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

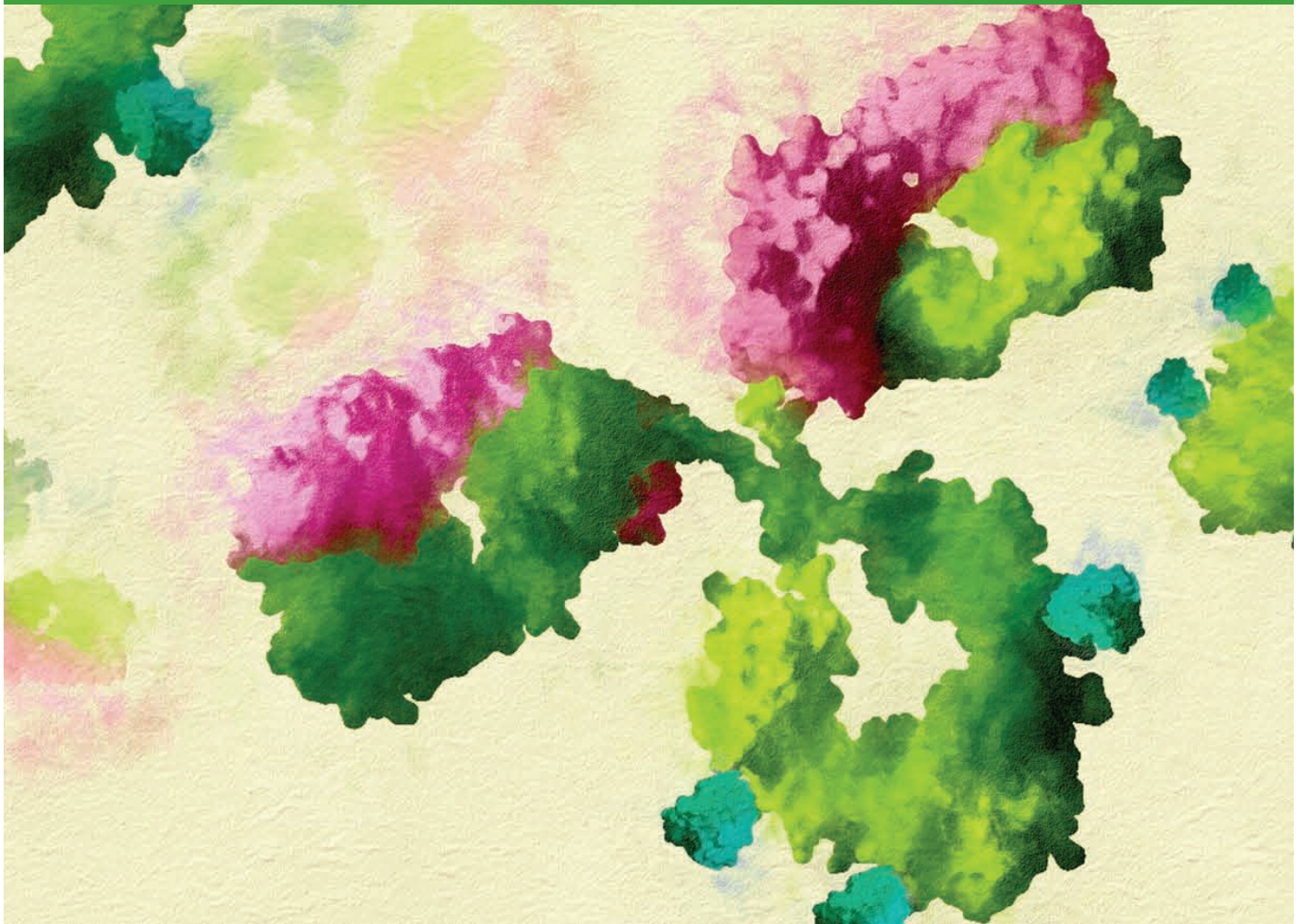
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INTRODUCTION

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.

RESEARCH ARTICLE

Bialucha et al.

