



Associate editor: Beverly Teicher

Antibody drug conjugates for treatment of breast cancer: Novel targets and diverse approaches in ADC design

Pamela A. Trail^{a,*}, Gene M. Dubowchik^b, Timothy B. Lowinger^c

^a Regeneron Pharmaceuticals, Tarrytown, NY 10591, United States

^b Bristol-Myers Squibb, Wallingford, CT 06492, United States

^c Mersana Therapeutics, Cambridge, MA 02139, United States



ARTICLE INFO

Keywords:

Antibody drug conjugate
Antibody-directed delivery
Breast cancer
Cancer therapy
Immunoconjugate
Monoclonal antibody

ABSTRACT

Breast cancer is a heterogeneous group of malignancies with a spectrum of molecular subtypes, pathologies and outcomes that together comprise the most common non-cutaneous cancer in women. Currently, over 80% of breast cancer patients are diagnosed at relatively early stages of disease where there are encouraging data on outcomes and long term survival. However, there is currently no curative option for those patients with metastatic disease and there is a substantial medical need to identify effective and safe treatment options for these patients. One approach to improve cancer therapy is by designing therapeutics directed against targets with differential levels of expression on malignant versus normal cells with the goal of improving tumor selectivity and reducing damage to normal tissues. Antibody drug conjugates (ADCs) are a rapidly evolving therapeutic class that exploits the target-selectivity of monoclonal antibodies (MAbs) to deliver cytotoxic drugs to antigen-expressing cells (Lambert & Morris, 2017; Senter, 2009; Thomas, Teicher, & Hassan, 2016; Trail, 2013). The regulatory approval of ADCs for both hematologic malignancies (brentuximab vedotin) (Younes et al., 2010) and solid tumors (ado-trastuzumab emtansine) (Amiri-Kordestani et al., 2014; Verma et al., 2012) clearly demonstrates the clinical potential of ADCs. This review will focus on targets under consideration for breast cancer directed ADCs and on the technology modifications being considered to improve ADC efficacy and safety.

1. Introduction

Antibody drug conjugates consist of a MAb chemically conjugated to a linker and a drug, that is generally cytotoxic (Fig. 1). This technology platform can be applied across therapeutic areas however the majority of ADCs are currently focused in oncology. Antibody-directed delivery has the potential to both improve the efficacy of therapy by delivering drug selectively to antigen expressing cells and to increase the safety of therapy by minimizing drug exposure to those cells that do not express the target antigen. Following tumor localization, ADCs bind to antigen expressing cells, and are internalized into endosomes/lysosomes where drug is released following cleavage of the linker or catabolism of the MAb (Lambert & Morris, 2017; Senter, 2009; Thomas, Teicher, & Hassan, 2016; Trail, 2013). The liberated drug enters the cytoplasm where it binds to its molecular target (e.g. tubulin, DNA, or topoisomerase 1) resulting in cell cycle arrest and apoptosis. The drug may also diffuse out of, or be released from, dying cells and, if membrane permeable, can enter neighboring cells (antigen positive or negative) and mediate antigen-independent bystander killing (Fig. 2).

2. ADC linkers

The ADCs that are the subject of this review fall into two broad categories based on their drug release mechanisms, generally termed cleavable or non-cleavable. Most of the ADCs currently being evaluated clinically employ cleavable linkers. These linkers are designed to be stable in systemic circulation and to release drug within antigen expressing cells via enzymatic cleavage, reduction by cytosolic thiols, or exposure to reduced pH, resulting in generation of a discrete biologically active payload. These ADCs typically release membrane-permeable drugs that can also cause bystander killing of neighboring antigen-negative cells. Brentuximab vedotin, one of the two clinically approved ADCs, incorporates a cleavable valine-citrulline (val-cit or vc) dipeptide linker (Dubowchik et al., 2002). A second set of ADCs utilize linkers termed “non-cleavable.” These rely on thorough catabolism of the ADC in the lysosome, resulting in the generation of payload attached to the linker and at least one amino acid residue, the one to which it was conjugated. For such an ADC to be active, this modified payload must retain significant cytotoxic activity and be able to exit the lysosome to cause cell death. Neither of these is a trivial assumption. Most small

* Corresponding author.

E-mail address: ptrail23@gmail.com (P.A. Trail).

<http://dx.doi.org/10.1016/j.pharmthera.2017.07.013>

Available online 27 July 2017

0163-7258/ © 2017 Elsevier Inc. All rights reserved.

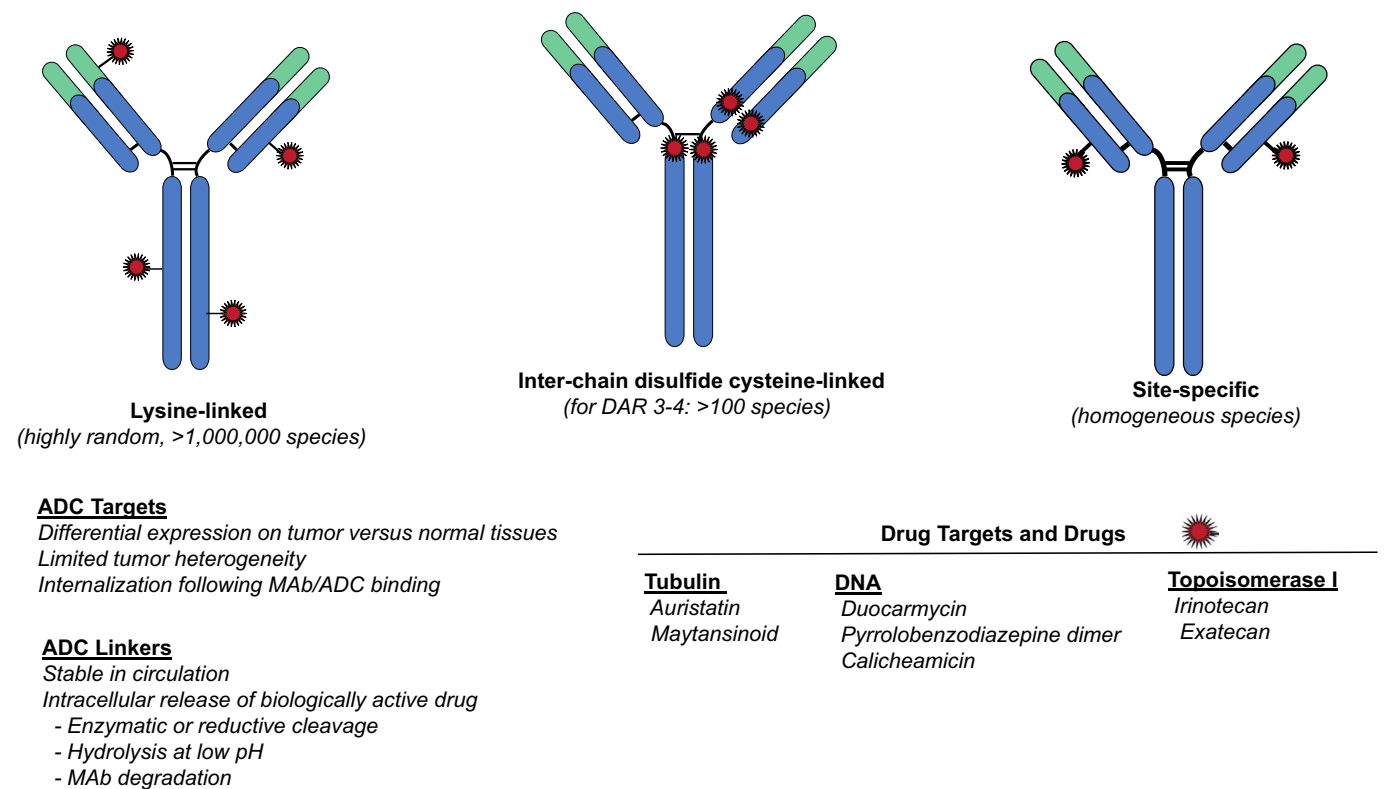


Fig. 1. Schematic of an antibody drug conjugate (ADC). An ADC consists of a MAb that binds a tumor-associated antigen. The antibody is chemically conjugated to a linker and drug, generally a highly potent cytotoxic compound. Conjugation to the MAb can be through endogenous lysine or cysteine residues or via one of a growing number of site-specific conjugation methodologies. Endogenous cysteine conjugation can result in a homogeneous conjugate if DAR of 8 is achieved.

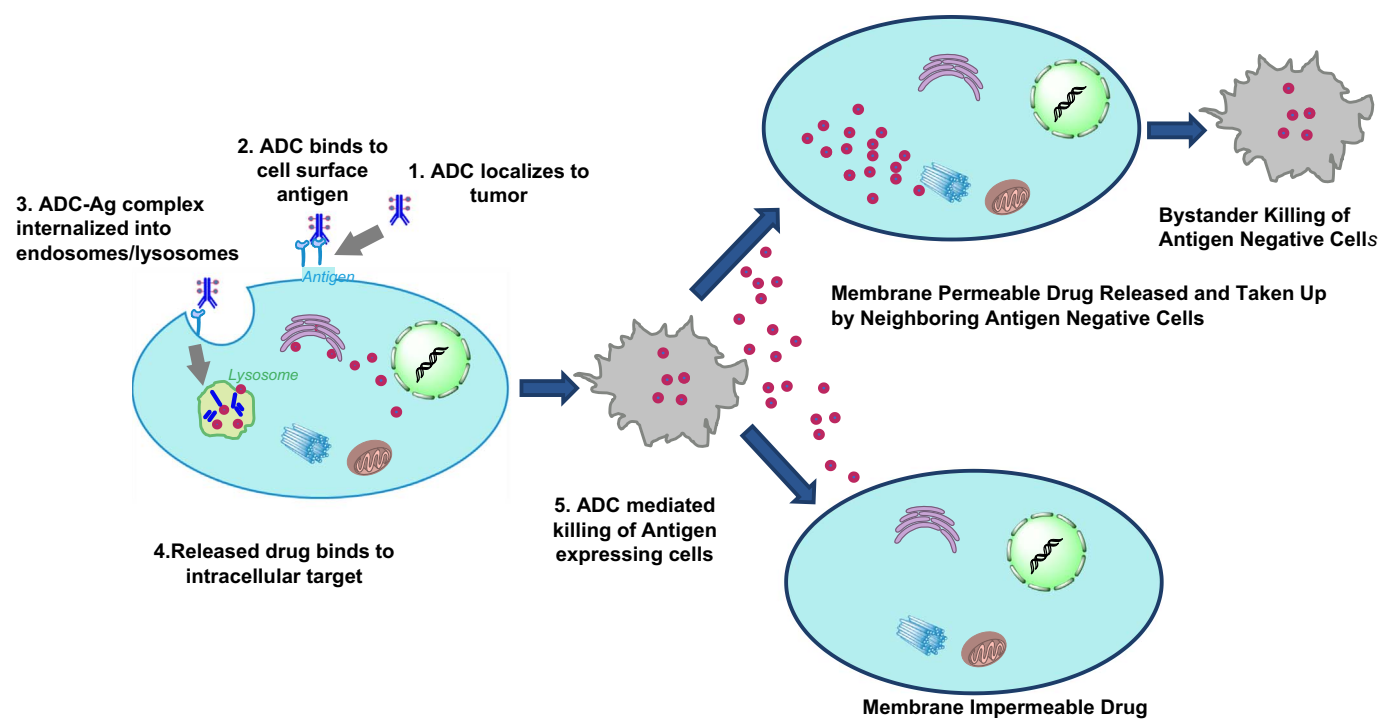


Fig. 2. Mechanism of ADC activity. Following tumor localization, the ADC binds to antigen expressing cells, is internalized into endosomes/lysosomes where drug is released following linker cleavage or catabolism of the MAb. The liberated drug enters the cytoplasm where it binds to its molecular target (typically tubulin, DNA, or Topoisomerase 1) resulting in cell death. The drug may also diffuse out of or be released from dying cells and if it is membrane permeable can enter cells (antigen positive or negative) in close proximity and mediate bystander killing. Ag = antigen.

molecule drugs have limited tolerance for this kind of drastic functionalization. In addition, both lysosomal permeability and a bystander effect that depends on passive diffusion through cell membranes is attenuated or abolished by having the polar residual amino acid attached to the drug (Kovtun & Goldmacher, 2007). Despite these challenges, it is notable that T-DM1, one of the two currently approved ADCs employs a non-cleavable linker (Lewis Phillips et al., 2008).

ADCs are generated through chemical conjugation of payload-linker compounds having reactive functionalities on the linker with complementary groups on the antibody. Drug payloads are typically much more hydrophobic than protein surface, and a drug-antibody ratio (DAR) > 4 often results in aggregation, poor pharmacokinetics, and increased toxicity (Adem et al., 2014; Hamblett et al., 2004). Two random conjugation methods have been used for most of the ADCs now in development (Fig. 1). The first results in attachment to lysine residues spread over the entire surface of a typical IgG (Chari, 2008). Because of the number of such residues, and the fact that only partial selectivity for a significant subset is usually achieved, this can result in generation of > 1 million discrete species in a given ADC batch. This is the conjugation method used in T-DM1. The second method involves selective opening of up to four relatively exposed inter-chain disulfides of the antibody (Lyon, Meyer, Setter, & Senter, 2012). This generates up to eight free thiol groups that are most often functionalized with maleimide-containing payload-linker compounds (giving ADCs shown in Figs. 5–10 and 12). This is less random than the lysine method, but can still result in > 100 species when a DAR of 3–4 is targeted. Emphasis in recent years has focused on truly site-specific conjugation methods that can yield homogeneous ADCs with superior properties (Schumacher, Hackenberger, Leonhardt, & Helma, 2016).

3. ADC payloads

The drug payloads currently used in ADCs target either tubulin or DNA. The FDA approved ADCs, Kadcyla® and Adcetris® both employ natural product-derived, microtubule-inhibitors of the maytansinoid (see Figs. 3 and 11) and auristatin (see Figs. 4, 6, 9 and 12) class, respectively. Tubulin inhibitors, which also includes the tubulysins (see

Fig. 5), have been the most extensively evaluated drug class used for ADCs (Klute et al., 2014). These compounds bind to tubulin, destabilize microtubules and cause mitotic arrest at sub-nanomolar concentrations. Tubulin inhibitors are most effective against highly proliferating cell populations, which describes most, but not all cancer cells. Like all current ADC payloads, these compounds are highly toxic to normal dividing cells and lack a therapeutic index when administered as “free” drugs.

