Antibody-Drug Conjugates: Possibilities and Challenges

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Abstract

The design of Antibody Drug Conjugates (ADCs) as efficient targeting agents for tumor cell is still in its infancy for clinical applications. This approach incorporates the antibody specificity and cell killing activity of chemically conjugated cytotoxic agents. Antibody in ADC structure acts as a targeting agent and a nanoscale carrier to deliver a therapeutic dose of cytotoxic cargo into desired tumor cells. Early ADCs encountered major obstacles including, low blood residency time, low penetration capacity to tumor microenvironment, low payload potency, immunogenicity, unusual off-target toxicity, drug resistance, and the lack of stable linkage in blood circulation. Although extensive studies have been conducted to overcome these issues, the ADCs based therapies are still far from having high-efficient clinical outcomes. This review outlines the key characteristics of ADCs including tumor marker, antibody, cytotoxic payload, and linkage strategy with a focus on technical improvement and some future trends in the pipeline.

Keywords: Antibody-Drug, Cancer therapy, Cytotoxic drugs, Monoclonal antibodies, Nanomedicine

Introduction

Similar to conventional cancer treatments such as chemotherapy and radiotherapy, antibody immunotherapy and targeted therapies based on nanoparticulate structures are not safe and efficacious as often claimed; therefore, alternative therapies are urgently needed. In this regard, Antibody Drug Conjugates (ADC) technology that could bring forth a new generation of cancer therapeutics was the main focus of this study. ADCs are monoclonal antibodies (mAbs) connected by a specific linkage to antitumor cytotoxic molecules. The main components of an ADC and mechanism of its action are further demonstrated in figure 1.

In ADC technology, the specificity of an antibody for its immunogenicity is exploited to home a chemically supertoxic agent into tumor cells, while administration of unconjugated drug alone is not suitable due to its high toxicity. Therefore, ADCs can be further defined as prodrugs requiring the release of their toxic agent for their activation that commonly happens after ADC internalization into the target cell 1. From the standpoint of nanomedicine, the antibody in ADC structure acts as a self-targeting nanoscale carrier 1,3, thus, it could overcome the issues associated with nanomedicines based on synthetic nanomaterials such as cellular internalization, clearance, sterical hindering of binding to the epitopes and failing to release into targeted cells 4.

The first experimental design on ADC subject dates back to more than 50 years ago 5. However, the use of ADCs for cancer therapy has achieved considerable success in recent years after the introduction of four clinically approved ADCs such as Brentuximab vedotin 6,7, Trastuzumab emtansine 8-11, Inotuzumab ozogamicin 12 and Gemtuzumab ozogamicin 12,13 used for the treatment of patients with lymphoma (HL and ALL), HER2-positive, CD22-positive AML and CD33-positive ALL cancers, respectively. Likewise, a great deal of effort has also been made by the pharmaceutical companies to overcome the technological barriers associated with ADCs 14,15, whereby there are 160 ADCs undergoing preclinical trials 16 and 70 more under various stages of clinical evaluation (Table 1).

Clinical efficacy of the ADCs arises following accurate selection of four parameters including tumor tar-
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The precise selection of each parameter can be achieved through the knowledge gained from the previous studies and established ADCs, and is discussed here.

**Tumor markers in ADCs**

The important aspects of tumor markers in ADCs are demonstrated in figure 2. An antigen with expression pattern slightly greater in tumor cells compared to healthy cells is sufficient to induce ADC activity. However, like other targeted drug delivery systems, the number of cell surface tumor markers can be a key determinant of ADC activity. The targets for ADC do not necessarily intervene in cell growth. ADCs tumor-suppressive function is mainly mediated through tumor marker potency for ADC internalization compared to the inhibition by blocking the cell growth. However, target biological roles such as those involved in cell division pathway (e.g., CD30 and CD70 tumor necrosis factor signaling) can be considered as an advantage for ADC efficacy. Accordingly, the currently employed targets and their biological roles are listed in table 1.

For instance, glembatumumab vedotin is an ADC against an extracellular domain of non-metastatic B melanoma-associated glycoprotein (GPNMB) that is aberrantly expressed in various carcinoma including hepatocellular, melanoma, gliomas, and two specific breast cancer types, Basal-Like Breast Cancer (BLBC) and Triple Negative Breast Cancer (TNBC). The GPNMB do not represent a high relative level of expression in all aforesaid carcinoma. One important property that may make GPNMB a potential therapeutic target for ADCs, originates from its biological role in MAPK/ERK pathway, as GPNMB expression can be upregulated by MAPK/ERK inhibitors.

From the structure standpoint, a relevant antigenic determinant on cell surface membranes, termed Extracellular Domain (ECD), is required as an immunizing agent for antibody generation. However, the potential of ECD to be shed into the circulation must be considered. The shed ECDs can potentially bind to ADC and consequently reduce the targeted delivery into the tumor cells.

A further concern in the selection of the target for ADC is related to the homogeneity or heterogeneity expression of the tumor marker on the tumor cell surface. Homogenous expression of the tumor targets has been demonstrated to be more in favor of ADC targeting than those expressed heterogeneously. However, heterogeneous antigen expression can particularly be beneficial for those ADCs that possess bystander killing activity. Bystander killing activity is referred to the potency of therapeutics delivery system in killing neighboring cells independently of targeted therapy assignment. This effect can be raised through reactive oxygen species or some cytotoxic metabolites that may be excreted from the tumor-targeted cells.

As a result, recycling capability of a tumor marker would enhance bystander killing activity as it may promote leakage of ADC and metabolites to the neighboring cells. However, according to the reports, an extra recycling property is not desirable as in further Bystander activity (Ba), the greater side effects are predicted.

Increasing the complexity of the targets can also be considered to improve the efficacy of the ADCs. The promising future of the ADCs supports extensive studies to look for a potent ADC target with a wide range of expression, from earliest cell recognizable lineage to maturation. This represents an exquisite-selective target that covers all types of malignancies. For example, CD19 is a good example of such target that is highly expressed in B-cell and the vast majority of Non-Hodgkin lymphomas (NHLs), and B-cell Acute Lymphoid Leukemia (B-ALL) (99%) as shown in table 1. CD19 has been marked as a target to produce ADCs, including SAR3419, SGN-CD19A, MDX-1206, and ADCT-402.
Table 1. Current ADCs in clinical development based on targeting antigens with an overview of their properties