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 N²-deacetyl-N²′-(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 N²-deacetyl-N²′-(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

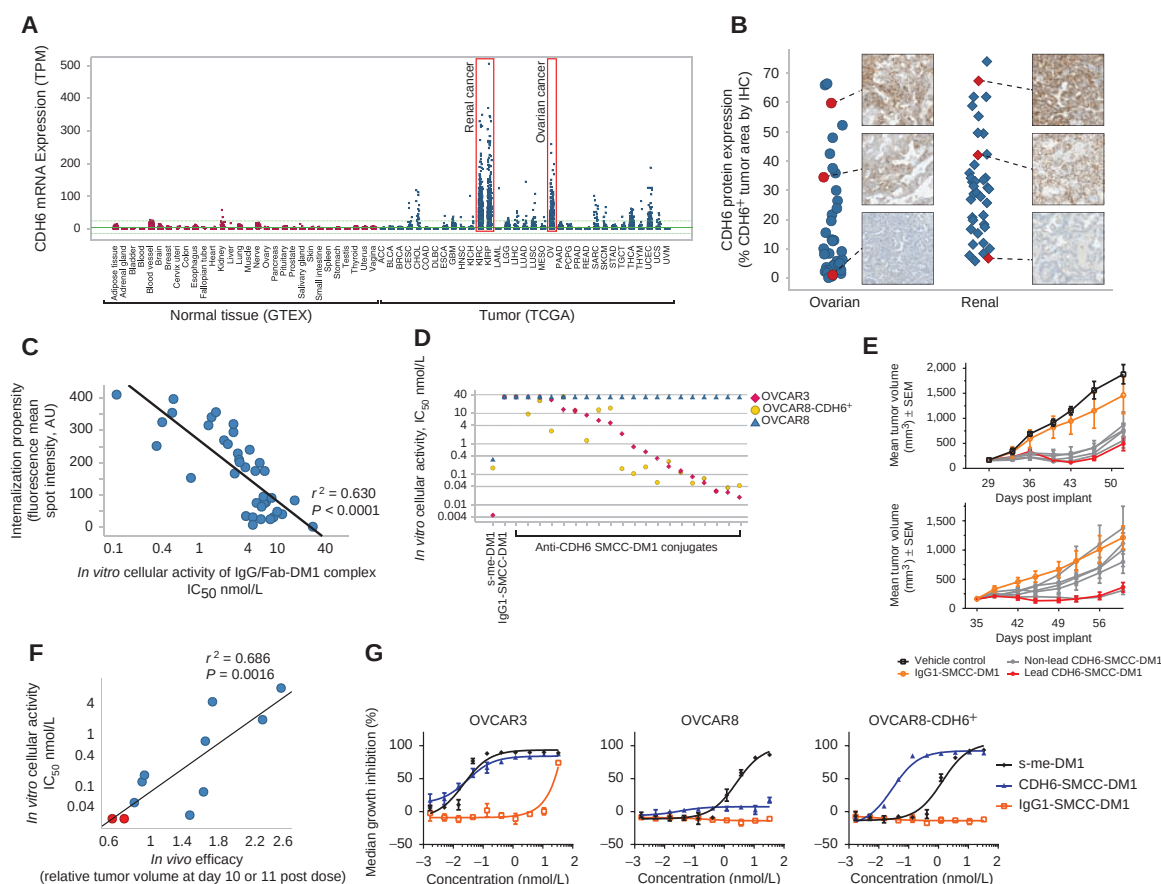


Figure 1. CDH6, a lineage gene frequently overexpressed in ovarian and renal cancer, is amenable to targeting using an ADC approach. **A**, CDH6 expression in transcripts per million reads (TPM) across normal tissue (pink) and cancer tissue (blue) samples. Renal and ovarian cancers are highlighted (red box) indications featuring frequent CDH6 overexpression. Green lines represent median expression $\pm$ 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. Insets 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 $\pm$ 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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Bialucha et al.