Unlike anti-mitotics, DNA damaging agents can work at any stage of the cell cycle and thus may be more effective against solid tumors where significant populations of quiescent cells exist. Compounds such as doxorubicin were used in early ADCs (Trail et al., 1993) but were found to be insufficiently potent. Topoisomerase I inhibitors of the camptothecin class (see Figs. 7 and 10) target an enzyme critical for controlling dynamic DNA structure (Martino et al., 2017). Highly potent anthracyclines, such as PNU-159682 (Stefan et al., 2017) are now being considered as ADC payloads. Calicheamicin (see Fig. 13), a member of the enediyne class of natural products was used in the first approved ADC, Mylotarg® (Hamann et al., 2002). Calicheamicin binds in the DNA minor groove and, when activated, simultaneously breaks both DNA strands. The duocarmycins (see Fig. 8) are also minor groove binders (MacMillan & Boger, 2009). In this case, activation leads to irreversible N3 alkylation of adenine. Pyrrolobenzodiazepine dimers (PBDs) are another class of highly potent minor groove binders being used in ADC development. (Jeffrey et al., 2013). While the ability of DNA damaging agents to kill quiescent tumor cells, including tumor stem cells is attractive from the perspective of tumor eradication it brings with it the obvious risk to of killing normal cells that express ADC targets at low density. The balance of drug potency and target selectivity remains a key consideration in designing safe and effective ADCs.

4. ADC targets

In contrast to small molecules and function-blocking MABs, the targets for ADCs do not need to be causal in tumor progression. Rather, the target antigen needs to be differentially expressed on the surface of

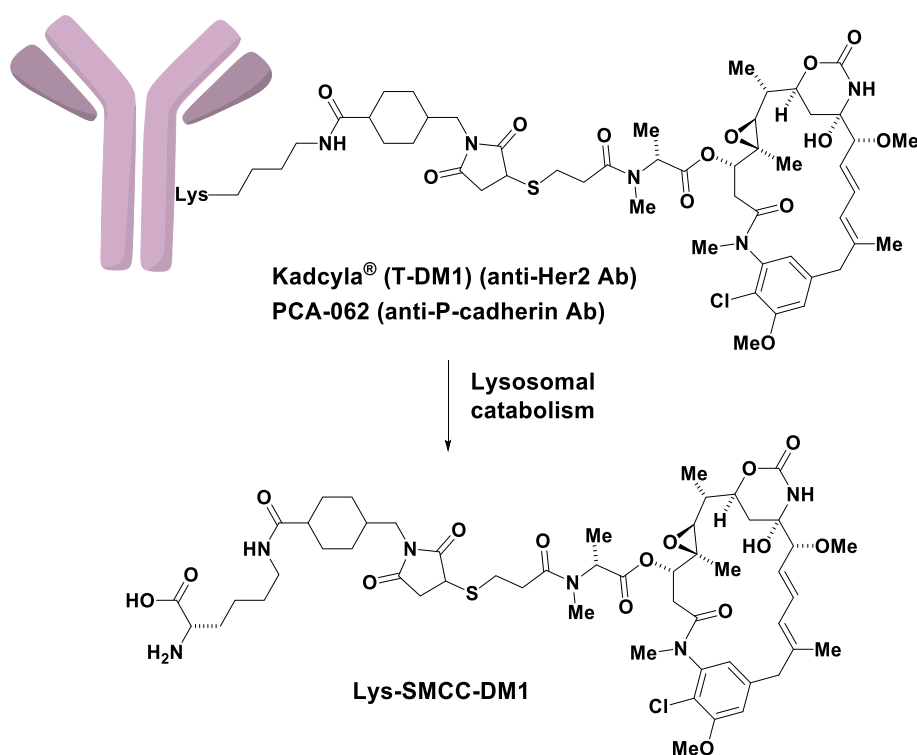


Fig. 3. Structures of Kadcyla® (T-DM1, Trastuzumab emtansine; DAR 3.5) and PCA-062, two ADCs that employ a non-cleavable linker, and the maytansinoid, DM1. The cytotoxic metabolite released within target cells shows no significant bystander effect.

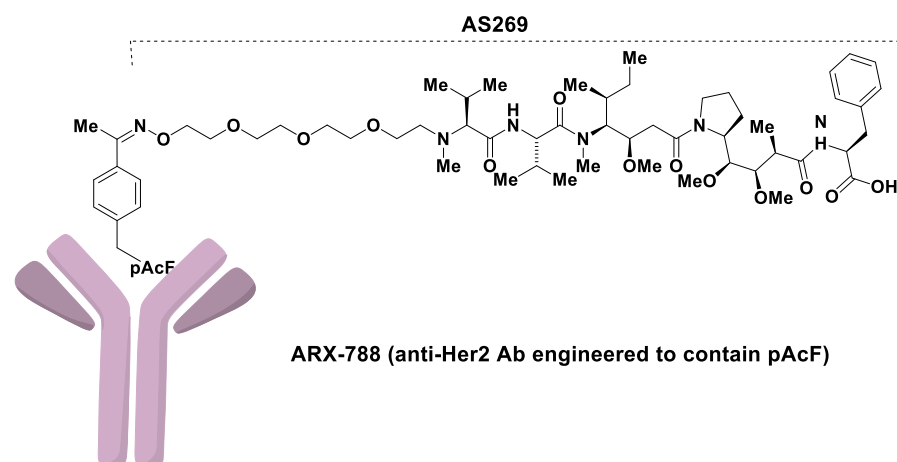


Fig. 4. Structure of ARX-788 (DAR 1.9), an ADC generated through site-specific conjugation of an anti-HER2 MAb that incorporates the non-natural amino acid, p-acetylphenylalanine (pAcF). ARX-788 employs a non-cleavable linker to the novel auristatin analog AS269.

the malignant cells relative to those of normal tissues, to be expressed on most tumor cells and typically to internalize rapidly following antibody binding. This increases the “target space” for ADC design to include not only tumor associated MABs such as trastuzumab with known clinical activity (Baselga, 2010; Verma et al., 2012), but also MABs such as SGN30 and LFA102 (anti-CD30 and anti-prolactin receptor, respectively) that are well tolerated but have shown minimal, if any, clinical activity (Agarwal et al., 2016; Younes, 2009) and targets identified from expression profiling of malignant versus normal tissues for which biologic function is not yet determined (Boonstra et al., 2016; Fauteux et al., 2016).

The choice of a suitable target antigen in terms of both selectivity and level of cell expression is critical to the selectivity and potency of an ADC as the intracellular concentration of drug that can be achieved is determined both by the level of antigen expression and the efficiency of ADC internalization and intracellular trafficking. Ideally, targets for ADCs would be cell surface antigens expressed homogeneously and at high density on malignant cells and not expressed on normal cells. The reality is that targets for ADCs are at best preferentially expressed on malignant cells, expressed at lower density on normal cells, and not expressed to any appreciable extent on cells of tissues that are vital and or lack renewal capacity. Current conjugation strategies rely on drug release following antigen specific internalization of the ADC complex and trafficking to endosomes and lysosomes. Consequently, ADC targets are selected based not only on expression analysis but the capacity to

internalize, ideally rapidly, following binding of the ADC. The cell surface antigens being evaluated as targets for breast cancer ADCs are in general tumor associated antigens preferentially expressed on tumor cells of several histologic types with limited normal tissue expression.

There are currently over 60 ADCs in various stages of clinical evaluation for hematologic malignancies or solid tumors, of which four are in late stages of clinical development for breast cancer and another 11 are being studied in solid tumor indications that include breast cancer specific cohorts. Of the 15 ADCs (Tables 1–3) currently being evaluated clinically in various breast cancer indications, 7 are directed against HER2 and several of these represent next generation approaches to this clinically validated ADC target.

5. Anti-HER2 ADCs

HER2 (erbB2, ERBB2) is a member of the human epidermal growth factor family of tyrosine kinase receptors which consists of four membrane bound receptor tyrosine kinases (RTKs); EGFR, HER2, HER3 and HER4 known to be involved in development and cancer progression. These RTKs contain an extracellular ligand binding domain, a trans-membrane domain and an intracellular tyrosine kinase domain making them therapeutically approachable by both small molecule tyrosine kinase inhibitors (TKIs) and MABs (Mendelsohn & Baselga, 2000; Yarden, 2001). HER2 is differentially expressed on tumor versus normal cells, is amplified and overexpressed in 20–25% of human breast

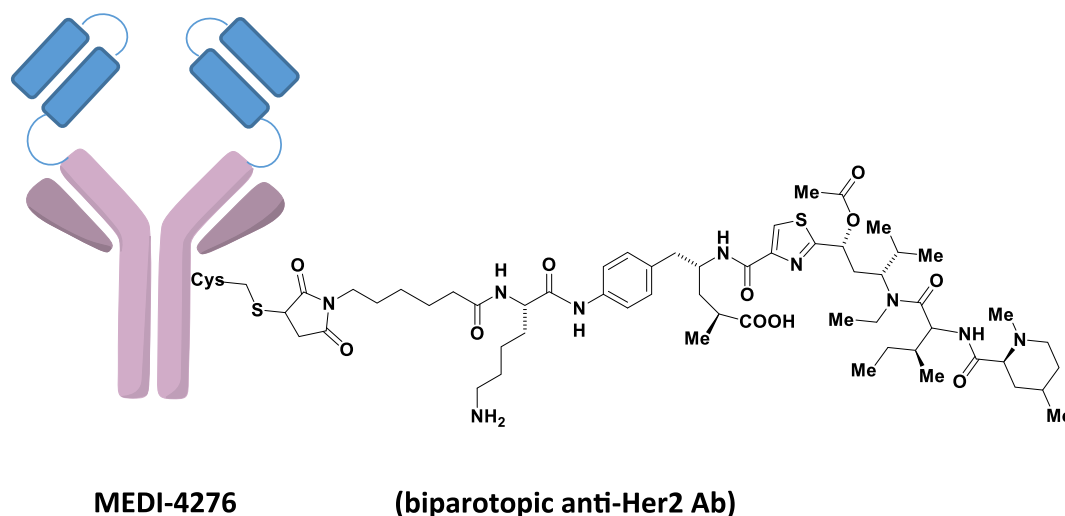


Fig. 5. Structure of MEDI-4276 (DAR 3.6), an ADC that consists of a biparotopic anti-Her2 MAB conjugated via engineered cysteine residues to a lysosomally-cleavable linker. MEDI-4276 delivers a novel tubulysin analog.

Table 1
Anti-HER2 antibody drug conjugates in clinical development for breast cancer indications.

Name	Anti-HER2 MAb	Isotype	Linker-drug	Drug class/target	Tumor indications ^a	Latest development stage ^a	Sponsor/Trial ID ^a
Kadcyla® T-DM1	Trastuzumab	Hu IgG1	SMCC-DM1	Maytansinoid/tubulin	HER2 + metastatic breast cancer prior to trastuzumab & taxane	Approved	Genentech/Roche
MEDI-4276	MAB 39S with Trastuzumab scFv at N terminus	39S Hu IgG1	Non-cleavable SC-Lys-AZ13599185	Tubulysin/tubulin	HER2 + advanced breast or gastric/stomach cancers	Phase 1/2	MedImmune/AZ NCT02576548
XMT-1522	XMT-1519	Hu IgG1	Cleavable Fleximer® polymer ester-aurostatin F-HPA	Auristatin/tubulin	HER2 + (≥ IHC 1 +) breast, gastric and lung cancers	Phase 1	Mersana NCT02952729
ARX788	Anti-HER2 MAb incorporating non-natural amino acids for site-specific conjugation	Undisclosed IgG1	Cleavable PEG4-AS-269 Non-cleavable	Auristatin/tubulin	Part 1: HER2 + breast or gastric cancers Part 2a: HER2 high (ISH + /IHC3 +) breast Part 2b: HER2 medium/low (ISH – / IHC2 +) breast	Phase 1/1b	Ambrx/Zhejiang Medicine Co NCT02512237
DS-8201a	Trastuzumab	Hu IgG1	Gly-Gly-Phe-Gly-Dxd Cleavable	Exatecan/topoisomerase I	Part 1: advanced solid tumors Part 2: breast cancer: 2a: HER2 overexpressed, prior T-DM1 2c: HER2 low	Phase 1	Daiichi Sankyo NCT02564900
SYD985	Trastuzumab	Hu IgG1	Val-Cit-PABC-CM-seco-DUBA Cleavable	Duocarmycin/DNA	Part 1: Advanced solid tumors of any histology Part 2: Breast, gastric, urothelial and endometrial tumors	Phase 1	Synthon NCT02277717
ADCT-502	Trastuzumab	Hu IgG1	Val-Ala-PABC Cleavable	PBD dimer/DNA	HER2 + breast, NSCLC, gastroesophageal, bladder cancer	Phase 1	ADC Therap. NCT03125200

Hu: humanized; Hu: fully human; PBD: pyrrolbenzodiazepine, NSCLC: non-small cell lung cancer.

^a Source: ClinicalTrials.gov; May 2017.

Table 2
Antibody drug conjugates in late stage clinical development for triple negative breast cancer.