<table>
<thead>
<tr>
<th>ADC names</th>
<th>Clinical phase, indication</th>
<th>Ab, kd, therapeutics activity</th>
<th>Payload</th>
<th>Linkage strategy</th>
<th>DAR, MTD, bystander effect</th>
<th>Sponsor, Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Targeting HER2 antigen, a transmembrane RTKs in the growth of some cancer cells:</strong></td>
<td></td>
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<tr>
<td>Kadeyla, Ado-Trastuzumab entansime, T-DM1</td>
<td>Approved in 2013, for treatment of her2+ positive breast cancer</td>
<td>hulgG1 (trastuzumab), n/a, ADCC and HER2-dependent PE1K/ACT signaling</td>
<td>DM1</td>
<td>Native lysine residues, SMCC nonreducible thioether linkage</td>
<td>~3.5, 3.6 mg/kg, no</td>
<td>Genentech, Inc. (8-11)</td>
</tr>
<tr>
<td>SYD985, Trastuzumab vc-seco-DUBA</td>
<td>Phase I, for treatment of USC and epithelial EOC</td>
<td>hulgG2 anti HER2 (Trastuzumab), n/a, no</td>
<td>DUO</td>
<td>VC-seco</td>
<td>~2.8, 1.88 mg/kg, yes</td>
<td>Synthony BV (37-39)</td>
</tr>
<tr>
<td>ADC XMT-1522</td>
<td>Phase I, for treatment of low HER+ breast, gastric and lung cancers</td>
<td>hulgG1anti-HER2 (HT-19), n/a, n/a</td>
<td>AF-HPA</td>
<td>Fleximer®</td>
<td>12, n/a, yes</td>
<td>Mersana Therapeutics (40)</td>
</tr>
<tr>
<td>ADC ARX788</td>
<td>Phase I, for treatment of low HER+ breast, ovarian, lung and gastric cancers</td>
<td>lgG1anti-HER2, n/a, n/a</td>
<td>MMAF</td>
<td>pAcF site-specific oxime linkage, AS269 noncleavable linker</td>
<td>2, n/a, n/a</td>
<td>Zhejiang Medicine/Ambrex (41)</td>
</tr>
<tr>
<td>ADC ADCT-502</td>
<td>Phase I, for treatment of low HER2+ expressing breast, NSCLC, gastroesophageal, bladder cancer</td>
<td>hulgG1anti-HER2 (trastuzumab)</td>
<td>PBD</td>
<td>Cysteine residues, VA-PABC</td>
<td>1.7, n/a, n/a</td>
<td>ADC Therapeutics S.A. (42)</td>
</tr>
<tr>
<td><strong>Targeting EGFR antigen, a RTKs that is essential for ductal and lobuloalveolar developlment:</strong></td>
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<tr>
<td>ABT-414, Deputaxizumab mafodotin</td>
<td>Phase II, for treatment of GBM</td>
<td>hulgG1 anti EGFR (ABT-806), 0.06 nM, inhibits EGFR signaling</td>
<td>MMAF</td>
<td>Native cysteine residues, MC noncleavable linker</td>
<td>~3.8, 1.5 mg/kg, no</td>
<td>Abbvie (43)</td>
</tr>
<tr>
<td>AMG 595</td>
<td>Phase I, for treatment of GBM</td>
<td>hulgG1anti-EGFRvIII, 0.61 nM, n/a</td>
<td>DM1</td>
<td>Native lysine residues, SMCC nonreducible thioether linkage</td>
<td>~3.5, n/a, no</td>
<td>Amgen (44)</td>
</tr>
<tr>
<td>IMGN289, Laprituximab entansime</td>
<td>Phase I, for treatment of NSCLC and HNSCC</td>
<td>hulgG anti-EGFR (J2898A), n/a, n/a</td>
<td>DM1</td>
<td>Native lysine residues, SMCC nonreducible thioether linker</td>
<td>n/a, n/a, no</td>
<td>ImmunoGen (45)</td>
</tr>
<tr>
<td>ABBV-221</td>
<td>Phase I, for treatment of solid tumor</td>
<td>hulgG1 anti-EGFR, n/a, n/a</td>
<td>MMAE</td>
<td>MC noncleavable linker</td>
<td>n/a, n/a, no</td>
<td>Abbvie (46)</td>
</tr>
<tr>
<td><strong>Targeting CD70 (CD27L) antigen a TP2 and member of the tumor necrosis factor family:</strong></td>
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<tr>
<td>SGN-75</td>
<td>Phase I, for treatment of CD70-positive NHL and metastatic RCC</td>
<td>hu anti-CD70 (hiF6), n/a, n/a</td>
<td>MMAF</td>
<td>Native cysteine residues, MC noncleavable linker</td>
<td>n/a, 3, n/a</td>
<td>Seattle Genetics (47)</td>
</tr>
<tr>
<td>MDX-1203, BMS-936561</td>
<td>Phase I, for treatment of ccRCC or B-NHL</td>
<td>hu anti-CD70, n/a, n/a</td>
<td>DUO</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a,15 mg/kg, yes</td>
<td>Bristol-Myers (48)</td>
</tr>
<tr>
<td>SGN-CD70A</td>
<td>Phase I, for treatment of RCC, MCLD, LBC, FL,</td>
<td>hu anti-CD70, n/a, n/a</td>
<td>PBD</td>
<td>VA linker</td>
<td>n/a, n/a, yes</td>
<td>Seattle Genetics (49)</td>
</tr>
<tr>
<td>AMG 172</td>
<td>Phase I, for treatment of ccRCC</td>
<td>hulgG1, n/a, n/a</td>
<td>DM1</td>
<td>Native lysine residues, MCC noncleavable linker</td>
<td>n/a, n/a, no</td>
<td>Amgen (50)</td>
</tr>
<tr>
<td><strong>Targeting CD33 antigen, an EGP:</strong></td>
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</tr>
<tr>
<td>Mylotarg, Gemtuzumab Ozogamicin (GO)</td>
<td>Withdrawn 2010 and approved in 2017, for treatment of CD33+ AML</td>
<td>huIgG4, n/a, n/a</td>
<td>Calich.</td>
<td>Native lysine residues, (AcBut)-N-acetyl acid-labile hydrazine linker</td>
<td>n/a, 0.25 mg/kg, yes</td>
<td>Pfizer (51)</td>
</tr>
<tr>
<td>SGN-CD33A</td>
<td>Phase I, for treatment of AML</td>
<td>hu anti-CD33 with engineered cysteines, n/a, n/a</td>
<td>PBD</td>
<td>Engineered cysteine residues, VA linker</td>
<td>n/a, n/a, yes</td>
<td>Seattle Genetics (12,13)</td>
</tr>
<tr>
<td>AVE9633</td>
<td>Phase I, for treatment of AML</td>
<td>anti-CD33, n/a, n/a</td>
<td>DM4</td>
<td>SPDB disulfide cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Sanofi (53)</td>
</tr>
</tbody>
</table>