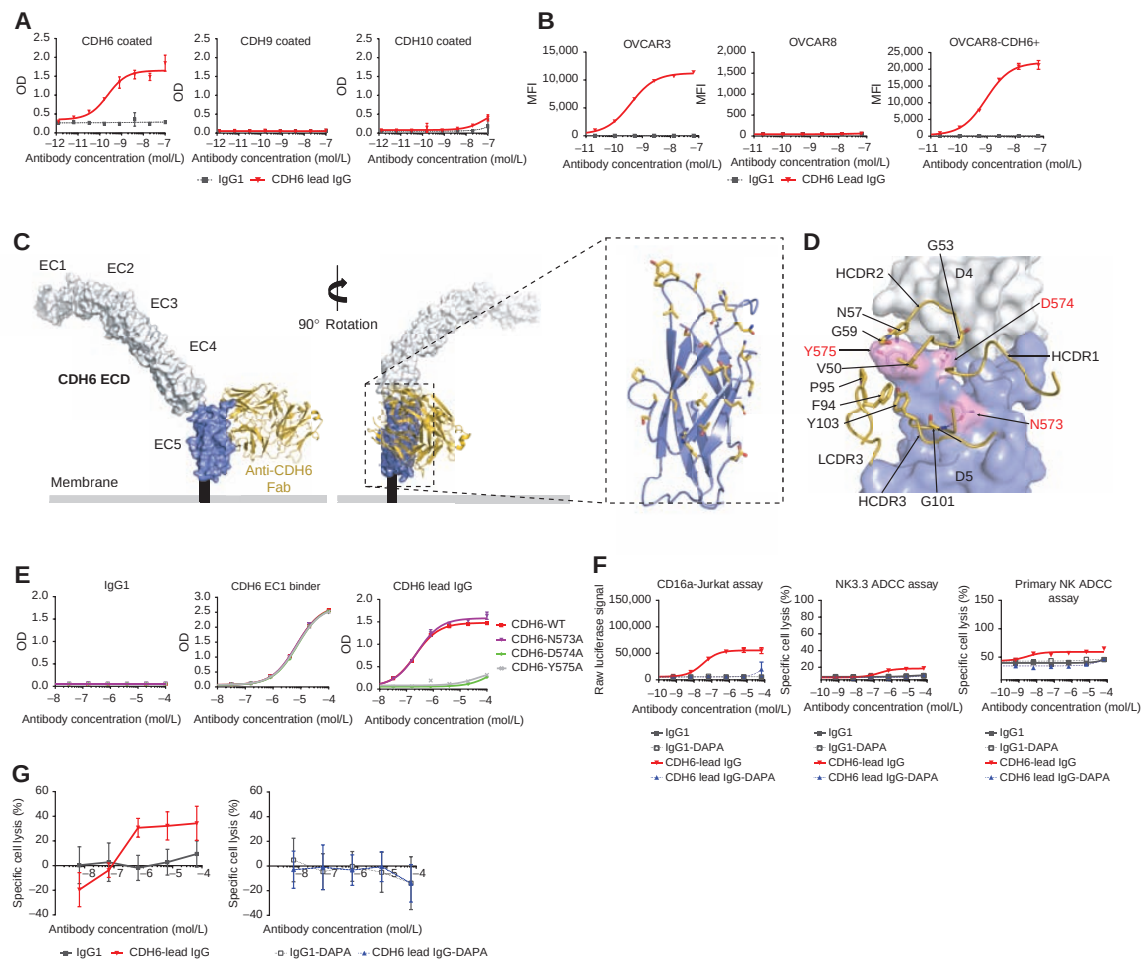


Figure 2. The lead CDH6-targeting mAb binds selectively to a conformational epitope conserved between rodents and primates and is competent of eliciting Fc-mediated effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) *in vitro*. **A**, Dose-dependent binding of control IgG1 (gray) or lead CDH6 antibody LTV977 (red) to recombinant human CDH6, CDH9, or CDH10 protein by ELISA (mean $\pm$ 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 $\pm$ standard deviation). **C**, Overall structure of lead CDH6 antibody Fab fragment (shown 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 $\pm$ 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 $\pm$ standard deviation is plotted for the reporter assay, whereas mean percent specific lysis $\pm$ 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 $\pm$ 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

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 anti-tumor 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

RESEARCH ARTICLE

Bialucha et al.