Name	ADC target	MAb/ Isotype	Linker-drug	Drug class/Target	Trial details ^a	Latest development stage ^a	Sponsor/Trial ID ^a
Sacituzumab govitecan IMMU-132	Trophoblast cell surface antigen 2 (TROP2)	hRS7 Hz IgG1	carbonate-SN-38 Cleavable	Irinotecan/ topoisomerase I	Metastatic TNBC refractory or relapsing. Patients randomized to IMMU-132 or physician's choice chemotherapy TROP2 expression not required	Phase 3	Immunomedics NCT02574455
Glembatumumab vedotin CDX-011 CR011-vc-MMAE	Glycoprotein non-metastatic b (GPNMB)	CR011 Hu IgG2	Val-Cit-PABC-MMAE Cleavable	Auristatin/tubulin	Metastatic TNBC Expression of GPNMB required	Phase 2	Celldex Therapeutics NCT01997333
SAR566658 anti-CA6-DM4	CA6 Sialoglycope of MUC-1	DS6 Hu IgG1	Sterically-stabilized disulfide-DM4 Cleavable	Maytansinoid/ tubulin	Measurable metastatic TNBC 1–3 prior chemotherapy regimens CA6 expression required	Phase 2	Sanofi NCT02984683

Hu: humanized; Hu: fully human; MMAE: monomethyl auristatin E.

^a Source: [ClinicalTrials.gov](https://clinicaltrials.gov); May 2017.

cancers (Slamon et al., 2011) and is internalized following binding of anti-HER2 MABs.

5.1. T-DM1

Trastuzumab (Herceptin®), a humanized MAb directed against the extracellular domain of HER2 has shown impressive activity in patients with HER2 amplified breast cancer and was approved for this population. Trastuzumab in combination with chemotherapy has also been shown to improve survival in the first line setting in patients with advanced disease. However, a significant proportion of patients do not respond to trastuzumab and those patients that initially respond frequently develop progression of their disease (Baselga, 2010).

Ado-trastuzumab emtansine (T-DM1, Kadcyla®) is to date, the only ADC that has received regulatory approval for treatment of solid tumors, in this case HER2 positive metastatic breast cancer in patients previously treated with the MAb trastuzumab and a taxane (Table 1). T-DM1 consists of trastuzumab (humanized IgG1) conjugated to DM1, a microtubule inhibitor, via a non-cleavable thioether (SMCC) linker (Table 1 and Fig. 3). T-DM1 has an average DAR of 3.5 (Burris, Tibbitts, Holden, Sliwkowski, & Lewis Phillips, 2011; Lewis Phillips et al., 2008). T-DM1 was selected over four other ADCs prepared with cleavable disulfide-linked derivatives of DM1 that differed in the degree of steric hindrance around the disulfide. In these studies, T-DM1 demonstrated potent antigen-specific activity in vitro and was more active, better tolerated and had a superior pharmacokinetic profile in human tumor xenograft models (Lewis Phillips et al., 2008). The active catabolite of

T-DM1 (Lys-SMCC-DM1, Fig. 3) demonstrates potent activity when released intracellularly following antigen-specific internalization and lysosomal degradation of the MAb. However, Lys-SMCC-DM1 has poor membrane permeability and as such produces minimal bystander killing (Fig. 2).

In the randomized Phase 3 trial (EMILIA), T-DM1 was administered at a dose of 3.6 mg/kg every 3 weeks, and compared to lapatinib plus capecitabine in patients with advanced HER2 positive breast cancer that were previously treated with trastuzumab and a taxane. Treatment with T-DM1 resulted in a significantly higher objective response rate (43.6% for T-DM1 versus 30.8% for lapatinib-capecitabine; $p < 0.001$), and increased progression free survival (9.6 months for T-DM1 versus 6.4 months for lapatinib-capecitabine; $p < 0.001$), and overall survival (30.9 months for T-DM1 versus 25.1 months for lapatinib-capecitabine; $p < 0.001$) in this patient population. Although adverse events of any grade were similar for both treatment arms (95.9% and 97.7% for T-DM1 and lapatinib-capecitabine, respectively), the incidence of grade 3 or 4 events was lower for T-DM1 (40.8%) than was observed for lapatinib-capecitabine (57%). It is important to note that these patients had previously progressed when treated with trastuzumab indicating that the mechanism of trastuzumab resistance could be overcome when trastuzumab was used as the vehicle to deliver the cytotoxic drug DM1. Despite the selection of patients with HER2-overexpressing tumors (by FISH or IHC staining), over 50% of patients with metastatic breast cancer treated in the EMILIA trial did not respond (Verma et al., 2012) and as such there remains significant need for additional therapeutics for breast cancer that address the HER2

Table 3
Antibody drug conjugates in early stage clinical development for breast cancer indications.

Name	ADC Target	MAB/ Isotype	Linker-Drug	Drug class/Target	Tumor indications ^a	Latest development stage ^a	Sponsor/Trial ID ^a
SGN-LIV1A	LIV1 SLC39A6	hLIV22 Hu IgG1	Val-Cit-PABC-MMAE Cleavable	Auristatin/tubulin	Locally advanced or metastatic breast cancer positive for LIV-1 expression	Phase 1	Seattle Genetics NCT01969643
PF-6647020	PTK7	h6M24 Hu IgG1	Val-Cit-PABC-auristatin0101 Cleavable	Auristatin/tubulin	Part 1: advanced solid tumors Part 2: includes TNBC with moderate to high PTK7 expression	Phase 1	Pfizer NCT02222922
SAR428926	LAMP-1	humAb-1 Hu IgG1	Disulfide-DM4 Cleavable	Maytansinoid/ tubulin	Advanced malignancies including HER2 negative breast Expansion in LAMP-1 positive TNBC	Phase 1	Sanofi NCT02575781
PCA062	P-Cadherin Cadherin3	IgG1	SMCC-DM1 Non-cleavable	Maytansinoid/ tubulin	Three cohorts including p-CAD positive TNBC	Phase 1	Novartis NCT02375958
PF-6647263	Ephrin-A4	huE22 Hu IgG1	Hydrazone calicheamicin Cleavable	Eneidyne/DNA	Part 1: advanced solid tumors Part 2: TNBC	Phase 1 active not recruiting	Pfizer NCT02078752

Hu: humanized; Hu: fully human; MMAE: monomethyl auristatin E; PTK7: Protein tyrosine kinase 7.

^a Source: [ClinicalTrials.gov](https://clinicaltrials.gov); May 2017.



Fig. 6. Structure of XMT-1522, an ADC that employs a unique anti-HER2 Ab, XMT-1518, and a Fleximer® polymer that allows high drug loading (DAR 12–15) of a novel auristatin.

positive patient population as well as those patients deemed HER2 low who are ineligible for T-DM1.

5.2. Next generation HER2 targeted ADCs

There are currently six HER2 based ADCs in early clinical development (Table 1) designed with the intention of improving the activity, maintaining or improving the safety of T-DM1 and in some cases expanding the target patient population to those patients whose tumors express lower levels of HER2. This includes three ADCs that like T-DM1 deliver microtubule inhibitors (MEDI-4276, XMT-1522, and ARX788) but vary the antibody, antibody valency, site of conjugation, linker, drug, and/or DAR. In addition, the use of cytotoxic drugs with non-overlapping mechanisms of action are being evaluated; DS-8201a delivers exatecan, a topoisomerase inhibitor (Nakada et al., 2016; Ogitani, Abe, et al., 2016; Ogitani, Aida, et al., 2016; Ogitani, Hagihara, et al., 2016; Wethington, Wright, & Herzog, 2008), SYD985 (Dokter et al., 2014; Elgersma et al., 2015; van der Lee et al., 2015) delivers duocarmycin a potent DNA alkylating agent (Ghosh, Sheldrake, Searcey, & Pors, 2009; Patil, Satam, & Lee, 2015) and ADCT-502 delivers a pyrrollobenzodiazepine (PBD) dimer that cross links in the minor groove of DNA (Zammarchi et al., 2016).

5.3. ARX788

The Ambryx ADC, **ARX788** (Fig. 4) employs site-specific conjugation, a non-cleavable linker and the payload-linker compound, Amberstatin (AS269), which consists of monomethyl auristatin F (MMAF) attached to a short polyethylene glycol (PEG) spacer terminated by an alkoxyamine (Humphreys et al., 2015). Conjugation to an anti-Her2 antibody via two defined sites using engineered non-natural amino acid p-acetylphenylalanine (pAF) residues, yields an ADC with a stable oxime linkage resulting from a selective reaction that occurs in the presence of all the other functionalities on the protein. The active payload released following lysosomal degradation of the ADC contains the pAF residue. Preclinical studies of ARX-788 demonstrated activity in ovarian, lung and gastric cancer xenograft models, as well as a trastuzumab-resistant breast cancer xenograft. The ADC is currently in Phase 1 (Table 1) and includes a dose escalation phase followed by expansion in two breast cancer cohorts in patients with medium/low HER2 expression (categorized as ISH negative, AND IHC 1⁺/IHC 2⁺) or patients with high HER2 expression (ISH positive or IHC 3⁺).

5.4. MEDI-4276

Another approach to HER2 as an ADC target has focused on modifications to both the targeting construct and the cytotoxic drug delivered (Li et al., 2016; Thompson et al., 2016). MAb 39S is a fully human IgG1 MAb that binds a HER2 epitope distinct from that of trastuzumab. Lin et al. designed a biparatopic ADC in which the scFv of trastuzumab was attached to the N-terminus of 39S. This biparatopic antibody contains four binding domains, with selectivity for the trastuzumab and 39S epitopes on each arm of the antibody. This biparatopic antibody was coupled with a novel antimetabolic payload, a tubulysin analogue (AZ13599185) functionalized on the tubuphenylalanine (Tup) portion with an amino group (Thompson et al., 2016). This provides a convenient attachment point for the linker, a succinimidylcaproyl-lysine (SC-Lys) group. Site-specific conjugation to four engineered cyteines in the Fc region of the anti-Her2 biparatopic antibody resulted in an ADC (Fig. 5) that showed superior mouse serum stability in comparison with one generated by conventional inter-chain disulfide conjugation (Li et al., 2016). The biparatopic ADC internalized more rapidly and to a greater extent than T-DM1 across a panel of cell lines that expressed different levels of HER2. The ADC also demonstrated potent HER2-specific activity in vitro. The potency of both MEDI-4276 and T-DM1 was related to the level of HER2 expressed by a given cell line, however,

the biparatopic ADC also demonstrated potent activity against cell lines that were 1 + and 2 + positive by HercepTest™, whereas T-DM1 did not. Similarly, the biparatopic ADC demonstrated HER2-specific anti-tumor activity against a panel of human tumor xenograft lines and patient-derived xenograft (PDX) models including those that were insensitive to T-DM1. A significant bystander effect was noted, suggesting discrete cleavage of the SC-Lys linker, possibly by cathepsins B or L, either from the ADC itself, or following lysosomal catabolism of the conjugate. In the preclinical models evaluated the biparatopic ADC in general showed activity that was superior to T-DM1. It is important to note that this biparatopic ADC incorporates multiple modifications relative to T-DM1: it binds both the trastuzumab and a second epitope of HER2, it delivers tubulysin, a distinct microtubule inhibitor, and it utilizes a cleavable linker that releases drug that is cell membrane permeable and able to mediate bystander killing. This ADC, termed MEDI4276 (Table 1) is currently in Phase I trials in patients with HER2 positive breast or gastric cancers.

5.5. XMT-1522

XMT-1522 (Fig. 6) utilizes a proprietary polyacetal-based polymer (Fleximer®) to significantly increase drug loading (DAR of 12–15) in comparison with commonly used random or site-specific conjugation methods (DAR of 2–4) (Yurkovetskiy et al., 2015). XMT-1522 incorporates a novel antibody, XMT-1519, selected specifically for use as an ADC. The XMT-1519 MAb binds to an epitope distinct from that of the HER2 MABs trastuzumab or pertuzumab.

The drug payload utilized in XMT-1522 is a novel auristatin which was designed to have unique pharmacological properties. Typically, payloads for ADCs are classified as either freely cell permeable and capable of bystander killing, such as MMAE, or non-cell permeable and not capable of bystander killing, such as MMAF (de Goeij & Lambert, 2016). In the case of XMT-1522, upon internalization the initial small molecule payload released upon cleavage of the ester bond is XMT-1267, which is freely cell permeable and thus is capable of diffusing through the tumor environment and effecting bystander killing. XMT-1267, however, is further metabolized within the tumor to the negatively charged auristatin XMT-1521, which has significantly diminished cell permeability and as a result accumulates in the tumor, with significant drug detectable in tumor tissue two weeks after a single dose of XMT-1522 (Yurkovetskiy et al., 2017).

XMT-1522 is an example of an ADC with a particularly high DAR, averaging 12–15 XMT-1267 payloads per antibody. The high DAR is achieved by conjugating the XMT-1267 payload to a highly hydrophilic, biodegradable polymer (Mikhail et al., 2005), resulting in a polymer-drug conjugate which is readily soluble in water. This polymer-drug conjugate is then conjugated to the antibody via a maleimide linkage to activated inter-chain cysteine residues, providing XMT-1522.

XMT-1522 has been shown to be active against a range of HER2 expressing tumor models, including tumors with as few as 22,000 copies of Her-2 per cell. In addition, non-clinical evaluation of tolerability in rats and non-human primates has demonstrated that the ADC is well tolerated, with no signs of the neutropenia commonly associated with auristatins that are cell permeable and mediate a bystander effect. XMT-1522, currently in Ph I development, is a HER2 targeted ADC with the potential for treatment of breast, gastric and lung cancer patients whose tumors express HER2 at the IHC 1 + or higher level (Table 1).

