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 specified 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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Antibody component in ADCs undertakes both roles including being a carrier and targeting agent. The main aspects of the antibody in ADCs are demonstrated in figure 3. High specificity of targeting and minimal immunogenicity are the main characteristics for Ab component in ADCs.

Antibodies in ADCs

Antibody component in ADCs undertakes both roles including being a carrier and targeting agent. The main aspects of the antibody in ADCs are demonstrated in figure 3. High specificity of targeting and minimal immunogenicity are the main characteristics for Ab component in ADCs.
<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>
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<th>Sponsor, Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kadyela, Ato-Trastuzumab entansine, T-DM1</td>
<td>Approved in 2013, for treatment of her2 positive breast cancer</td>
<td>hulgG1 (trastuzumab), n/a, ADCD and HER2-dependent PI3K/AKT 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>Synthon 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>Medicine/Ambrx (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>
</tbody>
</table>

| Targeting HER2 antigen, a transmembrane RTKs in the growth of some cancer cells: |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| ABT-414, Deputaxizumab mafodotin | Phase I, for treatment of GBM | hulgG1 anti EGRF (ABT-806), 0.06 nm, inhibits EGRF signaling | MMAF | Native cysteine residues, MC noncleavable linker | ~3.8,1.5 mg/kg, no | Abbvie (43) |
| AMG 595 | Phase I, for treatment of GBM | hulgG1anti-EGFRVIII, 0.61 nM, n/a | DM1 | Native lysine residues, SMCC noncleavable thioether linker | ~3.5, n/a, no | Amgen (44) |
| IMGN289, Laprituximab entansine | Phase I, for treatment of NSCLC and HNSCC | hulgG anti-EGFR (J2898A), n/a, n/a | DM1 | Native lysine residues, SMCC noncleavable thioether linker | n/a, n/a, no | ImmunoGen (45) |
| ABBV-221 | Phase I, for treatment of solid tumor | hulgG1 anti-EGFR, n/a, n/a | MMAE | Vc-protease-cleavable linker | n/a, n/a, no | Abbvie (46) |

| Targeting CD70 ( CD27L) antigen a TP2 and member of the tumor necrosis factor family: |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| SGN-75 | Phase I, for treatment of CD70-positive NHL and metastatic RCC | hu anti-CD70 (h1F6), n/a, n/a | MMAF | Native cysteine residues, MC noncleavable linker | n/a, 3, n/a | Seattle Genetics (47) |
| MDX-1203, BMS-936561 | Phase I, for treatment of ccRCC or B-NHL | hu anti-CD70, n/a, n/a | DUO | Native cysteine residues, VA-protease-cleavable linker | n/a,15 mg/kg, yes | Bristol-Myers (48) |
| SGN-CD70A | Phase I, for treatment of RCC, MCLD, LBC, FL, and NSCLC | hu anti-CD70, n/a, n/a | PBD | VA linker | n/a, n/a, yes | Seattle Genetics (49) |
| AMG 172 | Phase I, for treatment of ccRCC | hulgG1, n/a, n/a | DM1 | Native lysine residues, MCC noncleavable linker | n/a, n/a, no | Amgen (50) |

| Targeting CD33 antigen, a EGP: |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mylotarg, Gemtuzumab Ozogamicin (GO) | Withdrawn 2010 and approved in 2017, for treatment of CD33+ AML | hulgG4, n/a, n/a | Calich. | Native lysine residues, (AcBut)-N-acetyl acidlabile hydrazono linker | n/a, 0.25 mg/kg, yes | Pfizer (51) |
| SGN-CD33A | Phase I, for treatment of AML with engineered cytostesines, n/a, n/a | PBD | Engineered cysteine residues, VA linker | n/a, n/a, yes | Seattle Genetics (12,13) |
| AVE9633 | Phase I, for treatment of AML | anti-CD33, n/a, n/a | DM4 | SPDB disulfide cleavable linker | n/a, n/a, n/a | Sanofi (53) |

Not available (n/a), Relapsed B-cell non-Hodgkin's lymphoma (B-NHL), Acute myeloid leukemia (AML), Mertansine (DM1), Calicheamin (calich.), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), Hydrazone 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-hydroxypropylamide (AF-HPA), Polycetal-based polymer (Fleximer®), Non-natural amino acid linker para-acetyl-phenylalanine (pAcF), Ambrinatin, a short polyethylene glycol (PEG) spacer terminated by an alkoxysamine (AS269).
### Table 1: Antibody-Drug Conjugates: Possibilities and Challenges