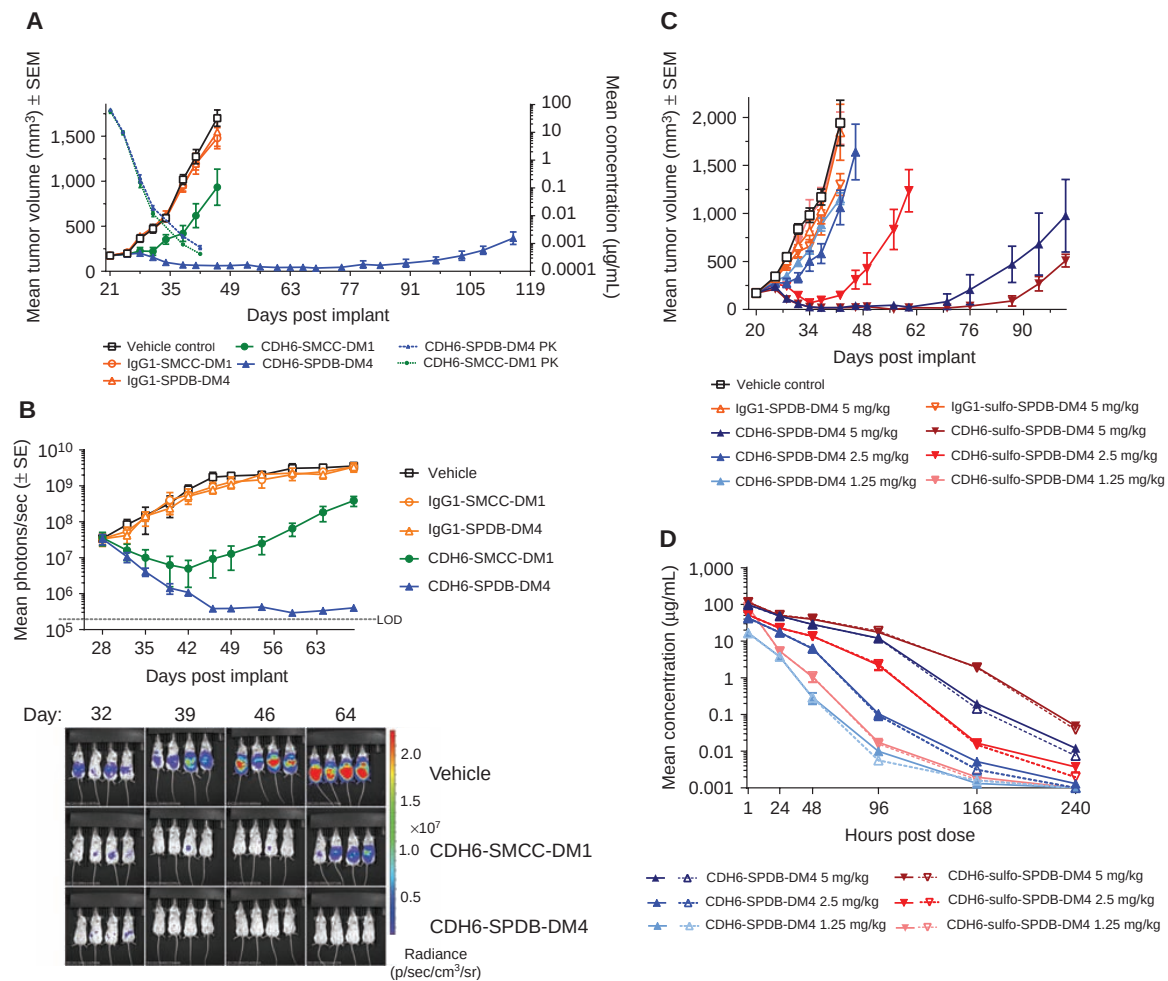


Figure 3. Comparative *in vivo* profiling identifies sulfo-SPDB-DM4 as the optimal L/P for CDH6-ADC. **A**, OVCAR3 xenografts were grown subcutaneously in NSG mice and treated with a single i.v. dose of 5 mg/kg control or CDH6-targeting antibodies linked to either SMCC-DM1 or SPDB-DM4 payloads. Mean 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