5.6. DS8201-a

A humanized anti-HER2 MAb synthesized from the published sequence of trastuzumab is used to deliver the topoisomerase I inhibitor, DXd, a camptothecin analogue. DXd was conjugated to the MAb through reduced inter-chain disulfides using a cleavable maleimide glycine-glycine-phenylalanine-glycine linker. DS8201-a (Fig. 7) is reported to have a DAR of 8 (Ogitani, Abe, et al., 2016; Ogitani, Aida,

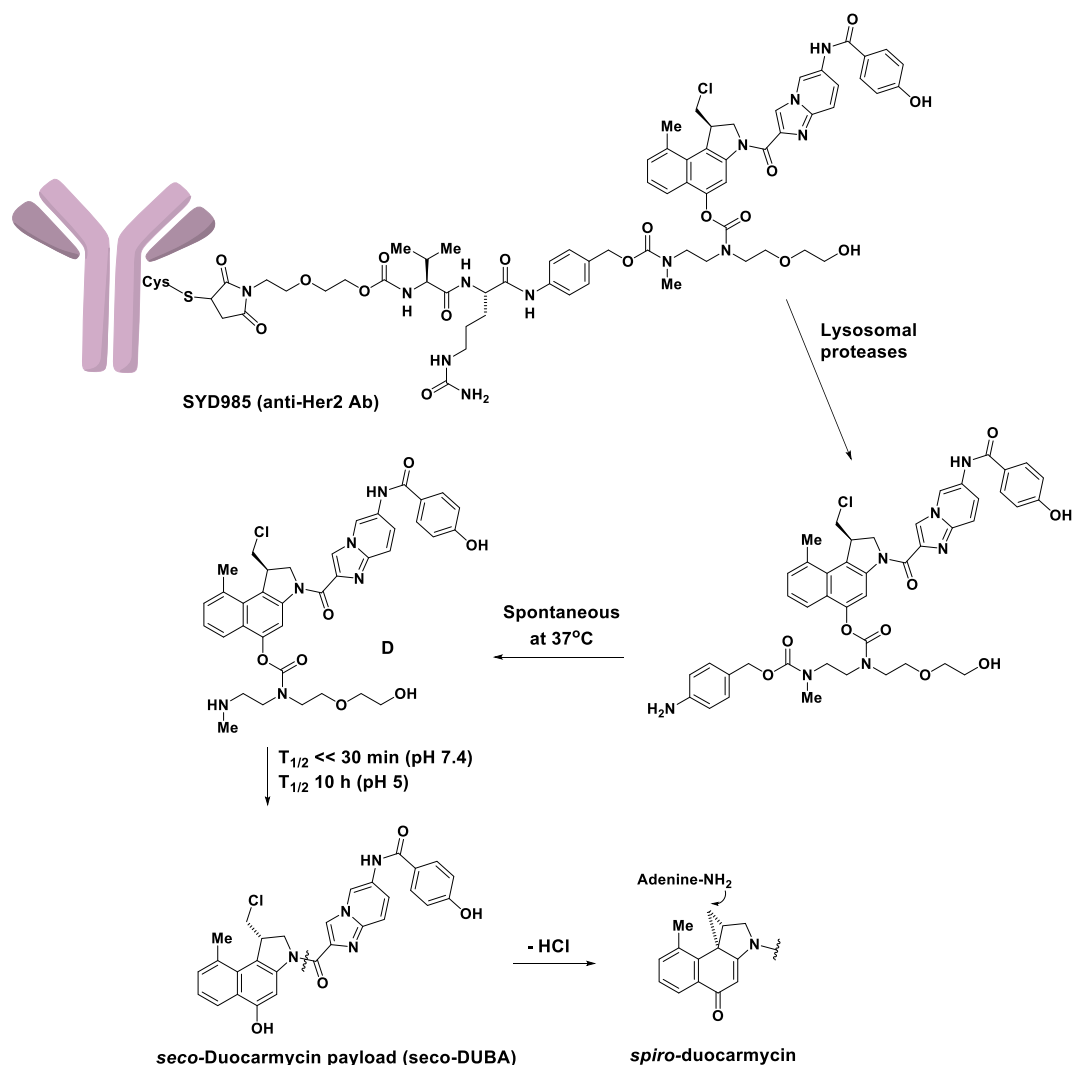


Fig. 8. Structure of SYD985 (DAR 2.8), an ADC employing trastuzumab and a lysosomally-cleavable linker to a DNA-damaging duocarmycin analogue, seco-DUBA. SYD985 is purified by hydrophobic interaction chromatography from SYD983 to remove higher DAR species. Also shown is the mechanism of activation of the payload.

is overexpressed on several tumor types including melanoma, breast, and small cell lung cancer and osteosarcoma with high levels relative to that seen on normal tissues (Maric, Rose, Annis, & Siegel, 2013; Maric et al., 2015; Roth et al., 2016; Turashvili et al., 2011). In breast cancer GPNMB expression has been shown to correlate with shorter progression free survival and reduced overall survival. Overexpression of GPNMB is seen on both triple negative breast cancer (TNBC) and basal type breast cancer and is associated with a poor prognosis (Rose et al.,

2010). In addition to overexpression on breast cancer cells, GPNMB has been reported to be highly expressed on the tumor stroma (Maric et al., 2013).

Glembatumumab vedotin (CDX-011, CR-11-vc-MMAE) referred to here as CDX-011 is an anti-GPNMB ADC which consists of an IgG2 MAbs conjugated to the microtubule inhibitor, monomethylauristatin E (MMAE) (Doronina et al., 2003) using a vc linker (Dubowchik et al., 2002) (Table 2 and Fig. 10). This is the same linker/drug combination

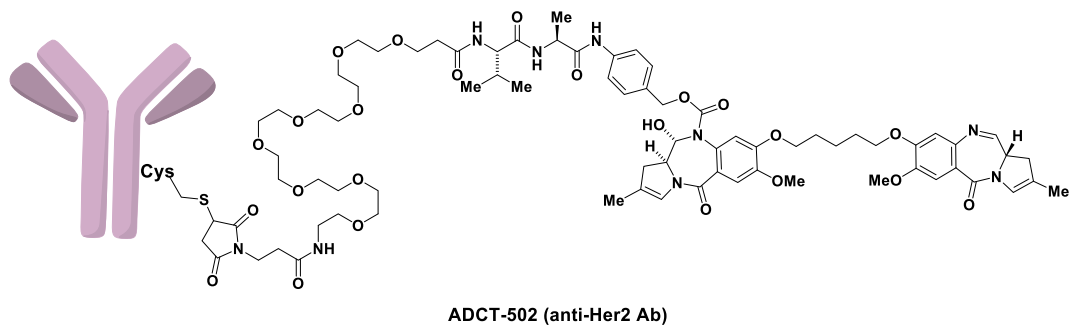


Fig. 9. Structure of ADCT-502 (DAR 2), an ADC consisting of an engineered version of trastuzumab conjugated to tesirine, a payload-linker compound comprising a DNA minor groove binding PBD dimer attached to a short PEG chain via a lysosomally-cleavable linker.

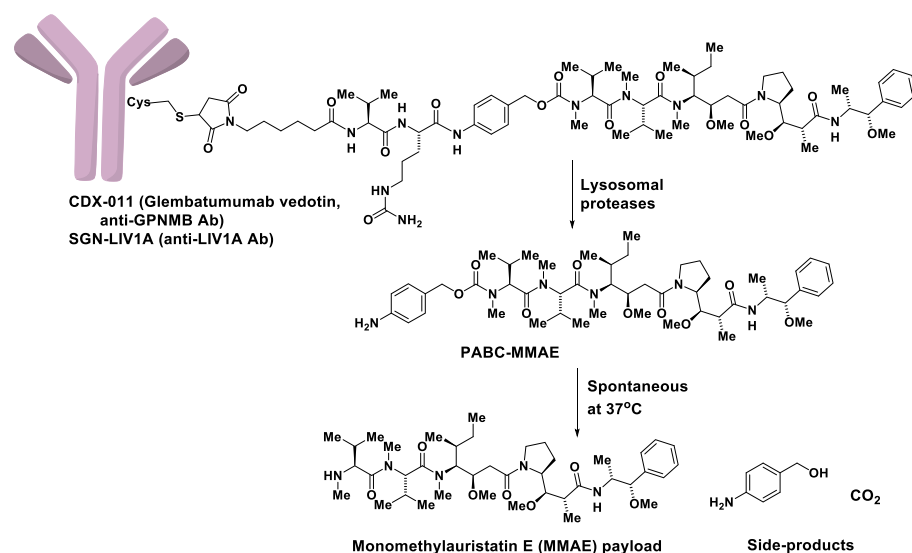


Fig. 10. Structure of CDX-011 (glembatumumab vedotin; DAR 4.5) and SGN-LIV1A (DAR 4), two ADCs employing lysosomally-cleavable linkers to the microtubule disrupting agent, MMAE.

that is used in Adcetris® the anti-CD30-vc-MMAE ADC approved for Hodgkin's lymphoma (Katz, Janik, & Younes, 2011; Younes et al., 2010).

Forty-two patients with locally advanced or metastatic breast cancer were treated with CDX-011 in a Phase 1/2 study, in which confirmed expression of GPNMB was not required. Exclusion of patients with baseline neuropathy > grade 1 defined a Phase 2 dose of 1.88 mg/kg administered every 3 weeks. The most common toxicities were fatigue, rash, nausea, peripheral neuropathy and neutropenia with treatment related rash potentially an “on target” toxicity as GPNMB is expressed in the skin. The ORR for all evaluable patients ($n = 34$) treated at the 1.88 mg/kg was 12% with a median PFS of 9.1 weeks and an ORR of 18% with a median PFS of 18 weeks was seen for patients with GPNMB expression demonstrated on archival samples. GPNMB expression was evaluated retrospectively and 84% of patients with available archival samples were found to express GPNMB, most frequently on tumor stroma. Interestingly, tumor responses were seen both in patients with GPNMB expressed on tumor cells and in patients in which GPNMB was detected only on the tumor stroma. These data suggest a role for bystander tumor cell killing following binding, internalization and drug release from the adjacent GPNMB expressing tumor stroma (Bendell et al., 2014).

A randomized phase II study (EMERGE, NCT01156753) of CDX-011 in heavily pre-treated patients with locally advanced or metastatic breast cancer, pre-selected for GPNMB expression, was initiated based on the results of the Phase I/II. Although GPNMB expression was required in EMERGE the threshold for expression was low ($\geq 5\%$ of

tumor or stromal cells). Patients were stratified into 3 groups based on GPNMB expression: Stratum 1: GPNMB expression on $\geq 5\%$ of tumor cells independent of stromal expression level, Stratum 2: GPNMB expression on $\geq 5\%$ of stromal cells with a 1 + − 2 + intensity score, Stratum 3: $\geq 5\%$ of stromal cells with 3 + intensity score and randomized 2:1 to receive CDX-011 (1.88 mg/kg) or investigators choice (IC) of chemotherapy. The ORR for all assigned patients did not differ for CDX-011 (6%) and IC (7%). There was no clear difference in ORR or PFS for patients that received CDX-011 in any of the three pre-specified strata. Retrospective analysis of patients in stratum 1 suggests an association of response rate to CDX-011 with higher levels of tumor expression of GPNMB. There were no responses in patients with TNBC treated with IC whereas CDX-011 demonstrated an ORR of 18%, with a suggestion of an increase in ORR in association with an increase in tumor GPNMB expression. However, the sample number was small making conclusions difficult (Yardley et al., 2015). To address this observation a second Phase II trial (NCT01997333) is ongoing in which patients with TNBC with GPNMB overexpressing tumors are randomized 2:1 to treatment with CDX-011 (1.88 mg/kg) or capecitabine.

6.2. IMMU-132

Trophoblast Cell Surface Antigen (TROP-2), also termed tumor associated calcium transducer (TACSTD2), epithelial glycoprotein-1 (EGP-1), gastrointestinal tumor-associated antigen (GA733-1) and surface marker 1 (M1S1) is a transmembrane glycoprotein that is over expressed on multiple tumor types including breast, gastric, lung,

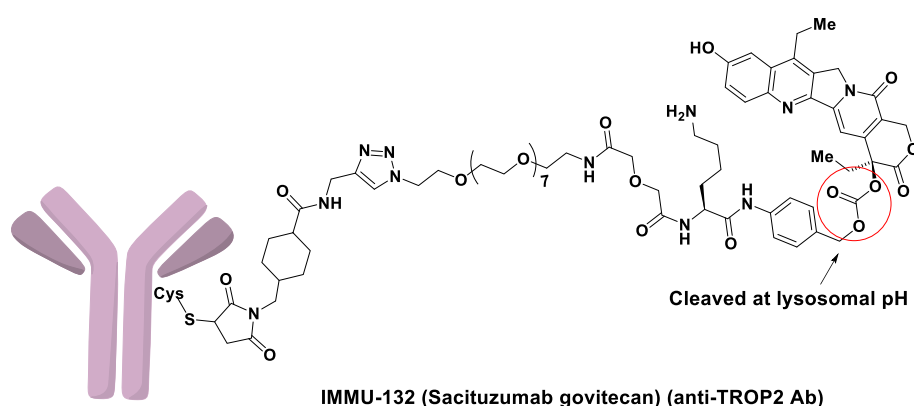


Fig. 11. Structure of IMMU-132 (Sacituzumab govitecan, DAR 7.6), an ADC that utilizes an acid-cleavable carbonate linkage (encircled) to the topoisomerase I inhibitor, SN-38.

colorectal, pancreatic, prostate, cervical and ovarian cancer relative to its expression on cells of normal tissues. Overexpression of TROP-2 is seen in more aggressive disease; it is associated with drug resistance and with a poor prognosis (Goldenberg, Cardillo, Govindan, Rossi, & Sharkey, 2015; Shvartsur & Bonavida, 2015; Zeng et al., 2016).

IMMU-132 (Sacituzumab govitecan, Fig. 11), is an anti-TROP2 ADC that incorporates a humanized IgG1 anti-TROP-2 MAb (hRS7) to deliver a topoisomerase inhibitor (Table 2). This ADC is different from the majority of current ADCs in several respects. Firstly, the payload, SN-38, is a topoisomerase I inhibitor with moderate potency (single-digit nM) in comparison with other drugs currently being used in ADCs (< 200 pM). Second, its release mechanism evolved from a lysosomally-cleavable dipeptide, Phe-Lys, but now relies on acid-mediated cleavage of the Lys-p-aminobenzylcarbonate highlighted in Fig. 11 (Cardillo, Govindan, Sharkey, Trisal, & Goldenberg, 2011). The linker contains a short PEG sequence and a lysine residue, making it fairly polar, and possibly explaining the high DAR level of 7.6 achieved which is nearly twice that typically seen for current ADCs. At a near lysosomal pH of 5.3 (37 °C), 50% of payload is released within 13 h. Interestingly, the rate of cleavage is reduced only two-fold in serum (pH of 7.4).