<table>
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<tr>
<th>ADC names</th>
<th>Clinical phase, indication</th>
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<tbody>
<tr>
<td><strong>Targeting CD9 by TNP on B cells as an accessory molecule for B-cell signal transduction and TAA:</strong></td>
<td></td>
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</tr>
<tr>
<td>SAR3419, coltuximab raviotanise</td>
<td>Phase II, for treatment of B-NHL and B-ALL</td>
<td>huLG1 anti-CD9 (hBu12), n/a, ADCD</td>
<td>MMAE</td>
<td>Native tyrosine residues, noncleavable linker</td>
<td>n/a, 6.0, no</td>
<td>Seattle Genetics (58)</td>
</tr>
<tr>
<td>SGN-CD19A</td>
<td>Phase I, for treatment of B-Cell Malignancies</td>
<td>huLG1 anti-CD9 (hBu12), n/a, ADCD</td>
<td>MMAE</td>
<td>Native tyrosine residues, noncleavable linker</td>
<td>n/a, 6.0, no</td>
<td>Seattle Genetics (32)</td>
</tr>
<tr>
<td>ADCT-402</td>
<td>Phase I, for treatment of relapsed or refractory B-ALL</td>
<td>huLG1anti-CD9, n/a, n/a</td>
<td>PBD</td>
<td>Native tyrosine residues, noncleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>ADC Therapeutics S.A. (33)</td>
</tr>
<tr>
<td><strong>Targeting Mesothelin antigen, a glycophorin anchored protein:</strong></td>
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</tr>
<tr>
<td>BAY 94-9343, anetumab raviotanise</td>
<td>Phase II, for treatment of MPM</td>
<td>hu anti-mesothelin, n/a, n/a</td>
<td>DM4</td>
<td>Lysine residues, noncleavable linker</td>
<td>n/a, 6.5 mg/kg, yes</td>
<td>Bayer (57)</td>
</tr>
<tr>
<td>BMS-966148</td>
<td>Phase I &amp; II, for treatment of Mesothelin-expressing cancers</td>
<td>Anti mesothelin</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a, n/a, n/a</td>
<td>Bristol-Myers (58)</td>
</tr>
<tr>
<td>DMO74039A</td>
<td>Phase I, for treatment of pancreatic and P-OC</td>
<td>hu anti-mesothelin (T9D,v5), 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><strong>Targeting CEACAM5 antigen, labetuzumab, CEA, CD66e, a EGP that has a role in cell adhesion and invasion:</strong></td>
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</tr>
<tr>
<td>IMMU-130, hMN14-SN38, labetuzumab goveicen, labetuzumab-SN38</td>
<td>Phase II, for treatment of mCRC</td>
<td>huLG1 anti-CEACAM5 (hMN14), 1.5 nm, ADCD</td>
<td>SN-38</td>
<td>Native tyrosine residues, noncleavable 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>huLG1 anti-CEACAM5, n/a, n/a</td>
<td>DM4</td>
<td>Lysine residues, noncleavable linker</td>
<td>n/a, n/a, yes</td>
<td>Sanofi (66)</td>
</tr>
<tr>
<td><strong>Targeting Trop-2 (M1S1, TACSTD2 or GA733-1) antigen, a EGP that transduces calcium signal has a role in ERK1/2 MAPK pathway which mediates cancer cell proliferation, migration, invasion, and survival:</strong></td>
<td></td>
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</tr>
<tr>
<td>IMMU-132, hr57-SN38, Sacituzumab goveicen, Sacituzumab-SN38</td>
<td>Phase III, for treatment of pancreatic cancers, SCLC and TNBC</td>
<td>huLG1 anti-trop-2 (R57 or Sacituzumab), 0.564 nm, ADCD</td>
<td>SN-38</td>
<td>Native tyrosine residues, noncleavable linker</td>
<td>~7.6, 8–10 mg/kg, yes</td>
<td>Immunomedics (67-72)</td>
</tr>
<tr>
<td>PF-06644178, Trop-2 ADC, RN927C</td>
<td>Phase I, for treatment of OC, SCLC and breast cancer</td>
<td>Engineered huLG1anti-trop-2, 14 nm, n/a</td>
<td>PF063801 01</td>
<td>Site-specific trastuzumab tag, Acl,lys-VC-PAB linker</td>
<td>2.0, n/a, n/a</td>
<td>Pfizer (73)</td>
</tr>
<tr>
<td><strong>Targeting PSMA antigen, a TP2 that has known enzymatic activity and acts as a glutamate-prefering carboxypeptidase:</strong></td>
<td></td>
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</tr>
<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 tyrosine residues, noncleavable 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 (hj591), n/a, n/a</td>
<td>DM1</td>
<td>Native tyrosine residues, noncleavable 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 733-1 (GA733-1), Malignant Pleural Mesothelioma (MPM), Platinum-resistant ovarian cancer (P-OC).