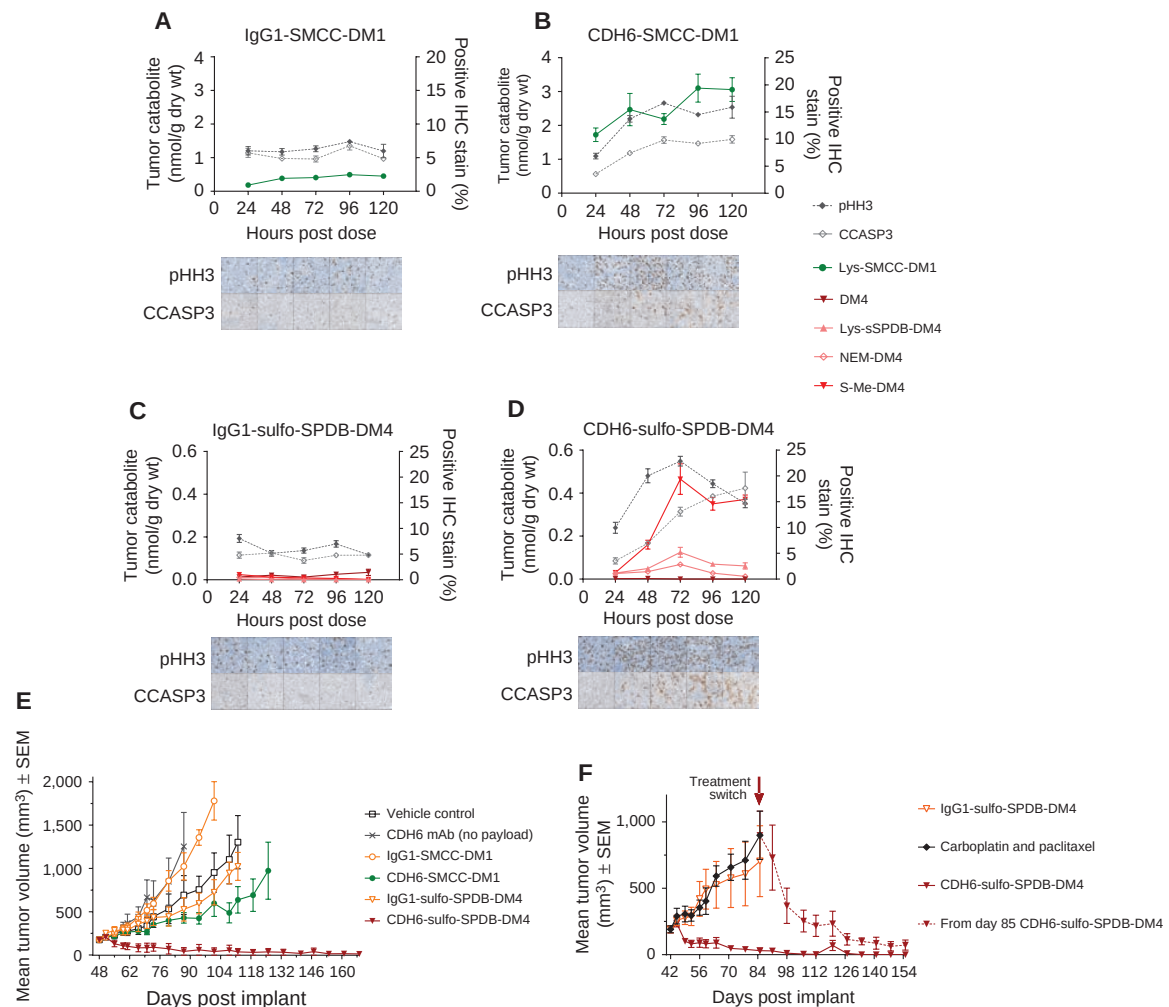


Figure 4. CDH6-sulfo-SPDB-DM4 has superior pharmacodynamic impact on OVCAR3 tumors, and robust antitumor response in ovarian PDX models. **A–D**, Established subcutaneous OVCAR3 tumors were treated with a single i.v. administration of either IgG1-SMCC-DM1 (**A**), CDH6-SMCC-DM1 5 mg/kg (**B**), IgG1-sulfo-SPDB-DM4 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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Bialucha et al.

