00, A61K31/4745, A61K39/395, A61K47/68, A61P13/12, A61P15/08, A61P35/00, A61P43/00, C07K16/28, C12N1/15, C12N1/19, C12N1/21, C12N5/10, C12P21/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan	1922-1996
Published unexamined utility model applications of Japan	1971-2018
Registered utility model specifications of Japan	1996-2018
Published registered utility model applications of Japan	1994-2018

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
JSTPlus/JMEDPlus/JST7580 (JDreamIII), CAplus/MEDLINE/EMBASE/BIOSIS (STN), WPIDS/WPIX (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/024195 A1 (NOVARTIS AG) 18 February 2016, claims 1-61, examples, in particular, example 7, fig. 4, etc. & US 2016/0046711 A1, claims 1-61, examples, in particular, example 7, fig. 4, etc.	1-53
A	WO 2014/057687 A1 (DAIICHI SANKYO CO., LTD.) 17 April 2014, claims 1-49, examples, etc. & US 2015/0297748 A1, claims 1-49, examples, etc.	1-53

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 July 2018 (30.07.2018)	07 August 2018 (07.08.2018)

Name and mailing address of the ISA/	Authorized officer
Japan Patent Office 3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2018/018572

CLASSIFICATION OF SUBJECT MATTER
C12N15/13(2006.01)i, A61K9/00(2006.01)i, A61K31/4745(2006.01)i, A61K39/395(2006.01)i, A61K47/68(2017.01)i, A61P13/12(2006.01)i, A61P15/08(2006.01)i, A61P35/00(2006.01)i, A61P43/00(2006.01)i, C07K16/28(2006.01)i, C12N1/15(2006.01)i, C12N1/19(2006.01)i, C12N1/21(2006.01)i, C12N5/10(2006.01)i, C12P21/08(2006.01)i

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国際調査報告

国際出願番号　ＰＣＴ／ＪＰ２０１８／０１８５７２

A.　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Int.Cl.　特別ページ参照

B.　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl.　C12N15/13, A61K9/00, A61K31/4745, A61K39/395, A61K47/68, A61P13/12, A61P15/08, A61P35/00, A61P43/00, C07K16/28, C12N1/15, C12N1/19, C12N1/21, C12N5/10, C12P21/08

最小限資料以外の資料で調査を行った分野に含まれるもの

日本国実用新案公報	１９２２－１９９６年
日本国公開実用新案公報	１９７１－２０１８年
日本国実用新案登録公報	１９９６－２０１８年
日本国登録実用新案公報	１９９４－２０１８年

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

JSTPlus/JMEDPlus/JST7580 (JDreamIII), CAplus/MEDLINE/EMBASE/BIOSIS (STN), WPIDS/WPIX (STN)

C.　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求項の番号
A	WO 2016/024195 A1 (NOVARTIS AG) 2016.02.18, 請求項 1-61, 実施例特に実施例 7, 図 4 等 & US 2016/0046711 A1, 請求項 1-61, 実施例特に実施例 7, 図 4 等	1-53
A	WO 2014/057687 A1 (第一三共株式会社) 2014.04.17, 請求項 1-49, 実施例等 & US 2015/0297748 A1, 請求項 1-49, 実施例等	1-53

☐　Ｃ欄の続きにも文献が列挙されている。

☐　パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「A」特に関連のある文献ではなく、一般的技術水準を示すもの
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「&」同一パテントファミリー文献

国際調査を完了した日 ３０．０７．２０１８	国際調査報告の発送日 ０７．０８．２０１８
国際調査機関の名称及びあて先 日本国特許庁（ＩＳＡ／ＪＰ） 郵便番号１００－８９１５ 東京都千代田区霞が関三丁目４番３号	特許庁審査官（権限のある職員）　４Ｂ　６１１５ 田ノ上　拓自 電話番号　０３－３５８１－１１０１　内線　３４４８

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（２０１５年１月）

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ２０１８／０１８５７２

発明の属する分野の分類
C12N15/13(2006.01)i, A61K9/00(2006.01)i, A61K31/4745(2006.01)i,
A61K39/395(2006.01)i, A61K47/68(2017.01)i, A61P13/12(2006.01)i, A61P15/08(2006.01)i,
A61P35/00(2006.01)i, A61P43/00(2006.01)i, C07K16/28(2006.01)i, C12N1/15(2006.01)i,
C12N1/19(2006.01)i, C12N1/21(2006.01)i, C12N5/10(2006.01)i, C12P21/08(2006.01)i

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RESEARCH ARTICLE

Discovery and Optimization of HKT288, a Cadherin-6–Targeting ADC for the Treatment of Ovarian and Renal Cancers

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ABSTRACT Despite an improving therapeutic landscape, significant challenges remain in treating the majority of patients with advanced ovarian or renal cancer. We identified the cell–cell adhesion molecule cadherin-6 (*CDH6*) as a lineage gene having significant differential expression in ovarian and kidney cancers. HKT288 is an optimized CDH6-targeting DM4-based antibody–drug conjugate (ADC) developed for the treatment of these diseases. Our study provides mechanistic evidence supporting the importance of linker choice for optimal antitumor activity and highlights CDH6 as an antigen for biotherapeutic development. To more robustly predict patient benefit of targeting CDH6, we incorporate a population-based patient-derived xenograft (PDX) clinical trial (PCT) to capture the heterogeneity of response across an unselected cohort of 30 models—a novel preclinical approach in ADC development. HKT288 induces durable tumor regressions of ovarian and renal cancer models *in vivo*, including 40% of models on the PCT, and features a preclinical safety profile supportive of progression toward clinical evaluation.

SIGNIFICANCE: We identify CDH6 as a target for biotherapeutics development and demonstrate how an integrated pharmacology strategy that incorporates mechanistic pharmacodynamics and toxicology studies provides a rich dataset for optimizing the therapeutic format. We highlight how a population-based PDX clinical trial and retrospective biomarker analysis can provide correlates of activity and response to guide initial patient selection for first-in-human trials of HKT288. *Cancer Discov; 7(9); 1030–45. ©2017 AACR.*

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Note: Supplementary data for this article are available at Cancer Discovery Online (http://cancerdiscovery.aacrjournals.org/).

C.U. Bialucha and S.D. Collins contributed equally to this article.

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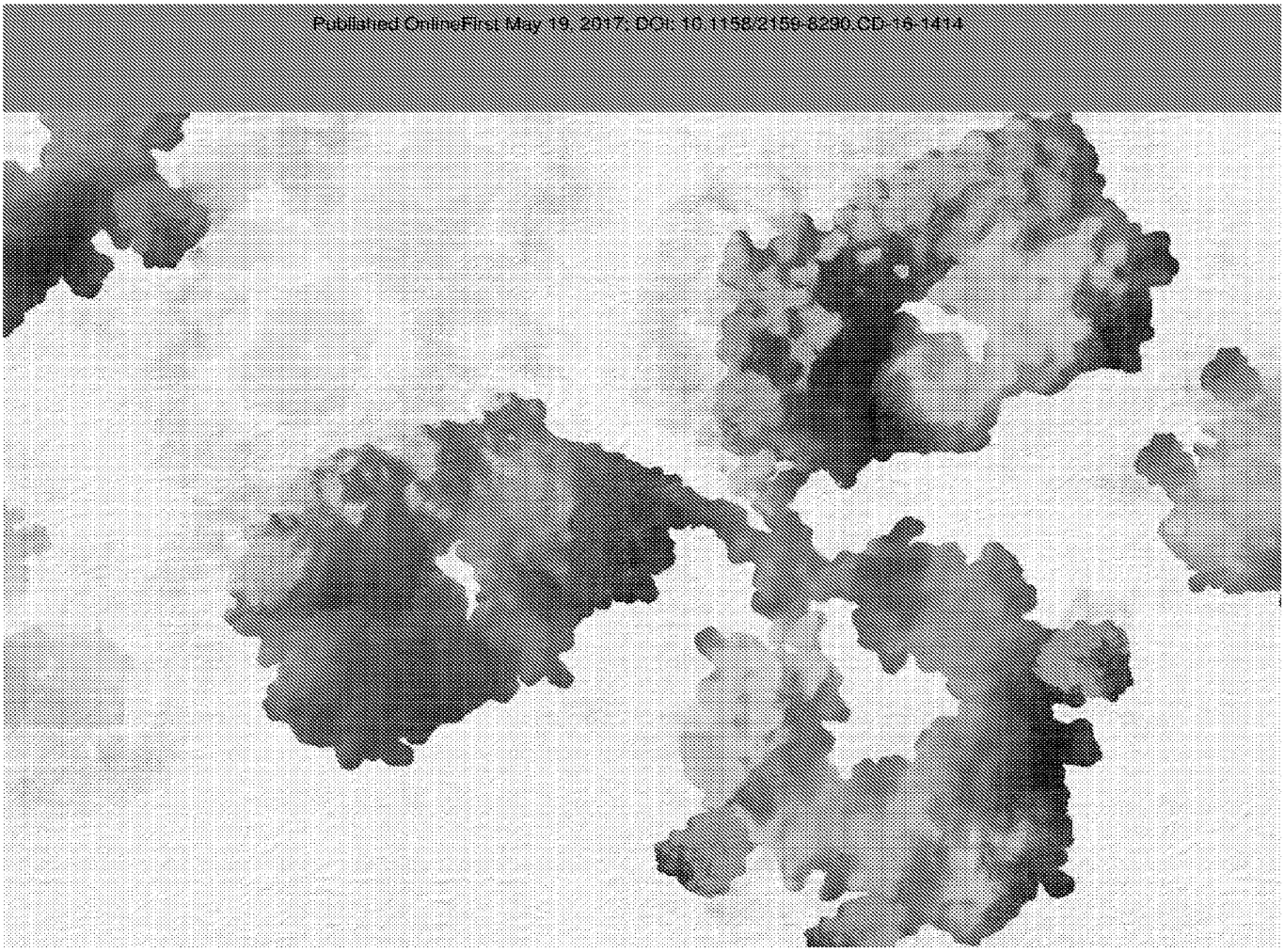
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INTRODUCTION

Despite recent therapeutic advances in both ovarian and renal cancers, there remains significant unmet medical need for patients suffering from these malignancies, especially in advanced settings. Unequivocally exemplifying this unmet need, most patients with ovarian cancer present with advanced-stage disease (70%) and face an associated low 5-year survival rate of 28% (1).

Antibody-drug conjugates (ADC) aim to leverage the specificity of monoclonal antibodies (mAb) to vectorize the delivery of highly potent cytotoxic agents preferentially to sites of antigen expression in tumor cells while attempting to limit the exposure to nontarget tissues. ADCs typically utilize a cytotoxic agent, such as monomethyl auristatin E (MMAE), maytansinoids (DM1 and DM4), calicheamicin, or a pyrrolobenzodiazepine dimer (PBD), linked to a target-specific mAb. There are two approved ADCs: brentuximab vedotin, a MMAE-based ADC targeting CD30 in lymphoma (2, 3), and ado-trastuzumab emtansine (T-DM1), a DM1-based ADC targeting HER2 approved for the treatment of patients with HER2-positive metastatic breast cancer (4). Multiple additional ADCs are currently in clinical development (reviewed in refs. 5–7).

To identify optimal cancer antigens for targeting with an ADC approach, we performed a genome-wide differential gene expression analysis across predicted cell-surface expressed genes from normal and cancer samples. Rather than selecting genes found overexpressed across many cancer types, albeit at lower frequency, we specifically aimed to identify genes with high-level, frequent overexpression in a specific indication, ovarian cancer. We hypothesized that such cell-surface expressed, lineage-linked genes might represent ideal ADC targets, based on their restricted normal tissue expression profile by definition and frequent, elevated expression in specific cancer indications. Such genes might further bias ADC targeting toward tumors and afford limited normal tissue exposure, while being maintained at sufficient frequency and level of expression in patient tumors covering select cancer indications, thus aiding patient selection.

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In our analysis, cadherin-6 (*CDH6*) was a top target candidate gene featuring frequent elevated mRNA expression in ovarian serous carcinoma and restricted expression across normal tissues. We also noticed extensive expression of *CDH6* in renal clear cell and papillary carcinoma, as well as evidence for elevated expression in thyroid cancer. Considering shared developmental pathways for these tissues involving the PAX8 lineage transcription factor, as well as evidence that *CDH6* is directly regulated by PAX8 (8, 9), CDH6 may itself be considered a lineage gene and its expression maintained in tumors arising from these tissues (10).

CDH6 is a type II, classic cadherin, first described as K-cadherin, which was found to be preferentially expressed in fetal kidney and kidney carcinoma (11, 12), as well as during normal renal development (13, 14). More recently, expression of CDH6 has also been described in ovarian and thyroid cancers (15–17). Like other members of the cadherin superfamily, CDH6 protein localizes to the basolateral membrane of epithelial cells and mediates calcium-dependent cell–cell adhesion (10, 18). Aside from the lineage-linked expression pattern of CDH6, other attributes of this class of proteins, including rapid internalization (19, 20) and reported altered membrane localization in tumor cells that have lost cellular polarity (21, 22), further highlighted the potential for CDH6 as a target for ADC development.

We here describe the identification and optimization of HKT288, a CDH6-targeting ADC comprising a fully human antibody selective for CDH6 conjugated to a maytansine-derived cytotoxic payload via a hindered disulfide-based linker, N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB), and $N^{2'}$-deacetyl-$N^{2'}$-(4-mercapto-4-methyl-1-oxopentyl)-maytansine (DM4). Our work provides a framework for knowledge-based ADC drug discovery, incorporating a hypothesis-driven target identification strategy, as well as the optimal design and preclinical evaluation of ADCs including broad assessment of efficacy across a heterogeneous population of PDX models.

RESULTS

CDH6, a Lineage Gene Frequently Overexpressed in Ovarian and Renal Cancers, Is Amenable to Targeting Using an ADC Approach

Genome-wide differential gene expression analysis across predicted cell-surface expressed genes using the publicly available mRNA expression datasets from The Cancer Genome Atlas (TCGA) and Gene-Tissue Expression (GTEx; refs. 23, 24) identified the *CDH6* gene as having frequent, elevated mRNA expression in ovarian serous carcinoma, renal clear cell carcinoma, and renal papillary carcinoma in conjunction with a restricted normal tissue expression profile (Fig. 1A; refs. 8, 9). CDH6 ranked in the top 0.3% of all surface protein genes for ovarian serous, renal clear cell, and papillary carcinoma (ranks of 8, 4, and 8, respectively, out of 2,475) based on expression differential between samples from a given tumor type and all available normal tissue samples, and requiring that maximum expression in normal tissues was in the lowest 25th percentile of expression values for all genes (details in Supplementary Methods). We validated CDH6 protein expression in clinical samples by performing immunohistochemistry (IHC) on 39 ovarian and 39 renal cancers. Homogeneous and heterogeneous cell-surface staining patterns of varying intensity were observed across both indications (Fig. 1B).

To identify an optimal CDH6-targeting therapeutic antibody for delivery of a cytotoxic payload to CDH6-positive tumors, a multipronged antibody generation campaign using a human combinatorial antibody library displayed on phage was conducted (HuCAL; MorphoSys; ref. 25). We identified 38 unique IgGs with selective binding to CDH6 from this screen. Efficient internalization of the ADC/antigen complex, followed by intracellular processing of the ADC and release of the cytotoxic payload, is thought to be a critical determinant of an ADC's activity (reviewed in refs. 5, 7), but is rarely assayed during antibody selection and optimization. To assess this process for each antibody, we developed a high-content immunofluorescence microscopy assay to measure antibody internalization independently of ADC cellular activity (Supplementary Fig. S1). In addition, as a surrogate for directly conjugating all 38 IgGs to a cytotoxic payload, we incubated DM1-conjugated anti-human Fab fragments with the unconjugated anti-CDH6 IgGs to form complexes and treated cells with these for 120 hours followed by measurement of cell viability. Both assays used the CDH6-positive cell line OVCAR3, which represents a relevant model system for high-grade serous ovarian cancer (26, 27). Antibody internalization propensity correlated positively with potency in the surrogate ADC assay ($r^2 = 0.630$; $P < 0.0001$), strongly implying that target-dependent, intracellular delivery of ADC payload drives ADC activity (Fig. 1C). These data were used to prioritize a subset of IgGs for subsequent direct conjugation to DM1 and activity profiling in cellular assays using CDH6-positive and CDH6-negative cell lines (Fig. 1D). In the CDH6-negative cell line OVCAR8, none of the CDH6-targeting ADCs were active over the assessed concentration range (1.7 pmol/L–33 nmol/L ADC). In contrast, CDH6-targeting ADCs featured cellular potencies ranging from double-digit picomolar to greater than 10 nmol/L IC_{50}s in the CDH6-positive cell line OVCAR3 and a clone of OVCAR8 engineered to overexpress human CDH6, OVCAR8-CDH6$^+$. A nontargeting isotype control ADC had no cytotoxic activity in any of the cell lines, whereas the cell-permeable, free maytansinoid compound s-me-DM1 was active across the cell line panel, further supporting the target-dependent activity of CDH6-binding ADCs.

On the basis of an integrated assessment of various parameters including cellular activity, antibody affinity, and epitope diversity, we selected 10 IgGs for *in vivo* efficacy testing as N-succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine (SMCC-DM1) conjugates in a subcutaneous OVCAR3 xenograft model. Following a single 10 mg/kg dose, a range of antitumor activity was observed across the panel of tested ADCs, with 6 of 10 ADCs inducing a transient tumor stasis and 4 of 10 yielding measurable antitumor activity (Fig. 1E; Supplementary Fig. S2). *In vitro* potency correlated positively with *in vivo* efficacy ($r^2 = 0.686$; $P = 0.0016$) and revealed that the most active ADC in cellular settings was also the most active *in vivo* (Fig. 1F). This

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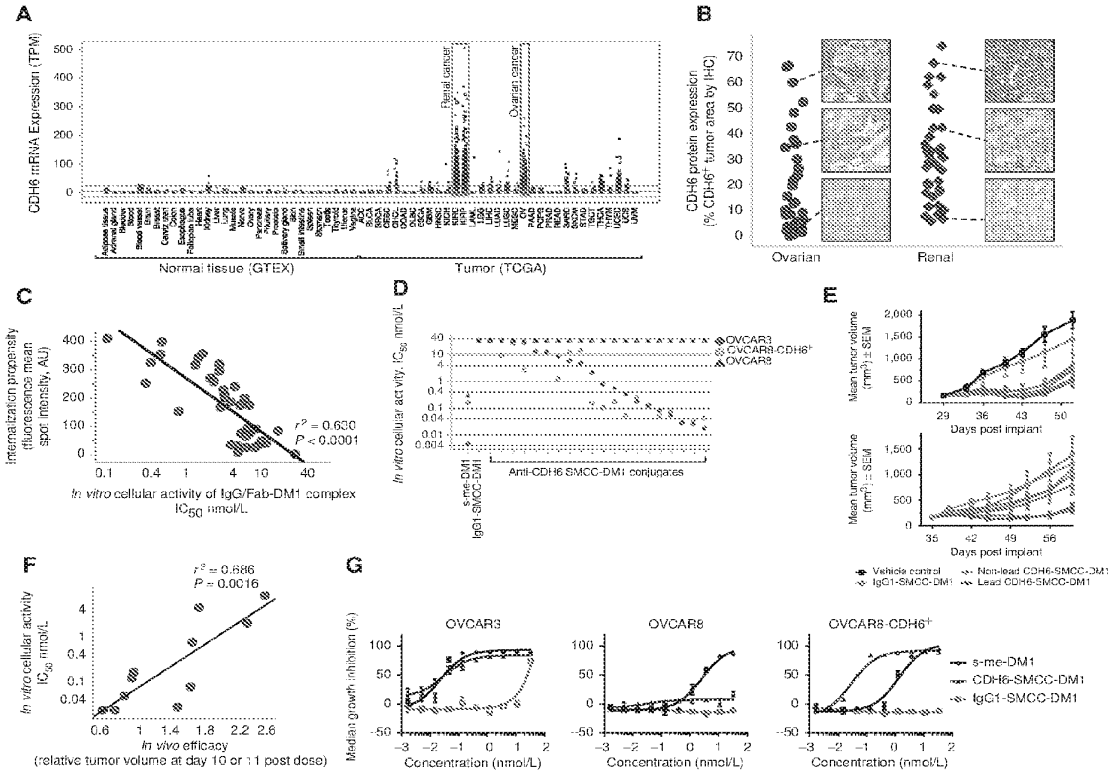


Figure 1. CDH6, a lineage gene frequently overexpressed in ovarian and renal cancer, is amenable to targeting using an ADC approach. **A,** CDH6 expression in transcripts per million reads (TPM) across normal tissue (pink) and cancer tissue (blue) samples. Renal and ovarian cancers are highlighted (red box) indications featuring frequent CDH6 overexpression. Green lines represent median expression ± standard deviation. **B,** CDH6 protein expression across clinical primary renal and ovarian cancer samples as assessed by IHC. Image analysis was performed to quantify the percent CDH6-positive tumor area for each sample. Inlays show IHC on sections of representative samples. **C,** Correlation of antibody internalization propensity quantified as mean fluorescence mean spot intensity in arbitrary units (AU) plotted versus IC_{50}s of antibody–Fab-DM1 complexes in a cellular cytotoxicity assay. **D,** Cellular activity summarized as IC_{50}s is shown for OVCAR3 (pink diamonds, CDH6-positive), OVCAR8 (blue triangles, CDH6-negative), and OVCAR8-CDH6$^+$ (yellow circles, CDH6-positive) treated with either CDH6-SMCC-DM1 ADCs, an isotype IgG1-SMCC-DM1, or a cell-permeable maytansinoid, S-me-DM1. **E,** OVCAR3 xenografts were grown subcutaneously in NSG mice and treated with a single intravenous dose of 10 mg/kg control IgG1 or CDH6-targeting antibodies conjugated to SMCC-DM1. Top and bottom plots represent two sets of antibodies that were profiled. The CDH6 lead antibody, LTV977, is highlighted in red and was included in both sets. Mean tumor volumes ±SEM per group over time are plotted. **F,** Correlation of *in vitro* cellular activity (IC_{50}) and *in vivo* efficacy in the OVCAR3 model. *In vivo* efficacy was calculated on the basis of tumor volume relative to control ADC treated at either day 10 (set 1) or day 11 (set 2) postdose. Red dots highlight the CDH6 lead antibody, LTV977. **G,** Cellular activity of lead CDH6 antibody as SMCC-DM1 conjugate (blue triangles), control IgG1-SMCC-DM1 (orange squares), or s-me-DM1 (black diamonds) titrated across OVCAR3, OVCAR8, or OVCAR8-CDH6$^+$ cells. Percent median inhibition relative to untreated is plotted.

antibody, henceforth designated the lead CDH6 antibody LTV977, demonstrated potent target- and concentration-dependent ADC activity *in vitro* (Fig. 1G) and was superior to other tested antibodies in an additional ovarian *in vivo* model (Supplementary Fig. S3). Of note, OVCAR3 cells efficiently internalize the ADCs and are exquisitely sensitive to the maytansinoid payload, as illustrated by the comparable activity of free drug and ADC. OVCAR8 cells are inherently less sensitive to payload, but high-level overexpression of CDH6 appears to compensate for this lower sensitivity through active delivery of the ADC.

The CDH6-Targeting mAb LTV977 Binds Selectively to a Conformational Epitope Conserved between Rodents and Primates and Is Capable of Eliciting Fc-Mediated Effector Functions Such as ADCC and CDC *In Vitro*

We next evaluated the binding profile of LTV977 and confirmed its selectivity using recombinantly produced and cell-surface expressed cadherin proteins. Biacore surface plasmon resonance measurements of LTV977 binding to CDH6 proteins revealed comparable, nanomolar affinities with K_D

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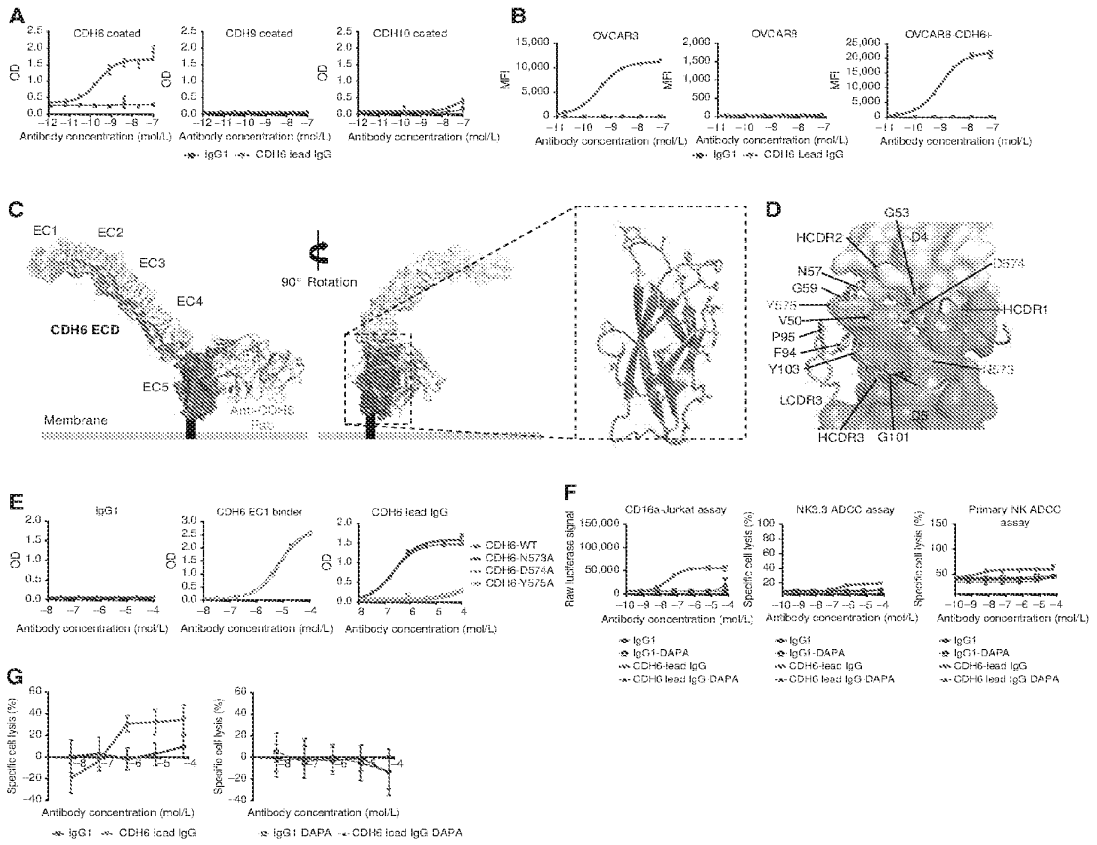


Figure 2. The lead CDH6-targeting mAb binds selectively to a conformational epitope conserved between rodents and primates and is competent of eliciting Fc-mediated effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) *in vitro*. **A,** Dose-dependent binding of control IgG1 (gray) or lead CDH6 antibody LTV977 (red) to recombinant human CDH6, CDH9, or CDH10 protein by ELISA (mean ± standard deviation). **B,** Dose-dependent binding of control IgG1 (gray) or lead CDH6 antibody LTV977 (red) to OVCAR3, OVCAR8, or OVCAR8-CDH6$^+$ cells as determined by FACS (mean ± standard deviation). **C,** Overall structure of lead CDH6 antibody Fab fragment (show as ribbon) binding to CDH6 (shown as surface). EC1–EC4 are modeled from N-cadherin (N-CDH) structure (PDB ID: 3Q2W, superimposed on the basis of EC5) and colored in white. EC5 of CDH6 (blue) and lead Fab (yellow) are shown. Epitope residues on CDH6 are shown as yellow sticks. **D,** Close-up view of critical interactions between CDH6 and lead Fab. Cadherin is shown as surface and antibody as ribbon. The three critical epitope residues N573, D574, and Y575 are colored in red. Important residues of the complementarity-determining region are also labeled and shown as sticks. **E,** Dose-dependent binding of control IgG1, a tool CDH6 antibody that binds EC1, or the lead CDH6 antibody LTV977 to recombinant human wild-type CDH6 (red), CDH6-N573A (pink), CDH6-D574A (green), or CDH6-Y575A (gray) protein by ELISA (mean ± standard deviation). **F,** Dose-dependent *in vitro* ADCC activity of control IgG1 (gray, solid line), IgG1-DAPA (gray, dotted line), or lead CDH6 antibody LTV977 (red) in wild-type Fc or DAPA (blue) format with OVCAR3 used as target cells. CD16-Jurkat-NFAT-luc reporter assay (left plot), NK3.3 ADCC assay (middle plot), or primary natural killer (NK)–cell assay (right plot) are shown. Mean raw luciferase signal ± standard deviation is plotted for the reporter assay, whereas mean percent specific lysis ± standard deviation is plotted for the NK3.3 and primary NK-cell ADCC assays. **G,** Dose-dependent *in vitro* CDC activity of control IgG1 (gray) or lead CDH6 antibody LTV977 in wild-type (red, left plot) or DAPA format (blue, right plot). OVCAR3 cells were used as target cells. Mean percent specific lysis from 5 individual runs ±SEM is plotted.

values of 8.8, 8.6, 7.7, and 9.0 nmol/L for human, cynomolgus, rat, and mouse CDH6, respectively (Supplementary Fig. S4). LTV977 bound to full-length CDH6 ECD protein by ELISA, but not to CDH9 or CDH10 ECDs, the most closely related cadherins in the human proteome with 74% amino acid homology to CDH6 in the ECD (Fig. 2A). LTV977 bound cell-surface CDH6 on OVCAR3 cells, which endogenously express CDH6, as well as on OVCAR8-CDH6–positive cells engineered to express the target. No binding was observed to wild-type, CDH6-negative OVCAR8 cells (Fig. 2B).

To gain a better understanding of how LTV977 interacts with its target, we solved the crystal structure of the corresponding Fab fragment in complex with CDH6. The E-cadherin homology domain 5 (EC5) was determined as sufficient for antibody binding by coimmunoprecipitation and was used to generate atomic-scale data on the epitope at 2.3 Å resolution (Supplementary Table S1). The structural similarity

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between the EC5 domains of CDH6 and N-cadherin (CDH2) enabled an overlay of the CDH6 EC5/Fab complex structure onto the full-length extracellular domain (ECD) structure of CDH2 based on the superposition of EC5 domains. In doing so, we find that the lead CDH6 antibody binds at the side of CDH6 EC5, with the long axis of the Fab nearly perpendicular to the long axis of CDH6 EC5 (Fig. 2A). The CDH6 binding surface for the antibody constitutes a three-dimensional, conformational epitope formed by several continuous and discontinuous sequences, namely residues 503, 520–527, 529, 532–534, 538–543, 550, 552, 569, and 571–577 (Fig. 2C, insert). Analysis of the CDH6 protein/lead Fab crystal structure highlighted several amino acid residues (e.g., Asn573, Asp574, and Tyr575) with high buried surface values, suggesting they might be important for mediating the interaction of the antibody with CDH6 (Fig. 2D). We produced recombinant mutant CDH6 protein, replacing residues 573, 574, and 575 with alanine, and performed ELISA binding assays. Mutation of Asp574 or Tyr575 abrogated antibody binding, whereas mutation of Asp573 did not. None of these mutations affected binding of an unrelated tool CDH6 antibody, which binds a distinct epitope in EC1—indicating the mutants did not alter the overall architecture of the proteins (Fig. 2E). These data further validate the proposed binding mode derived from the crystal structure and highlight the necessity of CDH6 residues Tyr575 and Asp574 for binding of the lead antibody.

LTV977 is of the IgG1/k isotype subclass and is hence potentially capable of triggering antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC). ADCC activity was assessed in a JURKAT-NFAT-luc reporter assay and coculture cytotoxicity assays using both primary and cell line–based natural killer cells. CDC activity assays were conducted using OVCAR3 cells in the presence of rabbit complement. *In vitro*, the lead antibody induced specific ADCC as well as CDC activity, whereas an Fc-mutant derivative containing two amino acid substitutions (D265A; P329A) previously shown to confer impaired binding to Fc-γ receptors and complement activation (28, 29) was inert (Fig. 2F and G). Together, these data indicate that the lead antibody is in principle capable of inducing Fc-dependent effector functions including ADCC and CDC.

Comparative *In Vivo* Profiling Identifies Sulfo-SPDB-DM4 as the Optimal Linker/Payload for CDH6-ADC

In an effort to identify the optimal linker and payload to pair with LTV977, we conducted head-to-head *in vivo* efficacy studies in the CDH6-expressing OVCAR3 xenograft comparing the activity of CDH6 ADCs using either a non-cleavable linker/payload (L/P), SMCC-DM1, or a disulfide-based cleavable L/P, SPDB-DM4. SMCC-DM1 yields a single nonpermeable cellular catabolite, whereas SPDB-DM4 is expected to produce a series of catabolites including cell-permeable products (30). As has been previously reported for other ADC targets (31), the SPDB-DM4 format demonstrated superior *in vivo* activity ($P < 0.001$, Supplementary Table S2) with a single intravenous 5 mg/kg dose for the CDH6 ADC. It elicited a robust durable regression lasting 82 days compared with modest tumor inhibition from an equivalent dose of its SMCC-DM1 counterpart, despite comparable pharmacokinetic (PK) profiles of the relevant conjugates (Fig. 3A). In a separate study, we were furthermore able to show that tumors which regrew following initial regression remained sensitive to the ADC for multiple cycles, suggesting that surviving cells retain CDH6 expression (Supplementary Fig. S5). In a pseudo-orthotopic, intraperitoneal luciferase-expressing OVCAR3 xenograft model (Fig. 3B; Supplementary Table S3), a single intravenous 5 mg/kg dose of a SPDB-DM4 CDH6-ADC elicited maximal tumor regression at day 69, an approximately 17-fold improvement over the maximal regression seen at day 42 from the SMCC-DM1 counterpart.

A sulfonate group-bearing, charged version of SPDB-DM4 (sulfo-SPDB-DM4) has been shown to have superior antitumor activity in the context of a folate receptor (FOLR1) targeting ADC (32). We performed a dose–response efficacy study in OVCAR3 comparing the lead CDH6 antibody conjugated to either SPDB-DM4 or sulfo SPDB-DM4 (Fig. 3C). In this study, CDH6-sulfo-SPDB-DM4 elicited significant regressions at 2.5 and 5 mg/kg doses, whereas the SPDB-DM4 format only yielded regression at the 5 mg/kg dose level and growth inhibition at 2.5 mg/kg (Supplementary Table S4). Comparison of the concentration profiles of each format revealed greater exposure of the sulfo-SPDB-DM4 ADC at each of the three dose levels assessed. These data suggest physicochemical properties of the linker, specifically the increased hydrophilicity provided by the sulfonate group may be responsible for enhanced exposure and activity of the sulfo-SPDB-DM4 format (Fig. 3D; Supplementary Table S5).

To further elucidate the molecular and mechanistic basis of the enhanced activity of the sulfo-SPDB-DM4 format, we conducted a pharmacodynamic study in the OVCAR3 model (Fig. 4A–D). Tumors were sampled across a time course following a single 5 mg/kg dose of CDH6-SMCC-DM1, CDH6-sulfo-SPDB-DM4, or the equivalent IgG control ADCs and assessed for catabolite levels along with markers for cell-cycle arrest [phosphohistone-H3 (pHH3)] and apoptosis [cleaved caspase-3 (CCASP3)]. A target-dependent kinetic profile of intratumoral catabolites was observed for both of the CDH6-targeting ADCs, but not the IgG control ADCs. Catabolite levels peaked at 72 hours for the sulfo-SPDB-DM4 format, but were still increasing for the SMCC-DM1 at the end of the time course. The presence of intratumoral ADC catabolites was followed by target-dependent increases in cell-cycle arrest and apoptosis as determined by IHC. DM1-driven apoptosis was measured at approximately 10% of cells 72 hours postdose and reached a plateau by 72 hours; however, the DM4-driven apoptosis continued to rise throughout the time course with a maximum measured of approximately 17% by 120 hours.

Emerging data have suggested that patient-derived xenografts (PDX) might represent human tumor biology better than cell line–based models, highlighting their utility in preclinical drug development (33–36). We therefore explored the efficacy of the CDH6-ADC in PDX models of ovarian carcinoma. In contrast with OVCAR3 xenografts, which feature high and homogenous CDH6 expression by IHC, PDX models including HOVX2263 commonly present a heterogeneous pattern of CDH6 expression more representative of that seen in ovarian cancer patient samples (Supplementary Fig. S6A–S6D). We

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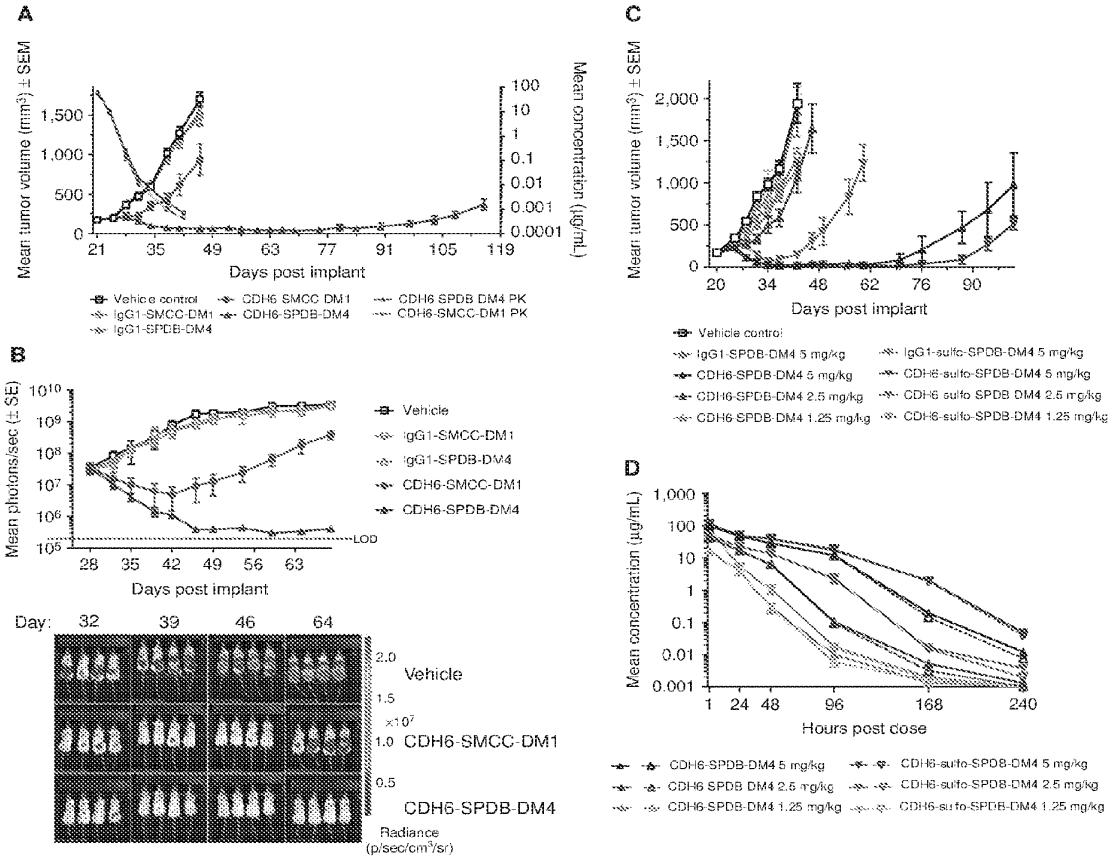


Figure 3. Comparative *in vivo* profiling identifies sulfo-SPDB-DM4 as the optimal L/P for CDH6-ADC. **A,** OVCAR3 xenografts were grown subcutaneously in NSG mice and treated with a single i.v. dose of 5 mg/kg control or CDH6-targeting antibodies linked to either SMCC-DM1 or SPDB-DM4 payloads. Mean tumor volumes and PK exposure of total ADC and total antibody over time ±SEM are plotted. **B,** OVCAR3-luc tumors were established intraperitoneally in SCID beige mice and treated with a single 5 mg/kg i.v. dose of control or CDH6-targeting antibodies linked to either SMCC-DM1 or SPDB-DM4 payloads. Mean tumor burden via bioluminescent imaging ±SEM is plotted over time. Images from day 46 post implant; LOD, limit of detection. **C,** OVCAR3 xenografts were grown subcutaneously in NSG mice and treated with a single i.v. dose of control or CDH6-targeting antibodies linked to either SPDB-DM4 or sulfo-SPDB-DM4 payloads. ADCs were dosed at 1.25, 2.5, and 5 mg/kg. Mean tumor volumes ±SEM are plotted. **D,** PK exposure of total ADC and total antibody are plotted from efficacy study plotted in **C**. Solid indicates total antibody, dotted total ADC.

first evaluated both linker/payload formats in the HOVX2263 model on a regimen of 5 mg/kg i.v. once every two weeks (q2w; Fig. 4E). Whereas CDH6-sulfo-SPDB-DM4 induced regressions and prevented tumor regrowth for 120 days after treatment initiation, CDH6-SMCC-DM1 elicited only a modest inhibition of tumor growth compared with vehicle control (treatment/control = 40.9%; Supplementary Table S6). To investigate the therapeutic potential of targeting CDH6 without delivering a cytotoxic moiety, we included the nonconjugated antibody in this experiment. The lack of efficacy observed with this agent after multiple doses (Fig. 4E) indicates that the antitumor efficacy of CDH6-sulfo-SPDB-DM4 is driven by the sulfo-SPDB-DM4 L/P and not the naked antibody component (ADCC/CDC) of the molecule.

Acquired resistance to platinum-based chemotherapy is commonly observed clinically (37) and is a feature linked to the poor 5-year survival of patients with advanced-stage ovarian cancer. We assessed antitumor efficacy of CDH6-sulfo-SPDB-DM4 in the heterogeneously CDH6-positive (Supplementary Fig. S6D) ovarian PDX model, HOVX4863. This model was known to be insensitive to combination carboplatin/paclitaxel standard-of-care (SoC) therapy from previous *in vivo* work (data not shown). As expected, the SoC therapy was unable to inhibit tumor growth and tracked

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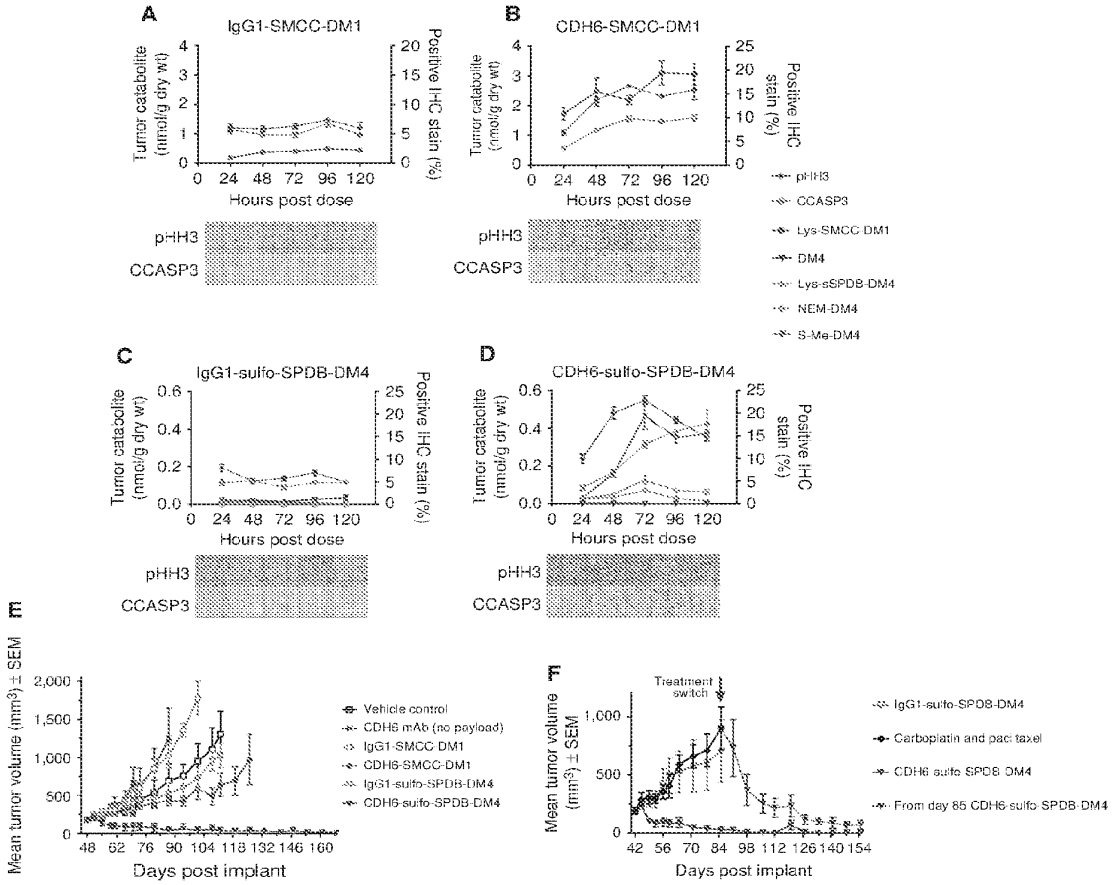


Figure 4. CDH6-sulfo-SPDB-DM4 has superior pharmacodynamic impact on OVCAR3 tumors, and robust antitumor response in ovarian PDX models. **A–D,** Established subcutaneous OVCAR3 tumors were treated with a single i.v. administration of either IgG1-SMCC-DM1 (**A**), CDH6-SMCC-DM1 5 mg/kg (**B**), IgG1-sulfo-SPDB-DM4 5 mg/kg (**C**), or CDH6-sulfo-SPDB-DM4 5 mg/kg (**D**). At each time point, 3 mice per group were euthanized and tumors excised. A section of tumor was sampled for IHC staining, and fragments were collected for catabolite profile analysis. For each treatment, representative IHC images of pHH3 CCASP3 across the time course are shown, and tumor catabolite and positive IHC stain values (±SEM) are shown for each treatment group. **E,** Tumors of the PDX model HOVX2263 were grown subcutaneously in female nude mice randomized into groups of equal mean tumor volume and treated q2w with a 5 mg/kg i.v. dose of either IgG1-SMCC-DM1, IgG1 sulfo-SPDB-DM4, CDH6-SMCC-DM1, CDH6-sulfo-SPDB-DM4, or the unconjugated CDH6 antibody. Mean tumor volumes ±SEM over time are plotted. **F,** Tumors of the PDX model HOVX4863 were grown subcutaneously in female nude mice randomized into groups of equal mean tumor volume. Mice were treated with either IgG1-sulfo-SPDB-DM4 5 mg/kg i.v. q2w, CDH6-sulfo-SPDB-DM4 5 mg/kg i.v. q2w, or with a combination of carboplatin (50 mg/kg i.p. weekly) and paclitaxel (12.5 mg/kg i.v. weekly) until day 85 when this group was switched to CDH6-sulfo-SPDB-DM4 treatment. Mean tumor volumes ± SEM over time are plotted.

with that of the control (Fig. 4F). At day 85 post-implant and a mean tumor volume of 900 mm³, SoC treatment was followed by CDH6-sulfo-SPDB-DM4 5 mg/kg i.v. q2w. Despite the increased tumor burden, CDH6-sulfo-SPDB-DM4 induced regressions beyond the starting volume of the experiment on day 42, to a mean of 72.5 mm³—a regression of 99.9% (Supplementary Table S7). This degree of efficacy in a model insensitive to a SoC therapy highlights the potential of CDH6-ADC in patients with CDH6-positive tumors who have progressed following first-line therapy.

Together, these data suggest that sulfo-SDPB-DM4 is the optimal linker payload for the lead CDH6-targeting antibody with regard to antitumor efficacy.

CDH6-Targeting ADCs Feature an Acceptable Tolerability Profile in Rats and Nonhuman Primates

In order to determine the preclinical tolerability profile of CDH6-ADCs, we conducted rat and nonhuman primate (NHP) toxicology studies. We first assessed whether rats and

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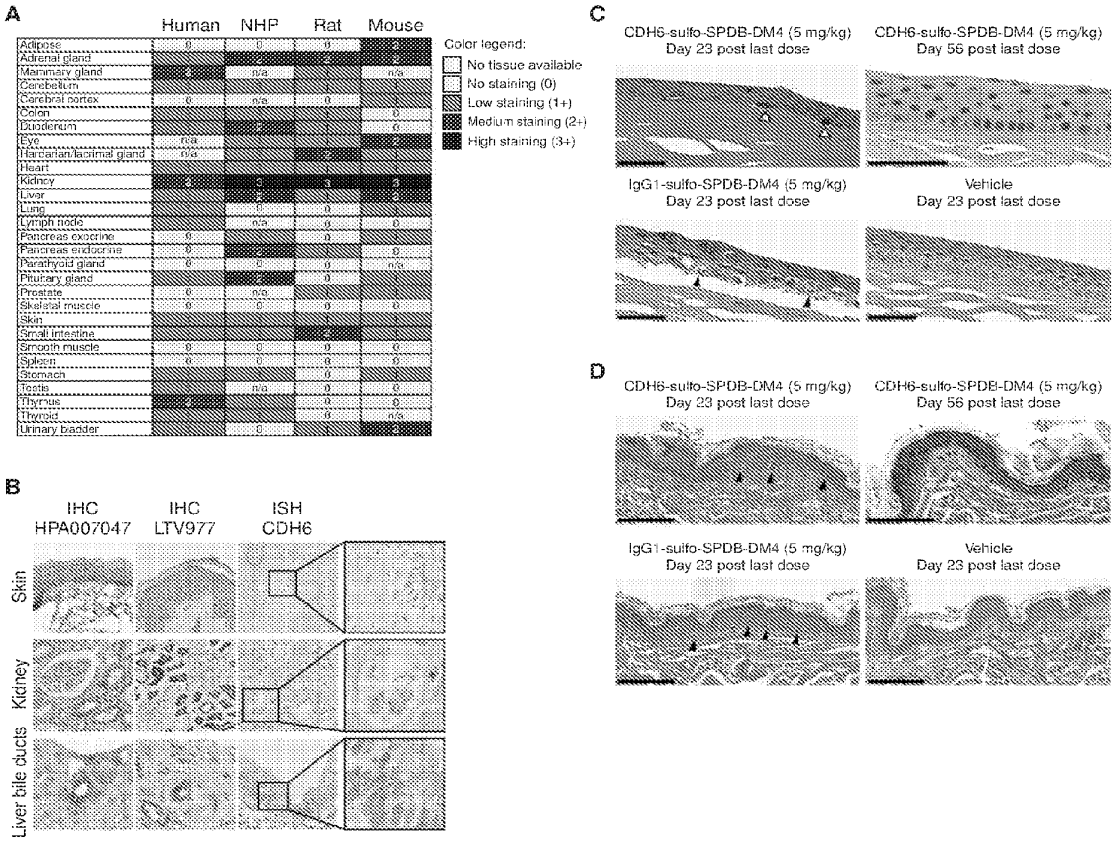


Figure 5. CDH6-targeting ADCs feature an acceptable tolerability profile in rats and nonhuman primates. **A,** Summary of CDH6 expression by IHC using the polyclonal CDH6 antibody HPA007047 in human, NHP, rat, and mouse tissue sections. Tissues were graded from no staining (0) to low (1+), medium (2+), and high (3+) staining intensity; n/a indicates that no tissue was available. **B,** Detail on CDH6 expression in skin, kidney, and liver bile ducts. IHC was performed using HPA007047 (left column) or LTV977 (middle column). *in situ* hybridization (ISH) using a CDH6-selective probe is shown in the right column with inserts showing magnified view. **C,** Representative images of hematoxylin/eosin-stained slides of corneal sections from NHPs treated with ADCs: CDH6-sulfo-SPDB-DM4 was dosed 3 × 5 mg/kg once weekly (qw) at day 23 after last dose (top left) or dosed 4 × 5 mg/kg qw at day 56 after last dose (top right). IgG-sulfo-SPDB-DM4 dosed 3 × 5 mg/kg qw and vehicle dosed qw both at 23 days after last dose are shown bottom left and right, respectively. White and black arrowheads indicate pigment deposits or single-cell necrosis, respectively. Scale bars represent 60 μm. **D,** Representative images of hematoxylin/eosin-stained slides of dorsal skin sections from NHPs treated with ADCs: CDH6-sulfo-SPDB-DM4 was dosed 3 × 5 mg/kg qw at day 23 after last dose (top left) or dosed 4 × 5 mg/kg qw at day 56 after last dose (top right). IgG-sulfo-SPDB-DM4 dosed 3 × 5 mg/kg qw and vehicle dosed qw both at 23 days after last dose are shown bottom left and right, respectively. Arrowheads indicate single-cell necrosis. Scale bars represent 100 μm.

cynomolgus monkeys are relevant species for assessing the safety of CDH6 targeting by examining the expression of CDH6 across normal rat, cynomolgus monkey, and human tissues. Using a species-crossreactive polyclonal antibody to CDH6, we found overall comparable staining patterns across species, with the most notable staining in the kidney (renal proximal tubule cells; Fig. 5A). Although this staining pattern appeared consistent with RNA expression data from normal tissues (Fig. 1A), we noted some low-level staining in tissues negative for CDH6 by RNA sequencing (RNA-seq; i.e., skin, adrenal gland). In particular, low-level IHC positivity in the basal layer of the skin (Fig. 5B; top right plot) was of concern based on severe on-target skin toxicities observed clinically for a CD44v6-targeting ADC (38). CD44v6 is expressed at high levels in normal skin (39). In directly stained fresh human tissue sections for RNA *in situ* hybridization of CDH6, prominent signals were observed in the kidney, as well as bile ducts, but not in the basal layer of the skin (Fig. 5B, right plots). To corroborate these findings, we directly stained fresh human tissue sections with the lead CDH6-sulfo-SPDB-DM4 conjugate. Although this reagent positively stained kidney and liver

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bile duct sections, no IHC signal was observed in the skin, suggesting the weak IHC signal in this tissue with the polyclonal CDH6 may be nonspecific (Fig. 5B, middle plots). Together, these data indicate that CDH6 is expressed in normal kidney and liver bile ducts, but not in the basal layer of the skin.

For the tolerability assessment, we dosed both rats and NHPs with CDH6-sulfo-SPDB-DM4 or nontargeting IgG1-sulfo-SPDB-DM4 ADCs at doses up to 15 mg/kg with various dosing regimens (Supplementary Table S8). Microscopic findings of increased mitotic figures and single-cell necrosis were observed across numerous tissues and were similar between animals treated with control and CDH6-targeting ADCs. These findings were considered to be consistent with the maytansinoid mechanism of action. Specific tissues exhibiting changes included sciatic nerve, testes, liver and the epithelia of the skin, eye (cornea), urinary bladder, mammary glands, uterus, and gingiva. In the spleen, lymph nodes, thymus, and bone marrow, decreased lymphoid or hypocellularity was observed. Various dosing frequencies were assessed including weekly, every 2 weeks, and every 3 weeks. Overall, weekly administration of CDH6-sulfo-SPDB-DM4 for up to 4 weeks was well tolerated in rats at doses up to 5 mg/kg, and at doses of 2 mg/kg in monkeys. Administration every 2 weeks was well tolerated in rats at 20 mg/kg and in monkeys at 5 mg/kg. Noteworthy toxicities occurred in the skin and in the corneal epithelium. In monkeys, weekly intravenous administration of 5 mg/kg CDH6-sulfo-SPDB-DM4 and IgG1-sulfo-SPDB-DM4 was associated with dose-related, reversible corneal changes that were most prominent peripherally (Fig. 5C). This is consistent with observations of non–target-mediated ocular toxicities in human clinical trials of ADCs that use microtubule-disrupting payloads (40). The most significant finding in NHP observed with both CDH6-sulfo-SPDB-DM4 and IgG1-sulfo-SPDB-DM4 was acanthosis/hyperkeratosis with epidermal cell necrosis leading to ulceration of the skin (Fig. 5D). These skin lesions were dose limiting at doses greater than 5 mg/kg (when dosed on a q2w schedule) and greater than 2 mg/kg (when dosed weekly). All findings reversed or showed evidence of reversal following cessation of treatment. Together, these data are supportive of an acceptable tolerability profile for CDH6-sulfo-SPDB-DM4 that lacks overt on-target, CDH6-mediated toxicities and establish sulfo-SPDB-DM4 as the final format for the ADC HKT288.

HKT288 Elicits Target-Dependent Antitumor Efficacy in PDX Models of Renal Clear Cell Carcinoma

In addition to ovarian tumors, elevated mRNA levels of CDH6 are observed in both the clear cell and papillary subtypes of renal cell carcinoma compared with normal tissue (Fig. 1A). For assessment of HKT288 efficacy in renal cancer, three PDX models in our collection were identified as displaying a range of CDH6 expression values relative to human renal clear cell carcinomas (Fig. 6A) and used to assess antitumor efficacy of HKT288. Representative CDH6 IHC images from the control PDX tumors display heterogeneous staining throughout the PDX (Fig. 6B). HKT288 demonstrated dose-dependent tumor growth inhibition relative to control-treated mice in all three models (Fig. 6C; Supplementary Tables S9–S11). Specifically, 5 mg/kg i.v. q2w caused significant inhibition in all models, whereas 2.5 mg/kg i.v. q2w elicited a significant antitumor effect in one model, HKIX3629, which had the greatest cell-surface protein expression of CDH6 via IHC. Congruently, the model with the lowest expression of CDH6, HKIX5374, was least sensitive to HKT288 treatment. These data suggest HKT288 has the potential to be efficacious in renal cancer and suggest that efficacy may track with CDH6 expression, although correlation analysis between CDH6 expression and response in this indication would be inappropriate based on the limited number of models tested.

HKT288 Induces Target-Dependent, Robust, and Durable Antitumor Response in over One Third of Subjects in an Unselected Ovarian PDX Clinical Trial

In order to assess preclinical efficacy across a broader heterogeneous population, HKT288 was next tested in an ovarian PDX clinical trial (PCT). This previously established 1 × 1 × 1 experimental format (41) utilized a panel of 30 ovarian cancer PDX models established from treatment-naïve patient tissue, with unknown CDH6 expression status, to assess the efficacy of HKT288 at a dose of 5 mg/kg i.v. q2w. Response was assessed by RECIST-style criteria of complete response (CR), partial response (PR), progressive disease (PD), or stable disease (SD). HKT288 displayed statistically significant ($P = 2.39E^{-6}$) benefit compared with an untreated xenograft patient population, enhancing probability of progression-free outcome (by tumor doubling; Fig. 7A). In this unselected population of ovarian cancer PDX models, 40% (12/30) responded with either CR or PR (Fig. 7B), and when efficacious, the responses to the HKT288 were robust and sustained for over 150 days after treatment initiation (Fig. 7C). Integration of the IHC, RNA-seq, and tumor response data sets demonstrated a positive correlation between sensitivity to HKT288 and CDH6 protein as well as RNA expression (Fig. 7C–F, $R^2 = 0.377$, $P = 0.000657$ and Supplementary Fig. S7A–S7B, $R^2 = 0.496$, $P = 0.000175$). Furthermore, selection of a subpopulation of models based on CDH6 expression (IHC) above the median value across models raises the response rate to 64% (9/14; Supplementary Fig. S7C). Representative IHC images of untreated control tumors from the PCT (Fig. 7E) illustrate the spectrum of CDH6 expression and response to HKT288, from a lack of target expression in model A (PD), to minimal staining in model B (SD → PD), and high staining intensity in models C and D (CR). Comparison of CDH6 IHC data from PDX models and primary human ovarian tumor samples shows a comparable distribution of CDH6 expression patterns. Furthermore, integration of PCT response data with IHC in PDX and primary human ovarian tumor samples indicates that a substantial fraction of patients with ovarian cancer have CDH6 expression patterns consistent with PDX tumors in which *in vivo* activity was demonstrated in the PCT (Fig. 7G).

DISCUSSION

There remains a significant need for improved therapy for patients with ovarian and renal cancers. Here, we describe the identification of a highly active ADC targeting CDH6 for the treatment of ovarian and renal cancers and present an

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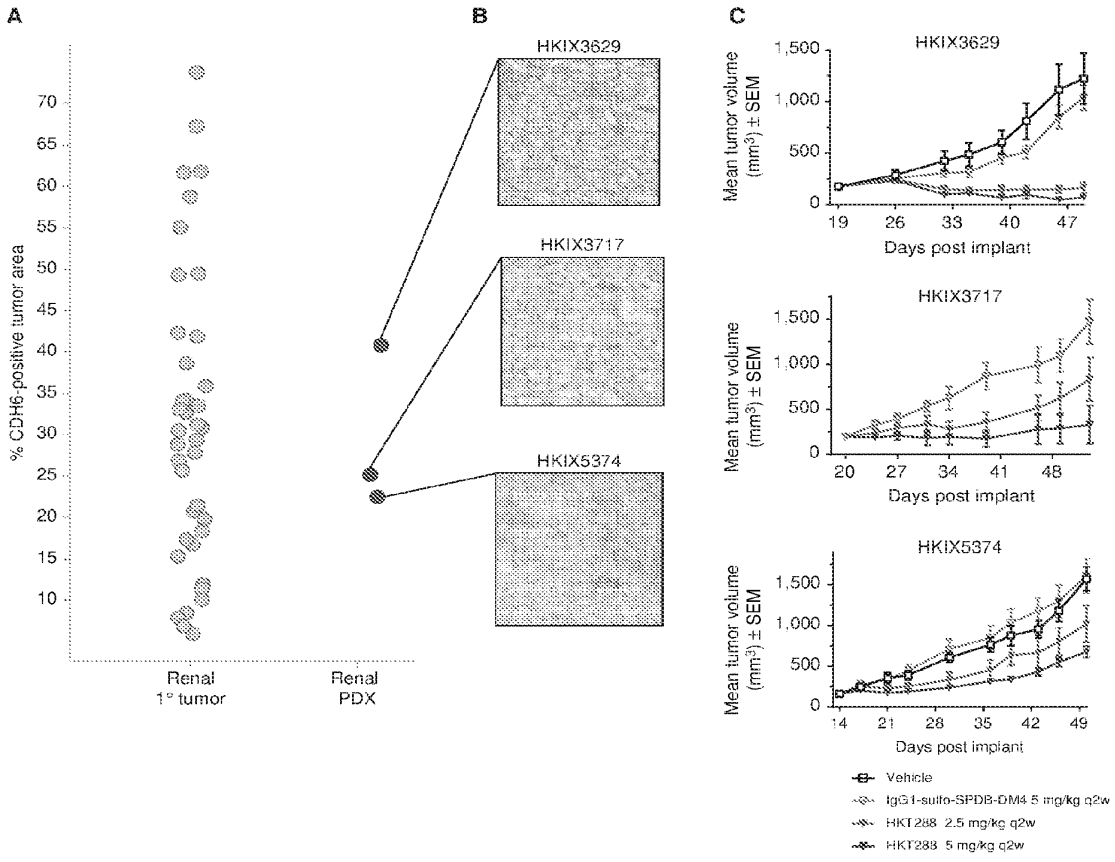


Figure 6. HKT288 elicits target-dependent antitumor efficacy in PDX models of renal clear cell carcinoma. **A,** Human renal clear cell carcinomas display a range of percent CDH6-positive tumor area as determined by quantitative IHC. Three examples of PDX models of renal clear cell carcinoma within this range are shown: HKIX3629, HKIX3717, and HKIX5374. **B,** Representative CDH6 IHC image of each renal cell carcinoma PDX model. **C,** Renal cell carcinoma PDX models HKIX3629, HKIX3717, and HKIX5374 were grown subcutaneously in female nude mice until they reached an appropriate tumor volume and then were treated q2w i.v. with either vehicle, IgG1-sulfo-SPDB-DM4 at 5 mg/kg, or HKT288 at 2.5 mg/kg or 5 mg/kg. Tumor size versus time post implant is shown.

integrated, pharmacology-driven paradigm for the discovery and optimization of ADCs.

A specifically designed bioinformatics strategy to uncover lineage-linked, cell-surface expressed cancer antigens identified CDH6 as having suitable characteristics for targeting with an ADC, including frequent, elevated expression in cancer with a concomitant restricted normal tissue expression profile (Fig. 1A). RNA-seq data and IHC studies further confirmed the restricted normal tissue distribution of CDH6 while highlighting ovarian and renal cancers as key target indications (Figs. 1A and B and 5A). We were particularly drawn to the observation that CDH6 overexpression is found in tumors originating from the developmentally related müllerian, renal, and thyroid lineages. Reports identifying CDH6 as a direct downstream target of the lineage transcription factor PAX8, key to the development of the aforementioned lineages, further indicate that CDH6 expression may be a characteristic feature of the cellular identity of these tumors and not easily lost under selective pressure. Consistent with this idea, we found tumors growing out after initial regression remained sensitive to subsequent doses of CDH6-ADC, and regressions under continuous treatment were durable beyond 150 days of treatment (Supplementary Fig. S5; Fig. 7C).

ADCs are considered modular drugs: The activity and safety profile are thought to be determined by a combination of the antibody target properties and the specific characteristics conferred by the linker and the payload (5). Consistent with data for other ADCs (32), in our study cleavable L/Ps producing cell-permeable catabolites were significantly more active than a noncleavable L/P

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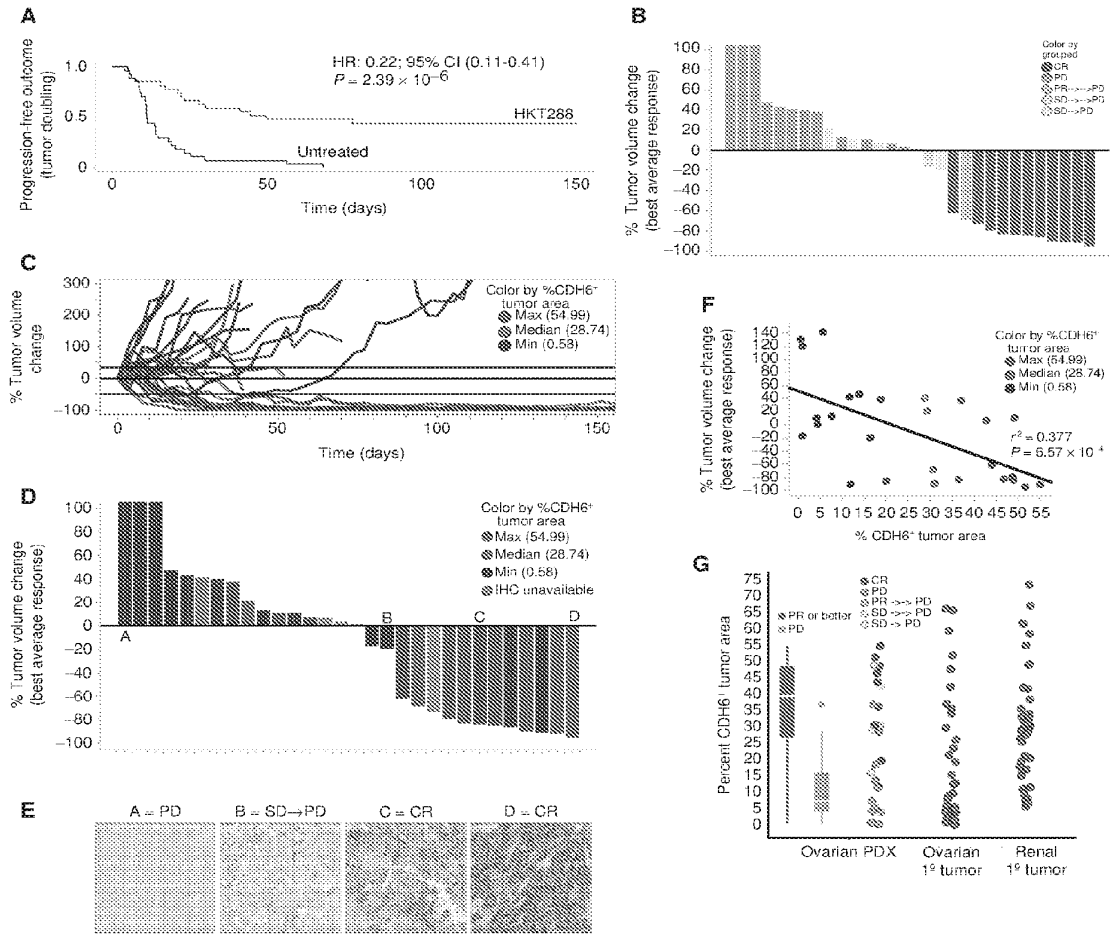


Figure 7. HKT288 induces target-dependent, robust, and durable antitumor response in over a third of an unselected ovarian PCT. **A,** Kaplan–Meier style plot comparing HKT288 to the untreated control arm. Progression-free outcome as determined by tumor volume doubling is plotted against time. **B,** Waterfall plot of percent best average response to HKT288 treatment in PCT. Color depicts response by RECIST-style criteria (blue, CR; green, PR progressing to PD; yellow, SD progressing to PD; pink, PD). **C,** Tumor growth kinetics of HKT288-treated mice are plotted. Color depicts the range in percent CDH6-positive tumor area as determined by quantitative IHC. **D,** Waterfall plot of percent best average response to HKT288 treatment in PCT. Color depicts the range in percent CDH6-positive tumor area as determined by quantitative IHC. IHC was unavailable for three models (green bars). **E,** Representative CDH6 IHC images from models labeled **A–D** in Fig. 4C annotated with their response category. **F,** Correlation plot between best average response and percent CDH6-positive tumor area as determined by quantitative IHC. **G,** Summary of PCT responses and CDH6 protein expression for ovarian PDX models compared with percent CDH6-positive tumor area as determined by quantitative IHC from sample human ovarian and renal tumors (tissue microarrays).