In preclinical models IMMU-132 showed antigen-specific activity against a panel of TROP-2 expressing cell lines of various tumor types in vitro and a panel of human tumor xenografts implanted in athymic mice. Immunohistochemistry (IHC) studies demonstrated that hRS7, the MAb used in IMMU-132 binds to TROP-2 expressed on normal tissues in humans and cynomolgus monkeys. In non-clinical toxicology studies in cynomolgus monkeys IMMU-132 was in general well tolerated and the major toxicities observed were similar to those of irinotecan (Cardillo et al., 2011; Goldenberg et al., 2015).

The results of a single arm study of IMMU-132 in heavily pre-treated advanced patients with metastatic TNBC were recently published (Bardia et al., 2017). Of the 69 patients treated, 41% developed grade > 3 adverse events and these were predominantly neutropenia. The ORR in this heavily treated TNBC population (n = 69) was 30% with 19 partial responses (PRs) and 2 complete responses (CRs). Tumor expression of TROP-2 was not pre-specified as an eligibility criterion, however 48 of the 69 patients treated had archival tumors available. TROP-2 expression on archival tumors was high, with 88% of tumor biopsies showing moderate (2⁺) to strong (3⁺) staining and importantly TROP-2 expression was in general seen on ≥ 50% of tumor cells. There was a trend toward improved activity with increased TROP-2 expression, however, the sample size was small. An open label randomized Phase 3 study in patients with metastatic TNBC refractory or relapsing after at least 2 prior chemotherapies (including a taxane) is ongoing (Table 2).

6.3. SAR566658

CA6 is a tumor-associated antigen and is a sialoglycotope of MUC1 thought to result from aberrant glycosylation (Boni et al., 2013). MUC1 is a transmembrane mucin family member with a very large extracellular domain characterized by tandem repeats with multiple O-linked sugars. MUC1 is typically expressed on apical mucosal surfaces. There are variations in the number of tandem repeats due to allelic and splice variations leading to complex differential glycosylation (Constantinou, Danysh, Dharmaraj, & Carson, 2011). The CA6 cancer-associated glycotype is expressed at low levels in most normal tissues, but overexpressed in many solid tumors including about 30% of breast cancer (Boni et al., 2013; Smith, Halliday, Finley, & Wennerberg, 2002). In IHC analysis of breast cancer specimens, CA6 was shown to localize predominantly to cell membranes or luminal surfaces with occasional cytoplasmic staining (Smith et al., 2002).

SAR566658 consists of an anti-CA6 antibody (huDS6) attached via a sterically-stabilized disulfide linkage to generate a relatively non-polar payload, S-methyl-DM4 (Fig. 12) (Widdison et al., 2006) that can mediate bystander killing. The two geminal methyl groups near the

disulfide in these linkers serve to sterically protect the linker from cleavage by the low levels of free thiols encountered in systemic circulation while allowing release in the presence of the much higher glutathione and cysteine levels within the cytosol or nucleus (Meister & Anderson, 1983).

In a Phase1 dose escalation study in advanced solid tumors, patients with tumors expressing CA6 in ≥ 30% tumor cells with an intensity 2⁺–3⁺ by IHC were selected (Boni et al., 2013; Gomez-Roca et al., 2016). SAR566658 was administered at doses ranging from 10 to 240 mg/square meter (mg/m²) Q3W and a recommended dose of 190 mg/m² Q3W was initially selected. However, a high frequency of keratopathy (36% grade 2/3 including 9 patients grade 3) led to modifications and two other dose schedules were tested: 90 mg/m² at days 1 and 8 of a Q3W schedule and 120 mg/m² Q2W. Keratopathy was reversible and could be prevented by prophylaxis with vasoconstrictor and steroid eyedrops in the modified dose schedules. Other frequent adverse events were fatigue, peripheral neuropathy, nausea, abdominal pain and diarrhea. There was one CR seen in an ovarian cancer patient, and eight PRs including three in breast cancer. A Phase 2 trial of SAR566658 is ongoing in patients with CA6 expressing TNBC (NCT02984683). This study consists of a first phase in which two different dose levels will be evaluated and a second phase with expansion at the selected dose (Table 2).

7. ADC targets in early stage clinical development that include breast cancer cohorts

There are several ADCs in Phase 1 trials that are directed to tumor associated antigens expressed on multiple tumor types, including breast cancer. Of these, five ADCs, are being evaluated in Phase 1 trials in which breast cancer specific cohorts are planned. These ADCs are discussed below.

7.1. LIV-1

LIV-1 (SLC39A6) is a multi-pass transmembrane protein involved in cellular zinc transport (Taylor, Morgan, Johnson, Hadley, & Nicholson, 2003; Taylor et al., 2007). LIV-1 was initially described as an estrogen regulated gene in breast cancer where its expression has been associated with tumor progression and lymph node metastasis (El-Tanani & Green, 1997; Taylor et al., 2003). In addition to breast cancer, LIV-1 is also highly expressed on several other tumor types including melanoma, prostate, pancreatic, ovarian and uterine cancers (Kasper et al., 2005; Ma et al., 2009; Sussman et al., 2014; Taylor et al., 2003; Taylor et al., 2007; Unno, Masamune, Hamada, & Shimosegawa, 2014; Unno et al., 2009). Expression of LIV-1 has been associated with downregulation of E-cadherin, and may have a role in promoting epithelial mesenchymal transition and increasing tumor metastasis (Grattan & Freake, 2012; Taylor, Morgan, Johnson, & Nicholson, 2005). LIV-1 is expressed to a limited extent on several normal tissues, most notably hormonally responsive tissues including breast, prostate, and testis (Sussman et al., 2014; Taylor et al., 2003).

The murine anti-LIV1 MAb mLIV22, that binds an epitope located in the extracellular N-terminus of LIV-1 was used to generate an IgG1 humanized anti-LIV-1 MAb termed hLIV22. An ADC directed to LIV1 (SGN-LIV1A) was produced by conjugating the hLIV22 MAb through endogenous cysteines to the auristatin analog MMAE using a cleavable vc linker (Fig. 10). SGN-LIV1A demonstrated potent in vitro killing of MCF7 breast cancer cells that express both the estrogen receptor (ER) and LIV1. Microtubule disruption was observed following treatment with SGN-LIV1A, consistent with the mechanism of action of auristatin. Antigen-specific antitumor activity was observed for both the MCF7 (ER⁺, LIV1⁺) a breast tumor xenograft line and BR0555 (ER⁺, LIV1⁺) a patient derived breast tumor xenograft (Sussman et al., 2014).

SGN-LIV1A, alone and in combination with trastuzumab, is currently in a Phase 1 trial in patients with LIV1 positive metastatic breast

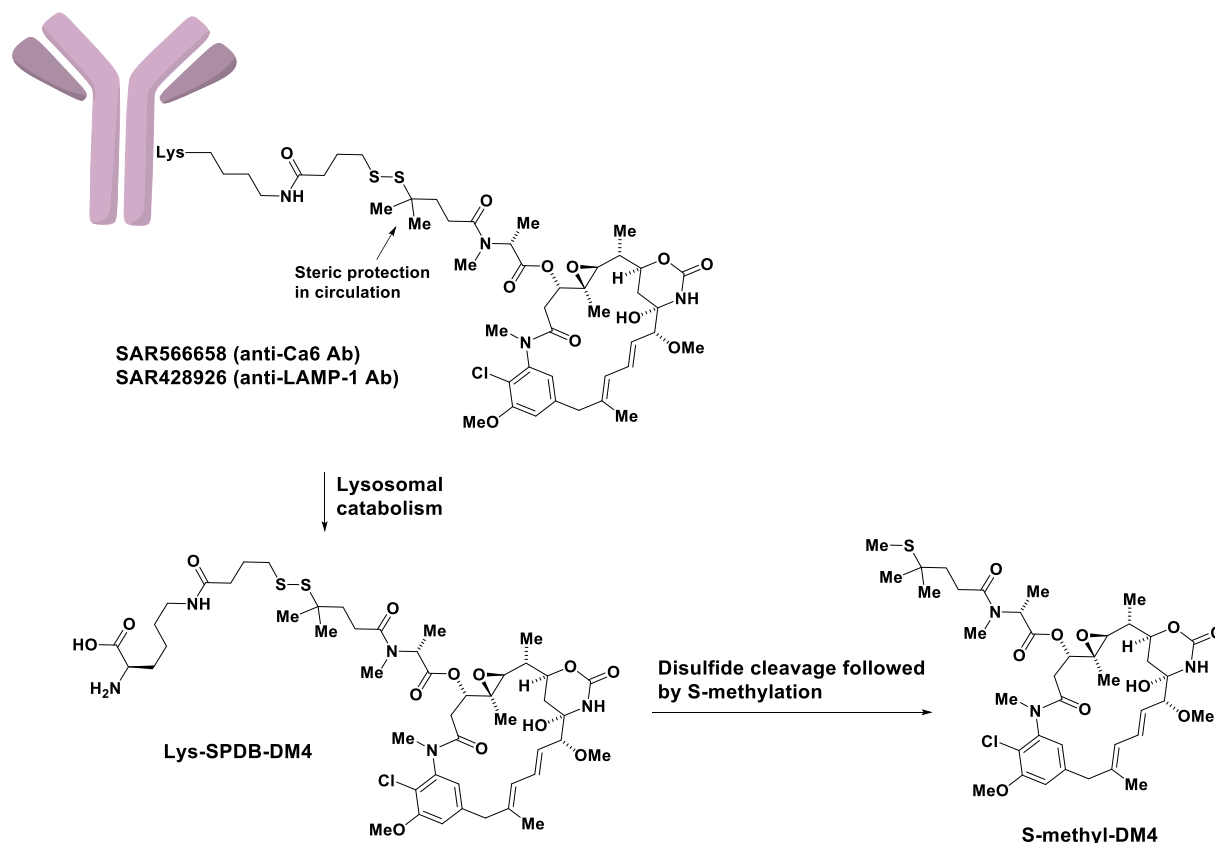


Fig. 12. Structure of SAR566658 and SAR428926, two ADCs that employ cleavable, sterically-stabilized disulfide linkers to the microtubule-disrupting agent, DM4, that is converted within cells to the S-methyl derivative.

cancer that have progressed on at least two prior regimens (Table 3).

7.2. Protein tyrosine kinase 7

Protein Tyrosine Kinase 7 (PTK7) also known as Colon Carcinoma Kinase 4 (CCK 4) is a highly-conserved RTK involved in the Wnt signaling pathway. PTK7 has been classified as a member of the pseudo-kinase family of RTKs and contains a catalytically inactive kinase domain. As PTK7 lacks a catalytically active kinase it is not a suitable target for small molecule kinase inhibitors. However, ADCs which require selective expression but not functional activity of a target provide a potential therapeutic approach to PTK7.

Gene expression profiling studies have demonstrated that PTK7 is overexpressed by a variety of tumor types including lung adenocarcinoma (Chen et al., 2014), colon cancer (Mossie et al., 1995), and breast cancer (Ataseven et al., 2013; Damelin et al., 2017; Speers et al., 2009). In breast cancer, PTK7 was more highly expressed in ER-negative relative to ER-positive tumors and siRNA knockdown of PTK7 resulted in substantial inhibition of growth of ER-negative human breast cancer lines but had minimal if any effect on ER-positive lines (Speers et al., 2009). Interestingly, studies using patient derived xenografts (PDXs) have demonstrated that PTK7 is over-expressed on tumor initiating cells (TICs) in PDXs derived from patients with TNBC, ovarian cancer and non-small cell lung cancer (NSCLC). PTK7, like most tumor associated antigens, while over expressed on tumors, is also expressed to some extent on cells of normal tissues including lung, GI and bladder (Human Protein Atlas). Evaluation of PDX tumors by IHC using anti-PTK7 MAbs revealed binding to both tumor cells and cells of the stromal compartment, where staining was consistent with the tumor vasculature suggesting the potential for both a direct anti-tumor and anti-angiogenic effects of PTK7 targeted therapeutics. Expression of PTK7 was

seen on plasmacytoid dendritic cells and to a lower extent on myeloid dendritic cells from human blood samples (Damelin et al., 2017). The increased expression of PTK7 on several tumor types and on TICs supports consideration of PTK7 as target for therapeutic intervention.

An anti-PTK7 ADC with a DAR of 4 was produced by conjugating h6 M24, a humanized (IgG1) Mab directed to PTK7, using a cleavable (vc-PABC) linker to the auristatin analog Aur0101. The binding of h6 M24 to human and cynomolgus monkey PTK7 was comparable and there was no binding to rodent PTK7. The anti-PTK7 ADC, referred to as h6 M24-vc-0101 or PF-06647020, is shown in Fig. 13. The novel auristatin analogue, Aur0101 was specifically designed to retain cellular potency and undergo more rapid oxidative metabolism in vivo than MMAE (Maderna et al., 2014). It was anticipated that enhanced in vivo clearance of this “soft payload” might reduce systemic toxicity and increase the therapeutic index of the ADC. PF-06647020 demonstrated potent antigen-specific cytotoxicity in vitro and PTK7 expressing cells showed evidence of mitotic arrest and microtubule disruption, effects consistent with the mechanism of action of this class of drugs. Studies in TNBC PDX models evaluated in NOD SCID mice demonstrated antigen-specific antitumor activity that was superior to that of the maximum tolerated dose of docetaxel. The nonclinical safety profile of PF-06647020, administered every 3 weeks at doses up to 5 mg/kg, was evaluated in cynomolgus monkeys. Target dependent toxicity was not observed in the tissues examined including those with known PTK7 expression. Myelosuppression was observed and considered to be target independent since it is typically observed with other ADCs that deliver microtubule inhibitors. The preclinical antitumor activity, acceptable non-clinical safety and pharmacokinetic profiles of PF-06647020 supported advancing this ADC into Phase I clinical studies initially in patients with advanced solid tumors not pre-selected for expression of PTK7 followed by expansion cohorts in TNBC, NSCLC and ovarian

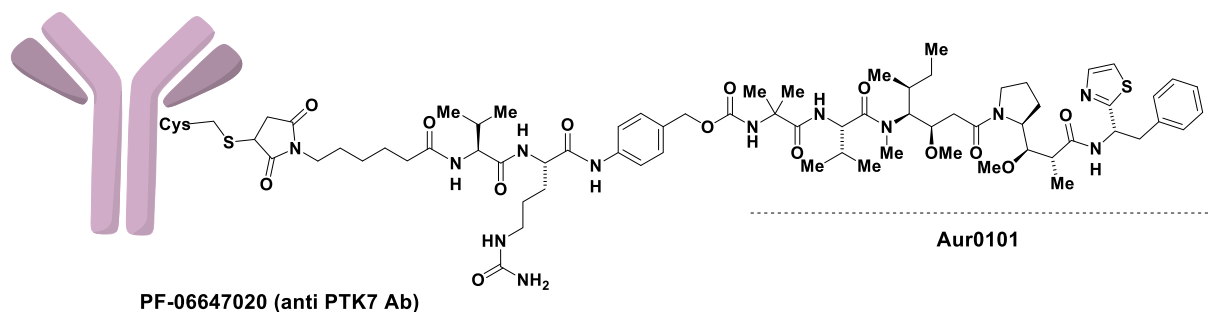


Fig. 13. Structure of PF-06647020 (DAR 4), an ADC that utilizes a lysosomally-cleavable linker to AUR0101, a novel auristatin analogue.

cancer (Table 3). Initial clinical data showed that of three patients with TNBC, two achieved a PR and one achieved stable disease at dose levels of 2.1 mg/kg and 2.8 mg/kg respectively, indicating evidence of activity at tolerated dose levels (Tolcher et al., 2015).