Not available (n/a), Relapsed B-cell non-Hodgkin's lymphoma (B-NHL), Acute myeloid leukemia (AML), Mertansine (DM1), Calicheamicin (calich.), N-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), Hydrazide acetyl butyrate (AcBut), Uterine Serous Carcinoma (USC), Tumor-Associated Antigen (TAA), Valine-citrulline-seco (vc-seco), Renal Cell Carcinoma (RCC), clear cell Renal Cell Carcinoma (ccRCC), Mantle-Cell Lymphoma Diffuse (MCLD), Non Small-Cell Lung Cancer (NSCLC), Receptor tyrosine kinases (RTKs), Recurrent Glioblastoma Multiforme (GBM), Transmembrane Protein (TP), CD27 ligand (CD27L), Epidermal growth factor receptor variant III (EGFRvIII), Glioblastoma multiforme (GBM), Epithelial Ovarian Cancer (EOC), Head and Neck Squamous Cell Carcinoma (HNSCC), Auristatin F-hydroxypyrrolamide (AF-HPA), Polyacetal-based polymer (Fleximer®), Non-natural amino acid linker para-acyetyl-phenylalanine (pAcF), Ambersinatia, a short polyethylene glycol (PEG) spacer terminated by an alkoxysamine (AS269).
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<th>Sponsor, Reference</th>
</tr>
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<tbody>
<tr>
<td>Targeting CD19 antigen, a TPI on B cells as an accessory molecule for B-cell signal transduction and TAA:</td>
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</tr>
<tr>
<td>SAR3419, coltuzumab ravtansine</td>
<td>Phase II, for treatment of B-NHL and B-ALL</td>
<td>hulgG1-anti-CD19 (huB4), n/a, ADCC</td>
<td>DM4</td>
<td>Native lysine residues, SPDB disulfide cleavable linker</td>
<td>~3.5, ~4.3 mg/kg, yes</td>
<td>ImmunoGen (7,34,35)</td>
</tr>
<tr>
<td>SGN-CD19A</td>
<td>Phase I, for treatment of B-Cell Malignancies</td>
<td>hulgG1-anti-CD19 (hBU12), n/a, ADCC</td>
<td>MMAF</td>
<td>Native cysteine residues, MC linker, noncleavable</td>
<td>n/a, 6.0, no</td>
<td>Seattle Genetics (32)</td>
</tr>
<tr>
<td>ADC-402</td>
<td>Phase I, for treatment of relapsed or refractory B-ALL</td>
<td>hulgG1anti-CD19, n/a, n/a</td>
<td>PBD</td>
<td>Native cysteine residues, VA and maleimide cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>ADC Therapeutics S.A. (33)</td>
</tr>
<tr>
<td>Targeting Mesothelin antigen, a glycoporphatidyl inositol anchored protein:</td>
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</tr>
<tr>
<td>BAY 94–9343, anetumab ravtansine</td>
<td>Phase I, for treatment of MPM</td>
<td>hu anti-mesothelin, n/a, n/a</td>
<td>DM4</td>
<td>Lysine residues, SPDB disulfide cleavable linker</td>
<td>n/a, 6.5 mg/kg, yes</td>
<td>Bayer (57)</td>
</tr>
<tr>
<td>BMS-986148</td>
<td>Phase I &amp; II, for treatment of Mesothelin-expressing cancers</td>
<td>anti mesothelin</td>
<td>n/a</td>
<td>n/a, n/a, n/a</td>
<td>n/a, 6.0, no</td>
<td>Bristol-Myers (58)</td>
</tr>
<tr>
<td>DMTO4039A</td>
<td>Phase I, for treatment of pancreatic and P-OC</td>
<td>hu anti-mesothelin (TD9.v3), n/a, n/a</td>
<td>MMAE</td>
<td>A noncleavable alkyl hydrazide linker</td>
<td>~3.5, 2.4 mg/kg, n/a</td>
<td>Genentech, Inc. (59,60)</td>
</tr>
<tr>
<td>Targeting CD22 antigen, a transmembrane sialoglycoprotein functions as an inhibitory receptor for BCR signaling and BCR-induced cell death:</td>
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</tr>
<tr>
<td>Inotuzumab, IO, Ozogamicin, CMC-544</td>
<td>Approved in 2017, for treatment of CD22+ ALL</td>
<td>hulgG4 anti CD29(G544),n/a, no</td>
<td>Calich.</td>
<td>Native lysine residues, (AcBut)-N-acyl, Acid-labile hydrazide linker</td>
<td>n/a, 0.05 mg/kg, yes</td>
<td>Pfizer (12)</td>
</tr>
<tr>
<td>Pinatuzumab vedotin, DCDT9298OS, RG7593</td>
<td>Phase II, for treatment of NHL and CLL</td>
<td>hulgG1anti-CD22 (Epratuzumab), n/a, n/a</td>
<td>MMAE</td>
<td>Native cysteines residues, MC-VC-PAB linker</td>
<td>~2.4, 2.4 mg/kg, yes</td>
<td>Genentech, Inc. (61)</td>
</tr>
<tr>
<td>Targeting CEACAM5 antigen, labetuzumab, CEA, CD66e, a EGP that has a role in cell adhesion and invasion:</td>
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</tr>
<tr>
<td>IMMU-130, hMN14-SN38, labetuzumab govtican, labetuzumab-SN38</td>
<td>Phase II, for treatment of mCRC</td>
<td>hulgG1 anti-CEACAM5 (hMN14), 1.5 nM, ADCC</td>
<td>SN-38</td>
<td>Native cysteine residues, CL2A pH sensitive (Benzylicarbonates site) carbonate linker</td>
<td>7-8, 6–10 mg/kg, yes</td>
<td>Immunomedics (63-65)</td>
</tr>
<tr>
<td>SAR40870</td>
<td>Phase I &amp; II, for treatment of B-Cell Malignancies</td>
<td>hulgG1 anti-CEACAM5, n/a, n/a</td>
<td>DM4</td>
<td>Lysine residues, SPDB disulfide cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Sanofi (66)</td>
</tr>
<tr>
<td>Targeting Trop-2 (M1S1, TACSTD2 or GA733-1) antigen, a EGP transduces calcium signal has a role in ERK1/2 MAPK pathway which mediates cancer cell proliferation, migration, invasion, and survival:</td>
<td></td>
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<tr>
<td>IMMU-132, hr57-SN-38, Sacituzumab govtican</td>
<td>Phase III, for treatment of pancreatic cancers, SLCCL and TNBC</td>
<td>hulgG1 anti-trop-2 (RS7 or Sacituzumab), 0.564 nM, ADCC</td>
<td>SN-38</td>
<td>Native cysteine residues, CL2A pH sensitive carbonate link Site-specific transglutaminase tag, AcLys-VC-PABC linker</td>
<td>~7.6, 8–10 mg/kg, yes</td>
<td>Immunomedics (67-72)</td>
</tr>
<tr>
<td>PF-06664178, Trop-2 ADC, RN927C</td>
<td>Phase I, for treatment of OC, NSCLC and breast cancer</td>
<td>Engineered hulgG1anti-Trop-2, 14 nM, n/a</td>
<td>PF063801</td>
<td>01</td>
<td>2.0, n/a, n/a</td>
<td>Pfizer (73)</td>
</tr>
<tr>
<td>Targeting PSMA antigen, a TP2 with known enzymatic activities and acts as a glutamate-prefering carbopxypeptidase:</td>
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<tr>
<td>PSMA ADC</td>
<td>Phase I &amp; II, for treatment of prostate cancer</td>
<td>hu anti-PSMA, 35.6-46.5 nM, n/a</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a, 2.5 mg/kg, yes</td>
<td>Progenics (74,75)</td>
</tr>
<tr>
<td>MLN2704</td>
<td>Phase I &amp; II, for treatment of prostate cancer</td>
<td>hu anti-PSMA (huj591), n/a, n/a</td>
<td>DM1</td>
<td>Lyseine residues, SPP disulfide cleavable linker</td>
<td>n/a, 60 mg/kg, yes</td>
<td>Millennium (76)</td>
</tr>
</tbody>
</table>

B Cell Receptor (BCR), Chronic Lymphocytic Leukemia (CLL), Prostate-specific membrane antigen (PSMA), Maleimido-[short PEG]-Lys-PABOCO-20-O (CL2A), Metastatic colorectal cancer (mCRC), Carcinoembryonic Antigen Related Cell Adhesion Molecule 5 (CEACAM5), Trophoblast cell-surface antigen 2 (Trop-2), Tumor-Associated Calcium Signal Transducer (TACSTD2), Gastric Antigen 733 (GA733-1), Malignant Pleural Mesothelioma (MPM), Platinum-resistant ovarian cancer (P-OC).

nalization. Receptor-mediated antibody internalization is a key mechanism underlying antibody endocytosis that is induced through antibody binding to its specific antigen 77. It has been reported that, alternative antibodies against the same immunogen can exhibit different rates of internalization 135. Rapid internalization can raise both ADC efficacy and safety simultaneously, since it reduces the opportunity of the ADC for off-target release 136. In addition to rapid internalization as a prerequisite for an antibody, the route by which antibody is internalized should be also considered, because it can potentially influence ADC processing 137. For instance, Clathrin-coated Pit-mediated receptor internalization...
Contd table 1.

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</tr>
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<tbody>
<tr>
<td><strong>Targeting CD37 (Tetraspanin-26) antigen, a Tp3 present on mature B cells, implicates as a signaling death receptor to regulate B/T-cell interactions/proliferation:</strong></td>
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</tr>
<tr>
<td>IMGN529, Naratuximab emtansine</td>
<td>Phase I or II, for treatment of BCL, CLL, NHL</td>
<td>huIgG1anti-CD37 (K7153A), n/a, ADCC and CDC, huIgG2 anti-CD37 (AGS67C or VCD37-9a73), n/a</td>
<td>DM1</td>
<td>Native lyseine residues, SMCC nonreducible thioether linkage</td>
<td>n/a, 1.0 mg/kg, no</td>
<td>ImmunoGen (78,79)</td>
</tr>
<tr>
<td>AGS67E</td>
<td>Phase I, trial for treatment of NHL, DLBLC with high level of CD37 expression</td>
<td></td>
<td>MMAE</td>
<td>Native cytoines residues, VC protease-cleavable linker</td>
<td>n/a, 1.2 mg/kg, yes</td>
<td>Agensys (80-81)</td>
</tr>
</tbody>
</table>

| **Targeting CD39 (TNFRSF8) antigen, a tumor necrosis factor:** |
| Adectis, brentuximab vedotin, SGN-35                  | Approved in 2011, for treatment of HL and ALL. | Chimeric IgG1anti-CD30 (eAC10 or SGN30), n/a | MMAE    | Native interchain cysteine, MC-VC- PABC linker | ~ 4, 1.8 mg/kg, yes | Seattle Genetics (6.7) |