(Figs. 3 and 4). We extended this observation by mechanistically linking the enhanced activity of the cleavable L/Ps to concomitantly elevated induction of G_2–M arrest and apoptosis in tumors. We also observed improved ADC exposure and activity with a charged sulfonate group-bearing cleavable linker, implying that more hydrophilic linkers may drive improved ADC PK and prompting us to select sulfo-SPDB-DM4 as the lead L/P format. These findings are consistent with reports highlighting the importance of optimizing the biophysical properties of ADCs toward increased hydrophilicity for an improved therapeutic index (42).

To assess the potential therapeutic index, we conducted safety studies in both rats and nonhuman primates (Fig. 5). Both species feature patterns of normal tissue CDH6 expression comparable with those in humans with notable CDH6 positivity in renal proximal tubule epithelia and liver bile ducts. We did not observe evidence for on-target toxicities originating in these tissues. This might be explained by a combination of factors including insufficient levels of

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CDH6 expression (Fig. 1A) and limited target accessibility in these polarized epithelia (43), as well as the low proliferative index of these tissues (as measured by Ki67 stain; refs. 44, 45). In addition, both tissues perform active excretion/elimination functions with hepatobiliary excretion having been described as the dominant route of catabolite elimination for maytansinoid ADCs in general (46, 47). On the basis of these findings, we hypothesize that high-level intracellular exposure to maytansinoids in these cells over extended periods of time is unlikely and not significantly affected by additive target-dependent uptake of CDH6-ADC. Noteworthy dose-dependent toxicities were observed in the corneal epithelium and skin of NHPs. These findings were present at comparable frequency and severity in animals treated with control IgG1-sulfo-SPDB-DM4 and therefore classed as non–CDH6-related, platform toxicities representative of the DM4 ADC technology used. Corneal toxicities have been commonly observed as part of the clinical experience with ADCs containing microtubule-disrupting payloads and are considered translatable from monkeys to humans (40), whereas non–target-mediated skin toxicities with DM4-based ADCs have not been described clinically. The FOLR1-targeting ADC mirvetuximab soravtansine, which employs the same L/P as HKT288, has demonstrated efficacy in humans in the absence of corneal toxicity. Taking into account that similar dose levels (≤ 5 mg/kg single dose; refs. 32, 48, 49) result in antitumor activity preclinically for both ADCs in ovarian cancer models, we believe these dose levels are clinically relevant and together with our overall tolerability profile support projection of a positive therapeutic index for HKT288.

With the intent to more robustly project how patient tumor CDH6 expression patterns may relate to preclinical HKT288 activity, we assessed HKT288 in an unbiased PDX clinical trial comprising 30 individual PDX models replicating the heterogeneity of CDH6 expression observed in clinical specimens and evaluated CDH6 expression retrospectively using IHC (Fig. 7). Without preselecting models based on CDH6 expression, durable regressions were observed in 12 of 30 models, representing an overall response rate of 40%. Positive correlations between best average response and CDH6 expression as determined by both IHC and mRNA imply the activity in this overall cohort is target-dependent with CDH6 expression as an important determinant of HKT288 activity (Supplementary Fig. S7). Comparing IHC from PDX and primary human ovarian tumor samples, we see that a substantial proportion of patients with ovarian cancer feature tumor CDH6 expression patterns consistent with *in vivo* activity in PDX. These data highlight the significant benefit of the population-based PCT approach for gaining a deeper understanding of molecular correlates of response and translation into biomarker-based patient selection strategies. These results are consistent with data in renal cancer PDX models, furthermore supporting CDH6 expression as an important correlate of response to HKT288.

Considering that first-in-human studies will be conducted in patients progressing on standard-of-care treatment, it is encouraging to observe HKT288-mediated regression of a carboplatin/paclitaxel-refractory ovarian PDX model at both low and high levels of tumor burden. We are furthermore encouraged by recently reported synergies between cytotoxic ADCs and immunotherapies (50, 51). Although it remains to be explored whether these data translate to clinically meaningful benefit without cumulative toxicities, single-agent immune checkpoint inhibitor activity has been confirmed in both ovarian and renal cancers, although response rates are low (52, 53). These data provide a rationale for combining HKT288 with checkpoint inhibitors and evaluate whether the combination can positively affect the response pattern.

Together, our study introduces CDH6 as a promising antigen for biotherapeutic targeting and exemplifies a new concept for ADC drug discovery by integrating cellular assays with empirical *in vivo* candidate screening, multispecies toxicology assessments, a population-based PDX clinical trial, and mechanistic xenograft studies. These preclinical data highlight the potential benefit of HKT288 as a therapeutic option for patients with multiple cancer types of high unmet medical need. HKT288 is currently being evaluated in a phase I clinical trial in patients with ovarian and renal cancers.

METHODS

RNA Expression Analysis of TCGA Data

RNA-sequence reads from TCGA and GTEx were aligned to the Human B37 genome using the Omicsoft Sequence Aligner by the Omicsoft Corporation. Details are described in the Supplementary Materials.

Recombinant Proteins

Recombinant monomeric CDH6 ECDs from human, rat, mouse, and cynomolgus monkey were cloned upstream of a C-terminal affinity tag, sequence-verified, expressed in HEK293-derived cells, and purified using an anti-tag antibody. Further details on the recombinant proteins can be found in the Supplementary Materials.

ELISA

Maxisorp plates (Nunc) were coated with the appropriate recombinant protein and blocked with BSA before incubating with the relevant test antibody for 2 hours at room temperature. Plates were washed and a peroxidase-linked goat anti-human antibody was used in conjunction with a colorimetric substrate for detection (Pierce).

Cell Lines

NIH-OVCAR3 (OVCAR3; cultured in RPMI + 20% FBS + 10 μg/mL insulin) was obtained from the ATCC (#HTB-161) in 2007. OVCAR8 (RPMI+10% FBS) was obtained from the NCI/DCTD Tumor/Cell Line Repository in 2012. Cell lines were acquired, maintained, and authenticated by SNP fingerprinting (Sequenom) as previously described (54). To generate an isogenic cell line featuring CDH6 expression, OVCAR8 cells were transduced with a lentiviral construct driving expression of a human CDH6 cDNA (Genecopoeia). Stable CHO cell lines featuring exogenous expression of CDH6 from mouse, rat, cynomolgus, and human origin were generated by transfection of CHO-K1 cells (for mouse, rat cyno CDH6) or CHO-TREx cells (for inducible human CDH6; Invitrogen, 2011) with the respective cDNAs cloned into a mammalian expression vector (pcDNA6.1; for mouse, rat, cyno or pcDNA-TO for human CDH6; Invitrogen). For the inducible human CDH6 CHO line, expression was induced with 1 μg/mL tetracycline for 20 to 24 hours. Jurkat E6-1 cells (ATCC #TIB-152, 2016), grown in RPMI-1640 +

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10% FBS (Gibco), were transfected with an NFAT-luciferase reporter vector (Biomyx Technology) as well as a synthesized expression vector encoding the CD16a gene corresponding to human FcγRIII V158 variant (Geneart). NK3.3 (obtained from J. Kornbluth; ref. 55; 2011) were cultured in RPMI containing 10% FBS, 15 mmol/L HEPES, 1.2 ng/mL IL2, and 8.5 ng/mL IL10. NK3.3 cells (55) were cultured in RPMI containing 10% FBS, 15 mmol/L HEPES, 1.2 ng/mL IL2, and 8.5 ng/mL IL10.

Antibody Internalization Assay

Cell internalization of IgGs by target-mediated endocytosis was assessed by microscopy using a VTI ArrayScan HC reader (Thermo Fisher). Briefly, OVCAR3 cells were seeded into a 96-well microtiter plate with transparent bottom and incubated for 24 hours at 37°C with 5% CO_2 followed by automated microscopy analysis as described in detail in the Supplementary Materials.

Cellular Cytotoxicity Assays

SMCC-DM1 and (sulfo-)SPDB-DM4 conjugates at microscale were prepared as previously described (56) and profiled as outlined in detail in the Supplementary Materials.

Protein Crystallography

A co-complex of CDH6 EC5 bound to a Fab-fragment of LTV977 was crystallized, and diffraction data were collected at beamline 17-ID at the Advanced Photon Source (Argonne National Laboratory). For details on data processing and modeling, refer to the Supplementary Materials and Supplementary Table S1.

Animal Welfare

Mice were maintained and handled in accordance with the Novartis Institutes for BioMedical Research (NIBR) Animal Care and Use Committee protocols and regulations. For toxicology studies, all in-life procedures were conducted in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

PDX Models and PDX Clinical Trial

For the PCT, a 1 × 1 × 1 experimental format was utilized as previously described (41). Details on this methodology and additional information on xenograft, syngeneic models, and PK methods are described in the Supplementary Materials.

Disclosure of Potential Conflicts of Interest

C.U. Bialucha has ownership interest (including patents) in Novartis. R. Mosher has ownership interest (including patents) in Novartis. M.J. Meyer has ownership interest (including patents) in Novartis. J.E. Faris is a consultant/advisory board member for Merrimack and has given expert testimony for N-of-One Therapeutics. M.J. Janatpour has ownership interest (including patents) in Novartis. J.A. Engelman reports receiving commercial research support from Novartis and is a consultant/advisory board member for the same. W.R. Sellers has ownership interest (including patents) in Novartis. No potential conflicts of interest were disclosed by the other authors.

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CANCER DISCOVERY

Discovery and Optimization of HKT288, a Cadherin-6–Targeting ADC for the Treatment of Ovarian and Renal Cancers

Carl U. Bialucha, Scott D. Collins, Xiao Li, et al.

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HEMOSTASIS, THROMBOSIS, AND VASCULAR BIOLOGY

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Monoclonal antibodies directed to different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability

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Vascular endothelial cadherin (VE-cadherin) is an endothelial cell–specific cadherin that plays an important role in the control of vascular organization. Blocking VE-cadherin antibodies strongly inhibit angiogenesis, and inactivation of VE-cadherin gene causes embryonic lethality due to a lack of correct organization and remodeling of the vasculature. Hence, inhibitors of VE-cadherin adhesive properties may constitute a tool to prevent tumor neovascularization. In this paper, we tested different monoclonal antibodies (mAbs) directed to human VE-cadherin ectodomain for their functional activity. Three mAbs (Cad 5, BV6, BV9) were able to increase paracellular permeability, inhibit VE-cadherin reorganization, and block angiogenesis in vitro. These mAbs could also induce endothelial cell apoptosis in vitro. Two additional mAbs, TEA 1.31 and Hec 1.2, had an intermediate or undetectable activity, respectively, in these assays. Epitope mapping studies show that BV6, BV9, TEA 1.31, and Hec 1.2 bound to a recombinant fragment spanning the extracellular juxtamembrane domains EC3 through EC4. In contrast, Cad 5 bound to the aminoterminal domain EC1. By peptide scanning analysis and competition experiments, we defined the sequences TIDLRY located on EC3 and KVFRVDAETGDVFAI on EC1 as the binding domain of BV6 and Cad 5, respectively. Overall, these results support the concept that VE-cadherin plays a relevant role on human endothelial cell properties. Antibodies directed to the extracellular domains EC1 but also EC3-EC4 affect VE-cadherin adhesion and clustering and alter endothelial cell permeability, apoptosis, and vascular structure formation. (Blood. 2001;97:1679-1684)

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Introduction

Vascular endothelial cadherin (VE-cadherin) is an endothelial cell–specific cadherin that plays a major role in the organization of intercellular adherens junctions.[1] A null mutation of VE-cadherin gene induces embryonic lethality at day 9.5 to 10 of development due to a lack of assembly and remodeling of the vasculature.[2,3] In VE-cadherin$^{-/-}$ mice, endothelial cells undergo apoptosis and lose the capacity to respond to protective signals induced by vascular endothelial cell growth factor (VEGF).[2] Consistent with these observations, VE-cadherin blocking antibodies are able to inhibit angiogenesis in vitro[4,5] and tumor vascularization in vivo in mice (F. Liao, P. Bohlen, and D. J. Hicklin, unpublished observations, 2000).

The molecular basis of cadherin homophilic binding has not yet been fully elucidated. Cadherins are single-pass transmembrane glycoproteins that associate as cis-dimers on the cell surface and then combine to form a linear zipperlike structure that promotes homophilic intercellular adhesion.[6-12]

The extracellular region of classical cadherins is composed of 5 homologous domains (numbered EC1 to EC5).[13] Several studies are consistent with the concept that the aminoterminal region corresponding to domain EC1 of cadherins is responsible for homophilic recognition. Blocking monoclonal antibodies (mAbs) directed to E-, P-, and N-cadherin have been found to bind within this region.[14] Studies using multiple-point mutations and chimeric proteins showed that the aminoterminal 113 amino acid (AA) region is responsible for homophilic adhesive specificity between cadherins.[14]

The crystal structure of N-cadherin EC1 supported the idea that homophilic adhesion of antiparallel cis-dimers occurs through N-terminal domains.[10] In addition, recent studies brought evidence that cis-dimers may form through the interaction of EC1 and EC2.[15]

At variance with these reports, others suggest that the ectodomain of cadherins may present multiple adhesive contacts and that other regions may influence cadherin-adhesive properties.[16]

In this paper, we analyze 5 mAbs directed to the human VE-cadherin extracellular region for their activity on a set of in vitro biologic assays using human endothelial cells. We found that 3 mAbs have a significant effect on endothelial cell permeability, in vitro angiogenesis, and endothelial cell apoptosis. One of these mAbs, Cad 5, bound to a peptidic region contained in the extracellular domain EC1; and BV6 and BV9, bound in EC3 or

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EC3-EC4, respectively. These data support the idea that VE-cadherin sequence contains multiple domains able to modulate its adhesive and clustering properties. VE-cadherin blocking mAbs may be useful tools to study the role of endothelial cell junctions in permeability control and angiogenesis.

Materials and methods

Cell culture

Endothelial cells were harvested from human umbilical veins and cultured as previously described.[17]

Antibodies and reagents

Mouse mAbs against the extracellular domain of human VE-cadherin were as follows: clone TEA 1.31,[18,19] clone BV6,[18,19] clone BV9,[18,19] Cad 5 (Transduction Laboratories, Lexington, KY), and Hec 1.2.[20] The mAbs were used as purified immunoglobulin G (IgG). A concentration of 50 μg/mL of each mAb was selected as saturating concentration. This was tested at flow cytometry using endothelial cells in suspension incubated with increasing concentrations of the mAbs. A total of 50 μg/mL of each mAb gave maximal fluorescence intensity, and higher concentrations did not further increase this parameter.[21] A polyclonal antiserum (VECR1-5) was raised against a bacterially expressed entire extracellular region of human VE-cadherin and affinity-purified using the same immunogen coupled to CNBr-Sepharose (Pharmacia, Piscataway, NJ).

Rhodamine-conjugated secondary antibodies (reactive with either mouse or rabbit IgG) were purchased from Dakopatts (Glostrup, Denmark). Antihistidine mAb was purchased from Amersham-Pharmacia (Uppsala, Sweden).

Peroxidase-conjugated goat antimouse IgG antibody was used for immunoblotting (Jackson Immunoresearch Laboratories, West Grove, PA).

After sodium dodecyl sulfate–polyacrylamide gel electrophoresis and blotting, the biotynilated proteins were detected by streptavidin–horseradish peroxidase (Biospa Division, Milan, Italy).

Development of peroxidase activity was performed using an enhanced chemiluminescence kit (Amersham-Pharmacia).

Immunofluorescence staining

The procedure for immunofluorescence microscopy analysis of endothelial cell monolayer has been described previously.[17] Briefly, endothelial cell monolayers were cultured on fibronectin-coated glass coverslips, fixed, permeabilized, and then labeled with a VE-cadherin antibody followed by rhodamine-conjugated secondary antibody. Fluorescence was detected with a Zeiss Axiophot microscope and photographed using T-Max 3200 film.

Fluorescence flow cytometric analysis was performed by a FACStar Plus apparatus (Becton Dickinson, Mountain View, CA) using a fluorescein isothiocynate (FITC)–conjugated goat antimouse antiserum (Jackson ImmunoResearch Laboratories) as described in detail elsewhere.[21]

Calcium switch assay

Cells grown to confluency on glass coverslips as described previously[22] were incubated with 5 mM ethyleneglycotetraacetic acid (EGTA) at 37°C for 30 minutes. EGTA was then removed and Ca^{++} restored adding fresh medium either in the presence or in absence of mAbs (50 μg/mL). One hour later, cells were fixed and processed for immunofluorescence as described above.

Permeability assay

Permeability across endothelial cell monolayers was measured in Transwell units (with polycarbonate filter, 0.4 μm pore; Corning Costar, Cambridge, MA) as described previously.[18,23] Briefly, endothelial cells were cultured for 3 to 4 days to confluency, and then mAbs (50 μg/mL) were added in the upper compartment followed by the addition of FITC-dextran (1 mg/mL, average molecular mass 40 000; Sigma Chemical). At the indicated time points, 50 μL samples were taken from the lower compartment and the fluorescence was measured (492/520 nm, absorption/emission wavelengths).

In some experiments, EGTA (5 mM, final concentration) was added both to the lower and upper compartments for 30 minutes at 37°C. Then, as described above, EGTA was removed and Ca^{++} restored in the presence or absence of mAbs (50 μg/mL). FITC-dextran was added 1 hour after addition of the mAbs, and aliquots were collected from the lower compartment at the indicated time points and assayed by fluorimetry.

Expression and purification of VE-cadherin recombinant fragments

VE1, VE2, VE3, and VE4 fragments, corresponding to the 1 to 486, 1 to 320, 281 to 486, and 48 to 218 AA residues, respectively, of human VE-cadherin (Figure 5) were cloned by polymerase chain reaction amplification. Complementary DNAs were subcloned into the bacterial pQE-32 expression vector (Qiagen, Germany) to produce HIS-tagged VE-cadherin fragments. The fragments were expressed and purified according to the published Qiagen protocol.

The same fragments were also expressed using the TNT Quick Coupled Transcription/Translation System (Promega, Madison, WI). Using this system, biotinylated lysine residues are incorporated into nascent protein during translation.

In few experiments (Figure 6), VE1 fragment was produced as a chimeric protein fused to the Fc portion of human IgG1. VE1-Ig chimera was constructed by cloning the complementary DNA encoding the extracellular portion corresponding to the 1-to-486 AA into the pIg1 eukaryotic expression vector[24] using a previously described method[24,25] for the expression and purification steps.

Epitope mapping

Peptide scanning was performed following the previously described procedure.[26] Peptides were chemically synthesized as arrays of N-terminally acetylated and C-terminally covalently immobilized products on cellulose sheets derivatized with amino–polyethylene glycol anchors by the SPOT synthesis technique using a model ASP222 spotting robot (Abimed Analysen-Technik, Langenfeld, Germany). Peptide arrays included overlapping pentadecapeptides, with an offset of 3 AA residues, spanning the entire extracellular domain of VE-cadherin. Binding of mAbs to peptide spots was detected using a goat antimouse IgG peroxidase-conjugated antibody and followed by enhanced chemiluminescence detection system.

Peptide competition experiments were described elsewhere.[24] Briefly, enzyme-linked immunosorbent assay (ELISA) plates were coated with recombinant VE1-Ig chimera (1 μg/mL) and then blocked with 2% bovine serum albumin. BV6 and BV9 mAbs (0.5 μg/mL) were preincubated for 1 hour with different peptides (range 1-500 μM) and were then added to coated plates, followed by peroxidase-conjugated goat antimouse IgG (Jackson ImmunoResearch Laboratories).

Apoptosis

To induce apoptosis, endothelial cells were seeded at 2×10^5 cells in 24-well dishes in 1 mL serum-free MCDB-131 medium (Life Technologies, Paisley, United Kingdom) supplemented with 1% bovine serum albumin and insulin-transferrin-selenium (Life Technologies). Cells were cultured for 72 hours either in the absence or in the presence of VEGF-A (R&D Systems, Minneapolis, MN), 30 ng/mL. Antibodies to VE-cadherin (50 μg/mL) were added 24 hours after seeding and daily thereafter. Apoptosis was quantitated by measuring DNA fragmentation (TUNEL detection method, Boehringer Mannheim, Germany).[2]

Capillary tube assay

Capillary tube formation was assessed as previously described.[4,27]

Briefly, human fibrinogen (5 mg/mL solution in distilled water, Sigma code F4883) was dialyzed against triethanolamine-buffered saline (10 mM Tris-HCl, pH 7.4; 150 mM NaCl) to eliminate sodium citrate and stored in aliquots at −20°C. To make the underlying fibrin gel, 500 μL fibrinogen solution (3 mg/mL in triethanolamine-buffered saline with one-tenth volume 10 × minimal essential medium, pH 8.5) was placed into each 24-well culture plate, and human thrombin (Sigma, code T6884) was added to a final concentration of 3 U/mL. Endothelial cells (2.5×10^5/mL per well) were seeded on the top of the polymerized gel and cultured for 24 hours in medium 199 (Life Technologies) with 20% newborn calf

serum (Life Technologies) containing endothelial cell growth supplement (50 μg/mL) and VEGF (30 ng/mL). Monclonal antibodies (50 μg/mL) were added for 30 minutes when indicated. The medium was then aspirated and another fibrin gel overlying the monolayer was prepared including mAbs (50 μg/mL) in the fibrinogen solution before clotting, as described.[4] Fresh medium containing the same concentration of mAbs was added over the gel, and the formation of tubes was followed by phase contrast microscopy.

Results

Effects of VE-cadherin mAbs on adherens junction organization and permeability

As shown in Figure 1A, mAbs BV6, BV9, and Cad-5 induced redistribution of VE-cadherin from intercellular junctions, while Hec 1.2 and TEA were inactive. Previous work[28] showed that VE-cadherin disappearance from junctions was due to diffusion of the molecule on the cell membrane and not to its internalization. Paracellular permeability of endothelial cell monolayers to FITC-dextran was significantly increased by mAbs Cad 5, BV9, and BV6 (Figure 1B). The time course of the effect showed a detectable increase in permeability within 1 hour after the addition of the mAbs, with maximal permeability reached at 5 hours. In contrast, mAb TEA 1.31 showed only a slight effect on permeability, and Hec 1.2 had no effect.

A saturating concentration of the mAbs (50 μg/mL) was used in all the assays. Higher concentrations did not produce a more marked effect (not shown).

The mAbs were further studied in a Ca^{++} switch assay. Endothelial cells were exposed to EGTA for 30 minutes to fully disrupt VE-cadherin clustering at cell-to-cell contacts[28] (Figure 2A). Calcium concentration was then restored, and the capacity of the cells to reorganize VE-cadherin at junctions was examined in the absence or presence of mAbs. As shown in Figure 2A, Hec 1.2 and TEA 1.31 did not prevent VE-cadherin reclustering, while the presence of BV9, Cad 5 and, to a lower extent, BV6 inhibited a full reorganization of intercellular adherens junctions.

This effect was quantified by measuring FITC-dextran passage through endothelial monolayers after Ca^{++} restoration (Figure 2B). The presence of BV6, BV9, or Cad 5 prevented a full reestablishment of paracellular barrier function. TEA 1.31 had a moderate but detectable effect, while Hec 1.2 had no activity.

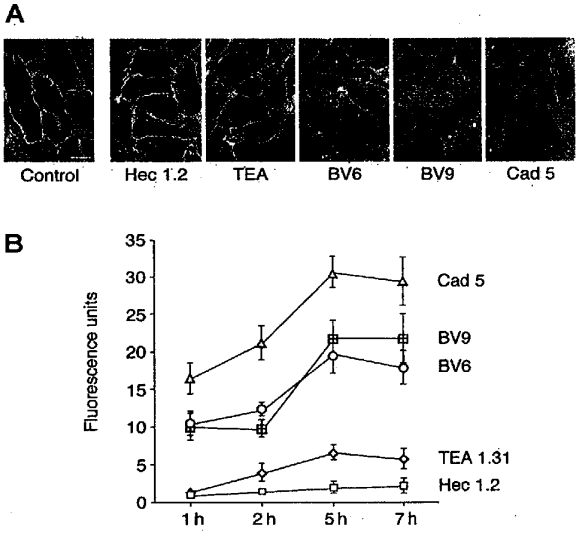


Figure 1. Antibodies directed to VE-cadherin increase endothelial permeability. (A) Endothelial cells grown to confluency on glass coverslips were exposed to mAbs (50 μg/mL). Seven hours later, cells were fixed and processed for immunofluorescence analysis of VE-cadherin distribution (bar = 20 μm). (B) FITC-dextran passage through endothelial cell monolayers on Transwell filters was measured at different times after incubation with VE-cadherin mAbs. The mAb code is on the right. The background permeability values measured in absence of the mAbs was subtracted from each point of the curves. Values are mean ± SEM of at least 4 replicates of a typical experiments out of 5 performed. The permeability values obtained with Cad 5, BV6, and BV9 were always significantly different ($P < .01$) from the values obtained with Hec 1.2 by analysis of variance and the Duncan test. The permeability values obtained with TEA 1.31 were significantly different ($P < .05$) from Hec 1.2 only at 5 hours.

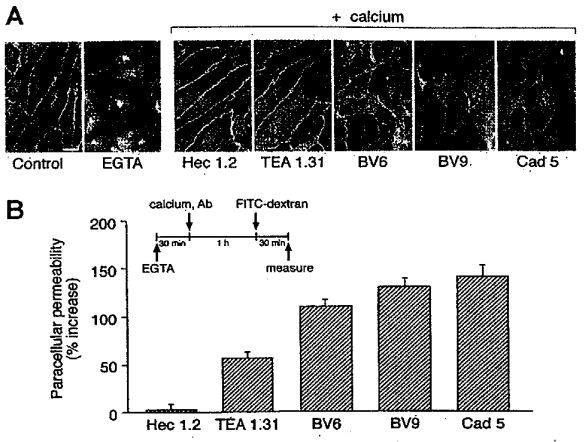


Figure 2. Antibodies to VE-cadherin inhibit VE-cadherin clustering after Ca^{++} switch. (A) Endothelial cell monolayers were exposed to 5 mM EGTA for 30 minutes. EGTA was then removed and Ca^{++} restored adding fresh culture medium either in the presence or in absence of mAbs. One hour later, cells were fixed and processed for immunofluorescence microscopy. The presence of VE-cadherin at junctions was detected by adding a polyclonal antibody VECR1-5. EGTA induced disappearance of VE-cadherin from cell-to-cell contacts. Addition of Ca^{++} restored junctional localizaton of VE-cadherin in cells cultured in absence of mAbs (not shown) or in the presence of Hec 1.2 or TEA 1.31. VE-cadherin remained in large part outside the intercellular junctions when cells were cultured in the presence of BV6, BV9, and Cad 5 (bar = 20 μm). (B) Endothelial cells grown on Transwell filters were incubated with EGTA for 30 minutes. To allow junction reorganization, Ca^{++} was then restored in the presence or absence of mAbs (see above). FITC-dextran was added 1 hour after Ca^{++} restoration, and permeability was measured 30 minutes later. Data are expressed as percentage increase in permeability in comparison to cells maintained in the absence of mAbs and are means ± SEM of 4 replicates from a typical experiment out of 3 performed.

Effect of VE-cadherin mAbs on endothelial cell apoptosis

We recently reported that blocking VE-cadherin mAbs prevented the protective effect of VEGF on endothelial cell apoptosis induced by the absence of serum.[2]

As reported in Figure 3, in the presence of VEGF, BV6, BV9, and Cad 5 strongly increased endothelial cell apoptosis. TEA 1.31 showed a poor but still measurable activity, while Hec 1.2 was inactive.

Effect of VE-cadherin mAbs on the formation of vascularlike structures in vitro

In fibrin gels, Cad 5, BV6, and BV9 blocked cord formation, and TEA 1.31 and Hec 1.2 did not induce any detectable change in comparison to control cultures (Figure 4). In this assay, the cells were kept in the presence of serum (see "Materials and methods") to reduce apoptosis induced by the mAbs (Figure 3). Within 48 hours, apoptosis induced by addition of BV6 or BV9 was still low and not significantly increased in this system (not shown).

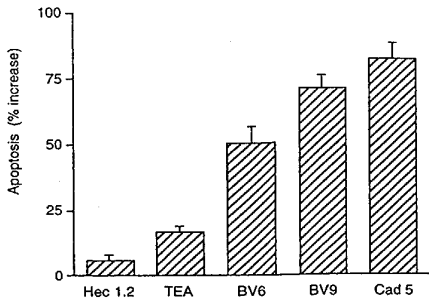


Figure 3. Monoclonal antibodies directed to VE-cadherin prevent the protective effect of VEGF on endothelial cell apoptosis. Endothelial cells were maintained without serum in the absence or presence of mAbs for 48 hours; 63% ± 7% control cells underwent apoptosis, and this percentage was not significantly modified by addition of the mAbs (not shown). When VEGF (30 ng/mL) was included in the absence of mAbs, apoptotic endothelial cells decreased to 31% ± 2%. Addition of the mAbs together with VEGF increased the apoptotic index. The values in the figure are expressed as percentage increase in apoptosis in comparison to cells cultured in absence of the mAbs and in the presence of VEGF. Data are means ± SEM of 4 replicates from a typical experiment out of 4 performed.

Identification of binding epitope of VE-cadherin mAbs

To identify the epitope of VE-cadherin mAbs, recombinant fragments of the protein were produced (Figure 5A). By Western blot and immunoprecipitation analysis (Figure 5B,C), TEA 1.31, BV6, and BV9 recognized a VE-cadherin fragment containing EC3 and EC4 (fragment VE3) but not EC1 and EC2 (fragments VE2 and VE4). In contrast, Cad 5 mAb bound only to EC1 and EC2 (VE2, VE4) and not to EC3 and EC4 (VE3) (Figure 5A). The boundaries among domains 1 to 5 reported in Figure 5A derive from alignment of the VE-cadherin AA sequence with that of other classic cadherins (E-, N-, and P-cadherins) as reported previously[29] and in a review by Yagi and Takeichi.[13]

To identify more precisely the epitopes of the mAbs, we studied their capacity to bind different peptidic sequences spanning the extracellular domain of VE-cadherin. As reported in Figure 6A, BV6 bound to 4 peptides sharing the same 6-AA sequence Thr-Ile-Asp-Leu-Arg-Tyr (TIDLRY). This sequence spans 343 to 348 AA of domain EC3 of VE-cadherin ectodomain.

Cad 5 bound to KVFRVDAETGDVFAI. This mAb was raised against a fragment spanning 26 to 194 AA of VE-cadherin EC1, and the identified peptidic epitope is contained in this sequence.

To confirm BV6 binding specificity for TIDLRY, 2 peptides containing this sequence and a scrambled peptide were tested in competition experiments. As reported in Figure 6Bi, TIDLRY-

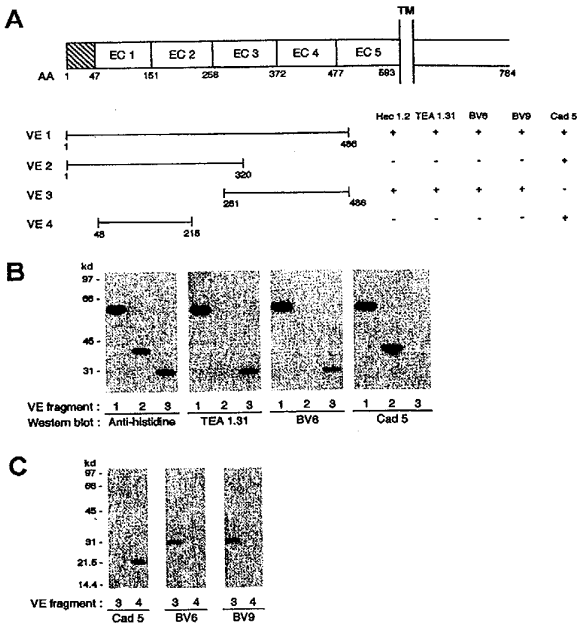


Figure 5. Different mAbs bind to different regions of VE-cadherin. (A) Schematic representation of VE-cadherin fragments spanning different regions of the protein ectodomain and the mAb binding domain. TM indicates transmembrane domain; EC, extracellular domain. (B) Western blot analysis of mAbs binding to VE-cadherin fragments produced in *Escherichia coli*. Antihistidine antibody recognized the 3 fragments VE1, VE2, and VE3, which migrated at molecular weights of 60, 40, and 30 kd, respectively. Monoclonal antibodies TEA 1.31 and BV6 bound to VE1 and VE3 but not VE2. mAb Cad 5 bound to VE1 and VE2. (C) Immunoprecipitation analysis of VE3 and VE4 obtained by protein synthesis in vitro. Biotinylated lysine residues are incorporated into nascent proteins during translation. Fragments VE3 and VE4 were immunoprecipitated with Cad 5, BV6, and BV9, and after sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotting, the biotinylated proteins were visualized by streptavidin–horseradish peroxidase. Cad 5 bound to VE4 while BV6 and BV9 bound to VE3 only.

containing peptides but not the scrambled peptide were able to block the binding of BV6 to a recombinant VE1-Ig chimera. Different concentrations of the peptides were used ranging from 1 to 500 μM, and the calculated inhibitory concentration of 50% for TIDLRY peptides was 12.6 to 13.4 μM.

The scrambled peptide did not show dose-dependent competition for BV6 binding. The 3 peptides were inactive on BV9 binding up to 500 μM.

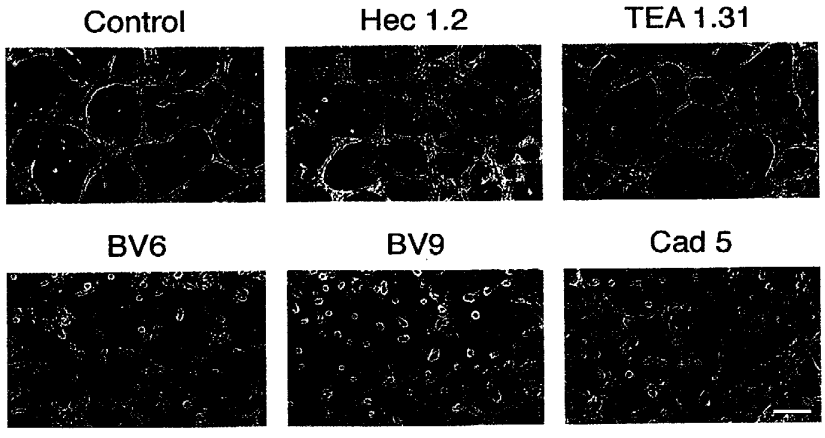


Figure 4. Monoclonal antibodies to VE-cadherin inhibit the organization of vascularlike structures in endothelial cells. Endothelial cell monolayers were cultured in a 3D fibrin gel (see "Materials and methods"). After 48 hours, cells reorganize in cords and tubes. In the presence of mAbs to VE-cadherin BV6, BV9, and Cad 5 (50 μg/mL), the formation of the cord network was blocked. Monoclonal antibodies TEA 1.31 or Hec 1.2 (50 μg/mL) did not change the reorganization pattern as compared with control (bar = 30 μm).

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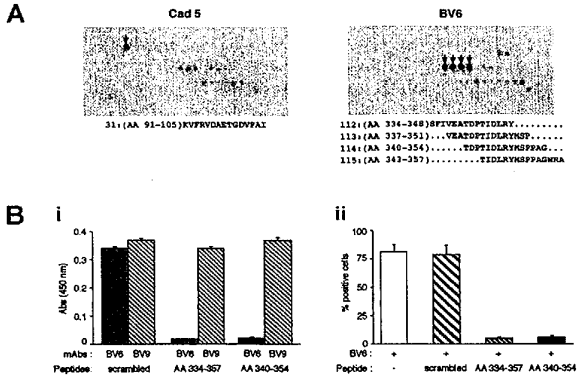


Figure 6. Identification of the peptidic sequence recognized by BV6. (A) Peptide scan of VE-cadherin extracellular region. Peptides of 15 AA spanning the entire extracellular domain of the protein were synthesized and tested for mAb recognition (arrows indicate the peptide spots recognized).[41] mAb Cad 5 bound to peptide 31: KVFRVDAETGDVFAI; mAb BV6 bound to peptides 112 to 115 containing the sequence TIDLRY. (B) The peptides containing TIDLRY corresponding to VE-cadherin sequences AA 334 to 357 and AA 340 to 354 and the scrambled peptide (APRTDIAEINDGYPVTSMRFPALS) were tested in competition ELISA experiments. (Bi) The binding of soluble BV6 and BV9 (0.5 μg/mL) to purified VE-cadherin recombinant fragment (VE1-Ig, 1 μg/mL) coated on plastic was tested in the presence or absence of the peptides (200 μM). TIDLRY peptides blocked BV6 but not BV9 binding. (Bii) Flow cytometric analysis of BV6 binding to endothelial cells expressing VE-cadherin. Peptides containing TIDLRY sequence but not scrambled peptides blocked the binding of BV6.

Besides purified recombinant fragments, TIDLRY-containing peptides blocked BV6 binding to native VE-cadherin, ie, expressed on cultured endothelial cells measured by flow cytometry analysis (Figure 6Bii).

The TIDLRY peptides or the scrambled peptide (up to a concentration of 500 μM) did not change paracellular permeability of endothelial monolayers (measured as in Figure 1) (data not shown), and they did not prevent VE-cadherin reassembly at intercellular contacts after Ca^{++} switch (measured as in Figure 2) (data not shown).

A general summary of the results is reported in Table 1.

Discussion

VE-cadherin has multiple functions in endothelial cells.[1,2] Different blocking antibodies may interfere with these activities to a different extent, and this may be related to the specific region of the molecule to which they bind.

In this paper, we first compared 5 available mAbs directed to the extracellular region of VE-cadherin for their effect on several endothelial cell biologic tests.

We found that 3 mAbs (Cad 5, BV9, and BV6) were significantly active in all the assays, while the 2 remaining mAbs, TEA 1.31 and Hec 1.2, showed moderate or no activity, respectively.

Comparing the effect of the mAbs on VE-cadherin clustering (Figures 1A, 2A), the most active (Cad 5, BV9, and BV6) were also those more effective in increasing permeability (Figures 1B and 2B). This was true measuring disruption of preorganized junctions (Figure 1) or measuring the ability of the mAbs to prevent the reorganization of EGTA-disrupted VE-cadherin clusters (Figure 2).

TEA 1.31 is an exception because it did not visibly inhibit VE-cadherin localization at junctions in both conditions (Figures 1A and 2A) yet slightly increased paracellular permeability. Possibly, immunofluorescence microscopy does not detect subtle changes in VE-cadherin clustering and organization, which may, however, lead to a significant increase in permeability to high molecular weight solutes. Also, in other conditions, as upon histamine activation of endothelial cells, a marked increase in paracellular permeability could be observed without major changes in VE-cadherin distribution at junctions.[23]

TEA 1.31 showed a slightly more marked effect on permeability when the cells were pretreated with EGTA. This means that this mAb is more effective in preventing correct homophilic interactions of VE-cadherin molecules during the organization of the cluster than in disrupting preconstituted junctional structures.

The mechanism of action of VE-cadherin in promoting angiogenesis and vasculogenesis in the embryo was explained in part by the capacity of this molecule to protect endothelial cells from apoptosis.[2] It was therefore important to test whether the mAbs could inhibit VE-cadherin antiapoptotic effect in human cells. We found that mAbs BV6, BV9, and Cad 5 were indeed able to prevent the protective effect of VEGF on apoptosis.

The mAbs also inhibited endothelial tube formation in fibrin gels. The effect of the mAbs may be due to inhibition of VE-cadherin adhesive properties but also to induction of apoptosis. The presence of serum in this assay significantly reduced the susceptibility of the cells to apoptosis, and BV6 and BV9 did not increase significantly this parameter. This suggests that the effect observed in this particular assay is mostly due to the block of VE-cadherin–mediated adhesion.

Others found that a VE-cadherin blocking mAb (Cad 5) was able to inhibit tube formation in a fibrin gel, and they brought direct evidence for VE-cadherin binding to fibrin.[4,30] It is possible that the block of this interaction leads to a lack of a correct anchorage of the cells to the matrix, causing disruption of vascularlike structures.

In the attempt to define the molecular basis of mAb activity, we analyzed their binding to different recombinant fragments of the protein. The mAbs TEA 1.31, Hec 1.2, BV6, and BV9 bound to a membrane domain of VE-cadherin spanning EC3 and EC4. Peptide scanning analysis showed that BV6 recognizes the peptidic sequence TIDLRY on EC3. In contrast, Cad 5 mAb bound to the fragment that includes EC1 and, by peptide scan, it recognizes a peptide contained in this region.

We have been unable to precisely define the epitope of the other blocking mAb BV9 by peptide scanning. A possibility is that BV9 binds to a conformational epitope that may be lost in short peptides of VE-cadherin. Competition experiments with peptides containing TIDLRY showed that BV9 binding to VE-cadherin was unaffected, indicating that it recognizes a different domain.

In general, the mechanisms underlying homophilic binding functions of cadherins are poorly understood.

The precise mechanism through which BV9 and BV6 binding to EC3-EC4 influences VE-cadherin homophilic adhesion is still unknown, but different possibilities may be considered.

Monoclonal antibody binding may change VE-cadherin conformation and, as a consequence, the availability of the aminoterminal region for homophilic binding. Conformational changes of other

Table 1. The effects of different VE-cadherin antibodies on endothelial cell activities

mAbs	Permeability	Ca^{++} switch	Apoptosis	Tube formation	Domain
Cad 5	+++	+++	+++	+++	EC1
BV9	++	+++	+++	+++	EC3-EC4
BV6	++	++	++	+++	EC3
TEA 1.31	+	+	+	–	EC3-EC4
Hec 1.2	–	–	–	–	EC3-EC4

– indicates no effect; +, barely detectable; ++, intermediate; +++, strong activity. For the actual data see the corresponding figures.

adhesive molecules by antibody/ligand binding were reported to induce activation or inhibition of their adhesive properties.[31-37]

BV6 or BV9 may also affect VE-cadherin association in cis by steric hindrance and alter the formation of the zipper structure promoting cell-to-cell recognition and binding. Indeed, data obtained in the Ca^{++} switch assay indicate that BV6 and BV9 may inhibit the reassembly of VE-cadherin at intercellular junctions.

Another interesting possibility comes from the recent paper of Sivasankar et al.[16] This work brings evidence that C-cadherin presents multiple adhesive contacts along the extracellular domain for the interdigitated antiparallel proteins. The block of one of these sites may affect the strength of recognition of cadherin molecules and facilitate the junctional rupture.

Other data in the literature may be accommodated in this last picture. The mAb DECMA directed to E-cadherin[38,39] or a mAb to chicken N-cadherin, which are strong inhibitors of intercellular adhesion,[40] both bind to regions outside EC1 and EC2 and closer to the cell membrane.

It is possible that EC1 interacts with EC3-EC4 and depending on their orientation form dual or multimeric aggregates. Thus, antibodies directed against either one of these epitopes may inhibit VE-cadherin–adhesive properties. Binding may involve a 2-stage process of initial docking or alignment of the 2 molecules along their length followed by specific engagement of the binding sites in domain EC1 and EC3-4.

In conclusion, inhibition of VE-cadherin adhesion properties by blocking antibodies affects several endothelial cell–specific functions, which may include the maintenance of barrier properties or formation of vascular structures in vitro. Interestingly, antibodies directed to different epitopes of the molecules present a comparable inhibitory effect, suggesting that the protein has multiple biologically important domains.

Acknowledgment

We thank A. Tiepold for help in the SPOT peptide synthesis.

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Date
19.01.21

Reference	Application No./Patent No.
PN833578EP	18802536.5 - 1111 / 3626825 PCT/JP2018018572
Applicant/Proprietor	
Daiichi Sankyo Company, Limited	

Communication

The extended European search report is enclosed.

The extended European search report includes, pursuant to Rule 62 EPC, the supplementary European search report (Art. 153(7) EPC) and the European search opinion.

Copies of documents cited in the European search report are attached.

☑ 0 additional set(s) of copies of such documents is (are) enclosed as well.

Refund of the search fee
If applicable under Article 9 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.

Should you wish to further prosecute this application in the examination phase, your attention is drawn to the provisions of Rule 70a EPC. An invitation to respond to the extended European search report will be issued shortly (R. 70(2) EPC).

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SUPPLEMENTARY EUROPEAN SEARCH REPORT

Application Number
EP 18 80 2536

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
Y	CORADA M ET AL: "Monoclonal antibodies directed to different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability", BLOOD, THE AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 97, no. 6, 15 March 2001 (2001-03-15), pages 1679-1684, XP002187985, ISSN: 0006-4971, DOI: 10.1182/BLOOD.V97.6.1679 * the whole document * -----	1-41	INV. C12N15/13 A61K9/00 A61K31/4745 A61K39/395 A61K47/68 A61P13/12 A61P15/08 A61P35/00 A61P43/00 C07K16/28 C12N1/15 C12N1/19 C12N1/21 C12N5/10 C12P21/08
Y,P	CARL U. BIALUCHA ET AL: "Discovery and Optimization of HKT288, a Cadherin-6-Targeting ADC for the Treatment of Ovarian and Renal Cancers", CANCER DISCOVERY, vol. 7, no. 9, 1 September 2017 (2017-09-01), pages 1030-1045, XP055484340, US ISSN: 2159-8274, DOI: 10.1158/2159-8290.CD-16-1414 * the whole document * -----	1-41	
E	WO 2018/185618 A1 (NOVARTIS AG [CH]) 11 October 2018 (2018-10-11) * the whole document * -----	1-41	

TECHNICAL FIELDS SEARCHED (IPC)

C07K
A61K
A61P
C12N
C12P

The supplementary search report has been based on the last set of claims valid and available at the start of the search.

Place of search	Date of completion of the search	Examiner
The Hague	11 January 2021	Hix, Rebecca

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

1

EPO FORM 1503 03.82 (P04C04)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 18 80 2536

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
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11-01-2021

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018185618 A1	11-10-2018	NONE	

Information on Search Strategy - Pilot phase (see OJ 2015, A86)
The type of information contained in this sheet may change during the pilot for improving the usefulness of this new service.

Application Number
EP 18 80 2536

TITLE: ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE

APPLICANT: Daiichi Sankyo Company, Limited

IPC CLASSIFICATION: C12N15/13, A61K9/00, A61K31/4745, A61K39/395, A61K47/68, A61P13/12, A61P15/08, A61P35/00, A61P43/00, C07K16/28, C12N1/15, C12N1/19, C12N1/21, C12N5/10, C12P21/08

EXAMINER: Hix, Rebecca

CONSULTED DATABASES: NPL, WPI

CLASSIFICATION SYMBOLS DEFINING EXTENT OF THE SEARCH:

IPC:

CPC:

FI/F-TERMS:

KEYWORDS OR OTHER ELEMENTS FEATURING THE INVENTION:
anti-Cadherin-6 (CDH6) antibody

EPO FORM P04A42

Datum / Date / Date	cf Form 1507	Blatt / Sheet / Feuille	1	Anmelde-Nr: / Application No: / Demande n°:	18 802 536.5

The examination is being carried out on the **following application documents**

Description, Pages

1-206 filed with entry into the regional phase before the EPO

Sequence listings, SEQ ID NO

1-86 filed with entry into the regional phase before the EPO

Claims, Numbers

1-41 filed in electronic form on 28-01-2020

Drawings, Sheets

1/29-29/29 filed with entry into the regional phase before the EPO

1 Observations are hereby presented which take into account the International Preliminary report on patentability (IPER) and the Applicants letter dated 28.01.2020 (L1).

2 **CORRECTIONS** (Rule 139 EPC)

2.1 With L1, the Applicant requests correction of pages 52, 145, 176 and 181. The corrections are acceptable according to Rule 139 EPC.

3 The following **documents** are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1 CORADA M ET AL: "Monoclonal antibodies directed to different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability",
BLOOD, THE AMERICAN SOCIETY OF HEMATOLOGY, US,
vol. 97, no. 6, 15 March 2001 (2001-03-15), pages 1679-1684, XP002187985,
ISSN: 0006-4971, DOI: 10.1182/BLOOD.V97.6.1679

Datum / Date / Date	cf Form 1507	Blatt / Sheet / Feuille	2	Anmelde-Nr: / Application No: / Demande n°:	18 802 536.5

D2 CARL U. BIALUCHA ET AL: "Discovery and Optimization of HKT288, a Cadherin-6-Targeting ADC for the Treatment of Ovarian and Renal Cancers",
CANCER DISCOVERY,
vol. 7, no. 9, 1 September 2017 (2017-09-01), pages 1030-1045, XP055484340,
US
ISSN: 2159-8274, DOI: 10.1158/2159-8290.CD-16-1414

D3 WO 2018/185618 A1 (NOVARTIS AG [CH]) 11 October 2018 (2018-10-11)

From the International search report:

D4 WO 2016/024195 A1 (NOVARTIS AG) 18 February 2016 (2016-02-18)

4 NOVELTY (Art. 54 EPC)

4.1 D1 discloses monoclonal antibodies directed to different regions of vascular endothelial Cadherin extracellular domain are shown to affect adhesion and clustering of the protein and modulate endothelial permeability. Three mAbs (Cad 5, BV6, BV9) were able to increase paracellular permeability, inhibit VE-cadherin reorganization, and block angiogenesis in vitro. These mAbs could also induce endothelial cell apoptosis in vitro. Epitope mapping studies show that some of the antibodies bound to a recombinant fragment spanning the extracellular juxtamembrane domains EC3 through EC4. By peptide scanning analysis and competition experiments, the sequences TIDLRY located on EC3 was identified.

4.2 D2 describes the identification of the cell–cell adhesion molecule cadherin-6 (CDH6) as a lineage gene having significant differential expression in ovarian and kidney cancers. HKT288 is an optimized CDH6-targeting DM4-based antibody–drug conjugate (ADC) developed for the treatment of these diseases.

4.3 D3 is published on 11.10.2018, claims the priority date of 03.04.2017. D3 discloses antibody drug conjugates comprising anti-CDH6 antibodies conjugates for use in the treatment of tumors.

4.4 D4 discloses anti-CDH6 antibodies, antibody fragments, antibody drug conjugates, and their uses for the treatment of cancer. The anti-CDH6 antibody NOV0712 disclosed in D4 is used as the comparative antibodies in the examples of the present application. The antibody-drug conjugate NOV0712-SPDB-DM4 elicited the greatest antitumor effect with regression 70 days post dose. NOV0710-SPDB-DM4 was the second most active anti-CDH6-SPDB-DM4, D4, Figure 15 and paragraph [00436].

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5 Priority

5.1 Since D2 is published on 01.09.2017, therefore before the priority date and the publication date of the present application and D3 is a potentially conflicting document under Article 54(3) EPC, the validity of the presently claimed priority should be assessed.

5.2 The present application claims a priority based on JP 2017-096749 dated 15.05.2017. At present the priority document is provided in Japanese. In order for the validity of the priority to claim to be assessed the Applicant is kindly requested to provide a translation of the said priority document.

5.3 Claim 1 defines in the antibody solely in terms of functional features of specifically binding to the amino acid sequence shown in SEQ ID NO: 4 and having internalization ability that permits cellular uptake, or a functional fragment of the antibody, without clearly and unambiguously defining the antibodies of the present application in terms of their technical, structural features.

5.4 Since the anti-CDH6 antibodies disclosed in D4 share the functional features defined in present independent claim 1 and discloses the features of claims 5, 13, 14, 18 to 20 and includes the same modifications and antibody-drug conjugates as claims 21 to 29, 32 and 33 and method of production according to claim 41 and the generally defined pharmaceutical compositions of claims 34 to 40 the subject-matter of said claims is not novel according to Article 54 EPC.

5.5 The subject-matter of claims 2 to 4, 6 to 12, 15 to 17, 30 and 31 is novel as the prior art does not disclose antibodies comprising the variable light and heavy chains or the CDR sequences having the amino acid sequences defined according to the SEQ ID NOs provided in said claims.

5.6 The present application does not meet the requirements of Article 52 (1) EPC because the subject-matter of claims 1, 5, 13, 14, 18 to 29, 32 to 40 is not new in the sense of Article 54 (1) and (2) EPC.

5.7 In view of the prior art cited, claims 2 to 4, 6 to 12, 15 to 17, 30 and 31 appear to be novel and meet therefore the requirements of Art. 54 EPC.

6 INVENTIVE STEP (Art. 56 EPC)

6.1 Document D4 is considered to represent the most relevant state of the art.

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6.2 In the light of the difference defined above, the effect is shown in the present application with respect to the four humanized anti-CDH6 antibodies of the present application as shown with their dissociation constant (KD) values in Table 2, page 175:

Antibody H01L02, VH amino acid SEQ ID NO: 69, VL amino acid SEQ ID NO: 61,

Antibody H02L02, VH amino acid SEQ ID NO: 73, VL amino acid SEQ ID NO: 61,

Antibody H02L03, VH amino acid SEQ ID NO: 73, VL amino acid SEQ ID NO: 65

Antibody H04L02, VH amino acid SEQ ID NO: 77, VL amino acid SEQ ID NO: 61.

6.3 Paragraph [0279] reports that the four humanized hG019 antibodies (clone Nos: H01L02, H02L02, H02L03 and H04L02) **bind to human CDH6 and cynomolgus monkey CDH6, but bind to neither mouse nor rat CDH6**. None of the antibodies bind to the cells transfected with the empty vector pcDNA3.1 as a negative control. Compared with the D4 antibody NOV0712 which exhibits **binding activity against all of human CDH6, cynomolgus monkey CDH6, mouse CDH6, and rat CDH6**.

6.4 Paragraph [0280] reports that the four humanized hG019antibodies (clone Nos: H01L02, H02L02, H02L03 and H04L02) bind to the full-length hCDH6, the EC1 deletion mutant,the EC2 deletion mutant, the EC4 deletion mutant, and the EC5 deletion mutant, **but do not bind to the EC3 deletion mutant**. Specifically, it was demonstrated that the 4 humanized hG019 antibodies **specifically bind to hCDH6 with EC3 as an epitope**. The anti-CDH6 antibody NOV0712 from D4 binds to the full-length hCDH6, the EC1deletion mutant, the EC2 deletion mutant, the EC3 deletion mutant, and the EC4 deletion mutant, but does not bind to the EC5 deletion mutant. Specifically, it was demonstrated that the anti-CDH6 antibody **NOV0712 specifically binds to hCDH6 with EC5 as an epitope**.

6.5 Consequently the anti-CDH6 antibodies disclosed in D4 and the present application bind specifically to different epitopes; EC5 and EC3, respectively, a supported by paragraph [0281] and the results shown in Figure 9. D4 antibody NOV0712 and the antibodies of the present application differ in epitope and thus do not compete with each other for binding.

6.6 Paragraph [0283] reports that the 4 humanized hG019 antibodies have an internalization rate of approximately 50 to 75% predicted from the cell survival rates for all of the 3 cell lines. Thus, the 4 humanized hG019 antibodies exhibit **very high internalization activity and exhibit much higher internalization activity** than that of NOV0712.

6.7 Example 8 and paragraph [0320] demonstrates that the 4 humanized hG019-drug conjugates **exhibit growth inhibition activity against tumor cells from a lower addition concentration** than that of the NOV0712-drug conjugate, and have high antitumor activity.

6.8 Example 9 and paragraph [0321] reports that the antibody drug conjugate from D4 NOV0712-DM4 exhibited no antitumor effect at any of the doses of 1 and 3 mg/kg in this tumor model. The antibody drug conjugate of the present application **H01L02-DXd significantly decreased tumor volume after administration at both the doses of 1 and 3mg/kg and exerted a tumor regression effect (Figure 13)**. The H01L02 antibody obtained in the present description and the NOV0712 antibody were conjugated to the same drug DXd, and the medicinal effects of the resulting samples were compared. As a result, H01L02-DXd exerted a stronger anti tumor effect than that of NOV0712-DXd at both the doses of 1 and 3 mg/kg. Specifically, it was demonstrated that the **H01L02 antibody of the present invention is a superior antibody for antibody-drug conjugates as anti tumor agents to the NOV0712 antibody (Figure 13).**

6.9 Paragraph [0327] reports that NOV0712-DM4 exhibited no anti tumor effect at the dose of 1 mg/kg, and exhibited an anti tumor effect at the dose of 3 mg/kg, though tumor regrowth was observed from 2 weeks after administration. **H01L02DXd significantly suppressed increase in tumor volume after administration at both the doses of 1 and 3 mg/kg, and sustained, particularly, at the dose of 3 mg/kg, the tumor growth inhibition effect over a long period of 31 days after administration (Figure 14).**

6.10 Paragraph [0330] reports that NOV0712-DM4 exhibited no significant anti tumor effect at any of the doses of 1 and 3 mg/kg in this tumor model. **H01L02-DXd decreased tumor volume after administration at both the doses of 1 and 3 mg/kg, and exerted, particularly, at the dose of 3 mg/kg significant tumor regression, and sustained the tumor regression effect for 20 days after administration (Figure 15).**

6.11 Paragraph [0332] reports that in the tumor model expressing no CDH6, H01L02-DXd and NOV0712-DM4 exhibited no anti tumor effect at any of the doses. The Applicants conclude that the anti tumor effect of the antibody-drug

conjugate in the CDH6-positive tumor model demonstrated in Examples 9)-1, 9)-2, 9)-3, and 9)-4 is an effect dependent on CDH6 expression in the tumor cells. The antibody-drug conjugate of the present application is considered as a selective and safe anti tumor drug that **specifically exhibits an antitumor effect on CDH6-positive tumor without causing cytotoxicity to CDH6negative normal tissues (Figure 16) .**

6.12 The problem to be solved by the present invention may therefore be regarded as providing alternative anti-CDH6 antibodies.

6.13 The proposed solution is the antibodies comprising the variable light and heavy chains or the CDR sequences having the amino acid sequences defined according to the SEQ ID NOs provided in claims 2 to 4, 6 to 12, 15 to 17, 30 and 31.

6.14 As discussed above antibody drug conjugate H01L02-DXd exhibits significant tumor regression compared to the antibody drug conjugate NOV0712-DM4, disclosed in the closest prior art D4.

6.15 The Examining Division is prepared to accept that the four humanized anti-CDH6 antibodies disclosed in the present application:

1. Antibody H01L02, VH amino acid SEQ ID NO: 69, VL amino acid SEQ ID NO: 61,

2. Antibody H02L02, VH amino acid SEQ ID NO: 73, VL amino acid SEQ ID NO: 61,

3. Antibody H02L03, VH amino acid SEQ ID NO: 73, VL amino acid SEQ ID NO: 65, and

4. Antibody H04L02, VH amino acid SEQ ID NO: 77, VL amino acid SEQ ID NO: 61 would exhibit the same surprising effects, specifically binding to epitope EC3 and therefore are considered to involve an inventive step according to Article 56 EPC.

6.16 However claims 2 to 4, 6 to 12, 15 to 17, 30 and 31 encompass a broad range of antibodies comprising variable light and heavy chains or the CDR sequences having the amino acid sequences defined according to the SEQ ID NOs., where only antibody H01L02, comprising VH amino acid SEQ ID NO: 69 and VL amino acid SEQ ID NO: 61 has been shown to exhibit a surprising and unexpected technical effect.

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6.17 In the absence of any technical facts or evidence to show the contrary, the broad range of antibodies encompassed by the various different combinations of CDRs and VH and VL sequences defined in claims 2 to 4, 6 to 12, 15 to 17, 30 and 31 can only be considered to to arbitrary alternatives that do not warrant recognition of an inventive step according to Article 56 EPC, particularly in the light of the teachings of D1 which discloses epitope mapping studies showing anti-CDH antibodies bind to a recombinant fragment spanning the extracellular juxtamembrane domains EC3.

6.18 In view of the above, the present application does not meet the requirements of Article 52(1) EPC, because the subject-matter of claims 2 to 4, 6 to 12, 15 to 17, 30 and 31 does not involve an inventive step in the sense of Article 56 EPC.

7 **CLARITY** (Art. 84 EPC)

7.1 It is clear from the description that the amino acid sequences of the VH and VL regions of the four humanized anti-CDH6 antibodies are essential to the performance of the invention. Since independent claim 1 does not contain these features it does not meet the requirement following from Article 84 taken in combination with Rules 43(1) and (3) EPC that any independent claim must contain all the technical features essential to the invention.

7.2 It is not sufficient to characterize an antibody by the six CDR sequences alone, since an antibody is structurally made of two light chains and two heavy chains, both necessary to confer antigen binding specificity. Unless the contrary is shown, either the six CDRs with the framework regions or the entire heavy and light chains (V_H and $V_{L)}$ are necessary to fully and unambiguously characterize an antibody.

7.3 In the present case the four humanized anti-CDH antibodies disclosed in the application: Antibody H01L02, Antibody H02L02, Antibody H02L03 and Antibody H04L02 should be clearly and unambiguously defined in terms of the amino acid sequences of the VH and VL regions for the sake of the clarity of the independent claim 1 according to Article 84 EPC.

7.4 Claim 1 does not meet the requirements of Article 84 EPC in that the matter for which protection is sought is not defined. The claim attempts to define the subject-matter in terms of the result to be achieved. Such a definition is only allowable under the conditions elaborated in the Guidelines F-IV, 4.10. In this instance, however, such a formulation is not allowable because it appears possible to define the subject-matter in more concrete terms, viz. in terms of how the effect is to be achieved.

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7.5 Furthermore independent claim 1 misses the essential technical feature that the antibodies specifically bind to cadherin-6, contrary to the requirements of clarity according to Article 84 EPC.

7.6 The vague and imprecise statement in the description on page 142, paragraph [0220] implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity of the claims (Article 84 EPC) when used to interpret them (see the Guidelines, F-IV, 4.4). This statement should therefore be amended to remove this inconsistency.

8 CONCLUSIONS

8.1 In the light of the above observations the Examining Division would appreciate the reformulation of independent claim 1 and claims dependent thereon, to include all the essential technical features as outlined above, such that the claims reflect the nature of the present invention and may be deemed to satisfy the requirements of the EPC.

Rebecca Hix

Bitte beachten Sie, dass angeführte Nichtpatentliteratur (wie z. B. wissenschaftliche oder technische Dokumente) je nach geltendem Recht dem Urheberrechtsschutz und/oder anderen Schutzarten für schriftliche Werke unterliegen könnte. Die Vervielfältigung urheberrechtlich geschützter Texte, ihre Verwendung in anderen elektronischen oder gedruckten Publikationen und ihre Weitergabe an Dritte ist ohne ausdrückliche Zustimmung des Rechtsinhabers nicht gestattet.

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XS CPRTENFRDE

Written notice of reasons for refusal

Patent application number 2019-518773
Draft date: September 29, 2020
Examiner, Japan Patent Office, Mr. Kiko Matsuura, 1585 4B00
Dr. Kouki Ishibashi, attorney for patent application (3 persons outside)
Applicable Section 29, Pillar Paper, Section 36

This application should be refused for the following reasons. If you have any comments on this, please submit a written opinion within 60 days from the date of dispatch of this notice.

Reasons

1. (Industrial applicability) The following claims of this application may not be granted a patent because the following requirements are not met in accordance with the requirements of Section 29(1) Pillar of the Patent Act.

2. (Enablement Requirement) In this application, the detailed explanation of the invention does not meet the requirements set forth in Article 36(4)(i) of the Patent Act in the following respects.

3. (Support Requirements) In this application, the description of the claims does not meet the requirements set forth in Article 36(6)(i) of the Patent Act in the following respects.

Description

●Reason 1 (Industrial Applicability)

•Claims 49-52
The invention according to claim 49 is "... a treatment method for tumors characterized by administering an antibody-drug conjugate... to an individual."
Here, since the "individual" being the subject of treatment usually includes humans, the invention according to claim 49 falls under the category of a method of treatment for the human body and is not considered to be an invention that can be applied industrially.
The invention according to claims 50 to 52 is also considered to be a method of treatment of the human body and is not considered to be an invention that can be applied industrially.

●Reasons 2 (Enablement Requirement), Reason 3 (Support Requirement)

•Claims 3, 5, 6, 13, 14, 20-36, 40-53

According to a third aspect of the invention, there is provided a light chain variable region comprising three CDRs: rG019 and rG055 and rG056 and rG061, and three CDRs in the heavy chain variable region: rG019 and rG055 and rG056, and rG061 and chG019 respectively, or a functional fraction of such an anticyclone ([0062] to [0065] and [0077]).

However, since it is understood that the binding ability of an antibody is exerted on the basis of an appropriate combination of CDRs, it is highly likely that an antibody composed of CDRs of rG019 antibody, rG055 antibody, rG056 antibody, rG061 antibody, or chG019 antibody in various combinations loses the binding ability that most of them specifically bind to the amino acid sequence set forth in SEQ ID NO: 4 specified in claim 1.

Also, when referring to the description in the detailed explanation of the invention, it is not found that an antibody comprising various combinations of CDRs derived from the above five antibodies as the heavy chain variable region and the light chain variable region respectively binds specifically to the amino acid sequence of SEQ ID NO:4.

Thereafter, it is not accepted that the antibodies contained in Claim 3, which consist of CDRs of the rG019 antibody, rG055 antibody, rG056 antibody, rG061 antibody, or chG019 antibody, are disclosed with sufficient support in the description of the detailed explanation of the invention, with "specific bonds to the amino acid sequence described in Sequence Number 4."

Similarly, obtaining an antibody that "specifically binds to the amino acid sequence set forth in SEQ ID NO: 4" from among antibodies composed of CDRs of rG019 antibody, rG055 antibody, rG056 antibody, rG061 antibody, or chG019 antibody in various combinations imposes an excessive burden on a person skilled in the art.

The invention according to claim 6 is similar in that the sequence of the light chain variable region comprises an antibody comprising various combinations of the sequence of the variable regions of hL02 or hL03, and the sequence of the heavy chain variable regions of hH01, hH02 or hH04 as the sequence of the heavy chain variable regions.

The inventions according to claims 5, 13, 14, 20-36, 40-53 citing claims 3 or 6 are also similar.

<Claims for which no reason for refusal is found>

As for the inventions according to claims 1, 2, 4, 7-12, 15-19 and 37-39, the reason for refusal is not found at this time. If a new reason for refusal is found, a notice of reasons for refusal is given.

<Record of Prior Art Literature Search Results>

•Fields investigated: IPC C12N 15/13, A61K 9/00, A61K 31/4745,
A61K 39/395, A61K 47/68, A61P 13/12,
A61P 15/08, A61P 35/00, A61P 43/00,
C07K 16/28, C12N 1/15-1/21, C12N 5/10,
C12P 21/08
DB name CAPLUS/MEDLINE/BIOSIS/MEDLINE/WPIDS

•Prior Art Document International Publication No. 2016/024195
International Publication No. 2014/057687

This record of the results of the search for prior art documents does not constitute a reason for refusal.

<Precautions when making amendments>
(1) When amending the description or claims, draw an underline at the amended location (Form No. 13 of the Implementing Regulations to the Patent Act, Notes 6 and 7).
Amendments to (2) shall be made within the scope of matters stated in the originally attached description, claims or drawings of this application. Also, please state in a written opinion the reasons why amendments are legitimate with respect to each amended matter after clearly indicating the places to be stated in the originally attached description, etc. as the grounds for filing of the application.
(3) Be careful not to make amendments to the claims that violate Section 17bis(4) of the Patent Act.

If any inquiry or interview is requested regarding the content of this notice of reasons for refusal, please contact the following address: If you wish to send an amendment, etc. via e-mail or fax, please contact us by telephone.