**Key Points:**

- Receptor-mediated antibody internalization is a key mechanism underlying antibody endocytosis that is induced through antibody binding to its specific antigen.
- It has been reported that, alternative antibodies against the same immunogen can exhibit different rates of internalization. Rapid internalization can raise both ADC efficacy and safety simultaneously, since it reduces the opportunity of the ADC for off-target release.
- 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. For instance, Clathrin-coated Pit-mediated receptor internalization.
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:

**ADC names**

- IMGN529, Naratuximab
- AGS67E

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<tr>
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</thead>
<tbody>
<tr>
<td>IMGN529, Naratuximab</td>
<td>Phase I or II, for treatment of BCL, CLL, NHL</td>
<td>huIgG1 anti-CD37 (K7153A), n/a, ADCC and CDC, huIgG2 anti-CD37 (AGS67C or vCD37-9a73), n/a, n/a</td>
<td>DM1</td>
<td>Native lysine 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, DLBCL with high level of CD37 expression</td>
<td></td>
<td>MMAE</td>
<td>Native cytoeines residues, VC protease-cleavable linker</td>
<td>n/a, 1.2 mg/kg, yes</td>
<td>Agenysys (80-81)</td>
</tr>
</tbody>
</table>

Targeting CD39 (TNFRSF8) antigen, a tumor necrosis factor:

- Adectris, brentuximab vedotin, SGN-35

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Adectris, brentuximab vedotin, SGN-35</td>
<td>Approved in 2011, for treatment of HL and ALL.</td>
<td>Chimeric IgGlanti-CD30 (eAC10 or SGN30), n/a, n/a</td>
<td>MMAE</td>
<td>Native interchain cytoeine, MC-VC- PABC linker</td>
<td>~4, 1.8 mg/kg, yes</td>
<td>Seattle Genetics (67)</td>
</tr>
</tbody>
</table>

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:

- DOX, IMMU-110, hLL1-
- DOX, VYER-1

<table>
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</thead>
<tbody>
<tr>
<td>DOX, IMMU-110, hLL1-</td>
<td></td>
<td>hLL1-DOX</td>
<td>DOX</td>
<td>Native lysine residues, Acid-labile hydrazide linker</td>
<td>n/a, n/a, yes</td>
<td>ImmunoMedic (95)</td>
</tr>
</tbody>
</table>

Targeting CD79b antigen, a TP1 on B cells mediates signal transduction cascade activated by BCR:

- Rovalpituzumab tesirine, Rova-T, RNIB0600A

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<tr>
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<th>DAR, MTD, by-stander effect</th>
<th>Sponsor, Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rovalpituzumab tesirine, Rova-T, RNIB0600A</td>
<td>Phase I &amp; II, for treatment of NSCLC and melanoma cancer</td>
<td>huIgG2 (CR011), n/a, no</td>
<td>MMAE</td>
<td>Cysteine residues, VC protease-cleavable linker</td>
<td>~4.5, 1.9 mg/kg, yes</td>
<td>Cellidex Therapeutics (84-87)</td>
</tr>
</tbody>
</table>

Targeting NaPi2b antigen, a sodium phosphate transporter 2b (NaPi2b), Transmembrane cell surface receptor guanylyl cyclase C (GCC), Delta-like protein 3 (DLL3), polyethylene glyc ol spacer (PEG8), Selective Catalytic Reduction (scr), Metastatic Urothelial Cancer (MUC), B-Cell Maturation Antigen (BCMA), DX-8951 a derivative of the camptothecin analog exatecan (DXd).

- GlaxoSmithKine
- Millenium
- Seattle Genetics
- Stemcentrx

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<tbody>
<tr>
<td>GlaxoSmithKine</td>
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<tr>
<td>Millenium</td>
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<tr>
<td>Seattle Genetics</td>
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<tr>
<td>Stemcentrx</td>
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</table>

Targeting HER3 antigen, a TP1 on B cells mediates signal transduction cascade activated by BCR:

- Lifiastuzumab vedotin, RG7599, RNIB0600A

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<th>Sponsor, Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifiastuzumab vedotin, RG7599, RNIB0600A</td>
<td>Phase I, for treatment of OC, breast, cervical, lung cancers</td>
<td>huIgG1 anti-CA6 (hDAS6 IgG1), n/a, n/a</td>
<td>DM4</td>
<td>Native lysine residues, SPDB disulfide cleavable linker</td>
<td>6.5 mg/kg</td>
<td>Sanofi (93,94)</td>
</tr>
</tbody>
</table>