| **Targeting HER3 antigen, a member of EGFR family RTK, frequently overexpressed in solid tumors, including breast, lung, and colorectal tumors of epithelial origin; it has no active kinase domain itself but is activated through heterodimerization with other members of the EGFR family:** |
| Glembatumumab vedotin, SGN30                         | Phase I, trial for treatment of HER3-positive metastatic breast cancer | huIgG1anti-HER3(Patritumab) | MMAE    | Native interchain cysteine, PEG8 va linker, cathepsin-B cleavable dipetide linker | ~ 2, 0.2 mg/kg, yes | Cellcentr (83)    |

| **Targeting CD79b antigen, a Tp on B cells mediates signal transduction cascade activated by BCR:** |
| Polatuzumab vedotin, RG7596, DCDS4501A                | Phase I, II, for treatment of NHLs and CLLs | anti-CD79b, n/a, n/a | MMAE    | Native cytoines residues, VC protease-cleavable linker | n/a, 2.4 mg/kg, yes | Genentech, Inc. (88) |

| **Targeting RCC antigen, a part of calcium negative feedback system and has a role in cGMP synthesizes from GTP:** |
| Indatuximab vedotin, MLN0264,TAK-264, 5F9-vcMMAE     | Phase II, for treatment of GI malignancies | IgG1 anti- GCC (TAK-264), n/a, n/a | MMAE    | Native cytoines residues, VC protease-cleavable linker | n/a, -1.8 mg/kg, yes | Millennium (89,90) |

| **Targeting NaPi2b antigen, a sodium phosphate transporter:** |
| Lifastuzumab vedotin, RG7599, DNB160600A               | Phase II, for treatment of NSCLC and ovarian cancer | huIgG1 anti-NaPi2b, 10.19 nM, n/a | MMAE    | Native cytoines residues, VC protease-cleavable linker | n/a, 2.4 mg/kg, yes | Genentech, Inc. (91,92) |

| **Targeting CA6 antigen, a sialoglycoprotein of MUC-1 is over-expressed in variety of solid tumors, including breast, ovarian, cervical, lung and pancreatic tumors:** |
| SAR566658                                            | Phase II, for treatment of OC, breast, cervical, lung cancers | huIgG1 anti-CA6 (huDS6 IgG1), n/a, n/a | DM4     | Native lysesine residues, SPDB disulfide cleavable linker | 6.5 mg/kg | Sanofi (93,94) |

| **Targeting CD74 antigen, a Tp2 on B cells involved in the formation and transport of MHC class II protein:** |
| Milatuzumab-doxorubicin, IMMU-110, HLL1-DOX           | Phase I & II, for treatment of MM | hu anti-CD74 | DOX     | Native lysesine residues, Acid-lable hydrazone linker | n/a, n/a, yes | Immunomedics (95) |

| **Targeting CD138 antigen, syndecan1, a type I transmembrane heparan sulfate proteoglycan participates in cell proliferation, cell migration and cell-matrix interactions:** |
| BT-062, Indatuximab ravnatisne                      | Phase I & II, for treatment of MM | Chimeric anti-CD138 (mBTO62), n/a, n/a | DM4     | Native lysesine residues, SPDB disulfide cleavable linker | n/a, 2.7 mg/kg, yes | Biotest (96) |

| **Targeting BCMA antigen, a receptor for a proliferation-inducing ligand and B-cell activating factor:** |
| GSK2857916                                           | Phase I, for treatment of MM | Engineered afucosylated huIgG1 anti-BCMA, 1 nM, ADCC | MMAF    | Native cytoines residues, MC noncleavable linker | n/a, n/a, no | GlaxoSmithKine (97) |

(caveolae pathway), at least in some cases, has been reported to traffic ADC to the cells. In caveolae pathway, ADC is directed to the Golgi or endoplasmic reticulum (Non-proteolytic compartments) instead of endosomes or lysosomes (Proteolytic compartment of the cells) \(^{118}\). ADC’s traffic to the non-proteolytic compartments may impede its proteolytic process to release effective metabolites \(^6\). Antibody capability to induce receptor mediated internalization is somewhat a mandatory requirement in design of new generation of ADCs.
ADC names | Clinical phase, indication | Ab, kd, therapeutic activity | Payload | Linkage strategy | DAR, MTD, by-stander effect | Sponsor, Reference
--- | --- | --- | --- | --- | --- | ---
**Targeting specific myeloma antigen:**
DFR4539A, RG7598 | Phase I, for treatment of MM | n/a, n/a, n/a | MMAE | n/a | n/a, n/a, n/a | Genentech, Inc. (100)
**Targeting SLAMF7 (CS1) antigen:**
ABBV-838 | Phase I, for treatment of MM | hulgGl anti-SLAMF7, n/a, n/a | MMAE | Native cysteine residues, VC protease-cleavable linker | n/a, n/a, n/a | Abbvie (101)
**Targeting CD56 antigen, associates with FGFR and stimulates RTKs to induce neurite outgrowth:**
IMGN901, Lorvotuzumab mertansine, huN901-DM1/BB-10901 | Phase I & II, for treatment of CD56+ MM | hulgGl anti-CD56 (Lorvotuzumab or N901), 0.002 nM, ADC | DM1 | Lysine residues, SPP disulfide cleavable linker | 3.7, 2.0 mg/kg, n/a | ImmunoGen (102)
**Targeting ENPP3 (CD203c) antigen, a TP2 belongs to a series of ectoenzymes, possess ATPase and ATP pyrophosphatase activities:**
AGS-16C3F | Phase I & II, for treatment of RRCC | hulgGl anti-ENPP3 (AGS16-7.8), 0.3-1.1 nM, no | MMAF | Native cysteine residues, MC noncleavable linker | ~ 4, 1.8 mg/kg, no | Astellas Pharma (103,104)
**Targeting TF (CD142) antigen, a TP and initiator of the coagulation cascade:**
Humax-TF-ADC, tisotumab vedotin | Phase I & II, for treatment of Multiple solid tumors | IgG1 anti-TF | MMAE | Native cysteine residues, VC protease-cleavable linker | n/a, 1.8 mg/kg, yes | Genmab (105)
**Targeting TIM1 antigen, a member of the T cell transmembrane IgG and mucin family, which plays critical roles in regulating immune cell activity especially regarding the host response to viral infection:**
CDX-014 | Phase I & II, for treatment of RCC | hulgGlanti-TIM1 | MMAE | Native cysteine residues, VC protease-cleavable linker | n/a, n/a, n/a | Celldex Therapeutics (106)
**Targeting FOLR1 antigen, a membrane-bound protein regulates transport of the vitamin B9 into cells:**
IMGN853, mirvetuximb soravtansine | Phase I, for treatment of folate receptor alpha (FRα)-positive cancer, e.g., relapsed EOC | FRα-binding antibody | DM4 | Native lysine residues, Sulfo-SPDB disulfide cleavable linker | n/a, 6 mg/kg, yes | ImmunoGen (17,107-110)
**Targeting MUC16 (CA-125) antigen, a member of the mucin family GP that acts as a lubricating barrier against foreign particles and infectious agents on the apical membrane of epithelial cells:**
RG7458, Sofituzumab Vedotin, DMUC5754A | Phase I, for treatment of ovarian and pancreatic cancer | IgG1anti-MUC16 (OC125), n/a, n/a | MMAE | Native cysteine residues, MC-VC-PABC linker | n/a, 2.4 mg/kg, yes | Genentech, Inc. (111)
**Targeting CanAg antigen, is a novel glycoform of mucin family GP:**
IMGN242, HuC242-DM4, cantuzumab ravtansine | Phase I, for treatment of Non-colorectal and Pancreatic Cancer | hu anti-CanAg (C242 or cantuzumab), n/a, n/a | DM4 | Native lysine residues, SPDB disulfide cleavable linker | n/a, n/a, yes | ImmunoGen (112)
**Targeting Kiat (CD117 or SCFR) antigen, a TP and RTKs having a key role in the regulation of cell differentiation and proliferation:**
LOP628, Anti c-KIT ADC | Phase I, for treatment of AML and solid tumors | hulgGlanti-c-(c-Ki), n/a, n/a | DM1 | Native lysine residues, SMCC noncleavable thioether linker | n/a, n/a, no | Novartis (113)
**Targeting EphA2 antigen, belonging to ephrin receptor subfamily of the RTKs family regulating cell migration, adhesion, proliferation and differentiation:**
MEDI-547, MI-CP177 | Phase I, for treatment of relapsed or refractory solid tumors associated with EphA2 expression | hulgGl anti-EphA2 (1C1), 1nM, n/a | MMAF | Native cysteine residues, MC noncleavable linker | 4, 6.0 mg/kg, no | Medimmune (114,115)
**Targeting Nectin 4 (PVR4L) antigen, a TPI and member of a family of cellular adhesion molecules, involved in Ca2+-independent cellular adhesion:**
ASG-22M6, AGS-22M6e, anti-nectin-4 ADC, Enfortumab vedotin | Phase I, for treatment of MUC | hulgGl anti-nectin-4 (AGS-22M6), 0.01 nM, n/a | MMAE | Native cysteine residues, VC protease-cleavable linker | n/a, 1-3 mg/kg, yes | Astellas Pharma (116,117)