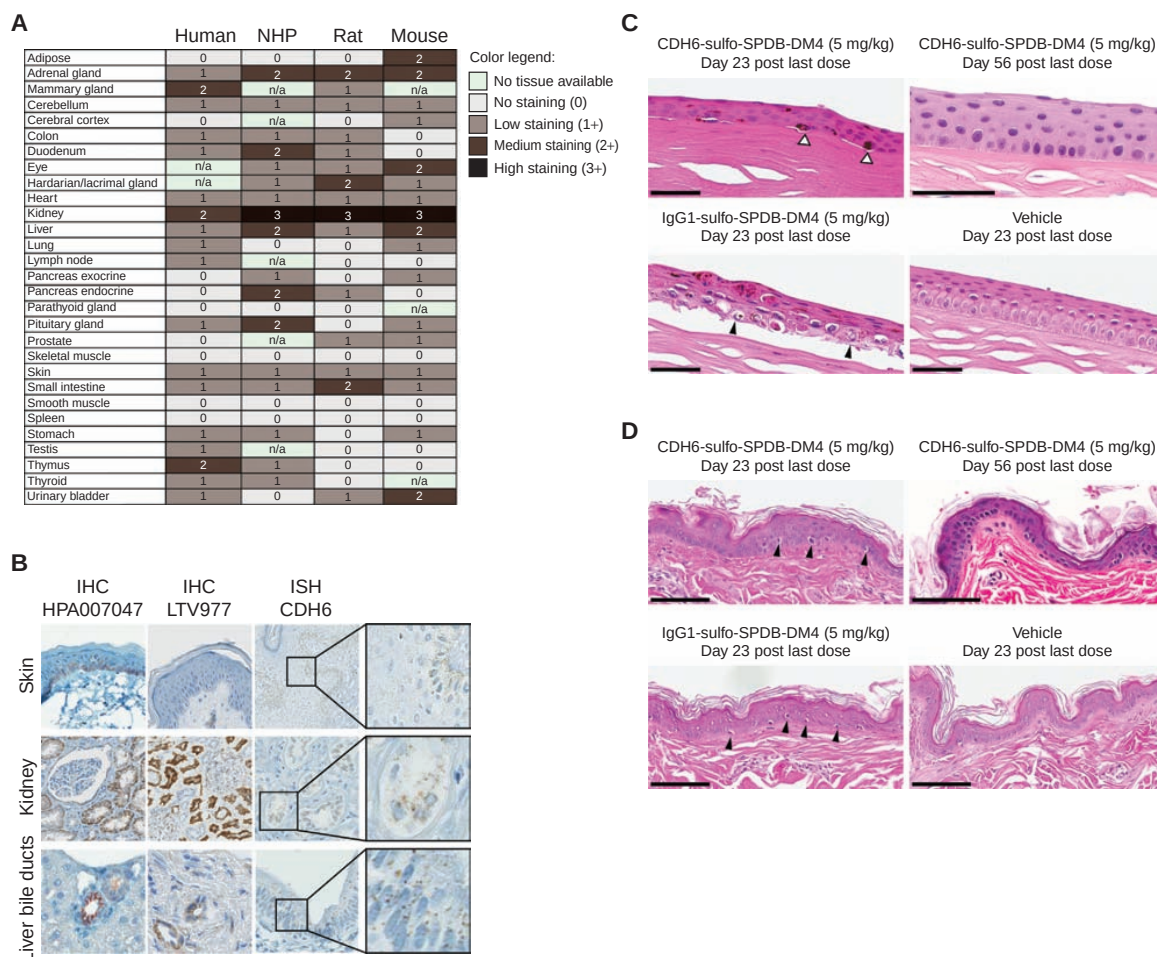


Figure 5. CDH6-targeting ADCs feature an acceptable tolerability profile in rats and nonhuman primates. **A**, Summary of CDH6 expression by IHC using the polyclonal CDH6 antibody HPA007047 in human, NHP, rat, and mouse tissue sections. Tissues were graded from no staining (0) to low (1+), medium (2+), 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 qw 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 $60 \mu\text{m}$. **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 $100 \mu\text{m}$.

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

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 $1 \times 1 \times 1$ 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 $\rightarrow$ 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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Bialucha et al.