Masato Tamai Tamai, Life Engineering Department III Examination Department
TEL.03-3581-1101 INTERNATIONAL 3448

拒絶理由通知書

特許出願の番号	特願２０１９－５１８７７３
起案日	令和　２年　９月２９日
特許庁審査官	松浦　安紀子　　１５８５　４Ｂ００
特許出願人代理人	石橋　公樹（外　３名）　様
適用条文	第２９条柱書、第３６条

この出願は、次の理由によって拒絶をすべきものです。これについて意見がありましたら、この通知書の発送の日から６０日以内に意見書を提出してください。

理由

１．（産業上の利用可能性）この出願の下記の請求項に係る発明は、下記の点で特許法第２９条第１項柱書に規定する要件を満たしていないから、特許を受けることができない。

２．（実施可能要件）この出願は、発明の詳細な説明の記載が下記の点で、特許法第３６条第４項第１号に規定する要件を満たしていない。

３．（サポート要件）この出願は、特許請求の範囲の記載が下記の点で、特許法第３６条第６項第１号に規定する要件を満たしていない。

記

●理由１（産業上の利用可能性）について

・請求項　４９～５２

請求項４９に係る発明は「…抗体－薬物コンジュゲート…を個体に投与することを特徴とする腫瘍の治療方法」である。

ここで、治療対象である「個体」は通常ヒトを含むから、請求項４９に係る発明は人体を治療する方法に該当するものであり、産業上利用することができる発明であるとは認められない。

請求項５０～５２に係る発明も同様に人体を治療する方法に該当するものであり、産業上利用することができる発明であるとは認められない。

●理由２（実施可能要件）、理由３（サポート要件）について

・請求項　３、５、６、１３、１４、２０～３６、４０～５３
　請求項３に係る発明は、軽鎖可変領域の３つのＣＤＲとして、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体又はｒＧ０６１抗体のいずれかのＣＤＲ、重鎖可変領域の３つのＣＤＲとして、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体又はｃｈＧ０１９抗体のいずれかのＣＤＲを含む抗体又は当該抗体の機能性断片である（［００６２］～［００６５］、［００７７］）。

　しかし、抗体の結合能はＣＤＲの適切な組み合わせに基づき発揮されるものと解されることから、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体又はｃｈＧ０１９抗体のＣＤＲを多様な組み合わせで含んで構成される抗体は、その大部分が請求項１で特定された「配列番号４に記載のアミノ酸配列に特異的に結合」するという結合能を喪失する蓋然性が高い。
　また、発明の詳細な説明の記載を参酌しても、重鎖可変領域、軽鎖可変領域それぞれのＣＤＲとして、上記の５種類の抗体に由来するものを様々な組み合わせで含む抗体が、いずれも配列番号４に記載のアミノ酸配列に特異的に結合するものとは認められない。

　そうすると、請求項３に包含されている抗体である、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体又はｃｈＧ０１９抗体のＣＤＲを多様な組み合わせで含んで構成された「配列番号４に記載のアミノ酸配列に特異的に結合」する抗体については、発明の詳細な説明の記載において十分な裏付けを伴って開示されているものとは認められない。
　同様に、ｒＧ０１９抗体、ｒＧ０５５抗体、ｒＧ０５６抗体、ｒＧ０６１抗体又はｃｈＧ０１９抗体のＣＤＲを多様な組み合わせで含んで構成された抗体の中から、「配列番号４に記載のアミノ酸配列に特異的に結合」する抗体を取得することは、当業者に過度の負担を課すものである。

　軽鎖可変領域の配列として、ｈＬ０２又はｈＬ０３の可変領域の配列、重鎖可変領域の配列としてｈＨ０１、ｈＨ０２又はｈＨ０４の重鎖可変領域の配列を様々な組み合わせで含んで構成される抗体を含む、請求項６に係る発明も同様である。
　また、請求項３又は６を引用する請求項５、１３、１４、２０～３６、４０～５３に係る発明も同様である。

＜拒絶の理由を発見しない請求項＞

請求項１、２、４、７～１２、１５～１９、３７～３９に係る発明については、現時点では、拒絶の理由を発見しない。拒絶の理由が新たに発見された場合には拒絶の理由が通知される。

－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－－

＜先行技術文献調査結果の記録＞

・調査した分野　　ＩＰＣ　C12N 15/13, A61K 9/00, A61K 31/4745, A61K 39/395, A61K 47/68, A61P 13/12, A61P 15/08, A61P 35/00, A61P 43/00, C07K 16/28, C12N 1/15-1/21, C12N 5/10, C12P 21/08

ＤＢ名　CAPLUS/MEDLINE/BIOSIS/MEDLINE/WPIDS

・先行技術文献　　国際公開第２０１６／０２４１９５号

国際公開第２０１４／０５７６８７号

この先行技術文献調査結果の記録は拒絶理由を構成するものではありません。

＜補正をする際の注意＞

（１）明細書、特許請求の範囲について補正をする場合は、補正により記載を変更した個所に下線を引いてください（特許法施行規則様式第１３備考６、７）。

（２）補正は、この出願の出願当初の明細書、特許請求の範囲又は図面に記載した事項の範囲内で行わなければなりません。また、意見書で、各補正事項について補正が適法なものである理由を、根拠となる出願当初の明細書等の記載箇所を明確に示したうえで主張してください。

（３）特許請求の範囲について補正をする際には、特許法第１７条の２第４項に違反する補正とならないよう、注意してください。

この拒絶理由通知の内容に関するお問合せ又は面接のご希望がありましたら次の連絡先までご連絡ください。電子メール又はＦＡＸで補正案等の送付を希望される場合は、その旨を電話でお知らせください。

審査第三部生命工学　玉井真人（たまいまこと）
ＴＥＬ．０３－３５８１－１１０１　内線３４４８

[Claim 1]

A antibody or a functional fragment of the antibody or that specifically binds to the amino acid sequence according to SEQ ID NO: 4 and has the endogenous capacity to be incorporated into cells.

[Claim 2]

The antibody of claim 1, or a functional fragment thereof, having competitive inhibitory activity with at least one antibody selected from the group consisting of the following (1)-(5):

(1) an antibody having a light chain comprising the amino acid sequence of SEQ ID NO: 53 at 21-233 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 56 at 20-471,

(2) an antibody having a light chain comprising the amino acid sequence of SEQ ID NO: 61-21-233 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 69-20-471,

(3) an antibody having a light chain comprising the amino acid sequence of SEQ ID NO: 61-21-233 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 73-20-471,

(4) an antibody having a light chain comprising the amino acid sequence of SEQ ID NO: 65-21-233 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 73-20-471; and

(5) an antibody having a light chain consisting of the amino acid sequence of SEQ ID NO: 61, 21 to 233, and an antibody having a heavy chain consisting of the amino acid sequence of SEQ ID NO: 77, 20 to 471.

[Claim 3]

The antibody or functional fragment of the antibody of claim 1 or 2, wherein the antibody comprises CDRL1, CDRL2 and CDRL3, and CDRH1, CDRH2 and CDRH3, selected from the group consisting of the following (1)-(5):

(i) CDRL1, consisting of the amino acid sequence set forth in SEQ ID NO: 12, CDRL2, comprising the amino acid sequence set forth in SEQ ID NO: 13, and CDRL3, comprising the amino acid sequence set forth in SEQ ID NO: 14, CDRH1, comprising the amino acid sequence set forth in SEQ ID NO: 17, CDRH2, comprising the amino acid sequence set forth in SEQ ID NO: 18, and CDRH3, comprising the amino acid sequence set forth in SEQ ID NO: 19,

(ii) CDRL1, consisting of the amino acid sequence set forth in SEQ ID NO: 22, CDRL2, comprising the amino acid sequence set forth in SEQ ID NO: 23, and CDRL3, comprising the amino acid sequence set forth in SEQ ID NO: 24, CDRH1, comprising the amino acid sequence set forth in SEQ ID NO: 27, CDRH2, comprising the amino acid sequence set forth in SEQ ID NO: 28, and CDRH3, comprising the amino acid sequence set forth in SEQ ID NO: 29,

(iii) CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO:32, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO:33 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO:34, and CDRH1

consisting of the amino acid sequence set forth in SEQ ID NO:37, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO:38 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO:39,

(iv) CDRL1, consisting of the amino acid sequence set forth in SEQ ID NO: 42, CDRL2, comprising the amino acid sequence set forth in SEQ ID NO: 43, and CDRL3, comprising the amino acid sequence set forth in SEQ ID NO: 44, CDRH1, comprising the amino acid sequence set forth in SEQ ID NO: 47, CDRH2, comprising the amino acid sequence set forth in SEQ ID NO: 48, and CDRH3, comprising the amino acid sequence set forth in SEQ ID NO: 49, and so on

(v) CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 13 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 14, and CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 60 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19.

[Claim 4]

The antibody of any one of claims 1-3, wherein the antibody is humanized, or a functional fragment of the antibody.

[Claim 5]

The antibody of any one of claims 1-4, comprising any light chain variable region and a heavy chain variable region, or a functional fragment of the antibody, comprising any one of the following (1)-(4):

(1) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 63 and heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 71,

(2) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 63 and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 75,

(3) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 67 and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 75; or

(4) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 63 and heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 79.

[Claim 6]

The antibody of any one of claims 1-5, or a functional fragment of the antibody, comprising any one of the following (1)-(4):

(1) a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 69.

(2) a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 73,

(3) a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 65 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 73; or

(4) a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 77.

[Claim 7]

The antibody or functional fragment of the antibody according to claim 6 having a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 69.

[Claim 8]

The antibody or functional fragment of the antibody according to claim 6, having a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 73.

[Claim 9]

The antibody or functional fragment of the antibody according to claim 6, having a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 65 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 73.

[Claim 10]

The antibody or functional fragment of the antibody according to claim 6 having a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 77.

[Claim 11]

The functional fragment of any one of claims 1-10, wherein the functional fragment is selected from the group consisting of Fab, F(ab')2, Fab' and Fv.

[Claim 12]

A polynucleotide encoding the antibody or functional fragment of the antibody according to any one of claims 1 to 11.

[Claim 13]

The polynucleotide of claim 12, wherein the polynucleotide comprises a polynucleotide according to any one being selected from the group consisting of the following (1)-(5):

(1) a polynucleotide encoding a CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 12, a CDRL2 comprising the amino acid sequence set forth in SEQ ID NO: 13, and a CDRL3 comprising the amino acid sequence set forth in SEQ ID NO: 14, and a heavy chain variable region comprising the CDRH1 comprising the amino acid sequence set forth in SEQ ID NO: 17, a CDRH2 comprising the amino acid sequence set forth in SEQ ID NO: 18, and a CDRH3 comprising the amino acid sequence set forth in SEQ ID NO: 19.

(2) a polynucleotide encoding a CDRL1 comprising the amino acid sequence set forth in SEQ ID NO: 22, a CDRL2 comprising the amino acid sequence set forth in SEQ ID NO: 23, and a CDRL3 comprising the amino acid sequence set forth in SEQ ID NO: 24; and a polynucleotide encoding a heavy chain variable region comprising the CDRH1 comprising the amino acid sequence set forth in SEQ ID NO: 27, a CDRH2 comprising the amino acid

sequence set forth in SEQ ID NO: 28, and a CDRH3 comprising the amino acid sequence set forth in SEQ ID NO: 29.

(3) A polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO:32, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO:33 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO:34, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO:37, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO:38, and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO:39.

(4) a polynucleotide encoding a light chain variable region comprising a CDRL1 comprising the amino acid sequence set forth in SEQ ID NO: 42, a CDRL2 comprising the amino acid sequence set forth in SEQ ID NO: 43, and a CDRL3 comprising the amino acid sequence set forth in SEQ ID NO: 44, and a polynucleotide encoding a heavy chain variable region comprising the CDRH1 comprising the amino acid sequence set forth in SEQ ID NO: 47, a CDRH2 comprising the amino acid sequence set forth in SEQ ID NO: 48, and a CDRH3 comprising the amino acid sequence set forth in SEQ ID NO: 49; and

(5) a polynucleotide encoding a CDRL1 comprising the amino acid sequence set forth in SEQ ID NO: 12, a CDRL2 comprising the amino acid sequence set forth in SEQ ID NO: 13, and a CDRL3 comprising the amino acid sequence set forth in SEQ ID NO: 14, and a polynucleotide encoding a heavy chain variable region comprising the CDRH1 comprising the amino acid sequence set forth in SEQ ID NO: 17, a CDRH2 comprising the amino acid sequence set forth in SEQ ID NO: 60, and a CDRH3 comprising the amino acid sequence set forth in SEQ ID NO: 19.

[Claim 14]

The polynucleotide of claim 12 or 13, comprising a polynucleotide encoding a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a polynucleotide encoding a overlapping chain comprising the 20-471 amino acid sequence of SEQ ID NO: 69.

[Claim 15]

The polynucleotide of claim 12 or 13, comprising a polynucleotide encoding a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a polynucleotide encoding a overlapping chain comprising the 20-471 amino acid sequence of SEQ ID NO: 73.

[Claim 16]

The polynucleotide of claim 12 or 13, comprising a polynucleotide encoding a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 65 and a polynucleotide encoding a overlapping chain comprising the 20-471 amino acid sequence of SEQ ID NO: 73.

[Claim 17]

The polynucleotide of claim 12 or 13, comprising a polynucleotide encoding a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a polynucleotide encoding a overlapping chain comprising the 20-471 amino acid sequence of SEQ ID NO: 77.

[Claim 18]

An expression vector comprising a polynucleotide according to any one of claims 12-17.

[Claim 19]

Host cell transformed by the expression vector of claim 18.

[Claim 20]

The host cell of claim 19, wherein the host cell is a eukaryotic cell.

[Claim 21]

The method of manufacturing a functional fragment of an antibody or antibody comprising the step of culturing the host cell of claim 19 or 20 and harvesting a functional fragment of the antibody or antibody of interest from a culture obtained in said step.

[Claim 22]

The antibody or functional fragment of any one of claims 1 to 11, wherein the heavy or light chain has undergone one or more modifications selected from the group consisting of: glycosylation to an N-bond, glycosylation to an O-bond, processing to an N-terminal, processing to an O-bond, isomerization to an aspartic acid, oxidation of methionine, addition to an N-terminal methionine residue, amidation of a proline residue, pyroglutamine oxidation of an N-terminal glutamine or N-terminal glutamic acid, and one or two amino acid deletions at the carboxyl terminus.

[Claim 23]

The antibody of claim 22, wherein one or two amino acids are deleted at the carboxyl terminus of the heavy chain.

[Claim 24]

The antibody of claim 23, wherein one amino acid is deleted at the carboxyl terminus on both sides of the heavy chain.

[Claim 25]

The antibody of any one of claims 22-24, wherein the carboxyl-terminal proline residue of the heavy chain is further amidated.

[Claim 26]

The antibody or functional fragment of an antibody according to any one of claims 1-11, wherein the carbohydrate modification is regulated to enhance antibody-dependent cytotoxic activity and 22-25.

[Claim 27]

An antibody-drug conjugate according to any one of claims 1-11 and 22-26, wherein the antibody or functional fragment of the antibody is associated with a drug.

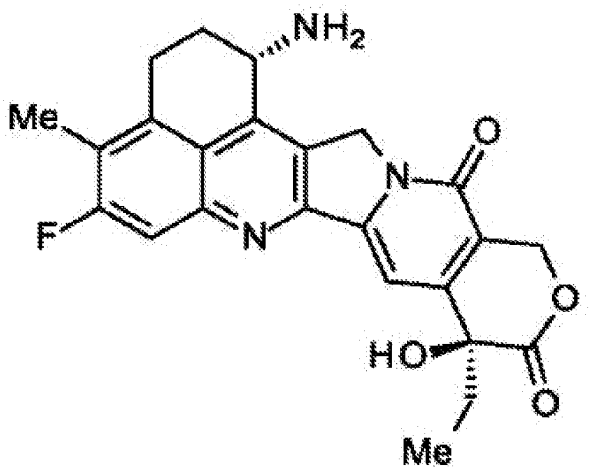
[Claim 28]

The antibody-drug conjugate of claim 27, wherein the drug is an antitumor compound.

[Claim 29]

The antibody-drug conjugate according to claim 38, wherein, the antitumor compound hae the following formula:

[化1]



[Claim 30]

The antibody-drug conjugate of any one of claims 27-29, wherein the antibody is coupled with the drug via a linker of any structure selected from the group consisting of the following (a)-(f):

(a)-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-、

(b)-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-、

(c)-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-、

(d)-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-、

(e)-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2O-CH_2CH_2O-CH_2CH_2-C(=O)-GGFG-NH-CH

$_2CH_2CH_2$-C (=O)-, and

(f)-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2O-CH_2CH_2O-CH_2CH_2O-CH_2CH_2O-CH_2CH

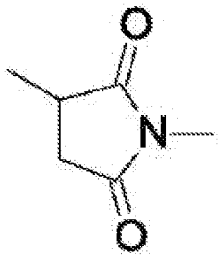
$_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-

(where the antibody binds at the end of-(Succinimid-3-yl-N). The antineoplastic compound is attached to the carbonyl group of-$CH_2CH_2CH_2$-C (=O)-moiety of (a), (b), (e) or (f), (c)-moiety of CH_2-O-CH_2-C

(=O)-moiety or CH_2CH_2-O-CH_2-C (=O)-moiety of (d) with the nitrogen atom of the amino group at position 1 as a binding site. The above formula GGFG shows the amino acid sequence linked by a peptide bond composed of glycine-glycine-phenylalanine-glycine.

-(Succinimid-3-yl-N)-

[화2]



Structure shown that binds to an antibody at position 3 of this molecule and binds to a methylene group within the linker structure comprising the antibody at position 1 of the nitrogen atom.)

[Claim 31]

The antibody-drug conjugate of any one of claims 27-30, wherein the linker is represented by any formula selected from the group consisting of (c), (d) and (e) below:

(c)-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-、

(d)-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2CH_2-O-CH_2-C(=O)-、

(e)-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2O-CH_2CH_2O-CH_2CH_2-C(=O)-GGFG-NH-CH

$_2CH_2CH_2$-C(=O)-。

[Claim 32]

The antibody-drug conjugate of any one of claims 27-31, wherein the linker is represented by the following formula (c) or (e):

(c)-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-CH_2-O-CH_2-C(=O)-、

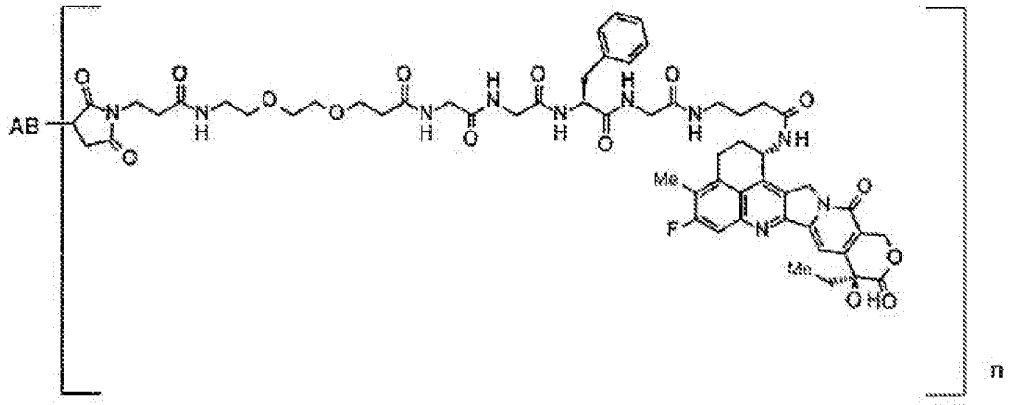
(e)-(Succinimid-3-yl-N)-CH_2CH_2-C(=O)-NH-CH_2CH_2O-CH_2CH_2O-CH_2CH_2-C(=O)-GGFG-NH-CH

$_2CH_2CH_2$-C(=O)-。

[Claim 33]

The antibody-drug conjugate of any one of claims 27-32, having the following formula:

[化3]

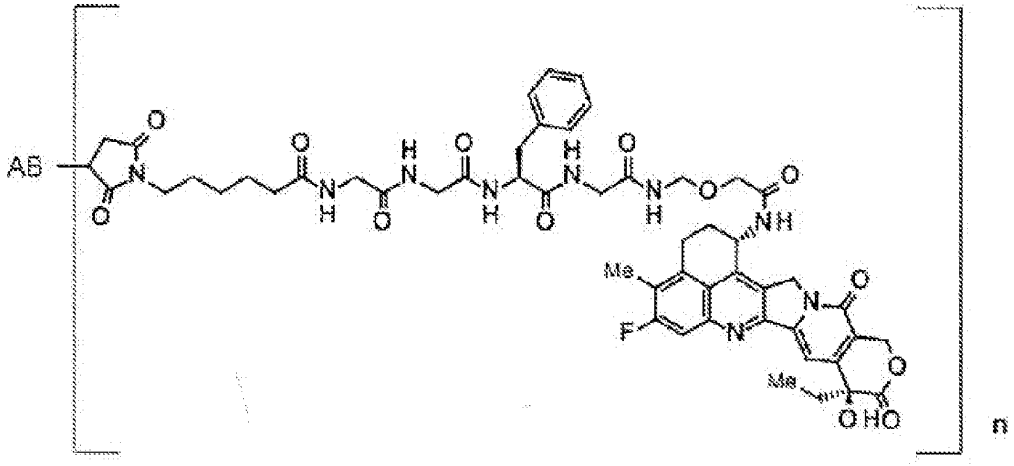


wherein AB indicates the antibody or functional fragment thereod. n shows the average number of drug-linker structures bound to an antibody per antibody. The antibody and linkers are linked via sulfhydryl groups derived from the antibody.

[Claim 34]

The antibody-drug conjugate of any one of claims 27-32, having the following formula:

[化4]



wherein AB indicates the antibody or a functional fragment thereof. n shows the average number of drug-linker structures bound to the antibody per the antibody. The Antibody and linkers are linked via sulfhydryl groups derived from the antibody.

[Claim 35]

The antibody-drug conjugate of any one of claims 27-34, wherein the antibody or a functional fragment of the antibody comprises a light chain and a heavy chain as defined in any one selected from the group consisting of the following (1)-(4):
(1) A light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 69.
(2) a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 73,
(3) a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 65 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 73; or
(4) a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 77.

[Claim 36]
The antibody-drug conjugate of claim 35, wherein the antibody is an antibody comprising a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 69 or a functional fragment of the antibody.

[Claim 37]
The antibody-drug conjugate of claim 35, wherein the antibody is an antibody comprising a light chain comprising the 21-233 amino acid sequence of SEQ ID NO: 61 and a heavy chain comprising the 20-471 amino acid sequence of SEQ ID NO: 77 or a functional fragment of the antibody.

[Claim 38]
The antibody-drug conjugate of any one of claims 27 to 37, wherein the heavy or the light chain receives 1 or 2 or more modifications from a group consisting of N-bond addition, sugar chain addition to O-bond, N-end processing, C-end processing, de-amidation, asparaginic acid differentiation, Methionine oxide, N-end addition of a methionine residual, amidization of a prolin residual, N-end glutamic acid pirotamine oxide or N-end glutamic acid with one or two amino acid deficiencies at the end of the pyroglutamine oxide and carvoxil.

[Claim 39]
The anti-drug conjugate of any one of claims 27-38, wherein the average number of bonds per one agent of a selected drug-linker structure is in the range of 1-10.

[Claim 40]
The antibody-drug conjugate of claim 39, wherein the average number of bonds per antibody of the selected one drug-linker structure ranges from 2 to 8.

[Claim 41]
The body-drug conjugate of claim 40, wherein the average number of bonds per one of the selected drug-linker structures is in the range of 5-8.

[Claim 42]

The antibody-drug conjugate of claim 41, wherein the average number of bonds per one of the selected drug-linker structures is between 7 and 8.

[Claim 43]

A pharmaceutical composition comprising the antibody-drug conjugate according to any one of claims 27 to 42, salt thereof, or hydrate thereof,.

[Claim 44]

The pharmaceutical composition of claim 43, wherein the drug is an antitumor agent.

[Claim 45]

The pharmaceutical composition of claim 44, wherein the tumor is a tumor expressing CDH6.

[Claim 46]

The pharmaceutical composition of claim 44 or 45, wherein the tumor is renal cell carcinoma, clear cell carcinoma of the kidney, papillary kidney cell carcinoma, ovarian cancer, serous adenocarcinoma of the ovary, thyroid cancer, bile duct cancer, lung cancer, small cell lung cancer, glioblastoma, mesothelioma, uterine cancer, pancreatic cancer, Wilms tumor or neuroblastoma.

[Claim 47]

The method of manufacturing an antibody-drug conjugate comprising the step of reacting a drug-linker intermediate compound with an antibody according to any one of claims 1-11 and 22-26 or a functional fragment of the antibody, or, a functional fragment of the antibody obtained by the method according to claim 21 or a functional fragment of the antibody.

【請求項１】
配列番号４に記載のアミノ酸配列に特異的に結合し、細胞内に取り込まれる内在化能を有する、抗体又は当該抗体の機能性断片。
【請求項２】
配列番号４に記載のアミノ酸配列への結合に対して、以下の（１）～（５）：
（１）配列番号５３の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号５６の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、
（２）配列番号６１の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、
（３）配列番号６１の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、
（４）配列番号６５の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、及び
（５）配列番号６１の２１～２３３番目に記載のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目に記載のアミノ酸配列からなる重鎖を有する抗体、
からなる群から選択される抗体のうち少なくともいずれか１つと競合阻害活性を有する請求項１に記載の抗体又は当該抗体の機能性断片。
【請求項３】
以下の（１）～（５）：
（１）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号１８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３、
（２）配列番号２２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号２３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号２４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号２７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号２８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号２９に記載のアミノ酸配列からなるＣＤＲＨ３、
（３）配列番号３２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号３３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号３４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号３７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号３８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号３９に記載のアミノ酸配列からなるＣＤＲＨ３、
（４）配列番号４２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号４３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号４４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号４７に記載のアミノ酸配列からなるＣＤＲＨ１、

配列番号４８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号４９に記載のアミノ酸配列からなるＣＤＲＨ３、及び
（５）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に記載のアミノ酸配列からなるＣＤＲＬ３、及び、配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号６０に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３、
からなる群から選択されるＣＤＲＬ１、ＣＤＲＬ２及びＣＤＲＬ３、並びに、ＣＤＲＨ１、ＣＤＲＨ２及びＣＤＲＨ３を含む、請求項１又は２に記載の抗体又は当該抗体の機能性断片。

【請求項４】

ヒト化されている請求項１～３のいずれか１項に記載の抗体又は当該抗体の機能性断片。

【請求項５】

以下の（１）～（４）：
（１）配列番号６３に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号７１に記載のアミノ酸配列からなる重鎖可変領域、
（２）配列番号６３に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号７５に記載のアミノ酸配列からなる重鎖可変領域、
（３）配列番号６７に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号７５に記載のアミノ酸配列からなる重鎖可変領域、又は
（４）配列番号６３に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号７９に記載のアミノ酸配列からなる重鎖可変領域、
のいずれかの軽鎖可変領域及び重鎖可変領域、を含む請求項１～４のいずれか１項に記載の抗体又は当該抗体の機能性断片。

【請求項６】

以下の（１）～（４）：
（１）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖、
（２）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖、
（３）配列番号６５の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖、又は
（４）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖、
のいずれかを有する請求項１～５のいずれか１項に記載の抗体又は当該抗体の機能性断片。

【請求項７】

配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖を有する請求項６に記載の抗体又は当該抗体の機能性断片。

【請求項８】

配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖を有する請求項６に記載の抗体又は当該抗体の機能性断片。

【請求項９】

配列番号６５の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖を有する請求項６に記載の抗体又は当該抗体の機能性断片。

【請求項１０】

配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖を有する請求項６に記載の抗体又は当該抗体の機能性断片。

【請求項１１】

機能性断片がＦａｂ、Ｆ（ａｂ’）２、Ｆａｂ’及びＦｖからなる群から選択される請求項１～１０のいずれか１項に記載の抗体の機能性断片。

【請求項１２】

請求項１～１１のいずれか１項に記載の抗体又は当該抗体の機能性断片をコードするポリヌクレオチド。

【請求項１３】

以下の（１）～（５）：

（１）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号１８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、

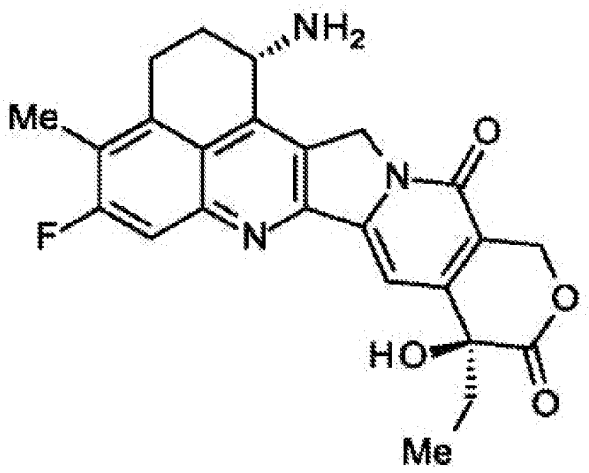
（２）配列番号２２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号２３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号２４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号２７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号２８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号２９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、

（３）配列番号３２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号３３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号３４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番

号３７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号３８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号３９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、
（４）配列番号４２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号４３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号４４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号４７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号４８に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号４９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、並びに
（５）配列番号１２に記載のアミノ酸配列からなるＣＤＲＬ１、配列番号１３に記載のアミノ酸配列からなるＣＤＲＬ２及び配列番号１４に記載のアミノ酸配列からなるＣＤＲＬ３を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号１７に記載のアミノ酸配列からなるＣＤＲＨ１、配列番号６０に記載のアミノ酸配列からなるＣＤＲＨ２及び配列番号１９に記載のアミノ酸配列からなるＣＤＲＨ３を含む重鎖可変領域をコードするポリヌクレオチド、
からなる群から選択されるいずれか１つに記載のポリヌクレオチドを含む請求項１２に記載のポリヌクレオチド。
【請求項１４】
配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項１２又は１３に記載のポリヌクレオチド。
【請求項１５】
配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項１２又は１３に記載のポリヌクレオチド。
【請求項１６】
配列番号６５の２１～２３３番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項１２又は１３に記載のポリヌクレオチド。
【請求項１７】
配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項１２又は１３に記載のポリヌクレオチド。
【請求項１８】
請求項１２～１７のいずれか１項に記載のポリヌクレオチドを含有する発現ベクター。
【請求項１９】
請求項１８に記載の発現ベクターにより形質転換された宿主細胞。

【請求項２０】
宿主細胞が真核細胞である請求項１９に記載の宿主細胞。
【請求項２１】
請求項１９又は２０に記載の宿主細胞を培養する工程、及び当該工程で得られた培養物から目的の抗体又は当該抗体の機能性断片を採取する工程を含むことを特徴とする当該抗体又は当該抗体の機能性断片の製造方法。
【請求項２２】
重鎖又は軽鎖が、Ｎ－結合への糖鎖付加、Ｏ－結合への糖鎖付加、Ｎ末のプロセッシング、Ｃ末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、Ｎ末にメチオニン残基の付加、プロリン残基のアミド化、Ｎ末グルタミンもしくはＮ末グルタミン酸のピログルタミン酸化、及びカルボキシル末端における１つ又は２つのアミノ酸欠失からなる群より選択される１又は２以上の修飾をうけた、請求項１～１１のいずれか１項に記載の抗体又は当該抗体の機能性断片。
【請求項２３】
重鎖のカルボキシル末端において１つ又は２つのアミノ酸が欠失している請求項２２に記載の抗体。
【請求項２４】
２本の重鎖の双方でカルボキシル末端において１つのアミノ酸が欠失している請求項２３に記載の抗体。
【請求項２５】
重鎖のカルボキシル末端のプロリン残基が更にアミド化されている請求項２２～２４のいずれか１項に記載の抗体。
【請求項２６】
抗体依存性細胞傷害活性を増強させるために糖鎖修飾が調節されている請求項１～１１及び請求項２２～２５からなる群から選択されるいずれか１項に記載の抗体又は当該抗体の機能性断片。
【請求項２７】
請求項１～１１及び請求項２２～２６からなる群から選択されるいずれか１項に記載の抗体又は当該抗体の機能性断片に薬物が結合している抗体－薬物コンジュゲート。
【請求項２８】
薬物が抗腫瘍性化合物である、請求項２７に記載の抗体－薬物コンジュゲート。
【請求項２９】
抗腫瘍性化合物が次式：

[化1]



で示される抗腫瘍性化合物である請求項２８に記載の抗体－薬物コンジュゲート。

【請求項３０】

抗体と薬物が、次式（ａ）～（ｆ）：

（ａ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

（ｂ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

（ｃ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、

（ｄ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、

（ｅ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＮＨ－CH_2CH_2O－CH_2CH_2O－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、及び

（ｆ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＮＨ－CH_2CH_2O－CH_2CH_2O－CH_2CH_2O－CH_2CH_2O－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－、

からなる群から選択されるいずれかの構造のリンカーを介して結合している、請求項２７～２９のいずれか１項に記載の抗体－薬物コンジュゲート。

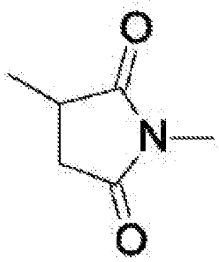
（ここで、抗体は、－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）の末端において結合する。抗腫瘍性化合物は、１位のアミノ基の窒素原子を結合部位として、

（ａ）、（ｂ）、（ｅ）又は（ｆ）の－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－部分、

（ｃ）のCH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－部分又は（ｄ）のCH_2CH_2－Ｏ－ＣＨ

$_2$－Ｃ（＝Ｏ）－部分のカルボニル基に結合する。上記式中ＧＧＦＧは、グリシン－グリシン－フェニルアラニン－グリシンからなるペプチド結合でつながっているアミノ酸配列を示す。
－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－は次式：

［化2］



で示される構造であり、このものの３位で抗体と結合し、１位の窒素原子上でこれを含むリンカー構造内のメチレン基と結合する。）。

【請求項３１】

リンカーが以下の（ｃ）、（ｄ）及び（ｅ）からなる群から選択されるいずれかの式で示される請求項２７～３０のいずれか１項に記載の抗体－薬物コンジュゲート：
（ｃ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、
（ｄ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、
（ｅ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＮＨ－CH_2CH_2Ｏ－CH_2CH_2Ｏ－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－。

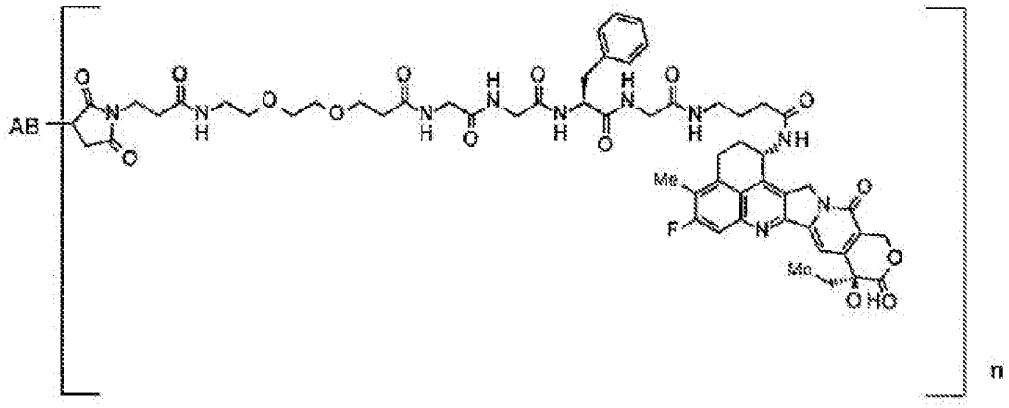
【請求項３２】

リンカーが以下の（ｃ）又は（ｅ）の式で示される請求項２７～３１のいずれか１項に記載の抗体－薬物コンジュゲート：
（ｃ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－$CH_2CH_2CH_2CH_2CH_2$－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－CH_2－Ｏ－CH_2－Ｃ（＝Ｏ）－、
（ｅ）－（Ｓｕｃｃｉｎｉｍｉｄ－３－ｙｌ－Ｎ）－CH_2CH_2－Ｃ（＝Ｏ）－ＮＨ－CH_2CH_2Ｏ－CH_2CH_2Ｏ－CH_2CH_2－Ｃ（＝Ｏ）－ＧＧＦＧ－ＮＨ－$CH_2CH_2CH_2$－Ｃ（＝Ｏ）－。

【請求項３３】

以下の式：

[化3]

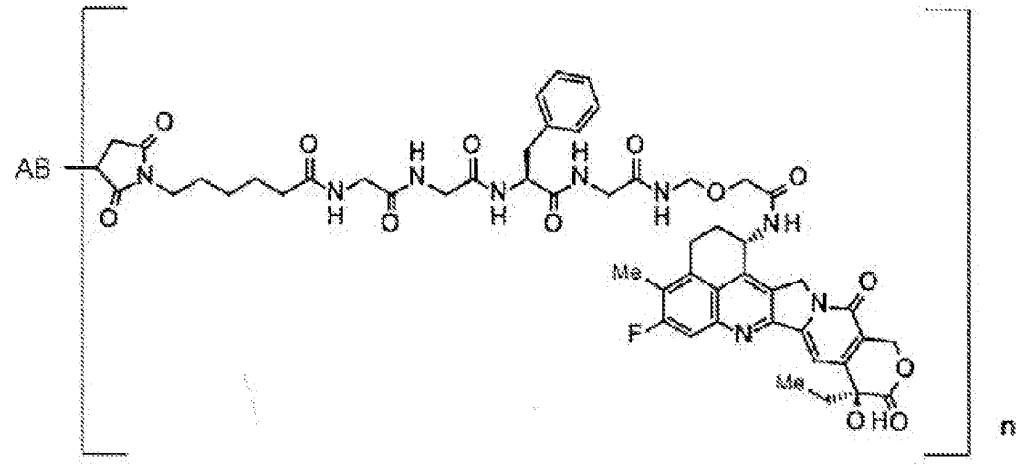


で示される構造を有する、請求項２７～３２のいずれか１項に記載の抗体－薬物コンジュゲート：
ここで、ＡＢは抗体又は該抗体の機能性断片を示す。ｎは抗体と結合している薬物－リンカー構造の１抗体あたりの平均結合数を示す。抗体とリンカーは抗体由来のスルフヒドリル基を介して結合している。

【請求項３４】

以下の式：

[化4]



で示される構造を有する、請求項２７～３２のいずれか１項に記載の抗体－薬物コンジュゲート：

ここで、ＡＢは抗体又は該抗体の機能性断片を示す。ｎは抗体と結合している薬物ーリンカー構造の１抗体あたりの平均結合数を示す。抗体とリンカーは抗体由来のスルフヒドリル基を介して結合している。

【請求項３５】

抗体が以下の（１）～（４）：

（１）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖、

（２）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖、

（３）配列番号６５の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７３の２０～４７１番目のアミノ酸配列からなる重鎖、又は

（４）配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖、

からなる群から選択されるいずれか１つに記載の軽鎖及び重鎖を含む抗体又は当該抗体の機能性断片である、請求項２７～３４のいずれか１項に記載の抗体ー薬物コンジュゲート。

【請求項３６】

抗体が、配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号６９の２０～４７１番目のアミノ酸配列からなる重鎖を含む抗体又は当該抗体の機能性断片である、請求項３５に記載の抗体ー薬物コンジュゲート。

【請求項３７】

抗体が、配列番号６１の２１～２３３番目のアミノ酸配列からなる軽鎖及び配列番号７７の２０～４７１番目のアミノ酸配列からなる重鎖を含む抗体又は当該抗体の機能性断片である、請求項３５に記載の抗体ー薬物コンジュゲート。

【請求項３８】

重鎖又は軽鎖が、Ｎー結合への糖鎖付加、Ｏー結合への糖鎖付加、Ｎ末のプロセッシング、Ｃ末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、Ｎ末にメチオニン残基の付加、プロリン残基のアミド化、Ｎ末グルタミンもしくはＮ末グルタミン酸のピログルタミン酸化及びカルボキシル末端における１つ又は２つのアミノ酸欠失からなる群から選択される１又は２以上の修飾をうけた、請求項２７～３７のいずれか１項に記載の抗体ー薬物コンジュゲート。

【請求項３９】

選択された１種の薬物ーリンカー構造の１抗体あたりの平均結合数が１～１０個の範囲である請求項２７～３８のいずれか１項に記載の抗体ー薬物コンジュゲート。

【請求項４０】

選択された１種の薬物ーリンカー構造の１抗体あたりの平均結合数が２～８個の範囲である請求項３９に記載の抗体ー薬物コンジュゲート。

【請求項４１】

選択された１種の薬物－リンカー構造の１抗体あたりの平均結合数が５～８個の範囲である請求項４０に記載の抗体－薬物コンジュゲート。

【請求項４２】

選択された１種の薬物－リンカー構造の１抗体あたりの平均結合数が７～８個である請求項４１に記載の抗体－薬物コンジュゲート。

【請求項４３】

請求項２７～４２のいずれか１項に記載の抗体－薬物コンジュゲート、その塩、又はそれらの水和物を含むことを特徴とする医薬組成物。

【請求項４４】

抗腫瘍薬であることを特徴とする、請求項４３に記載の医薬組成物。

【請求項４５】

腫瘍がＣＤＨ６を発現する腫瘍であることを特徴とする、請求項４４に記載の医薬組成物。

【請求項４６】

腫瘍が、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巣癌、卵巣漿液性腺癌、甲状腺癌、胆管癌、肺癌、小細胞肺癌、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫であることを特徴とする、請求項４４又は４５に記載の医薬組成物。

【請求項４７】

請求項１～１１及び請求項２２～２６からなる群から選択されるいずれか１項に記載の抗体又は当該抗体の機能性断片或いは請求項２１に記載の製造方法で得られる抗体又は当該抗体の機能性断片と、薬物－リンカー中間化合物を反応させる工程を含むことを特徴とする、抗体－薬物コンジュゲートの製造方法。

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Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	098065-0249-IDS_C_SB08.pdf	391753 f91aef1bd4dd088c169febe4860c721267074ae2	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	C1--WO_2018_185618A1.pdf	10395469 d812826942 99e07dd8bba6a9b5b49766b2f6b1b8	no	197
Warnings:					
Information:					
4	Foreign Reference	C2--WO_2018_212136A1.pdf	17433170 fcdb1b627edf544ef4dbd666e1e597c8bf00d217	no	465
Warnings:					
Information:					
5	Non Patent Literature	C3--CARL_U__BIALUCHA_ET_AL__Discovery_and_O.pdf	2836754 957568999d2a046a2124e5898fa85f0d951dd99a	no	17
Warnings:					
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6	Non Patent Literature	C4--CORADA_M_ET_AL___Monoclonal_antibodies_d.pdf	718730 f464bfc1b0b48d955d49d80d383dbe48c21669b7	no	6
Warnings:					
Information:					
7	Non Patent Literature	C5--Extended_European_Search_Report_dated_Ja.pdf	501670 ca4da30304b8abc339ff2786ba293d6e53af4869	no	13
Warnings:					
Information:					
8	Non Patent Literature	C6--Office_Action_issued_on_October_7__2020_.pdf	1101744 2d1fb32491419ae7f272cff2f2094a683e420b9f	no	26
Warnings:					
Information:					

Total Files Size (in bytes):	33748566

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Atty. Dkt. No. 098065-0249

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Atsuko SAITO
Title:	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE
Application No.:	16/613,203
International Filing Date:	5/14/2018
371(c) Date:	11/13/2019
Examiner:	NOT YET ASSIGNED
Art Unit:	1643
Confirmation No.:	6337

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR §1.56

Commissioner:

Applicant submits herewith documents for the Examiner's consideration in accordance with 37 CFR §§1.56, 1.97 and 1.98.

Applicant respectfully requests that each listed document be considered by the Examiner and be made of record in the present application and that an initialed copy of Form PTO/SB/08 be returned in accordance with MPEP §609.

The submission of any document herewith is not an admission that such document constitutes prior art against the claims of the present application or that such document is considered material to patentability as defined in 37 CFR §1.56(b). Applicant does not waive any rights to take any action which would be appropriate to antedate or otherwise remove as a competent reference any document submitted herewith.

Atty. Dkt. No. 098065-0249

CONCISE EXPLANATION OF RELEVANCE

Reference C1, C3, and C4 were cited in an Extended European Search Report ("EESR") dated January 19, 2021 for corresponding European Patent Application No. 18802536.5. The EESR is submitted as Reference C5.

Reference C6 is the Office Action, along with English translations, issued on October 7, 2020 for corresponding Japanese Patent Application No. 2019-518773.

TIMING OF THE DISCLOSURE

The listed documents are being submitted in compliance with 37 CFR §1.97(b), before the mailing date of the first Office Action on the merits.

Although Applicant believes that no fee is required, the Commissioner is hereby authorized to charge any additional fees that may be due to Deposit Account No. 19-0741.

Respectfully submitted,

Date 2021-02-16

By /Michael D. Kaminski/

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Telephone: (202) 672-5490
Facsimile: (202) 672-5399

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/613,203	11/13/2019	Atsuko SAITO	098065-0249	6337

22428 7590 11/12/2021

FOLEY & LARDNER LLP
3000 K STREET N.W.
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WASHINGTON, DC 20007-5109

EXAMINER	
CENTRAL, DOCKET	

ART UNIT	PAPER NUMBER
OPAP	

NOTIFICATION DATE	DELIVERY MODE
11/12/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipdocketing@foley.com

Office Action Summary	Application No. 16/613,203	Applicant(s) SAITO et al.	
	Examiner Patricia Duffy	Art Unit 1645	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-28 and 53-54 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☐ Claim(s) _____ is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☑ Claim(s) 1-28 and 53-54 are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claims 1-28, 53 and 54 are pending.

Election/Restriction

REQUIREMENT FOR UNITY OF INVENTION

As provided in 37 CFR 1.475(a), a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in a national stage application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

The determination whether a group of inventions is so linked as to form a single general inventive concept shall be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim. See 37 CFR 1.475(e).

When Claims Are Directed to Multiple Categories of Inventions:

As provided in 37 CFR 1.475 (b), a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

(1) A product and a process specially adapted for the manufacture of said product; or

(2) A product and a process of use of said product; or

(3) A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or

(4) A process and an apparatus or means specifically designed for carrying out the said process; or

(5) A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

Otherwise, unity of invention might not be present. See 37 CFR 1.475 (c).

Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 1-13 and 24-28, drawn to antibodies that bind SEQ ID NO:4.

Group II, claim(s) 14-26, drawn to polynucleotides, vectors, host cells encoding antibodies that bind SEQ ID NO:4.

Group III, claim(s) 53 and 54, drawn to methods of making antibody drug conjugates.

Species Election

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

The species are as follows:

For Groups I, III and III: Applicants are required to elect a single antibody species defined by light chain and heavy chain CDRs 1, 2 and 3 by SEQ ID NO. Applicants are also directed to indicate what light and heavy chain SEQ ID NOS read on the elected combination of CDRs.

Applicant is required, in reply to this action, to elect a single species to which the claims shall be restricted if no generic claim is finally held to be allowable. The reply must also identify the claims readable on the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered non-responsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise require all the limitations of an allowed generic claim. Currently, the following claim(s) are generic: 1, 24-28, 53 and 54.

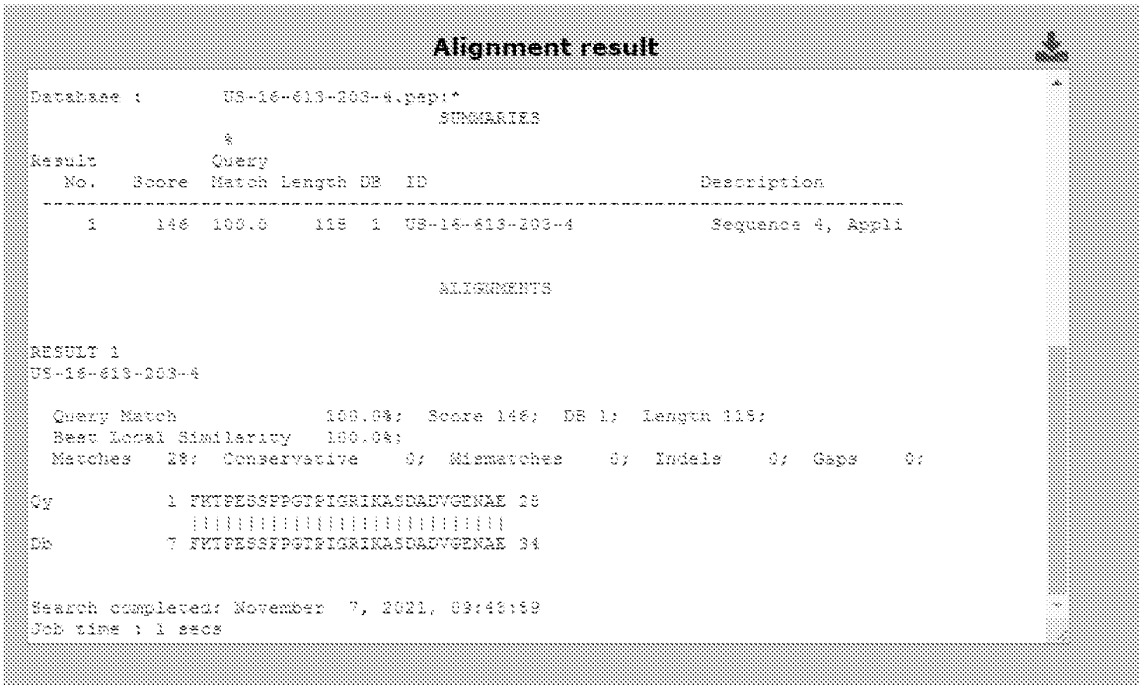
The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I and II lack unity of invention because the groups do not share the same or corresponding technical feature. In the instant case the groups are drawn to different chemical structures.

Groups I and III also lack unity of invention because even though the inventions of these groups require the technical feature of an antibody that binds SEQ ID NO:4, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of WO 2016/024195.

WO 2016/024195 A1 teaches an anti-CDH6 antibody drug conjugate (see, e.g., Abstract). The anti-CDH6 antibody binds specifically to the extracellular domain of human CDH6 (Table 1 on page 97), which comprise the amino acid sequence of SEQ ID NO: 4 of the present application (see sequence alignment below). In particular, WO 2016/024195 A1 teaches an anti-CDH6 antibody, NOV0719, which binds to the epitope of FKTPESSPPGTPIGRIKASDADVGENAE (SEQ ID NO: 342). The epitope bound by NOV0719

falls within the amino acid sequence of SEQ ID NO: 4 and corresponds to amino acid residues 7-34 of SEQ ID NO: 4 of the present application.



WO 2016/024195 A1 also teaches expression of CDH6 in renal cell carcinoma and ovarian cancer (page 2, paragraph [0004]). WO 2016/024195 A1 further teaches that an antibody drug conjugate can be used for the delivery of cytotoxic agent for cancer therapy (page 2, paragraph [0005]). As such, the technical feature does not define over the art and the groups of inventions are not linked by a “special technical feature”.

Joint Inventorship

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be corrected in compliance with 37 CFR 1.48(a) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. A request to correct inventorship under 37 CFR 1.48(a) must be accompanied by an

application data sheet in accordance with 37 CFR 1.76 that identifies each inventor by his or her legal name and by the processing fee required under 37 CFR 1.17(i).

Rejoinder

The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Patricia Duffy whose telephone number is (571)272-0855. The examiner can normally be reached 8:00 am - 4 pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Patricia Duffy/
Primary Examiner, Art Unit 1645

Notice of References Cited	Application/Control No. 16/613,203	Applicant(s)/Patent Under Reexamination SAITO et al.	
	Examiner Patricia Duffy	Art Unit 1645	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A					
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N	2016024195	02-2016	WIPO	Bialucha et al	
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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International Bureau

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WIPO | PCT

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A61K 39/395 (2006.01) *A61K 31/00* (2006.01)

(21) International Application Number:
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(22) International Filing Date:
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(25) Filing Language: English

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62/036,382 12 August 2014 (12.08.2014) US

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(74) Agent: **NOVARTIS AG;** Lichtstrasse 35, 4056 Basel (CH).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2016/024195 A1

(54) **Title:** ANTI-CDH6 ANTIBODY DRUG CONJUGATES

(57) **Abstract:** The present invention relates to anti-CDH6 antibodies, antibody fragments, antibody drug conjugates, and their uses for the treatment of cancer.

ANTI-CDH6 ANTIBODY DRUG CONJUGATES

FIELD OF THE INVENTION

[0001] The present disclosure is directed to anti-CDH6 antibodies, antibody fragments, antibody drug conjugates, and their uses for the treatment of cancer.

BACKGROUND OF THE INVENTION

[0002] Cadherins are a family of cell adhesion molecules involved in cell-cell contact. There are more than 30 cadherin molecules in the family which consists of subclasses with individual binding specificities. There are well studied classic cadherins such as epithelial (E) cadherin, neural (N) cadherin and placental (P) cadherin which play key roles in the development and maintenance of tissues such as the epithelium. The cadherin family has also been classified by their amino acid similarities, which separate the cadherins into two groups, type I and type II. Then there are tissue specific cadherins such as cadherin-6 (CDH6).

[0003] CDH6 was first cloned and characterized in 1995 (Shimoyama et al., Cancer Res. 1995: 55 (10)2206-221 1). The CDH6 cDNA isolated by Shimoyama was 4315 nucleotides and coded for a cadherin protein of 790 amino acids and exhibited 97% homology with rat K-cadherin (Shimoyama, *supra).* CDH6 is a type II cadherin with five extracellular cadherin repeats, a transmembrane domain and a cytoplasmic domain. In probing for normal tissue expression, CDH6 was detected in brain, cerebellum and kidney, with weaker expression in the lung, pancreas and gastric mucosa. In the nervous system, CDH6 was found to demarcate the auditory and somatosensory systems (Inoue et al., Mol. Cell Neuro. 2008: (39) 95-104). It was demonstrated that CDH6 is expressed by a subset of retinal ganglion cells (RGCs) in the eye responsible for such vision qualities as brightness, direction of motion or edges (Osterhout et al., Neuron 201 1: 71(4)632-639). In the CDH6 knockout mouse, the absence of CDH6 cause loss of axon targeting. The CDH6 mutant axons elongated properly and were appropriately guided to their targets, but then grew through and past their connections (Osterhout, *supra).* In another study, CDH6 knockout mice demonstrated defects in vocalization (Nakagawa eta la., PLOS 2012: 7(1 1) e49233).

[0004] Turning to its expression in cancer, the CDH6 cDNA was cloned from a hepatocellular carcinoma cell line, which was an early indication it may be involved in this type of cancer (Shimoyama, *supra).* The early work found CDH6 expression in several hepatoma lines, but

no expression in normal liver. In later work, a group analyzed 216 patients with renal cell carcinoma and found that CDH6 expression correlated with this type of cancer (Paul et al., J Urology 2004 (171): 97-101). Shimazui found that renal cell carcinoma patients who expressed CDH6 and lacked E-cadherin expression had a poor prognosis (Shimazui et al., Clin. Cancer Res. 1998 (4): 2419-2424). It was also discovered that CDH6 was a TGF-B target and plays a role in thyroid cancer (Sancisi et al., PLoS One 2013; 8(9): e75489). Lastly, CDH6 was shown to be a biomarker for ovarian cancer (Kobel et al., PLoS One 2008 5(12): e232).

[0005] Antibody drug conjugates ("ADCs") have been used for the local delivery of cytotoxic agents in the treatment of cancer (see *e.g.,* Lambert, Curr. Opinion In Pharmacology 5:543-549, 2005). ADCs allow targeted delivery of the drug moiety where maximum efficacy with minimal toxicity may be achieved. As more ADCs show promising clinical results, there is an increased need to develop new therapeutics for cancer therapy.

SUMMARY OF THE INVENTION

[0006] An antibody drug conjugate of the formula:

$$Ab\text{-}(L\text{-}(D)_m)_n$$

or a pharmaceutically acceptable salt thereof; wherein

Ab is an antibody or antigen binding fragment thereof that specifically binds to an epitope of human CDH6;

L is a linker;

D is a drug moiety;

m is an integer from 1 to 8; and

n is an integer from 1 to 10.

[0007] The antibody drug conjugate according of any of the preceding embodiments, wherein said n is 3 or 4.

[0008] The antibody drug conjugate of any of the preceding embodiments, wherein said antibody or antigen binding fragment thereof specifically binds the extracellular domain of CDH6 (SEQ ID NO:4).

[0009] The antibody drug conjugate of any of the preceding embodiments, wherein said antibody or antigen binding fragment specifically binds to an epitope of human CDH6 at SEQ ID NO:534.

[0010] The antibody drug conjugate of any of the preceding embodiments, wherein said antibody or antigen binding fragment thereof comprises:

(i) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:224, (b) a LCDR2 of SEQ ID NO:225, (c) a LCDR3 of SEQ ID NO:226; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 227, (e) a HCDR2 of SEQ ID NO: 228, and (f) a HCDR3 of SEQ ID NO:229;

(ii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:210, (b) a LCDR2 of SEQ ID NO:211, (c) a LCDR3 of SEQ ID NO:212; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:213, (e) a HCDR2 of SEQ ID NO: 214, and (f) a HCDR3 of SEQ ID NO:215;

(iii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:266, (b) a LCDR2 of SEQ ID NO:267, (c) a LCDR3 of SEQ ID NO:268; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 269, (e) a HCDR2 of SEQ ID NO:270, and (f) a HCDR3 of SEQ ID NO: 271;

(iv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:308, (b) a LCDR2 of SEQ ID NO:309, (c) a LCDR3 of SEQ ID NO:310; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:311, (e) a HCDR2 of SEQ ID NO:312, and (f) a HCDR3 of SEQ ID NO:313;

(v) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 14, (b) a LCDR2 of SEQ ID NO: 15, (c) a LCDR3 of SEQ ID NO: 16; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 17, (e) a HCDR2 of SEQ ID NO: 18, and (f) a HCDR3 of SEQ ID NO: 19;

(vi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:28, (b) a LCDR2 of SEQ ID NO:29, (c) a LCDR3 of SEQ ID NO:30; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:31, (e) a HCDR2 of SEQ ID NO:32, and (f) a HCDR3 of SEQ ID NO:33;

(vii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:42, (b) a LCDR2 of SEQ ID NO:43, (c) a LCDR3 of SEQ ID NO:44; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:45, (e) a HCDR2 of SEQ ID NO:46, and (f) a HCDR3 of SEQ ID NO:47;

(viii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:56, (b) a LCDR2 of SEQ ID NO:57, (c) a LCDR3 of SEQ ID NO:58; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO:59, (e) a HCDR2 of SEQ ID NO:60, and (f) a HCDR3 of SEQ ID NO:61;

(ix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:70, (b) a LCDR2 of SEQ ID NO:71, (c) a LCDR3 of SEQ ID NO:72; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO:73, (e) a HCDR2 of SEQ ID NO:74, and (f) a HCDR3 of SEQ ID NO:75;

(x) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:84, (b) a LCDR2 of SEQ ID NO:85, (c) a LCDR3 of SEQ ID NO:86; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO:87, (e) a HCDR2 of SEQ ID NO: 88, and (f) a HCDR3 of SEQ ID NO: 89;

(xi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:98, (b) a LCDR2 of SEQ ID NO:99, (c) a LCDR3 of SEQ ID NO: 100; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 101, (e) a HCDR2 of SEQ ID NO: 102, and (f) a HCDR3 of SEQ ID NO: 103;

(xii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 112, (b) a LCDR2 of SEQ ID NO: 113, (c) a LCDR3 of SEQ ID NO: 114; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 115, (e) a HCDR2 of SEQ ID NO: 116, and (f) a HCDR3 of SEQ ID NO: 117;

(xiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 126, (b) a LCDR2 of SEQ ID NO: 127, (c) a LCDR3 of SEQ ID NO: 128; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 129, (e) a HCDR2 of SEQ ID NO: 130, and (f) a HCDR3 of SEQ ID NO: 131;

(xiv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 140, (b) a LCDR2 of SEQ ID NO: 141, (c) a LCDR3 of SEQ ID NO: 142; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 143, (e) a HCDR2 of SEQ ID NO: 144, and (f) a HCDR3 of SEQ ID NO: 145;

(xv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 154, (b) a LCDR2 of SEQ ID NO: 155, (c) a LCDR3 of SEQ ID NO: 156; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 157, (e) a HCDR2 of SEQ ID NO: 158, and (f) a HCDR3 of SEQ ID NO: 159;

(xvi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 168, (b) a LCDR2 of SEQ ID NO: 169, (c) a LCDR3 of SEQ ID NO: 170; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 171, (e) a HCDR2 of SEQ ID NO: 172, and (f) a HCDR3 of SEQ ID NO: 173;

(xvii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 182, (b) a LCDR2 of SEQ ID NO: 183, (c) a LCDR3 of SEQ ID NO: 184; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 185, (e) a HCDR2 of SEQ ID NO: 186, and (f) a HCDR3 of SEQ ID NO: 187;

(xviii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 196, (b) a LCDR2 of SEQ ID NO: 197, (c) a LCDR3 of SEQ ID NO: 198; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 199, (e) a HCDR2 of SEQ ID NO:200, and (f) a HCDR3 of SEQ ID NO:201;

(xix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:238, (b) a LCDR2 of SEQ ID NO:239, (c) a LCDR3 of SEQ ID NO:240; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:241, (e) a HCDR2 of SEQ ID NO:242, and (f) a HCDR3 of SEQ ID NO:243;

(xx) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:252, (b) a LCDR2 of SEQ ID NO:253, (c) a LCDR3 of SEQ ID NO:254; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:255, (e) a HCDR2 of SEQ ID NO:256, and (f) a HCDR3 of SEQ ID NO:257;

(xxi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:280, (b) a LCDR2 of SEQ ID NO:281, (c) a LCDR3 of SEQ ID NO:282; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:283, (e) a HCDR2 of SEQ ID NO:284, and (f) a HCDR3 of SEQ ID NO:285;

(xxii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:294, (b) a LCDR2 of SEQ ID NO:295, (c) a LCDR3 of SEQ ID NO:296; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:297, (e) a HCDR2 of SEQ ID NO:298, and (f) a HCDR3 of SEQ ID NO:299; or

(xxiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:322, (b) a LCDR2 of SEQ ID NO:323, (c) a LCDR3 of SEQ ID NO:324; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:325, (e) a HCDR2 of SEQ ID NO:326, and (f) a HCDR3 of SEQ ID NO:327.

[0011] The antibody drug conjugate of any of the preceding embodiments, in which at least one amino acid within a CDR is substituted by a corresponding residue of a corresponding CDR of another anti-CDH6 antibody in Table 5 or 6.

[0012] The antibody drug conjugate of any of the preceding embodiments, in which one or two amino acids within a CDR have been modified, deleted or substituted.

[0013] The antibody drug conjugate of any of the preceding embodiments, wherein said antibody or antigen binding fragment thereof comprises:

(i) a heavy chain variable region (vH) that comprises SEQ ID NO: 230, and a light chain variable region (vL) that comprises SEQ ID NO:23 1;

(ii) a heavy chain variable region (vH) that comprises SEQ ID NO: 216, and a light chain variable region (vL) that comprises SEQ ID NO:217;

(iii) a heavy chain variable region (vH) that comprises SEQ ID NO: 272, and a light chain variable region (vL) that comprises SEQ ID NO:273;

(iv) a heavy chain variable region (vH) that comprises SEQ ID NO:3 14, and a light chain variable region (vL) that comprises SEQ ID NO:3 15;

(v) a heavy chain variable region (vH) that comprises SEQ ID NO: 20, and a light chain variable region (vL) that comprises SEQ ID NO:21;

(vi) a heavy chain variable region (vH) that comprises SEQ ID NO: 34, and a light chain variable region (vL) that comprises SEQ ID NO:35;

(vii) a heavy chain variable region (vH) that comprises SEQ ID NO:48, and a light chain variable region (vL) that comprises SEQ ID NO:49;

(viii) a heavy chain variable region (vH) that comprises SEQ ID NO:62, and a light chain variable region (vL) that comprises SEQ ID NO:63;

(ix) a heavy chain variable region (vH) that comprises SEQ ID NO: 76. and a light chain variable region (vL) that comprises SEQ ID NO:77;

(x) a heavy chain variable region (vH) that comprises SEQ ID NO:90, and a light chain variable region (vL) that comprises SEQ ID NO:91;

(xi) a heavy chain variable region (vH) that comprises SEQ ID NO: 104. and a light chain variable region (vL) that comprises SEQ ID NO: 105;

(xii) a heavy chain variable region (vH) that comprises SEQ ID NO: 118, and a light chain variable region (vL) that comprises SEQ ID NO: 119;

(xiii) a heavy chain variable region (vH) that comprises SEQ ID NO: 132, and a light chain variable region (vL) that comprises SEQ ID NO: 133;

(xiv) a heavy chain variable region (vH) that comprises SEQ ID NO: 146, and a light chain variable region (vL) that comprises SEQ ID NO: 147;

(xv) a heavy chain variable region (vH) that comprises SEQ ID NO: 160, and a light chain variable region (vL) that comprises SEQ ID NO:161;

(xvi) a heavy chain variable region (vH) that comprises SEQ ID NO: 174, and a light chain variable region (vL) that comprises SEQ ID NO: 175;

(xvii) a heavy chain variable region (vH) that comprises SEQ ID NO: 188, and a light chain variable region (vL) that comprises SEQ ID NO: 189;

(xviii) a heavy chain variable region (vH) that comprises SEQ ID NO: 202. and a light chain variable region (vL) that comprises SEQ ID NO:203;

(xix) a heavy chain variable region (vH) that comprises SEQ ID NO: 244, and a light chain variable region (vL) that comprises SEQ ID NO:245;

(xx) a heavy chain variable region (vH) that comprises SEQ ID NO:258, and a light chain variable region (vL) that comprises SEQ ID NO:259;

(xxi) a heavy chain variable region (vH) that comprises SEQ ID NO:286, and a light chain variable region (vL) that comprises SEQ ID NO:287;

(xxii) a heavy chain variable region (vH) that comprises SEQ ID NO: 300, and a light chain variable region (vL) that comprises SEQ ID NO:301; or

(xxiii) a heavy chain variable region (vH) that comprises SEQ ID NO:328, and a light chain variable region (vL) that comprises SEQ ID NO:329.

[0014] The antibody drug conjugate of any of the preceding embodiments, wherein said antibody or antigen binding fragment thereof comprises:

(i) a heavy chain that comprises SEQ ID NO: 234, and a light chain that comprises SEQ ID NO:235;

(ii) a heavy chain that comprises SEQ ID NO: 220, and a light chain that comprises SEQ ID NO:22 1;

(iii) a heavy chain that comprises SEQ ID NO: 276, and a light chain that comprises SEQ ID NO:277;

(iv) a heavy chain that comprises SEQ ID NO:3 18, and a light chain that comprises SEQ ID NO:3 19;

(v) a heavy chain that comprises SEQ ID NO:24, and a light chain that comprises SEQ ID NO:25;

(vi) a heavy chain that comprises SEQ ID NO: 38, and a light chain that comprises SEQ ID NO:39;

(vii) a heavy chain that comprises SEQ ID NO:52, and a light chain that comprises SEQ ID NO:53;

(viii) a heavy chain that comprises SEQ ID NO:66, and a light chain that comprises SEQ ID NO:67;

(ix) a heavy chain that comprises SEQ ID NO:80, and a light chain that comprises SEQ ID NO:81;

(x) a heavy chain that comprises SEQ ID NO:94, and a light chain that comprises SEQ ID NO:95;

(xi) a heavy chain that comprises SEQ ID NO: 108, and a light chain that comprises SEQ ID NO: 109;

(xii) a heavy chain that comprises SEQ ID NO: 122, and a light chain that comprises SEQ ID NO: 123;

(xiii) a heavy chain that comprises SEQ ID NO: 136, and a light chain that comprises SEQ ID NO: 137;

(xiv) a heavy chain that comprises SEQ ID NO: 150, and a light chain that comprises SEQ ID NO: 151;

(xv) a heavy chain that comprises SEQ ID NO: 164, and a light chain that comprises SEQ ID NO: 165;

(xvi) a heavy chain that comprises SEQ ID NO: 178, and a light chain that comprises SEQ ID NO: 179;

(xvii) a heavy chain that comprises SEQ ID NO: 192, and a light chain that comprises SEQ ID NO: 193;

(xviii) a heavy chain that comprises SEQ ID NO:206, and a light chain that comprises SEQ ID NO:207;

(xix) a heavy chain that comprises SEQ ID NO:248, and a light chain that comprises SEQ ID NO:249;

(xx) a heavy chain that comprises SEQ ID NO:262, and a light chain that comprises SEQ ID NO:263;

(xxi) a heavy chain that comprises SEQ ID NO:290, and a light chain that comprises SEQ ID NO:291;

(xxii) a heavy chain that comprises SEQ ID NO:304, and a light chain that comprises SEQ ID NO:305; or

(xxiii) a heavy chain that comprises SEQ ID NO:332, and a light chain that comprises SEQ ID NO:333.