**ADCs. Antibody with low internalization rate has no desired therapeutic activity even for the tumors expressing high levels of surface antigen.** To compensate inefficient internalizing of ADC, a much more potent drug and high stable linkage chemistry (linkage between the antibody and drug moiety) are required that would be discussed in next sections.

Optimal pharmacokinetic (PK) properties including longer half-life is another aspect of the antibody component in ADC design. It has been reported that Ab with longer half-life show high elimination and rapid clearance of the ADC in plasma. As shown in table 1, it is not compulsory for a mAb itself to represent therapeutic activity in the ADC. However, therapeuti-
Targeting LIV1 (SLC39A6 or ZIP6) antigen, a member of the zinc transporter family playing a key role in tumor cell progression and metastasis:

- PF-06647263
  - Phase I, for treatment of metastatic breast
  - hulgGi-anti-LIV1(hLIV22), 4.6 nM, n/a
  - MMAE
  - Native cytotoxic residues, VC protease-cleavable linker
  - n/a, n/a, yes
  - ABBvie (120-123)

Targeting Ephrin-A4 (EFNA4) antigen, RTKs modulate signaling pathways that impact cell fate decisions during embryogenesis and adult tissue homeostasis:

- ABBV-399
  - Phase I, for treatment of c-Met-expressing NSCLC
  - Engineered hulgG1 without the agonist activity associated with c-Met (ABT-700), 0.2 to 1.5 nM, ADC and c-Met inhibition & downstream signaling molecules
  - MMAE
  - Native cytotoxic residue, VC protease-cleavable linker
  - n/a, n/a, yes
  - Abbvie (120-123)

Targeting FGFR2 (cMet) antigen, type 2 RTKs with a role in both embryonic development and tissue repair:

- BAY1129890, Luponatuzumab amadotin, anti-C4.4a ADC
  - Phase I, for treatment of LSCC
  - hulgGi1-FGFR2 isoforms FGFR2-IIb
  - MMAE
  - Lysine side chains and a noncleavable linker
  - n/a, n/a, yes
  - Bayer (124)

Targeting P-Cadherin (Caderhin 3) antigen, a cell-surface protein and member of the cadherin family plays a role in cell adhesion, motility, invasion, and proliferation:

- PCA062
  - Phase I, for treatment of TNBC, head and neck & esophageal cancers
  - IgG1 anti-P-cadherin, n/a, n/a
  - DM1
  - Native lysine residues, SMCC noncleavable thioether linker
  - n/a, n/a, yes
  - Novartis (126)

Targeting STEAP1 antigen, cell-surface protein is predominantly expressed in prostate tissue:

- RG7450, DSTP3086S, Vandortuzumab vedotin, STEAP1 ADC
  - Phase I, for treatment of mCRPC
  - hulgGi1-anti-TEAP1(MSTP2109A), 2.4 nM, n/a
  - MMAE
  - Native cytotoxic residue, MC-vc-PAB linker
  - 1.8-2.0, 2.4 mg/kg, yes
  - Genentech, Inc. (128-131)

Targeting LIV1 (SLC39A6 or ZIP6) antigen, a member of the zinc transporter family playing a key role in tumor cell progression and metastasis:

- PF-06647263
  - Phase I, for treatment of metastatic breast
  - hulgGi1-anti-LIV1(hLIV22), 4.6 nM, n/a
  - MMAE
  - Native cytotoxic residues, VC protease-cleavable linker
  - n/a, n/a, yes
  - Seattle Genetics (135)

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Antibody therapeutic activity is usually mediated via immune-mediated effector functions such as Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody-Dependent Cell-Mediated Cytotoxicity (CDC), and cytokine signaling modulation in terms of inhibition or induction (Table 1). Such therapeutic activities can be further employed to design ADCs with enhanced cell killing activity. According to the obtained data in table 1, isotype 1 immunoglobulin (IgG1) seems to be prone to induce immunotherapeutic activity.