7.3. Lysosomal membrane associate protein 1

The lysosomal membrane associated proteins LAMP-1 (CD107a) and LAMP-2 are type I transmembrane proteins that together represent about 50% of all the proteins of the lysosomal membrane (Eskelinen, 2006). LAMP-1 is a highly-glycosylated protein involved in protecting the lysosomal membrane from hydrolytic enzymes and thereby maintaining its structural integrity (Eskelinen, 2006; Lu et al., 2016). In normal cells, LAMP-1 is expressed in late endosomes and lysosomes, however LAMP-1 has been shown to translocate to the cell surface of tumor cells, where the level of expression has been correlated with invasiveness and metastasis in colorectal, melanoma and laryngeal squamous cell carcinomas (Lu et al., 2016; Saitoh, Wang, Lotan, & Fukuda, 1992; Sarafian et al., 1998). Membrane expression of LAMP-1 has also been reported on cytotoxic T-lymphocytes and natural killer cells (Alter, Malenfant, & Altfield, 2004; Sarafian et al., 1998).

SAR428926 is an anti-LAMP1 ADC (Fig. 12) currently in Phase I trials with a dose escalation phase in advanced malignancies and a planned expansion cohort in LAMP-1 positive TNBC (Table 3). The humanized MAb used in this ADC, termed Ab-1, binds to the luminal domain of LAMP1. The MAb identified LAMP-1 expressed on breast, colorectal, gastric, prostate, lung and ovarian tumors but displayed limited binding to cells of normal tissues.

SAR428926 is conjugated to DM4 via the cleavable linker, *N*-succinimidyl-4-(2-pyridyldithio) butanoate (SPDB) (Fig. 12). Evaluation of SAR428926 in subcutaneous PDX models in mice demonstrated antigen-specific antitumor activity, including regressions of established breast, colon, lung, prostate, gastric, and ovarian tumors. Antitumor activity was reported to be correlated both with the level of LAMP-1 expression and the inherent sensitivity of the tumor models to DM4 (Baudat et al., 2016; Calvet et al., 2016).

7.4. P-cadherin

P-cadherin is a cell surface glycoprotein involved in Ca^{2+} -dependent cell to cell adhesion. P-cadherin is expressed during development and in adults P-cadherin is expressed on normal myoepithelial/basal cells. P-cadherin is expressed in normal breast where it has a role in maintaining breast epithelial architecture and function, in the basal layer of multiple adult tissues and in hair follicles (Li & Feng, 2011; Paredes et al., 2008; Turashvili et al., 2011; Vieira & Paredes, 2015). P-cadherin is frequently highly over-expressed on epithelial tumors. In breast cancer, overexpression of P-cadherin is strongly associated with basal subtypes, indicative of highly aggressive tumors and serves as a marker of poor prognosis (Turashvili et al., 2011).

PCA062 is an anti-P-cadherin ADC in which an IgG1 MAb is

conjugated to the maytansinoid DM1 using the same linker/drug employed in T-DM1 (Fig. 3). PCA062 was shown to rapidly internalize and localize to lysosomes following binding to P-cadherin expressing cells, resulting in antigen-specific cytotoxicity. The PCA062 ADC demonstrated activity in breast, head and neck and bladder carcinoma xenograft models (Menezes et al., 2015). PCA062 is currently being evaluated in a Phase I trial in P-cadherin expressing TNBC (Table 3).

7.5. Ephrin A4

Ephrin receptors are a family of RTKs, classified into two subgroups EphA and EphB based on the structure of their extracellular domains and ligand-binding affinity. Their membrane anchored ligands, the ephrins are also subdivided into two classes: ephrins A and ephrins B, each of which preferentially binds to EphA or EphB receptors, respectively. The Eph/ephrin signaling network is complex, with both forward signaling to the Eph receptor expressing cell and reverse signaling to the ephrin-ligand expressing cell. Eph receptors are over expressed on tumors and have been associated with progression and metastasis in several tumor types including breast, lung and pancreatic cancer (Giaginis et al., 2014; Liu, Huang, Wang, Kong, & Zhang, 2014). Profiling of a series of PDX models revealed elevated expression of EphA4 on TNBC tumors relative to that on normal adjacent breast tissue and other breast cancer subtypes. In particular, TICs were enriched for Ephrin A4 (EphA4) expression relative to both non-tumorigenic cells and cells of normal tissues (Damelin et al., 2015).

PF-06647263 (Fig. 14), is an anti-EphA4 ADC in which calicheamicin, a highly potent DNA-damaging agent, is conjugated to an IgG1 anti-EphA4 MAb through a two-stage linker system to MAb lysines as used in the first marketed ADC, Mylotarg® (Gemtuzumab ozogamicin) (Hamann et al., 2002). Following internalization, the critical first step of drug release relies on selective hydrolysis of the acylhydrazone in the mildly acidic conditions of the lysosome (Trail, King, & Dubowchik, 2003). Acylhydrazones had been used in other ADCs with doxorubicin as the payload, showing good systemic stability (pH 7.4) with reasonably rapid release in lysosomes (pH 4.8), although specific structural characteristics are very important in the practical use of these linkers (Trail et al., 1993). The acylhydrazide intermediate A must either diffuse or be transported out of the lysosome where, upon disulfide cleavage, intermediate B is set up to undergo the so-called Bergman cyclization of the highly-strained enediyne (Fig. 14). The resulting diradical intermediate C, if bound in the DNA minor groove causes double strand DNA breaks by hydrogen abstraction from the deoxyribose backbone (Walker, Landovitz, Ding, Ellestad, & Kahne, 1992). PF-06647263 with an average DAR of 4.6 was evaluated in breast PDX models and demonstrated dose dependent, antigen-specific antitumor activity in TNBC PDX models, including long term tumor regressions. PF-06647263 was also evaluated in nonclinical safety studies in cynomolgus monkeys and the primary organ toxicities were attributed to the off-target effects of calicheamicin as they were similar to those seen previously for this drug class. Based on the preclinical efficacy and

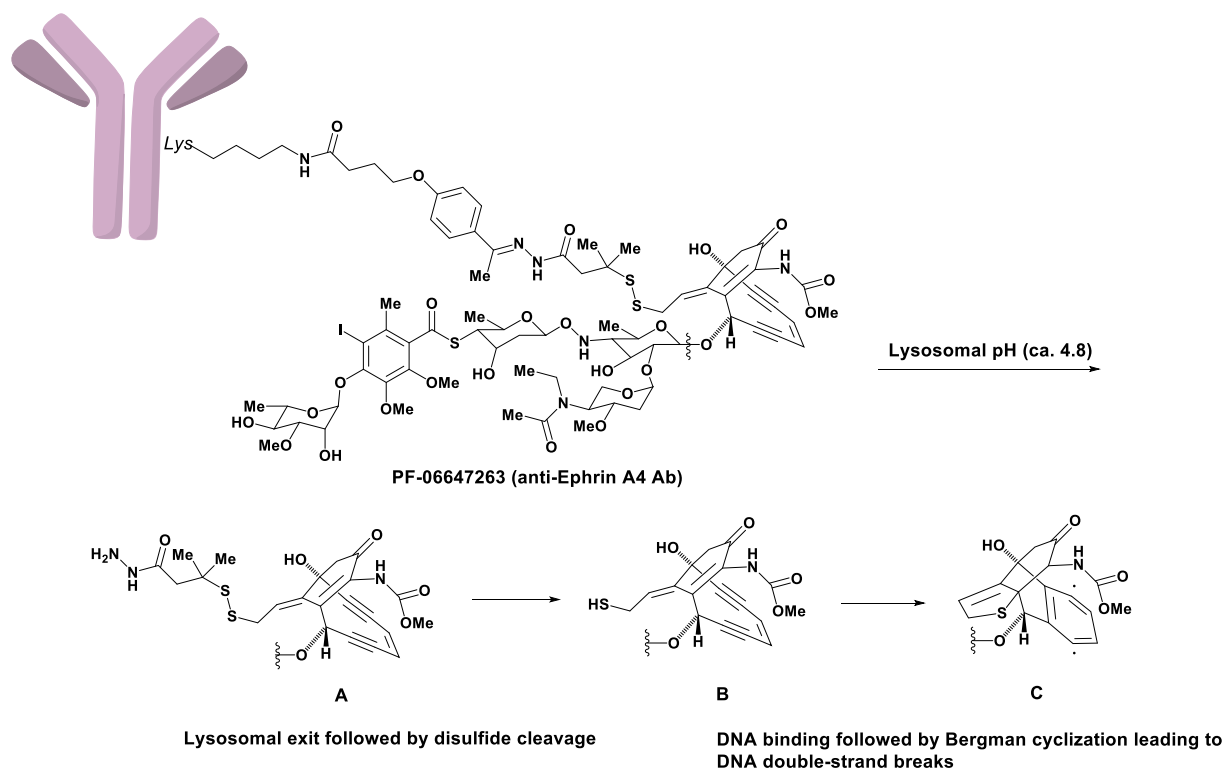


Fig. 14. Structure of PF-06647263 (DAR 4.6), an ADC utilizing an acid-cleavable acylhydrazone linker to the DNA-damaging agent, calicheamicin. Also shown is the mechanism of activation of the payload.

safety profile, PF-06647263 was progressed into a Phase I trial (Table 3) in patients with advanced solid tumors and an expansion cohort in TNBC.

8. Concluding remarks

The regulatory approval of both Adcetris® and Kadcyla® clearly demonstrate the potential of antibody-directed delivery in both hematologic malignancies and solid tumors (Verma et al., 2012; Younes et al., 2010). As the field continues to evolve, the selection of suitable ADC targets remains a critical challenge and it is likely that the target and its biology (e.g. selectivity, heterogeneity, level of expression and internalization rate) will drive many choices in ADC design. In addition, there are a variety of opportunities to further optimize ADCs using engineered antibodies and innovative linkers, conjugation methods and drug payloads. The next generation of clinical-stage ADCs includes the use of biparatopic MAbs, site-selective conjugation, linkers that improve target cell selectivity and the number of molecules of drug delivered, as well as cytotoxic payloads that impart distinct mechanisms of action and potencies of cell killing. There are > 60 ADCs currently in the clinic, with 7 of them interrogating the same target, HER2, using different optimization approaches to ADC design. The clinical data emerging from these next-generation ADCs will provide important insights into the mechanistic basis of ADC design, and the opportunity to better understand the impact of changes in ADC properties on therapeutic activity and safety. These important efforts will inform expansion of clinical use of ADCs in combination with other treatment modalities including cytotoxic drugs (e.g. NCT01874054, NCT02658084), targeted small molecule inhibitors (e.g. NCT02164006, NCT02236000), and immunomodulatory antibodies (e.g. NCT02572167, NCT02581631).

Abbreviations

ADC	antibody drug conjugate
CR	complete response
DAR	drug/antibody ratio
ER	estrogen receptor
FISH	fluorescence in situ hybridization
GGFG	glycine-glycine-phenylalanine-glycine
GPNMB	glycoprotein non-metastatic B
HER2	human epidermal growth factor receptor 2
IgG	immunoglobulin G
IHC	Immunohistochemistry
ISH	in situ hybridization
LAMP	lysosomal associated membrane protein
MAb	monoclonal antibody
mg/m ²	milligram/square meter
MMAE	monomethyl auristatin E
MMAF	monomethyl auristatin F
MUC1	mucin-1
ORR	objective response rate
PABC	p-aminobenzyloxycarbonyl
pAF	p-acetylphenylalanine
PBD	pyrrolobenzodiazepine dimer
PDX	patient derived xenograft
PFS	progression-free survival
PR	partial response
PTK7	protein tyrosine kinase 7
RTK	receptor tyrosine kinase
SC	succinimidylcaproyl
SMCC	succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate
SPDB	N-succinimidyl-4-(2-pyridyldithio) butanoate
TNBC	triple negative breast cancer
Tup	tubuphenylalanine

vc valine-citrulline or Val-Cit

Conflict of interest statements

Pamela Trail is an employee of Regeneron Pharmaceuticals.
Gene Dubowchik is an employee of Bristol Myers Squibb.
Timothy Lowinger is an employee of Mersana Therapeutics and holds stock options in Mersana.

Acknowledgements

The authors wish to thank Brandy Bennett and Tracey Rowlands for their thoughtful and insightful comments on the manuscript.