[0015] The antibody drug conjugate of any of the preceding embodiments that retains at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% identity over either the variable light or variable heavy region.

[0016] The antibody drug conjugate of any of the preceding embodiments, wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human engineered antibody, a human antibody, a single chain antibody (scFv) or an antibody fragment.

[0017] The antibody drug conjugate according to any of the preceding embodiments, wherein said linker (L) is selected from the group consisting of a cleavable linker, a non-cleavable linker, a hydrophilic linker, a procharged linker and a dicarboxylic acid based linker.

[0018] The antibody drug conjugate according to any of the preceding embodiments, wherein the linker is derived from a cross-linking reagent selected from the group consisting of: N-succinimidyl-4-(2-py ridyldithio)2-sulfo-butanoate (sulfo-SPDB), N-succinimidy 1-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl iodoacetate (SIA), N-

succimmidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N- sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC) or 2,5-dioxopyrrolidin-l-yl 17-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)-5,8, 11,14-tetraoxo-4,7, 10,13-tetraazaheptadecan-l-oate (CXl-1).

[0019] The antibody drug conjugate according to any of the preceding embodiments, wherein said linker is derived from N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanoate (sulfo-SPDB).

[0020] The antibody drug conjugate of any of the preceding embodiments, wherein said drug moiety (D) is selected from a group consisting of: a maytansinoid, a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

[0021] The antibody drug conjugate of any of the preceding embodiments, wherein the drug moiety is a maytansinoid.

[0022] The antibody drug conjugate of any of the preceding embodiments, wherein the maytansinoid is N(2')-deacetyl-N2-(4- mercapto-4-methyl- 1 -oxopentyl)-maytansine (DM4) or N(2')-deacetyl-N(2')-(3-mercapto-l-oxopropyl)-maytansine (DM1).

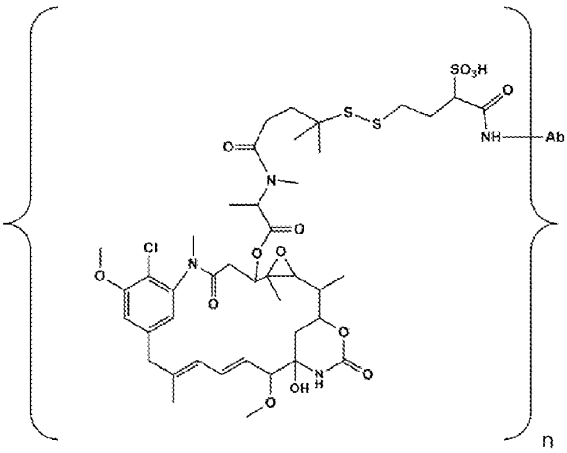
[0023] The antibody drug conjugate of any of the preceding embodiments in combination with another therapeutic agent.

[0024] The antibody drug conjugate of any of the preceding embodiments in combination with a therapeutic agent listed in Table 18.

[0025] The antibody drug conjugate of any of the preceding embodiments in combination with a BCL2 inhibitor, a BCL-XL inhibitor, a BCL2/BCL-XL inhibitor, an IAP inhibitor or a MEK inhibitor.

[0026] The antibody drug conjugate of any of the preceding embodiments in combination with an immune modulatory molecule.

[0027] An antibody drug conjugate of the formula:



Ab-suifo-SPDB-DM4

or a pharmaceutically acceptable salt thereof; wherein;

Ab is an antibody or antigen binding fragment thereof that specifically binds to human CDH6, and n is an integer from 1 to 10.

[0028] The antibody drug conjugate of any of the preceding embodiments, wherein said antibody or antigen binding fragment specifically binds to an epitope of human CDH6 at (SEQ ID NO:4).

[0029] The antibody drug conjugate of any of the preceding embodiments, wherein said antibody or antigen binding fragment thereof specifically binds human CDH6 at (SEQ ID NO:534).

[0030] The antibody drug conjugate of any of the preceding embodiments, wherein said Ab is an antibody or antigen binding fragment thereof comprises:

(i) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:224, (b) a LCDR2 of SEQ ID NO:225, (c) a LCDR3 of SEQ ID NO:226; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 227, (e) a HCDR2 of SEQ ID NO: 228, and (f) a HCDR3 of SEQ ID NO:229;

(ii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:210, (b) a LCDR2 of SEQ ID NO:211, (c) a LCDR3 of SEQ ID NO:212; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:213, (e) a HCDR2 of SEQ ID NO: 214, and (f) a HCDR3 of SEQ ID NO:215;

(iii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:266, (b) a LCDR2 of SEQ ID NO:267, (c) a LCDR3 of SEQ ID NO:268; and

a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 269, (e) a HCDR2 of SEQ ID NO:270, and (f) a HCDR3 of SEQ ID NO: 271;

(iv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:308, (b) a LCDR2 of SEQ ID NO:309, (c) a LCDR3 of SEQ ID NO:310; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:311, (e) a HCDR2 of SEQ ID NO:312, and (f) a HCDR3 of SEQ ID NO:313;

(v) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 14, (b) a LCDR2 of SEQ ID NO: 15, (c) a LCDR3 of SEQ ID NO: 16; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 17, (e) a HCDR2 of SEQ ID NO: 18, and (f) a HCDR3 of SEQ ID NO: 19;

(vi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:28, (b) a LCDR2 of SEQ ID NO:29, (c) a LCDR3 of SEQ ID NO:30; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:31, (e) a HCDR2 of SEQ ID NO:32, and (f) a HCDR3 of SEQ ID NO:33;

(vii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:42, (b) a LCDR2 of SEQ ID NO:43, (c) a LCDR3 of SEQ ID NO:44; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:45, (e) a HCDR2 of SEQ ID NO:46, and (f) a HCDR3 of SEQ ID NO:47;

(viii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:56, (b) a LCDR2 of SEQ ID NO:57, (c) a LCDR3 of SEQ ID NO:58; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:59, (e) a HCDR2 of SEQ ID NO:60, and (f) a HCDR3 of SEQ ID NO:61;

(ix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:70, (b) a LCDR2 of SEQ ID NO:71, (c) a LCDR3 of SEQ ID NO:72; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:73, (e) a HCDR2 of SEQ ID NO:74, and (f) a HCDR3 of SEQ ID NO:75;

(x) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:84, (b) a LCDR2 of SEQ ID NO:85, (c) a LCDR3 of SEQ ID NO:86; and a

heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO:87, (e) a HCDR2 of SEQ ID NO: 88, and (f) a HCDR3 of SEQ ID NO: 89;

(xi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:98, (b) a LCDR2 of SEQ ID NO:99, (c) a LCDR3 of SEQ ID NO: 100; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 101, (e) a HCDR2 of SEQ ID NO: 102, and (f) a HCDR3 of SEQ ID NO: 103;

(xii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 112, (b) a LCDR2 of SEQ ID NO: 113, (c) a LCDR3 of SEQ ID NO: 114; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 115, (e) a HCDR2 of SEQ ID NO: 116, and (f) a HCDR3 of SEQ ID NO: 117;

(xiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 126, (b) a LCDR2 of SEQ ID NO: 127, (c) a LCDR3 of SEQ ID NO: 128; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 129, (e) a HCDR2 of SEQ ID NO: 130, and (f) a HCDR3 of SEQ ID NO: 131;

(xiv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 140, (b) a LCDR2 of SEQ ID NO: 141, (c) a LCDR3 of SEQ ID NO: 142; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 143, (e) a HCDR2 of SEQ ID NO: 144, and (f) a HCDR3 of SEQ ID NO: 145;

(xv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 154, (b) a LCDR2 of SEQ ID NO: 155, (c) a LCDR3 of SEQ ID NO: 156; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 157, (e) a HCDR2 of SEQ ID NO: 158, and (f) a HCDR3 of SEQ ID NO: 159;

(xvi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 168, (b) a LCDR2 of SEQ ID NO: 169, (c) a LCDR3 of SEQ ID NO: 170; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 171, (e) a HCDR2 of SEQ ID NO: 172, and (f) a HCDR3 of SEQ ID NO: 173;

(xvii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 182, (b) a LCDR2 of SEQ ID NO: 183, (c) a LCDR3 of SEQ ID NO: 184; and

a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 185, (e) a HCDR2 of SEQ ID NO: 186, and (f) a HCDR3 of SEQ ID NO: 187;

(xviii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 196, (b) a LCDR2 of SEQ ID NO: 197, (c) a LCDR3 of SEQ ID NO: 198; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 199, (e) a HCDR2 of SEQ ID NO:200, and (f) a HCDR3 of SEQ ID NO:201;

(xix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:238, (b) a LCDR2 of SEQ ID NO:239, (c) a LCDR3 of SEQ ID NO:240; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:241, (e) a HCDR2 of SEQ ID NO:242, and (f) a HCDR3 of SEQ ID NO:243;

(xx) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:252, (b) a LCDR2 of SEQ ID NO:253, (c) a LCDR3 of SEQ ID NO:254; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:255, (e) a HCDR2 of SEQ ID NO:256, and (f) a HCDR3 of SEQ ID NO:257;

(xxi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:280, (b) a LCDR2 of SEQ ID NO:281, (c) a LCDR3 of SEQ ID NO:282; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:283, (e) a HCDR2 of SEQ ID NO:284, and (f) a HCDR3 of SEQ ID NO:285;

(xxii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:294, (b) a LCDR2 of SEQ ID NO:295, (c) a LCDR3 of SEQ ID NO:296; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:297, (e) a HCDR2 of SEQ ID NO:298, and (f) a HCDR3 of SEQ ID NO:299; or

(xxiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:322, (b) a LCDR2 of SEQ ID NO:323, (c) a LCDR3 of SEQ ID NO:324; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:325, (e) a HCDR2 of SEQ ID NO:326, and (f) a HCDR3 of SEQ ID NO:327.

[0031] The antibody drug conjugate of any of the preceding embodiments in which at least one amino acid within a CDR is substituted by a corresponding residue of a corresponding CDR of another anti-CDH6 antibody in Table 5 or 6.

[0032] The antibody drug conjugate of any of the preceding embodiments in which one or two amino acids within a CDR have been modified, deleted or substituted.

[0033] The antibody drug conjugate of any of the preceding embodiments, wherein said antibody or antigen binding fragment thereof comprises:

(i) a heavy chain variable region (vH) that comprises SEQ ID NO: 230, and a light chain variable region (vL) that comprises SEQ ID NO:23 1;

(ii) a heavy chain variable region (vH) that comprises SEQ ID NO: 216, and a light chain variable region (vL) that comprises SEQ ID NO:217;

(iii) a heavy chain variable region (vH) that comprises SEQ ID NO: 272, and a light chain variable region (vL) that comprises SEQ ID NO:273;

(iv) a heavy chain variable region (vH) that comprises SEQ ID NO:3 14, and a light chain variable region (vL) that comprises SEQ ID NO: 3 15;

(v) a heavy chain variable region (vH) that comprises SEQ ID NO: 20, and a light chain variable region (vL) that comprises SEQ ID NO:21;

(vi) a heavy chain variable region (vH) that comprises SEQ ID NO: 34, and a light chain variable region (vL) that comprises SEQ ID NO:35;

(vii) a heavy chain variable region (vH) that comprises SEQ ID NO:48, and a light chain variable region (vL) that comprises SEQ ID NO:49;

(viii) a heavy chain variable region (vH) that comprises SEQ ID NO:62, and a light chain variable region (vL) that comprises SEQ ID NO:63;

(ix) a heavy chain variable region (vH) that comprises SEQ ID NO:76, and a light chain variable region (vL) that comprises SEQ ID NO:77;

(x) a heavy chain variable region (vH) that comprises SEQ ID NO: 90, and a light chain variable region (vL) that comprises SEQ ID NO:91;

(xi) a heavy chain variable region (vH) that comprises SEQ ID NO: 104, and a light chain variable region (vL) that comprises SEQ ID NO: 105;

(xii) a heavy chain variable region (vH) that comprises SEQ ID NO: 118, and a light chain variable region (vL) that comprises SEQ ID NO: 119;

(xiii) a heavy chain variable region (vH) that comprises SEQ ID NO: 132, and a light chain variable region (vL) that comprises SEQ ID NO: 133;

(xiv) a heavy chain variable region (vH) that comprises SEQ ID NO: 146. and a light chain variable region (vL) that comprises SEQ ID NO: 147;

(xv) a heavy chain variable region (vH) that comprises SEQ ID NO: 160, and a light chain variable region (vL) that comprises SEQ ID NO: 161;

(xvi) a heavy chain variable region (vH) that comprises SEQ ID NO: 174. and a light chain variable region (vL) that comprises SEQ ID NO: 175;

(xvii) a heavy chain variable region (vH) that comprises SEQ ID NO: 188, and a light chain variable region (vL) that comprises SEQ ID NO: 189;

(xviii) a heavy chain variable region (vH) that comprises SEQ ID NO: 202. and a light chain variable region (vL) that comprises SEQ ID NO:203;

(xix) a heavy chain variable region (vH) that comprises SEQ ID NO: 244, and a light chain variable region (vL) that comprises SEQ ID NO:245;

(xx) a heavy chain variable region (vH) that comprises SEQ ID NO:258, and a light chain variable region (vL) that comprises SEQ ID NO:259;

(xxi) a heavy chain variable region (vH) that comprises SEQ ID NO:286, and a light chain variable region (vL) that comprises SEQ ID NO:287;

(xxii) a heavy chain variable region (vH) that comprises SEQ ID NO:300, and a light chain variable region (vL) that comprises SEQ ID NO:301; or

(xxiii) a heavy chain variable region (vH) that comprises SEQ ID NO:328, and a light chain variable region (vL) that comprises SEQ ID NO:329.

[0034] The antibody drag conjugate of any of the preceding embodiments that retains at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% identity over either the variable light or variable heavy region.

[0035] The antibody drug conjugate of any of the preceding embodiments, wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human engineered antibody, a human antibody, a single chain antibody(scFv) or an antibody fragment.

[0036] The antibody drag conjugate of any of the preceding embodiments, wherein said n is an integer from 2 to 8.

[0037] The antibody drag conjugate of any of the preceding embodiments, wherein said n is an integer from 3 to 4.

[0038] The antibody drug conjugate of any of the preceding embodiments in combination with another therapeutic agent.

[0039] The antibody drug conjugate of any of the preceding embodiments in combination with a therapeutic agent listed in Table 18.

[0040] The antibody drug conjugate of any of the preceding embodiments in combination with a BCL2 inhibitor, a BCL-XL inhibitor, a BCL2/BCL-XL inhibitor, an IAP inhibitor or a MEK inhibitor.

[0041] The antibody drag conjugate of any of the preceding embodiments in combination with an immune modulatory molecule.

[0042] A pharmaceutical composition comprising the antibody drag conjugate of any of the preceding embodiments and a pharmaceutically acceptable carrier.

[0043] The pharmaceutical composition of any of the preceding embodiments wherein said composition is prepared as a lyophilisate.

[0044] The pharmaceutical composition of any of the preceding embodiments, wherein said lyophilisate comprises the antibody drag conjugate of any of the preceding embodiments, sodium succinate, and polysorbate 20.

[0045] A method of treating an CDH6 positive cancer in a patient in need thereof, comprising administering to said patient the antibody drag conjugate or the pharmaceutical composition.

[0046] The method wherein said cancer is selected from the group consisting of: ovarian cancer, renal cancer, hepatic cancer, soft tissue cancer, CNS cancers, thyroid cancer and cholangiocarcinoma.

[0047] The method wherein the antibody drag conjugate or the pharmaceutical composition is administered in combination with another therapeutic agent.

[0048] The method wherein the antibody drag conjugate or the pharmaceutical composition is administered in combination with a therapeutic listed in Table 18.

[0049] The antibody drug conjugate of any of the preceding embodiments in combination with a BCL2 inhibitor, a BCL-XL inhibitor, a BCL2/BCL-XL inhibitor, an IAP inhibitor or a MEK inhibitor.

[0050] The antibody drug conjugate of any of the preceding embodiments in combination with an immune modulatory molecule.

[0051] The antibody drug conjugate of any of the preceding embodiments for use as a medicament.

[0052] The antibody drug conjugate or the pharmaceutical composition thereof, for use in the treatment of a CDH6 positive cancer.

[0053] The antibody drug conjugate of any of the preceding embodiments, administered in combination with another therapeutic agent.

[0054] The antibody drug conjugate of any of the preceding embodiments administered in combination with a therapeutic agent listed in Table 18.

[0055] The antibody drug conjugate of any of the preceding embodiments in combination with a BCL2 inhibitor, a BCL-XL inhibitor, a BCL2/BCL-XL inhibitor, an IAP inhibitor or a MEK inhibitor.

[0056] The antibody drug conjugate of any of the preceding embodiments in combination with an immune modulatory molecule.

[0057] A nucleic acid that encodes the antibody or antigen binding fragment of any of the preceding embodiments.

[0058] A vector comprising the nucleic acid of any of the preceding embodiments.

[0059] A host cell comprising the vector according to any of the preceding embodiments.

[0060] A process for producing an antibody or antigen binding fragment comprising cultivating the host cell and recovering the antibody from the culture.

[0061] A process for producing an anti-CDH6 antibody drug conjugate, the process comprising:

(a) chemically linking N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanoate (sulfo-SPDB) to a drug moiety DM-4;

(b) conjugating said linker-drug to the antibody recovered from the cell culture; and

(c) purifying the antibody drug conjugate.

[0062] The antibody drug conjugate made according to any of the preceding embodiments having an average maytansinoid to antibody ratio (MAR), measured with a UV spectrophotometer, about 3.5.

[0063] An antibody or antigen binding fragment thereof that comprises:

(i) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:224, (b) a LCDR2 of SEQ ID NO:225, (c) a LCDR3 of SEQ ID NO:226; and

a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 227, (e) a HCDR2 of SEQ ID NO: 228, and (f) a HCDR3 of SEQ ID NO:229;

(ii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:210, (b) a LCDR2 of SEQ ID NO:211, (c) a LCDR3 of SEQ ID NO:212; and aheavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:213, (e) a HCDR2 of SEQ ID NO: 214, and (f) a HCDR3 of SEQ ID NO:215;

(iii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:266, (b) a LCDR2 of SEQ ID NO:267, (c) a LCDR3 of SEQ ID NO:268; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 269, (e) a HCDR2 of SEQ ID NO:270, and (f) a HCDR3 of SEQ ID NO: 271;

(iv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:308, (b) a LCDR2 of SEQ ID NO:309, (c) a LCDR3 of SEQ ID NO:310; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:311, (e) a HCDR2 of SEQ ID NO:312, and (f) a HCDR3 of SEQ ID NO:313;

(v) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 14, (b) a LCDR2 of SEQ ID NO: 15, (c) a LCDR3 of SEQ ID NO: 16; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 17, (e) a HCDR2 of SEQ ID NO: 18, and (f) a HCDR3 of SEQ ID NO: 19;

(vi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:28, (b) a LCDR2 of SEQ ID NO:29, (c) a LCDR3 of SEQ ID NO:30; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:31, (e) a HCDR2 of SEQ ID NO:32, and (f) a HCDR3 of SEQ ID NO:33;

(vii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:42, (b) a LCDR2 of SEQ ID NO:43, (c) a LCDR3 of SEQ ID NO:44; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:45, (e) a HCDR2 of SEQ ID NO:46, and (f) a HCDR3 of SEQ ID NO:47;

(viii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:56, (b) a LCDR2 of SEQ ID NO:57, (c) a LCDR3 of SEQ ID NO:58; and a

heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO:59, (e) a HCDR2 of SEQ ID NO:60, and (f) a HCDR3 of SEQ ID NO:61;

(ix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:70, (b) a LCDR2 of SEQ ID NO:71, (c) a LCDR3 of SEQ ID NO:72; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO:73, (e) a HCDR2 of SEQ ID NO:74, and (f) a HCDR3 of SEQ ID NO:75;

(x) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:84, (b) a LCDR2 of SEQ ID NO:85, (c) a LCDR3 of SEQ ID NO:86; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO:87, (e) a HCDR2 of SEQ ID NO: 88, and (f) a HCDR3 of SEQ ID NO: 89;

(xi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:98, (b) a LCDR2 of SEQ ID NO:99, (c) a LCDR3 of SEQ ID NO: 100; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 101, (e) a HCDR2 of SEQ ID NO: 102, and (f) a HCDR3 of SEQ ID NO: 103;

(xii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 112, (b) a LCDR2 of SEQ ID NO: 113, (c) a LCDR3 of SEQ ID NO: 114; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 115, (e) a HCDR2 of SEQ ID NO: 116, and (f) a HCDR3 of SEQ ID NO: 117;

(xiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 126, (b) a LCDR2 of SEQ ID NO: 127, (c) a LCDR3 of SEQ ID NO: 128; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 129, (e) a HCDR2 of SEQ ID NO: 130, and (f) a HCDR3 of SEQ ID NO: 131;

(xiv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 140, (b) a LCDR2 of SEQ ID NO: 141, (c) a LCDR3 of SEQ ID NO: 142; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 143, (e) a HCDR2 of SEQ ID NO: 144, and (f) a HCDR3 of SEQ ID NO: 145;

(xv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 154, (b) a LCDR2 of SEQ ID NO: 155, (c) a LCDR3 of SEQ ID NO: 156; and

a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 157, (e) a HCDR2 of SEQ ID NO: 158, and (f) a HCDR3 of SEQ ID NO: 159;

(xvi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 168, (b) a LCDR2 of SEQ ID NO: 169, (c) a LCDR3 of SEQ ID NO: 170; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 171, (e) a HCDR2 of SEQ ID NO: 172, and (f) a HCDR3 of SEQ ID NO: 173;

(xvii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 182, (b) a LCDR2 of SEQ ID NO: 183, (c) a LCDR3 of SEQ ID NO: 184; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 185, (e) a HCDR2 of SEQ ID NO: 186, and (f) a HCDR3 of SEQ ID NO: 187;

(xviii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 196, (b) a LCDR2 of SEQ ID NO: 197, (c) a LCDR3 of SEQ ID NO: 198; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO: 199, (e) a HCDR2 of SEQ ID NO:200, and (f) a HCDR3 of SEQ ID NO:201;

(xix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:238, (b) a LCDR2 of SEQ ID NO:239, (c) a LCDR3 of SEQ ID NO:240; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:241, (e) a HCDR2 of SEQ ID NO:242, and (f) a HCDR3 of SEQ ID NO:243;

(xx) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:252, (b) a LCDR2 of SEQ ID NO:253, (c) a LCDR3 of SEQ ID NO:254; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:255, (e) a HCDR2 of SEQ ID NO:256, and (f) a HCDR3 of SEQ ID NO:257;

(xxi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:280, (b) a LCDR2 of SEQ ID NO:281, (c) a LCDR3 of SEQ ID NO:282; and a heavy chain variable region that comprises: (d) a HCDRl of SEQ ID NO:283, (e) a HCDR2 of SEQ ID NO:284, and (f) a HCDR3 of SEQ ID NO:285;

(xxii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:294, (b) a LCDR2 of SEQ ID NO:295, (c) a LCDR3 of SEQ ID NO:296; and

a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:297, (e) a HCDR2 of SEQ ID NO:298, and (f) a HCDR3 of SEQ ID NO:299; or

(xxiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:322, (b) a LCDR2 of SEQ ID NO:323, (c) a LCDR3 of SEQ ID NO:324; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:325, (e) a HCDR2 of SEQ ID NO:326, and (f) a HCDR3 of SEQ ID NO:327.

[0064] The antibody or antigen binding fragment thereof of any of the preceding embodiments in which at least one amino acid within a CDR is substituted by a corresponding residue of a corresponding CDR of another anti-CDH6 antibody in Table 5 or 6.

[0065] The antibody or antigen binding fragment thereof of any of the preceding embodiments, wherein said antibody or antigen binding fragment thereof comprises:

(i) a heavy chain variable region (vH) that comprises SEQ ID NO: 230, and a light chain variable region (vL) that comprises SEQ ID NO:23 1;

(ii) a heavy chain variable region (vH) that comprises SEQ ID NO: 216, and a light chain variable region (vL) that comprises SEQ ID NO:217;

(iii) a heavy chain variable region (vH) that comprises SEQ ID NO: 272, and a light chain variable region (vL) that comprises SEQ ID NO:273;

(iv) a heavy chain variable region (vH) that comprises SEQ ID NO:3 14, and a light chain variable region (vL) that comprises SEQ ID NO:3 15;

(v) a heavy chain variable region (vH) that comprises SEQ ID NO: 20, and a light chain variable region (vL) that comprises SEQ ID NO:21;

(vi) a heavy chain variable region (vH) that comprises SEQ ID NO: 34, and a light chain variable region (vL) that comprises SEQ ID NO:35;

(vii) a heavy chain variable region (vH) that comprises SEQ ID NO:48, and a light chain variable region (vL) that comprises SEQ ID NO:49;

(viii) a heavy chain variable region (vH) that comprises SEQ ID NO:62, and a light chain variable region (vL) that comprises SEQ ID NO:63;

(ix) a heavy chain variable region (vH) that comprises SEQ ID NO:76, and a light chain variable region (vL) that comprises SEQ ID NO:77;

(x) a heavy chain variable region (vH) that comprises SEQ ID NO:90, and a light chain variable region (vL) that comprises SEQ ID NO:91;

(xi) a heavy chain variable region (vH) that comprises SEQ ID NO: 104, and a light chain variable region (vL) that comprises SEQ ID NO: 105;

(xii) a heavy chain variable region (vH) that comprises SEQ ID NO: 118, and a light chain variable region (vL) that comprises SEQ ID NO: 119;

(xiii) a heavy chain variable region (vH) that comprises SEQ ID NO: 132. and a light chain variable region (vL) that comprises SEQ ID NO: 133;

(xiv) a heavy chain variable region (vH) that comprises SEQ ID NO: 146, and a light chain variable region (vL) that comprises SEQ ID NO: 147;

(xv) a heavy chain variable region (vH) that comprises SEQ ID NO: 160. and a light chain variable region (vL) that comprises SEQ ID NO: 161;

(xvi) a heavy chain variable region (vH) that comprises SEQ ID NO: 174, and a light chain variable region (vL) that comprises SEQ ID NO: 175;

(xvii) a heavy chain variable region (vH) that comprises SEQ ID NO: 188, and a light chain variable region (vL) that comprises SEQ ID NO: 189;

(xviii) a heavy chain variable region (vH) that comprises SEQ ID NO: 202, and a light chain variable region (vL) that comprises SEQ ID NO:203;

(xix) a heavy chain variable region (vH) that comprises SEQ ID NO: 244, and a light chain variable region (vL) that comprises SEQ ID NO:245;

(xx) a heavy chain variable region (vH) that comprises SEQ ID NO:258, and a light chain variable region (vL) that comprises SEQ ID NO:259;

(xxi) a heavy chain variable region (vH) that comprises SEQ ID NO:286, and a light chain variable region (vL) that comprises SEQ ID NO:287;

(xxii) a heavy chain variable region (vH) that comprises SEQ ID NO: 300, and a light chain variable region (vL) that comprises SEQ ID NO:301; or

(xxiii) a heavy chain variable region (vH) that comprises SEQ ID NO:328, and a light chain variable region (vL) that comprises SEQ ID NO:329.

[0066] A diagnostic reagent comprising the antibody or antigen binding fragment thereof of any of the preceding embodiments which is labeled.

[0067] The diagnostic reagent of any of the preceding embodiments, wherein the label is selected from the group consisting of a radiolabel, a fluorophore, a chromophore, an imaging agent, and a metal ion.

Definitions

[0068] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[0069] The term "alkyl" refers to a monovalent saturated hydrocarbon chain having the specified number of carbon atoms. For example, C_{1-6} alkyl refers to an alkyl group having from 1 to 6 carbon atoms. Alkyl groups may be straight or branched. Representative branched alkyl groups have one, two, or three branches. Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl (n-propyl and isopropyl), butyl (n-butyl, isobutyl, sec-butyl, and t-butyl), pentyl (n-pentyl, isopentyl, and neopentyl), and hexyl.

[0070] The term "antibody" as used herein refers to a polypeptide of the immunoglobulin family that is capable of binding a corresponding antigen non-covalently, reversibly, and in a specific manner. For example, a naturally occurring IgG antibody is a tetramer comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CHI, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL

regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system *(e.g.,* effector cells) and the first component (Clq) of the classical complement system.

[0071] The term "antibody" includes, but is not limited to, monoclonal antibodies, human antibodies, humanized antibodies, camelid antibodies, chimeric antibodies, and anti-idiotypic (anti-Id) antibodies (including, *e.g.,* anti-Id antibodies to antibodies of the present disclosure). The antibodies can be of any isotype/class *(e.g.,* IgG, IgE, IgM, IgD, IgA and IgY), or subclass *(e.g.,* IgGl, IgG2, IgG3, IgG4, IgAl and IgA2).

[0072] "Complementarity -determining domains" or "complementary -determining regions" ("CDRs") interchangeably refer to the hypervariable regions of VL and VH. The CDRs are the target protein-binding site of the antibody chains that harbors specificity for such target protein. There are three CDRs (CDRl-3, numbered sequentially from the N-terminus) in each human VL or VH, constituting about 15-20% of the variable domains. CDRs can be referred to by their region and order. For example, "VHCDRl" or "HCDR1" both refer to the first CDR of the heavy chain variable region. The CDRs are structurally complementary to the epitope of the target protein and are thus directly responsible for the binding specificity. The remaining stretches of the VL or VH, the so-called framework regions, exhibit less variation in amino acid sequence (Kuby, Immunology, 4th ed., Chapter 4. W.H. Freeman & Co., New York, 2000).

[0073] The positions of the CDRs and framework regions can be determined using various well known definitions in the art, *e.g.,* Kabat, Chothia, and AbM (see, *e.g.,* Johnson *et al.,* Nucleic Acids Res., 29:205-206 (2001); Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987); Chothia *et al,* Nature, 342:877-883 (1989); Chothia *et al ,* J. Mol. Biol., 227:799-817 (1992); Al-Lazikani *et al ,* J.Mol.Biol., 273:927-748 (1997)). Definitions of antigen combining sites are also described in the following: Ruiz *et al ,* Nucleic Acids Res., 28:219-221 (2000); and Lefranc, M.P., Nucleic Acids Res., 29:207-209 (2001); MacCallum *et al ,* J. Mol. Biol., 262:732-745 (1996); and Martin *et al,* Proc. Natl. Acad. Sci. USA, 86:9268-9272 (1989); Martin *et al ,* Methods Enzymol., 203: 121-153 (1991); and

Rees *et al* , In Sternberg M.J.E. (ed.), Protein Structure Prediction, Oxford University Press, Oxford, 141-172 (1996).

[0074] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CHI, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention, the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminus is a variable region and at the C-terminus is a constant region; the CH3 and CL domains actually comprise the carboxy-terminal domains of the heavy and light chain, respectively.

[0075] The term "antigen binding fragment", as used herein, refers to a polypeptide including one or more portions of an antibody that retain the ability to specifically interact with *(e.g.,* by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. Examples of binding fragments include, but are not limited to, single-chain Fvs (scFv), disulfide-linked Fvs (sdFv), Fab fragments, F(ab') fragments, a monovalent fragment consisting of the VL, VH, CL and CHI domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CHI domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment (Ward *et al.,* Nature 341:544-546, 1989), which consists of a VH domain; and an isolated complementarity determining region (CDR), or other epitope-binding fragments of an antibody.

[0076] Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv ("scFv"); see, *e.g.,* Bird *et al,* Science 242:423-426, 1988; and Huston *et al,* Proc. Natl. Acad. Sci. 85:5879-5883, 1988). Such single chain antibodies are also intended to be encompassed within the term "antigen binding fragment." These antigen binding fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0077] Antigen binding fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-

scFv (see, *e.g.*, Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005). Antigen binding fragments can be grafted into scaffolds based on polypeptides such as fibronectin type III (Fn3) (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies).

[0078] Antigen binding fragments can be incorporated into single chain molecules comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata *et al,* Protein Eng. 8:1057-1062, 1995; and U.S. Pat. No. 5,641,870).

[0079] The term "monoclonal antibody" or "monoclonal antibody composition" as used herein refers to polypeptides, including antibodies and antigen binding fragments that have substantially identical amino acid sequence or are derived from the same genetic source. This term also includes preparations of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0080] The term "human antibody", as used herein, includes antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, *e.g.*, human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik *et al*, J. Mol. Biol. 296:57-86, 2000.

[0081] The human antibodies of the present disclosure can include amino acid residues not encoded by human sequences *(e.g.*, mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo, or a conservative substitution to promote stability or manufacturing).

[0082] The term "recognize" as used herein refers to an antibody or antigen binding fragment thereof that finds and interacts *(e.g.*, binds) with its epitope, whether that epitope is linear or conformational. The term "epitope" refers to a site on an antigen to which an antibody or antigen binding fragment of the disclosure specifically binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include techniques in the art, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance (see, *e.g.,*

Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, G. E. Morris, Ed. (1996)). A "paratope" is the part of the antibody which recognizes the epitope of the antigen.

[0083] The phrase "specifically binds" or "selectively binds," when used in the context of describing the interaction between an antigen *(e.g.,* a protein) and an antibody, antibody fragment, or antibody-derived binding agent, refers to a binding reaction that is determinative of the presence of the antigen in a heterogeneous population of proteins and other biologies, *e.g.,* in a biological sample, *e.g.,* a blood, serum, plasma or tissue sample. Thus, under certain designated immunoassay conditions, the antibodies or binding agents with a particular binding specificity bind to a particular antigen at least two times the background and do not substantially bind in a significant amount to other antigens present in the sample. In one aspect, under designated immunoassay conditions, the antibody or binding agent with a particular binding specificity binds to a particular antigen at least ten (10) times the background and does not substantially bind in a significant amount to other antigens present in the sample. Specific binding to an antibody or binding agent under such conditions may require the antibody or agent to have been selected for its specificity for a particular protein. As desired or appropriate, this selection may be achieved by subtracting out antibodies that cross-react with molecules from other species *(e.g.,* mouse or rat) or other subtypes. Alternatively, in some aspects, antibodies or antibody fragments are selected that cross-react with certain desired molecules.

[0084] The term "affinity" as used herein refers to the strength of interaction between antibody and antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody "arm" interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity.

[0085] The term "isolated antibody" refers to an antibody that is substantially free of other antibodies having different antigenic specificities. An isolated antibody that specifically binds to one antigen may, however, have cross-reactivity to other antigens. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0086] The term "corresponding human germline sequence" refers to the nucleic acid sequence encoding a human variable region amino acid sequence or subsequence that shares the highest determined amino acid sequence identity with a reference variable region amino acid sequence or subsequence in comparison to all other all other known variable region amino acid sequences encoded by human germline immunoglobulin variable region sequences. The corresponding human germline sequence can also refer to the human variable region amino acid sequence or subsequence with the highest amino acid sequence identity with a reference variable region amino acid sequence or

subsequence in comparison to all other evaluated variable region amino acid sequences. The corresponding human germline sequence can be framework regions only, complementarity determining regions only, framework and complementary determining regions, a variable segment (as defined above), or other combinations of sequences or subsequences that comprise a variable region. Sequence identity can be determined using the methods described herein, for example, aligning two sequences using BLAST, ALIGN, or another alignment algorithm known in the art. The corresponding human germline nucleic acid or amino acid sequence can have at least about 90%, 91, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference variable region nucleic acid or amino acid sequence.

[0087] A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, *e.g.,* Harlow & Lane, Using Antibodies, A Laboratory Manual (1998), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective binding reaction will produce a signal at least twice over the background signal and more typically at least 10 to 100 times over the background.

[0088] The term "equilibrium dissociation constant (KD, M)" refers to the dissociation rate constant (kd, time-1) divided by the association rate constant (ka, time-1, M-l). Equilibrium dissociation constants can be measured using any known method in the art. The antibodies of the present disclosure generally will have an equilibrium dissociation constant of less than about 10^{-7} or 10^{-8} M, for example, less than about 10^{-9} M or 10^{-10} M, in some aspects, less than about 10^{-11} M, 10^{-12} M or 10^{-13} M.

[0089] The term "bioavailability" refers to the systemic availability (i.e., blood/plasma levels) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates measurement of both the time (rate) and total amount (extent) of drug that reaches the general circulation from an administered dosage form.

[0090] As used herein, the phrase "consisting essentially of refers to the genera or species of active pharmaceutical agents included in a method or composition, as well as any excipients inactive for the intended purpose of the methods or compositions. In some aspects, the phrase "consisting essentially of expressly excludes the inclusion of one or more additional active agents other than an antibody drug conjugate of the present disclosure. In some aspects, the phrase "consisting essentially

of expressly excludes the inclusion of one or more additional active agents other than an antibody drug conjugate of the present disclosure and a second co-administered agent.

[0091] The term "amino acid" refers to naturally occurring, synthetic, and unnatural amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.,* hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.,* homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups *(e.g.,* norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0092] The term "conservatively modified variant" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0093] For polypeptide sequences, "conservatively modified variants" include individual substitutions, deletions or additions to a polypeptide sequence which result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing

functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles. The following eight groups contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, *e.g.,* Creighton, Proteins (1984)). In some aspects, the term "conservative sequence modifications" are used to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence.

[0094] The term "optimized" as used herein refers to a nucleotide sequence that has been altered to encode an amino acid sequence using codons that are preferred in the production cell or organism, generally a eukaryotic cell, for example, a yeast cell, a Pichia cell, a fungal cell, a Trichoderma cell, a Chinese Hamster Ovary cell (CHO) or a human cell. The optimized nucleotide sequence is engineered to retain completely or as much as possible the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the "parental" sequence.

[0095] The terms "percent identical" or "percent identity," in the context of two or more nucleic acids or polypeptide sequences, refers to the extent to which two or more sequences or subsequences that are the same. Two sequences are "identical" if they have the same sequence of amino acids or nucleotides over the region being compared. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 30 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

[0096] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the

percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0097] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482c (1970), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, *e.g.*, Brent *et al.*, Current Protocols in Molecular Biology, 2003).

[0098] Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al,* Nuc. Acids Res. 25:3389-3402, 1977; and Altschul *et al*, J. Mol. Biol. 215:403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide

sequences) uses as defaults a word length (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff, (1989) Proc. Natl. Acad. Sci. USA 89: 10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0099] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.*, Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5787, 1993). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[00100] The percent identity between two amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller, Comput. Appl. Biosci. 4:11-17, 1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch, J. Mol. Biol. 48:444-453, 1970) algorithm which has been incorporated into the GAP program in the GCG software package using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[00101] Other than percentage of sequence identity noted above, another indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[00102] The term "nucleic acid" is used herein interchangeably with the term "polynucleotide" and refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-

stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[00103] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof *(e.g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, as detailed below, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al,* (1991) Nucleic Acid Res. 19:5081; Ohtsuka *et al ,* (1985) J. Biol. Chem. 260:2605-2608; and Rossolini *et al,* (1994) Mol. Cell. Probes 8:91-98).

[00104] The term "operably linked" in the context of nucleic acids refers to a functional relationship between two or more polynucleotide *(e.g.*, DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, i.e., they are cis-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

[00105] The terms "polypeptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Unless otherwise indicated, a particular polypeptide sequence also implicitly encompasses conservatively modified variants thereof.

[00106] The term "immunoconjugate" or "antibody drug conjugate" as used herein refers to the linkage of an antibody or an antigen binding fragment thereof with another agent, such as a payload, drug moiety, chemotherapeutic agent, a toxin, an immunotherapeutic agent, an imaging probe, and the like. The linkage can be covalent bonds, or non-covalent interactions such as through

electrostatic forces. Various linkers, known in the art, can be employed in order to form the immunoconjugate. Additionally, the immunoconjugate can be provided in the form of a fusion protein that may be expressed from a polynucleotide encoding the immunoconjugate. As used herein, "fusion protein" refers to proteins created through the joining of two or more genes or gene fragments which originally coded for separate proteins (including peptides and polypeptides). Translation of the fusion gene results in a single protein with functional properties derived from each of the original proteins.

[00107] The term "subject" includes human and non-human animals. Non-human animals include all vertebrates, *e.g.* , mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

[00108] The term "toxin," "cytotoxin" or "cytotoxic agent" as used herein, refers to any agent that is detrimental to the growth and proliferation of cells and may act to reduce, inhibit, or destroy a cell or malignancy.

[00109] The term "anti-cancer agent" as used herein refers to any agent that can be used to treat a cell proliferative disorder such as cancer, including but not limited to, cytotoxic agents, chemotherapeutic agents, radiotherapy and radiotherapeutic agents, targeted anti-cancer agents, and immunotherapeutic agents.

[00110] The term "drug moiety" or "payload" as used herein refers to a chemical moiety that is conjugated to an antibody or antigen binding fragment, and can include any therapeutic or diagnostic agent, for example, an anti-cancer, anti-inflammatory, anti-infective *(e.g.,* anti-fungal, antibacterial, anti-parasitic, anti-viral), or an anesthetic agent. In certain aspects, a drug moiety is selected from a V-ATPase inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, an inhibitor of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a proteasome inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor. Methods for attaching each of these to a linker compatible with the antibodies and method of the present disclosure are known in the art. See, e.g., Singh et al., (2009) Therapeutic Antibodies: Methods and Protocols, vol. 525, 445-457. In addition, a payload can be a biophysical probe, a fluorophore, a spin label, an infrared probe, an affinity probe, a chelator, a spectroscopic probe, a radioactive probe, a lipid molecule, a polyethylene glycol, a polymer, a spin

label, DNA, RNA, a protein, a peptide, a surface, an antibody, an antibody fragment, a nanoparticle, a quantum dot, a liposome, a PLGA particle, a saccharide or a polysaccharide.

[00111] The term "maytansinoid drug moiety" means the substructure of an antibody-drug conjugate that has the structure of a maytansinoid compound. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Pat. No. 3,896,1 11). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and maytansinol analogues have been reported. See U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, and Kawai et al (1984) Chem. Pharm. Bull. 3441-3451, each of which are expressly incorporated by reference. Specific examples of maytansinoids useful for conjugation include DM1, DM3 and DM4.

[00112] "Tumor" refers to neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

[00113] The term "anti-tumor activity" means a reduction in the rate of tumor cell proliferation, viability, or metastatic activity. A possible way of showing anti-tumor activity is to show a decline in growth rate of tumor cells, tumor size stasis or tumor size reduction. Such activity can be assessed using accepted in vitro or in vivo tumor models, including but not limited to xenograft models, allograft models, MMTV models, and other known models known in the art to investigate anti-tumor activity.

[00114] The term "malignancy" refers to a non-benign tumor or a cancer. As used herein, the term "cancer" includes a malignancy characterized by deregulated or uncontrolled cell growth. Exemplary cancers include: carcinomas, sarcomas, leukemias and lymphomas.

[00115] The term "cancer" includes primary malignant tumors *(e.g.,* those whose cells have not migrated to sites in the subject's body other than the site of the original tumor) and secondary malignant tumors *(e.g.,* those arising from metastasis, the migration of tumor cells to secondary sites that are different from the site of the original tumor).

[00116] The term "Cadherin 6" or "CDH6" refers to a cell adhesion molecule that is a member of the cadherin family of cell-cell adhesion molecules. The nucleic acid and amino acid sequences of CDH6 are known, and have been published in GenBank Accession Nos. AK291290 (protein accession number BAF83979.1) See also SEQ ID NO: 1 for the human CDH6 cDNA sequence and SEQ ID NO:2 for the human CDH6 protein sequence. Structurally, CDH6 receptor is a type II cadherin with

five extracellular cadherin repeats and has over its full length at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO:2. Structurally, a CDH6 nucleic acid sequence has over its full length at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the nucleic acid sequence of SEQ ID NO 1.

[00117] The terms "CDH6 expressing cancer" or "CDH6 positive cancer" refers to a cancer that express CDH6 and/or a mutant form of CDH6 on the surface of cancer cells.

[00118] As used herein, the terms "treat," "treating," or "treatment" of any disease or disorder refer in one aspect, to ameliorating the disease or disorder (i.e., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another aspect, "treat," "treating," or "treatment" refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another aspect, "treat," "treating," or "treatment" refers to modulating the disease or disorder, either physically, *(e.g.,* stabilization of a discernible symptom), physiologically, *(e.g.*, stabilization of a physical parameter), or both. In yet another aspect, "treat," "treating," or "treatment" refers to preventing or delaying the onset or development or progression of the disease or disorder.

[00119] The term "therapeutically acceptable amount" or "therapeutically effective dose" interchangeably refers to an amount sufficient to effect the desired result (i.e., a reduction in tumor size, inhibition of tumor growth, prevention of metastasis, inhibition or prevention of viral, bacterial, fungal or parasitic infection). In some aspects, a therapeutically acceptable amount does not induce or cause undesirable side effects. A therapeutically acceptable amount can be determined by first administering a low dose, and then incrementally increasing that dose until the desired effect is achieved. A "prophylactically effective dosage," and a "therapeutically effective dosage," of the molecules of the present disclosure can prevent the onset of, or result in a decrease in severity of, respectively, disease symptoms, including symptoms associated with cancer.

[00120] The term "co-administer" refers to the simultaneous presence of two active agents in the blood of an individual. Active agents that are co-administered can be concurrently or sequentially delivered.

BRIEF DESCRIPTION OF THE DRAWINGS

[00121] Figure 1 is a graphic depiction of anti-CDH6 antibodies cellular binding EC50 on OVCAR3 cells.

[00122] Figure 2 shows epitope binning of anti-CDH6 antibodies.

[00123] Figure 3 represents deuterium mapping of the five EC domains of CDH6.

[00124] Figure 4 is the crystal structure of NOV07 10 binding to CDH6 EC5 domain overlaid onto the known structure of the CDH2 ECD.

[00125] Figure 5 depicts the crystal structure of NOV0712 binding to CDH6.

[00126] Figure 6 depicts the crystal structure of NOV0710 binding to CDH6.

[00127] Figure 7 is an ELISA assay showing the ability of three anti-CDH6 antibodies to bind to mutant forms of CDH6.

[00128] Figure 8 is FACS data of anti-CDH6 antibodies either unconjugated or conjugated with a sulfo-SPDB-DM4 linker/payload binding to CHO cell lines expressing human, cynomolgus, rat or mouse CDH6.

[00129] Figure 9 are FACS graphics showing the levels of CDH6 expression on ovarian cancer cell lines, including CDH6 transfected lines and lines with CDH6 siRNA knockdown.

[00130] Figure 10 shows anti-CDH6 antibodies either unconjugated or conjugated with a sulfo-SPDB-DM4 linker/payload in a panel of ovarian cancer cell lines.

[00131] Figure 11 shows in vitro activity of anti-CDH6 antibodies when conjugated to DM1 or DM4 on ovarian cancer cell lines.

[00132] Figure 12 shows *in vitro* activity of unconjugated anti-CDH6 antibodies or anti-CDH6 antibodies conjugated to sulfo-SPDB-DM4.

[00133] Figure 13 demonstrates the efficacy of anti-CDH6 antibodies when conjugated to DM1 and tested in an ovarian cancer xenograft mouse model.

[00134] Figure 14 demonstrates the efficacy of anti-CDH6 antibodies when conjugated to DM1 and tested in an ovarian cancer xenograft mouse model.

[00135] Figure 15 shows the efficacy of anti-CDH6 antibodies when SPDB-DM4 conjugated and tested in an ovarian cancer xenograft mouse model.

[00136] Figure 16 compares the efficacy of NOV0712-SPDB-DM4 and NOV0712-SMCC-DM1 in an ovarian cancer xenograft mouse model.

[00137] Figure 17 compares the efficacy of NOV07 12 with SPDB-DM4, sulfo-SPDB-DM4 and SMCC-DMl linker/payload combinations in patient-derived primary tumor xenograft (PTX) mouse model of ovarian cancer.

[00138] Figure 18 shows the efficacy of different doses of NOV0712 when conjugated to SPDB-DM4 or sulfo-SPDB-DM4 in an ovarian cancer xenograft mouse model.

[00139] Figure 19 compares NOV0712 with sulfo-SPDB-DM4 with SMCC-DM1 linker payload combinations in a patient-derived primary ovarian tumor xenograft mouse model.

[00140] Figure 20 demonstrates the in vivo efficacy of anti-CDH6 antibodies conjugated to sulfo-SPDB-DM4 in a patient derived primary xenograft mouse model of ovarian cancer.

[00141] Figure 21 shows in vivo efficacy of anti-CDH6 antibodies conjugated to sulfo-SPDB-DM4 in a patient derived primary xenograft mouse model of renal cancer.

[00142] Figure 22 shows in vivo efficacy of anti-CDH6 antibodies conjugated to sulfo-SPDB-DM4 in a patient derived primary xenograft mouse model of renal cancer.

[00143] Figure 23 shows in vivo efficacy of anti-CDH6 antibodies conjugated to sulfo-SPDB-DM4 in a patient derived primary xenograft mouse model of renal cancer.

[00144] Figure 24 provides the Loewe Synergy scores for combination activity of CDH6-targeting ADCs with BCL2/BCL-X1, BCL-X1, IAP and MEK inhibitors.

[00145] Figure 25 is a graphic representation of CDH6 mRNA expression in cancerous tissues.

[00146] Figure 26 is a graphical representation of CDH6 protein expression by immunohistochemistry in ovarian cancer, endometrial cancer, renal cancer and cholangiocarcinoma tissue.

DETAILED DESCRIPTION

[00147] The present disclosure provides for antibodies, antibody fragments *(e.g.,* antigen binding fragments), and antibody drug conjugates that bind to CDH6. In particular, the present disclosure is directed to antibodies and antibody fragments *(e.g.*, antigen binding fragments) that bind to CDH6, and internalize upon such binding. The antibodies and antibody fragments *(e.g.,* antigen binding fragments) of the present disclosure can be used for producing antibody drug conjugates. Furthermore, the present disclosure provides antibody drug conjugates that have desirable pharmacokinetic characteristics and other desirable attributes, and thus can be used for treating cancer expressing CDH6, without limitation, for example: ovarian cancer, renal cancer, hepatic cancer, soft tissue cancer, CNS cancers, thyroid cancer and cholangiocarcinoma. The present disclosure further provides pharmaceutical compositions comprising the antibody drug conjugates, and methods of making and using such pharmaceutical compositions for the treatment of cancer.

Antibody Drug Conjugates

[00148] The present disclosure provides antibody drug conjugates, where an antibody, antigen binding fragment or its functional equivalent that specifically binds to CDH6 is linked to a drug

moiety. In one aspect, the antibodies, antigen binding fragments or their functional equivalents are linked, via covalent attachment by a linker, to a drug moiety that is an anti-cancer agent. The antibody drug conjugates can selectively deliver an effective dose of an anti-cancer agent *(e.g.,* a cytotoxic agent) to tumor tissues expressing CDH6, whereby greater selectivity (and lower efficacious dose) may be achieved.

[00149] In one aspect, the disclosure provides for an immunoconjugate of Formula (I):

$$Ab—(L—(D)_m)_n$$

Wherein Ab represents an CDH6 binding antibody or antibody fragment *(e.g.,* antigen binding fragment) described herein;

L is a linker;

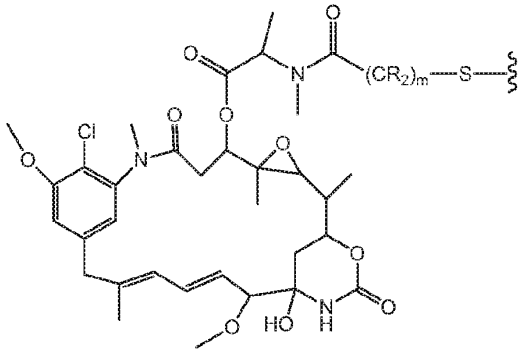
D is a drug moiety;

m is an integer from 1-8; and

n is an integer from 1-20. In one aspect, n is an integer from 1 to 10, 2 to 8, or 2 to 5. In a specific aspect, n is 3 to 4. In some aspects, m is 1. In some aspects, m is 2, 3 or 4.

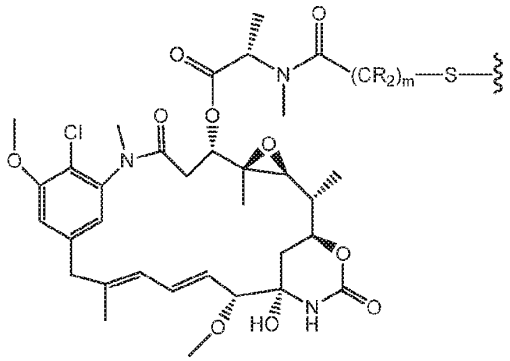
[00150] While the drug moiety to antibody ratio has an exact integer value for a specific conjugate molecule (e.g., n multiplied by m in Formula (I)), it is understood that the value will often be an average value when used to describe a sample containing many molecules, due to some degree of inhomogeneity, typically associated with the conjugation step. The average loading for a sample of an immunoconjugate is referred to herein as the drug moiety to antibody ratio, or "DAR." In the aspect of maytansinoids, this can be referred to as maytansinoid to antibody ratio or "MAR." In some aspects, the DAR is between about 1 and about 5, and typically is about 3, 3.5, 4, 4.5, or 5. In some aspects, at least 50% of a sample by weight is compound having the average DAR plus or minus 2, and preferably at least 50% of the sample is a conjugate that contains the average DAR plus or minus 1. Other aspects include immunoconjugates wherein the DAR is about 3.5. In some aspects, a DAR of 'about n' means the measured value for DAR is within 20% of n.

[00151] The present disclosure provides immunoconjugates comprising the antibodies, antibody fragments *(e.g.,* antigen binding fragments) and their functional equivalents as disclosed herein, linked or conjugated to a drug moiety. In one aspect, the drug moiety D is a maytansinoid drug moiety, including those having the structure:

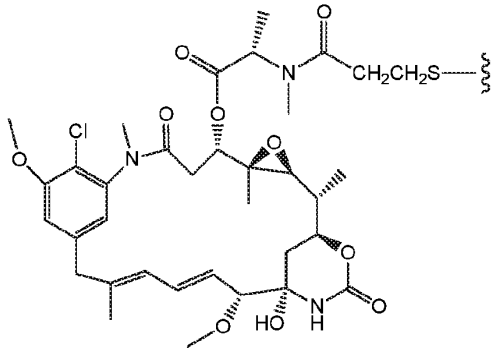


where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid to a linker of an antibody drug conjugate. R at each occurrence is independently H or a C_1-Cg alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propanyl, i.e. m is 1, 2, or 3. (U. S. Pat. No. 633,4 10, U.S. Pat. No. 5,208,020, Chari *et a/.* (1992) Cancer Res. 52; 127-13 1, Lui *et al.* (1996) Proc. Natl. Acad. Sci. 93:8618-8623).

[00152] All stereoisomers of the maytansinoid drug moiety are contemplated for the immunoconjugates disclosed, *i.e.* any combination of R and S configurations at the chiral carbons of the maytansinoid. In one aspect the maytansinoid drug moiety has the following stereochemistry.

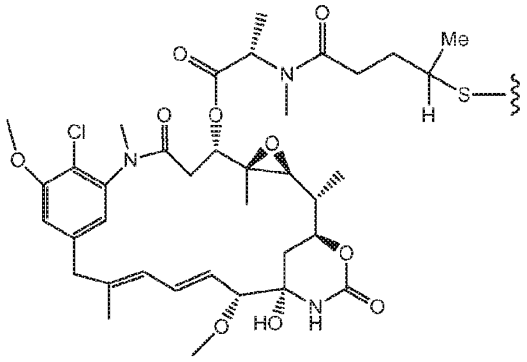


[00153] In one aspect, the maytansinoid drug moiety is $N^{2'}$-deacetyl-$A^{2'}$-(3-mercapto-l-oxopropyl)-maytansine (also known as DM1). DM1 is represented by the following structural formula.



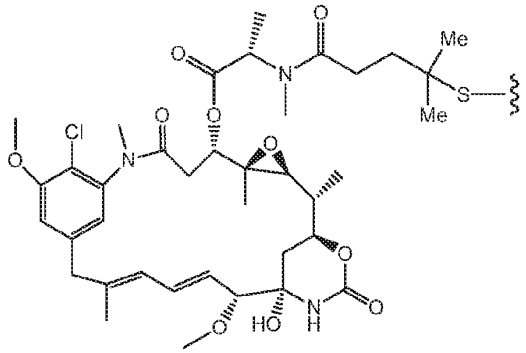
DM1

[00154] In another aspect the maytansinoid drug moiety is $N^{2'}$-deacetyl-$N^{2'}$-(4-mercapto-1-oxopentyl)-maytansine (also known as DM3). DM3 is represented by the following structural formula.



DM3

[00155] In another aspect the maytansinoid drug moiety is $N^{2'}$-deacetyl-$A^{2'}$-(4-methyl-4-mercapto-1-oxopentyl)-maytansine (also known as DM4). DM4 is represented by the following structural formula.



DM4

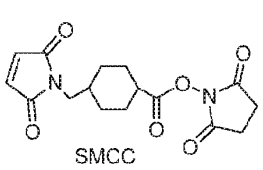
[00156] The drag moiety D can be linked to the antibody Ab through a linker L. L is any chemical moiety that is capable of linking the antibody Ab to the drag moiety D. The linker, L attaches the antibody Ab to the drag moiety D through covalent bond(s). The linker reagent is a bifunctional or multifunctional moiety which can be used to link a drag moiety D and an antibody Ab to form antibody drag conjugates. Antibody drag conjugates can be prepared using a linker having a reactive functionality for binding to the drag moiety D and to the antibody Ab. A cysteine, thiol or an amine, e.g. N-terminus or amino acid side chain such as lysine of the antibody can form a bond with a functional group of a linker reagent.

[00157] In one aspect, L is a cleavable linker. In another aspect, L is a non-cleavable linker. In some aspects, L is an acid-labile linker, photo-labile linker, peptidase cleavable linker, esterase cleavable linker, a disulfide bond reducible linker, a hydrophilic linker, a procharged linker, or a dicarboxylic acid based linker.

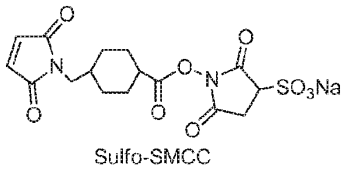
[00158] Suitable cross-linking reagents that form a non-cleavable linker between the drug moiety D, for example maytansinoid, and the antibody Ab are well known in the art, and can form non-cleavable linkers that comprise a sulfur atom (such as SMCC) or those that are without a sulfur atom. Preferred cross-linking reagents that form non-cleavable linkers between the drag moiety D, for example maytansinoid, and the antibody Ab comprise a maleimido- or haloacetyl-based moiety. According to the present disclosure, such non-cleavable linkers are said to be derived from maleimido- or haloacetyl-based moieties.

[00159] Cross-linking reagents comprising a maleimido-based moiety include but not limited to, N^{j}-succinimidyl-4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-l-carboxylate (sulfo-SMCC), jV-succinimidyl-4-(maleimidomethyl)cyclohexane-l-carboxy-(6-amidocaproate), which is a "long chain" analog of

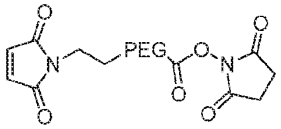
SMCC (LC-SMCC), κ-maleimidoundeconoic acid N-succinimidyl ester (KMUA), γ-maleimidobutyric acid jV-succinimidyl ester (GMBS), ε-maleimidocaproic acid A^-succinimidyl ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(a-maleimidoacetoxy)-succinimide ester (AMSA), succinimidyl-6 -(β-maleimidopropionamido)hexanoate (SMPH), jV-succinimidyl-4-(p-maleimidophenyl)-butyrate (SMPB), N-(-p-maleomidophenyl)isocyanate (PMIP) and maleimido-based cross-linking reagents containing a polyethythene glycol spacer, such as MAL-PEG-NHS. These cross-linking reagents form non-cleavable linkers derived from maleimido-based moieties. Representative structures of maleimido-based cross-linking reagents are shown below.



SMCC



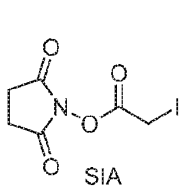
Sulfo-SMCC



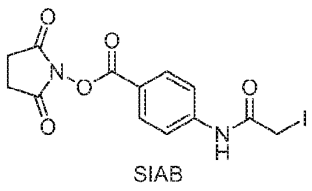
MAL-PEG-NHS

[00160] In another aspect, the linker L is derived from iV-succinimidyl-4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-l-carboxylate (sulfo-SMCC) or MAL-PEG-NHS.

[00161] Cross-linking reagents comprising a haloacetyle-based moiety include N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), N-succinimidyl bromoacetate (SBA) and N-succinimidyl 3-(bromoacetamido)propionate (SBAP). These cross-linking reagents form a non-cleavable linker derived from haloacetyl-based moieties. Representative structures of haloacetyl-based cross-linking reagents are shown below.



SIA

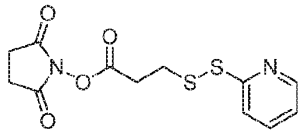


SIAB

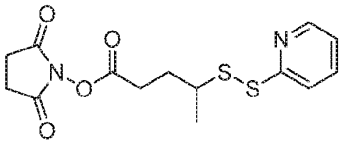
or

[00162] In one aspect, the linker L is derived from N-succinimidyl iodoacetate (SIA) or N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB).

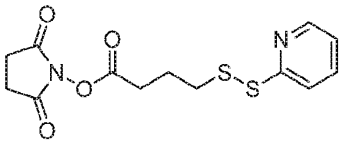
[00163] Suitable cross-linking reagents that form a cleavable linker between the drug moiety D, for example maytansinoid, and the antibody Ab are well known in the art. Disulfide containing linkers are linkers cleavable through disulfide exchange, which can occur under physiological conditions. According to the present disclosure, such cleavable linkers are said to be derived from disulfide-based moieties. Suitable disulfide cross-linking reagents include N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl-4-(2-pyridyldithio)butanoate (SPDB) and iV-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanoate (sulfo-SPDB), the structures of which are shown below. These disulfide cross-linking reagents form a cleavable linker derived from disulfide-based moieties.



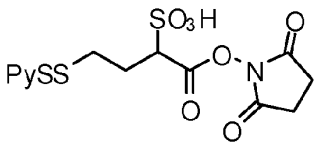
N-succinimidyl-3 -(2-pyridyldithio)propionate (SPDP),



N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP),



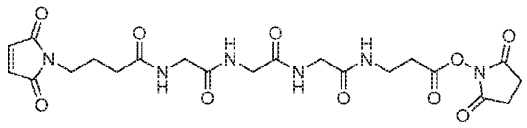
N-succimmidyl-4-(2-pyridyldithio)butanoate (SPDB) and



N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanoate (sulfo-SPDB)

[00164] In one aspect, the linker L is derived from N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanoate (sulfo-SPDB).

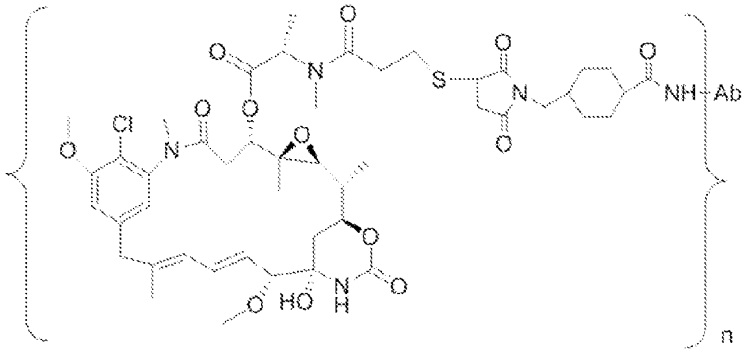
[00165] Suitable cross-linking reagents that form a charged linker between the drug moiety D, for example maytansinoid, and the antibody Ab are known as procharged cross-linking reagents. In one aspect, the linker L is derived from the procharged cross-linking reagent is CXl-1. The structure of CXl-1 is below.



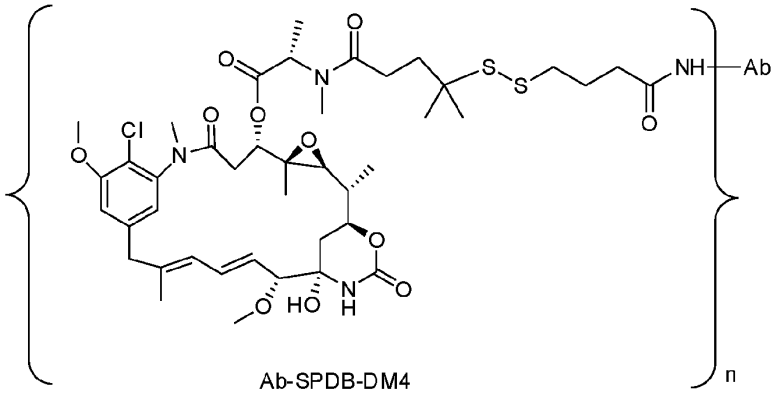
2,5-dioxopyrrolidin-l-yl 17-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)-5,8,l 1,14-tetraoxo-4,7,10,13-tetraazaheptadecan-l-oate (CXl-1)

[00166] In one aspect provided by the disclosure, the conjugate is represented by any one of the following structural formulae:

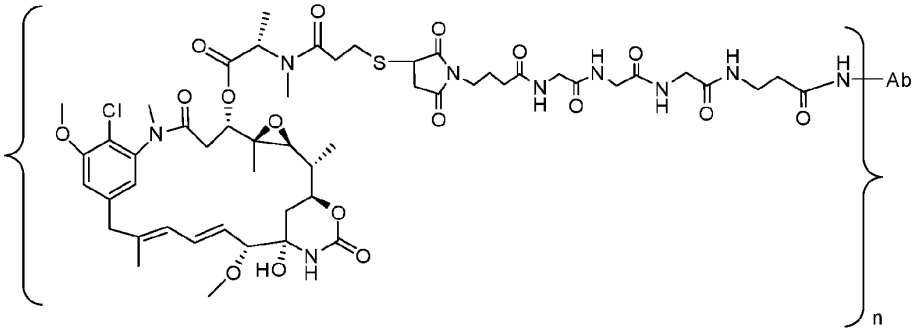
OnCusp
Ex. 1002



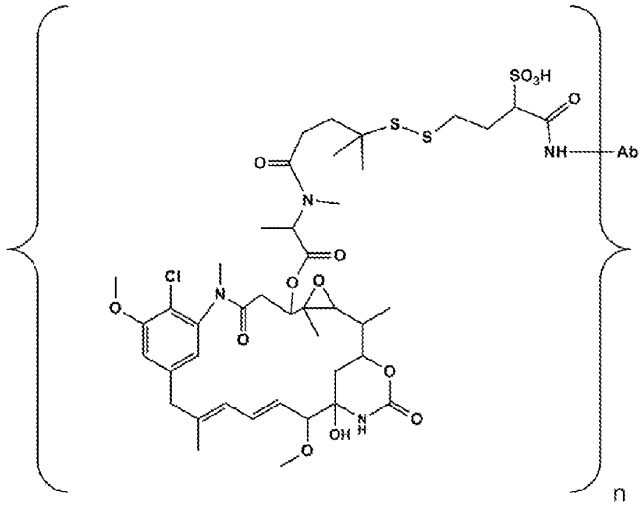
Ab-MCC-DM1



Ab-SPDB-DM4



Ab-CX1 -1-DM1



Ab-suifo-SPDB-DM4

[00167] wherein:

[00168] Ab is an antibody or antigen binding fragment thereof that specifically binds to human CDH6;

[00169] n, which indicates the number of D-L groups attached the Ab through the formation of an amide bond with a primary amine of the Ab, is an integer from 1 to 20. In one aspect, n is an integer from 1 to 10, 2 to 8 or 2 to 5. In a specific aspect, n is 3 or 4.

[00170] In one aspect, the average molar ratio of drug moiety *(e.g.,* DM1 or DM4) to the antibody in the conjugate *(i.e.,* average w value, also known as Maytansinoid Antibody Ratio (MAR)) is about 1 to about 10, about 2 to about 8 *(e.g.,* 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, or 8.1), about 2.5 to about 7, about 3 to about 5, about 2.5 to about 4.5 *(e.g.,* about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3.0, about 3.1, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5), about 3.0 to about 4.0, about 3.2 to about 4.2, or about 4.5 to 5.5 *(e.g.,* about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, or about 5.5).