Hepatocyte Growth Factor Receptor (HGFR), Structural homolog of the urokinase-type Plasminogen Activator Receptor (uPAR), Tumor-associated antigen (C4.4a), Lung Squamous Cell Carcinoma (LSCC), Fibroblast growth factor receptor type 2 (FGFR2), Ovarian Cancers (OC), Trophoblast Glycoprotein (TPBG), metastatic Castration-Resistant Prostate Cancer (mCRPC), transmembrane epithelial antigen of the prostate-1 (STEAP1), Anti-solute carrier family 39 zinc transporter member 6 (SLC39A6, LIV-1; ZIP6), Anti-Endothelin B Receptor (ETBR), Auristatin. 0101 (Aur0101).
<table>
<thead>
<tr>
<th>ADC names</th>
<th>Clinical phase, indication</th>
<th>Ab, kd, therapeutics activity</th>
<th>Payload</th>
<th>Linkage strategy</th>
<th>DAR, MTD, by-stander effect</th>
<th>Sponsor, Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TENB2 ADC</td>
<td>Phase I, for treatment of prostate cancer</td>
<td></td>
<td></td>
<td>Native lysine residues,</td>
<td>2, n/a, n/a</td>
<td>Seattle Genetics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>prostate-labile VC-PABC-</td>
<td></td>
<td>(131,139).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ThioMab version of the</td>
<td>MAAE</td>
<td>linker</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>anti-TENB2 antibody (Pr1), 2.3 nM, n/a</td>
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</tr>
<tr>
<td>Targeting ETBR antigen, a G-protein coupled receptor that can activate RAF/MEK signaling:</td>
<td></td>
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</tr>
<tr>
<td>RG7636,</td>
<td>Phase I, for treatment of melanoma</td>
<td></td>
<td></td>
<td>Native lysine residues,</td>
<td>n/a, 2.4 mg/kg, n/a</td>
<td>Genentech, Inc.</td>
</tr>
<tr>
<td>DEDN-6526A</td>
<td></td>
<td>hulgG1 anti-ETBR, n/a, n/a</td>
<td>MAAE</td>
<td>prostate-labile VC-PABC-</td>
<td></td>
<td>(140).</td>
</tr>
<tr>
<td>Targeting integrin v3 antigen:</td>
<td></td>
<td></td>
<td></td>
<td>linker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMGN-388</td>
<td>Phase I, for treatment of NSCLC and prostate cancer</td>
<td></td>
<td></td>
<td>Native lysine residues,</td>
<td>n/a, 3.5 mg/kg, n/a</td>
<td>ImmunoGen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hulgG1anti-Integrin v3</td>
<td>DM4</td>
<td>prostate-labile VC-PABC-</td>
<td></td>
<td>(141).</td>
</tr>
<tr>
<td>Targeting crypto antigen, belonging to the EGF-CFC family of growth factor-like molecules, playing a key role in signaling pathways of certain transforming growth factor-beta super-family members:</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BIIB-015</td>
<td>Phase I, for treatment of breast, ovary, stomach, lung, and pancreas CRIPTO-expressing tumor cells</td>
<td></td>
<td></td>
<td>Native lysine residues,</td>
<td>n/a, n/a, n/a</td>
<td>Biogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hulgG1 anti-Cripto</td>
<td>DM4</td>
<td>prostate-labile VC-PABC-</td>
<td></td>
<td>(142).</td>
</tr>
<tr>
<td>Targeting AGS-5 (SLC44A4) antigen, a sodium-dependent transmembrane transport protein:</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>ASG-5ME</td>
<td>Phase I, for treatment of pancreatic, prostate and gastric cancers</td>
<td></td>
<td></td>
<td>Native cysteine residues,</td>
<td>n/a, n/a, n/a</td>
<td>Seattle Genetics/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hulgG2 anti-AGS-5, n/a, n/a</td>
<td>MAAE</td>
<td>VC-protease-cleavable</td>
<td></td>
<td>Astellas</td>
</tr>
<tr>
<td>Targeting LYM6E antigen, an interferon (IFN)-inducible glycosylphosphatidyl inositol (GPI)-linked cell membrane protein:</td>
<td></td>
<td></td>
<td></td>
<td>linker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RG7841,</td>
<td>Phase I, for treatment of HER2- breast cancer and NSCLC</td>
<td></td>
<td></td>
<td>Native cysteine residues,</td>
<td>n/a, n/a, n/a</td>
<td>Genentech, Inc.</td>
</tr>
<tr>
<td>DLYE5953A</td>
<td></td>
<td>n/a, n/a, n/a</td>
<td>MAAE</td>
<td>VC-protease-cleavable</td>
<td></td>
<td>(141).</td>
</tr>
<tr>
<td>Targeting AXL (UFO antigen, a member of the TAM (TYRO3, AXL and MER) family of RTK, playing a key role in tumor cell proliferation, survival, invasion and metastasis:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HuMax-Axl-ADC</td>
<td>Phase I, for treatment of multiple solid tumors</td>
<td></td>
<td></td>
<td>Native cysteine residues,</td>
<td>n/a, n/a, n/a</td>
<td>Gennab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hulgG1anti-Axl, n/a, n/a</td>
<td>DM4</td>
<td>VC-protease-cleavable</td>
<td></td>
<td>(145).</td>
</tr>
<tr>
<td>Targeting CD205 antigen, a type I C-type lectin receptor normally expressed on various APC and some leukocyte sub-populations:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MEN1309/OBT076</td>
<td>Phase I, for treatment of NHL</td>
<td></td>
<td></td>
<td>Native lysine residues,</td>
<td>n/a, n/a, yes</td>
<td>Menarini Ricerche</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hulgG1 anti-CD205, n/a, n/a</td>
<td>DM4</td>
<td>prostate-labile VC-PABC-</td>
<td></td>
<td>(146).</td>
</tr>
<tr>
<td>Targeting CD25 (IL-2R alpha) antigen, a TP and tumor-associated antigen (TAA), expressed on certain cancer cells:</td>
<td></td>
<td></td>
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<tr>
<td>ADCT-301, anti-CD25-PBD ADC</td>
<td>Phase I, for treatment of AML, ALL, relapsed HL and NSCL with CD25-positive HER2-negative breast expansion in LAMP-1 positive TNBC</td>
<td></td>
<td></td>
<td>PBD</td>
<td>Cleavable linker</td>
<td>ADC Therapeutics S.A.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hulgG1against CD25, n/a, n/a</td>
<td>PBD</td>
<td></td>
<td></td>
<td>(147).</td>
</tr>
<tr>
<td>Targeting LAMP-1 antigen, playing a key role in cell-cell adhesion and migration:</td>
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<td></td>
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<tr>
<td>SAR428926</td>
<td>Phase I, for treatment of HER2 negative breast expansion in LAMP-1 positive TNBC</td>
<td></td>
<td></td>
<td>Lysine residues, SPDB</td>
<td>n/a, n/a, n/a</td>
<td>Sanofi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hulgG1anti-LAMP1(AB-1)</td>
<td>DM4</td>
<td></td>
<td></td>
<td>(148).</td>
</tr>
<tr>
<td>Targeting MN/CA IX antigen, a TGP expressed in some human carcinomas and appears to be involved in cancer cell proliferation and transformation:</td>
<td></td>
<td></td>
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<tr>
<td>ADC BAY79-4620, MN-IC</td>
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<td></td>
<td></td>
<td>Native cysteine residues,</td>
<td>n/a, n/a, n/a</td>
<td>Bayer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hulgG1 anti-MN/CA IX, n/a, n/a</td>
<td>MAAE</td>
<td>VC-protease-cleavable</td>
<td></td>
<td>(149).</td>
</tr>
</tbody>
</table>

In this regard, many attempts have been made to engineer mAbs with therapeutic activity. For instance, the Fe domain affinity of anti-CD19 targeting antibodies for the FcyRIII has been enhanced, either by Fe glycoengineering approaches, e.g. MEDI-55 150 and MDX-1342 151 or amino acid substitution, e.g. XmAb5574 152 and XmAb 5871 or MOR-208 153, 154. Such modification resulted in an increase of ADCC activity in antibody. In the best of our knowledge, the above engineered antibodies have not been used for designing ADCs yet. However, there are some reports of ADCs which have employed a combination/fusion of two engineered antibody fragments. Such fusion antibodies are termed as bispecific Antibody (bsAb), while ADCs designed from the bsAbs were named bispecific ADC (bsADC) 154.

Blinatumomab and AFM11 are typical bispecific antibodies, two fusions of anti-CD19 scFv and anti-CD3 scFv, which were engineered to enhance CD19-positive cells killing activity through induction of T or NK cytotoxic immune effector cells. 35,155 A derivative of blinatumomab has been also constructed to induce the controlled T cell activation, named ZW38 156. The ZW38 was conjugated to a microtubule cytotoxic agent for the preparation of a novel class of bsADC capable of mediating T cell cytotoxicity. 156 Another bsADC, B10v5x225-H-vc-MMAE (Monomethyl auristatin E-MMAE), has been recently developed to simultaneously target EGFR and c-MET which are two tyrosine kinases receptors correlated with tumor growth and metastasis 157,158. B10v5x225-H-vc-MMAE contains a bsAb...
from fusion of anti c-MET Fab fragment and anti-EGFR scFv that was engineered to represent low affinity to EGFR which is a ubiquitous tissue antigen. The side effect of B10v5x225-H-vc-MMAE can be avoided to some extent due to attenuated affinity toward EGFR receptors in healthy cells. Bridging a rapidly internalizing protein with a tumor specific marker is also another recent method to construct bsAb, e.g., anti HER2 crosslink to prolactin cytoplasmic domain receptor with the ability to improve internalization and cell killing activity of the bsADC.

**Cytotoxic payloads in ADCs**

Briefly, cytotoxic payloads for new generation of ADCs should meet many of the criteria as outlined in figure 5. Antibody component in ADCs is incapable of carrying a large number of cytotoxic payload due to its structure. Therefore, the cytotoxic payload in the new generation of ADCs must be highly super-toxic to eradicate majority of the tumor cells even with minimal payload delivery. The rate of mAb uptake by tumor cells is approximately less than 0.003-0.08% of injected dose per gram in a tumor. Furthermore, low expression and poor internalizing activity of the most tumor-associated antigens can cause negligible ADC delivery to the tumor target cells. Hence, ADCs equipped with highly super-cytotoxic payload are imperative, because they must show therapeutic effect while having limited release. According to the reports, a highly cytotoxic agent should exhibit an IC50 of about 10 nM or less obtained from an examination with KB cells upon a 24-hr exposure time. A highly super cytotoxic payload can be originated from plant, animal or microorganisms; in this regard, the most important issue can be the finding of cytotoxic payloads with negligible immunogenic potential in the body. In new generation of ADCs, such cytotoxic payloads are likely to be chemical anti-cancer drugs since experimental evidence confirmed that they are less immunogenic than glycol/peptide cytotoxic agents when circulating in the blood. Some anticancer drugs such as doxorubicin (DOX), mitoxantrone, and etoposide are impaired under hypoxic condition; a condition appeared in solid cancer cell population. Hence, needless to say, those drugs may not be considered as cytotoxic payloads.