References

- Adem, Y. T., Schwarz, K. A., Duenas, E., Patapoff, T. W., Galush, W. J., & Esue, O. (2014). Auristatin antibody drug conjugate physical instability and the role of drug payload. *Bioconjugate Chemistry*, 25(4), 656–664.
- Agarwal, N., Machiels, J. P., Suarez, C., Lewis, N., Higgins, M., Wisinski, K., ... Elmiegy, M. (2016). Phase I study of the prolactin receptor antagonist LFA102 in metastatic breast and castration-resistant prostate cancer. *The Oncologist*, 21(5), 535–536.
- Alter, G., Malenfant, J. M., & Altfeld, M. (2004). CD107a as a functional marker for the identification of natural killer cell activity. *Journal of Immunological Methods*, 294(1–2), 15–22.
- Amiri-Kordestani, L., Blumenthal, G. M., Xu, Q. C., Zhang, L., Tang, S. W., Ha, L., ... Cortazar, P. (2014). FDA approval: Ado-trastuzumab emtansine for the treatment of patients with HER2-positive metastatic breast cancer. *Clinical Cancer Research*, 20(17), 4436–4441.
- Ataseven, B., Angerer, R., Kates, R., Gunesch, A., Knyazev, P., Hogel, B., ... Harbeck, N. (2013). PTK7 expression in triple-negative breast cancer. *Anticancer Research*, 33(9), 3759–3763.
- Bardia, A., Mayer, I. A., Diamond, J. R., Moroos, R. L., Isakoff, S. J., Starodub, A. N., ... Vahdat, L. T. (2017). Efficacy and safety of anti-Trop-2 antibody drug conjugate sacituzumab govitecan (IMMU-132) in heavily pretreated patients with metastatic triple-negative breast cancer. *Journal of Clinical Oncology* (Jco2016708297). Retrieved from.
- Baselga, J. (2010). Treatment of HER2-overexpressing breast cancer. *Annals of Oncology*, 21(Suppl. 7), vii36–40.
- Baudat, Y., Cameron, B., Dabdoubi, T., Lefebvre, A.-M., Merino-Trigo, A., Thomas, C., ... Blanc, V. (2016). Abstract 1198: characterization of a novel maytansinoid-antibody drug conjugate targeting LAMP1 expressed at the surface of tumor cells. *Proceedings of the 107th annual meeting of the American Association for Cancer Research, New Orleans, LA*.
- Bendell, J., Saleh, M., Rose, A. A., Siegel, P. M., Hart, L., Sirpal, S., ... Vahdat, L. (2014). Phase I/II study of the antibody-drug conjugate glembatumumab vedotin in patients with locally advanced or metastatic breast cancer. *Journal of Clinical Oncology*, 32(32), 3619–3625.
- Boni, V., Rixe, O., Rasco, D., Gomez-Roca, C., Calvo, E., Morris, J. C., ... Delord, J.-P. (2013). Abstract A73: A phase I first-in-human (FIH) study of SAR566658, an anti CA6-antibody drug conjugate (ADC), in patients (pts) with CA6-positive advanced solid tumors (STs). *Proceedings of the AACR-NCI-EORTC international conference: Molecular targets and cancer therapeutics; 2013 Boston, MA*.
- Boonstra, M. C., de Geus, S. W., Prevoo, H. A., Hawinkels, L. J., van de Velde, C. J., Kuppen, P. J., ... Sier, C. F. (2016). Selecting targets for tumor imaging: An overview of cancer-associated membrane proteins. *Biomarker Cancer*, 8, 119–133.
- Burris, H. A., 3rd, Tibbitts, J., Holden, S. N., Sliwkowski, M. X., & Lewis Phillips, G. D. (2011). Trastuzumab emtansine (T-DM1): A novel agent for targeting HER2 + breast cancer. *Clinical Breast Cancer*, 11(5), 275–282.
- Calvet, L., Lefebvre, A.-M., Nicolazzi, C., Blot, L., Thomas, C., Baudat, Y., ... Sidhu, S. (2016). Abstract 1197: Outstanding preclinical efficacy of a novel maytansinoid-antibody-drug conjugate targeting LAMP1 in patient-derived xenograft solid tumors. *Proceedings: AACR 107th annual meeting, New Orleans, LA*.
- Cardillo, T. M., Govindan, S. V., Sharkey, R. M., Trisal, P., & Goldenberg, D. M. (2011). Humanized anti-Trop-2 IgG-SN-38 conjugate for effective treatment of diverse epithelial cancers: Preclinical studies in human cancer xenograft models and monkeys. *Clinical Cancer Research*, 17(10), 3157–3169.
- Carl, P. L., Chakravarty, P. K., & Katzenellenbogen, J. A. (1981). A novel connector linkage applicable in prodrug design. *Journal of Medicinal Chemistry*, 24(5), 479–480.
- Chari, R. V. (2008). Targeted cancer therapy: Conferring specificity to cytotoxic drugs. *Accounts of Chemical Research*, 41(1), 98–107.
- Chen, R., Khatri, P., Mazur, P. K., Polin, M., Zheng, Y., Vaka, D., ... Sweet-Cordero, E. A. (2014). A meta-analysis of lung cancer gene expression identifies PTK7 as a survival gene in lung adenocarcinoma. *Cancer Research*, 74(10), 2892–2902.
- Constantinou, P. E., Danysh, B. P., Dharmaraj, N., & Carson, D. D. (2011). Transmembrane mucins as novel therapeutic targets. *Expert Review of Endocrinology and Metabolism*, 6(6), 835–848.
- Damelin, M., Bankovich, A., Bernstein, J., Lucas, J., Chen, L., Williams, S., ... Dylla, S. J. (2017). A PTK7-targeted antibody-drug conjugate reduces tumor-initiating cells and induces sustained tumor regressions. *Science Translational Medicine*, 9(372).
- Damelin, M., Bankovich, A., Park, A., Aguilar, J., Anderson, W., Santaguida, M., ... Dylla, S. J. (2015). Anti-EFNA4 calicheamicin conjugates effectively target triple-negative breast and ovarian tumor-initiating cells to result in sustained tumor regressions. *Clinical Cancer Research*, 21(18), 4165–4173.
- Dokter, W., Ubink, R., van der Lee, M., van der Vleuten, M., van Achterberg, T., Jacobs, D., ... Timmers, M. (2014). Preclinical profile of the HER2-targeting ADC SYD983/ SYD985: Introduction of a new duocarmycin-based linker-drug platform. *Molecular Cancer Therapeutics*, 13(11), 2618–2629.
- Doronina, S. O., Toki, B. E., Torgov, M. Y., Mendelsohn, B. A., Cerveny, C. G., Chace, D. F., ... Senter, P. D. (2003). Development of potent monoclonal antibody auristatin conjugates for cancer therapy. *Nature Biotechnology*, 21(7), 778–784.
- Dubowchik, G. M., Firestone, R. A., Padilla, L., Willner, D., Hofstead, S. J., Masure, K., ... Trail, P. A. (2002). Cathepsin B-labile dipeptide linkers for lysosomal release of doxorubicin from internalizing immunoconjugates: Model studies of enzymatic drug release and antigen-specific in vitro anticancer activity. *Bioconjugate Chemistry*, 13(4), 855–869.
- Elgersma, R. C., Coumans, R. G., Huijbregts, T., Menge, W. M., Joosten, J. A., Spijker, H. J., ... Beusker, P. H. (2015). Design, synthesis, and evaluation of linker-duocarmycin payloads: Toward selection of HER2-targeting antibody-drug conjugate SYD985. *Molecular Pharmaceutics*, 12(6), 1813–1835.
- El-Tanani, M. K., & Green, C. D. (1997). Interaction between estradiol and growth factors in the regulation of specific gene expression in MCF-7 human breast cancer cells. *The Journal of Steroid Biochemistry and Molecular Biology*, 60(5–6), 269–276.
- Eskelinen, E. L. (2006). Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. *Molecular Aspects of Medicine*, 27(5–6), 495–502.
- Fauteux, F., Hill, J. J., Jaramillo, M. L., Pan, Y., Phan, S., Famili, F., & O'Connor-McCourt, M. (2016). Computational selection of antibody-drug conjugate targets for breast cancer. *Oncotarget*, 7(3), 2555–2571.
- Ghosh, N., Sheldrake, H. M., Searcey, M., & Pors, K. (2009). Chemical and biological explorations of the family of CC-1065 and the duocarmycin natural products. *Current Topics in Medicinal Chemistry*, 9(16), 1494–1524.
- Giagnin, C., Tsoukalas, N., Boumakis, E., Alexandrou, P., Kavantzis, N., Patsouris, E., & Theocharis, S. (2014). Ephrin (Eph) receptor A1, A4, A5 and A7 expression in human non-small cell lung carcinoma: Associations with clinicopathological parameters, tumor proliferative capacity and patients' survival. *BMC Clinical Pathology*, 14(1), 8.
- de Goeij, B. E., & Lambert, J. M. (2016). New developments for antibody-drug conjugate-based therapeutic approaches. *Current Opinion in Immunology*, 40, 14–23.
- Goldenberg, D. M., Cardillo, T. M., Govindan, S. V., Rossi, E. A., & Sharkey, R. M. (2015). Trop-2 is a novel target for solid cancer therapy with sacituzumab govitecan (IMMU-132), an antibody-drug conjugate (ADC). *Oncotarget*, 6(26), 22496–22512.
- Gomez-Roca, C., V. B., V. M., J. C., J.-P. D., E. C., ... AW, T. (2016). A phase I study of SAR566658, an anti CA6-antibody drug conjugate (ADC), in patients (pts) with CA6-positive advanced solid tumors (STs)(NCT01156870). *Molecular targets and cancer therapeutics, Boston, MA*.
- Grattan, B. J., & Freaque, H. C. (2012). Zinc and cancer: Implications for LIV-1 in breast cancer. *Nutrients*, 4(7), 648–675.
- Hamann, P. R., Hinman, L. M., Hollander, I., Beyer, C. F., Lindh, D., Holcomb, R., ... Bernstein, I. (2002). Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. *Bioconjugate Chemistry*, 13(1), 47–58.
- Hamblett, K. J., Senter, P. D., Chace, D. F., Sun, M. M., Lenox, J., Cerveny, C. G., ... Francisco, J. A. (2004). Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. *Clinical Cancer Research*, 10(20), 7063–7070.
- Humphreys, R. C., Kirtely, J., Hewit, A., Biroc, S., Knudsen, N., Skidmore, L., & Wahl, A. (2015). Abstract 639: Site specific conjugation of ARX-788, an antibody drug conjugate (ADC) targeting HER2, generates a potent and stable targeted therapeutic for multiple cancers. *Proceedings of the 106th annual meeting of the American Association for Cancer Research, Philadelphia, PA*.
- Jeffrey, S. C., Burke, P. J., Lyon, R. P., Meyer, D. W., Sussman, D., Anderson, M., ... Senter, P. D. (2013). A potent anti-CD70 antibody-drug conjugate combining a dimeric pyrrolbenzodiazepine drug with site-specific conjugation technology. *Bioconjugate Chemistry*, 24(7), 1256–1263.
- Kasper, G., Weiser, A. A., Rump, A., Sparbier, K., Dahl, E., Hartmann, A., ... Lehmann, K. (2005). Expression levels of the putative zinc transporter LIV-1 are associated with a better outcome of breast cancer patients. *International Journal of Cancer*, 117(6), 961–973.
- Katz, J., Janik, J. E., & Younes, A. (2011). Brentuximab vedotin (SGN-35). *Clinical Cancer Research*, 17(20), 6428–6436.
- Klute, K., Nackos, E., Tasaki, S., Nguyen, D. P., Bander, N. H., & Tagawa, S. T. (2014). Microtubule inhibitor-based antibody-drug conjugates for cancer therapy. *OncoTargets and Therapy*, 7, 2227–2236.
- Kovtun, Y. V., & Goldmacher, V. S. (2007). Cell killing by antibody-drug conjugates. *Cancer Letters*, 255(2), 232–240.
- Lambert, J. M., & Morris, C. Q. (2017). Antibody-drug conjugates (ADCs) for personalized treatment of solid tumors: A review. *Advances in Therapy*, 34(5), 1015–1035.
- van der Lee, M. M., Groothuis, P. G., Ubink, R., van der Vleuten, M. A., van Achterberg, T. A., Loosveld, E. M., ... Dokter, W. H. (2015). The preclinical profile of the duocarmycin-based HER2-targeting ADC SYD985 predicts for clinical benefit in low HER2-expressing breast cancers. *Molecular Cancer Therapeutics*, 14(3), 692–703.
- Lewis Phillips, G. D., Li, G., Dugger, D. L., Crocker, L. M., Parsons, K. L., Mai, E., ... Sliwkowski, M. X. (2008). Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Research*, 68(22), 9280–9290.
- Li, D. M., & Feng, Y. M. (2011). Signaling mechanism of cell adhesion molecules in breast cancer metastasis: Potential therapeutic targets. *Breast Cancer Research and Treatment*, 128(1), 7–21.
- Li, J. Y., Perry, S. R., Muniz-Medina, V., Wang, X., Wetzel, L. K., Rebelatto, M. C., ... Coats, S. R. (2016). A biparatopic HER2-targeting antibody-drug conjugate induces tumor regression in primary models refractory to or ineligible for HER2-targeted therapy. *Cancer Cell*, 29(1), 117–129.
- Liu, C., Huang, H., Wang, C., Kong, Y., & Zhang, H. (2014). Involvement of ephrin receptor A4 in pancreatic cancer cell motility and invasion. *Oncology Letters*, 7(6), 2165–2169.
- Lu, M., Zhu, H., Wang, X., Zhang, D., Xiong, L., Zhu, J., ... Qiang, J. (2016). LAMP1 expression is associated with malignant behaviours and predicts unfavourable prognosis in laryngeal squamous cell carcinoma. *Pathology*, 48(7), 684–690.