[00171] In one aspect provided by the disclosure, the conjugate has substantially high purity and has one or more of the following features: (a) greater than about 90% *(e.g.,* greater than or equal to

about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%), preferably greater than about 95%, of conjugate species are monomeric, (b) unconjugated linker level in the conjugate preparation is less than about 10% (*e.g.*, less than or equal to about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0%) (relative to total linker), (c) less than 10% of conjugate species are crosslinked (*e.g.*, less than or equal to about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0%), (d) free drug moiety (*e.g.*, DM1 or DM4) level in the conjugate preparation is less than about 2% (*e.g.*, less than or equal to about 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or 0%) (mol/mol relative to total cytotoxic agent).

[00172] As used herein, the term "unconjugated linker" refers to the antibody that is covalently linked with a linker derived from a cross-linking reagent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1), wherein the antibody is not covalently coupled to the drug moiety (*e.g.*, DM1 or DM4) through a linker (i.e., the "unconjugated linker" can be represented by Ab-SMCC, Ab-SPDB, Ab-sulfo-SPDB, or Ab-CXl-1).

1. Drug Moiety

[00173] The present disclosure provides immunoconjugates that specifically bind to CDH6. The immunoconjugates of the present disclosure comprise anti-CDH6 antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents that are conjugated to a drug moiety, *e.g.*, an anti-cancer agent, anti-hematological disorder agent, an autoimmune treatment agent, an anti¬inflammatory agent, an antifungal agent, an antibacterial agent, an anti-parasitic agent, an anti-viral agent, or an anesthetic agent. The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents can be conjugated to several identical or different drug moieties using any methods known in the art.

[00174] In certain aspects, the drug moiety of the immunoconjugates of the present disclosure is selected from a group consisting of: a maytansinoid, a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, an inhibitor of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

[00175] In one aspect, the drag moiety of the immunoconjugates of the present disclosure is a maytansinoid drug moiety, such as but not limited to, DM1, DM3, or DM4.

[00176] Further, the antibodies, antibody fragments *(e.g.*, antigen binding fragments) or functional equivalents of the present disclosure may be conjugated to a drag moiety that modifies a given biological response. Drag moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein, peptide, or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, cholera toxin, or diphtheria toxin, a protein such as tumor necrosis factor, a-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a cytokine, an apoptotic agent, an anti-angiogenic agent, or, a biological response modifier such as, for example, a lymphokine.

[00177] In one aspect, the antibodies, antibody fragments *(e.g.,* antigen binding fragments) or functional equivalents of the present disclosure are conjugated to a drag moiety, such as a cytotoxin, a drag *(e.g.*, an immunosuppressant) or a radiotoxin. Examples of cytotoxin include but are not limited to, taxanes (see, *e.g.,* International (PCT) Patent Application Nos. WO 01/383 18 and PCT/US03/02675), DNA-alkylating agents *(e.g.,* CC-1065 analogs), anthracyclines, tubuly sin analogs, duocarmycin analogs, auristatin E, auristatin F, maytansinoids, and cytotoxic agents comprising a reactive polyethylene glycol moiety (see, *e.g.*, Sasse *et al* , J. Antibiot. (Tokyo), 53, 879-85 (2000), Suzawa *et al* , Bioorg. Med. Chem., 8, 2175-84 (2000), Ichimura *et al* , J. Antibiot. (Tokyo), 44, 1045-53 (1991), Francisco *et al,* Blood 2003 15; 102(4): 1458-65), U.S. Pat. Nos. 5,475,092, 6,340,701, 6,372,738, and 6,436,93 1, U.S. Patent Application Publication No. 2001/0036923 Al, Pending U.S. patent application Ser. Nos. 10/024,290 and 10/1 16,053, and International (PCT) Patent Application No. WO 01/49698), taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorabicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1 -dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, anti-metabolites *(e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), ablating agents *(e.g.,* mechlorethamine, thiotepa chlorambucil, meiphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin, anthracyclines *(e.g.,* daunorabicin (formerly daunomycin) and doxorubicin), antibiotics *(e.g.,* dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin

(AMC)), and anti-mitotic agents *(e.g.,* vincristine and vinblastine). (See *e.g.,* Seattle Genetics US20090304721).

[00178] Other examples of cytotoxins that can be conjugated to the antibodies, antibody fragments (antigen binding fragments) or functional equivalents of the present disclosure include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof.

[00179] Various types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies are known in the art, see, *e.g.,* Saito *et al,* (2003) Adv. Drug Deliv. Rev. 55: 199-215; Trail *et al ,* (2003) Cancer Immunol. Immunother. 52:328-337; Payne, (2003) Cancer Cell 3:207-212; Allen, (2002) Nat. Rev. Cancer 2:750-763; Pastan and Kreitman, (2002) Curr. Opin. Investig. Drugs 3:1089-1091; Senter and Springer, (2001) Adv. Drug Deliv. Rev. 53:247-264.

[00180] The antibodies, antibody fragments *(e.g.,* antigen binding fragments) or functional equivalents of the present disclosure can also be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine-131, indium-111, yttrium-90, and lutetium-177. Methods for preparing radioimmunoconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including Zevalin™ (IDEC Pharmaceuticals) and Bexxar™ (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies disclosed herein. In certain aspects, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N''' -tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo *et al ,* (1998) Clin Cancer Res. 4(10):2483-90; Peterson *et al,* (1999) Bioconjug. Chem. 10(4):553-7; and Zimmerman *et al ,* (1999) Nucl. Med. Biol. 26(8):943-50, each incorporated by reference in their entireties.

[00181] The antibodies, antibody fragments *(e.g.,* antigen binding fragments) or functional equivalents of the present disclosure can also conjugated to a heterologous protein or polypeptide (or fragment thereof, preferably to a polypeptide of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids) to generate fusion proteins. In particular, the present disclosure provides fusion proteins comprising an antibody fragment *(e.g.,* antigen binding fragment) described herein *(e.g.,* a Fab fragment, Fd fragment, Fv fragment, F(ab)2 fragment, a VH domain, a VH CDR, a VL domain or a VL CDR) and a heterologous protein, polypeptide, or peptide.

[00182] Additional fusion proteins may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to alter the activities of antibodies of the present disclosure or fragments thereof *(e.g.* , antibodies or fragments thereof with higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793, 5,81 1,238, 5,830,721, 5,834,252, and 5,837,458; Patten *et al,* (1997) Curr. Opinion Biotechnol. 8:724-33; Harayama, (1998) Trends Biotechnol. 16(2):76-82; Hansson *et al,* (1999) J. Mol. Biol. 287:265-76; and Lorenzo and Blasco, (1998) Biotechniques 24(2):308- 313 (each of these patents and publications are hereby incorporated by reference in its entirety). Antibodies or fragments thereof, or the encoded antibodies or fragments thereof, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. A polynucleotide encoding an antibody or fragment thereof that specifically binds to an antigen may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[00183] Moreover, the antibodies, antibody fragments *(e.g.,* antigen binding fragments) or functional equivalents of the present disclosure can be conjugated to marker sequences, such as a peptide, to facilitate purification. In preferred aspects, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Chatsworth, CA), among others, many of which are commercially available. As described in Gentz *et al* , (1989) Proc. Natl. Acad. Sci. USA 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin ("HA") tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al,* (1984) Cell 37:767), and the "FLAG" tag (A. Einhauer *et al,* J. Biochem. Biophys. Methods 49: 455^165, 2001). As described in the present disclosure, antibodies or antigen binding fragments can also be conjugated to tumor-penetrating peptides in order to enhance their efficacy.

[00184] In other aspects, the antibodies, antibody fragments *(e.g.,* antigen binding fragments) or functional equivalents of the present disclosure are conjugated to a diagnostic or detectable agent. Such immunoconjugates can be useful for monitoring or prognosing the onset, development, progression and/or severity of a disease or disorder as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Such diagnosis and detection can be accomplished by coupling the antibody to detectable substances including, but not limited to, various enzymes, such as, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidinfoiotin and

avidin/biotin; fluorescent materials, such as, but not limited to, Alexa Fluor 350, Alexa Fluor 405, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 500, Alexa Fluor 514, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 610, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, Alexa Fluor 750, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as, but not limited to, iodine (^{131}I, ^{125}I, ^{123}I, and ^{m}I,), carbon (^{14}C), sulfur (^{35}S), tritium (3H), indium (^{115}In, ^{113}In, ^{112}In, and ^{111}In,), technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{64}Cu, ^{113}Sn, and ^{117}Sn; and positron emitting metals using various positron emission tomographies, and non-radioactive paramagnetic metal ions.

[00185] The antibodies, antibody fragments *(e.g.*, antigen binding fragments) or functional equivalents of the present disclosure may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

2. Linker

[00186] As used herein, a "linker" is any chemical moiety that is capable of linking an antibody, antibody fragment *(e.g.*, antigen binding fragments) or functional equivalent to another moiety, such as a drug moiety. Linkers can be susceptible to cleavage (cleavable linker), such as, acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage, at conditions under which the compound or the antibody remains active. Alternatively, linkers can be substantially resistant to cleavage *(e.g.,* stable linker or noncleavable linker). In some aspects, the linker is a procharged linker, a hydrophilic linker, or a dicarboxylic acid based linker.

[00187] In one aspect, the linker used is derived from a crosslinking reagent such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), 7V-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl)

cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC) or 2,5-dioxopyrrolidin-l-yl 17-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)-5,8,l 1,14-tetraoxo-4,7, 10, 13-tetraazaheptadecan-l-oate (CXl-1). In another aspect, the linker used is derived from a cross-linking agent such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC), N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB) or 2,5-dioxopyrrolidin-l-yl 17-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)-5,8, 11, 14-tetraoxo-4,7, 10,13-tetraazaheptadecan-l-oate (CXl-1).

[00188] Non-cleavable linkers are any chemical moiety capable of linking a drug moiety, such as a maytansinoid, to an antibody in a stable, covalent manner and does not fall off under the categories listed above for cleavable linkers. Thus, non-cleavable linkers are substantially resistant to acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage and disulfide bond cleavage. Furthermore, non-cleavable refers to the ability of the chemical bond in the linker or adjoining to the linker to withstand cleavage induced by an acid, photolabile-cleaving agent, a peptidase, an esterase, or a chemical or physiological compound that cleaves a disulfide bond, at conditions under which the drug moiety, such as maytansinoid or the antibody does not lose its activity.

[00189] Acid-labile linkers are linkers cleavable at acidic pH. For example, certain intracellular compartments, such as endosomes and lysosomes, have an acidic pH (pH 4-5), and provide conditions suitable to cleave acid-labile linkers.

[00190] Photo-labile linkers are linkers that are useful at the body surface and in many body cavities that are accessible to light. Furthermore, infrared light can penetrate tissue.

[00191] Some linkers can be cleaved by peptidases, i.e. peptidase cleavable linkers. Only certain peptides are readily cleaved inside or outside cells, see e.g. Trout et al., 79 Proc. Natl. Acad.Sci. USA, 626-629 (1982) and Umemoto et al. 43 Int. J. Cancer, 677-684 (1989). Furthermore, peptides are composed of a-amino acids and peptidic bonds, which chemically are amide bonds between the carboxylate of one amino acid and the amino group of a second amino acid. Other amide bonds, such as the bond between a carboxylate and the ε-amino group of lysine, are understood not to be peptidic bonds and are considered non-cleavable.

[00192] Some linkers can be cleaved by esterases, i.e. esterase cleavable linkers. Again, only certain esters can be cleaved by esterases present inside or outside of cells. Esters are formed by the

condensation of a carboxylic acid and an alcohol. Simple esters are esters produced with simple alcohols, such as aliphatic alcohols, and small cyclic and small aromatic alcohols.

[00193] Procharged linkers are derived from charged cross-linking reagents that retain their charge after incorporation into an antibody drug conjugate. Examples of procharged linkers can be found in US 2009/0274713.

3. Conjugation and Preparation of ADCs

[00194] The conjugates of the present disclosure can be prepared by any methods known in the art, such as those described in US Patent Nos. 7,81 1,572, 6,41 1,163, 7,368,565, and 8,163,888, and US application publications 201 1/0003969, 201 1/0166319, 2012/0253021 and 2012/0259100. The entire teachings of these patents and patent application publications are herein incorporated by reference.

One-Step Process

[00195] In one aspect, the conjugates of the present disclosure can be prepared by a one-step process. The process comprises combining the antibody, drug and cross-linking agent in a substantially aqueous medium, optionally containing one or more co-solvents, at a suitable pH. In one aspect, the process comprises the step of contacting the antibody of the present disclosure with a drug *(e.g.,* DM1 or DM4) to form a first mixture comprising the antibody and the drug, and then contacting the first mixture comprising the antibody and the drug with a cross-linking agent *(e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate *(e.g.*, Ab-MCC-DMl, Ab-SPDB-DM4, Sulfo-SPDB-DM4, or Ab-CXl-l-DMl), (ii) free drug *(e.g.*, DM1 or DM4), and (iii) reaction by-products.

[00196] In one aspect, the one-step process comprises contacting the antibody with the drug *(e.g.,* DM1 or DM4) and then the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) in a solution having a pH of about 6 or greater *(e.g.*, about 6 to about 9, about 6 to about 7, about 7 to about 9, about 7 to about 8.5, about 7.5 to about 8.5, about 7.5 to about 8.0, about 8.0 to about 9.0, or about 8.5 to about 9.0). For example, the process comprises contacting a cell-binding agent with the drug (DM1 or DM4) and then the cross-linking agent *(e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) in a solution having a pH of about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, about 8.1, about 8.2, about 8.3, about 8.4, about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, or about 9.0. In another aspect, the process comprises contacting a cell-binding agent with the drug *(e.g.*, DM1 or DM4) and then the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) in a solution having a pH of about 7.8 *(e.g.*, a pH of 7.6 to 8.0 or a pH of 7.7 to 7.9).

[00197] The one-step process (i.e., contacting the antibody with the drug *(e.g.*, DM1 or DM4) and then the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) can be carried out at any suitable temperature known in the art. For example, the one-step process can occur at about 20°C or less *(e.g.,* about -10°C (provided that the solution is prevented from freezing, *e.g.,* by the presence of organic solvent used to dissolve the cytotoxic agent and the bifunctional crosslinking reagent) to about 20°C, about 0°C to about 18°C, about 4°C to about 16°C), at room temperature *(e.g.,* about 20°C to about 30°C or about 20°C to about 25°C), or at an elevated temperature *(e.g.,* about 30°C to about 37°C). In one aspect, the one-step process occurs at a temperature of about 16°C to about 24°C *(e.g.,* about 16°C, about 17°C, about 18°C, about 19°C, about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, or about 25°C). In another aspect, the one-step process is carried out at a temperature of about 15°C or less *(e.g.,* about -10°C to about 15°C, or about 0°C to about 15°C). For example, the process comprises contacting the antibody with the drug *(e.g.,* DM1 or DM4) and then the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) at a temperature of about 15°C, about 14°C, about 13°C, about 12°C, about 11°C, about 10°C, about 9°C, about 8°C, about 7°C, about 6°C, about 5°C, about 4°C, about 3°C, about 2°C, about 1°C, about 0°C, about -1°C, about -2°C, about -3°C, about -4°C, about -5°C, about -6°C, about -7°C, about -8°C, about -9°C, or about -10°C, provided that the solution is prevented from freezing, *e.g.,* by the presence of organic solvent(s) used to dissolve the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, Sulfo-SPDB SPDB, or CXl-1). In one aspect, the process comprises contacting the antibody with the drug *(e.g.,* DM1 or DM4) and then the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) at a temperature of about -10°C to about 15°C, about 0°C to about 15°C, about 0°C to about 10°C, about 0°C to about 5°C, about 5°C to about 15°C, about 10°C to about 15°C, or about 5°C to about 10°C. In another aspect, the process comprises contacting the antibody with the drug *(e.g.,* DM1 or DM4) and then the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) at a temperature of about 10°C *(e.g.,* a temperature of 8°C to 12°C or a temperature of 9°C to 11°C).

[00198] In one aspect, the contacting described above is effected by providing the antibody, then contacting the antibody with the drug *(e.g.*, DM1 or DM4) to form a first mixture comprising the antibody and the drug *(e.g.,* DM1 or DM4), and then contacting the first mixture comprising the antibody and the drug *(e.g.*, DM1 or DM4) with the cross-linking agent *(e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1). For example, in one aspect, the antibody is provided in a reaction vessel, the drug *(e.g.*, DM1 or DM4) is added to the reaction vessel (thereby contacting the antibody), and then the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) is added to the mixture comprising the antibody and the drug *(e.g.,* DM1 or DM4) (thereby contacting the mixture comprising the antibody and the drug). In one aspect, the antibody is provided in a reaction vessel, and the drug *(e.g.*, DM1 or DM4) is added to the reaction vessel immediately following

providing the antibody to the vessel. In another aspect, the antibody is provided in a reaction vessel, and the drug (*e.g.*, DM1 or DM4) is added to the reaction vessel after a time interval following providing the antibody to the vessel (*e.g.*, about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 1 hour, about 1 day or longer after providing the cell-binding agent to the space). The drug (*e.g.*, DM1 or DM4) can be added quickly (i.e., within a short time interval, such as about 5 minutes, about 10 minutes) or slowly (such as by using a pump).

[00199] The mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) can then be contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) either immediately after contacting the antibody with the drug (*e.g.*, DM1 or DM4) or at some later point (*e.g.*, about 5 minutes to about 8 hours or longer) after contacting the antibody with the drug (*e.g.*, DM1 or DM4). For example, in one aspect, the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) is added to the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) immediately after the addition of the drug (*e.g.*, DM1 or DM4) to the reaction vessel comprising the antibody. Alternatively, the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) can be contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) at about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, or longer after contacting the antibody with the drug (*e.g.*, DM1 or DM4).

[00200] After the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) is contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) the reaction is allowed to proceed for about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 2 1 hours, about 22 hours, about 23 hours, about 24 hours, or longer (*e.g.*, about 30 hours, about 35 hours, about 40 hours, about 45 hours, or about 48 hrs).

[00201] In one aspect, the one-step process further comprises a quenching step to quench any unreacted drug (*e.g.*, DM 1 or DM4) and/or unreacted cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1). The quenching step is typically performed prior to purification of the conjugate. In one aspect, the mixture is quenched by contacting the mixture with a quenching reagent. As used herein, the "quenching reagent" refers to a reagent that reacts with the free drug (*e.g.*, DM1 or DM4) and/or cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1). In one aspect, maleimide or haloacetamide quenching reagents, such as 4-maleimidobutyric acid, 3-maleimidopropionic acid, N-ethylmaleimide, iodoacetamide, or iodoacetamidopropionic acid, can be used to ensure that any unreacted group (such as thiol) in the drug (*e.g.*, DM1 or DM4) is quenched. The quenching step can help prevent the dimerization of the drug (*e.g.*, DM1). The dimerized DM1

can be difficult to remove. Upon quenching with polar, charged thiol-quenching reagents (such as 4-maleimidobutyric acid or 3-maleimidopropionic acid), the excess, unreacted DM1 is converted into a polar, charged, water-soluble adduct that can be easily separated from the covalently- linked conjugate during the purification step. Quenching with non-polar and neutral thiol-quenching reagents can also be used. In one aspect, the mixture is quenched by contacting the mixture with a quenching reagent that reacts with the unreacted cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1). For example, nucleophiles can be added to the mixture in order to quench any unreacted SMCC. The nucleophile preferably is an amino group containing nucleophile, such as lysine, taurine and hydroxylamine.

[00202] In another aspect, the reaction (i.e., contacting the antibody with the drug *(e.g.*, DM1 or DM4) and then cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1)) is allowed to proceed to completion prior to contacting the mixture with a quenching reagent. In this regard, the quenching reagent is added to the mixture about 1 hour to about 48 hours *(e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, or about 25 hours to about 48 hours) after the mixture comprising the antibody and the drug *(e.g.*, DM1 or DM4) is contacted with the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1).

[00203] Alternatively, the mixture is quenched by lowering the pH of the mixture to about 5.0 *(e.g.,* 4.8, 4.9, 5.0, 5.1 or 5.2). In another aspect, the mixture is quenched by lowering the pH to less than 6.0, less than 5.5, less than 5.0, less than 4.8, less than 4.6, less than 4.4, less than 4.2, less than 4.0. Alternatively, the pH is lowered to about 4.0 *(e.g.,* 3.8, 3.9, 4.0, 4.1 or 4.2) to about 6.0 *(e.g.,* 5.8, 5.9, 6.0, 6.1 or 6.2), about 4.0 to about 5.0, about 4.5 *(e.g.,* 4.3, 4.4, 4.5, 4.6 or 4.7) to about 5.0. In one aspect, the mixture is quenched by lowering the pH of the mixture to 4.8. In another aspect, the mixture is quenched by lowering the pH of the mixture to 5.5.

[00204] In one aspect, the one-step process further comprises a holding step to release the unstably bound linkers from the antibody. The holding step comprises holding the mixture prior to purification of the conjugate *(e.g.*, after the reaction step, between the reaction step and the quenching step, or after the quenching step). For example, the process comprises (a) contacting the antibody with the drug (e.g., DM1 or DM4) to form a mixture comprising the antibody and the drug *(e.g.,* DM1 or DM4); and then contacting the mixture comprising the antibody and drug (e.g., DM1 or DM4) with the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1), in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate (e.g., Ab-MCC-DM1, Ab-SPDB-DM4, Sulfo-SPDB-DM4 or Ab-CXl-l-DMl), (ii) free drug (e.g., DM1 or DM4), and (iii) reaction by-products, (b) holding the mixture prepared in step (a) to release the unstably

bound linkers from the cell-binding agent, and (c) purifying the mixture to provide a purified conjugate.

[00205] In another aspect, the process comprises (a) contacting the antibody with the drug (*e.g.*, DM1 or DM4) to form a mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4); and then contacting the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1), in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate, (ii) free drug (*e.g.*, DM1 or DM4), and (iii) reaction by-products, (b) quenching the mixture prepared in step (a) to quench any unreacted drug (*e.g.*, DM 1 or DM4) and/or unreacted cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1), (c) holding the mixture prepared in step (b) to release the unstably bound linkers from the cell-binding agent, and (d) purifying the mixture to provide a purified conjugate (*e.g.*, Ab-MCC-DMl, Ab-SPDB-DM4, Ab-Sulfo-SPDB-DM4 or Ab-CXl-l-DMl).

[00206] Alternatively, the holding step can be performed after purification of the conjugate, followed by an additional purification step.

[00207] In another aspect, the reaction is allowed to proceed to completion prior to the holding step. In this regard, the holding step can be performed about 1 hour to about 48 hours (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 2 1 hours, about 22 hours, about 23 hours, about 24 hours, or about 24 hours to about 48 hours) after the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) is contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1).

[00208] The holding step comprises maintaining the solution at a suitable temperature (*e.g.*, about 0°C to about 37°C) for a suitable period of time (*e.g.*, about 1 hour to about 1 week, about 1 hour to about 24 hours, about 1 hour to about 8 hours, or about 1 hour to about 4 hours) to release the unstably bound linkers from the antibody while not substantially releasing the stably bound linkers from the antibody. In one aspect, the holding step comprises maintaining the solution at about 20 °C or less (*e.g.*, about 0°C to about 18°C, about 4°C to about 16°C), at room temperature (*e.g.*, about 20°C to about 30°C or about 20°C to about 25°C), or at an elevated temperature (*e.g.*, about 30°C to about 37°C). In one aspect, the holding step comprises maintaining the solution at a temperature of about 16°C to about 24°C (*e.g.*, about 15°C, about 16°C, about 17°C, about 18°C, about 19°C, about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, or about 25°C). In another aspect, the holding step comprises maintaining the solution at a temperature of about 2°C to about 8°C (*e.g.*, about 0°C, about 1°C, about 2°C, about 3°C, about 4°C, about 5°C, about 6°C, about 7°C, about 8°C, about 9°C, or about 10°C). In another aspect, the holding step comprises maintaining the solution at a temperature of

about 37°C (*e.g.*, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, or about 40°C).

[00209] The duration of the holding step depends on the temperature and the pH at which the holding step is performed. For example, the duration of the holding step can be substantially reduced by performing the holding step at elevated temperature, with the maximum temperature limited by the stability of the cell-binding agent-cytotoxic agent conjugate. The holding step can comprise maintaining the solution for about 1 hour to about 1 day (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, or about 24 hours), about 10 hours to about 24 hours, about 12 hours to about 24 hours, about 14 hours to about 24 hours, about 16 hours to about 24 hours, about 18 hours to about 24 hours, about 20 hours to about 24 hours, about 5 hours to about 1 week, about 20 hours to about 1 week, about 12 hours to about 1 week (*e.g.*, about 12 hours, about 16 hours, about 20 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days), or about 1 day to about 1 week.

[00210] In one aspect, the holding step comprises maintaining the solution at a temperature of about 2 °C to about 8 °C for a period of at least about 12 hours for up to a week. In another aspect, the holding step comprises maintaining the solution at a temperature of about 2 °C to about 8 °C overnight (*e.g.*, about 12 to about 24 hours, preferably about 20 hours).

[00211] The pH value for the holding step preferably is about 4 to about 10. In one aspect, the pH value for the holding step is about 4 or more, but less than about 6 (*e.g.*, 4 to 5.9) or about 5 or more, but less than about 6 (*e.g.*, 5 to 5.9). In another aspect, the pH values for the holding step range from about 6 to about 10 (*e.g.*, about 6.5 to about 9, about 6 to about 8). For example, pH values for the holding step can be about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10.

[00212] In other aspects, the holding step can comprise incubating the mixture at 25°C at a pH of about 6-7.5 for about 12 hours to about 1 week, incubating the mixture at 4°C at a pH of about 4.5-5.9 for about 5 hours to about 5 days, or incubating the mixture at 25°C at a pH of about 4.5-5.9 for about 5 hours to about 1 day.

[00213] The one-step process can optionally include the addition of sucrose to the reaction step to increase solubility and recovery of the conjugates. Desirably, sucrose is added at a concentration of about 0.1% (w/v) to about 20% (w/v) (*e.g.*, about 0.1% (w/v), 1% (w/v), 5% (w/v), 10% (w/v), 15% (w/v), or 20% (w/v)). Preferably, sucrose is added at a concentration of about 1% (w/v) to about 10% (w/v) (*e.g.*, about 0.5% (w/v), about 1% (w/v), about 1.5% (w/v), about 2% (w/v), about 3% (w/v), about 4% (w/v), about 5% (w/v), about 6% (w/v), about 7% (w/v), about 8% (w/v), about 9% (w/v), about 10% (w/v), or about 11% (w/v)). In addition, the reaction step also can

comprise the addition of a buffering agent. Any suitable buffering agent known in the art can be used. Suitable buffering agents include, for example, a citrate buffer, an acetate buffer, a succinate buffer, and a phosphate buffer. In one aspect, the buffering agent is selected from the group consisting of HEPPSO (N-(2-hydroxyethyl)piperazine-N'-(2-hydroxypropanesulfonic acid)), POPSO (piperazine-1,4-bis-(2-hydroxy-propane-sulfonic acid) dehydrate), HEPES (4-(2-hydroxyethyl)piperazine-l-ethanesulfonic acid), HEPPS (EPPS) (4-(2-hydroxyethyl)piperazine-l-propanesulfonic acid), TES (N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid), and a combination thereof.

[00214] The one-step process can further comprise the step of purifying the mixture to provide purified conjugate *(e.g.,* Ab-MCC-DMl, Ab-SPDB-DM4, Ab-Sulfo-SPDB-DM4 or Ab-CXl-l-DMl). Any purification methods known in the art can be used to purify the conjugates of the present disclosure. In one aspect, the conjugates of the present disclosure use tangential flow filtration (TFF), non-adsorptive chromatography, adsorptive chromatography, adsorptive filtration, selective precipitation, or any other suitable purification process, as well as combinations thereof. In another aspect, prior to subjecting the conjugates to purification process described above, the conjugates are first filtered through one or more PVDF membranes. Alternatively, the conjugates are filtered through one or more PVDF membranes after subjecting the conjugates to the purification process described above. For example, in one aspect, the conjugates are filtered through one or more PVDF membranes and then purified using tangential flow filtration. Alternatively, the conjugates are purified using tangential flow filtration and then filtered through one or more PVDF membranes.

[00215] Any suitable TFF systems may be utilized for purification, including a Pellicon® type system (MiUipore, Billerica, MA), a Sartocon® Cassette system (Sartorius AG, Edgewood, NY), and a Centrasette® type system (Pall Corp., East Hills, NY).

[00216] Any suitable adsorptive chromatography resin may be utilized for purification. Preferred adsorptive chromatography resins include hydroxyapatite chromatography, hydrophobic charge induction chromatography (HCIC), hydrophobic interaction chromatography (HIC), ion exchange chromatography, mixed mode ion exchange chromatography, immobilized metal affinity chromatography (DVIAC), dye ligand chromatography, affinity chromatography, reversed phase chromatography, and combinations thereof. Examples of suitable hydroxyapatite resins include ceramic hydroxyapatite (CHT Type I and Type II, Bio-Rad Laboratories, Hercules, CA), HA Ultrogel® hydroxyapatite (Pall Corp., East Hills, NY), and ceramic fluoroapatite (CFT Type I and Type II, Bio-Rad Laboratories, Hercules, CA). An example of a suitable HCIC resin is MEP Hypercel® resin (Pall Corp., East Hills, NY). Examples of suitable HIC resins include Butyl-Sepharose, Hexyl-Sepaharose, Phenyl-Sepharose, and Octyl Sepharose resins (all from GE Healthcare, Piscataway, NJ), as well as Macro-prep® Methyl and Macro-Prep® t-Butyl resins (Biorad Laboratories, Hercules, CA). Examples of suitable ion exchange resins include SP-Sepharose®, CM-Sepharose®, and Q-Sepharose® resins (all from GE Healthcare, Piscataway, NJ), and Unosphere® S

resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable mixed mode ion exchangers include Bakerbond® ABx resin (JT Baker, Phillipsburg NJ). Examples of suitable IMAC resins include Chelating Sepharose ® resin (GE Healthcare, Piscataway, NJ) and Profinity® IMAC resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable dye ligand resins include Blue Sepharose resin (GE Healthcare, Piscataway, NJ) and Affi-gel Blue resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable affinity resins include Protein A Sepharose resin *(e.g.,* MabSelect, GE Healthcare, Piscataway, NJ) and lectin affinity resins, *e.g.* Lentil Lectin Sepharose® resin (GE Healthcare, Piscataway, NJ), where the antibody bears appropriate lectin binding sites. Examples of suitable reversed phase resins include C4, C8, and C18 resins (Grace Vydac, Hesperia, CA).

[00217] Any suitable non-adsorptive chromatography resin may be utilized for purification. Examples of suitable non-adsorptive chromatography resins include, but are not limited to, SEPHADEX™ G-25, G-50, G-100, SEPHACRYL™ resins *(e.g.,* S-200 and S-300), SUPERDEX™ resins *(e.g.,* SUPERDEX™ 75 and SUPERDEX™ 200), BIO-GEL® resins *(e.g.,* P-6, P-10, P-30, P-60, and P-100), and others known to those of ordinary skill in the art.

Two-Step Process and One-Pot Process

[00218] In one aspect, the conjugates of the present disclosure can be prepared as described in the U.S. Patent 7,81 1,572 and U.S. Patent Application Publication No. 2006/0182750. The process comprises the steps of (a) contacting the antibody of the present disclosure with the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1) to covalently attach the linker (i.e., Ab-SMCC, Ab-SPDB or Ab-CXl-1) to the antibody and thereby prepare a first mixture comprising the antibody having the linker bound thereto; (b) optionally subjecting the first mixture to a purification process to prepare a purified first mixture of the antibody having the linker bound thereto; (c) conjugating the drug *(e.g.,* DM1 or DM4) to the antibody having the linker bound thereto in the first mixture by reacting the antibody having the linker bound thereto with the drug *(e.g.,* DM1 or DM4) in a solution having a pH of about 4 to about 9 to prepare a second mixture comprising (i) conjugate *(e.g.,* Ab-MCC-DMl, Ab-SPDB-DM4, Ab-Sulfo-SPDB-DM4 or Ab-CXl-l-DMl), (ii) free drug *(e.g.,* DM1 or DM4); and (iii) reaction by-products; and (d) subjecting the second mixture to a purification process to purify the conjugate from the other components of the second mixture. Alternatively, the purification step (b) can be omitted. Any purification methods described herein can be used for steps (b) and (d). In one embodiment, TFF is used for both steps (b) and (d). In another embodiment, TFF is used for step (b) and absorptive chromatography *(e.g.,* CHT) is used for step (d).

One-Step Reagent and In-situ Process

[00219] In one aspect, the conjugates of the present disclosure can be prepared by conjugating pre-formed drug-linker compound *(e.g.,* SMCC-DM1, Sulfo-SMCC-DMl, SPDB-DM4, Sulfo-SPDB-

DM4 or CXl-l-DMl) to the antibody of the present disclosure, as described in U.S. Patent 6,441, 163 and U.S. Patent Application Publication Nos. 201 1/0003969 and 2008/0145374, followed by a purification step. Any purification methods described herein can be used. The drug-linker compound is prepared by reacting the drug *(e.g.,* DM1 or DM4) with the cross-linking agent *(e.g.,* SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CXl-1). The drug-linker compound *(e.g.,* SMCC-DM1, Sulfo-SMCC-DM1, SPDB-DM4, Sulfo-SPDB-DM4 or CXl-l-DMl) is optionally subjected to purification before being conjugated to the antibody.

4. Characterization and Selection of Desirable Antibodies and Antibody Drug Conjugates

[00220] The antibodies, antibody fragments *(e.g.,* antigen binding fragments) or antibody drug conjugates of the present disclosure can be characterized and selected for their physical/chemical properties and/or biological activities by various assays known in the art.

[00221] For example, an antibody of the present disclosure can be tested for its antigen binding activity by known methods such as ELISA, FACS, Biacore or Western blot.

[00222] Transgenic animals and cell lines are particularly useful in screening antibody drug conjugates (ADCs) that have potential as prophylactic or therapeutic treatments of cancer overexpression of tumor-associated antigens and cell surface receptors. Screening for a useful ADC may involve administering a candidate ADC over a range of doses to the transgenic animal, and assaying at various time points for the effect(s) of the ADC on the disease or disorder being evaluated. Alternatively, or additionally, the drug can be administered prior to or simultaneously with exposure to an inducer of the disease, if applicable. The candidate ADC may be screened serially and individually, or in parallel under medium or high-throughput screening format.

[00223] One aspect is a screening method comprising (a) transplanting cells from a stable cancer cell line or human patient tumor expressing CDH6 *(e.g.,* an ovarian cell line or tumor fragment, a renal cell line or tumor fragment, a hepatic cell line or tumor fragment, a thyroid cell line or tumor fragment, a CNS cancer cell line or tumor fragment, a cholangiocarcinoma cancer cell line or tumor fragment, ovarian, renal, hepatic, soft tissue, CNS, thyroid, or cholangiocarcinoma primary cells) into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to inhibit the growth of tumors from the transplanted cell line. The present disclosure also encompasses a method of screening ADC candidates for the treatment of a disease or disorder characterized by the overexpression of CDH6 comprising (a) contacting cells from a stable cancer cell line expressing CDH6 with a drug candidate, and (b) evaluating the ability of the ADC candidate to inhibit the growth of the stable cell line.

[00224] A further aspect is a screening method comprising (a) contacting cells from a stable cancer cell line expressing CDH6 with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one aspect the ability of the ADC candidate to induce apoptosis is evaluated.

[00225] Candidate ADC can be screened by being administered to the transgenic animal over a range of doses, and evaluating the animal's physiological response to the compounds over time. In some cases, it can be appropriate to administer the compound in conjunction with co-factors that would enhance the efficacy of the compound. If cell lines derived from the subject transgenic animals are used to screen for ADCs useful in treating various disorders associated with overexpression of CDH6, the test ADCs are added to the cell culture medium at an appropriate time, and the cellular response to the ADCs is evaluated over time using the appropriate biochemical and/or histological assays.

[00226] Thus, the present disclosure provides assays for identifying ADC which specifically target and bind to CDH6, and CDH6 expressed on tumor cells.

CDH6 Antibodies

[00227] The present disclosure provides for antibodies or antibody fragments ***(e.g.,*** antigen binding fragments) that specifically bind to human CDH6. Antibodies or antibody fragments ***(e.g.,*** antigen binding fragments) of the present disclosure include, but are not limited to, the human monoclonal antibodies or fragments thereof, isolated as described, in the Examples below.

[00228] The present disclosure in certain aspects provides antibodies or antibody fragments ***(e.g.,*** antigen binding fragments) that specifically bind CDH6, said antibodies or antibody fragments ***(e.g.,*** antigen binding fragments) comprise a VH domain having an amino acid sequence of SEQ ID NO: 20, 34, 48, 62, 76, 90, 104, 118, 132, 146, 160, 174, 188, 202, 216, 230, 244, 258, 272, 286, 300, 314 or 328 (Table 5 and 6). The present disclosure also provides antibodies or antibody fragments ***(e.g.,*** antigen binding fragments) that specifically bind to CDH6, said antibodies or antibody fragments ***(e.g.,*** antigen binding fragments) comprise a VH CDR having an amino acid sequence of any one of the VH CDRs listed in Table 5 and 6. In particular aspects, the present disclosure provides antibodies or antibody fragments ***(e.g.,*** antigen binding fragments) that specifically bind to CDH6, said antibodies comprising (or alternatively, consist of) one, two, three, four, five or more VH CDRs having an amino acid sequence of any of the VH CDRs listed in Table 5 and 6.

[00229] The present disclosure provides antibodies or antibody fragments *(e.g.*, antigen binding fragments) that specifically bind to CDH6, said antibodies or antibody fragments *(e.g.*, antigen binding fragments) comprise a VL domain having an amino acid sequence of SEQ ID NO: 21, 35, 49, 63, 77, 91, 105, 119, 133, 147, 161, 175, 189, 203, 217, 231, 245, 259, 273, 287, 301, 315 or 329 (Table 5 and 6). The present disclosure also provides antibodies or antibody fragments *(e.g.*, antigen binding fragments) that specifically bind to CDH6, said antibodies or antibody fragments *(e.g.*, antigen binding fragments) comprise a VL CDR having an amino acid sequence of any one of the VL CDRs listed in Table 5 and 6, *infra.* In particular, the disclosure provides antibodies or antibody fragments *(e.g.*, antigen binding fragments) that specifically bind to CDH6, said antibodies or antibody fragments *(e.g.*, antigen binding fragments) comprise (or alternatively, consist of) one, two, three or more VL CDRs having an amino acid sequence of any of the VL CDRs listed in Table 5 and 6.

[00230] Other antibodies or antibody fragments *(e.g.*, antigen binding fragments) of the present disclosure include amino acids that have been mutated, yet have at least 60, 70, 80, 90 or 95 percent identity in the CDR regions with the CDR regions depicted in the sequences described in Table 5 and 6. In some aspects, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the CDR regions when compared with the CDR regions depicted in the sequence described in Table 5 and 6.

[00231] The present disclosure also provides nucleic acid sequences that encode VH, VL, the full length heavy chain, and the full length light chain of the antibodies that specifically bind to CDH6. Such nucleic acid sequences can be optimized for expression in mammalian cells.

[00232] Other antibodies of the present disclosure include those where the amino acids or nucleic acids encoding the amino acids have been mutated, yet have at least 60, 70, 80, 90 or 95 percent identity to the sequences described in 5 and 6. In some aspects, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the variable regions when compared with the variable regions depicted in the sequence described in 5 and 6, while retaining substantially the same therapeutic activity.

[00233] Since each of these antibodies can bind to CDH6, the VH, VL, full length light chain, and full length heavy chain sequences (amino acid sequences and the nucleotide sequences encoding the amino acid sequences) can be "mixed and matched" to create other CDH6-binding antibodies. Such "mixed and matched" CDH6 -binding antibodies can be tested using the binding assays known in the art *(e.g.*, ELISAs, and other assays described in the Example section). When these chains are mixed and matched, a VH sequence from a particular VH/VL pairing should be replaced with a

structurally similar VH sequence. Likewise a full length heavy chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length heavy chain sequence. Likewise, a VL sequence from a particular VH/VL pairing should be replaced with a structurally similar VL sequence. Likewise, a full length light chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length light chain sequence. Accordingly, in one aspect, the disclosure provides for an isolated monoclonal antibody or antigen binding region thereof having: a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 20, 34, 48, 62, 76, 90, 104, 118, 132, 146, 160, 174, 188, 202, 216, 230, 244, 258, 272, 286, 300, 314 or 328(Table 5 and 6); and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, 35, 49, 63, 77, 91, 105, 119, 133, 147, 161, 175, 189, 203, 217, 231, 245, 259, 273, 287, 301, 315 or 329 (Table 5 and 6); wherein the antibody specifically binds to CDH6.

[00234] In another aspect, the disclosure provides (i) an isolated monoclonal antibody having: a full length heavy chain comprising an amino acid sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NO: 24, 38, 52, 66, 80, 94, 108, 122, 136, 150, 164, 178, 192, 206, 220, 234, 248, 262, 276, 290, 304, 318 or 332; and a full length light chain comprising an amino acid sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NO: 25, 39, 53, 67, 81, 95, 109, 123, 137, 151, 165, 179, 193, 207, 221, 235, 249, 263, 277, 291, 305, 319 or 333 ; or (ii) a functional protein comprising an antigen binding portion thereof.

[00235] In another aspect, the present disclosure provides CDH6 -binding antibodies that comprise the heavy chain and light chain CDRls, CDR2s and CDR3s as described in Table 5 and 6, or combinations thereof. The amino acid sequences of the VH CDRls of the antibodies are shown in SEQ ID NOs: 17, 31, 45, 59, 73, 87, 101, 115, 129, 143, 157, 171, 185, 199, 213, 227, 241, 255, 269, 283, 297, 311 and 325. The amino acid sequences of the VH CDR2s of the antibodies and are shown in SEQ ID NOs: 18, 32, 46, 60, 74, 88, 102, 116, 130, 144, 158, 172, 186, 200, 214, 228, 242, 256, 270, 284, 298, 312 and 326. The amino acid sequences of the VH CDR3s of the antibodies are shown in SEQ ID NOs: 19, 33, 47, 61, 75, 89, 103, 117, 131, 145, 159, 173, 187, 201, 215, 229, 243, 257, 271, 285, 299, 313 and 327. The amino acid sequences of the VL CDRls of the antibodies are shown in SEQ ID NOs: 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182, 196, 210, 224, 238, 252, 266, 280, 294, 308 and 322. The amino acid sequences of the VL CDR2s of the antibodies are shown in SEQ ID NOs: 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253, 267,

281, 295, 309 and 323. The amino acid sequences of the VL CDR3s of the antibodies are shown in SEQ ID NOs: 16, 30, 44, 58, 72, 86, 100, 114, 128, 142, 156, 170, 184, 198, 212, 226, 240, 254, 268, 282, 296, 310 and 324.

[00236] Given that each of these antibodies can bind to CDH6 and that antigen-binding specificity is provided primarily by the CDR1, 2 and 3 regions, the VH CDR1, 2 and 3 sequences and VL CDR1, 2 and 3 sequences can be "mixed and matched" (i.e., CDRs from different antibodies can be mixed and match, although each antibody must contain a VH CDR1, 2 and 3 and a VL CDR1, 2 and 3 to create other CDH6-binding binding molecules. Such "mixed and matched" CDH6-binding antibodies can be tested using the binding assays known in the art and those described in the Examples (*e.g.*, ELISAs). When VH CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VH sequence should be replaced with a structurally similar CDR sequence(s). Likewise, when VL CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VL sequence should be replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel VH and VL sequences can be created by substituting one or more VH and/or VL CDR region sequences with structurally similar sequences from the CDR sequences shown herein for monoclonal antibodies of the present disclosure.

[00237] Accordingly, the present disclosure provides an isolated monoclonal antibody or antigen binding region thereof comprising a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 31, 45, 59, 73, 87, 101, 115, 129, 143, 157, 171, 185, 199, 213, 227, 241, 255, 269, 283, 297, 311 and 325; a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 32, 46, 60, 74, 88, 102,116, 130, 144, 158, 172, 186, 200, 214, 228, 242, 256, 270, 284, 298, 312 and 326; a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 33, 47, 61, 75, 89, 103, 117, 131, 145, 159, 173, 187, 201, 215, 229, 243, 257, 271, 285, 299, 313 and 327; a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182, 196, 210, 224, 238, 252, 266, 280, 294, 308 and 322; a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253, 267, 281, 295, 309 and 323; and a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 30, 44, 58, 72, 86, 100, 114, 128, 142, 156, 170, 184, 198, 212, 226, 240, 254, 268, 282, 296, 310 and 324; wherein the antibody specifically binds CDH6.

[00238] In a specific aspect, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to CDH6 comprising:

(i) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:224, (b) a LCDR2 of SEQ ID NO:225, (c) a LCDR3 of SEQ ID NO:226; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 227, (e) a HCDR2 of SEQ ID NO: 228, and (f) a HCDR3 of SEQ ID NO:229;

(ii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:210, (b) a LCDR2 of SEQ ID NO:211, (c) a LCDR3 of SEQ ID NO:212; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:213, (e) a HCDR2 of SEQ ID NO: 214, and (f) a HCDR3 of SEQ ID NO:215;

(iii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:266, (b) a LCDR2 of SEQ ID NO:267, (c) a LCDR3 of SEQ ID NO:268; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 269, (e) a HCDR2 of SEQ ID NO:270, and (f) a HCDR3 of SEQ ID NO: 271;

(iv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:308, (b) a LCDR2 of SEQ ID NO:309, (c) a LCDR3 of SEQ ID NO:310; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:311, (e) a HCDR2 of SEQ ID NO:312, and (f) a HCDR3 of SEQ ID NO:313;

(v) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 14, (b) a LCDR2 of SEQ ID NO: 15, (c) a LCDR3 of SEQ ID NO: 16; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 17, (e) a HCDR2 of SEQ ID NO: 18, and (f) a HCDR3 of SEQ ID NO: 19;

(vi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:28, (b) a LCDR2 of SEQ ID NO:29, (c) a LCDR3 of SEQ ID NO:30; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:31, (e) a HCDR2 of SEQ ID NO:32, and (f) a HCDR3 of SEQ ID NO:33;

(vii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:42, (b) a LCDR2 of SEQ ID NO:43, (c) a LCDR3 of SEQ ID NO:44; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:45, (e) a HCDR2 of SEQ ID NO:46, and (f) a HCDR3 of SEQ ID NO:47;

(viii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:56, (b) a LCDR2 of SEQ ID NO:57, (c) a LCDR3 of SEQ ID NO:58; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:59, (e) a HCDR2 of SEQ ID NO:60, and (f) a HCDR3 of SEQ ID NO:61;

(ix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:70, (b) a LCDR2 of SEQ ID NO:71, (c) a LCDR3 of SEQ ID NO:72; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:73, (e) a HCDR2 of SEQ ID NO:74, and (f) a HCDR3 of SEQ ID NO:75;

(x) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:84, (b) a LCDR2 of SEQ ID NO:85, (c) a LCDR3 of SEQ ID NO:86; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO:87, (e) a HCDR2 of SEQ ID NO: 88, and (f) a HCDR3 of SEQ ID NO: 89;

(xi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:98, (b) a LCDR2 of SEQ ID NO:99, (c) a LCDR3 of SEQ ID NO: 100; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 101, (e) a HCDR2 of SEQ ID NO: 102, and (f) a HCDR3 of SEQ ID NO: 103;

(xii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 112, (b) a LCDR2 of SEQ ID NO: 113, (c) a LCDR3 of SEQ ID NO: 114; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 115, (e) a HCDR2 of SEQ ID NO: 116, and (f) a HCDR3 of SEQ ID NO: 117;

(xiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 126, (b) a LCDR2 of SEQ ID NO: 127, (c) a LCDR3 of SEQ ID NO: 128; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 129, (e) a HCDR2 of SEQ ID NO: 130, and (f) a HCDR3 of SEQ ID NO: 131;

(xiv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 140, (b) a LCDR2 of SEQ ID NO: 141, (c) a LCDR3 of SEQ ID NO: 142; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 143, (e) a HCDR2 of SEQ ID NO: 144, and (f) a HCDR3 of SEQ ID NO: 145;

(xv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 154, (b) a LCDR2 of SEQ ID NO: 155, (c) a LCDR3 of SEQ ID NO: 156; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 157, (e) a HCDR2 of SEQ ID NO: 158, and (f) a HCDR3 of SEQ ID NO: 159;

(xvi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 168, (b) a LCDR2 of SEQ ID NO: 169, (c) a LCDR3 of SEQ ID NO: 170; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 171, (e) a HCDR2 of SEQ ID NO: 172, and (f) a HCDR3 of SEQ ID NO: 173;

(xvii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 182, (b) a LCDR2 of SEQ ID NO: 183, (c) a LCDR3 of SEQ ID NO: 184; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 185, (e) a HCDR2 of SEQ ID NO: 186, and (f) a HCDR3 of SEQ ID NO: 187;

(xviii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO: 196, (b) a LCDR2 of SEQ ID NO: 197, (c) a LCDR3 of SEQ ID NO: 198; and a heavy chain variable region that comprises: (d) a HCDRI of SEQ ID NO: 199, (e) a HCDR2 of SEQ ID NO:200, and (f) a HCDR3 of SEQ ID NO:201;

(xix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:238, (b) a LCDR2 of SEQ ID NO:239, (c) a LCDR3 of SEQ ID NO:240; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:241, (e) a HCDR2 of SEQ ID NO:242, and (f) a HCDR3 of SEQ ID NO:243;

(xx) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:252, (b) a LCDR2 of SEQ ID NO:253, (c) a LCDR3 of SEQ ID NO:254; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:255, (e) a HCDR2 of SEQ ID NO:256, and (f) a HCDR3 of SEQ ID NO:257;

(xxi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:280, (b) a LCDR2 of SEQ ID NO:281, (c) a LCDR3 of SEQ ID NO:282; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:283, (e) a HCDR2 of SEQ ID NO:284, and (f) a HCDR3 of SEQ ID NO:285;

(xxii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:294, (b) a LCDR2 of SEQ ID NO:295, (c) a LCDR3 of SEQ ID NO:296; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:297, (e) a HCDR2 of SEQ ID NO:298, and (f) a HCDR3 of SEQ ID NO:299; or

(xxiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:322, (b) a LCDR2 of SEQ ID NO:323, (c) a LCDR3 of SEQ ID NO:324; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:325, (e) a HCDR2 of SEQ ID NO:326, and (f) a HCDR3 of SEQ ID NO:327.

[00239] In certain aspects, an antibody that specifically binds to CDH6 is an antibody or antibody fragment *(e.g.,* antigen binding fragment) that is described in Table 5 and/or 6.

1. Identification of Epitopes and Antibodies That Bind to the Same Epitope

[00240] The present disclosure provides antibodies and antibody fragments *(e.g.,* antigen binding fragments) that bind to an epitope of within the extracellular domain of CDH6. In certain aspects the antibodies and antibody fragments can bind to epitopes within domains of the CDH6 extracellular domain.

[00241] The present disclosure also provides antibodies and antibody fragments *(e.g.,* antigen binding fragments) that bind to the same epitope as do the anti- CDH6 antibodies described in Table 5 and 6. Additional antibodies and antibody fragments *(e.g.,* antigen binding fragments) can therefore be identified based on their ability to cross-compete *(e.g.,* to competitively inhibit the binding of, in a statistically significant manner) with other antibodies in CDH6 binding assays. The ability of a test antibody to inhibit the binding of antibodies and antibody fragments *(e.g.,* antigen binding fragments)

of the present disclosure to a CDH6 protein *(e.g.*, human CDH6) demonstrates that the test antibody can compete with that antibody or antibody fragment *(e.g.*, antigen binding fragments) for binding to CDH6; such an antibody may, according to non-limiting theory, bind to the same or a related *(e.g.*, a structurally similar or spatially proximal) epitope on the CDH6 protein as the antibody or antibody fragment *(e.g.,* antigen binding fragments) with which it competes. In a certain aspect, the antibody that binds to the same epitope on CDH6 as the antibodies or antibody fragments *(e.g.,* antigen binding fragments) of the present disclosure is a human or humanized monoclonal antibody. Such human or humanized monoclonal antibodies can be prepared and isolated as described herein.

2. Further Alteration of the Framework of Fc Region

[00242] The present disclosure provides site-specific labeled immunoconjugates. These immunoconjugates can comprise modified antibodies or antigen binding fragments thereof that further comprise modifications to framework residues within VH and/or VL, *e.g.* to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "back-mutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived. To return the framework region sequences to their germline configuration, the somatic mutations can be "back-mutated" to the germline sequence by, for example, site-directed mutagenesis. Such "back-mutated" antibodies are also intended to be encompassed.

[00243] Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T-cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 2003/0153043 by Carr *et al.*

[00244] In addition or alternative to modifications made within the framework or CDR regions, antibodies can be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody can be chemically modified *(e.g.,* one or more chemical moieties can be attached to the antibody) or be modified to alter

its glycosylation, again to alter one or more functional properties of the antibody. Each of these aspects is described in further detail below.

[00245] In one aspect, the hinge region of CHI is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.* The number of cysteine residues in the hinge region of CHI is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

[00246] In another aspect, the Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcyl protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.*

[00247] In yet other aspects, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the CI component of complement. This approach is described in, *e.g.*, U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

[00248] In another aspect, one or more amino acids selected from amino acid residues can be replaced with a different amino acid residue such that the antibody has altered Clq binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in, *e.g.*, U.S. Patent Nos. 6,194,551 by Idusogie *et al.*

[00249] In another aspect, one or more amino acid residues are altered to thereby alter the ability of the antibody to fix complement. This approach is described in, *e.g.*, the PCT Publication WO 94/29351 by Bodmer *et al.* In a specific aspect, one or more amino acids of an antibody or antigen binding fragment thereof of the present disclosure are replaced by one or more allotypic amino acid residues, for the IgGl subclass and the kappa isotype. Allotypic amino acid residues also include, but are not limited to, the constant region of the heavy chain of the IgGl, IgG2, and IgG3 subclasses as well as the constant region of the light chain of the kappa isotype as described by Jefferis *et al.*, MAbs. 1:332-338 (2009).

[00250] In yet another aspect, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fey receptor by modifying one or more amino acids. This approach is described in, *e.g.,* the PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgGl for FcyRl, FcyRII, FcyRIII and FcRn have been mapped and variants with improved binding have been described (see Shields *et al.,* J. Biol. Chem. 276:6591-6604, 2001).

[00251] In still another aspect, the glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for "antigen." Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in, *e.g.,* U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

[00252] Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lecl3 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields *et al.,* (2002) J. Biol. Chem. 277:26733-26740). PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases *(e.g.,* beta(l,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.,* Nat. Biotech. 17: 176-180, 1999).

[00253] In another aspect, the antibody is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half-life, the antibody can be altered within the CHI or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

[00254] In order to minimize the ADCC activity of an antibody, specific mutations in the Fc region result in "Fc silent" antibodies that have minimal interaction with effector cells. In general, the "IgG Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain, including native sequence Fc region and variant Fc regions. The human IgG heavy chain Fc region is generally defined as comprising the amino acid residue from position C226 or from P230 to the carboxyl-terminus of the IgG antibody. The numbering of residues in the Fc region is that of the EU index of Kabat. The C- terminal lysine (residue K447) of the Fc region may be removed, for example, during production or purification of the antibody.

[00255] Silenced effector functions can be obtained by mutation in the Fc region of the antibodies and have been described in the art: LALA and N297A (Strohl, W., 2009, Curr. Opin. Biotechnol. vol. 20(6):685-691); and D265A (Baudino et al., 2008, J. Immunol. 181 : 6664-69) see also Heusser et al., WO20 12065950. Examples of silent Fc lgGl antibodies are the LALA mutant comprising L234A and L235A mutation in the lgGl Fc amino acid sequence. Another example of a silent lgGl antibody is the DAPA (D265A, P329A) mutation (US 6,737,056). Another silent lgGl antibody comprises the N297A mutation, which results in aglycosylated/non-glycosylated antibodies.

[00256] Fc silent antibodies result in no or low ADCC activity, meaning that an Fc silent antibody exhibits an ADCC activity that is below 50% specific cell lysis, No ADCC activity means that the Fc silent antibody exhibits an ADCC activity (specific cell lysis) that is below 1%.

3. Production of the CDH6 Antibodies

[00257] Anti- CDH6 antibodies and antibody fragments *(e.g.* , antigen binding fragments) thereof can be produced by any means known in the art, including but not limited to, recombinant expression, chemical synthesis, and enzymatic digestion of antibody tetramers, whereas full-length monoclonal antibodies can be obtained by, *e.g.* , hybridoma or recombinant production. Recombinant expression can be from any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc.

[00258] The disclosure further provides polynucleotides encoding the antibodies described herein, *e.g.,* polynucleotides encoding heavy or light chain variable regions or segments comprising the complementarity determining regions as described herein. In some aspects, the polynucleotide encoding the heavy chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide selected from the group consisting of SEQ ID NOs: 22, 36, 50, 64, 78, 92, 106, 120, 134, 148, 162, 176, 190, 204, 218, 232, 246, 260, 274, 288, 302, 316, and 330. In some aspects, the polynucleotide encoding the light chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide selected from the group consisting of SEQ ID NOs: 23, 37, 51, 65, 79, 93, 107, 121, 135, 149, 163, 177, 191, 205, 219, 233, 247, 261, 275, 289, 303, 317, and 331.

[00259] In some aspects, the polynucleotide encoding the heavy chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 26, 40, 54, 68, 82, 96, 110, 124, 138, 152, 166, 180, 194, 208, 222, 236, 250, 264, 278, 292, 306, 320, and 334. In some aspects, the polynucleotide encoding the light chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 27, 41, 55, 69, 83, 97, 111, 125, 139, 153, 167, 181, 195, 209, 223, 237, 251, 265, 279, 293, 307, 321, and 335.

[00260] The polynucleotides of the present disclosure can encode only the variable region sequence of an anti- CDH6 antibody. They can also encode both a variable region and a constant region of the antibody. Some of the polynucleotide sequences encode a polypeptide that comprises variable regions of both the heavy chain and the light chain of one of an exemplified anti- CDH6 antibody. Some other polynucleotides encode two polypeptide segments that respectively are substantially identical to the variable regions of the heavy chain and the light chain of one of the mouse antibodies.

[00261] The polynucleotide sequences can be produced by de novo solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence *(e.g.,* sequences as described in the Examples below) encoding an anti-CDH6 antibody or its binding fragment. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang *et al.,* Meth. Enzymol. 68:90, 1979; the phosphodiester method of Brown *el al,* Meth. Enzymol. 68: 109, 1979; the diethylphosphoramidite method of Beaucage *et al ,* Tetra. Lett., 22: 1859, 1981; and the solid support method of U.S. Patent No. 4,458,066. Introducing mutations to a polynucleotide sequence by

PCR can be performed as described in, *e.g.,* PCR Technology: Principles and Applications for DNA Amplification, H.A. Erlich (Ed.), Freeman Press, NY, NY, 1992; PCR Protocols: A Guide to Methods and Applications, Innis *et al.* (Ed.), Academic Press, San Diego, CA, 1990; Mattila *et al ,* Nucleic Acids Res. 19:967, 1991; and Eckert *et al,* PCR Methods and Applications 1:17, 1991.

[00262] Also provided in the present disclosure are expression vectors and host cells for producing the anti-CDH6 antibodies described above. Various expression vectors can be employed to express the polynucleotides encoding the anti- CDH6 antibody chains or binding fragments. Both viral-based and nonviral expression vectors can be used to produce the antibodies in a mammalian host cell. Nonviral vectors and systems include plasmids, episomal vectors, typically with an expression cassette for expressing a protein or RNA, and human artificial chromosomes (see, *e.g.,* Harrington *et al,* Nat Genet 15:345, 1997). For example, nonviral vectors useful for expression of the anti-CDH6 polynucleotides and polypeptides in mammalian *(e.g.,* human) cells include pThioHis A, B & C, pcDNA3. 1/His, pEBVHis A, B & C (Invitrogen, San Diego, CA), MPSV vectors, and numerous other vectors known in the art for expressing other proteins. Useful viral vectors include vectors based on retroviruses, adenoviruses, adenoassociated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent *et al,* supra; Smith, Annu. Rev. Microbiol. 49:807, 1995; and Rosenfeld *et al,* Cell 68: 143, 1992.

[00263] The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences *(e.g.,* enhancers) that are operably linked to the polynucleotides encoding an anti-CDH6 antibody chain or fragment. In some aspects, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, *e.g.,* arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under noninducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of an anti-CDH6 antibody chain or fragment. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, *e.g.,* Scharf *et al.,* Results Probl. Cell Differ. 20: 125, 1994; and Bittner *et al.,* Meth. Enzymol., 153 :5 16, 1987). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

[00264] The expression vectors may also provide a secretion signal sequence position to form a fusion protein with polypeptides encoded by inserted anti-CDH6 antibody sequences. More often, the inserted anti-CDH6 antibody sequences are linked to a signal sequences before inclusion in the vector. Vectors to be used to receive sequences encoding anti-CDH6 antibody light and heavy chain variable domains sometimes also encode constant regions or parts thereof. Such vectors allow expression of the variable regions as fusion proteins with the constant regions thereby leading to production of intact antibodies or fragments thereof. Typically, such constant regions are human.

[00265] The host cells for harboring and expressing the anti-CDH6 antibody chains can be either prokaryotic or eukaryotic. E. coli is one prokaryotic host useful for cloning and expressing the polynucleotides of the present disclosure. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilis, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which typically contain expression control sequences compatible with the host cell *(e.g.* , an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation. Other microbes, such as yeast, can also be employed to express anti-CDH6 polypeptides. Insect cells in combination with baculovirus vectors can also be used.

[00266] In other aspects, mammalian host cells are used to express and produce the anti-CDH6 polypeptides of the present disclosure. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes *(e.g.* , a myeloma hybridoma clone) or a mammalian cell line harboring an exogenous expression vector *(e.g.* , SP2/0 myeloma cells). These include any normal mortal or normal or abnormal immortal animal or human cell. For example, a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed, including the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, *e.g.* , Winnacker, From Genes to Clones, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (see, *e.g.,* Queen *et al* , Immunol. Rev. 89:49-68, 1986), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites,

and transcriptional terminator sequences. These expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPSV promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

[00267] Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts (see generally Sambrook *et al,* supra). Other methods include, *e.g.,* electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycatiomnucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, Cell 88:223, 1997), agent-enhanced uptake of DNA, and ex vivo transduction. For long-term, high-yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express anti-CDH6 antibody chains or binding fragments can be prepared using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate to the cell type.

Therapeutic and Diagnostic Uses

[00268] The antibodies, antibody fragments *(e.g.* , antigen binding fragments), and antibody drug conjugates of the present disclosure are useful in a variety of applications including, but not limited to, treatment of cancer, such as solid cancers. In certain aspects, the antibodies, antibody fragments *(e.g.,* antigen binding fragments), and antibody drug conjugates are useful for inhibiting tumor growth, inducing differentiation, reducing tumor volume, and/or reducing the tumorigenicity of a tumor. The methods of use can be in vitro, ex vivo, or in vivo methods.

[00269] In one aspect, the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates are useful for detecting the presence of CDH6 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain aspects, a biological sample comprises a cell or tissue. In certain aspects, such tissues include normal and/or cancerous tissues that express CDH6 at higher levels relative to other tissues.

[00270] In one aspect, the present disclosure provides a method of detecting the presence of CDH6 in a biological sample. In certain aspects, the method comprises contacting the biological sample with an anti-CDH6 antibody under conditions permissive for binding of the antibody to the antigen, and detecting whether a complex is formed between the antibody and the antigen.

[00271] Also included is a method of diagnosing a disorder associated with increased expression of CDH6. In certain aspects, the method comprises contacting a test cell with an anti-CDH6 antibody; determining the level of expression (either quantitatively or qualitatively) of CDH6 on the test cell by detecting binding of the anti-CDH6 antibody to the CDH6 antigen; and comparing the level of expression of CDH6 in the test cell with the level of expression of CDH6 in a control cell (*e.g.*, a normal cell of the same tissue origin as the test cell or a cell that expresses CDH6 at levels comparable to such a normal cell), wherein a higher level of expression of CDH6 on the test cell as compared to the control cell indicates the presence of a disorder associated with increased expression of CDH6. In certain aspects, the test cell is obtained from an individual suspected of having a disorder associated with increased expression of CDH6. In certain aspects, the disorder is a cell proliferative disorder, such as a cancer or a tumor.

[00272] In certain aspects, a method of diagnosis or detection, such as those described above, comprises detecting binding of an anti-CDH6 antibody to CDH6 expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing CDH6 on its surface. An exemplary assay for detecting binding of an anti-CDH6 antibody to CDH6 expressed on the surface of a cell is a "FACS" assay.

[00273] Certain other methods can be used to detect binding of anti-CDH6 antibodies to CDH6. Such methods include, but are not limited to, antigen-binding assays that are well known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

[00274] In certain aspects, anti-CDH6 antibodies are labeled. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-

dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.,* through an enzymatic reaction or molecular interaction.

[00275] In certain aspects, anti-CDH6 antibodies are immobilized on an insoluble matrix. Immobilization entails separating the anti-CDH6 antibody from any CDH6 proteins that remains free in solution. This conventionally is accomplished by either insolubilizing the anti-CDH6 antibody before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al, U.S. Patent No. 3,720,760), or by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the anti-CDH6 antibody after formation of a complex between the anti-CDH6 antibody and CDH6 protein, *e.g.,* by immunoprecipitation.

[00276] Any of the above aspects of diagnosis or detection can be carried out using an immunoconjugate of the present disclosure in place of or in addition to an anti-CDH6 antibody.

[00277] In one aspect, the disclosure provides for a method of treating, preventing or ameliorating a disease comprising administering the antibodies, antibody fragments *(e.g.,* antigen binding fragments), and antibody drug conjugates to a patient, thereby treating the disease. In certain aspects, the disease treated with the antibodies, antibody fragments *(e.g.,* antigen binding fragments), and antibody drug conjugates is a cancer. Examples of diseases which can be treated and/or prevented include, but are not limited to, ovarian cancer, renal cancer, hepatic cancer, soft tissue cancer, CNS cancers, thyroid cancer and cholangiocarcinoma. In certain aspects, the cancer is characterized by CDH6 expressing cells to which the antibodies, antibody fragments *(e.g.,* antigen binding fragments), and antibody drug conjugates can specifically bind.

[00278] The present disclosure provides for methods of treating cancer comprising administering a therapeutically effective amount of the antibodies, antibody fragments *(e.g.,* antigen binding fragments), or antibody drug conjugates. In certain aspects, the cancer is a solid cancer. In certain aspects, the subject is a human.

[00279] In certain aspects, the method of inhibiting tumor growth comprises administering to a subject a therapeutically effective amount of the antibodies, antibody fragments *(e.g.,* antigen binding fragments), or antibody drug conjugates. In certain aspects, the subject is a human. In certain aspects, the subject has a tumor or has had a tumor removed.

[00280] In certain aspects, the tumor expresses the CDH6 to which the anti-CDH6 antibody binds. In certain aspects, the tumor overexpresses the human CDH6.

[00281] For the treatment of the disease, the appropriate dosage of the antibodies, antibody fragments *(e.g.,* antigen binding fragments), or antibody drug conjugates depend on various factors,

such as the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, previous therapy, patient's clinical history, and so on. The antibody or agent can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved *(e.g.,* reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual antibody, antibody fragment *(e.g.,* antigen binding fragment), or antibody drug conjugates. In certain aspects, dosage is from O.Olmg to 10 mg *(e.g.,* 0.01 mg, 0.05mg, O.lmg, 0.5mg, lmg, 2mg, 3mg, 4mg, 5mg, 7mg, 8mg, 9mg, or lOmg) per kg of body weight, and can be given once or more daily, weekly, monthly or yearly. In certain aspects, the antibody, antibody fragment *(e.g.,* antigen binding fragment), or antibody drug conjugate of the present disclosure is given once every two weeks or once every three weeks. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues.

Combination Therapy

[00282] In certain instances, an antibody, antibody fragment *(e.g.,* antigen binding fragment), or antibody drug conjugate of the present disclosure is combined with other therapeutic agents, such as other anti-cancer agents, anti-allergic agents, anti-nausea agents (or anti-emetics), pain relievers, cytoprotective agents, and combinations thereof.