Taking a look at current cytotoxic drugs (Table 1) shows that they generally affect DNA synthesis or cell division to block cell proliferation (mitosis). Monomethyl auristatin derivatives which bind to tubulin and are able to inhibit microtubule assembly/polymerization (IC50=10-500 pM) are the most commonly used cytotoxic drugs in ADC design with approximately 50% share of the field. Maytansinoids derivatives (~30%), pyrrolobenzodiazepine (~7%), camptothecin analogs (~6%), n-acetyl-γ-calicheamicin (4%), duocarmycin (DUO) (~3%) and doxorubicin (~1%) are the other abundant cytotoxic payloads. The above cytotoxic compounds are 100 to10000 folds more potent in vitro than typical chemotherapeutic agents and are chosen based on their...
Antibody-Drug Conjugates: Possibilities and Challenges

Different actions on cancer and noncancerous cells. DNA modulators have significant effects on malignant cells as they are divided more rapidly than normal cells. Furthermore, a cytotoxic agent of the ADC is better to be studied in an *in vitro* condition to determine whether it is a substrate, inhibitor or inducer of metabolizing enzymes (e.g., cytochrome P-450 isozymes (CYPs), and some transporter enzymes like P-glycoprotein). Such studies help to elucidate the *in vitro* factors that may be contributed to the elimination/enhancement of the cytotoxic agent. New studies to introduce new payloads focused on agents against Tumor-Initiating Cells (TICs). Such payloads assist to widen the target area and to circumvent potential resistance of cancer cells. Pyrrolobenzodiazepines (PBDs), derivatives of naturally occurring tricyclic antibiotics, duocarmycins, anthracyclines, α-amanitin (a bicyclic octapeptide from the fungus Amanita), and topoisomerase inhibitors including SN-38 are categorized as TIC payloads.

Rovalpituzumab tesirine is one example of ADC with PBD as a payload (Table 1), that has been reported to have a potency to eliminate pulmonary neuroendocrine TICs at subpicomolar level *in vivo*. The cytotoxic payload should be also stable during preparation or storage and circulation in the blood. Cytotoxic payloads that are not fully stable can potentially be converted to undesirable drug forms during conjugation or storage. Solubility of the cytotoxic agent in aqueous solution is another important criterion in ADC design. Antibody is considered a protein and its conjugation to the cytotoxic agent must be performed in aqueous solutions with minimal organic cosolvents. Extreme hydrophobicity of payload can potentially change antibodies biological properties, resulting in hydrophobic aggregation of the antibody either during conjugation process or storage. The hydrophilicity of cytotoxic payloads will affect cell membrane permeability of parent ADC or its metabolites which may also be beneficial in term of bystander activity. However, the ability of cytotoxic payloads to form hydrophobic metabolite after intercellular cleavage of ADC is preferable since the metabolites with more hydrophobic group show better blood clearance and safety. According to the reports, about 95-99% of ADC molecules are metabolized before binding to tumor cells. This may raise safety concern as it can enhance the potential cytotoxic side effects of ADC. Thereby, the use of cytotoxic payloads with well-characterized metabolite profiles can be an essential consideration in choosing cytotoxic payloads for ADC design and development.

![Figure 4. Kd frequency distribution (a) and histogram data (b) of current ADC in clinical development (Table S1, n=13). Antibody affinities (Kd) that have been used in current ADC in clinical development were classified into either ≤10 nM or ≥10 nM groups. The average Kd and standard deviation of ≤10 nM group was 1.12 and 1.3 and for ≥10 nM group was 39.9 and 28.2, respectively. Median Kd of ≤10 nM group and ≥10 nM groups was 0.7 and 40.5, respectively. Average Kd was significantly different between two groups (p<0.05). The frequency distributions of Kd in ≥10 nM groups are more than ≤10 nM groups (a).](image)

![Figure 5. Main considerations in choosing cytotoxic payloads for ADC design and development.](image)
Cytotoxic payload should present a dominant functional group suitable for linkage to the antibody component of ADC. If a dominant functional group does not exist on the cytotoxic agent, at least, it should be amenable to modification, in which a desired substituent is introduced on appropriate sites.

The copy number and heterogeneity of antigen expression are the other important issues that must be considered in the selection of cytotoxic agent. More expression of target antigen may be a reason to apply a cytotoxic agent with low potency. Typically, payloads that promote the bystander effect in cancer cells are more desirable to design ADCs directed for the antigens expressed heterogeneously.

The ability to choose specified cytotoxic payloads with mechanism of action compatible with standard of care has been reported to facilitate clinical success of the ADCs in biopharmaceutical market. For instance, microtubule disrupting payloads are commonly chemotherapeutic drugs that are used for the treatment of cancers, including breast, ovarian and prostate cancer. Both availability in the market and reasonable cost can be alternative rationale for choosing a cytotoxic payload in ADC design.

**Linking cytotoxic payloads to antibodies in ADCs**

One of the dynamic research fields in ADC design is the study of the methods that are correlated with antibody conjugation to cytotoxic payloads, as it has a great role on balancing between ADC therapeutic efficacy and toxicity. The key concerns in linkage chemistry are demonstrated in figure 6. Conjugation site on antibody component, a well-defined Drug to Antibody Ratio (DAR), homogeneity and linkage stability are the important parameters that need to be considered in conjugation.

In general, interchain disulfide bridges and surface-exposed lysines are the most currently used residues on the antibody for conjugation to cytotoxic payloads, respectively (>50 vs. >30%) (Table 1). Hydroxyl groups on carbohydrate structures are the other residues in antibodies that have been rarely used as conjugation sites for ADC (The schematic linkage in figure 6 is an example of this strategy). Theoretically, the linkage of cytotoxic payloads to the surface-exposed lysine of mAb occurs after reduction of ~40 lysine residues on both heavy and light chain of mAb and it results in 0-8 cytotoxic payload linkages per antibody and heterogeneity with about one million different ADC species. Cysteine conjugation occurs after reduction of four interchain disulfide bonds and results in eight exposed sulfhydryl groups. Linking drugs per antibody can differ from zero to 8 molecules, generating a heterogeneous population of ADC (Greater than one hundred different ADC species).

Due to low stability and safety properties of the pharmaceutical products with heterogeneous contents, they are complex to be accurately predicted in terms of efficacy or therapeutic window. Therefore, improvement of conjugation methods to achieve homogeneous ADC is very crucial.

In this case, it is possible to reduce just two of four interchain mAb’s disulfide bonds of cysteine residues through carefully mild reduction conditions, as interchain disulfide bridges are more prone to reduction than intrachain disulfide bridges. However, such mild reduction is not easily possible in practice and a diverse number of cysteines may be reduced (0-4), resulting in a heterogeneous mixture of ADC. Hence, the production of homogeneous ADCs through payload conjugation with native residues can be laborious. To overcome this limitation, many site-specific conjugation approaches have been developed, in which a known number of cytotoxic payloads are constantly conjugated to defined sites on mAbs. Some of the approaches are explained below:

1. A conjugation through engineered cysteine residues that neither damages antibody fab region nor interferes with Fc-mediated effector functions, called THIOMAB technology. In THIOMAB technology, the heavy chain alanine 114 is substituted with two or more reactive cysteine residues at a predefined site for conjugation with cytotoxic payload. Anti-TENB2 ADC is an example that is prepared by THIOMAB technology and is currently in phase I trial (Table 1).
2. Re-engineering of mAb is able to incorporate with unnatural amino acids, e.g. selenocysteine \(^{177}\), acetyl-phenylalanine \(^{179}\), and para-azidomethyl-l-phenylalanine \(^{42}\).
3. Site-specific enzyme-mediated conjugation to genetically engineered antibody is as follows: Incorporating a thiolted sugar analogue, 6-thiofucose, to the antibody carbohydrate that introduces new chemically active thiol groups using fucosyltransferase VIII \(^{176}\).

Providing a ketone reactive group on antibody glycosylation site by glycotransferases \(^{180}\).

Introducing an aldehyde reactive group on the antibody using sialyltransferase \(^{181}\) or formylglycine-generating enzyme \(^{182}\). Genetically introducing specific glutamine tags to antibody whereby payloads with a primary amine group can be linked to the \(\gamma\)-carbonyl amide group of glutamine tags. Such reaction is catalyzed by a microbial transglutaminase which is capable of recognizing glutaminine tags from naturally glutamines residues \(^{175,183}\).