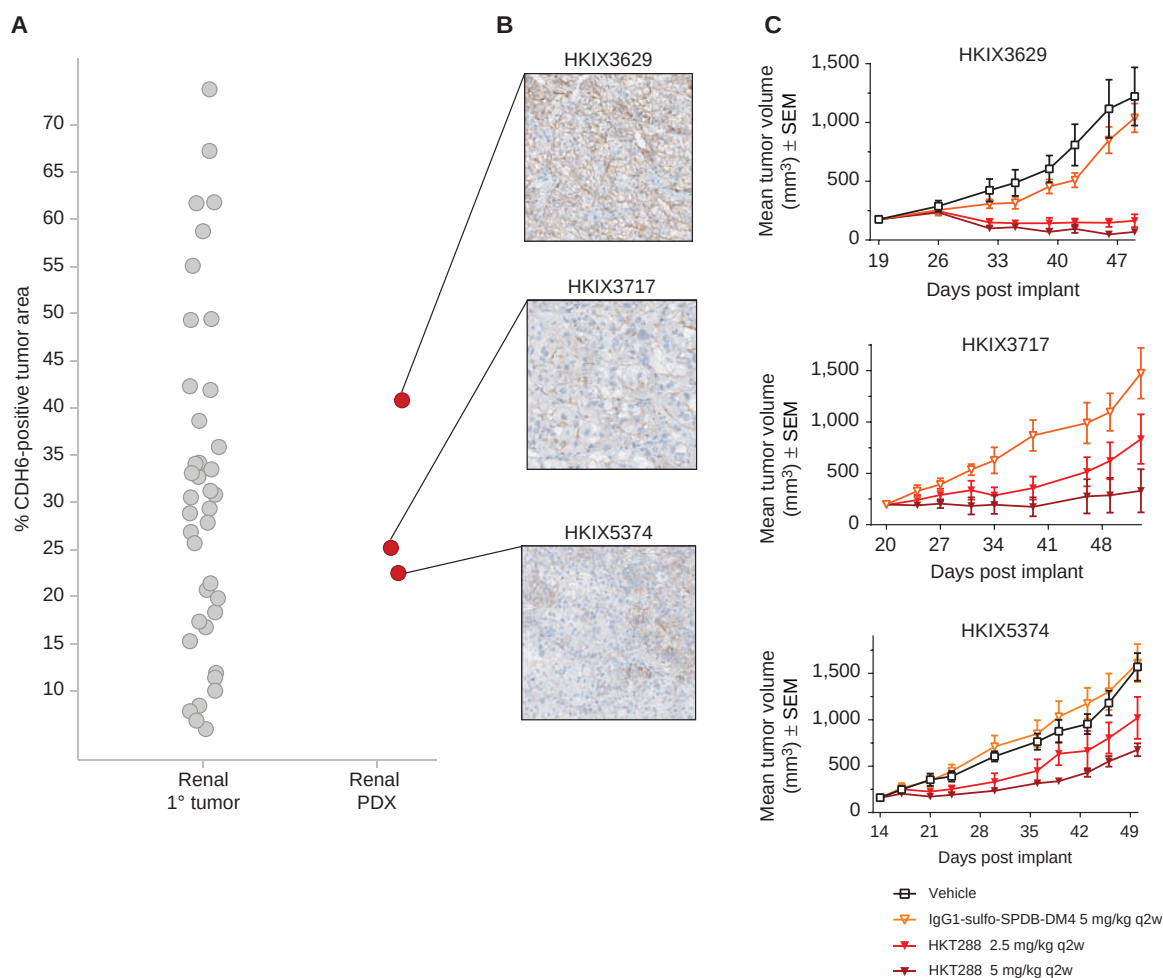


Figure 6. HKT288 elicits target-dependent antitumor efficacy in PDX models of renal clear cell carcinoma. **A**, Human renal clear cell carcinomas display a range of percent CDH6-positive tumor area as determined by quantitative IHC. Three examples of PDX models of renal clear cell carcinoma within this 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