[00283] General chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan®), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine

implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

[00284] In one aspect, an antibody, antibody fragment *(e.g.* , antigen binding fragment), or antibody drug conjugate of the present disclosure is combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with a second compound having anti-cancer properties. The second compound of the pharmaceutical combination formulation or dosing regimen can have complementary activities to the antibody or immunoconjugate of the combination such that they do not adversely affect each other. For example, an antibody, antibody fragment *(e.g.* , antigen binding fragment), or antibody drug conjugate of the present disclosure can be administered in combination with, but not limited to, a chemotherapeutic agent, a tyrosine kinase inhibitor, for example, Imatinib.

[00285] The term "pharmaceutical combination" as used herein refers to either a fixed combination in one dosage unit form, or non-fixed combination or a kit of parts for the combined administration where two or more therapeutic agents may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, *e.g.* synergistic effect.

[00286] The term "combination therapy" refers to the administration of two or more therapeutic agents to treat a therapeutic condition or disorder described in the present disclosure. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of active ingredients. Alternatively, such administration encompasses co-administration in multiple, or in separate containers *(e.g.,* capsules, powders, and liquids) for each active ingredient. Powders and/or liquids may be reconstituted or diluted to a desired dose prior to administration. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner, either at approximately the same time or at different times. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the conditions or disorders described herein.

[00287] The combination therapy can provide "synergy" and prove "synergistic", *i.e.,* the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect can be attained when the active ingredients are: **(1)** co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some

other regimen. When delivered in alternation therapy, a synergistic effect can be attained when the compounds are administered or delivered sequentially, e.g., by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, *i.e.,* serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

[00288] In one aspect, the present disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with one or more tyrosine kinase inhibitors, including but not limited to, EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGFR inhibitors, and Met inhibitors.

[00289] For example, tyrosine kinase inhibitors include but are not limited to, Erlotinib hydrochloride (Tarceva®); Linifanib (N-[4-(3-amino-lH-indazol-4-yl)phenyl]-N'-(2-fluoro-5-methylphenyl)urea, also known as ABT 869, available from Genentech); Sunitinib malate (Sutent®); Bosutinib (4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methylpiperazin-l-yl)propoxy]quinoline-3-carbonitrile, also known as SKI-606, and described in US Patent No. 6,780,996); Dasatinib (Sprycel®); Pazopanib (Votrient®); Sorafenib (Nexavar®); Zactima (ZD6474); nilotinib (Tasigna®); Regorafenib (Stivarga®) and Imatinib or Imatinib mesylate (Gilvec® and Gleevec®).

[00290] Epidermal growth factor receptor (EGFR) inhibitors include but are not limited to, Erlotinib hydrochloride (Tarceva®), Gefitnib (Iressa®); N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[(3"S")4etrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide, Tovok®); Vandetanib (Caprelsa®); Lapatinib (Tykerb®); (3R,4R)-4-Amino-l-((4-((3-methoxyphenyl)amino)pyrrolo[2, 1-f] [l,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS6905 14); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-l-piperazinyl)methyl]phenyl]-N-[(lR)-l-phenylethyl]- 7H-Pyrrolo[2,3-d]pyrimidin-4-amine (AEE788, CAS 497839-62-0); Mubritinib (TAK165); Pelitinib (EKB569); Afatinib (BIBW2992); Neratinib (HKI-272); N-[4-[[l-[(3-Fluorophenyl)methyl]-lH-indazol-5-yljamino]-5-methylpyrrolo[2, 1-f] [1,2,4]triazin-6-yl]-carbamic acid, (38)-3^ oφηo liny lπ̃ethy 1ester (BMS599626); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aa,5p,6aa)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]- 4-quinazolinamine (XL647, CAS 781613-23-8); and 4-[4-[[(lR)-l-Phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrirnidin-6-yl]-phenol (PKI166, CAS 187724-61-4).

[00291] EGFR antibodies include but are not limited to, Cetuximab (Erbitux®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); Nimotuzumab (hR3); Zalutumumab; TheraCIM h-R3; MDX0447 (CAS 339151-96-1); and ch806 (mAb-806, CAS 946414-09-1).

[00292] Human Epidermal Growth Factor Receptor 2 (HER2 receptor) (also known as Neu, ErbB-2, CD340, or pl85) inhibitors include but are not limited to, Trastuzumab (Herceptin®); Pertuzumab (Omnitarg®); Neratinib (HKI-272, (2E)-N-[4-[[3-chloro-4-[(pyridin-2-yl)methoxy]phenyl]amino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide, and described PCT Publication No. WO 05/028443); Lapatinib or Lapatinib ditosylate (Tykerb®); (3R,4R)-4-amino- 1-((4-((3-methoxyphenyl)amino)pyrrolo [2, 1-f] [1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); (2E)-N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[(3S)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-2-butenamide (BIBW-2992, CAS 850140-72-6); N-[4-[[l-[(3-Fluorophenyl)methyl]-lH-indazol-5-yljamino]-5-methylpyrrolo [2, 1-f] [1,2,4]triazin-6-yl]-carbamic acid, (38)-3^ oφholinylmethyl ester (BMS 599626, CAS 714971-09-2); Canertinib dihydrochloride (PD 183805 or CI-1033); and N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aa,5p,6aa)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]- 4-quinazolinamine (XL647, CAS 781613-23-8).

[00293] HER3 inhibitors include but are not limited to, LJM716, MM-121, AMG-888, RG7116, REGN-1400, AV-203, MP-RM-1, MM-111, and MEHD-7945A.

[00294] MET inhibitors include but are not limited to, Cabozantinib (XL184, CAS 849217-68-1); Foretinib (GSK1363089, formerly XL880, CAS 849217-64-7); Tivantinib (ARQ197, CAS 1000873-98-2); l-(2-Hydroxy-2-methylpropyl)-^-(5-(7-methoxyquinolin-4-yloxy)pyridin-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-l/f-pyrazole-4-carboxamide (AMG 458); Cryzotinib (Xalkori®, PF-02341066); (3Z)-5-(2,3-Dihydro-lH-indol-l-ylsulfonyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-l-yl)carbonyl]-lH-pyrrol-2-yl}methylene)-l,3-dihydro-2H-indol-2-one (SU11271); (3Z)-N-(3-Chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-l-yl)carbonyl]-lH-pyrrol-2-yl}methylene)-N-methyl-2-oxoindoline-5-sulfonamide (SU11274); (3Z)-N-(3-Chlorophenyl)-3-{ [3,5-dimethyl-4-(3-moφholin-4-ylpropyl)-lH-pyrrol-2-yl]methylene}-N-methyl-2-oxoindoline-5-sulfonamide (SU11606); 6-[Difluoro[6-(l-methyl-lH-pyrazol-4-yl)-l,2,4-triazolo[4,3-b]pyridazin-3-yl]methyl]-quinoline (JNJ38877605, CAS 943540-75-8); 2-[4-[l-(Quinolin-6-ylmethyl)-lH-[l,2,3]triazolo[4,5-b]pyrazin-6-yl]-lH-pyrazol-l-yl]ethanol (PF04217903, CAS 956905-27-4); N-((2R)-l,4-Dioxan-2-ylmethyl)-N-methyl-N'-[3-(l-methyl-lH-pyrazol-4-yl)-5-oxo-5H-benzo[4,5]cyclohepta[l,2-b]pyridin-7-yl]sulfamide (MK2461, CAS 917879-39-1); 6-[[6-(l-Methyl-l/f-pyrazol-4-yl)-l,2,4-triazolo[4,3-

£]pyridazin-3-yl]thio]-quinoline (SGX523, CAS 1022150-57-7); and (3Z)-5-[[(2,6-DicUorophenyl)methyl]sulfonyl]-3-[[3,5-dimethyl-4-[[(2i?)-2-(l-pyrrolidinylmethyl)-l-pyrrolidinyl]carbonyl]-l/i-pyrrol-2-yl]methylene]-l,3-dihydro-2/i-indol-2-one (PHA665752, CAS 477575-56-7).

[00295] IGF1R inhibitors include but are not limited to, BMS-754807, XL-228, OSI-906, GSK0904529A, A-928605, AXL1717, KW-2450, MK0646, AMG479, IMCA12, MEDI-573, and BI836845. See *e.g.,* Yee, JNCI, 104; 975 (2012) for review.

[00296] In another aspect, the present disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with one or more FGF downstream signaling pathway inhibitors, including but not limited to, MEK inhibitors, Braf inhibitors, PI3K/Akt inhibitors, SHP2 inhibitors, and also mTor inhibitors.

[00297] For example, mitogen-activated protein kinase (MEK) inhibitors include but are not limited to, XL-5 18 (also known as GDC-0973, Cas No. 1029872-29-4, available from ACC Corp.); 2-[(2-Chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluoro-benzamide (also known as CI-1040 or PD184352 and described in PCT Publication No. WO2000035436); N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]- benzamide (also known as PD0325901 and described in PCT Publication No. WO2002006213); 2,3-Bis[amino[(2-aminophenyl)thio] methylene] -butanedinitrile (also known as U0126 and described in US Patent No. 2,779,780); N-[3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]-6-methoxyphenyl]-l-[(2R)-2,3-dihydroxypropyl]- cyclopropanesulfonamide (also known as RDEAl 19 or BAY869766 and described in PCT Publication No. WO200701401 1); (3S,4R,5Z,8S,9S, 1 lE)-14-(Ethylamino)-8,9, 16-trihydroxy-3,4-dimethyl-3,4,9, 19-tetrahydro-lH-2-benzoxacyclotetradecine-l,7(8H)-dione] (also known as E6201 and described in PCT Publication No. WO2003076424); 2'-Amino-3 '-methoxyflavone (also known as PD98059 available from Biaffin GmbH & Co., KG, Germany); Vemurafenib (PLX-4032, CAS 918504-65-1); (R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione (TAK-733, CAS 1035555-63-5); Pimasertib (AS-703026, CAS 120453 1-26-9); and Trametinib dimethyl sulfoxide (GSK-1 120212, CAS 120453 1-25-80).

[00298] Phosphoinositide 3-kinase (PI3K) inhibitors include but are not limited to, 4-[2-(lH-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-l-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]mo ϕ holine (also known as GDC 0941 and described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(qmnolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-l-

yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimo ϕ holinopyrimidin-4-yl)pyridin-2 -amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. WO2007/084786); Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (lE,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-l-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro- 11-hydroxy -4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho [1,2-c]pyran-2,7, 10(lH)-trione (PX866, CAS 502632-66-8); and 8-Phenyl-2-(mo ϕ holin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6).

[00299] mTor inhibitors include but are not limited to, Temsirolimus (Torisel®); Ridaforolimus (formally known as deferolimus, *(lR,2R,4S)-4-[(2R)-2 [(1R,9S,12S, 15R, 16E, 18R,19R,21R,* 23S,24£,26£,28Z,30S,32S,35i?)-l,18-dihydroxy-19,30-dimethoxy-15,17,2 1,23, 29,35-hexamethyl-2,3,10, 14,20-pentaoxo-l l,36-dioxa-4-azatricyclo[30.3. 1.0^{4,9}] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); Everolimus (Afinitor® or RADOOl); Rapamycin (AY22989, Sirolimus®); Simapimod (CAS 164301-5 1-3); (5-{2,4-Bis[(3S)-3-methylmo ϕ holin-4-yl]pyrido[2,3-d]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[/ra «*s*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-^pyrirnidin-7(8/f)-one (PF04691502, CAS 1013 101-36-4); and N^2-[l,4-dioxo-4-[[4-(4-oxo-8-phenyl-4/ *1*-l-benzopyran-2-yl)mo ϕ holinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-a-aspartylL-serine-, inner salt (SF1 126, CAS 936487-67-1).

[00300] In yet another aspect, the present disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with one or more pro-apoptotics, including but not limited to, IAP inhibitors, Bcl2 inhibitors, MCll inhibitors, Trail agents, Chk inhibitors.

[00301] For examples, IAP inhibitors include but are not limited to, NVP-LCL161, GDC-0917, AEG-35 156, AT406, and TL3271 1. Other examples of IAP inhibitors include but are not limited to those disclosed in WO04/005284, WO 04/007529, WO05/097791, WO 05/069894, WO 05/069888, WO 05/094818, US2006/00 14700, US2006/0025347, WO 06/069063, WO 06/0101 18, WO 06/017295, and WO08/134679, all of which are incoϕ orated herein by reference.

[00302] BCL-2 inhibitors include but are not limited to, 4-[4-[[2-(4-Chlorophenyl)-5,5-dimethyl-l-cyclohexen-l-yl]methyl]-l-piperazinyl]-N-[[4-[[(lR)-3-(4-mo ϕ holinyl)-l-

[(phenyltWo)methyl]propyl]amino]-3-[(trifluorom ethyl)sulfonyl]phenyl]sulfonyl]benzamide (also known as ABT-263 and described in PCT Publication No. WO 09/155386); Tettocarcin A; Antimycin; Gossypol ((-)BL-193); Obatoclax; Ethyl-2-amino-6-cyclopentyl-4-(l-cyano-2-ethoxy-2-oxoethyl)-4Hchromone-3-carboxylate (HA14 - 1); Oblimersen (G3 139, Genasense®); Bak BH3 peptide; (-)-Gossypol acetic acid (AT-101); 4-[4-[(4'-Chloro[l,l'-biphenyl]-2-yl)methyl]-l-piperazinyl]-N-[[4-[[(lR)-3-(dimethylamino)-l-[(phenyltWo)methyl]propyl]amino]-3-nitrophenyl]sulfonyl]-benzamide (ABT-737, CAS 852808-04-9); and Navitoclax (ABT-263, CAS 923564-5 1-6).

[00303] Proapoptotic receptor agonists (PARAs) including DR4 (TRAILRl) and DR5 (TRAILR2), including but are not limited to, Dulanermin (AMG-95 1, RhApo2L/TRAIL); Mapatumumab (HRS-ETR1, CAS 658052-09-6); Lexatumumab (HGS-ETR2, CAS 845816-02-6); Apomab (Apomab®); Conatumumab (AMG655, CAS 89673 1-82-1); and Tigatuzumab (CS1008, CAS 946415-34-5, available from Daiichi Sankyo).

[00304] Checkpoint Kinase (CHK) inhibitors include but are not limited to, 7-Hydroxystaurosporine (UCN-01); 6-Bromo-3-(l-methyl-l//-pyrazol-4-yl)-5-(3i?)-3-piperidinyl-pyrazolo[l,5-a]pyrimidin-7-amine (SCH900776, CAS 891494-63-6); 5-(3-Fluorophenyl)-3-ureidothiophene-2-carboxylic acid N-[(S)-piperidin-3-yl]amide (AZD7762, CAS 860352-01-8); 4-[((3S)-l-Azabicyclo[2.2.2]oct-3-yl)amino]-3-(lH-benzimidazol-2-yl)-6-cUoroquinolin-2(lH)-one (CHIR 124, CAS 405 168-58-3); 7-Aminodactinomycin (7-AAD), Isogranulatimide, debromohymenialdisine; N-[5-Bromo-4-methyl-2-[(2S)-2-mo ϕ holinylmethoxy]-phenyl]-N'-(5-methyl-2-pyrazinyl)urea (LY2603618, CAS 911222-45-2); Sulforaphane (CAS 4478-93-7, 4-Methylsulfinylbutyl isothiocyanate); 9,10,11,12-Tetrahydro- 9,12-epoxy-l//-diindolo[l,2,3-¾:3',2',l'-A:/]pyrrolo[3,4-/] [l,6]benzodiazocine-l,3(2/f)-dione (SB-218078, CAS 135897-06-2); and TAT-S216A (Sha et al., Mol. Cancer. Ther 2007; 6(1): 147-153), and CBP501.

[00305] In one aspect, the present disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with one or more FGFR inhibitors. For example, FGFR inhibitors include but are not limited to, Brivanib alaninate (BMS-582664, (5)-((i?)-l-(4-(4-Fluoro-2-methyl-l//-indol-5-yloxy)-5-methylpyrrolo[2,l-*f*][l,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Vargatef (BIBF1 120, CAS 928326-83-4); Dovitinib dilactic acid (TKI258, CAS 852433-84-2); 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-l-{6-[4-(4-ethyl-piperazin-l-yl)-phenylamino]-pyrimidin-4-yl}-l-methyl-urea (BGJ398, CAS 8725 11-34-7); Danusertib (PHA-739358); and (PD173074, CAS 219580-1 1-7). In a specific aspect, the present

disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with an FGFR2 inhibitor, such as 3-(2,6-dichloro-3,5-dimethoxyphenyl)- 1-(6((4-(4-ethylpiperazin- 1-yl)phenyl)amino)pyrimidin-4-yl)- 1-methylurea (also known as BGJ-398); or 4-amino-5-fluoro-3-(5-(4-methylpiperazinl-yl)-l/i-benzo [*i*/]imidazole-2-yl)quinolin-2(l//)-one (also known as dovitinib or TKI-258). AZD4547 (Gavine *et al,* 2012, Cancer Research 72, 2045-56, N-[5-[2-(3,5-Dimethoxyphenyl)ethyl]-2H-pyrazol-3-yl]-4-(3R,5S)-diemthylpiperazin-l-yl)benzamide), Ponatinib (AP24534; Gozgit *et a/.*, 2012, Mol Cancer Ther., 1 1; 690-99; 3-[2-(imidazo[l,2-b]pyridazin-3-yl)ethynyl]-4-methyl-N-{4-[(4-methylpiperazin-l-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide, CAS 9433 19-70-8)

Pharmaceutical Compositions

[00306] To prepare pharmaceutical or sterile compositions including immunoconjugates, the immunoconjugates of the present disclosure are mixed with a pharmaceutically acceptable carrier or excipient. The compositions can additionally contain one or more other therapeutic agents that are suitable for treating or preventing cancer, for example, ovarian cancer, renal cancer, hepatic cancer, soft tissue cancer, CNS cancers, thyroid cancer and cholangiocarcinoma.

[00307] Formulations of therapeutic and diagnostic agents can be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, *e.g.* , lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, *e.g.* , Hardman *et al.* , Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y., 2001; Gennaro, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y., 2000; Avis, *et al.* (eds.), Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY, 1993; Lieberman, *et al.* (eds.), Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY, 1990; Lieberman, *et al.* (eds.) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY, 1990; Weiner and Kotkoskie, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y., 2000).

[00308] In a specific aspect, the clinical service form (CSF) of the antibody drug conjugates of the present disclosure is a lyophilisate in vial containing the ADC, sodium succinate, and polysorbate 20. The lyophilisate can be reconstitute with water for injection, the solution comprises the ADC, sodium succinate, sucrose, and polysorbate 20 at a pH of about 5.0. For subsequent intravenous administration, the obtained solution will usually be further diluted into a carrier solution.

[00309] Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. In certain aspects, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, *e.g.,* Wawrzynczak, Antibody Therapy, Bios Scientific Pub. Ltd, Oxfordshire, UK, 1996; Kresina (ed.), Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, N.Y., 1991; Bach (ed.), Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, N.Y., 1993; Baert *et al* , New Engl. J. Med. 348:601-608, 2003; Milgrom *et al,* New Engl. J. Med. 341 :1966-1973, 1999; Slamon *et al,* New Engl. J. Med. 344:783-792, 2001; Beniaminovitz *et al,* New Engl. J. Med. 342:613-619, 2000; Ghosh *et al* , New Engl. J. Med. 348:24-32, 2003; Lipsky *et al* , New Engl. J. Med. 343 :1594-1602, 2000).

[00310] Determination of the appropriate dose is made by the clinician, *e.g.,* using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, *e.g.,* the inflammation or level of inflammatory cytokines produced.

[00311] Actual dosage levels of the active ingredients in the pharmaceutical compositions antibody drug conjugates can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors known in the medical arts.

[00312] Compositions comprising antibodies or fragments thereof can be provided by continuous infusion, or by doses at intervals of, *e.g.*, one day, one week, or 1-7 times per week. Doses can be provided intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, or by inhalation. A specific dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects.

[00313] For the immunoconjugates of the present disclosure, the dosage administered to a patient may be 0.0001 mg/kg to 100 mg/kg of the patient's body weight. The dosage may be between 0.0001 mg/kg and 20 mg/kg, 0.0001 mg/kg and 10 mg/kg, 0.0001 mg/kg and 5 mg/kg, 0.0001 and 2 mg/kg, 0.0001 and 1 mg/kg, 0.0001 mg/kg and 0.75 mg/kg, 0.0001 mg/kg and 0.5 mg/kg, 0.000 1 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.0 1 to 0.25 mg/kg or 0.01 to 0.10 mg/kg of the patient's body weight. The dosage of the antibodies or fragments thereof can be calculated using the patient's weight in kilograms (kg) multiplied by the dose to be administered in mg/kg.

[00314] Doses of the immunoconjugates the can be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months. In a specific aspect, doses of the immunoconjugates of the present disclosure are repeated every 3 weeks.

[00315] An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method, route and dose of administration and the severity of side effects (see, *e.g.,* Maynard *et al,* A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla., 1996; Dent, Good Laboratory and Good Clinical Practice, Urch Publ., London, UK, 2001).

[00316] The route of administration may be by, *e.g.*, topical or cutaneous application, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional, or by sustained release systems or an implant (see, *e.g.*, Sidman *et al,* Biopolymers 22:547-556, 1983; Langer *et al*., J. Biomed. Mater. Res. 15: 167-277, 1981; Langer, Chem. Tech. 12:98-105, 1982; Epstein *et al.*, Proc. Natl. Acad. Sci. USA 82:3688-3692, 1985; Hwang *et al*, Proc. Natl. Acad. Sci. USA 77:4030-4034, 1980; U.S. Pat. Nos. 6,350,466 and 6,3 16,024). Where necessary, the composition may also include a solubilizing agent or a local anesthetic such as lidocaine to ease pain at the site of the injection, or both. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

See, *e.g.*, U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/3 1346, and WO 99/66903, each of which is incorporated herein by reference their entirety.

[00317] A composition of the present disclosure can also be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Selected routes of administration for the immunoconjugates include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. Parenteral administration may represent modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, a composition of the present disclosure can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. In one aspect, the immunoconjugates of the present disclosure are administered by infusion. In another aspect, the immunoconjugates are administered subcutaneously.

[00318] If the immunoconjugates of the present disclosure are administered in a controlled release or sustained release system, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, CRC Crit. Ref Biomed. Eng. 14:20, 1987; Buchwald *et al*, Surgery 88:507, 1980; Saudek *et al*, N. Engl. J. Med. 321:574, 1989). Polymeric materials can be used to achieve controlled or sustained release of the therapies of the immunoconjugates (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla., 1974; Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York, 1984; Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61, 1983; see also Levy *et al*, Science 228: 190, 1985; During *et al*, Ann. Neurol. 25:35 1, 1989; Howard *et al*, J. Neurosurg. 7 1:105, 1989; U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U.S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacry lie acid), polyglycolides (PLG),

polyanhydrides, poly(N-vinyl pyrrolidone), polyvinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In one aspect, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. A controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138, 1984).

[00319] Controlled release systems are discussed in the review by Langer, Science 249: 1527-1533, 1990). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more immunoconjugates of the present disclosure. See, *e.g.*, U.S. Pat. No. 4,526,938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning *et al,* Radiotherapy & Oncology 39: 179-189, 1996; Song *et al*, PDA Journal of Pharmaceutical Science & Technology 50:372-397, 1995; Cleek *et al.*, Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, 1997; and Lam *et al*, Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, 1997, each of which is incorporated herein by reference in their entirety.

[00320] If the immunoconjugates of the disclosure are administered topically, they can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, *e.g.*, Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity, in some instances, greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents *(e.g.,* preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, in some instances, in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile *(e.g.,* a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

[00321] If the compositions comprising the immunoconjugates are administered intranasally, it can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present disclosure can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant *(e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, *e.g.,* gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00322] Methods for co-administration or treatment with a second therapeutic agent, *e.g.,* a cytokine, steroid, chemotherapeutic agent, antibiotic, or radiation, are known in the art (see, *e.g.*, Hardman *et al.*, (eds.) (200 1) Goodman and Gilman's The Pharmacological Basis of Therapeutics, lO.sup.th ed., McGraw-Hill, New York, N.Y.; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice: A Practical Approach, Lippincott, Williams & Wilkins, Phila., Pa.; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., Pa.). An effective amount of therapeutic may decrease the symptoms by at least 10%; by at least 20%; at least about 30%; at least 40%, or at least 50%.

[00323] Additional therapies *(e.g.*, prophylactic or therapeutic agents), which can be administered in combination with the immunoconjugates may be administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours apart from the immunoconjugates of the present disclosure. The two or more therapies may be administered within one same patient visit.

[00324] In certain aspects, immunoconjugates can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds.

To ensure that the therapeutic compounds of the disclosure cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.,* U.S. Pat. Nos. 4,522,81 1; 5,374,548; and 5,399,33 1. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.,* Ranade, (1989) J. Clin. Pharmacol. 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.,* U.S. Pat. No. 5,416,016 to Low *et al);* mannosides (Umezawa *et al,* (1988) Biochem. Biophys. Res. Commun. 153: 1038); antibodies (Bloeman *et al.,* (1995) FEBS Lett. 357: 140; Owais *et al.,* (1995) Antimicrob. Agents Chemother. 39: 180); surfactant protein A receptor (Briscoe *et al.,* (1995) Am. J. Physiol. 1233:134); p 120 (Schreier et al, (1994) J. Biol. Chem. 269:9090); see also K. Keinanen; M. L. Laukkanen (1994) FEBS Lett. 346: 123; J. J. Killion; I. J. Fidler (1994) Immunomethods 4:273.

[00325] The present disclosure provides protocols for the administration of pharmaceutical composition comprising immunoconjugates alone or in combination with other therapies to a subject in need thereof. The combination therapies *(e.g.,* prophylactic or therapeutic agents) can be administered concomitantly or sequentially to a subject. The therapy *(e.g.,* prophylactic or therapeutic agents) of the combination therapies can also be cyclically administered. Cycling therapy involves the administration of a first therapy *(e.g.,* a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy *(e.g.,* a second prophylactic or therapeutic agent) for a period of time and repeating this sequential administration, i.e., the cycle, in order to reduce the development of resistance to one of the therapies *(e.g.,* agents) to avoid or reduce the side effects of one of the therapies *(e.g.,* agents), and/or to improve, the efficacy of the therapies.

[00326] The therapies *(e.g.,* prophylactic or therapeutic agents) of the combination therapies of the disclosure can be administered to a subject concurrently.

[00327] The term "concurrently" is not limited to the administration of therapies *(e.g.,* prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising antibodies or fragments thereof are administered to a subject in a sequence and within a time interval such that the immunoconjugates can act together with the other therapy(ies) to provide an increased benefit than if they were administered otherwise. For example, each therapy may be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy

can be administered to a subject separately, in any appropriate form and by any suitable route. In various aspects, the therapies *(e.g.,* prophylactic or therapeutic agents) are administered to a subject less than 15 minutes, less than 30 minutes, less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, 24 hours apart, 48 hours apart, 72 hours apart, or 1 week apart. In other aspects, two or more therapies *(e.g.* , prophylactic or therapeutic agents) are administered to a within the same patient visit.

[00328] The prophylactic or therapeutic agents of the combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

EXAMPLES

Example 1: Identification of CDH6 antibodies by phase-display technology

HuCAL PLATINUM® Pannings

[00329] Antibodies were identified by the selection of clones that bound to CDH6-ECD. As a source of antibody variants a commercially available phage display library, the Morphosys HuCAL PLATINUM® library, was used. The phagemid library is based on the HuCAL® concept (Knappik et al., . J Mol Biol. 2000: 296(l):57-86; Prassler et al., J Mol Biol. 201 1: 413(l):261-78 201 1) and employs the CysDisplayTM technology for displaying the Fab on the phage surface (Rothe et al., J Mol Biol. 2008: 376(4): 1182-200). For isolation of anti-CDH6 antibodies, standard panning strategies were performed using solid phase and solution panning approaches.

Overview of antigen (recombinant protein and cell lines) and antibody reagents used for pannings and screenings

[00330] Pannings were done on various forms of CDH6 antigens. An overview of recombinant CDH6 proteins and CDH6-expressing cell lines used as antigens for pannings and subsequent screening and characterization is given in Table 1.

[00331] Human, mouse and rat CDH6 extracellular domains were gene synthesized based on amino acid sequences from the GenBank or Uniprot databases (see Table 1). Cynomolgus CDH6

cDNA template was gene synthesized based on amino acid sequences and homology information generated using mRNA from various cyno tissues (*e.g.* Zyagen Laboratories, San Diego, CA). All synthesized DNA fragments were cloned into appropriate expression vectors listed affinity tags to allow for purification or expression vectors Zyagen Laboratories for mammalian cell surface expression.

[00332] For the generation of a stable CHO cell line exogenously expressing human CDH6 the TREX expression system (Invitrogen, Carlsbad, CA) was used according to the manufacturer's instructions. Briefly, CHO-TREx cells (Invitrogen, R718-07, Carlsbad, CA) were grown in DMEM media (Invitrogen 11995-085) withl0% FBS (Invitrogen, 10082-147, Carlsbad, CA), and 10 μg/mΐ Blasticidin (Invitrogen A1 1139-02, Carlsbad, CA). Transfection was performed in 6 well plates when cell reach 90% confluence. 4 μg of linearized CDH6 plasmid DNA was mixed with 100 μΐ DMEM media (no FBS) in a sterile Eppendorf tube, 12 μΐ of Lipofectamine® 2000 (Invitrogen 11668-019, Carlsbad, CA) were mixed with 100 μΐ DMEM media in another sterile Eppendorf tube. DNA and Lipofectamine® were mixed together, and incubated in room temperature for 15 min. The culture media of CHO-TREx cells was changed to 1 ml DMEM media without FBS for each well; half above DNA/Lipofectamine® mix were added into each well, incubated for 2 hours. Then 2 ml growth media (with FBS) were added into each well, and the cells were incubated overnight. The transfected cells were split from 6 well plate to T175 flask next day and grew in selection DMEM media with 10% FBS, 10 μg/ml Blasticidin, 800-1000 μg/ηιΐ Geneticin (Invitrogen 1013 1-027). CDH6 expression was induced with final 1 μg/ml Tetracycline in selection media for 20-24 hours. Positive cells were labeled with final 5 μg/ml anti-CDH6 primary monoclonal antibody (MAB2715 from R&D Systems, Minneapolis, MN), and then labeled with PE conjugated anti-mouse secondary antibody (cat #12-4010-87, eBioscience, San Diego, CA) and sorted by FACS.

[00333] Stable CHO cell lines featuring exogenous expression of CDH6 from mouse, rat and cynomolgus origin were generated by transfection of CHO-K1 cells (Invitrogen, Carlsbad, CA) with the respective cDNAs cloned into a mammalian expression vector (pcDNA6.1, Invitrogen, Carlsbad, CA). Transfection was performed in 6 well plates when cell reach 90% confluence. 4 μg of linearized CDH6 plasmid DNA were mixed with 100 μΐ DMEM media without FBS in a sterile Eppendorf tube, 12 μΐ of Lipofectamine® 2000 (Invitrogen 11668-019, Carlsbad, CA) were mixed with 100 μΐ DMEM media in another sterile Eppendorf tube. The DNA and Lipofectamine® solution were mixed together, and incubated at room temperature for 15 min. The culture media of CHO cells was changed to 1 ml DMEM media without FBS for each well; half above DNA/Lipofectamine® mix were added into each well, incubated for 2 hours. Then 2 ml growth media (with FBS) were added into each well, and the cells were incubated overnight. The transfected cells were split from 6 well plate to T175 flask next day and grew in selection DMEM media with 10% FBS, 10 μg/ml Blasticidin, 800-1000 μg/ml Geneticin (Invitrogen 1013 1-027 Carlsbad, CA). CDH6 expression was induced with final 1 μg/ml

Tetracycline in selection media for 20-24 hours. Positive cells were labeled with final 5 μg/ml anti-CDH6 primary monoclonal antibody (MAB2715 from R&D Systems, Minneapolis, MN), and then labeled with PE conjugated anti-mouse secondary antibody (cat #12-4010-87, eBioscience, San Diego, CA) and sorted by FACS. An alternative commercially available anti-CDH6 antibody is the 2B6 antibody (#GWB-E8FDF3 - Genway, San Diego, CA). This antibody was also used to confirm CDH6 expression in cells.

Table 1

Overview of antigen, antibody and cell line reagents for pannings and screenings

Antigen/Antibody	Accession/Source	Description	Mature sequence
His6-SUMO-CDH6 aa54-260-APP-Avi	NM_004932.3	human EC 1/2 domain variant (SEQ ID NO:3)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAINRRTGRP VEPESEFIIKIHDINDNEPIFTKEVYTATVP EMSDVGTFWQVTATDADDPTYGNSAK WYSILQGQPYFSVESETGIIKTALLNMD RENREQYQWIQAKDMGGQMGGLSGTT TVNITLTDGGGGSEFRHDSGLNDIFEAQK IEWHE
hCDH6aa54-615 APPavi	NM_004932.3	human full-length ECD (SEQ ID NO:4)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAINRRTGRP VEPESEFIIKIHDINDNEPIFTKEVYTATVP EMSDVGTFWQVTATDADDPTYGNSAK WYSILQGQPYFSVESETGIIKTALLNMD RENREQYQWIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTYQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG LDMFDVITDQETQEGIITVKKLLDFEKKK WTLKVE ASNPYVEPRFLYLGPFKD SAT VRIWEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLLWHNIT VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFVCEKAKADQLIQTLHAVDK DDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPWIS DNDYPVQSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGA
hCDH6aa54-615 APPavi-biotin	NM_004932.3	human full-length ECD, biotinylated (SEQ ID NO:5)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAINRRTGRP VEPESEFIIKIHDINDNEPIFTKEVYTATVP EMSDVGTFWQVTATDADDPTYGNSAK WYSILQGQPYFSVESETGIIKTALLNMD RENREQYQWIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTYQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG

			LDMFDVITDQETQEGIITVKKLLDFEKKK VYTLKVEASNPYVEPRFLYLGPFKDSAT VRIWEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLLWHNIT VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFVCEKAKADQLIQTLHAVDK DDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPVVIS DNDYPVQSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGAGSEFRHDSGLND IFEAQK(BIOTIN)IEWHE
hCDH6aa54-615 Fc	NM_004932.3	human full-length ECD, dimerized (SEQ ID NO:6)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAINRRTGRP VEPESEFIIKIHDINDNEPIFTKEVYTATVP EMSDVGTFWQVTATDADDPTYGNSAK WYSILQGQPYFSVESETGIIKTALLNMD RENREQYQWIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTYQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG LDMFDVITDQETQEGIITVKKLLDFEKKK VYTLKVEASNPYVEPRFLYLGPFKDSAT VRIWEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLLWHNIT VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFVCEKAKADQLIQTLHAVDK DDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPVVIS DNDYPVQSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGAGSDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVT CVWDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
cynoCDH6 FL	See above description on cynomolgus sequence derivation	Cynomolgus *(Macaca fascicularis)* full-length CDH6 (SEQ ID NO:7)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAINRRTGRP VEPESEFIIKIHDINDNEPIFTKEVYTATVP EMSDVGTFWQVTATDADDPTYGNSAK WYSILQGQPYFSVESETGIIKTALLNMD RENREQYQWIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTYQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG LDMFDVITDQETQEGIITVKKLLDFEKKK WTLKVE ASNPHVEPRFLYLGPFKDSAT VRIWEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLLWHNIT

			VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFVCEKAKADQLIQTLRAVDK DDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPWIS DNDYPVQSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGAGSEFRHD SGLND IFEAQKIEWHE
moCDH6 FL	NM_007666.3	Mouse *(Mus musculus)* full-length CDH6 (SEQ ID NO:8)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAVNRRTGRP VEPESEFIIKIHDINDNEPIFTKDVYTATVP EMADVGTFWQVTATDADDPTYGNSAK WYSILQGQPYFSVESETGIIKTALLNMD RENREQYQWIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTYQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG HEMFDVITDQETQEGIITVKKLLDFEKKK VYTLKVEASNPHVEPRFLYLGPFKD SAT VRIWDDVDEPPVFSKLAYILQIREDARIN TTIGSVAAQDPDAARNPVKYSVDRHTD MDRIFNIDSGNGSIFTSKLLDRETLLWHNI TVIATEINNPKQ SSRVPLYIKVLDVNDNA PEFAEFYETFVCEKAKADQLIQTLRAVD KDDPYSGHQFSFSLAPEAASSSNFTIQDN KDNTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCACDHHGNM QSCHAEALIHPTGLSTGAGSEFRHDSGLN DIFEAQKIEWHE
ratCDH6 FL	NM_0 12927. 1	Rat *(Rattus norvegicus)* full-length CDH6 (SEQ ID NO:9)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAINRRTGRP VEPESEFIIKIHDINDNEPIFTKDVYTATVP EMADVGTFWQVTATDADDPTYGNSAK WYSILQGQPYFSVESETGIIKTALLNMD RENREQYQWIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTYQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG HDMFDVITDQETQEGIITVKKLLDFEKKR VYTLKVEASNPHIEPRFLYLGPFKDSATV RIWDDVDEPPVFSKLAYILQIREDAQINT TIGSVAAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLLWHNIT VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFVCEKAKADQLIQTLHAVDK DDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPWIS DNDYPVQSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGAGSEFRHD SGLND IFEAQKIEWHE

Recombinant protein expression and purification:

His6-SUMO-CDH6 aa54-260-APP-Avi

[00334] Protein was expressed in Rosetta® 2 De3 pLysS cells (Millipore, Billerica, MA) in pET vector using 2xYT + 50 μg/ml Kanamycin and 30 μg/ml chloramphenicol. Cells were induced with 0.1 mM IPTG at 30°C. The pellet resuspended in 400 ml 20mM Tris-Cl, pH 8.0, 150 mM NaCl, 3mM CaC12, 20 mM Imidazole, + PI tabs (1 per 50 ml) + lmg/ml lysozyme added fresh, homogenized to break up clumps and passed through microfluidizer 3 times. Following centrifugation at 20K x g for 30 min the soluble fraction of supernatant was used for purification over a 2 ml Ni-NTA (Qiagen superflow resin, Qiagen, Venlo, Netherlands). The lysate was passed over the column at 2.0 ml/min, washed with 10 CV (column volume) 20mM Tris-Cl, pH 8.0, 150 mM NaCl, 3mM CaC12, 20 mM Imidazole and protein was eluted with 4 x 3.0 ml elution fractions using 20mM Tris-Cl, pH 8.0, 500 mM NaCl, 3mM CaC12, 250 mM Imidazole. Fractions were pooled. 200 units of SUMO Protease (Invitrogen) were added with ~15ml protein (~20mgs) and dialyzed into 5L of 20 mM Tris-Cl, pH 8.0, 150 mM NaCl, 3 mM CaC12 overnight at 4°C using Snakeskin® pleated tubing with 10K cutoff (Thermo Scientific, Rockford, IL). Dialyzed material was collected and re-purified over 2.0 ml Ni-NTA resin to remove uncleaved protein and SUMO Protease. Cleaved protein was collected from flow through and wash fractions. Concentrated flow through and wash from reverse Ni-NTA to 5 ml volume, added NaCl to final concentration of 500mM and injected onto S200 column, buffer = 20mM Tris-Cl, pH 8.0, 150 mM NaCl, 3mM CaC12.

hCDH6aa54-615APPavi, hCDH6aa54-615 Fc, cynoCDH6 FL, moCDH6 FL, ratCDH6 FL

[00335] The protein was expressed in HEK293 (ATCC CRL-1573) derived cell lines previously adapted to suspension culture and grown in a Novartis proprietary serum-free medium. Small scale expression verification was undertaken in transient 6-well-plate transfection assays on the basis of lipofection. Large-scale protein production via transient transfection and was performed at the 10-20 L scale in the Wave™ bioreactor system (GE Healthcare, Pittsburgh, PA). DNA Polyethylenimine (Polysciences, Warrington, PA) was used as a plasmid carrier at a ratio of 1:3 (w:w). The cell culture supernatants were harvested 7-10 days post transfection and concentrated by cross-flow filtration and diafiltration prior to purification.

[00336] For purification, clarified and filtered conditioned supernatant from the transient transfection was passed over a 20 ml affinity column at 5ml/min. Wash 20 CV PBS, 1% TX100, 0.3% t-n-butylphospate buffer, 20CV PBS. Elute using 100 mM sodium citrate, 150 mM NaCl pH3.0, protein collecting 2 ml fractions. Pooled fractions were neutralized and then dialyzed overnight at 4°C with 5L 50 mM Tris, 150 mM NaCl pH 7.4, 3 mM CaC12. Sample was then subjected to size exclusion chromatography on a Superdex® S200 16/60 column (GE Healthcare, Pittsburgh, PA) equilibrated with dialysis buffer.

[00337] An overview of the various panning strategies is indicated in Table 2

Table 2

Overview of panning strategies

Pancode	Phage subpool	Target and conditions
1038.1	VH1A/B/5 λ+κ	
1038.2	VH3 λ+κ	
1038.3	VH2/6 λ+κ	SP on hCDH6aa54-615 APPavi, without $CaCl_2$
1038.4	VH6, κ	
1038.5	VH1A/B/5 λ+κ	SP on hCDH6aa54-615 APPavi, with $CaCl_2$
1038.6	VH3 λ+κ	
1038.7	VH2/6 λ+κ	
1038.8	VH6. κ	
1038.9	VH1A/B/5 λ+κ	SP on His6-SUMO-CDH6 aa54-260-APP-Avi, with lniM $CaCl_2$
1038.10	VH3 λ+κ	
1038.11	VH2/6 λ+κ	
1038.12	VH6, κ	
1038.13	VH1A/B/5 λ+κ	SP on His6-SUMO-CDH6 aa54-260-APP-Avi, with ImM $CaCl_2$
1038.14	VH3 λ+κ	Binding competition using tool antibody MAB2715
1038.15	VH2/6 λ+κ	
1038.16	VH6, κ	
1038.17	VH1A/B/5 λ+κ	LP on biotinylated hCDH6aa54-615 APPavi
1038.18	VH3 λ+κ	
1038.19	VH2/6 λ+κ	
1038.20	VH1A/B/5 λ+κ	LP on hCDH6aa54-615 APPavi
1038.21	VH3 λ+κ	Pull-down panning using biotinylated tool antibody 2B6
1038.22	VH2/6 λ+κ	
1038.23	VH1A/B/5 λ+κ	LP on Biotinylated hCDH6aa54-615-Fc
1038.24	VH3 λ+κ	
1038.25	VH2/6 λ+κ	
1038.26	VH1A/B/5 λ+κ	SP on hCDH6aa54-615-Fc
1038.27	VH3 λ+κ	
1038.28	VH2/6 λ+κ	

Biotinylation of proteins

[00338] Avi-tagged proteins were biotinylated using BirA enzyme following manufacture (Avidity, Aurora. CO) standard protocol for protein biotinylation on an Avi-tagged protein. After

biotinylation the protein was subjected to size exclusion chromatography on a Superdex® S200 16/60 column into 50 mM Tris, 150 mM NaCl pH 7.4, 3 niM CaC12.

[00339] Non-Avi-tagged proteins were dialyzed using Slide-A-Lyzer Mini Dialysis units, 10000 MWCO (#69576 Thermo Scientific, Rockford, IL) twice for 2h against 5L of 1x PBS under stirring at room temperature prior to biotinylation. EZ-link Sulfo-NHS-LC-biotin (#21327 Thermo Scientific, Rockford, IL) was reconstituted according to the manufacturer's protocol. The proteins were incubated with a 20-fold molar excess of biotin reagent for 30 min at room temperature. To remove non-reacted biotin reagent the sample was again dialyzed twice for 2h against 5L of 1x PBS under stirring at room temperature. Protein concentrations were determined by means of the Nanodrop® device (Thermo Scientific, Rockford, IL). Samples were stored at 4°C until usage.

Solid phase panning

[00340] In solid phase pannings the Fab fragment displaying phages are incubated with antigen that has been bound to a surface support by direct immobilization.

[00341] Prior to the antigen selection process, a coating check ELISA was performed to determine the optimal antigen coating concentration. For this purpose a 2 fold dilution series of hCDH6aa54-615 APPavi (see Table 1) covering 24 to 0.19 µg/mL was generated. The individual dilutions were used to coat wells on a 96-well Maxisorp™ plate (#442404 Nunc, Rochester, NY) via direct immobilization. After coating the plate was washed thrice with 300 µĩ˛ PBS and subsequently blocked with 1x blocking buffer (2.5% milk, 2.5% BSA, 0.05% Tween 20) for 2h at room temperature.

[00342] To detect bound antigen, an anti-APP antibody (generated in-house) was added to 1 µM, 0.18 µM or 0.04 µM. Secondary detection was done using AP-labeled anti-mouse IgG-F(ab)2 specific antibody in a 1:5000 dilution Ref. 115-056-006 Jackson-Immunoresearch, West Grove, PA). The antigen concentration at which signal saturation was observed was chosen as coating concentrations for SP pannings.

Pancodes 1038.1-8

[00343] For SP pannings 1038.1-8 (see Table 2) hCDH6aa54-615 APPavi (see Table 1, SEQ ID NO:4) was coated on a 96-well Maxisorp™ plate (Nunc) via direct immobilization. Two wells were coated with 300 µL antigen solution (10 µg/mL in PBS) per phage subpool combination. Coating was done at 4°C overnight with (pannings 1038.1-4) or without (pannings 1038.4-8) the presence of 1 mM CaC12. The wells were subsequently washed twice with 400 µL PBS and blocked with 400 µĩ˛ blocking buffer for 2h at room temperature at 400 rpm. After blocking the cells were washed twice with 400 µL PBS.

[00344] Prior to binding phage subpools were blocked and depleted with unrelated FLT2-APPavi at 10 µg/mL for 1h at room temperature to eliminate unspecific and tag binders. 300 µL of blocked and depleted phage pools were transferred per coated well and incubated for 2h at room temperature and 400 rpm. Non-specific bound phages were removed by the washing steps listed below in Table 3.

Table 3

First Round	Second Round	Third round (only pancodes 1038.9-16)
3x PBST (quick)	1x PBST (quick)	lOx PBST (quick)
2x PBST / 5min	4x PBST / 5min	5x PBST / 5min
3x PBS (quick)	1x PBS (quick)	lOx PBS (quick)
2x PBS / 5min	4x PBS / 5min	5x PBS / 5min

[00345] The specifically bound phages were eluted with 300 µL elution buffer for 10 min at room temperature. Eluted phages were used to reinfect 14 mL of E. coli TG1F+ (Stratagene/Agilent, Santa Clara, CA),at an optical density of OD600= 0.6-0.8. The mix of E. coli TG1F+ and phage eluate was incubated for 45 min in a water bath at 37°C for phage infection. The bacterial pellets were re-suspended in 2xYT medium, plated on LB agar plates supplemented with 34 µg/mL chloramphenicol and incubated overnight at 37°C. Colonies were scraped off the plates and were used for polyclonal amplification of enriched clones and phage production. With purified phage the next panning round was started. The second round of pannings was performed according to the protocol of the first round except for a more stringent washing condition. A third panning round was omitted due to the low second round output titers of -104 cfu/mL.

Pancodes 1038.9-16

[00346] For SP pannings 1038.9-16 (Table 2) His6-SUMO-CDH6 aa54-260-APP-Avi (see Table 1) was coated on a 96-well Maxisorp™ plate (Nunc) via direct immobilization as described for pancodes 1038. 1-8. For pannings 1038. 13-16 300 µL of a 100 nM solution of tool antibody MAB2715 was added per well and incubated for 1 h at room temperature to avoid selection of binders recognizing the same epitope as MAB2715. Besides the antigen coated and a third panning round the pannings were done as described for pancodes 1038. 1-8.

Pancodes 1038.26-28

[00347] For SP pannings 1038.26-28 (see Table 2) hCDH6aa54-615-Fc (see Table 1, SEQ ID NO:6) was coated on a 96-well Maxisorp™ plate (Nunc) via direct immobilization as described for pancodes 1038. 1-8. Besides the antigen coated and the washing stringencies the pannings were done as described for pancodes 1038. 1-8.

Liquid phase panning

[00348] During solution panning, the Fab fragment displaying phage and biotinylated antigens are incubated in solution which is expected to increase accessibility of the antigen by the phage. Antigen biotinylation was done as described above.

Pancodes 1038.17-22

[00349] Each phage library subpool was blocked with an equal volume of 2x Chemiblocker for 2h at room temperature on a turning wheel. To avoid selection of antibodies against the APPavi and biotin tag unrelated FLT3-APPAvi and biotinylated anti-Cyclosporin msIgG was added to 7.5 and 18 µg/mL in the blocking step, respectively. For removal of phage particles binding to Streptavidin-beads, pre-adsorption of blocked phage particles was performed using blocked Streptavidin beads (# 112.06D Invitrogen, Carlsbad, CA). Blocked and pre-adsorbed phages were incubated with biotinylated hCDH6aa54-615 APPavi (see Table 1, SEQ ID NO:4) for 1h at room temperature on a turning wheel. Phage-antigen complexes were captured either with washed beads (Pancodes 1038.17-19) or with beads precoated with biotinylated anti-CDH6 antibody 2B6 (Genway, San Diego, CA) to enrich binders for non-2B6 epitopes (Pancodes 1038.20-22). Non-specific phage particles were removed by several washing steps (see below). Elution of specifically bound phage particles, infection, and amplification were done as described for pancodes 1038.1-8.

Table 4

Washing conditions Pancodes 1038.17-25

First Round	Second Round	Third round (only pancodes 1038.9-16)
5x PBST (quick)	lOx PBST (quick)	lOx PBST (quick)
2x PBST / 5min	3x PBST / 5min	5x PBST / 5min
3x PBS (quick)	5x PBS (quick)	5x PBS (quick)

Pancodes 1038.23-25

[00350] Pannings were done as described for pancodes 1038.1-8 but using biotinylated hCDH6aa54-615-Fc (see Table 1, SEQ ID NO:6) as antigen.

Subcloning

Conversion for FAB-FH expression

[00351] To facilitate rapid expression of soluble Fab fragments, the Fab encoding inserts of the selected HuCAL® PLATINUM phage particles were subcloned from the pMORPH®30 display vector into the pMORPH® 11_FH expression vector. Glycerol stocks containing E. coli that were

infected with the final panning round output phages were used to inoculate cultures for pMORPH®30 DNA purification using the Nucleobond® Xtra Midi Plus kit according to the manufacturers manual (#740.412.50 Machery Nagel, Bethlehem, PA). 5 µg of each pMORPH®30 DNA output pool was triple digested via EcoRI/Xbal/Bmtl (all restriction enzymes were purchased from New England Biolabs, Ipswich, MA). The resulting EcoRI/Xbal Fab encoding 1485 bp fragment was gel purified using the Wizard SV Gel/PCR clean-up kit according to the manufacturers manual (A9282, Promega, Madison, WI) and ligated into the EcoRI/Xbal cut pMORPH® 11_FH vector backbone.

Conversionfor IgG expression

[00352] In order to express full length IgG, variable domain fragments of VH and VL were subcloned from Fab expression vectors into pMORPH®4_hIgGlf vectors following the RapCLONE® protocol (Morphosys, Martinsried/Planegg, Germany). RapCLONE® is a two-step cloning method for the batch conversion of a large amount of Fab expression vectors into IgG expression vectors. In a first cloning step, a eukaryotic expression cassette was introduced into the pMORPH®l 1 expression vectors via BsiWI/Mfel (for κ pools) or Hpal/Mfel (for λ pools) digestion and subsequent ligation. This was followed by a second cloning step, in which the Fab pools containing the expression cassette were digested using EcoRV/BlpI (both κ and λ pools) and subsequently cloned into the pMORPH®4_IgGlf acceptor vector for expression in mammalian cells.

Expression and Purification of Fab Fragments

Transformation of E. coli TG1F

Electroporation-competent E. coli TG1F- aliquots of 50 µL were thawed on ice, mixed with 50 pg/µï pMORPH® 11_FH plasmid DNA and transferred into pre-cooled electroporation cuvettes. The cells were electroporated (Bio-Rad Gene Pulser; settings: 1.75 kV, 200 Ω, and 25 µF (Bio-Rad, Hercules, CA), transferred into 950 µï pre-warmed SOB medium and incubated for 1 h at 37 °C shaking at 220 rpm. An appropriate volume of the transformation samples were plated onto LB agar plates supplemented with 34 µg/mL Chloramphenicol and incubated overnight at 37°C to obtain single colonies.

Generation of Master plates

[00353] Chloramphenicol resistant single clones were picked into the wells of a sterile 384-well microtiter plate (Nunc) pre-filled with 60 µï 2xYT medium supplemented with 34 µg/ml of Chloramphenicol and 1 % glucose and grown overnight at 37°C. Next morning, 20 µï sterile 2xYT media containing 60 % glycerol and 1 % glucose were added into each well of the master plates. Plates were sealed with aluminum foil and stored at -8 °C.

Generation of Fab-Containing Bacterial Lysatesfor ELISA Screening

[00354] 5 µĩ of each well of a Master plate was transferred to a sterile 384-well microtiter plate pre-filled with 40 µĩ 2xYT medium per well supplemented with 34 µg/ml of Chloramphenicol and 0.1 % glucose. Plates were incubated at 37°C at 480 rpm and 80% humidity until the cultures were slightly turbid. 10 µĩ of 2xYT medium supplemented with 34 µg/ml Chloramphenicol and 5 mM IPTG were added per well for induction of Fab fragment expression. Plates were sealed with a gas-permeable tape and incubated overnight at 22°C at 500 rpm and 80% humidity. The next day 15 µĩ BEL lysis buffer (2.5 mg/ml lysozyme (#10837059001, Roche, Nutley, NJ), 4 mM EDTA, 10 U/µI Benzonase (# 1.01654.0001 Merck, White House Station, NJ) was added to each well and plates were incubated for 1 h at 500 rpm and 80% humidity. Resulting Fab lysates were blocked by adding 15 µL of 2x Chemiblocker per well followed by incubation for 30 min at 22°C, 500 rpm and 80% humidity.

Generation of Fab-Containing Bacterial Lysates for FACS Screening

[00355] 5 µĩ of each well of a compression plate was transferred to a sterile 96-well round bottom microtiter plate pre-filled with 100 µĩ 2xYT medium per well supplemented with 34 µg/ml of Chloramphenicol. Plates were incubated at 37°C at 500 rpm and 80% humidity until the cultures were slightly turbid. 10 µĩ of 2xYT medium supplemented with 34 µg/ml Chloramphenicol and 5 mM IPTG were added per well for induction of Fab fragment expression. Plates were sealed with a gas-permeable tape and incubated overnight at 22°C at 500 rpm and 80% humidity. The next day the plate was centrifuged for 10 min at 1200g and the supernatant discarded. The bacterial pellets were frozen overnight at -20°C to facilitate the lysis step. The thawed pellets were re-suspended in 200 µL of BEL lysis buffer (see above) and incubated for 1 h at 22°C and 250 rpm. Resulting Fab lysates were centrifuged to remove cellular debris for 10 min at 1200 g. Fab containing supernatants were used for screening purposes.

Expression and Purification of His6-tagged Fab Fragments in E. coli

[00356] E. coli TG1F- transformants containing pMORPH®l 1Fab FH DNA were singled out on 2xYT supplemented with 34 µg/mL Chloramphenicol and 1% glucose. Individual clones were picked and transferred into 3 mL 2xYT seed cultures supplemented with 34 µg/mL Chloramphenicol and 1% glucose and incubated at 37°C, 220 rpm for 4h. The seed culture was used to inoculate 50 mL main cultures with 2xYT supplemented with 34 µg/mL Chloramphenicol and 1% glucose and incubated in 250 mL shake flasks at 30°C until the OD600 reached a value of 0.6. Fab expression was induced by addition of IPTG to a final concentration of 0.75 mM and cultures were further incubated overnight at 25°C and 220 rpm. The next day cells were harvested and the cell pellets frozen overnight at -20°C. Cells pellets were disrupted by re-suspending and incubating in lysis buffer ((25 mM TRIS/pH=8, 500 mM NaCl, 2 mM $MgCl_2$, 10 U/µI Benzonase (# 1.01654.0001 Merck), 0.1% Lysozyme (# 10837059001, Roche), Protease Inhibitor Complete w/o EDTA 1 tablet/ 50 mL of buffer (#1 1873580001 Roche)) for 1h at room temperature on a rocking table. Lysates were clarified by

centrifugation for 30 min at 15000 g and filtration of the supernatant through a 200 nm pore sized filter. His6-tagged Fab fragments were isolated via immobilized metal ion affinity chromatography (Ni-NTA Superflow® beads, #30430 Qiagen, Venlo, Netherlands) and eluted using imidazole. Buffer exchange to 1x PBS was performed using PD-10 columns (# 17-085 1-01 GE Healthcare, Pittsburgh, PA). Samples were sterile-filtered and the protein concentrations were determined by UV-spectrophotometry. The purity of the samples was analyzed in denaturing, reducing 15% SDS-PAGE. The identity of the samples was confirmed by MS.

Expression and purification of IgGs

Expression of IgGs at screening scale

[00357] Eukaryotic HEK293cl8 cells (ATCC CRL-10852) were used in a 96-well expression system for the generation of conditioned cell culture supematants containing full-length IgG for the subsequent use in specificity and/or functional assays. Eukaryotic HEK293 cl8 cells were re-suspended in transfection medium (D-MEM supplemented with 2% L-glutamine (#25030-024 Gibco, Grand Island, NY) 10 % FCS (#3302 PAN Biotech, Aidenbach, Germany) and 1 % penicillin/streptomycin (#15 140-122 Gibco, Grand Island, NY)) and seeded in 96-well F-bottom plates to a density of approximately 4xl0 ^{4} cells in 50 µī per well the day before. A transfection master mix was prepared by mixing 0.6 µī Lipofectamine® 2000 (#1 1668 Invitrogen, Carlsbad, CA) per well with 25 µL Opti-MEM® I medium. (#3 1985-047 Invitrogen, Carlsbad, CA). The master mix was incubated for 15 min at room temperature. On a new plate 20 µL of Opti- MEM® I medium was added to wells containing 300 ng DNA and gently mixed. After that, the DNA was combined with the pre-incubated Lipofectamine® 2000, mixed gently and incubated for 20 min at room temperature. IOOµI of the pre-incubated Lipofectamine® 2000/DNA complexes were then transferred to each well of the plates with the cells and gently mixed. Plates were incubated for 40 h at 37°C and 6 % C02 for transient expression. The culture supematants were transferred to 96-well V-bottom plates and cleared by centrifugation. The resulting IgG-containing supematants were tested by an anti-Fd capture ELISA for assessment of IgG protein concentration in reference to a known standard and stored at -80°C for later use.

IgG expression check by anti-Fd ELISA

[00358] To assess IgG levels in screening scale derived supematants Maxisorp ® 96-well plates (#4371 11 Nunc) were coated with 50 µī/well Fd-fragment-specific sheep anti-human IgG (#PC075 The Binding Site, San Diego, CA) diluted 1:1000 in PBS. After coating, the plates were washed twice with TBST and blocked for 1h with 5 % skim milk powder in TBST. In the meantime the IgG containing supematants were diluted 1:50 in 2.5% skim milk powder in TBST. After washing the ELISA plates three times with TBST, 100 µL of the diluted supematants were transferred per well and plates were incubated for 1 h at room temperature. Subsequently, the plates were washed five

times with TBST and the captured IgGs were detected by incubation with 50 µĩ F(ab')2-specific goat anti-human IgG (#109-055-097 Dianova, Hamburg, Germany) (diluted 1:5000) in 0.5 % skim milk powder in TBST for lh. After washing the plates five times with TBST the AttoPhos fluorescence substrate (#1484281 Roche, Nutley, NJ) was added according to the manufacturer's instructions and the fluorescence emission at 535 nm was recorded with excitation at 430 nm with an ELISA reader.

Expression and Purification of IgGs in Microscale

[00359] CAP-T® cells (CEVEC Pharmaceuticals, Cologne, Germany) were transiently transfected with pMORPH®4 IgG expression plasmid in FreeStyle293® expression medium (#12338 Invitrogen, Carlsbad, CA) using 40 kDa linear PEI ((PEI Max (#24765-2 Polysciences Warrington, PA)) as gene delivery vehicle.

[00360] One day prior to the transfection cells were diluted to ~0.8E+06 cells/ml to ensure exponential growth. At the day of transfection cells were diluted in 9 niL pre-warmed Freestyle® (Invitrogen, Carlsbad, CA) medium to ~0.5xl0 ^{7} cells/ml and transferred into a 125ml shake flask. 30 µg DNA was diluted in 500 µĩ OptiMEM. 1200 µg PEI Max (#24765-2 Polysciences Warrington, PA) was diluted in 8.80 niL OptiMEM medium. The DNA solution was added drop wise to the cells and gently mixed. After that, 500 µL PEI Max solution was added to the cells and gently mixed.

[00361] The cells were incubated at 37°C, 6 % C02, 85 % humidity with agitation at 100 rpm. After 4 h 10 mL pre-warmed PEM supplemented with 4 mM L-Glutamine and 5µg/ml blasticidin (#R21001 Invitrogen, Carlsbad, CA) was added. Simultaneously, 400 µL valproic acid (#P4543-25G Sigma Aldrich, St. Louis, MO) was added to a final concentration of 4 mM. Cells were incubated for 6 days at 37°C, 6 % C02, 85 % humidity with agitation at 100 rpm.

[00362] After centrifugation for 5 min at 1200 g at 4°C the supernatant was sterile-filtered (0.2 µm pore size) and subjected to a Protein A affinity chromatography (MabSelect® SURE, GE Healthcare, Pittsburgh, PA) using a liquid handling station. Buffer exchange was performed to 1x PBS and samples were sterile filtered (0.2 µm pore size). Protein concentrations were determined by UV spectrophotometry and purity of IgGs was analyzed under denaturing, reducing conditions in SDS-PAGE.

Screening of Fab-containing raw bacterial lysates

ELISA screening

[00363] Using ELISA screening, single Fab clones were identified from panning outputs for binding to the target antigen. Fab fragments were tested using Fab containing crude E. coli lysates.

ELISA screening on directly coated antigen

[00364] Maxisorp® (#442404 Nunc, Rochester, NY) 384-well plates were coated overnight at 4°C with huCDH6 proteins at a concentration of 3 μg/ml in PBS. After washing plates were blocked for 2 h with 5% skimmed milk in lxPBST. Fab-containing E. coli lysates were added and binding allowed for 1 h at room temperature.

[00365] To detect bound Fab fragments plates were washed 5x with TBST and AP-anti human IgG F(ab')2 (#109-055-097 Jackson Immunoresearch, West Grove, PA)was added in a 1/2500 dilution. After 1h at room temperature plates were washed 5x with TBST and AttoPhos substrate was added according to the manufacturer's specifications. Plates were read in an ELISA reader 5 minutes after adding the substrate.

ELISA Screening on BiotinylatedAntigen using NeutrAvidin® Plates

[00366] Maxisorp® (#442404 Nunc, Rochester, NY) 384-well plates were coated overnight at 4°C with NeutrAvidin® (#3 1000 Thermo Scientific, Rockford, IL) at a concentration of 10 μg/ml in PBS. After washing, plates were blocked for 2 h with 1x Chemiblocker. Fab-containing E. coli lysates were dispensed into a fresh 384-well microtiter plate and biotinylated CDH6 antigens added at a concentration of 2.5 μg/mL. Fab-antigen complexes were then transferred to the NeutrAvidin® coated plates and capturing allowed for 1h at room temperature and detected as described above.

FACS screening

[00367] In FACS screening, Fab fragments binding to cell surface expressed CDH6 antigen were identified from the panning output. $1x10^5$ cells/well were transferred into U bottom 96 well plates and mixed with 40 μΐ/well of the Fab-containing bacterial lysates. Plates were incubated shaking at 4°C for 1 h. After the incubation, 100 μΐ/well ice-cold FACS buffer (3% FCS, 0.02% NaN_3, 2 mM EDTA in PBS) was added, cells were spun down at 4°C for 5 min at 250 g and washed twice with 180 μΐ/well ice-cold FACS buffer. After each washing step, cells were centrifuged and carefully re-suspended. After the last washing step cells were re-suspended in 50 μL of 1/200 diluted secondary detection antibody (PE-conjugated goat anti-human IgG (#109-1 16-088, Jackson Immunoresearch, West Grove, PA). After 1h incubation at 4°C cells were again washed twice in 180 μL/weII ice-cold FACS buffer. Finally, cell pellets were re-suspended in 120 μΐ/well FACS buffer with 0.4 % paraformaldehyde and analyzed in a FACSCalibur® (BD Biosciences, San Jose, CA) equipped with an HTS plate reader.

Clone sequencing

Fab clone sequencing

[00368] Confirmed cell binding Fab hits were subjected to VL and VH sequencing. 1.2 mL 2xYT supplemented with 34 μg/mL Chloramphenicol and 1% glucose were inoculated with 5 μL of bacterial glycerol stock deriving from the compression plates and grown in 96-well deep well

microtiter plates overnight at 37°C and 500 rpm. The next day the bacteria were harvested and the pMORPH® 11FH plasmids purified using a 96-well DNA purification kit (Nucleospin® 96 Plasmid Purification Kit #740625.24, Machery Nagel, Bethlehem, PA) according to the manufacturers protocol. To sequence the VL and VH primers M13rev (5' CAGGAAACAGCTATGAC 3' (SEQ ID NO: 10) and HuCAL_VH_for ((5' GATAAGCATGCGTAGGAGAAA 3 (SEQ ID NO: 11)) were used, respectively.

IgG clone sequencing

[00369] Unique cell binding Fab clones were converted in the IgGl format in a polyclonal manner and afterwards retrieved by VL and VH sequencing. 1.5 mL 2xYT medium supplemented with 100 µg/mL Amp and 1% glucose were inoculated with single clones and grown in 96-well deep well microtiter plates overnight at 37°C, 450 rpm and 80% humidity. The next day the bacteria were harvested and the IgG encoding pMORPH®4 plasmids purified using a 96-well DNA purification kit according to the manufacturer's protocol (Nucleospin® 96 Plasmid Purification Kit #740625.24, Machery Nagel, Bethlehem, PA). To sequence the VL and VH primers ((T7 5' TAATACGACTCACTATAGGG 3' (SEQ ID NO: 12) and CMV HC for (5' CTCTAGCGCCACCATGAAACA 3' (SEQ ID NO: 13)) were used, respectively.

[00370] ***In vitro* assays**

Affinity assessment using Octet® QK

[00371] Affinity assessments by determining kinetic parameters were performed via Bio-Layer Interferometry technology. All Fab samples were measured using Streptavidin Dip and Read biosensors (#18-0009 ForteBio, Menlo Park, CA). The plate was placed in an Octet® QK instrument (ForteBio, Menlo Park, CA) and allowed to equilibrate to 27°C in the chamber. The run was initiated by placing the sensors in the wells containing 150 nM of biotinylated CDH6 protein for 300s. After that the sensors were placed in the wells containing 250 nM Fab sample. Fab association and dissociation were each recorded for 600s by measuring the change in layer thickness (in nanometers, nm) with time, all under computer control. Data were processed automatically using the Octet® User Software version 3.0.

Assessment of antibody cellular internalization propensity

[00372] Cell Internalization of IgGs by target mediated endocytosis was assessed by microscopy using a VTI Array Scan® HC reader (ThermoFischer, Waltham, MA) as described below. The underlying analysis protocol was the Spot Detector V4 algorithm (Thermo Scientific Cellomics®, Thermo Scientific, Rockford, IL). In brief, this analysis protocol provides a fast and generic spot analysis that identifies intracellular punctuate objects, such as IgG containing lysosomal vesicles after

immunofluorescent staining. The total punctuate fluorescent intensity can then be averaged over the number of cells analyzed.

[00373] OVCAR3 cells (ATCC HTB-161) were grown in T150 tissue culture flasks to approximately 90% confluency. Media was discarded and cells flushed with 10 mL PBS. Cells were detached by incubation with 4 mL cell dissociation buffer for 5 min at 37°C. Detached cells were re-suspended thoroughly in growth medium and transferred to a 50 mL tube. After counting the cells on a Vi-Cell® analyzer (Beckman Coulter, Brea, CA) the suspension was adjusted to 10^5 cells/mL in growth medium. 100 µL of the cell suspension was seeded per well of a 96-well microtiter plate with transparent bottom. Plates were incubated for 24 h at 37°C in 5% C02 to allow the cells to adhere and to expand.