Providing LPXTG tagged antibodies (A penta-peptide as a substrate for transpeptidation reaction) as specific linkage sites for the oligo-glycine-containing payloads, which are mediated by Staphylococcus aureus Sortase A enzyme \(^{184}\).

Conjugation of phosphopantetheine-linked payloads to the serine residues of the peptide-tagged antibodies via phosphopantetheinyl transferases catalysis \(^{185}\).

4. Chemoenzymatic site direct conjugation, e.g., providing two azide groups at asparagine 297 (Asn-297) residue in antibody constant region (Fc) is linked with cytotoxic payloads using copper-mediated click reaction \(^{186}\). The azide functional groups are formed in a selective hydrolysis reaction that is mediated by an Endo-beta-N-acetylglucosaminidase (EndoS) chemo-enzyme.

ADC as a potential targeted delivery system must be passed through all hurdles, including blood circulation, antigen binding, internalization, payload release, and eventual payload action. An unstable linkage can lead to premature release of the payload, before reaching the site of action \(^{188}\). Therefore, reasonable chemical stability must be considered in the design of chemical linkage between cytotoxic payload and antibody.

Although a direct linkage between cytotoxic and antibody components has generally shown more stability in circulation \(^{190}\), conjugation reactions are mostly created with linkers in comparison with direct linkage between cytotoxic and antibody component (Table 1). The choice of proper linkers has been discussed in the related publications devoted to the progress of ADCs \(^{30,31,54,189,190}\). As shown in table 1, about 50% of the ADCs are using Valine-Citrulline peptidyl (VC) linker. N-succinimidyl 4-(2-pyridyldithio) butyrate (SPDB) (18%), acid-labile hydrazine (10%), maleimidomethyl cyclohexane-1-carboxylate (MCC), maleimidocaproyl (MC) (10%), N-succinimidyl 4-(2-pyridyldithio pentanoate (SPP) and carbonate (3%) linkers are other employed linkers.

Limited drug-linker designs for more than 70 current ADC clinical trials (Table 1) are a dilemma regarding linkage chemistry that may restrict simultaneous development of ADCs against both hematological and solid tumors. Generally, the properties of linkers can be altered by the cytotoxic payload release mechanism \(^{191}\). Cytotoxic payload in ADC technology must be released into the cell to exert its therapeutic activity, thus ADC linkers should be chosen based on their stability to keep ADC intact during circulation and capable of cleaving inside the targeted cell \(^{191,192}\). Linker stability is defined based on lack/low level of cleaving agents (e.g., protease or reductive agents) in the bloodstream compared to the cytoplasm \(^{193}\).

The current linkers used in ADCs are also broadly classified as cleavable and noncleavable linkers based on where they are cleaved into the cytoplasm. Cleavable linkers are those containing a conditional cleavage sites sensitive to be cleaved immediately after ADC internalization, such as VC, SPDB, SPP, and hydrazine which can be triggered through protease reactions, glutathione reduction, and acidic pH, respectively \(^{185,186}\). Noncleavable linkers are stable from early to late endosome transition and their cytotoxic partner is just released by degradation of antibody in lysosomes, e.g. MCC and MC linkers that link Ab to the payload via thioether linkage \(^{190}\).

Characteristics of ADC target such as copy number, internalization rate and level of homogeneity should be considered in conjugation method and linker selection. For instance, ADC with disulfide-linkage has been shown to have more cytotoxic activity than the same ADC with thioether linkage when they were directed to the tumor cell lines expressing a low copy number of targeted antigen \(^{17}\).

Cleavable linkers may increase the possibility of bystander effect \(^{195}\). Hence, it is logical to use cleavable linkers in designing ADCs directed for the antigen that is heterogeneously expressed in tumors \(^{196}\).

In vivo adverse effects of ADCs are influenced by the use of cleavable or noncleavable linkers. As in the case of tubulin inhibitor payloads, which is linked through cleavable linkers to the antibody component, e.g. SPDB-DM4 (Ravtansine-DM4), or VC-MMAE, peripheral neuropathy can be frequently observed, whereas noncleavable linkers often trigger hematological toxicity, possibly due to an increased dose and interactions with Fc receptors on hematopoietic cells \(^{197}\).

The type of linker plays an important role in ADC catabolite products with regard to processing into target cells or metabolizing by clearance mechanisms. The type of ADC catabolites may influence some ADC features such as IC50, Maximum Tolerated Dose (MTD) \(^{192,193}\), and kill Multidrug Resistance (MDR) expressing cells \(^{192,194}\).
Conclusion

ADC is considered exciting and promising antibody-based therapeutics to improve cancer therapy. Growth in the number of registered ADCs in clinical trials (Table 1) represents the pharmaceutical industry interest in investment for research and development in the field, as it has been stated by others. The design of an ADC might seem to be not very complex, while several issues must be taken into consideration to complete ADC’s potential as a therapeutic agent for cancer. This might be the main reason for the condition that small number of ADCs have reached the market (Table 1). The major issues associated with the development of ADCs seem to be originated from the factors that interfere with ADCs efficacy and off-target cytotoxicity. The precise selection of all four parameters, i.e. tumor marker, antibody, cytotoxic payload, and linkage strategy would be required to prepare a successful ADC.

With regard to ADC tumor markers, they do not have to be involved in tumor growth. Therefore, ADC can present therapeutic application in a broad range of tumors. However, an ADC tumor marker should meet at least three criteria of considerable expression level in tumor cells vs. normal cells, presenting cell surface immunogen, and being capable of performing ADC internalization.

High specificity, adequate affinity, and receptor-mediated internalization are the major aspects of antibody choice. Efforts to optimize antibody component would be a great idea to translate into improved ADCs. In fact, some major ADCs’ weaknesses including, low efficiency, low internalization, off-target effect due to the target expression in normal tissues, and heterogeneity expression of the target in the tumors can be overcome via antibody improvement. Antibody engineering technology for production of alternative bsAbs to design more efficient ADCs (bsADCs) has been proven in several preclinical models. The rationale behind this technology is the fact that the aforesaid ADC’s weaknesses can be solved through ADC designs (bsADCs) operating from improved antibody (bsAb) in terms of affinity, specificity, internalization activity, by enhancing the therapeutic activity or decreasing ADC’s side effects.

Another main concern in the development of ADCs is related to the study of finding cytotoxic payloads that are potent enough with confined DAR (Up to 7 drugs per antibody) to exert therapeutic activity. Having reasonable aqueous solubility, non-immuno-genic, as well as stability in storage and bloodstream is a common criterion for choosing cytotoxic payloads.

In contrast, the introduction of innovative methods to modify ADCs cytotoxic payloads with versatile functional groups (e.g. thiol, amine groups) is the other interesting subject, as it eases the conjugation process. One further challenge of ADCs is associated with the limitation of linkage and conjugation chemistry to link an optimized number of the payloads to the antibody in predefined location homogeneously.

Interdisciplinary and multidisciplinary works and related studies such as recombinant DNA technology, bioconjugation, and chemistry are the hopeful strategies to get the purpose of achievement in site-specific conjugation and homogeneous ADCs.

Based on promising reports from research to synthesize homogeneous ADCs, it is likely that the first ADC products constructed using site-specific conjugation will be made for cancer therapy that may hold the promise about the future use of ADCs.

Taken together, despite challenges in ADC design, the future of ADCs seems to be much promising as more clinical trials and basic researches conducted on existing ADCs would pave the way to tackle issues regarding tumor marker, antibody, cytotoxic payload, and linkage strategy.

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Conflict of Interest

The authors declare that they have no competing interests.

References


75. Wang X, Ma D, Olson WC, Heston WD. In vitro and in vivo responses of advanced prostate tumors to PSMA ADC, an auristatin-conjugated antibody to prostate specific membrane antigen. Mol Cancer Ther 2011;10(9):1728-1739.


147. Horwitz SM, Fanale MA, Spira AI, Havenith K, He S, Feingold JM, et al. Interim data from the first clinical study of ADCT-301, a novel pyrroplomenzodia-pine-