- Lyons, R. P., Meyer, D. L., Setter, J. R., & Senter, P. D. (2012). Conjugation of anticancer drugs through endogenous monoclonal antibody cysteine residues. *Methods in Enzymology*, 502, 123–138.
- Ma, X., Ma, Q., Liu, J., Tian, Y., Wang, B., Taylor, K. M., ... Zhou, J. (2009). Identification of LIV1, a putative zinc transporter gene responsible for HDACi-induced apoptosis, using a functional gene screen approach. *Molecular Cancer Therapeutics*, 8(11), 3108–3116.
- MacMillan, K. S., & Boger, D. L. (2009). Fundamental relationships between structure, reactivity, and biological activity for the duocarmycins and CC-1065. *Journal of Medicinal Chemistry*, 52(19), 5771–5780.
- Maderna, A., Doroski, M., Subramanyam, C., Porte, A., Leverett, C. A., Vetelino, B. C., ... O'Donnell, C. J. (2014). Discovery of cytotoxic dolastatin 10 analogues with N-terminal modifications. *Journal of Medicinal Chemistry*, 57(24), 10527–10543.
- Maric, G., Annis, M. G., Dong, Z., Rose, A. A., Ng, S., Perkins, D., ... Siegel, P. M. (2015). GPNMB cooperates with neuropilin-1 to promote mammary tumor growth and engages integrin $\alpha 5 \beta 1$ for efficient breast cancer metastasis. *Oncogene*, 34(43), 5494–5504.
- Maric, G., Rose, A. A., Annis, M. G., & Siegel, P. M. (2013). Glycoprotein non-metastatic b (GPNMB): A metastatic mediator and emerging therapeutic target in cancer. *OncoTargets and Therapy*, 6, 839–852.
- Martino, E., Della Volpe, S., Terribile, E., Benetti, E., Sakaj, M., Centamore, A., ... Collina, S. (2017). The long story of camptothecin: From traditional medicine to drugs. *Bioorganic & Medicinal Chemistry Letters*, 27(4), 701–707.
- Meister, A., & Anderson, M. E. (1983). Glutathione. *Annual Review of Biochemistry*, 52, 711–760.
- Mendelsohn, J., & Baselga, J. (2000). The EGF receptor family as targets for cancer therapy. *Oncogene*, 19(56), 6550–6565.
- Menezes, D., Abrams, T. J., Karim, C., Tang, Y., Ying, C., Miller, K., ... Damiano, J. (2015). Abstract 1682: Development and activity of a novel antibody-drug conjugate for the treatment of P-cadherin expressing cancers. *Cancer Research*, 75(15 Supplement), 1682.
- Mikhail, I., Papisov, A., Hiller, A., Yurkovetskiy, M., Yin, M., Barzana, M., ... Fischman, A. (2005). Semi-synthetic hydrophilic polyals. *Biomacromolecules*, 6, 2659–2670.
- Mossie, K., Jallal, B., Alves, F., Sures, I., Plowman, G. D., & Ullrich, A. (1995). Colon carcinoma kinase-4 defines a new subclass of the receptor tyrosine kinase family. *Oncogene*, 11(10), 2179–2184.
- Nakada, T., Masuda, T., Naito, H., Yoshida, M., Ashida, S., Morita, K., ... Honda, T. (2016). Novel antibody drug conjugates containing exatecan derivative-based cytotoxic payloads. *Bioorganic & Medicinal Chemistry Letters*, 26(6), 1542–1545.
- Ogitani, Y., Abe, Y., Iguchi, T., Yamaguchi, J., Terauchi, T., Kitamura, M., ... Agatsuma, T. (2016). Wide application of a novel topoisomerase I inhibitor-based drug conjugation technology. *Bioorganic & Medicinal Chemistry Letters*, 26(20), 5069–5072.
- Ogitani, Y., Aida, T., Hagihara, K., Yamaguchi, J., Ishii, C., Harada, N., ... Agatsuma, T. (2016). DS-8201a, a novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1. *Clinical Cancer Research*, 22(20), 5097–5108.
- Ogitani, Y., Hagihara, K., Oitate, M., Naito, H., & Agatsuma, T. (2016). Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. *Cancer Science*, 107(7), 1039–1046.
- Paredes, J., Correia, A. L., Ribeiro, A. S., Milanezi, F., Cameselle-Teijeiro, J., & Schmitt, F. C. (2008). Breast carcinomas that co-express E- and P-cadherin are associated with p120-catenin cytoplasmic localisation and poor patient survival. *Journal of Clinical Pathology*, 61(7), 856–862.
- Patil, P. C., Satam, V., & Lee, M. (2015). A short review on the synthetic strategies of duocarmycin analogs that are powerful DNA alkylating agents. *Anti-Cancer Agents in Medicinal Chemistry*, 15(5), 616–630.
- Rose, A. A., Grosset, A. A., Dong, Z., Russo, C., Macdonald, P. A., Bertos, N. R., ... Siegel, P. M. (2010). Glycoprotein nonmetastatic B is an independent prognostic indicator of recurrence and a novel therapeutic target in breast cancer. *Clinical Cancer Research*, 16(7), 2147–2156.
- Roth, M., Barris, D. M., Piperdi, S., Kuo, V., Everts, S., Geller, D., ... Gorlick, R. (2016). Targeting glycoprotein NMB with antibody-drug conjugate, glembatumumab vedotin, for the treatment of osteosarcoma. *Pediatric Blood & Cancer*, 63(1), 32–38.
- Saitoh, O., Wang, W. C., Lotan, R., & Fukuda, M. (1992). Differential glycosylation and cell surface expression of lysosomal membrane glycoproteins in sublines of a human colon cancer exhibiting distinct metastatic potentials. *The Journal of Biological Chemistry*, 267(8), 5700–5711.
- Sarafian, V., Jadot, M., Foidart, J. M., Letesson, J. J., Van den Brule, F., Castronovo, V., ... Coninck, S. W. (1998). Expression of Lamp-1 and Lamp-2 and their interactions with galectin-3 in human tumor cells. *International Journal of Cancer*, 75(1), 105–111.
- Schumacher, D., Hackenberger, C. P., Leonhardt, H., & Helma, J. (2016). Current status: Site-specific antibody drug conjugates. *Journal of Clinical Immunology*, 36(Suppl. 1), 100–107.
- Senter, P. D. (2009). Potent antibody drug conjugates for cancer therapy. *Current Opinion in Chemical Biology*, 13(3), 235–244.
- Shvartsur, A., & Bonavida, B. (2015). Trop2 and its overexpression in cancers: Regulation and clinical/therapeutic implications. *Genes & Cancer*, 6(3–4), 84–105.
- Slamon, D., Eiermann, W., Robert, N., Pienkowski, T., Martin, M., Press, M., ... Crown, J. (2011). Adjuvant trastuzumab in HER2-positive breast cancer. *The New England Journal of Medicine*, 365(14), 1273–1283.
- Smith, N. L., Halliday, B. E., Finley, J. L., & Wennerberg, A. E. (2002). The spectrum of immunohistochemical reactivity of monoclonal antibody DS6 in nongynecologic neoplasms. *Applied Immunohistochemistry & Molecular Morphology*, 10(2), 152–158.
- Speers, C., Tsimelzon, A., Sexton, K., Herrick, A. M., Gutierrez, C., Culhane, A., ... Brown, P. (2009). Identification of novel kinase targets for the treatment of estrogen receptor-negative breast cancer. *Clinical Cancer Research*, 15(20), 6327–6340.
- Stefan, N., Gebleux, R., Waldmeier, L., Hell, T., Escher, M., Wolter, F. I., ... Beerli, R. R. (2017). Highly potent, anthracycline-based antibody-drug conjugates generated by enzymatic, site-specific conjugation. *Molecular Cancer Therapeutics*, 879–892.
- Sussman, D., Smith, L. M., Anderson, M. E., Duniho, S., Hunter, J. H., Kostner, H., ... Benjamin, D. R. (2014). SGN-LIV1A: A novel antibody-drug conjugate targeting LIV-1 for the treatment of metastatic breast cancer. *Molecular Cancer Therapeutics*, 13(12), 2991–3000.
- Taylor, K. M., Morgan, H. E., Johnson, A., Hadley, L. J., & Nicholson, R. I. (2003). Structure-function analysis of LIV-1, the breast cancer-associated protein that belongs to a new subfamily of zinc transporters. *The Biochemical Journal*, 375(Pt 1), 51–59.
- Taylor, K. M., Morgan, H. E., Johnson, A., & Nicholson, R. I. (2005). Structure-function analysis of a novel member of the LIV-1 subfamily of zinc transporters, ZIP14. *FEBS Letters*, 579(2), 427–432.
- Taylor, K. M., Morgan, H. E., Smart, K., Zahari, N. M., Pumford, S., Ellis, I. O., ... Nicholson, R. I. (2007). The emerging role of the LIV-1 subfamily of zinc transporters in breast cancer. *Molecular Medicine*, 13(7–8), 396–406.
- Thomas, A., Teicher, B. A., & Hassan, R. (2016). Antibody-drug conjugates for cancer therapy. *The Lancet Oncology*, 17(6), e254–262.
- Thompson, P., Fleming, R., Bezabeh, B., Huang, F., Mao, S., Chen, C., ... Dimasi, N. (2016). Rational design, biophysical and biological characterization of site-specific antibody-tubulysin conjugates with improved stability, efficacy and pharmacokinetics. *Journal of Controlled Release*, 236, 100–116.
- Tolcher, A. W., Calvo, E., Maitland, M. L., Gibson, B., Xuan, D., Joh, T., ... Sachdev, J. C. (2015). 28LBA A phase 1 study of PF-06647020, an antibody-drug conjugate targeting PTK7, in patients with advanced solid tumors. *European Journal of Cancer*, 51(Supplement 3), S724.
- Trail, P. A. (2013). Antibody drug conjugates as cancer therapeutics. *Antibodies*, 2, 113–129.
- Trail, P. A., King, H. D., & Dubowchik, G. M. (2003). Monoclonal antibody drug immunoconjugates for targeted treatment of cancer. *Cancer Immunology, Immunotherapy*, 52(5), 328–337.
- Trail, P. A., Willner, D., Lasch, S. J., Henderson, A. J., Hofstead, S., Casazza, A. M., ... Hellstrom, K. E. (1993). Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. *Science*, 261(5118), 212–215.
- Turashvili, G., McKinney, S. E., Goktepe, O., Leung, S. C., Huntsman, D. G., Gelmon, K. A., ... Aparicio, S. A. (2011). P-cadherin expression as a prognostic biomarker in a 3992 case tissue microarray series of breast cancer. *Modern Pathology*, 24(1), 64–81.
- Unno, J., Masamune, A., Hamada, S., & Shimosegawa, T. (2014). The zinc transporter LIV-1 is a novel regulator of stemness in pancreatic cancer cells. *Scandinavian Journal of Gastroenterology*, 49(2), 215–221.
- Unno, J., Satoh, K., Hirota, M., Kanno, A., Hamada, S., Ito, H., ... Shimosegawa, T. (2009). LIV-1 enhances the aggressive phenotype through the induction of epithelial to mesenchymal transition in human pancreatic carcinoma cells. *International Journal of Oncology*, 35(4), 813–821.
- Verma, S., Miles, D., Gianni, L., Krop, I. E., Welslau, M., Baselga, J., ... Blackwell, K. (2012). Trastuzumab emtansine for HER2-positive advanced breast cancer. *The New England Journal of Medicine*, 367(19), 1783–1791.
- Vieira, A. F., & Paredes, J. (2015). P-cadherin and the journey to cancer metastasis. *Molecular Cancer*, 14, 178.
- Walker, S., Landovitz, R., Ding, W. D., Ellestad, G. A., & Kahne, D. (1992). Cleavage behavior of calicheamicin gamma 1 and calicheamicin T. *Proceedings of the National Academy of Sciences of the United States of America*, 89(10), 4608–4612.
- Wethington, S. L., Wright, J. D., & Herzog, T. J. (2008). Key role of topoisomerase I inhibitors in the treatment of recurrent and refractory epithelial ovarian carcinoma. *Expert Review of Anticancer Therapy*, 8(5), 819–831.
- Widdison, W. C., Wilhelm, S. D., Cavanagh, E. E., Whiteman, K. R., Leece, B. A., Kovtun, Y., ... Chari, R. V. (2006). Semisynthetic maytansine analogues for the targeted treatment of cancer. *Journal of Medicinal Chemistry*, 49(14), 4392–4408.
- Yarden, Y. (2001). Biology of HER2 and its importance in breast cancer. *Oncology*, 61(Suppl. 2), 1–13.
- Yardley, D. A., Weaver, R., Melisko, M. E., Saleh, M. N., Arena, F. P., Forero, A., ... Vahdat, L. T. (2015). EMERGE: A randomized phase II study of the antibody-drug conjugate glembatumumab vedotin in advanced glycoprotein NMB-expressing breast cancer. *Journal of Clinical Oncology*, 33(14), 1609–1619.
- Younes, A. (2009). Novel treatment strategies for patients with relapsed classical Hodgkin lymphoma. *Hematology. American Society of Hematology. Education Program*, 507–519.
- Younes, A., Bartlett, N. L., Leonard, J. P., Kennedy, D. A., Lynch, C. M., Sievers, E. L., & Forero-Torres, A. (2010). Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *The New England Journal of Medicine*, 363(19), 1812–1821.
- Yurkovetskiy, A., Gumerov, D., Ter-Ovanesyan, E., Conlon, P., Devit, M., Bu, C., ... Bergstrom, D. (2017). Non-clinical pharmacokinetics of XMT-1522, a HER2 targeting auristatin-based antibody drug conjugate. *Proceedings of the American Association for Cancer Research, Washington, DC*.
- Yurkovetskiy, A. V., Yin, M., Bodyak, N., Stevenson, C. A., Thomas, J. D., Hammond, C. E., ... Lowinger, T. B. (2015). A polymer-based antibody-Vinca drug conjugate platform: Characterization and preclinical efficacy. *Cancer Research*, 75(16), 3365–3372.
- Zammarchi, F., Chivers, S., Williams, D. G., Adams, L., Mellinas-Gomez, M., Tyrer, P., ... Van Berkel, P. H. (2016). 62 - ADCT-502, a novel pyrrolbenzodiazepine (PBD)-based antibody-drug conjugate (ADC) targeting low HER2-expressing solid cancers. *European Journal of Cancer*, 69(Supplement 1), S28.
- Zeng, P., Chen, M. B., Zhou, L. N., Tang, M., Liu, C. Y., & Lu, P. H. (2016). Impact of TROP2 expression on prognosis in solid tumors: A systematic review and meta-analysis. *Scientific Reports*, 6, 33658.