[00374] IgG containing cell culture supernatants were generated as described and diluted in PBS in a fourfold dilution series covering dilutions ranging from 1:4 to 1:128. 100 µî of each sample dilution was dispensed per well of the cell containing microtiter plate and incubated for 2h at 37°C to permit IgG internalization. Subsequently, cells were fixed by adding 100 µî 1x CellFix® reagent (#340181 BD Biosciences, San Jose, CA) per well. After 10 minutes plates were washed twice with PBS and cells then permeabilized by adding 100 µî 0.1% Triton X-100 per well. After 10 min plates were washed again twice with PBS and blocked with 100 µî 1x Odyssey blocking buffer (#927-40000 LiCor, Lincoln, NE) for 2 hours at room temperature. To detect internalized IgGs 100 µî of a 1:000 dilution of secondary antibody Alexa Fluor® 488 goat anti-human IgG (#1 1001 Molecular Probes, Grand Island, NY) supplemented with a 1:10000 dilution of Hoechst nucleus staining reagent (#B2261 Sigma, St. Louis, Mo) were added per well and incubated for 1 hours at room temperature in the dark. Cells were then washed twice with PBS without final aspiration. Plates were then loaded into the Cellomics® VTI Array Scan HC reader (ThermoFischer, Waltham, MA) and analyzed. The extent of IgG internalization was assessed by the mean average spot intensity (MSAI) per cell.

Surrogate ADC assay using anti-human FAB-DM1 conjugate

[00375] To test the ability of CDH6 antibodies to internalize after receptor binding and deliver cytotoxic payload, a surrogate ADC assay was performed mixing anti-human Fab-DMl reagent (AffiniPure® Fab Fragment Goat Anti-Human IgG H+L conjugated with SMCC-DM1) with purified IgGs at a fixed 1:2 ratio. Cytotoxic potential was tested on the cancer cell line OVCAR3 (ovarian serous carcinoma, cultured in McCoys + 20% FCS) as these cells show high expression of CDH6.

[00376] Cells in culture were counted and diluted in medium to a concentration of $lxlO^5$ cells/ml. 1000 cells/well were transferred to 384-well plates (Corning Costar#3707, Corning, Tewksbury, MA). Primary antibody/Fab-DMl solution was prepared in 1.4ml Matrix tubes (Thermo, # 3790, Rockford, IL) by combining 666nM Fab-DMl with 333nM primary human IgG diluted in cell

culture media and incubated at 37°C for 30 minutes. A 10-point, 1:3 serial dilution was prepared in a 384-well deep-well plate (Brandtech Scientific Inc #701355, Essex, CT) and 25µ1were transferred per assay plate (triplicates) to yield a highest starting concentration of FAB-DMl/human IgG of 66nM and 33nM, respectively. For controls, wells with cells only (=100% viability control) and cells only incubated with Fab-DMl (to check for unspecific killing of the secondary reagent) were prepared. Plates were incubated for 120 h at 37°C and 5% C02. Cellular activity of the primary antibody/Fab-DMl complexes was determined using CellTiter-Glo® reagent (#G7571 Promega, Madison, WI) according to the manufacturer's instructions. Viability was normalized to the cells only control.

Antibody screening summary

[00377] The HuCAL® PLATINUM phagemid library was used to select specific Fab fragments against human CDH6-ECD antigens. Recombinant human APPAvi and Fc fusion as well as truncated domain APPAvi fusion proteins were used for the pannings. HuCAL® PLATINUM antibody-phage particles were subjected in different subpool combinations to a total of 8 different panning strategies resulting in 28 panning output pools. In summary, six out of eight strategies have been productive and resulted in 77 1ELISA positive screening hits.

[00378] FACS screening on CHO cells expressing human CDH6 as well as OVCAR3 cells resulted in 271 confirmed cell binding hits. To consolidate these cell binding hits VL and VH sequencing was performed leading to the identification of 53 unique Fab antibody clones.

[00379] To allow for the functional characterization, the Fab antibody clones were converted into the IgG format, yielding 47 unique IgG clones of which 44 were successfully expressed at screening scale. The purified, unique IgGs were subjected to a series of characterization assays including human/cyno/rat/mouse cross-reactivity by FACS, affinity ranking by Octet, cellular internalization assays as well as surrogate ADC assays using an anti-human IgG Fab-SMCC-DMl secondary reagent. Based on these assays, human IgGs were selected for scaled up production, subsequent direct conjugation to ADC linker/payloads and testing as ADCs in *in vitro* and *in vivo* experiments.

[00380] The sequence information for the anti-CDH6 IgGs selected for further in-depth characterization are shown in Table 5.

Table 5

Sequence information of the IgGs selected for in-depth characterization.

NOV ID	Category	Value
NOV0670	LCDR1	SGSSSNIGSQYVY

	(Kabat) (SEQ ID NO:14)	
	LCDR2 (Kabat) (SEQ ID NO:15)	YNSERPS
	LCDR3 (Kabat) (SEQ ID NO:16)	QTWDASSQSFV
	HCDR1 (Kabat) (SEQ ID NO:17)	SYAIS
	HCDR2 (Kabat) (SEQ ID NO:18)	GIIPIFGTANYAQKFQG
	HCDR3 (Kabat) (SEQ ID NO:19)	KFPGRGPFAY
	vH full sequence (SEQ ID NO:20)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSL RSEDTAVYYCARKFPGRGPFAYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:21)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSQYVYWYQQLPGTA PKLLIYYNSERPSGMPDRFSGSKSGTSASLAITGLQAEDEADYY CQTWDASSQSFVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:22)	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAAAC CGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCGGAGG GACGTTTTCTTCTTACGCTATCTCTTGGGTGCGCCAGGCCCC GGGCCAGGGCCTCGAGTGGATGGGCGGTATCATCCCGATCT TCGGCACTGCGAACTACGCCCAGAAATTTCAGGGCCGGGTG ACCATTACCGCCGATGAAAGCACCAGCACCGCCTATATGGA ACTGAGCAGCCTGCGCAGCGAAGATACGGCCGTGTATTATT GCGCGCGTAAATTCCCGGGTCGTGGTCCGTTCGCTTACTGGG GCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:23)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTCAGTACGTGTACTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACTACAACTCTGAACG CCCGAGCGGCATGCCGGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAA GACGAAGCGGATTATTACTGCCAGACTTGGGACGCTTCTTCT CAGTCTTTCGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO:24)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSL RSEDTAVYYCARKFPGRGPFAYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR

		VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:25)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSQYVYWYQQLPGTA PKLLIYYNSERPSGMPDRFSGSKSGTSASLAITGLQAEDEADYY CQTWDASSQSFVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:26)	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAAAC CGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCGGAGG GACGTTTTCTTCTTACGCTATCTCTTGGGTGCGCCAGGCCCC GGGCCAGGGCCTCGAGTGGATGGGCGGTATCATCCCGATCT TCGGCACTGCGAACTACGCCCAGAAATTTCAGGGCCGGGTG ACCATTACCGCCGATGAAAGCACCAGCACCGCCTATATGGA ACTGAGCAGCCTGCGCAGCGAAGATACGGCCGTGTATTATT GCGCGCGTAAATTCCCGGGTCGTGGTCCGTTCGCTTACTGGG GCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAAG GGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTA CTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCC TGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCT CAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGC ACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACG CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:27)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTCAGTACGTGTACTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACTACAACTCTGAACG CCCGAGCGGCATGCCGGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAA GACGAAGCGGATTATTACTGCCAGACTTGGGACGCTTCTTCT CAGTCTTTCGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA GGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCC TCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTG

		TCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCT GGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC CACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCA GCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCAC AGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGT GGAGAAGACAGTGGCCCCTACAGAATGTTCA
NOV0672	LCDR1 (Kabat) (SEQ ID NO:28)	TGTSSDVGAYNYVS
	LCDR2 (Kabat) (SEQ ID NO:29)	GVSKRPS
	LCDR3 (Kabat) (SEQ ID NO:30)	QSYDHLLHVV
	HCDR1 (SEQ ID NO:31)	TYGIH
	HCDR2 (Kabat) (SEQ ID NO:32)	YIHYSGSSTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:33)	HAYGYMDF
	vH full sequence (SEQ ID NO:34)	QVQLLESGGGLVQPGGSLRLSCAASGFTFNTYGIHWVRQAPG KGLEWVSYIHYSGSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARHAYGYMDFWGQGTLVTVSS
	vL full sequence (SEQ ID NO:35)	DIALTQPASVSGSPGQSITISCTGTSSDVGAYNYVSWYQQHPGK APKLMIYGVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCQSYDHLLHVVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:36)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTAACACTTACGGTATCCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCTACATCCATTACTCTGGTT CTTCTACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACCA TCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTCATGCTTACGGTTACATGGATTTCTGGGGCCAAGGCA CCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:37)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGGCACCAGCAGCG ATGTGGGCGCTTACAACTACGTGTCTTGGTACCAGCAGCAT CCGGGCAAGGCGCCGAAACTGATGATCTACGGTGTTTCTAA ACGTCCGAGCGGCGTGAGCAACCGTTTTAGCGGATCCAAAA GCGGCAACACCGCGAGCCTGACCATTAGCGGCCTGCAAGCG GAAGACGAAGCGGATTATTACTGCCAGTCTTACGACCATCT

		GCTGCATGTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT A
	HC full sequence (SEQ ID NO:38)	QVQLLESGGGLVQPGGSLRLSCAASGFTFNTYGIHWVRQAPG KGLEWVSYIHYSGSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARHAYGYMDFWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:39)	DIALTQPASVSGSPGQSITISCTGTSSDVGAYNYVSWYQQHPGK APKLMIYGVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCQSYDHLLHWFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:40)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTAACACTTACGGTATCCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCTACATCCATTACTCTGGTT CTTCTACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACCA TCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTCATGCTTACGGTTACATGGATTTCTGGGGCCAAGGCA CCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCCATCG GTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGC ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGA ACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCG GCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTG GGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCG TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTG GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGG CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAG CCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGA GATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT GGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGT GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGGCACCAGCAGCG

	(SEQ ID NO:41)	ATGTGGGCGCTTACAACTACGTGTCTTGGTACCAGCAGCAT CCGGGCAAGGCGCCGAAACTGATGATCTACGGTGTTTCTAA ACGTCCGAGCGGCGTGAGCAACCGTTTTAGCGGATCCAAAA GCGGCAACACCGCGAGCCTGACCATTAGCGGCCTGCAAGCG GAAGACGAAGCGGATTATTACTGCCAGTCTTACGACCATCT GCTGCATGTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT AGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCA CAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACC GTGGAGAAGACAGTGGCCCCTACAGAATGTTCA
NOV0674	LCDR1 (Kabat) (SEQ ID NO:42)	SGSSSNIGYNYVS
	LCDR2 (Kabat) (SEQ ID NO:43)	RDNQRPS
	LCDR3 (SEQ ID NO:44)	AAWTSGSIGWV
	HCDR1 (Kabat) (SEQ ID NO:45)	SYAMT
	HCDR2 (Kabat) (SEQ ID NO:46)	GISGGGSNTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:47)	GGGQYFDY
	vH full sequence (SEQ ID NO:48)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMTWVRQAPG KGLEWVSGISGGGSNTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARGGGQYFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:49)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGYNYVSWYQQLPGTA PKLLIYRDNQRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYY CAAWTSGSIGWVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:50)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTATGACTTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGGTATCTCTGGTGGTGGTT CTAACACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTGGTGGTGGTCAGTACTTCGATTACTGGGGCCAAGGC ACCCTGGTGACTGTTAGCTCA

	vL DNA sequence (SEQ ID NO:51)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTACAACTACGTGTCTTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACCGTGACAACCAGCG CCCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAA GACGAAGCGGATTATTACTGCGCTGCTTGGACTTCTGGTTCT ATCGGTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT A
	HC full sequence (SEQ ID NO:52)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMTWVRQAPG KGLEWVSGISGGGSNTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARGGGQYFDYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:53)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGYNYVSWYQQLPGTA PKLLIYRDNQRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYY CAAWTSGSIGWVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:54)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTATGACTTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGGTATCTCTGGTGGTGGTT CTAACACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTGGTGGTGGTCAGTACTTCGATTACTGGGGCCAAGGC ACCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCCATC GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTC TACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTG GGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCG TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTG GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGG CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAG CCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGA GATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG

		GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT GGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGT GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:55)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTACAACTACGTGTCTTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACCGTGACAACCAGCG CCCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAA GACGAAGCGGATTATTACTGCGCTGCTTGGACTTCTGGTTCT ATCGGTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT AGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCA CAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACC GTGGAGAAGACAGTGGCCCCTACAGAATGTTCA
NOV0682	LCDR1 (Kabat) (SEQ ID NO:56)	RASQTINSYLN
	LCDR2 (Kabat) (SEQ ID NO:57)	RASNLQS
	LCDR3 (SEQ ID NO:58)	QQGDSSWT
	HCDR1 (Kabat) (SEQ ID NO:59)	SYAIS
	HCDR2 (Kabat) (SEQ ID NO:60)	FIKSNADGYTTNYAAPVKG
	HCDR3 (Kabat) (SEQ ID NO:61)	IRYFRNWDY
	vH full sequence (SEQ ID NO:62)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYAISWVRQAPGK GLEWVGFIKSNADGYTTNYAAPVKGRFTISRDDSKNTLYLQM NSLKTEDTAVYYCARIRYFRNWDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:63)	DIQMTQSPSSLSASVGDRVTITCRASQTINSYLNWYQQKPGKA PKLLIYRASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQGDSSWTFGQGTKVEIK
	vH DNA	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC

	sequence (SEQ ID NO:64)	AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTTCTTCTTACGCTATCTCTTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTGGGCTTCATCAAATCTAACGCT GACGGTTACACTACTAACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCGCGATGATTCGAAAAACACCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTATCCGTTACTTCCGTAACTGGGATTACTGGG GCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:65)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGA CTATTAACTCTTACCTGAACTGGTACCAGCAGAAACCGGGC AAAGCGCCGAAACTATTAATCTACCGTGCTTCTAACCTGCA AAGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGATCCGGCA CCGATTTCACCCTGACCATTAGCTCTCTGCAACCGGAAGACT TTGCGACCTATTATTGCCAGCAGGGTGACTCTTCTTGGACCT TTGGCCAGGGCACGAAAGTTGAAATTAAA
	HC full sequence (SEQ ID NO:66)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYAISWVRQAPGK GLEWVGFIKSNADGYTTNYAAPVKGRFTISRDDSKNTLYLQM NSLKTEDTAVYYCARIRYFRNWDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSW TVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:67)	DIQMTQSPSSLSASVGDRVTITCRASQTINSYLNWYQQKPGKA PKLLIYRASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQGDSSWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASW CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:68)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTTCTTCTTACGCTATCTCTTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTGGGCTTCATCAAATCTAACGCT GACGGTTACACTACTAACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCGCGATGATTCGAAAAACACCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTATCCGTTACTTCCGTAACTGGGATTACTGGG GCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAAG GGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTA CTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCC TGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCT CAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGC ACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG

		GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACG CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:69)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGA CTATTAACTCTTACCTGAACTGGTACCAGCAGAAACCGGGC AAAGCGCCGAAACTATTAATCTACCGTGCTTCTAACCTGCA AAGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGATCCGGCA CCGATTTCACCCTGACCATTAGCTCTCTGCAACCGGAAGACT TTGCGACCTATTATTGCCAGCAGGGTGACTCTTCTTGGACCT TTGGCCAGGGCACGAAAGTTGAAATTAAACGTACGGTGGCC GCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGCTG AAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTT CTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTCACCGAGC AGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTG ACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACG CCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGACC AAGAGCTTCAACCGGGGCGAGTGT
NOV0685	LCDR1 (Kabat) (SEQ ID NO:70)	SGSSSNIGSYYVS
	LCDR2 (Kabat) (SEQ ID NO:71)	YNTKRPS
	LCDR3 (Kabat) (SEQ ID NO:72)	QSWDKLGKGYV
	HCDR1 (Kabat) (SEQ ID NO:73)	GNSAAWN
	HCDR2 (Kabat) (SEQ ID NO:74)	IIYYRSKWYNDYAVSVKS
	HCDR3 (Kabat) (SEQ ID NO:75)	SSYSGGFDY
	vH full	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSGNSAAWNWIRQSP

	sequence (SEQ ID NO:76)	SRGLEWLGIIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLN SVTPEDTAVYYCARSSYSGGFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:77)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSYYVSWYQQLPGTA PKLLIYYNTKRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYY CQSWDKLGKGYVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:78)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCGGTAACTCTGCTGCTTGGAACTGGATTCGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCATCATCTACTA CCGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTTCTTCTTACTCTGGTGGTTTCGATTAC TGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:79)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTTACTACGTGTCTTGGTACCAGCAGCTGCCGG GCACGGCGCCGAAACTGCTGATCTACTACAACACTAAACGC CCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCGG CACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAAG ACGAAGCGGATTATTACTGCCAGTCTTGGGACAAACTGGGT AAAGGTTACGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT A
	HC full sequence (SEQ ID NO:80)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSGNSAAWNWIRQSP SRGLEWLGIIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLN SVTPEDTAVYYCARSSYSGGFDYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSWTVPS SSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLD SD GSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSL SL SPGK
	LC full sequence (SEQ ID N0:81)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSYYVSWYQQLPGTA PKLLIYYNTKRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYY CQSWDKLGKGYVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:82)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCGGTAACTCTGCTGCTTGGAACTGGATTCGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCATCATCTACTA CCGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTTCTTCTTACTCTGGTGGTTTCGATTAC TGGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCAC CAAGGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG

		ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGC GCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCC CTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGA ATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGA GCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCC CAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCC CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCC TGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAG CACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGG ACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGC CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC CATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACC TGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGA GTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTAC AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC ACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:83)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTTACTACGTGTCTTGGTACCAGCAGCTGCCGG GCACGGCGCCGAAACTGCTGATCTACTACAACACTAAACGC CCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCGG CACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAAG ACGAAGCGGATTATTACTGCCAGTCTTGGGACAAACTGGGT AAAGGTTACGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT AGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCA CAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACC GTGGAGAAGACAGTGGCCCCTACAGAATGTTCA
NOV0689	LCDR1 (Kabat) (SEQ ID NO:84)	SGSSSNIGSNFVS
	LCDR2 (Kabat) (SEQ ID NO:85)	DNSNRPS
	LCDR3 (Kabat) (SEQ ID NO:86)	SSYDSFDHSWV
	HCDR1 (Kabat) (SEQ ID NO:87)	SFAMN

	HCDR2 (Kabat) (SEQ ID NO:88)	VISSSGSNTNYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:89)	PSYFQAMDY
	vHfull sequence (SEQ ID NO:90)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSFAMNWVRQAPG KGLEWVSVISSSGSNTNYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARPSYFQAMDYWGQGTLVTVSS
	vL full sequence (SEQ ID N0:91)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNFVSWYQQLPGTAP KLLIYDNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYC SSYDSFDHSWVFGGGTKLTVL
	vHDNA sequence (SEQ ID NO:92)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTTCGCTATGAACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCTCTTCTTCTGGTT CTAACACCAACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTCCGTCTTACTTCCAGGCTATGGATTACTGGGGCCAAG GCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:93)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTAACTTCGTGTCTTGGTACCAGCAGCTGCCGG GCACGGCGCCGAAACTGCTGATCTACGACAACTCTAACCGC CCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCGG CACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAAG ACGAAGCGGATTATTACTGCTCTTCTTACGACTCTTTCGACC ATTCTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO:94)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSFAMNWVRQAPG KGLEWVSVISSSGSNTNYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARPSYFQAMDYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSWTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:95)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNFVSWYQQLPGTAP KLLIYDNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYC SSYDSFDHSWVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTTCGCTATGAACTGGGTGCGCCAGGCCCCGG

	NO:96)	GCAAAGGTCTCGAGTGGGTTTCCGTTATCTCTTCTTCTGGTT CTAACACCAACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTCCGTCTTACTTCCAGGCTATGGATTACTGGGGCCAAG GCACCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCCA TCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCC CGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCA GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC CAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTT GTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAA CTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCC AAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATG CGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGT TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG ACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGG TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC AGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC CCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA AGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCA ATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG CTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGA AGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:97)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTAACTTCGTGTCTTGGTACCAGCAGCTGCCGG GCACGGCGCCGAAACTGCTGATCTACGACAACTCTAACCGC CCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCGG CACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAAG ACGAAGCGGATTATTACTGCTCTTCTTACGACTCTTTCGACC ATTCTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA GGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCC TCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTG TCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCT GGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC CACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCA GCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCAC AGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGT GGAGAAGACAGTGGCCCCTACAGAATGTTCA
NOV0690	LCDR1 (Kabat) (SEQ ID NO:98)	SGDAIGTKFAH
	LCDR2 (Kabat) (SEQ ID NO:99)	YDHERPS

	LCDR3 (Kabat) (SEQ ID NO:100)	YSRASSNLV
	HCDR1 (Kabat) (SEQ ID NO:101)	DHAID
	HCDR2 (Kabat) (SEQ ID NO:102)	VIAGDGSITYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:103)	DTGVYREYMDV
	vH full sequence (SEQ ID NO:104)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSDHAIDWVRQAPGK GLEWVSVIAGDGSITYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCARDTGVYREYMDVWGQGTLVTVSS
	vL full sequence (SEQ ID NO:105)	DIELTQPPSVSVSPGQTASITCSGDAIGTKFAHWYQQKPGQAPV LVIYYDHERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCY SRASSNLVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:106)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTGACCATGCTATCGACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCGCTGGTGACGGTT CTATCACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTGACACTGGTGTTTACCGTGAATACATGGATGTTTGG GGCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:107)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATGCTATCG GTACTAAATTCGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACTACGACCATGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCTACTCTCGTGCTTCTTCTAACCTGGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO:108)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSDHAIDWVRQAPGK GLEWVSVIAGDGSITYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCARDTGVYREYMDVWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK

	LC full sequence (SEQ ID NO: 109)	DIELTQPPSVSVSPGQTASITCSGDAIGTKFAHWYQQKPGQAPV LVIYYDHERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCY SRASSNLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATL VCLISDFYPGAVT VAWKAD SSPVKAGVETTTP SKQSNNKYAA SSYL SLTPEQWKSHRS Y SCQVTHEGST VEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 110)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTGACCATGCTATCGACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCGCTGGTGACGGTT CTATCACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTGACACTGGTGTTTACCGTGAATACATGGATGTTTGG GGCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAA GGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCAC CTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT ACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGC ACCTGAACTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACG CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO: 111)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATGCTATCG GTACTAAATTCGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACTACGACCATGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCTACTCTCGTGCTTCTTCTAACCTGGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCCCAA GGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGA GCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG ACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGAT AGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTC CAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG AGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAG CTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACA GTGGCCCCTACAGAATGTTCA

NOV0691	LCDR1 (Kabat) (SEQ ID NO:112)	TGTSSDVGRYNFVS
	LCDR2 (Kabat) (SEQ ID NO:113)	RVSNRPS
	LCDR3 (Kabat) (SEQ ID NO: 114)	QSWTTYSNVV
	HCDR1 (Kabat) (SEQ ID NO:115)	SYALN
	HCDR2 (Kabat) (SEQ ID NO:116)	RIKSKTYGGSTDYAAPVKG
	HCDR3 (Kabat) (SEQ ID NO:117)	DRGGYVGFDS
	vH full sequence (SEQ ID NO:118)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYALNWVRQAPG KGLEWVGRIKSKTYGGSTDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARDRGGYVGFDSWGQGTLVTVSS
	vL full sequence (SEQ ID NO:119)	DIALTQPASVSGSPGQSITISCTGTSSDVGRYNFVSWYQQHPGK APKLMIYRVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCQSWTTYSNVVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:120)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTTCTTCTTACGCTCTGAACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAACT TACGGTGGTTCTACTGACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCGCGATGATTCGAAAAACACCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTGACCGTGGTGGTTACGTTGGTTTCGATTCTT GGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:121)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGGCACCAGCAGCG ATGTGGGCCGTTACAACTTCGTGTCTTGGTACCAGCAGCATC CGGGCAAGGCGCCGAAACTGATGATCTACCGTGTTTCTAAC CGTCCGAGCGGCGTGAGCAACCGTTTTAGCGGATCCAAAAG CGGCAACACCGCGAGCCTGACCATTAGCGGCCTGCAAGCGG AAGACGAAGCGGATTATTACTGCCAGTCTTGGACTACTTAC TCTAACGTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYALNWVRQAPG KGLEWVGRIKSKTYGGSTDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARDRGGYVGFDSWGQGTLVTVSSASTK

	NO: 122)	GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSWTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEWCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVF S CSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 123)	DIALTQPASVSGSPGQSITISCTGTSSDVGRYNFVSWYQQHPGK APKLMIYRVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCQSWTTYSNWFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 124)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTTCTTCTTACGCTCTGAACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAACT TACGGTGGTTCTACTGACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCGCGATGATTCGAAAAACACCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTGACCGTGGTGGTTACGTTGGTTTCGATTCTT GGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCACC AAGGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGA CTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCG CCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGT CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCT CCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGC CCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCA GCACCTGAACTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCC CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTG AGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA CGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGAC TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCA AAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCA TCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGT GGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAC GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO: 125)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGGCACCAGCAGCG ATGTGGGCCGTTACAACTTCGTGTCTTGGTACCAGCAGCATC CGGGCAAGGCGCCGAAACTGATGATCTACCGTGTTTCTAAC CGTCCGAGCGGCGTGAGCAACCGTTTTAGCGGATCCAAAAG CGGCAACACCGCGAGCCTGACCATTAGCGGCCTGCAAGCGG AAGACGAAGCGGATTATTACTGCCAGTCTTGGACTACTTAC TCTAACGTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA

		GGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCC TCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTG TCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCT GGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC CACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCA GCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCAC AGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGT GGAGAAGACAGTGGCCCCTACAGAATGTTCA
NOV0692	LCDR1 (Kabat) (SEQ ID NO:126)	SGDSIGSKYAQ
	LCDR2 (Kabat) (SEQ ID NO:127)	YNSERPS
	LCDR3 (Kabat) (SEQ ID NO:128)	QSWDGQSTIRV
	HCDR1 (Kabat) (SEQ ID NO:129)	RYWMD
	HCDR2 (Kabat) (SEQ ID NO:130)	RIKSKANGGITDYAAPVKG
	HCDR3 (Kabat) (SEQ ID NO:131)	GMTFLGI
	vH full sequence (SEQ ID NO:132)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSRYWMDWVRQAP GKGLEWVGRIKSKANGGITDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARGMTFLGIWGQGTLVTVSS
	vL full sequence (SEQ ID NO:133)	DIELTQPPSVSVSPGQTASITCSGDSIGSKYAQWYQQKPGQAPV LVIYYNSERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQS WDGQSTIRVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:134)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTTCTCGTTACTGGATGGACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAGCT AACGGTGGTATCACTGACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCGCGATGATTCGAAAAACACCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTGGTATGACTTTCCTGGGTATCTGGGGCCAA GGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATTCTATCG GTTCTAAATACGCTCAGTGGTACCAGCAGAAACCGGGCCAG

	NO:135)	GCGCCGGTGCTGGTGATCTACTACAACTCTGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACGGTCAGTCTACTATC CGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO: 136)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSRYWMDWVRQAP GKGLEWVGRIKSKANGGITDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARGMTFLGIWGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EWCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 137)	DIELTQPPSVSVSPGQTASITCSGDSIGSKYAQWYQQKPGQAPV LVIYYNSERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQS WDGQSTIRVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 138)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTTCTCGTTACTGGATGGACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAGCT AACGGTGGTATCACTGACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCGCGATGATTCGAAAAACACCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTGGTATGACTTTCCTGGGTATCTGGGGCCAA GGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCC ATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCC CCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACC AGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGG ACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG CCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAAT CTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCT GAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAA CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCA AGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC GGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT CCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGC AGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGT CAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCC GTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC

		AGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:139)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATTCTATCG GTTCTAAATACGCTCAGTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACTACAACTCTGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACGGTCAGTCTACTATC CGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCA GCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTC TGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCA TAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAG GCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA CACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAG CTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAA GCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCTACAGAATGTTCA
NOV0693	LCDR1 (Kabat) (SEQ ID NO:140)	RASQSISFYLA
	LCDR2 (Kabat) (SEQ ID NO:141)	GASTLQS
	LCDR3 (Kabat) (SEQ ID NO:142)	HQYSYWLRT
	HCDR1 (Kabat) (SEQ ID NO:143)	SYALH
	HCDR2 (Kabat) (SEQ ID NO:144)	YIFYDSSSTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:145)	FLYSAYGVAN
	vH full sequence (SEQ ID NO:146)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYALHWVRQAPG KGLEWVSYIFYDSSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARFLYSAYGVANWGQGTLVTVSS
	vL full sequence (SEQ ID NO:147)	DIQMTQSPSSLSASVGDRVTITCRASQSISFYLAWYQQKPGKAP KLLIYGASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCH QYSYWLRTFGQGTKVEIK
	vH DNA sequence (SEQ ID NO:148)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTCTGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCTACATCTTCTACGACTCTT CTTCTACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACCA

		TCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTTTCCTGTACTCTGCTTACGGTGTTGCTAACTGGGGCCA AGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO: 149)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGT CTATTTCTTTCTACCTGGCTTGGTACCAGCAGAAACCGGGCA AAGCGCCGAAACTATTAATCTACGGTGCTTCTACTCTGCAA AGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGATCCGGCAC CGATTTCACCCTGACCATTAGCTCTCTGCAACCGGAAGACTT TGCGACCTATTATTGCCATCAGTACTCTTACTGGCTGCGTAC CTTTGGCCAGGGCACGAAAGTTGAAATTAAA
	HC full sequence (SEQ ID NO: 150)	QVQLLES GGGL VQPGGSLRL SCAAS GFTFS SYALHW VRQAPG KGLEWVS YIFYDSSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARFLYSAYGVANWGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQS SGL YSL SSVVTVPS SSLGTQTYICNVNHKPSNTKVD KRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EWCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHE ALHNH YTQKSL SL SPGK
	LC full sequence (SEQ ID NO: 151)	DIQMTQSPSSLSASVGDRVTITCRASQSISFYLAWYQQKPGKAP KLLIYGASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCH QYSYWLRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASW CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTL SKADYEKHKVYACE VTHQGL SSP VTKSFNRGEC
	HC DNA sequence (SEQ ID NO: 152)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTCTGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCTACATCTTCTACGACTCTT CTTCTACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACCA TCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTTTCCTGTACTCTGCTTACGGTGTTGCTAACTGGGGCCA AGGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTC CATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTG GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC CCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGAC CAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAG GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAA GCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACC TGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAA ACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCA CATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC CGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCT GAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCC

		CTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCC GGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTG GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGA GAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAG CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACA CGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:153)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGT CTATTTCTTTCTACCTGGCTTGGTACCAGCAGAAACCGGGCA AAGCGCCGAAACTATTAATCTACGGTGCTTCTACTCTGCAA AGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGATCCGGCAC CGATTTCACCCTGACCATTAGCTCTCTGCAACCGGAAGACTT TGCGACCTATTATTGCCATCAGTACTCTTACTGGCTGCGTAC CTTTGGCCAGGGCACGAAAGTTGAAATTAAACGTACGGTGG CCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGC TGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAAC TTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAA CGCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTCACCGAG CAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCT GACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTAC GCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGAC CAAGAGCTTCAACCGGGGCGAGTGT
NOV0695	LCDR1 (Kabat) (SEQ ID NO:154)	RASQGIFTYLN
	LCDR2 (Kabat) (SEQ ID NO:155)	AASTLQS
	LCDR3 (Kabat) (SEQ ID NO:156)	QQYYSTSLT
	HCDR1 (Kabat) (SEQ ID NO:157)	SNSAAWN
	HCDR2 (Kabat) (SEQ ID NO:158)	RIYYRSKWYNDYAVSVKS
	HCDR3(Kabat) (SEQ ID NO:159)	ERSYRDYFDY
	vH full sequence (SEQ ID NO:160)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCARERSYRDYFDYWGQGTLVTVSS
	vL full	DIQMTQSPSSLSASVGDRVTITCRASQGIFTYLNWYQQKPGKA

	sequence (SEQ ID NO: 161)	PKLLISAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQYYSTSLTFGQGTKVEIK
	vH DNA sequence (SEQ ID NO: 162)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGCTTGGAACTGGATTCGTCAGA GCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTAC CGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAAG CCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGTG TATTATTGCGCGCGTGAACGTTCTTACCGTGACTACTTCGAT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO: 163)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGG GTATTTTCACTTACCTGAACTGGTACCAGCAGAAACCGGGC AAAGCGCCGAAACTATTAATCTCTGCTGCTTCTACTCTGCAA AGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGATCCGGCAC CGATTTCACCCTGACCATTAGCTCTCTGCAACCGGAAGACTT TGCGACCTATTATTGCCAGCAGTACTACTCTACTTCTCTGAC CTTTGGCCAGGGCACGAAAGTTGAAATTAAA
	HC full sequence (SEQ ID NO: 164)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCARERSYRDYFDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVWDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPP VLD SD GSFFLYSKLTVDKSRWQQGNVF S CSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 165)	DIQMTQSPSSLSASVGDRVTITCRASQGIFTYLNWYQQKPGKA PKLLISAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQYYSTSLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO: 166)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGCTTGGAACTGGATTCGTCAGA GCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTAC CGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAAG CCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGTG TATTATTGCGCGCGTGAACGTTCTTACCGTGACTACTTCGAT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTC CACCAAGGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAA GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCA GGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCG TGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAG

		TTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG TGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAG ACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACA ACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCAC CAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTC CAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCA AAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCC TGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACA AGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC AACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAA A
	LC DNA sequence (SEQ ID NO:167)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGG GTATTTTCACTTACCTGAACTGGTACCAGCAGAAACCGGGC AAAGCGCCGAAACTATTAATCTCTGCTGCTTCTACTCTGCAA AGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGATCCGGCAC CGATTTCACCCTGACCATTAGCTCTCTGCAACCGGAAGACTT TGCGACCTATTATTGCCAGCAGTACTACTCTACTTCTCTGAC CTTTGGCCAGGGCACGAAAGTTGAAATTAAACGTACGGTGG CCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGC TGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAAC TTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAA CGCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTCACCGAG CAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCT GACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTAC GCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGAC CAAGAGCTTCAACCGGGGCGAGTGT
NOV0699	LCDR1 (Kabat) (SEQ ID NO:168)	SGDNIRKYVVH
	LCDR2 (Kabat) (SEQ ID NO:169)	RDNNRPS
	LCDR3 (Kabat) (SEQ ID NO:170)	QSWDSFLAVV
	HCDR1 (Kabat) (SEQ ID NO:171)	SYAMH
	HCDR2 (Kabat) (SEQ ID NO:172)	FISSLGSYTYYADSVKG

	HCDR3 (Kabat) (SEQ ID NO: 173)	ETAGYGYAFDP
	vHfull sequence (SEQ ID NO: 174)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPG KGLEWVSFISSLGSYTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARETAGYGY AFDPWGQGTL VTVSS
	vL full sequence (SEQ ID NO: 175)	DIELTQPPSVSVSPGQTASITCSGDNIRKYWHWYQQKPGQAP VLVIYRDNNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYC QSWDSFLAWFGGGTKLTVL
	vH DNA sequence (SEQ ID NO: 176)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTATGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCTTCATCTCTTCTCTGGGTT CTTACACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTGAAACTGCTGGTTACGGTTACGCTTTCGATCCGTGGG GCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO: 177)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATAACATCC GTAAATACGTTGTTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACCGTGACAACAACCGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACTCTTTCCTGGCTGTT GTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO: 178)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPG KGLEWVSFISSLGSYTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARETAGYGY AFDPWGQGTL VTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSW TVPSSSLGTQTYICN VNHKP SNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 179)	DIELTQPPSVSVSPGQTASITCSGDNIRKYWHWYQQKPGQAP VLVIYRDNNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYC QSWDSFLAVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKY AASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 180)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTATGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCTTCATCTCTTCTCTGGGTT CTTACACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG

		CGCGTGAAACTGCTGGTTACGGTTACGCTTTCGATCCGTGGG GCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAAG GGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTA CTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCC TGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCT CAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGC ACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACG CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO: 181)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATAACATCC GTAAATACGTTGTTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACCGTGACAACAACCGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACTCTTTCCTGGCTGTT GTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCC CAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGA GGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAA GTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGCA GATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACAC CCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTAT CTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTA CAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAG ACAGTGGCCCCTACAGAATGTTCA
NOV0705	LCDR1 (Kabat) (SEQ ID NO: 182)	SGSSSNIGLDYVN
	LCDR2 (Kabat) (SEQ ID NO: 183)	RNKQRPS
	LCDR3 (Kabat) (SEQ ID NO: 184)	QAWAGRTNYVV

	HCDR1 (Kabat) (SEQ ID NO: 185)	NYWIG
	HCDR2 (Kabat) (SEQ ID NO: 186)	FIDPGVSYTRYSPSFQG
	HCDR3 (Kabat) (SEQ ID NO: 187)	VLAHSTEYNWPAF
	vHfull sequence (SEQ ID NO: 188)	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWIGWVRQMPG KGLEWMGFIDPGVSYTRYSPSFQGQVTISADKSISTAYLQWSSL KASDTAMYYCARVLAHSTEYNWP AFWGQGTLVTVS S
	vL full sequence (SEQ ID NO: 189)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGLDYVNWYQQLPGTA PKLLIYRNKQRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYY CQAWAGRTNYWFGGGTKLTVL
	vHDNA sequence (SEQ ID NO: 190)	CAGGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAAAAAAC CGGGCGAAAGCCTGAAAATTAGCTGCAAAGGCTCCGGATAT AGCTTCACTAACTACTGGATCGGTTGGGTGCGCCAGATGCC GGGCAAAGGTCTCGAGTGGATGGGCTTCATCGACCCGGGTG TTAGCTACACCCGTTATAGCCCGAGCTTTCAGGGCCAGGTG ACCATTAGCGCGGATAAAAGCATCAGCACCGCGTATCTGCA ATGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTATTATT GCGCGCGTGTTCTGGCTCATTCTACTGAATACAACTGGCCGG CTTTCTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:191)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTCTGGACTACGTGAACTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACCGTAACAAACAGCG CCCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAA GACGAAGCGGATTATTACTGCCAGGCTTGGGCTGGTCGTAC TAACTACGTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT A
	HC full sequence (SEQ ID NO: 192)	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWIGWVRQMPG KGLEWMGFIDPGVSYTRYSPSFQGQVTISADKSISTAYLQWSSL KASDTAMYYCARVLAHSTEYNWP AFWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVWDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPP VLD SD GSFFLYSKLTVDKSRWQQGNVF S CSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 193)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGLDYVNWYQQLPGTA PKLLIYRNKQRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYY CQAWAGRTNYWFGGGTKLTVLGQPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSN

		NKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 194)	CAGGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAAAAAAC CGGGCGAAAGCCTGAAAATTAGCTGCAAAGGCTCCGGATAT AGCTTCACTAACTACTGGATCGGTTGGGTGCGCCAGATGCC GGGCAAAGGTCTCGAGTGGATGGGCTTCATCGACCCGGGTG TTAGCTACACCCGTTATAGCCCGAGCTTTCAGGGCCAGGTG ACCATTAGCGCGGATAAAAGCATCAGCACCGCGTATCTGCA ATGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTATTATT GCGCGCGTGTTCTGGCTCATTCTACTGAATACAACTGGCCGG CTTTCTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGCCT CCACCAAGGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCA AGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTC AGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCC TACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGA GTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACC GTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGA AGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA CAACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGT CTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCT CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACAC CCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCA GCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACT ACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT TCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG GTAAA
	LC DNA sequence (SEQ ID NO: 195)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTCTGGACTACGTGAACTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACCGTAACAAACAGCG CCCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAA GACGAAGCGGATTATTACTGCCAGGCTTGGGCTGGTCGTAC TAACTACGTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT AGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCA CAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACC GTGGAGAAGACAGTGGCCCCTACAGAATGTTCA
NOV0709	LCDR1 (Kabat) (SEQ ID	TGTSSDVGSYNYVS

	NO:196)	
	LCDR2 (Kabat) (SEQ ID NO:197)	YVSNRPS
	LCDR3 (Kabat) (SEQ ID NO:198)	ASYTHQGSWV
	HCDR1 (Kabat) (SEQ ID NO:199)	TYYMH
	HCDR2 (Kabat) (SEQ ID NO:200)	VISSDGSFTFYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:201)	HGYGAFDY
	vH full sequence (SEQ ID NO:202)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSTYYMHWVRQAPG KGLEWVSVISSDGSFTFYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARHGYGAFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:203)	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVSWYQQHPGK APKLMIYYVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCASYTHQGSWVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:204)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTACTTACTACATGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCTCTTCTGACGGTT CTTTCACCTTCTATGCGGATAGCGTGAAAGGCCGCTTTACCA TCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTCATGGTTACGGTGCTTTCGATTACTGGGGCCAAGGCA CCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:205)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGGCACCAGCAGCG ATGTGGGCTCTTACAACTACGTGTCTTGGTACCAGCAGCATC CGGGCAAGGCGCCGAAACTGATGATCTACTACGTTTCTAAC CGTCCGAGCGGCGTGAGCAACCGTTTTAGCGGATCCAAAAG CGGCAACACCGCGAGCCTGACCATTAGCGGCCTGCAAGCGG AAGACGAAGCGGATTATTACTGCGCTTCTTACACTCATCAG GGTTCTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT A
	HC full sequence (SEQ ID NO:206)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSTYYMHWVRQAPG KGLEWVSVISSDGSFTFYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARHGYGAFDYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR

		VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:207)	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVSWYQQHPGK APKLMIYYVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCASYTHQGSWVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:208)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTACTTACTACATGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCTCTTCTGACGGTT CTTTCACCTTCTATGCGGATAGCGTGAAAGGCCGCTTTACCA TCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTCATGGTTACGGTGCTTTCGATTACTGGGGCCAAGGCA CCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCCATCG GTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGC ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGA ACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCG GCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTG GGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTC CTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCG TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTG GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGG CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAG CCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGA GATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT GGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGT GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:209)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGGCACCAGCAGCG ATGTGGGCTCTTACAACTACGTGTCTTGGTACCAGCAGCATC CGGGCAAGGCGCCGAAACTGATGATCTACTACGTTTCTAAC CGTCCGAGCGGCGTGAGCAACCGTTTTAGCGGATCCAAAAG CGGCAACACCGCGAGCCTGACCATTAGCGGCCTGCAAGCGG AAGACGAAGCGGATTATTACTGCGCTTCTTACACTCATCAG GGTTCTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT AGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT

		GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCA CAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACC GTGGAGAAGACAGTGGCCCCTACAGAATGTTCA
NOV0710	LCDR1 (Kabat) (SEQ ID NO:210)	RASQSISLWLN
	LCDR2 (Kabat) (SEQ ID NO:211)	AASTLQS
	LCDR3 (Kabat) (SEQ ID NO:212)	QQYYTSPYT
	HCDR1 (Kabat) (SEQ ID NO:213)	SYAMS
	HCDR2 (Kabat) (SEQ ID NO:214)	VIRSSGSSTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:215)	GGGYFDY
	vH full sequence (SEQ ID NO:216)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSVIRSSGSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARGGGYFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:217)	DIQMTQSPSSLSASVGDRVTITCRASQSISLWLNWYQQKPGKA PKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQYYTSPYTFGQGTKVEIK
	vH DNA sequence (SEQ ID NO:218)	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAACC TGGCGGATCCCTGAGGCTGAGCTGCGCTGCTAGTGGCTTCA CCTTCTCTAGCTACGCTATGAGCTGGGTCCGCCAGGCCCCTG GTAAAGGCCTCGAGTGGGTGTCAGTGATTAGATCTAGCGGC TCTAGCACCTACTACGCCGATAGCGTGAAGGGCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCTGTACCTGCAGA TGAACTCCCTGAGGGCCGAGGACACCGCCGTCTACTACTGC GCTAGAGGCGGAGGCTACTTCGACTACTGGGGTCAAGGCAC CCTGGTCACCGTGTCTAGC
	vL DNA sequence (SEQ ID NO:219)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATTAGCCTGTGGCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGCCTCTACCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATCTCTAGCCTGCAGCCCGAGGACT

		TCGCTACCTACTACTGTCAGCAGTACTACACTAGCCCCTACA CCTTCGGTCAGGGCACTAAGGTCGAGATTAAG
	HC full sequence (SEQ ID NO:220)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSVIRSSGSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARGGGYFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSWTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:221)	DIQMTQSPSSLSASVGDRVTITCRASQSISLWLNWYQQKPGKA PKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQYYTSPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:222)	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAACC TGGCGGATCCCTGAGGCTGAGCTGCGCTGCTAGTGGCTTCA CCTTCTCTAGCTACGCTATGAGCTGGGTCCGCCAGGCCCCTG GTAAAGGCCTCGAGTGGGTGTCAGTGATTAGATCTAGCGGC TCTAGCACCTACTACGCCGATAGCGTGAAGGGCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCTGTACCTGCAGA TGAACTCCCTGAGGGCCGAGGACACCGCCGTCTACTACTGC GCTAGAGGCGGAGGCTACTTCGACTACTGGGGTCAAGGCAC CCTGGTCACCGTGTCTAGCGCTAGCACTAAGGGCCCAAGTG TGTTTCCCCTGGCCCCCAGCAGCAAGTCTACTTCCGGCGGAA CTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTCCCCGAGC CCGTGACAGTGTCCTGGAACTCTGGGGCTCTGACTTCCGGC GTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTA CAGCCTGAGCAGCGTGGTGACAGTGCCCTCCAGCTCTCTGG GAACCCAGACCTATATCTGCAACGTGAACCACAAGCCCAGC AACACCAAGGTGGACAAGAGAGTGGAGCCCAAGAGCTGCG ACAAGACCCACACCTGCCCCCCCTGCCCAGCTCCAGAACTG CTGGGAGGGCCTTCCGTGTTCCTGTTCCCCCCCAAGCCCAAG GACACCCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGT GGTGGTGGACGTGTCCCACGAGGACCCAGAGGTGAAGTTCA ACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACC AAGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGG TGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGC AAAGAATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCAGC CCCAATCGAAAAGACAATCAGCAAGGCCAAGGGCCAGCCA CGGGAGCCCCAGGTGTACACCCTGCCCCCCAGCCGGGAGGA GATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGG GCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGCAAC GGCCAGCCCGAGAACAACTACAAGACCACCCCCCCAGTGCT GGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACCG TGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTCAGCTGC AGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAA GTCCCTGAGCCTGAGCCCCGGCAAG
	LC DNA sequence	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT

	(SEQ ID NO:223)	CTATTAGCCTGTGGCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGCCTCTACCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATCTCTAGCCTGCAGCCCGAGGACT TCGCTACCTACTACTGTCAGCAGTACTACACTAGCCCCTACA CCTTCGGTCAGGGCACTAAGGTCGAGATTAAGCGTACGGTG GCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCA GCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACA ACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGAC AACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCG AGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACC CTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAGGTGTA CGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGA CCAAGAGCTTCAACAGGGGCGAGTGC
NOV0712	LCDR1 (Kabat) (SEQ ID NO:224)	RASQSISSYLN
	LCDR2 (Kabat) (SEQ ID NO:225)	AVSTLQS
	LCDR3 (Kabat) (SEQ ID NO:226)	QQSGTFPPTT
	HCDR1 (Kabat) (SEQ ID NO:227)	SHGMH
	HCDR2 (Kabat) (SEQ ID NO:228)	VISGSGSNTGYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:229)	QWGSYAFDS
	vH full sequence (SEQ ID NO:230)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSHGMHWVRQAPG KGLEWVSVISGSGSNTGYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARQWGSYAFDSWGQGTLVTVSS
	vL full sequence (SEQ ID NO:231)	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAP KLLIYAVSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSGTFPPTTFGQGTKVEIK
	vH DNA sequence (SEQ ID NO:232)	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAGCC TGGCGGATCCCTGAGGCTGAGCTGCGCTGCTAGTGGCTTCA CCTTTAGCTCTCACGGAATGCACTGGGTCCGCCAGGCCCCTG GTAAAGGCCTCGAGTGGGTGTCAGTGATTAGCGGTAGCGGC TCTAACACCGGCTACGCCGATAGCGTGAAGGGCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCTGTACCTGCAGA TGAACTCCCTGAGGGCCGAGGACACCGCCGTCTACTACTGC GCTAGACAGTGGGGCTCCTACGCCTTCGATAGCTGGGGTCA

		AGGCACCCTGGTCACCGTGTCTAGC
	vL DNA sequence (SEQ ID NO:233)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATCTCTAGCTACCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGTGTCTACCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATTAGTAGCCTGCAGCCCGAGGAC TTCGCTACCTACTACTGTCAGCAGTCAGGCACCTTCCCCCCT ACTACCTTCGGTCAGGGCACTAAGGTCGAGATTAAG
	HC full sequence (SEQ ID NO:234)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSHGMHWVRQAPG KGLEWVSVISGSGSNTGYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARQWGSYAFDSWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSWTVPS SSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:235)	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAP KLLIYAVSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSGTFPPTTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASW CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:236)	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAGCC TGGCGGATCCCTGAGGCTGAGCTGCGCTGCTAGTGGCTTCA CCTTTAGCTCTCACGGAATGCACTGGGTCCGCCAGGCCCCTG GTAAAGGCCTCGAGTGGGTGTCAGTGATTAGCGGTAGCGGC TCTAACACCGGCTACGCCGATAGCGTGAAGGGCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCTGTACCTGCAGA TGAACTCCCTGAGGGCCGAGGACACCGCCGTCTACTACTGC GCTAGACAGTGGGGCTCCTACGCCTTCGATAGCTGGGGTCA AGGCACCCTGGTCACCGTGTCTAGCGCTAGCACTAAGGGCC CAAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTACTTCCG GCGGAACTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTC CCCGAGCCCGTGACAGTGTCCTGGAACTCTGGGGCTCTGAC TTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCG GCCTGTACAGCCTGAGCAGCGTGGTGACAGTGCCCTCCAGC TCTCTGGGAACCCAGACCTATATCTGCAACGTGAACCACAA GCCCAGCAACACCAAGGTGGACAAGAGAGTGGAGCCCAAG AGCTGCGACAAGACCCACACCTGCCCCCCCTGCCCAGCTCC AGAACTGCTGGGAGGGCCTTCCGTGTTCCTGTTCCCCCCCAA GCCCAAGGACACCCTGATGATCAGCAGGACCCCCGAGGTGA CCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGTG AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGC CAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTAC AGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCT GAACGGCAAAGAATACAAGTGCAAAGTCTCCAACAAGGCC CTGCCAGCCCCAATCGAAAAGACAATCAGCAAGGCCAAGG GCCAGCCACGGGAGCCCCAGGTGTACACCCTGCCCCCCAGC CGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCT

		GGTGAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGG AGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCC CCCAGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCA AGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGT GTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACT ACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
	LC DNA sequence (SEQ ID NO:237)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATCTCTAGCTACCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGTGTCTACCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATTAGTAGCCTGCAGCCCGAGGAC TTCGCTACCTACTACTGTCAGCAGTCAGGCACCTTCCCCCCT ACTACCTTCGGTCAGGGCACTAAGGTCGAGATTAAGCGTAC GGTGGCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGA GCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGA ACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTG GACAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCA CCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGC ACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAGGT GTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCG TGACCAAGAGCTTCAACAGGGGCGAGTGC
NOV0713	LCDR1 (Kabat) (SEQ ID NO:238)	SGDNLRSYYVH
	LCDR2 (Kabat) (SEQ ID NO:239)	GNNKRPS
	LCDR3 (Kabat) (SEQ ID NO:240)	GVYTLSSVV
	HCDR1 (Kabat) (SEQ ID NO:241)	SNSAAWN
	HCDR2 (Kabat) (SEQ ID NO:242)	RIYYRSKWYNDYAVSVKS
	HCDR3 (Kabat) (SEQ ID NO:243)	GLVGRYGQPYHFDV
	vH full sequence (SEQ ID NO:244)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCARGLVGRYGQPYHFDVWGQGTLVTVSS
	vL full sequence (SEQ ID NO:245)	DIELTQPPSVSVSPGQTASITCSGDNLRSYYVHWYQQKPGQAP VLVIYGNNKRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYC GVYTLSSVVFGGGTKLTVL

	vH DNA sequence (SEQ ID NO:246)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGCTTGGAACTGGATTCGTCAGA GCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTAC CGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAAG CCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGTG TATTATTGCGCGCGTGGTCTGGTTGGTCGTTACGGTCAGCCG TACCATTTCGATGTTTGGGGCCAAGGCACCCTGGTGACTGTT AGCTCA
	vL DNA sequence (SEQ ID NO:247)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATAACCTGC GTTCTTACTACGTTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGGTAACAACAAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCGGTGTTTACACTCTGTCTTCTGTTGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO:248)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCARGLVGRYGQPYHFDVWGQGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSWTVPSSSLGTQTYICNVNHKP SNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:249)	DIELTQPPSVSVSPGQTASITCSGDNLRSYYVHWYQQKPGQAP VLVIYGNNKRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYC GVYTLS SWFGGGTKLTVLGQPKAAPS VTLFPPS SEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:250)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGCTTGGAACTGGATTCGTCAGA GCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTAC CGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAAG CCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGTG TATTATTGCGCGCGTGGTCTGGTTGGTCGTTACGGTCAGCCG TACCATTTCGATGTTTGGGGCCAAGGCACCCTGGTGACTGTT AGCTCAGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCA CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGG CTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTC CCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTA CATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGG ACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACA TGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTC

		AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT CTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACC GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG TACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCG ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT CCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT CTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:251)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATAACCTGC GTTCTTACTACGTTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGGTAACAACAAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCGGTGTTTACACTCTGTCTTCTGTTGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCCCAA GGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGA GCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG ACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGAT AGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTC CAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG AGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAG CTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACA GTGGCCCCTACAGAATGTTCA
NOV0718	LCDR1 (Kabat) (SEQ ID NO:252)	SGDKIPTYTVH
	LCDR2 (Kabat) (SEQ ID NO:253)	DDNKRPS
	LCDR3 (Kabat) (SEQ ID NO:254)	QSTASGTVV
	HCDR1 (Kabat) (SEQ ID NO:255)	SYALH
	HCDR2 (Kabat) (SEQ ID NO:256)	RIKSKTNGGTTDYAAPVKG
	HCDR3 (Kabat)	VDATYSYSGYYYPMDY

	(SEQ ID NO:257)	
	vHfull sequence (SEQ ID NO:258)	QVQLVESGGGLVKPGGSLRLSCAASGFTFNSYALHWVRQAPG KGLEWVGRIKSKTNGGTTDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARVDATYSYSGYYYPMDYWGQGTLVT VSS
	vL full sequence (SEQ ID NO:259)	DIELTQPPSVSVSPGQTASITCSGDKIPTYTVHWYQQKPGQAPV LVIYDDNKRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQ STASGTWFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:260)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTAACTCTTACGCTCTGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAACT AACGGTGGTACTACTGACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCGCGATGATTCGAAAAACACCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTGTTGACGCTACTTACTCTTACTCTGGTTACT ACTACCCGATGGATTACTGGGGCCAAGGCACCCTGGTGACT GTTAGCTCA
	vL DNA sequence (SEQ ID NO:261)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATAAAATCC CGACTTACACTGTTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACGACAACAAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTACTGCTTCTGGTACTGTTGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO:262)	QVQLVESGGGLVKPGGSLRLSCAASGFTFNSYALHWVRQAPG KGLEWVGRIKSKTNGGTTDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARVDATYSYSGYYYPMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:263)	DIELTQPPSVSVSPGQTASITCSGDKIPTYTVHWYQQKPGQAPV LVIYDDNKRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQ STASGTWFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKAT LVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:264)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTAACTCTTACGCTCTGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAACT AACGGTGGTACTACTGACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCGCGATGATTCGAAAAACACCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT

		ATTGCGCGCGTGTTGACGCTACTTACTCTTACTCTGGTTACT ACTACCCGATGGATTACTGGGGCCAAGGCACCCTGGTGACT GTTAGCTCAGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTG GCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCT GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGAC CTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG TGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCAC ACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACC GTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCAT GATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACG TGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTG GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG AGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTC ACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGA AAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACA GGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGA ACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGAC GGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAG CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCC CTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:265)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATAAAATCC CGACTTACACTGTTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACGACAACAAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTACTGCTTCTGGTACTGTTGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCCCAA GGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGA GCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG ACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGAT AGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTC CAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG AGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAG CTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACA GTGGCCCCTACAGAATGTTCA
NOV0719	LCDR1 (Kabat) (SEQ ID NO:266)	RASQSIVSYLN
	LCDR2 (Kabat) (SEQ ID NO:267)	DASSLQS
	LCDR3 (Kabat) (SEQ ID	QQSGSHSIT

	NO:268)	
	HCDR1 (Kabat) (SEQ ID NO:269)	SHWVH
	HCDR2 (Kabat) (SEQ ID NO:270)	VISYMGSSTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:271)	GSYDMAFDV
	vHfull sequence (SEQ ID NO:272)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSHWVHWVRQAPG KGLEWVSVISYMGSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARGSYDMAFDVWGQGTLVTVSS
	vL full sequence (SEQ ID NO:273)	DIQMTQSPSSLSASVGDRVTITCRASQSIVSYLNWYQQKPGKAP KLLIYDASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSGSHSITFGQGTKVEIK
	vHDNA sequence (SEQ ID NO:274)	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAGCC TGGCGGTAGCCTGAGACTGAGCTGCGCTGCTAGTGGCTTCA CCTTTAGCTCTCACTGGGTGCACTGGGTCAGACAGGCCCCTG GTAAAGGCCTGGAGTGGGTGTCAGTGATTAGCTATATGGGC TCTAGCACCTACTACGCCGATAGCGTGAAGGGCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCTGTACCTGCAGA TGAATAGCCTGAGAGCCGAGGACACCGCCGTCTACTACTGC GCTAGAGGCTCCTACGATATGGCCTTCGACGTGTGGGGTCA GGGCACCCTGGTCACCGTGTCTAGC
	vL DNA sequence (SEQ ID NO:275)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATCGTCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGACGCCTCTAGCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATTAGTAGCCTGCAGCCCGAGGAC TTCGCTACCTACTACTGTCAGCAGTCAGGCTCTCACTCTATC ACCTTCGGTCAGGGCACTAAGGTCGAGATTAAG
	HC full sequence (SEQ ID NO:276)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSHWVHWVRQAPG KGLEWVSVISYMGSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARGSYDMAFDVWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSWTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE WCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:277)	DIQMTQSPSSLSASVGDRVTITCRASQSIVSYLNWYQQKPGKAP KLLIYDASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSGSHSITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS

		STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:278)	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAGCC TGGCGGTAGCCTGAGACTGAGCTGCGCTGCTAGTGGCTTCA CCTTTAGCTCTCACTGGGTGCACTGGGTCAGACAGGCCCCTG GTAAAGGCCTGGAGTGGGTGTCAGTGATTAGCTATATGGGC TCTAGCACCTACTACGCCGATAGCGTGAAGGGCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCTGTACCTGCAGA TGAATAGCCTGAGAGCCGAGGACACCGCCGTCTACTACTGC GCTAGAGGCTCCTACGATATGGCCTTCGACGTGTGGGGTCA GGGCACCCTGGTCACCGTGTCTAGCGCTAGCACTAAGGGCC CAAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTACTTCCG GCGGAACTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTC CCCGAGCCCGTGACAGTGTCCTGGAACTCTGGGGCTCTGAC TTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCG GCCTGTACAGCCTGAGCAGCGTGGTGACAGTGCCCTCCAGC TCTCTGGGAACCCAGACCTATATCTGCAACGTGAACCACAA GCCCAGCAACACCAAGGTGGACAAGAGAGTGGAGCCCAAG AGCTGCGACAAGACCCACACCTGCCCCCCCCTGCCCAGCTCC AGAACTGCTGGGAGGGCCTTCCGTGTTCCTGTTCCCCCCCAA GCCCAAGGACACCCTGATGATCAGCAGGACCCCCGAGGTGA CCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGTG AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGC CAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTAC AGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCT GAACGGCAAAGAATACAAGTGCAAAGTCTCCAACAAGGCC CTGCCAGCCCCAATCGAAAAGACAATCAGCAAGGCCAAGG GCCAGCCACGGGAGCCCCAGGTGTACACCCTGCCCCCCAGC CGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCT GGTGAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGG AGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCC CCCAGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCA AGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGT GTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACT ACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
	LC DNA sequence (SEQ ID NO:279)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATCGTCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGACGCCTCTAGCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATTAGTAGCCTGCAGCCCGAGGAC TTCGCTACCTACTACTGTCAGCAGTCAGGCTCTCACTCTATC ACCTTCGGTCAGGGCACTAAGGTCGAGATTAAGCGTACGGT GGCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGC AGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAAC AACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGA CAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACC GAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAGGTG TACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGT GACCAAGAGCTTCAACAGGGGCGAGTGC
NOV0720	LCDR1 (Kabat) (SEQ ID NO:280)	SGDNIGSMTAH

	LCDR2 (Kabat) (SEQ ID NO:281)	DKNERPS
	LCDR3 (Kabat) (SEQ ID NO:282)	QSWDDSYNSVV
	HCDR1 (Kabat) (SEQ ID NO:283)	SNSAGWN
	HCDR2 (Kabat) (SEQ ID NO:284)	RIYYRSKWYNDYAVSVKS
	HCDR3 (Kabat) (SEQ ID NO:285)	EKYTVSFYDFFDY
	vH full sequence (SEQ ID NO:286)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAGWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCAREKYTVSFYDFFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:287)	DIELTQPPSVSVSPGQTASITCSGDNIGSMTAHWYQQKPGQAPV LVIYDKNERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQ SWDDSYNSVVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:288)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGGTTGGAACTGGATTCGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTA CCGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTGAAAAATACACTGTTTCTTTCTACGA CTTCTTCGATTACTGGGGCCAAGGCACCCTGGTGACTGTTAG CTCA
	vL DNA sequence (SEQ ID NO:289)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATAACATCG GTTCTATGACTGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACAAAAACGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACGACTCTTACAACTCT GTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO:290)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAGWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCAREKYTVSFYDFFDYWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL

		MISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:291)	DIELTQPPSVSVSPGQTASITCSGDNIGSMTAHWYQQKPGQAPV LVIYDKNERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQ SWDDSYNSVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKY AASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:292)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGGTTGGAACTGGATTCGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTA CCGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTGAAAAATACACTGTTTCTTTCTACGA CTTCTTCGATTACTGGGGCCAAGGCACCCTGGTGACTGTTAG CTCAGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCACC CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCT GCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGT GGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATG CCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAG TCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT CCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG CAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCGT CCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA AGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTA CACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGG TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGAC ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA ACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC TTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTG GCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTC CGGGTAAA
	LC DNA sequence (SEQ ID NO:293)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGGCGATAACATCG GTTCTATGACTGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACAAAAACGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACGACTCTTACAACTCT GTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCA GCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTC TGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCA

		TAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAG GCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA CACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAG CTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAA GCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCTACAGAATGTTCA

Example 2: Antibody engineering by site-directed mutagenesis

[00381] Site-directed mutagenesis for the removal of the potential sites for post-translational modifications and germlining was performed for a subset of anti-CDH6 antibodies using the QuikChange® Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA). NOV690 sequence was changed $D_{53}S$ and VL (3-J-germline) resulting in antibody NOV1 126. NOV695 sequence was changed $N_{31}Q$ and $S_{49}Y$, resulting in antibody NOV1 127. NOV0720 sequence was changed $N_{31}Q$, $N_{95}Q$ and VL (3-J-germline), resulting in antibody NOV1 132. Sequence information for the engineered antibodies is described in Table 6.