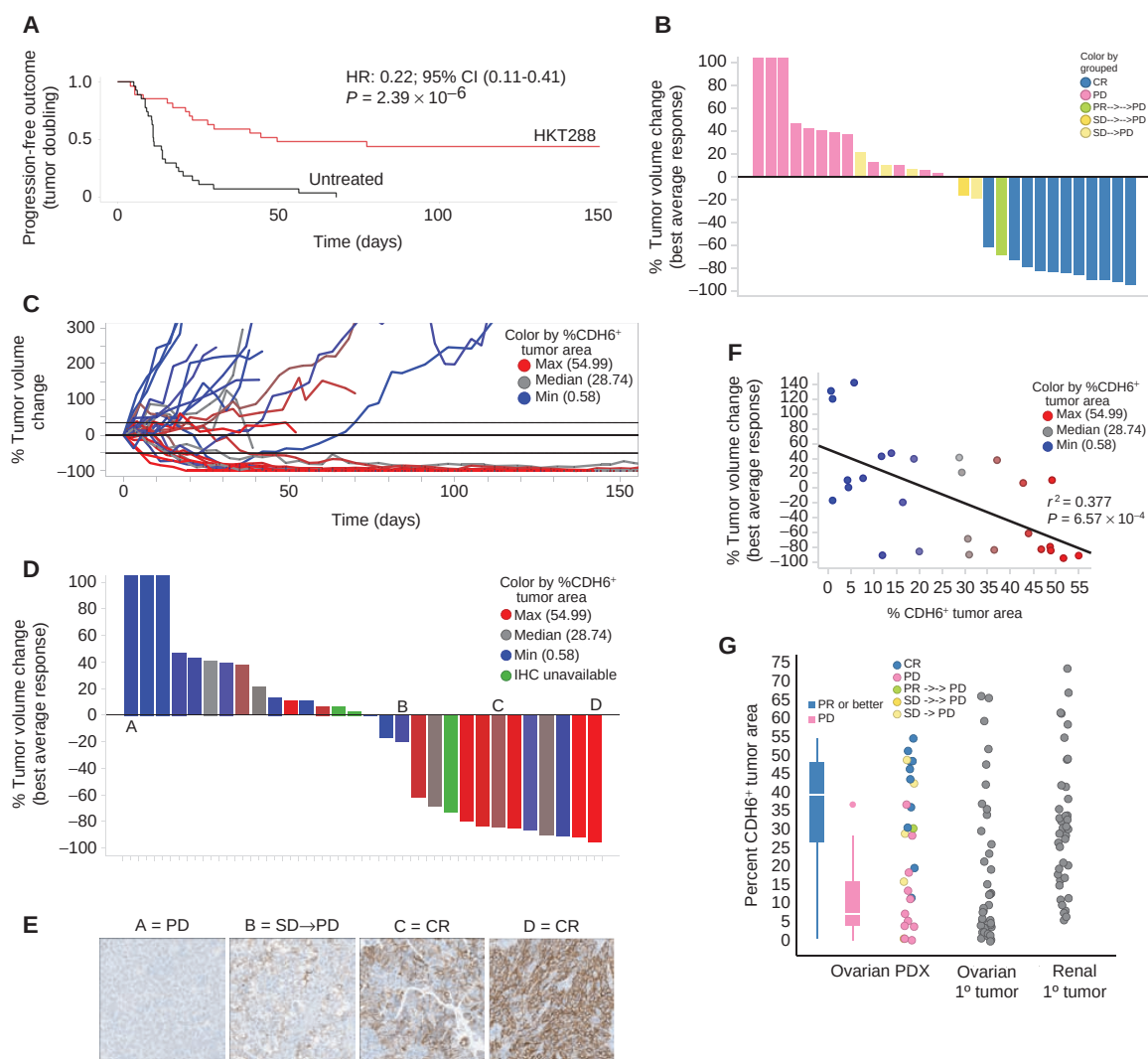


Figure 7. HKT288 induces target-dependent, robust, and durable antitumor response in over a third of an unselected ovarian PCT. **A**, Kaplan-Meier style plot comparing HKT288 to the untreated control arm. Progression-free outcome as determined by tumor volume doubling is plotted against time. **B**, 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 G₂-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

RESEARCH ARTICLE

Bialucha et al.

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 μ g/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 μ g/mL tetracycline for 20 to 24 hours. Jurkat E6-1 cells (ATCC #TIB-152, 2016), grown in RPMI-1640 +

10% FBS (Gibco), were transfected with an NFAT-luciferase reporter vector (Biomx 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% CO₂ 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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