Table 6

Sequence information of engineered antibodies

NOV1126	LCDR1 (Kabat) (SEQ ID NO:294)	SGDAIGTKFAH
	LCDR2 (Kabat) (SEQ ID NO:295)	YDHERPS
	LCDR3 (Kabat) (SEQ ID NO:296)	YSRASSNLV
	HCDR1 (Kabat) (SEQ ID NO:297)	DHAID
	HCDR2 (Kabat) (SEQ ID NO:298)	VIAGSGSITYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:299)	DTGVYREYMDV
	vH full sequence	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDHAIDWVRQAPGK GLEWVSVIAGSGSITYYADSVKGRFTISRDNSKNTLYLQMNSL

	(SEQ ID NO:300)	RAEDTAVYYCARDTGVYREYMDVWGQGTLVTVSS
	vL full sequence (SEQ ID NO:301)	SYELTQPLSVSVALGQTARITCSGDAIGTKFAHWYQQKPGQAP VLVIYYDHERPSGIPERFSGSNSGNTATLTISRAQAGDEADYYC YSRAS SNLVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:302)	GAAGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTGACCATGCTATCGACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCGCTGGTAGCGGTT CTATCACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTGACACTGGTGTTTACCGTGAATACATGGATGTTTGG GGCCAAGGCACCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:303)	AGCTATGAACTGACCCAGCCGCTGAGCGTGAGCGTGGCGCT GGGCCAGACCGCGCGCATTACCTGTAGCGGCGATGCTATCG GTACTAAATTCGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACTACGACCATGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCCGCGCGCAGGCGGGCGACGAA GCGGATTATTACTGCTACTCTCGTGCTTCTTCTAACCTGGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO:304)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDHAIDWVRQAPGK GLEWVSVIAGSGSITYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCARDTGVYREYMDVWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSW TVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:305)	SYELTQPLSVSVALGQTARITCSGDAIGTKFAHWYQQKPGQAP VLVIYYDHERPSGIPERFSGSNSGNTATLTISRAQAGDEADYYC YSRASSNLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:306)	GAAGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTGACCATGCTATCGACTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCGCTGGTAGCGGTT CTATCACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTGACACTGGTGTTTACCGTGAATACATGGATGTTTGG GGCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAA GGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCAC CTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT ACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCC

		TCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGC ACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACG CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:307)	AGCTATGAACTGACCCAGCCGCTGAGCGTGAGCGTGGCGCT GGGCCAGACCGCGCGCATTACCTGTAGCGGCGATGCTATCG GTACTAAATTCGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACTACGACCATGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCCGCGCGCAGGCGGGCGACGAA GCGGATTATTACTGCTACTCTCGTGCTTCTTCTAACCTGGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCCCAA GGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGA GCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG ACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGAT AGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTC CAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG AGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAG CTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACA GTGGCCCCTACAGAATGTTCA
NOV1127	LCDR1 (Kabat) (SEQ ID NO:308)	RASQGIFTYLN
	LCDR2 (Kabat) (SEQ ID NO:309)	AASTLQS
	LCDR3 (Kabat) (SEQ ID NO:310)	QQYYSTSLT
	HCDR1 (Kabat) (SEQ ID NO:311)	SQSAAWN
	HCDR2 (Kabat)	RIYYRSKWYNDYAVSVKS

	(SEQ ID NO:312)	
	HCDR3 (Kabat) (SEQ ID NO:313)	ERSYRDYFDY
	vHfull sequence (SEQ ID NO:314)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSQSAAWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCARERSYRDYFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:315)	DIQMTQSPSSLSASVGDRVTITCRASQGIFTYLNWYQQKPGKA PKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQYYSTSLTFGQGTKVEIK
	vHDNA sequence (SEQ ID NO:316)	CAGGTGCAGCTGCAGCAGTCAGGCCCTGGCCTGGTCAAGCC TAGTCAGACCCTGAGCCTGACCTGCGCTATTAGCGGCGATA GTGTGTCTAGTCAGTCAGCCGCCTGGAACTGGATTAGACAG TCACCCTCTAGGGGCCTGGAGTGGCTGGGTAGAATCTACTA TAGGTCTAAGTGGTATAACGACTACGCCGTCAGCGTGAAGT CTAGGATCACTATTAACCCCGACACCTCTAAGAATCAGTTTA GCCTGCAGCTGAATAGCGTGACCCCCGAGGACACCGCCGTC TACTACTGCGCTAGAGAGCGGTCCTATAGAGACTACTTCGA CTACTGGGGTCAGGGCACCCTGGTCACCGTGTCTAGC
	vL DNA sequence (SEQ ID NO:317)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGG GAATCTTCACCTACCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGCCTCTACCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATCTCTAGCCTGCAGCCCGAGGACT TCGCTACCTACTACTGTCAGCAGTACTACTCTACTAGCCTGA CCTTCGGTCAGGGCACTAAGGTCGAGATTAAG
	HC full sequence (SEQ ID NO:318)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSQSAAWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCARERSYRDYFDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVWDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:319)	DIQMTQSPSSLSASVGDRVTITCRASQGIFTYLNWYQQKPGKA PKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQYYSTSLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:320)	CAGGTGCAGCTGCAGCAGTCAGGCCCTGGCCTGGTCAAGCC TAGTCAGACCCTGAGCCTGACCTGCGCTATTAGCGGCGATA GTGTGTCTAGTCAGTCAGCCGCCTGGAACTGGATTAGACAG TCACCCTCTAGGGGCCTGGAGTGGCTGGGTAGAATCTACTA TAGGTCTAAGTGGTATAACGACTACGCCGTCAGCGTGAAGT

		CTAGGATCACTATTAACCCCGACACCTCTAAGAATCAGTTTA GCCTGCAGCTGAATAGCGTGACCCCCGAGGACACCGCCGTC TACTACTGCGCTAGAGAGCGGTCCTATAGAGACTACTTCGA CTACTGGGGTCAGGGCACCCTGGTCACCGTGTCTAGCGCTA GCACTAAGGGCCCAAGTGTGTTTCCCCTGGCCCCCAGCAGC AAGTCTACTTCCGGCGGAACTGCTGCCCTGGGTTGCCTGGTG AAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACTC TGGGGCTCTGACTTCCGGCGTGCACACCTTCCCCGCCGTGCT GCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACAG TGCCCTCCAGCTCTCTGGGAACCCAGACCTATATCTGCAACG TGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGT GGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCCCCCCT GCCCAGCTCCAGAACTGCTGGGAGGGCCTTCCGTGTTCCTGT TCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCAGGACC CCCGAGGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGA CCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGG TGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAA CAGCACCTACAGGGTGGTGTCCGTGCTGACCGTGCTGCACC AGGACTGGCTGAACGGCAAAGAATACAAGTGCAAAGTCTCC AACAAGGCCCTGCCAGCCCCAATCGAAAAGACAATCAGCA AGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACCCTG CCCCCCAGCCGGGAGGAGATGACCAAGAACCAGGTGTCCCT GACCTGTCTGGTGAAGGGCTTCTACCCCAGCGATATCGCCG TGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAA GACCACCCCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCC TGTACAGCAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAG GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCA CAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCA AG
	LC DNA sequence (SEQ ID NO:321)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGG GAATCTTCACCTACCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGCCTCTACCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATCTCTAGCCTGCAGCCCGAGGACT TCGCTACCTACTACTGTCAGCAGTACTACTCTACTAGCCTGA CCTTCGGTCAGGGCACTAAGGTCGAGATTAAGCGTACGGTG GCCGCTCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCA GCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACA ACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGAC AACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCG AGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACC CTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAGGTGTA CGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGA CCAAGAGCTTCAACAGGGGCGAGTGC
NOV1132	LCDR1 (Kabat) (SEQ ID NO:322)	SGDNIGSMTAH
	LCDR2 (Kabat) (SEQ ID NO:323)	DKNERPS
	LCDR3	QSWDDSYTSW

	(Kabat) (SEQ ID NO:324)	
	HCDR1 (Kabat) (SEQ ID NO:325)	SQSAGWN
	HCDR2 (Kabat) (SEQ ID NO:326)	RIYYRSKWYNDYAVSVKS
	HCDR3 (Kabat) (SEQ ID NO:327)	EKYTVSFYDFFDY
	vH full sequence (SEQ ID NO:328)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSQSAGWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCAREKYTVSFYDFFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:329)	SYELTQPLSVSVALGQTARITCSGDNIGSMTAHWYQQKPGQAP VLVIYDKNERPSGIPERFSGSNSGNTATLTISRAQAGDEADYYC QSWDDSYTSVVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:330)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTCAGTCTGCTGGTTGGAACTGGATTCGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTA CCGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTGAAAAATACACTGTTTCTTTCTACGA CTTCTTCGATTACTGGGGCCAAGGCACCCTGGTGACTGTTAG CTCA
	vL DNA sequence (SEQ ID NO:331)	AGCTATGAACTGACCCAGCCGCTGAGCGTGAGCGTGGCGCT GGGCCAGACCGCGCGCATTACCTGTAGCGGCGATAACATCG GTTCTATGACTGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACAAAAACGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCCGCGCGCAGGCGGGCGACGAA GCGGATTATTACTGCCAGTCTTGGGACGACTCTTACACCTCT GTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO:332)	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSQSAGWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCAREKYTVSFYDFFDYWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK

	LC full sequence (SEQ ID NO:333)	SYELTQPLSVSVALGQTARITCSGDNIGSMTAHWYQQKPGQAP VLVIYDKNERPSGIPERFSGSNSGNTATLTISRAQAGDEADYYC QSWDDSYTSWFGGGTKLTVLGQPKAAPSVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:334)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTCAGTCTGCTGGTTGGAACTGGATTCGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTA CCGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTGAAAAATACACTGTTTCTTTCTACGA CTTCTTCGATTACTGGGGCCAAGGCACCCTGGTGACTGTTAG CTCAGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCACC CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCT GCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGT GGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATG CCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAG TCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT CCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG CAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCGT CCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA AGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTA CACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGG TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGAC ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA ACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC TTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTG GCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTC CGGGTAAA
	LC DNA sequence (SEQ ID NO:335)	AGCTATGAACTGACCCAGCCGCTGAGCGTGAGCGTGGCGCT GGGCCAGACCGCGCGCATTACCTGTAGCGGCGATAACATCG GTTCTATGACTGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACAAAAACGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCCGCGCGCAGGCGGGCGACGAA GCGGATTATTACTGCCAGTCTTGGGACGACTCTTACACCTCT GTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCA GCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTC TGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCA TAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAG GCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA CACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAG CTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAA GCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG

		AAGACAGTGGCCCCTACAGAATGTTCA

Example 3: Determination of anti-CDH6 IgG binding to cells featuring expression of CDH6 from human, cynomolgus. rat and mouse origins

[00382] To determine anti-CDH6 binding to cells featuring expression of CDH6 from different species, FACS (Fluorescence Activated Cell sorting) analysis was performed on OVCAR3 cells, which express CDH6 endogenously, as well as on CHO cells engineered to express CDH6 from human, cynomolgus, rat and mouse origins.

[00383] OVCAR3 (ovarian serous carcinoma,) were obtained from ATCC (#HTB-161). The generation of the CHO cell lines featuring CDH6 expression is described in Example 1 above.

[00384] A cell suspension was prepared by treating cells in culture with Accutase® Cell Dissociation Reagent (#A1 11050 1 Gibco, Grand Island, NY) according to the manufacturer's instructions followed by washing the cells in FACS buffer (PBS/1%BSA, Gibco). Cells were resuspended in FACS buffer at $lxlO^6$ cells/ml and aliquoted into a 96-well round bottom plate (Corning #CLS3360 at IOOμI/well. An 8-point, 1:5 serial dilution of the primary antibodies was prepared to yield a highest final starting concentration of 10 μg/ml and IOOμI/well were added to the cell suspension. As a negative control antibody human isotype control IgG (R&D systems #1-001-A Minneapolis, MN) was used. Cells were incubated with the primary antibody solutions for 30 minutes on ice, followed by three washes in 200μ1of cold FACS buffer. The cells were resuspended in 100μï of PE-conjugated anti-human Human Fc used at 1/500 dilution (Jackson Immuno Research #109-1 16-098 West Grove, PA) and cells were incubated for 30 minutes on ice. Following three washes in 200μ1 cold FACS buffer, cells were analyzed on a BD FACS Canto II® (BD Biosciences, San Jose, CA). Geomean of signal per sample was determined using FlowJo® software and EC50s were determined by plotting the geomean of signal versus concentration and non-linear regression curve fitting using Tibco Spotfire® (Tibco, Boston, MA). All of the 20 analyzed anti-CDH6 antibodies displayed dose-dependent, target-specific binding on OVCAR3 cells (Figure 1) and CHO cells expressing CDH6. No reactivity was observed on wild-type CHO cells, which do not express CDH6. EC50 values are summarized in Table **7.**

Table 7

Cellular binding affinity (EC50 fnMJ) of CDH6 IgGs on OVCAR3 cells and CHO cells negative for CDH6 expression (CHO-TREX) or engineered to express human, cynomolgus, rat or mouse CDH6.

Antibody	OVCAR3	CHO-cyCDH6	CHO-huCDH6	CHO-moCDH6	CHO-ratCDH6	CHO-TREX
hlgG	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

NOV0670	4.092	0.281	0.205	0.3 12	0.521	n.a.
NOV0672	7.524	0.294	0.339	0.525	0.53	n.a.
NOV0674	13.398	0.32	0.494	0.527	0.537	n.a.
NOV0682	17.028	0.624	0.556	0.758	0.558	n.a.
NOV0685	5.214	0.178	0.179	0.23	0.321	n.a.
NOV0689	0.396	0.116	0.257	0.177	0.12	n.a.
NOV0690	0.924	0.777	0.136	3.041	16.595	n.a.
NOV0691	1.056	0.042	0.493	0.104	0.102	n.a.
NOV0692	1.056	0.08	0.107	0.221	0.155	n.a.
NOV0693	4.686	0.528	0.556	0.66	0.55	n.a.
NOV0695	2.64	0.306	0.267	0.427	0.659	n.a.
NOV0699	1.188	1.254	0.972	1.554	1.43	n.a.
NOV0705	0.858	0.005	0.5 11	0.018	0.646	n.a.
NOV0709	1.5 18	0.53	n.a.	0.669	0.53 1	n.a.
NOV0710	0.858	0.15	0.15 1	0.22	0.152	n.a.
NOV0712	1.122	0.202	0.21	0.537	0.523	n.a.
NOV0713	11.22	4.779	3.115	7.034	8.369	n.a.
NOV0718	0.66	0.3 13	0.187	0.52	0.322	n.a.
NOV0719	1.914	0.171	0.248	0.527	0.222	n.a.
NOV0720	0.66	0.543	0.428	0.338	0.493	n.a.

Example 4: Determination of binding affinity and cross-reactivity to recombinant CDH6 from human, cynomolgus, rat and mouse by Biacore measurement

[00385] Affinity of the antibodies to recombinant CDH6 protein from human, cynomolgus, rat and mouse origin was determined using SPR technology on a Biacore® T100 instrument (GE Healthcare, Pittsburgh, PA) and with CM5 sensor chips.

[00386] Briefly, HBS-P (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 0.005% Surfactant P20) supplemented with 0.5% Bovine Albumin Fraction V (7.5% solution) (Gibco 15260-037) was used as the running buffer for all the experiments. The immobilization level and analyte interactions were measured by response unit (RU). Pilot experiments were performed to test and confirm the feasibility of the immobilization of the anti-human Fc antibody (Jackson ImmunoResearch 109-006-098 West Grove, PA) and the capture of the test antibodies.

[00387] For kinetic measurements, the experiments were performed in which the antibodies were captured to the sensor chip surface via the immobilized anti-human Fc antibody and the ability of the CDH6 proteins to bind in free solution was determined. Briefly, 30 µg/ml of anti-human Fc antibody at pH 5 was immobilized on a CM5 sensor chip through amine coupling at flow rate of 12 µï/minute on all four flow cells to reach 7500 RUs. 5-10µg/ml of test antibodies were then injected at 10 µï/min for 12 seconds to flow cell 2,3 and 4. Subsequently, 0.78-50 nM of CDH6 receptor

extracellular domains (ECD) were diluted in a 2-fold series and injected at a flow rate of 80 µï/min for 100 seconds over reference (flow cell 1) and test flow cells 2,3 and4. Table of tested ECDs is listed below. Dissociation of the binding was followed for 10 minutes. After each injection cycle, the chip surface was regenerated with 10 mM Glycine pH2.0 at 60 µï/min for 30 s. All experiments were performed at 25 °C and the response data were globally fitted with a simple 1:1 interaction model using Biacore® T100 evaluation software version 2.0.3 to obtain estimates of on rate (k_a), off-rate (k_d) and affinity (K_D). The results suggest most of the CDH6 antibodies assayed in this panel are cross-reactive to human, cynomolgus, rat and mouse (Table 8).

Table 8

Binding affinity of anti-CDH6 IgGs to recombinant CDH6from human, cynomolgus, rat and mouse by Biacore measurement.

Antibody	KD [M] human CDH6	KD [M] cynomolgus CDH6	KD [M] rat CDH6	KD [M] mouse CDH6
NOV0670	9.329E-09	7.378E-09	7.232E-09	1.903E-08
NOV0672	2.463E-09	4.589E-09	3.291E-09	4.283E-09
NOV0674	1.954E-09	6.995E-09	2.829E-09	4.77E-09
NOV0682	6.188E-09	1.273E-08	5.554E-09	1.776E-07
NOV0685	4.551E-09	4.115E-09	4.636E-09	5.321E-09
NOV0689	5.648E-09	6.402E-09	6.083E-09	8.854E-09
NOV0690	6.969E-09	n/a	n/a	1.111E-09
NOV0691	8.343E-09	9.797E-09	4.332E-09	n/a
NOV0692	5.082E-09	5.554E-09	2.502E-09	2.494E-09
NOV0693	1.184E-09	1.966E-09	1.078E-09	1.843E-09
NOV0695	3.023E-12	n/a	n/a	1.67E-11
NOV0705	2.479E-09	n/a	2.334E-09	4.926E-11
NOV0709	8.174E-10	1.983E-09	6.977E-10	2.139E-09
NOV0710	2.065E-09	3.84E-09	2.385E-09	4.009E-09
NOV0712	2.904E-09	3.784E-09	5.719E-09	4.835E-09
NOV0713	1.419E-08	9.049E-09	7.147E-09	2.006E-08
NOV0718	5.455E-09	6.903E-09	6.608E-09	5.941E-09
NOV0719	1.899E-09	4.944E-09	8.789E-09	5.172E-09
NOV0720	2.47E-09	3.335E-09	1.406E-09	2.268E-09

Example 5: Epitope binning by Biacore

[00388] Epitope Mapping of CDH6 antibodies was determined using Surface Plasmon Resonance (SPR) technology on a Biacore® A-100 instrument (GE Healthcare, Pittsburgh, PA) with a CM5 (s) sensor chip. Briefly, HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM;

EDTA, 0.005% Surfactant P20 supplemented with 0.25% BSA and lOmM calcium) was used as the running buffer for all the experiments. The immobilization level and analyte interactions were measured by response unit (RU). Pilot experiments were performed to test and confirm the feasibility of the immobilization of the anti-human Fc antibody (Catalog number Jackson Immuno Research 109-006-098 West Grove, PA) and the capture of the test antibodies.

[00389] For epitope mapping, anti-human Fc antibody was immobilized on spot 1,2 and 4,5 of all four flow cells at 10,000RU. Spot 5 was used as reference. Each of the primary testing CDH6 antibodies was then captured via the immobilized anti-human Fc on the biosensor chip at a response level greater than 300 RU, followed by injection of the CDH6 protein in spot 1 and 5 at binding levels above 20 RU. Spots 2 and 4 were used as additional reference surface. All five spots of each flow cell were blocked with two injections of human IgG at 1 mg/ml to block any free binding sites of anti-human Fc. Each of the secondary testing antibodies was then injected over spots 1,2 or 4,5 to assess the degree of binding to the complex of primary testing antibody and CDH6 protein. Human IgG isotype control was used as primary and secondary testing negative control antibody. The antibodies were tested in parallel in all four flow cells until all possible combinations of antibody pairs had been evaluated. Regeneration of all flow cell surfaces after each primary and secondary antibody binding cycle was done with injection of lOmM glycine pH2.0.

[00390] The results were evaluated using the epitope mapping module in the Biacore® 4000 evaluation software and presented as a matrix, with primary antibodies in rows and secondary in columns (Figure 2). Additional binding of the secondary testing antibody to the complex of the primary testing antibody and CDH6 protein is indicated by positive response values. Negative pairs are marked and compare to human IgG isotype control. The matrix is then used to map which antibodies recognize which epitopes based on how they can form pairs with other antibodies. Antibody NOV0710 and NOV0712 compete with each other indicating they recognize and overlapping epitope on the antigen. Antibody NOV1 127 shows positive binding when it was used as primary and secondary binding antibody, suggesting it may bind to multiple sites on CDH6 or that initial binding alters the conformation and opens up additional binding sites. NOV0719, NOV0692, NOV1 126 and NOV1 132 all appear to bind distinct epitopes based on this analysis.

Example 6: Epitope mapping by hydrogen-deuterium exchange/mass spectrometry

[00391] Hydrogen-deuterium exchange (HDx) in combination with mass spectrometry (MS) (Woods et al., J. Cell Biochem. 200 1 S37: 89-98)) was used to map the binding site of antibodies NOV1 127, NOV0719, NOV0710 and NOV07 12 on the full-length extra-cellular domain (ECD) of hCDH6 full-length (aa54-615), which includes cadherin domains (EC) 1 through 5 (Table 9). In addition, HDx was also performed on a truncated ECD consisting of EC 1 through 3 using antibodies NOV0710 and NOV0712 (Table 10). In HDx exchangeable amide hydrogens of proteins are replaced

by deuterium. This process is sensitive to protein structure/dynamics and solvent accessibility and, therefore, able to report on locations that undergo a decrease in deuterium uptake upon ligand binding. The goal of these experiments was to identify the potential epitopes and understand the dynamics of hCDH6 when bound to our therapeutic antibodies. It is important to note that changes in deuterium uptake are sensitive to both direct binding and allosteric events; in order to precisely determine the epitope HDx has to be combined with orthogonal technologies (e.g. X-ray crystallography).

[00392] Automated HDx/MS experiments were performed using methods similar to those described in the literature (Chalmers et al., Anal Chem. 2006: 78(4): 1005-14). The experiments were performed on a Waters HDx-MS® platform, which includes a LEAP autosampler, nanoACQUITY UPLC System, and Synapt G2 mass spectrometer (Waters *Corp,* Milford MA). The deuterium buffer used to label the protein backbone of the full length hCDH6(54-615) with deuterium was 50 mM D-Tris, 150 mM NaCl pH 7.4 + 3 mM CaC12; the overall percentage of deuterium in the solution was 89.5%. For hCDH6(54-615) deuterium labeling experiments in the absence of hCDH6 antibody, 600 pmol of hCDH6(54-615), volume of 5 µĩ, was diluted using 45 µĩ of the deuterium buffer in a chilled tube and incubated for 25 minutes on a rotator at 4 °C. The labeling reaction was then quenched with 75 µĩ of chilled quench buffer on ice for three minutes followed by injected onto the LC-MS system for automated pepsin digestion and peptide analysis. For hCDH6(54-615) deuterium labeling experiments in the presence of bound hCDH6 antibody, 600 pmol of the hCDH6 antibody was first immobilized on Thermo Protein G Plus beads and cross-linked using disuccinimidyl suberate (DSS). To perform the labeling experiments, the antibody beads (containing 600 pmol antibody) were incubated with 600 pmol hCDH6(54-615) for 30 minutes at 4 °C. After 30 minutes the beads were washed with 200 µĩ of Tris buffer (50mM Tris, 150 mM NaCl pH 7.4 +3 mM CaC12). Then 200 µĩ of chilled deuterium buffer (80.6% deuterium) was added and the complex was incubated for 25 minutes at 4 °C. After 25 minutes, the labeling reaction was quenched with 125 µĩ of chilled quench buffer on ice for 2.5 minutes. After spinning the sample for 30 seconds in a centrifuge, the quenched solution was injected onto the LC-MS system for automated pepsin digestion and peptide analysis. Similar experiments were also performed on a hCDH6(267-615) construct that contains only EC 3 through 5. In these experiments the buffer was 25 mM HEPES pH = 7.4, 150 mM NaCl, 3 mM CaC12 and a deuterium version of the buffer containing 94.2% deuterium.

[00393] All measurements were carried out using a minimum of three analytical triplicates. All deuterium exchange experiments were quenched using 0.5 M TCEP and 3 M urea (pH = 2.5). After quenching, the exchanged antigen was subjected to on-line pepsin digestion using a Poroszyme® Immobilized Pepsin column (2. 1 x 30 mm) at 12 °C followed by trapping on a Waters Vanguard® HSS T3 (Waters Corp. Milford MA) trapping column. Peptides were eluted from the trapping column and separated on a Waters CSH C18 1 x 100 mm column (maintained at 1 °C) at a flow rate of 40

µï/min using a binary eight minute gradient of 2 to 35% B (mobile phase A was 99.9% water and 0.1% formic acid; mobile phase B was 99.9% acetonitrile and 0.1% formic acid).

[00394] For hCDH6(54-615) protein 73% of the sequence was monitored by the deuterium exchange experiments; a complete list of these peptides appears in Table 9. Figure 3 provides a summary of the on-exchange characteristics of the hCDH6 (54-615) protein. Regions that have a greater uptake in deuterium, such as EC3, are either more solvent exposed or have a weaker hydrogen bonding network that regions that exhibit less deuterium uptake, such as regions EC4 and EC5. Overall, it is observed that ECl through EC3 domains have greater on-exchange of deuterium that the EC4 of EC5 domains. For example, the EC2 peptide 181-199 VTATDADDPTYGNSAKVVY (SEQ ID NO:336) mean deuterium uptake is 3.61 Da; in contrast the EC5 peptide 506-525 IQTLHAVDKDDPYSGHQFSF (SEQ ID NO:337) mean deuterium uptake is only 1.18 Da. Limited deuterium uptake can make the determination of protection regions, those that undergo a shift of -0.5 Da or less upon antibody binding, more challenging due to limitations in the dynamic range.

[00395] For ECl (54-159) the only peptides that exhibits a decrease in deuterium uptake upon binding of all four antibodies is 61-70 FLLEEYTGSD (SEQ ID NO:338) . In ECl the antibodies NOVI 127 and NOV0719 demonstrate significant destabilization in the structure with changes in deuterium uptake greater or equal to 0.5 Da. Binding of NOV0710 and NOV0712 does not significantly alter the deuterium exchange behavior of the antigen in the ECl domain.

[00396] For EC2 (160-268) the region 195-202 AKVVYSIL (SEQ ID NO:339) has a borderline decrease in deuterium uptake by all antibodies. More interestingly, the region 203-221 QGQPYFSVESETGIIKTAL (SEQ ID NO:340) is destabilized substantially (i.e. undergoes an increase in deuterium uptake) by NOV0719 and slightly less by NOV0712. Neither NOV1 127 nor NOV0710 destabilize this region with a change in deuterium incorporation of +0.5 Da or greater. Lastly, the region 225-237 DRENREQYQWIQ (SEQ ID NO:341) is only destabilized upon NOV0719 binding to hCDH6 antigen.

[00397] For EC3 (269-383) the region 275-302 FKTPESSPPGTPIGRIKASDADVGENAE (SEQ ID NO:342) is destabilized by NOV0719. The region 303-315 IEYSITDGEGLDM (SEQ ID NO:343) exhibits a decrease in deuterium uptake by all antibodies, but the decrease is more pronounced by NOV0710 and NOV0712 relative to the others two antibodies. The region 316-330 FDVITDQETQEGIIT (SEQ ID NO:344) exhibits a decrease in deuterium uptake for all antibodies, but the decrease is the least by NOV0719. In contrast, the region 331-336 VKKLLD (SEQ ID NO:345) is destabilized by NOV0719 and less so by NOV0712. The region 337-358 FEKKKVYTLKVEASNPYVEPRF (SEQ ID NO:346) exhibits a decrease in deuterium uptake with all antibodies except NOV0719; in contrast NOV0719 is the only antibody that destabilizes the region

337-345 FEKKKVYTL (SEQ ID NO:347). Lastly, it observed that NOV0719 also destabilizes the region 370-376 VRIWED (SEQ ID NO:348).

[00398] For EC4 (384-486) and EC5 (487-608) the deuterium on-exchange of the antigen (Figure 3) was overall relatively low making detection of decreases in deuterium uptake challenging. For EC4 the region 407-427 AQDPDAARNPVKYSVDRHTDM (SEQ ID NO:349) exhibits a borderline decrease in deuterium uptake with NOV0710. There are no other regions in EC4 that decrease in deuterium uptake upon binding with any of the four antibodies. Overall, these observations strongly suggest that EC4 is not involved in the epitope. In contrast, 459-488 IATEINNPKQSSRVPLYIKVLDVNDNAPEF (SEQ ID NO:350) exhibits various degrees of destabilization with NOV0719, NOV1 127, NOV0712, and NOV0710 (listed in decreasing order of destabilization).

[00399] For EC5 only the region 564-586 YLLPWISDNDYPVQSSTGTVTV (SEQ ID NO:35 1) exhibits a borderline decrease in deuterium uptake when hCDH6 complexes with either NOV0710 or NOV0712. It should be noted that of the five extracellular domains that only EC5 does not exhibit any regions of destabilization when hCDH6 interacts with any of the four studied antibodies.

[00400] HDx experiments were also performed on hCDH6 construct consisting of only EC3-EC5, hCDH6(267-615). In these experiments the antibodies NOV0710 and NOV0712 were studied. This construct is same construct is used in crystallography experiments. The sequence coverage in these experiments was 86%. Table 10 provides a comprehensive list of all the peptides.

[00401] With this construct, in EC3 a decrease in deuterium uptake is observed in the region 295-3 15 ADVGENAEIEYSITDGEGLDM (SEQ ID NO:352) for both NOV0710 and NOV0712; this is very similar behavior to the full-length antigen where the region 303-3 15 IEYSITDGEGLDM (SEQ ID NO:343) exhibits a decrease in deuterium uptake by all antibodies, but more so by NOV0712 and NOV07 10. Interestingly, there are regions in EC3 that exhibit differential behavior between the two constructs. For example, in the hCDH6(54-615) NOV0719 caused significant destabilization in many regions while the other antibodies did not. In contrast, with the hCDH6(267-615) protein both NOV0710 and NOV0712 also exhibit significant destabilization in regions that are very similar to the regions observed to be destabilized by NOV0719 in the full length hCDH6(54-615) protein.

[00402] In EC4 a similar trend is observed for destabilizaton. In the hCDH6(54-615) NOV0719 caused the most significant destabilization on the C-terminal side of this domain and NOV0710 and NOV0712 had little effect. In contrast, when using the hCDH6(267-615) construct both NOV0710 and NOV712 significantly destabilize the C-terminal side of EC4. The origins for the differences in destabilization behavior are not well understood. It is important to note that in the EC4

domain neither construct had substantial decrease in deuterium uptake with NOV07 10 or NOV07 12 suggesting that this region is not involved in binding NOV0710 or NOV0712.

[00403] In EC5 we observe the most consistent deuterium exchange behavior between the hCDH6 (54-615) and hCDH6 (267-615) constructs. Neither constructs exhibited significant destabilization with NOV0712 or NOV0710. Moreover, the slight and often insignificant decrease in deuterium uptake (not less than -0.5 Da) with the hCD6 (54-615) becomes more pronounced in hCDH6 (267-615). In the EC5 of hCDH6 (267-615) regions that exhibit the most significant decrease in deuterium uptake upon binding either NOV0710 or NOV0712 include the regions 492-505 YETFVCEKAKADQL (SEQ ID NO:353), 551-563 TRKNGYNRHEMST (SEQ ID NO:354), and 572-586 DNDYPVQSSTGTVTV (SEQ ID NO:355). The substantial protection of 572-586 DNDYPVQSSTGTVTV(SEQ ID NO:355) is in excellent agreement with the epitope data from X-ray crystallography for both NOV0710 and NOV0712. The amino acids N573, D574, and Y575 exhibit a large accessible surface area and upon formation of a complex with either antibody all three are substantially buried. V577 is also buried in both structures indicating further agreement. Crystallography data does not indicate that 492-505 YETFVCEKAKADQL (SEQ ID NO:353) is buried so this protection appears to be allosteric in nature, but the protection of 551-563 TRKNGYNRHEMST (SEQ ID NO:354) appears to be of significance especially in the NOV0712 complex where R552 is substantially buried.

[00404] Lastly, it is important to note that there are other regions in the EC5 domain that exhibit significant (-0.5 Da or less) decrease in deuterium uptake upon complex formation with either of the two antibodies. For NOV0710 these regions include 510-525 HAVDKDDPYSGHQFSF(SEQ ID NO:356), 542-550 NKDNTAGIL (SEQ ID NO:357), and 591-604 CDHHGNMQSCHAEA (SEQ ID NO:358). For NOV0712 these regions include 512-522 VDKDDPYSGHQ (SEQ ID NO:359), 542 -550 NKDNTAGIL (SEQ ID NO:357), and 591-604 CDHHGNMQSCHAEA (SEQ ID NO:358).

[00405] Overall, the HDx data suggest that binding of CDH6 by the identified CDH6-binding antibodies results in several regions of the CDH6 protein becoming more prone to deuterium exchange, likely reflecting conformational changes induced by the binding of the CDH6 antibodies. The regions identified as protected from deuterium exchange in the presence of CDH6 antibodies are consistent with the x-ray crystallography data for NOV0710 and NOV0712 and form part of the epitope of these antibodies.

Table 9

Effect of NOV1127, NOV0719, NOV0710, and NOV0712 on hCDH6(54-615) containing cadherin 1-5 domains.

Peptide	Sequence	Change in Deuterium

Name		Incorporation (Daltons)			
		NOV 1127	NOV 0719	NOV 0710	NOV 0712
61-70	FLLEEYTGSD (SEQ ID NO:338)	-1.1	-1.2	-1.1	-1.3
63-70	LEEYTGSD (SEQ ID NO:360)	-0.1	-0.3	-0.4	-0.2
71-77	YQYVGKL (SEQ ID NO:361)	0.2	0.2	-0.1	0.1
71-88	YQYVGKLHSDQDRGDGSL (SEQ ID NO:362)	0.2	0.2	-0.1	0.1
71-100	YQYVGKLHSDQDRGDGSLKYILSGDGAGDL (SEQ ID NO:363)	1.0	0.9	-0.1	0.2
90-100	YILSGDGAGDL (SEQ ID NO:364)	0	0	0	0.1
101-112	FIINENTGDIQA (SEQ ID NO:365)	0.4	0.4	0.1	0.3
101-124	FIINENTGDIQATKRLDREEKPVY (SEQ ID NO:366)	0.4	0.6	-0.3	0
101-126	FIINENTGDIQATKRLDREEKPVYIL (SEQ ID NO:367)	0.6	0.7	-0.2	0
101-128	FIINENTGDIQATKRLDREEKPVYILRA (SEQ ID NO:368)	1.1	1.1	-0.2	0.2
102-124	IINENTGDIQATKRLDREEKPVY (SEQ ID NO:369)	0.4	0.5	-0.3	0.1
106-124	NTGDIQATKRLDREEKPVY (SEQ ID NO:370)	0.1	0.3	-0.4	0
106-126	NTGDIQATKRLDREEKPVYIL (SEQ ID NO:371)	0.6	0.6	-0.2	0
113-124	TKRLDREEKPVY (SEQ ID NO:372)	0.1	0.1	-0.2	-0.1

125-144	ILRAQAINRRTGRPVEPESE (SEQ ID NO:373)	0.3	0.4	-0.2	0.1
125-145	ILRAQAINRRTGRPVEPESEF (SEQ ID NO:374)	0.7	0.9	0	0.3
127-144	RAQAINRRTGRPVEPESE (SEQ ID NO:375)	0.3	0.4	-0.3	-0.1
127-145	RAQAINRRTGRPVEPESEF (SEQ ID NO:376	0.3	0.4	0	0.2
129-144	QAINRRTGRPVEPESE (SEQ ID NO:377)	0.1	0.2	-0.3	-0.2
129-145	QAINRRTGRPVEPESEF (SEQ ID NO:378)	0.2	0.3	-0.1	0.1
145-159	FIIKIHDINDNEPIF (SEQ ID NO:379)	0.9	0.9	0.4	0.5
145-162	FIIKIHDINDNEPIFTKE (SEQ ID NO:380)	0.7	0.7	0	0.2
146-159	IIKIHDINDNEPIF (SEQ ID NO:381)	0.5	0.6	0.2	0.3
146-160	IIKIHDINDNEPIFT (SEQ ID NO:382)	0.4	0.1	0.1	0.2
146-162	IIKIHDINDNEPIFTK (SEQ ID NO:383)	0.3	0.3	0	0.1
160-176	TKEVYTATVPEMSDVGT (SEQ ID NO:384)	-0.4	0.5	-0.3	-0.2
160-177	TKEVYTATVPEMSDVGTF (SEQ ID NO:385)	-0.5	0.3	-0.6	-0.4
163-171	VYTATVPEMSDVGTF (SEQ ID NO:386)	-0.1	0.6	0.2	0.2
177-184	FVVQVTAT (SEQ ID NO:387)	0.4	0.8	0.2	0.6
178-184	WQVTAT (SEQ ID NO:388)	0.3	0.5	0.2	0.3
178-194	VVQVTATDADDPTYGNS (SEQ ID NO:389)	-0.1	0	-0.2	-0.1
178-199	WQVTATDADDPTYGNSAKWY (SEQ ID NO:390)	-0.6	-0.1	-0.8	-0.5

181-199	VTATDADDPTYGNSAKVVY (SEQ ID NO:336)	-0.7	-0.3	-0.9	-0.6
185-199	DADDPTYGNSAKWY (SEQ ID NO:391)	-0.3	-0.5	-0.4	-0.4
200-208	SILQGQPYF (SEQ ID NO:392)	-0.3	-0.1	-0.3	-0.3
203-211	QGQPYFSVE (SEQ ID NO:393)	-0.1	0.5	0	0.1
203-221	QGQPYFSVESETGIIKTAL (SEQ ID NO340)	0.1	2.6	0.4	1.1
209-211	SVESETGIIKTAL (SEQ ID NO:394)	-0.1	2.1	0.3	0.8
212-221	SETGIIKTAL (SEQ ID NO:395)	0	1.9	0.4	0.8
214-222	TGIIKTALL (SEQ ID NO:396)	0.1	0.8	-0.1	0
222-232	LNMDRENREQY (SEQ ID NO:397)	-0.1	0	-0.1	-0.1
222-233	LNMDRENREQYQ (SEQ ID NO:398)	-0.2	0.1	-0.2	-0.1
225-233	DRENREQYQ (SEQ ID NO:399)	-0.4	-0.3	-0.4	-0.4
225-237	DRENREQYQWIQ (SEQ ID N0341)	-0.4	1.1	-0.1	0.2
275-294	FKTPESSPPGTPIGRIKASD (SEQ ID NO:400)	0.1	1.0	-0.1	0.2
275-300	FKTPESSPPGTPIGRIKASDADVGEN (SEQ ID NO:401)	0.1	1.3	0	0.3
275-302	FKTPESSPPGTPIGRIKASDADVGENAE (SEQ ID NO:342)	0.1	1.4	0	0.3
303-315	IEYSITDGEGLDM (SEQ ID NO:343)	-0.9	-1.1	-1.3	-1.3
305-315	YSITDGEGLDM (SEQ ID NO:402)	-0.6	-0.7	-0.8	-0.8

306-315	SITDGEGLDM (SEQ ID NO:403)	-0.2	-0.1	-0.2	-0.1
316-326	FDVITDQETQE (SEQ ID NO:404)	-1.0	-0.6	-0.9	-0.8
316-330	FDVITDQETQEGIIT (SEQ ID NO:344)	-1.2	-0.7	-1.4	-1.5
316-336	FDVITDQETQEGIITVKKLLD (SEQ ID NO:405)	-0.6	2.2	-0.8	-0.2
316-337	FDVITDQETQEGIITVKKLLDF (SEQ ID NO:406)	-0.6	2.3	-0.8	-0.2
316-345	FDVITDQETQEGIITVKKLLDFEKKKVYTL (SEQ ID NO:407)	-0.2	3.3	-1.1	-0.5
317-336	DVITDQETQEGIITVKKLLD (SEQ ID NO:408)	-0.2	2.2	-0.1	0.6
317-337	DVITDQETQEGIITVKKLLDF (SEQ ID NO:409)	-0.2	2.3	-0.1	0.6
318-337	VITDQETQEGIITVKKLLDF (SEQ ID NO:410)	-0.2	2.3	-0.3	0.3
327-336	GIITVKKLLD (SEQ ID N0:411)	0.1	0.9	-0.1	0.1
327-337	GIITVKKLLDF (SEQ ID NO:412)	0.3	1.1	-0.1	0.1
331-358	VKKLLDFEKKKVYTLKVEASNPYVEPRF (SEQ ID NO:413)	-0.4	1.3	-1.0	-0.7
337-358	FEKKKVYTLKVEASNPYVEPRF (SEQ ID NO:346)	-0.9	0.5	-1.1	-0.9
338-345	EKKKVYTL (SEQ ID NO:414)	-0.1	-0.6	-0.2	-0.2
346-358	KVEASNPYVEPRF (SEQ ID NO:415)	-0.7	-0.4	-0.5	-0.4
370-376	VRIWED (SEQ ID NO:348)	0.3	1.6	0	0.2

370-386	VRIWEDVDEPPVFSKL (SEQ ID NO:416)	0.3	1.6	0.1	0.4
376-383	DVDEPPVF (SEQ ID NO:417)	0.2	0.3	0.1	0.1
376-386	DVDEPPVFSKL (SEQ ID NO:418)	-0.1	0	-0.2	-0.2
377-383	VDEPPVF (SEQ ID NO:419)	0.3	0.6	0	0.1
377-386	VDEPPVFSKL (SEQ ID NO:420)	-0.2	-0.1	-0.4	-0.3
407-427	AQDPDAARNPVKYSVDRHTDM (SEQ ID NO:349)	-0.3	-0.3	-0.6	-0.4
413-427	ARNPVKYSVDRHTDM (SEQ ID NO:421)	-0.2	-0.2	-0.4	-0.4
420-427	SVDRHTDM (SEQ ID NO:422)	-0.1	-0.2	-0.1	-0.1
428-441	DRIFNIDSGNGSIF (SEQ ID NO:423)	1.0	0.1	-0.3	-0.3
442-451	TSKLLDRETL (SEQ ID NO:424)	0	-0.2	-0.1	-0.1
446-452	LDRETLL (SEQ ID NO:425)	-0.2	-0.2	-0.2	-0.2
459-475	lATEINNPKQSSRVPLY (SEQ ID NO:426)	0.6	1.0	0.2	0.4
461-474	TEINNPKQS SRVPL (SEQ ID NO:427)	0.3	0.6	0.1	0.2
461-475	TEINNPKQS SRVPLY (SEQ ID NO:428)	0.6	0.9	0.2	0.4
462-474	EINNPKQ SSRVPL (SEQ ID NO:429)	0.2	0.3	-0.1	0.1
462-475	EINNPKQ SSRVPLY (SEQ ID NO:430)	0.6	0.9	0.3	0.5
462-488	EINNPKQSSRVPLYIKVLDVNDNAPEF (SEQ ID NO:431)	1.4	2.2	0.7	1.0
463-475	INNPKQS SRVPLY (SEQ ID NO:432)	0.6	0.8	0.2	0.4

475-488	YIKVLDVNDNAPEF (SEQ ID NO:433)	0.5	0.8	0.3	0.4
476-488	IKVLDVNDNAPEF (SEQ ID NO:434)	0.5	0.7	0.2	0.4
496-505	VCEKAKADQL (SEQ ID NO:435)	-0.4	-0.4	-0.6	-0.6
506-522	IQTLHAVDKDDPYSGHQ (SEQ ID NO:436)	0.1	0	-0.1	0
506-525	IQTLHAVDKDDPYSGHQFSF (SEQ ID NO:337)	0.2	0.2	-0.1	-0.1
510-522	HAVDKDDPYSGHQ (SEQ ID NO:437)	-0.2	-0.4	-0.3	-0.3
510-524	HAVDKDDPYSGHQFS (SEQ ID NO:438)	-0.3	-0.5	-0.3	-0.3
510-525	HAVDKDDPYSGHQFSF (SEQ ID NO:356)	-0.2	-0.2	-0.3	-0.3
512-522	VDKDDPYSGHQ (SEQ ID NO:359)	-0.2	-0.4	-0.3	-0.4
512-525	VDKDDPYSGHQFSF (SEQ ID NO:439)	-0.1	-0.1	-0.3	-0.3
551-563	TRKNGYNRHEMST (SEQ ID NO:354)	-0.2	-0.1	-0.4	-0.4
587-602	RVCACDHHGNMQSCHA (SEQ ID NO:440)	-0.1	-0.3	-0.3	-0.4
591-602	CDHHGNMQSCHA (SEQ ID NO:441)	-0.2	-0.6	-0.4	-0.6

Table 10

Effect ofNOV0710 and NOV0712 on hCDH6 (267-615) containing cadherin 3-5 domains.

Peptide Name	**Sequence**	**Change in Deuterium Incorporation (Daltons)**	
		NOV0710	**NOV0712**

274-283	QFKTPESSPP (SEQ ID NO:442)	-0.4	-0.3
275-294	FKTPES SPPGTPIGRIKASD (SEQ ID NO:443)	0.3	0.3
275-294	FKTPESSPPGTPIGRIKASDA (SEQ ID NO:444)	0.3	0.4
282-294	PPGTPIGRIKASD (SEQ ID NO:445)	0.2	0.1
295-302	ADVGENAE (SEQ ID NO:446)	-0.9	-0.9
303-312	IEYSITDGEG (SEQ ID NO:447)	-0.7	-0.6
303-315	IEYSITDGEGLDM (SEQ ID NO:343)	-1.1	-1.1
306-312	SITDGEG (SEQ ID NO:448)	-0.6	-0.5
308-315	TDGEGLDM (SEQ ID NO:449)	-0.7	-0.8
316-323	FDVITDQE (SEQ ID NO:450)	0.1	0.1
316-326	FDVITDQETQE (SEQ ID NO:451)	-0.2	-0.3
316-336	FDVITDQETQEGIITVKKLLD (SEQ ID NO:452)	2.2	2.0
316-337	FDVITDQETQEGIITVKKLLDF (SEQ ID NO:453)	2.5	2.2
317-330	DVITDQETQEGIIT (SEQ ID NO:454)	-0.8	-0.8
317-336	DVITDQETQEGIITVKKLLD (SEQ ID NO:455)	2.0	1.8
318-336	VITDQETQEGIITVKKLLD (SEQ ID NO:456)	2.0	1.8

320-326	TDQETQE (SEQ ID NO:457)	-0.6	-0.6
321-336	DQETQEGIITVKKLLD (SEQ ID NO:458)	2.3	2.1
321-337	DQETQEGIITVKKLLDF (SEQ ID NO:459)	2.5	2.3
323-336	ETQEGIITVKKLLD (SEQ ID NO:460)	2.1	1.9
324-336	TQEGIITVKKLLD (SEQ ID NO:461)	1.8	1.8
327-336	GIITVKKLLD (SEQ ID NO:462)	1.3	1.2
327-337	GIITVKKLLDF (SEQ ID NO:463)	1.5	1.4
328-337	IITVKKLLDF (SEQ ID NO:464)	1.4	1.3
329-336	ITVKKLLD (SEQ ID NO:465)	1.1	1.0
329-337	ITVKKLLDF (SEQ ID NO:466)	1.3	1.2
330-336	TVKKLLD (SEQ ID NO:467)	1.1	0.9
330-337	TVKKLLDF (SEQ ID NO:468)	1.3	1.0
331-337	VKKLLDF (SEQ ID NO:469)	0.5	0.5
337-344	FEKKKVYT (SEQ ID NO:470)	-0.2	-0.1
337-345	FEKKKVYTL (SEQ ID NO:347)	0.5	0.6
338-345	EKKKVYTL (SEQ ID NO:471)	0.3	0.3
346-358	KVEASNPYVEPRF (SEQ ID NO:472)	-0.7	-0.4
348-358	EASNPYVEPRF (SEQ ID NO:473)	-0.9	-0.6

359-369	LYLGPFKDSAT (SEQ ID NO:474)	-0.5	-0.3
359-370	LYLGPFKDSATV (SEQ ID NO:475)	-0.2	-0.1
361-369	LGPFKDSAT (SEQ ID NO:476)	-0.4	-0.2
370-383	VRIVVEDVDEPPVF (SEQ ID NO:477)	1.8	1.7
370-386	VRIVVEDVDEPPVFSKL (SEQ ID NO:478)	2.0	1.9
373-383	VVEDVDEPPVF (SEQ ID NO:479)	0.5	0.5
376-383	DVDEPPVF (SEQ ID NO:480)	0.4	0.4
376-386	DVDEPPVFSKL (SEQ ID NO:481)	-0.1	0.0
376-388	DVDEPPVFSKLAY (SEQ ID NO:482)	-0.3	-0.1
377-383	VDEPPVF (SEQ ID NO:483)	0.4	0.4
377-386	VDEPPVFSKL (SEQ ID NO:484)	-0.2	-0.1
407-419	AQDPDAARNPVKY (SEQ ID NO:485)	-0.1	0.1
407-427	AQDPDAARNPVKYSVDRHTDM (SEQ ID NO:349)	-0.2	-0.1
413-427	ARNPVKYSVDRHTDM (SEQ ID NO:486)	0.1	0
421-427	VDRHTDM (SEQ ID NO:487)	-0.2	-0.3
428-441	DRIFNIDSGNGSIF (SEQ ID NO:488)	0.1	0.2
442-451	TSKLLDRETL (SEQ ID NO:489)	0	0
446-452	LDRETLL (SEQ ID NO:490)	-0.3	-0.1

459-474	IATEINNPKQSSRVPL (SEQ ID NO:491)	1.0	1.1
459-475	IATEINNPKQS SRVPLY (SEQ ID NO:492)	1.2	1.4
461-475	TEINNPKQSSRVPLY (SEQ ID NO:493)	1.0	1.0
461-478	TEINNPKQSSRVPLYIKV (SEQ ID NO:494)	2.5	2.6
462-474	EINNPKQSSRVPL (SEQ ID NO:495)	0.7	0.8
462-475	EINNPKQSSRVPLY (SEQ ID NO:496)	1.1	1.2
463-475	INNPKQS SRVPLY (SEQ ID NO:497)	1.2	1.2
464-474	NNPKQSSRVPL (SEQ ID NO:498)	1.7	1.7
464-476	NNPKQSSRVPLYI (SEQ ID NO:499)	1.2	1.2
475-488	YIKVLDVNDNAPEF (SEQ ID NO:500)	1.1	1.3
476-483	IKVLDVND (SEQ ID NO:501)	1.1	1.1
476-488	IKVLDVNDNAPEF (SEQ ID NO:502)	0.8	1.0
477-488	KVLDVNDNAPEF (SEQ ID NO:503)	0.4	0.4
479-488	LDVNDNAPEF (SEQ ID NO:504)	-0.1	0.1
492-505	YETFVCEKAKADQL (SEQ ID N0353)	-1.1	-1.1
495-505	FVCEKAKADQL (SEQ ID NO: 505)	-0.7	-0.6
496-505	VCEKAKADQL (SEQ ID NO:506)	-0.8	-0.7
499-505	KAKADQL (SEQ ID NO:507)	-0.7	-0.7

506-513	IQTLHAVD (SEQ ID NO:508)	0.3	0.4
506-522	IQTLHAVDKDDPYSGHQ (SEQ ID NO:509)	0	0.1
506-524	IQTLHAVDKDDPYSGHQFS (SEQ ID NO:510)	0.1	0.1
506-525	IQTLHAVDKDDPYSGHQFSF (SEQ ID NO:511)	-0.1	0
510-522	HAVDKDDPYSGHQ (SEQ ID NO:512)	-0.4	-0.3
510-524	HAVDKDDPYSGHQFS (SEQ ID NO:513)	-0.3	-0.2
510-525	HAVDKDDPYSGHQFSF (SEQ ID NO:356)	-0.5	-0.4
512-522	VDKDDPYSGHQ (SEQ ID NO:359)	-0.6	-0.6
512-525	VDKDDPYSGHQFSF (SEQ ID NO:514)	-0.5	-0.4
542-550	NKDNTAGIL (SEQ ID NO:357)	-0.5	-0.6
549-563	ILTRKNGYNRHEMST (SEQ ID NO:515)	-0.5	-0.4
551-563	TRKNGYNRHEMST (SEQ ID NO:354)	-0.6	-0.5
552-563	RKNGYNRHEMST (SEQ ID NO:516)	-0.7	-0.8
564-571	YLLPWIS (SEQ ID NO:517)	-0.1	0.1
564-575	YLLPWISDNDY (SEQ ID NO:518)	-0.5	-0.4
564-578	YLLPWISDNDYPVQ (SEQ ID NO:519)	-1.0	-1.0
567-586	PWISDNDYPVQSSTGTVTV (SEQ ID NO:520)	-1.5	-0.8
587-600	RVCACDHHGNMQSC (SEQ ID NO:521)	-0.5	-0.5

587-602	RVCACDHHGNMQSCHA (SEQ ID NO:522)	-0.3	-0.3
591-600	CDHHGNMQSC (SEQ ID NO:523)	-0.7	-0.7
591-602	CDHHGNMQSCHA (SEQ ID NO:524)	-0.4	-0.4
591-604	CDHHGNMQSCHAEA (SEQ ID NO:358)	-0.6	-0.8
592-602	DHHGNMQSCHA (SEQ ID NO:525)	-0.6	-0.6
605-618	LIHPTGLSTGAGSE (SEQ ID NO:526)	-0.5	-0.4
619-625	FRHDSGL (SEQ ID NO:527)	-0.3	-0.2
619-627	FRHDSGLND (SEQ ID NO:528)	-0.4	-0.4
630-637	EAQKIEWH (SEQ ID NO:529)	-0.6	-0.5
630-638	EAQKIEWHE (SEQ ID NO:530)	-0.8	-0.7

Example 7: Epitope mapping by x-ray crystallography

[00406] The crystal structures of a human CDH6 fragment (extracellular domain 5, or EC5, SEQ ID NO: 443, Table 11) bound to Fab fragment of NOV0712 or NOV0710 (SEQ ID NO: 444-447, Table 12 and 13) were determined. As detailed below, CDH6 EC5 was co-expressed with NOV0712 or NOV0710 Fab in mammalian cells to produce a purified complex. Protein crystallography was then employed to generate atomic resolution data for CDH6 EC5 bound to NOV712 or NOV710 Fab and define the epitopes.

Protein production for epitope mapping

[00407] The sequences of CDH6 EC5, NOV0712 Fab and NOV0710 Fab produced for crystallography are shown in Table 9 above. The CDH6 EC5 construct comprises residues 490 to 608 shown as underlined in the context of human CDH6 (UniProt identifier P55285, SEQ ID NO: 533, Table 11), and shown below in Table 11 as SEQ ID NO: 534. The N-terminal signal sequence from mouse IgG kappa light chain is used for secreted expression of CDH6 EC5 and is cleaved during expression, leaving intact N-terminus of CDH6 EC5. The C-terminus of CDH6 EC5 is fused with a

purification tag derived from β amyloid (APP tag, amino acids: EFRHDS (SEQ ID NO:53 1)), preceded by a PreScission® protease (GE Healthcare, Piscataway, NJ) recognition site (amino acids: LEVLFQGP (SEQ ID NO:532)) to facilitate cleavage and removal of tag after purification. For NOV0712 and NOV0710 Fab, the sequences of heavy and light chains are shown (SEQ ID NO:535-538).

Table 11

Proteins usedfor structural biology studies

Construct	Amino acid sequence in one letter code	SEQ ID NO
Human CDH6 (P55285)	MRTYRYFLLLFWVGQPYPTLSTPLSKRTSGFPAKKRALEL SGNSKNELNRSKRSWMWNQFFLLEEYTGSDYQYVGKLHS DQDRGDGSLKYILSGDGAGDLFIINENTGDIQATKRLDREE KPVYILRAQArNRRTGRPVEPESEFIIKIHDINDNEPIFTKEV YTATVPEMSDVGTFWQVTATDADDPTYGNSAKWYSIL QGQPYFSVESETGIIKTALLNMDRENREQYQVVIQAKDMG GQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPP GTPIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQET QEGIITVKKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFK DSATVRIWEDVDEPPVFSKLAYILQIREDAQINTTIGSVTA QDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLD RETLLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAPEF AEFYETFVCEKAKADQLIQTLHAVDKDDPYSGHQFSFSLA PEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPV VISDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHP TGLSTGALVAILLCIVILLVTWLFAALRRQRKKEPLIISKE DIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIEDNKLRRD IVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPY DSLATYAYEGTGSVADSLSSLESVTTDADQDYDYLSDWG PRFKKLADMYGGVDSDKDS	(SEQ ID NO:533)
CDH6 EC5 construct	EFYETFVCEKAKADQLIQTLHAVDKDDPYSGHQFSFSLAP EAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPW ISDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHP	(SEQ ID NO:534)
NOV0712 Fab heavy chain	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSHGMHWVRQ APGKGLEWVSVISGSGSNTGYADSVKGRFTISRDNSKNTL YLQMNSLRAEDTAVYYCARQWGSYAFDSWGQGTLVTVS	(SEQ ID NO:535)

	SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSWTVPSSSLGTQTY ICNVNHKPSNTKVDKRVEPKSCDKTH	
NOV0712 Fab light chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPG KAPKLLIYAVSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSGTFPPTTFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC	(SEQ ID NO:536)
NOV0710 Fab heavy chain	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQ APGKGLEWVSVIRSSGSSTYYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCARGGGYFDYWGQGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSWTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTH	(SEQ ID NO:537)
NOV0710 Fab light chain	DIQMTQSPSSLSASVGDRVTITCRASQSISLWLNWYQQKPG KAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQYYTSPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC	(SEQ ID NO:538)
CDH6-D574A	MRTYRYFLLLFWVGQPYPTLSTPLSKRTSGFPAKKRALEL SGNSKNELNRSKRSWMWNQFFLLEEYTGSDYQYVGKLHS DQDRGDGSLKYILSGDGAGDLFIINENTGDIQATKRLDREE KPVYILRAQAINRRTGRPVEPESEFIIKIHDINDNEPIFTKEV YTATVPEMSDVGTFWQVTATDADDPTYGNSAKWYSIL QGQPYFSVESETGIIKTALLNMDRENREQYQWIQAKDMG GQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPP GTPIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQET QEGIITVKKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFK DSATVRIWEDVDEPPVFSKLAYILQIREDAQINTTIGSVTA QDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLD RETLLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAPEF AEFYETFVCEKAKADQLIQTLHAVDKDDPYSGHQFSFSLA PEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPV VISDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHP	(SEQ ID NO:539

	TGLSTGALVAILLCIVILLVTWLFAALRRQRKKEPLIISKE DIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIEDNKLRRD IVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPY DSLATYAYEGTGSVADSLSSLESVTTDADQDYDYLSDWG PRFKKLADMYGGVDSDKDS	
CDH6-Y575A	MRTYRYFLLLFWVGQPYPTLSTPLSKRTSGFPAKKRALEL SGNSKNELNRSKRSWMWNQFFLLEEYTGSDYQYVGKLHS DQDRGDGSLKYILSGDGAGDLFIINENTGDIQATKRLDREE KPVYILRAQAINRRTGRPVEPESEFIIKIHDINDNEPIFTKEV YTATVPEMSDVGTFWQVTATDADDPTYGNSAKWYSIL QGQPYFSVESETGIIKTALLNMDRENREQYQWIQAKDMG GQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPP GTPIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQET QEGIITVKKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFK DSATVRIWEDVDEPPVFSKLAYILQIREDAQINTTIGSVTA QDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLD RETLLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAPEF AEFYETFVCEKAKADQLIQTLHAVDKDDPYSGHQFSFSLA PEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPV VISDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHP TGLSTGALVAILLCIVILLVTWLFAALRRQRKKEPLIISKE DIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIEDNKLRRD IVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPY DSLATYAYEGTGSVADSLSSLESVTTDADQDYDYLSDWG PRFKKLADMYGGVDSDKDS	(SEQ ID NO:540)
CDH6-N573A	MRTYRYFLLLFWVGQPYPTLSTPLSKRTSGFPAKKRALEL SGNSKNELNRSKRSWMWNQFFLLEEYTGSDYQYVGKLHS DQDRGDGSLKYILSGDGAGDLFIINENTGDIQATKRLDREE KPVYILRAQAINRRTGRPVEPESEFIIKIHDINDNEPIFTKEV YTATVPEMSDVGTFWQVTATDADDPTYGNSAKWYSIL QGQPYFSVESETGIIKTALLNMDRENREQYQWIQAKDMG GQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPP GTPIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQET QEGIITVKKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFK DSATVRIWEDVDEPPVFSKLAYILQIREDAQINTTIGSVTA QDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLD	(SEQ ID NO:541

	P^TLLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAPEF AEFYETFVCEKAKADQLIQTLHAVDKDDPYSGHQFSFSLA PEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPV VISDNDYPVQSSTGTVTVRVCACDHHGNMQSCHAEALIHP TGLSTGALVAILLCIVILLVTWLFAALRRQRKKEPLIISKE DIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIEDNKLRRD IVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPY DSLATYAYEGTGS VAD SL SSLES VTTD ADQD Y DYL SDWG PRFKKL ADM YGG VD SDKD S	

[00408] CDH6 EC5 was co-expressed with NOV0712 or NOV0710 Fab in Expi293® cells to produce complex for crystallography. In detail, 0.5 mg of plasmid encoding CDH6 EC5 was mixed with 0.5 mg of NOV0712 or NOV0710 Fab plasmid, diluted into 50ml of OptiMEM I medium (Life Technologies, Grand Island, NY), and incubated with 2.5mg of PEI (Polysciences, Warrington, PA) in 50ml of the same medium for 30 minutes. The mixture was then added into 1L of Expi293® cells growing in suspension in Expi293® Expression medium (Life Technologies Grand Island, NY) at 1 million cells/ml at 37 °C with 8% of C02 for transfection. After 72 hours, the medium which contains CDH6 EC5-Fab complex was harvested by centrifugation, and supplemented with CaC12 to 3mM. 10ml of Sepharose® 4B beads (GE Healthcare Pittsburgh, PA) conjugated with an anti-APP tag IgG (generated in-house) were then added into the medium and kept stirring at 4 °C overnight. The next day the beads were packed into a gravity column and washed with 25mM Hepes pH 7.4 + 150mM NaCl (HBS), and 3mM CaC12. The target complex was eluted with 3 column volumes (CV) of 100 mM glycine pH 2.5, 150mM NaCl, 3mM CaC12, into 1/10 (v/v) of 1 M Tris pH 8.5. The complex was incubated with 1/100 (w/w) GST-tagged PreScission® protease (GE Healthcare, Pittsburgh, PA) at 4 °C overnight to cleave APP tag. The next day, PreScission® protease was removed by passing the mixture through a 1ml GSTrap® HP column (GE Healthcare, Pittsburgh, PA). The flow-through was then incubated with 1/10 (w/w) of PNGaseF (purified in house) at 37 °C overnight to remove N-linked glycosylation. After deglycosylation, the mixture was concentrated and loaded onto HiLoad 16/600 Superdex® 200 pg (GE Healthcare, Pittsburgh, PA) equilibrated in HBS plus 3 mM CaC12. Peak fractions containing purified CDH6-Fab complex were analyzed by SDS-PAGE and LCMS, pooled and concentrated for crystallization.

Crystallization and structure determination

[00409] CDH6 EC5/NOV07 12 and CDH6 EC5/NOV07 10 complexes were concentrated to 9.3 mg/ml and 14.7 mg/ml, respectively, centrifuged at 20,000 g for 5 minutes, and screened for crystallization. Crystals for data collection were grown by hanging drop vapor diffusion at 20 °C.

Crystals of CDH6 EC5/NOV07 12 complex were grown by mixing 1.2 μl of the complex with 1 μl of reservoir solution containing 20% (v/v) PEG3350 and 0.2 M di-ammonium hydrogen citrate, and equilibrating the drop against 450 μl of the same reservoir solution plus 50 μl water. Crystals of CDH6 EC5/NOV0710 complex are grown by mixing 0.8 μl of the complex with 1 μl of reservoir solution containing 0.7 M tri-sodium citrate and 0.1 M Tris'HCl pH 8.5, and equilibrating the drop against 425 μl of the same reservoir solution plus 75 μl water. Before data collection, the crystals were transferred to 75% of reservoir solutions plus 25% glycerol and flash cooled in liquid nitrogen.

[00410] Diffraction data were collected at beamline 17-ID at the Advanced Photon Source (Argonne National Laboratory, USA). Data are processed and scaled using Autoproc (Global Phasing, LTD). The data of CDH6 EC5/NOV0712 was processed to 2.3 A in space group P21212 1 with cell dimensions a=78.83 A, b=88.86 A, c=186.73 A, alpha=90°, beta=90°, gamma=90°. The data of CDH6 EC5/NOV0710 complex was processed to 3.5 A in space group P43212 with cell dimensions a=101.48 A, b=101.48 A, c=240.96 A, alpha=90°, beta=90°, gamma=90°. The structures of the complexes were solved by molecular replacement using Phaser (McCoy et al., (2007) J. Appl. Cryst. 40:658-674) with in-house Fab structures as search models. The structure of CDH6 EC5 was built from scratch using Buccaneer (K. Cowtan (2006) Acta Cryst. D62: 1002-101 1) in the CCP4 program suite (Winn et al., (201 1) Acta. Cryst. D67:235-242). The final models were built in COOT (Emsley & Cowtan (2004) Acta Cryst. D60:2126-2132) and refined with Buster (Global Phasing, LTD, Cambridge, UK). For the CDH6 EC5/NOV0712 complex, the Rwork and Rfree values are 21.2% and 25.2%, respectively; and root-mean-square (r.m.s) deviation values of bond lengths and bond angles are 0.0 IθA and 1.24°, respectively. For the CDH6 EC5/NOV0710 complex, the Rwork and Rfree values are 18.2% and 24.6%, respectively; and r.m.s deviation values of bond lengths and bond angles are QOIθA and 1.28°, respectively.

[00411] Residues of CDH6 EC5 that are in contact with NOV0712 or NOV0710 Fab, the types of interactions, and the buried surface areas are all identified by PISA (Krissinel et al., (2007) J Mol Biol. 372:774-97) and listed in Tables 12 and 13. For the structures that contain more than one copy of complex in the asymmetric unit (the smallest unique unit in the crystal), only those antibody-contacting residues that are common in all copies are listed as epitope residues.

Epitopes ofNOV0712 and NOV0710 on CDH6

Overall structures

[00412] There is no reported crystal structure of CDH6 EC5 at this time. The overall folding of CDH6 EC5 is similar to that of CDH2 (N-Cadherin) EC5, both composed by one three-strand β sheet stacking against another four-strand β sheet. Overlay of the two structures results in a small root-mean-square distance (RMSD) of 2.4A and similar orientation of the N- and C-terminus. Thus one can overlay the CDH6 EC5/Fab complex structure onto the full-length ECD structure of CDH2 based on

the superposition of EC5 domains, in order to estimate the orientations and NOV0712 and NOV0710 Fab. As shown in Figure 4, NOV0712 and NOV0710 bind the same side of CDH6 EC5, and share a significantly overlapped epitope. NOV0712 binds slightly shifted away from the EC4-EC5 linker compared to NOV07 10.

Epitope of NOV0712

[00413] The crystal structure of the CDH6 EC5/NOV07 12 complex is used to identify the NOV0712 epitope on CDH6. The interaction surface on CDH6 EC5 by NOV0712 Fab is formed by several continuous and discontinuous (i.e. noncontiguous) sequences: namely residues 503, 520-527, 529, 532-534, 538-543, 550, 552, 569, and 571-577, as detailed in Table 12. These residues form the three-dimensional conformational epitope that is recognized by NOV0712 Fab (Figure 5 upper panel). This epitope defined by crystallography is in good agreement with that defined by hydrogen deuterium exchange mass spectrometry (HDx-MS), in which residues 572-586 are substantially protected by NOV0712. Interestingly, a potential Ca2+-binding loop of CDH6 EC5 (residues 571-579) has majority of it (8 out 10 residues) in contact with NOV0712, especially Tyr575 and Asn573 which are almost completely buried by the antibody (Table 12 and Figure 5 lower panel). Compared with CDH2 structure, this Ca2+-binding loop of CDH6 EC5 is in an "out" conformation induced by NOV0712 binding (e.g. Asp572 of CDH6 is flipped out compared with its corresponding residue Asp525 in CDH2).

Table 12

NOV0712 epitope on CDH6. All residues of CDH6 that are in contact with NOV0712 in the crystal structure are identified by PISA, listed and sorted by their buried surface area by NOV0712. Types of interaction are also listed where applicable.

Residue name	Residue number	Accessible surface area (ASA, Å^2)	Buried surface area (BSA) by heavy chain (Å^2)	BSA by light chain (Å^2)	Total BSA by NOV0712 (Å^2)	BSA/ASA (%)	Type of interaction
TYR	575	205.77	13 1.73	47. 11	178.84	86.91	H-SC[1], H-MC[2], Hydrophobic
ASN	573	106.72	103.67	0	103.67	97. 14	H-SC, H-MC
LYS	543	147.48	65.S4	37.33	102. I	69.96	Salt bridge
ARG	552	148.25	0	78.9	78.9	53.22	H-SC
ASP	574	104.57	68.99	0	68.99	65.97	H-SC, H-MC
GLN	540	99.9 1	0	68.33	68.33	68.35	H-SC
PRO	529	122.44	0	65.25	65.25	53.29	
ALA	532	67.0 1	0	64.9	64.9	96.85	H-MC
SER	533	103.84	0	59.58	59.58	57.38	H-SC

VAL	577	97.82	53.24	0	53.24	54.43	H-MC
SER	526	52.04	0	4"7. i:	4"7. i:	90.55	
ASP	54 1	45.28	8.8(.	36.42	45. : s	100.00	H-MC
GLN	522	100.04	45. 14	0	45. 14	45. 12	
SER	524	43.52	:<. 9s	16. 54	43.52	100.00	
GLY	520	64.48	32.ss	0	32.ss	50.99	H-MC
ASP	503	113.22	0	32.06	32.06	28.32	
ILE	539	31.09	0	29.09	29.09	93.57	H-MC
HIS	52 1	104.3	23.91	0	23.91	22.92	
THR	538	39.69	0	23	23	57.95	
LEU	527	24.5 1	0	: 3.3 i	: 0.0 i	8 1.64	H-MC
PRO	576	111.16	17.57	0	17.57	15.81	
ASN	542	72.05	0	17.39	17.39	24. 14	
PHE	525	15.47	0.95	14.52	15.4"7	100.00	
SER	57 1	16. 16	6.9(.	"7. 1	14. (c.	87.00	
VAL	569	23. 13	0	S.54	S.54	36.92	
LEU	550	23.71	0	7.9	7.9	33.32	
GLY	534	71.3	0	5.13	5.13	7.19	
PHE	523	9.8 1	4.5	0	4.5	45.87	
ASP	572	23.71	2.26	0	2.26	9.53	

[1]Hydrogen bond by side-chain atoms; [2]Hydrogen bond by main-chain atoms

Epitope of NOV0710

[00414] The crystal structure of the CDH6 EC5/NOV0710 complex is used to identify the NOV0710 epitope on CDH6. The interaction surface on CDH6 EC5 by NOV0712 Fab is formed by several continuous and discontinuous (i.e. noncontiguous) sequences: namely residues 520-527, 529, 541, 543, 569, and 571-579, as detailed in Table 13. These residues form the three-dimensional conformational epitope that is recognized by NOV0710 Fab (Figure 6 upper panel). This epitope defined by crystallography is in good agreement with that defined by hydrogen deuterium exchange mass spectrometry (HDx-MS), in which residues 572-586 are substantially protected by NOV0710. Interestingly, similar as in the CDH6 EC5/NOV0712 complex structure, all residues in the potential Ca2+-binding loop of CDH6 EC5 (residues 571-579) are in contact with NOV0710; among which Tyr575, Asn573 and Asp574 are almost completely buried by the antibody (Table 13 and Figure 6 lower panel). Compared with CDH2 structure, this Ca2+-binding loop of CDH6 EC5 is in an "out" conformation induced by NOV0710 binding (e.g. Asp572 of CDH6 is flipped out compared with its corresponding residue Asp525 in CDH2).

Table 13

NOV0710 epitope on CDH6. All residues of CDH6 that are in contact with NOV0710 in the crystal structure are identified by PISA, listed and sorted by their buried surface area by NOV0710. Types of interaction are also listed where applicable.