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

<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>Milatuzumab-doxorubicin, IMMU-110, HLL1-DOX</td>
<td></td>
<td>hu-anti-CD74</td>
<td>DOX</td>
<td>Native lysine residues, Acid-labile hydrazine linker</td>
<td>n/a, n/a, yes</td>
<td>Immunomedics (95)</td>
</tr>
</tbody>
</table>

Targeting CD138 antigen, syndecan1, a type I transmembrane heparan sulfate proteoglycan participates in cell proliferation, cell migration and cell-matrix interactions:

- BT-062, Indatuximab ravtansine

<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>BT-062, Indatuximab ravtansine</td>
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</tbody>
</table>

Targeting BCMA antigen, a receptor for a proliferation-inducing ligand and B-cell activating factor:

- GSK2857916

<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>
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</thead>
<tbody>
<tr>
<td>GSK2857916</td>
<td>Phase I, for treatment of MM</td>
<td>Engineered afucosylated huIgG1 anti-BCMA, 1 nM, ADCC</td>
<td>MMAF</td>
<td>Native cytoeines residues, MC noncleavable linker</td>
<td>n/a, n/a, no</td>
<td>GlaxoSmithKine (97)</td>
</tr>
</tbody>
</table>

Target sodium phosphate transporter 2b (NaPi2b), Transmembrane cell surface receptor guanylyl cyclase C (GCC), Delta-like protein 3 (DLL3), polyethylene glycol spacer (PEG8), Selective Catalytic Reduction (scr), Metastatic Urothelial Cancer (MUC), B-Cell Maturation Antigen (BCMA), DX-8951 a derivative of the camptothecin analog exatecan (DXd).
Antibody-Drug Conjugates: Possibilities and Challenges

Contd table 1.

<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>Targeting specific myeloma antigen:</td>
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</tr>
<tr>
<td>DFRF4539A, RG7598</td>
<td>Phase I, for treatment of MM</td>
<td>n/a, n/a, n/a</td>
<td>MMAE</td>
<td>n/a</td>
<td>n/a, n/a, n/a</td>
<td>Genentech, Inc. (100)</td>
</tr>
<tr>
<td>Targeting SLAMF7 (CS1) antigen:</td>
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<tr>
<td>ABBV-838</td>
<td>Phase I, for treatment of MM</td>
<td>hulgGl anti-SLAMF7, n/a, n/a</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Abbbvie (101)</td>
</tr>
<tr>
<td>Targeting CD56 antigen, associates with FGFR and stimulates RTKs to induce neurite outgrowth:</td>
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<tr>
<td>IMGN901, Lorotuzumab mertansine, huN901-DM1/BB-10901</td>
<td>Phase I &amp; II, for treatment of CD56+ MM</td>
<td>hulgGl anti-CD56 (Lorotuzumab or N901), 0.002 nM, ADC</td>
<td>DM1</td>
<td>Lysine residues, SPP disulfide cleavable linker</td>
<td>3.7, 2.0 mg/kg, n/a</td>
<td>ImmunoGen (102)</td>
</tr>
<tr>
<td>Targeting ENPP3 (CD203c) antigen, a TP2 belongs to a series of ectoenzymes, possess ATPase and ATP pyrophosphatase activities:</td>
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<tr>
<td>AGS-16C3F</td>
<td>Phase I &amp; II, for treatment of RCRCC</td>
<td>hulgGl2k anti-ENPP3 (AGS16-7.8), 0.3-1.1 nM, no</td>
<td>MMAF</td>
<td>Native cysteine residues, MC noncleavable linker</td>
<td>~ 4, 1.8 mg/kg, no</td>
<td>Astellas Pharma (103,104)</td>
</tr>
<tr>
<td>Targeting TF (CD142) antigen, a TP and initiator of the coagulation cascade:</td>
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<tr>
<td>Humax-TF-ADC, tisotumab vedotin</td>
<td>Phase I &amp; II, for treatment of Multiple solid tumors</td>
<td>IgG1 anti-TF</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a,1.8 mg/kg, yes</td>
<td>Gemnab (105)</td>
</tr>
<tr>
<td>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:</td>
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<tr>
<td>CDX-014</td>
<td>Phase I &amp; II, for treatment of RCC</td>
<td>hulgG1anti-TIM1</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Celldice Therapeutics (106)</td>
</tr>
<tr>
<td>Targeting FOLR1 antigen, a membrane-bound protein regulates transport of the vitamin B9 into cells:</td>
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<tr>
<td>IMGN853, mirvetuximab soravatansine</td>
<td>Phase I, for treatment of folate receptor alpha (FRα)-positive cancer, e.g., relapsed EOC</td>
<td>FRa-binding antibody</td>
<td>DM4</td>
<td>Native lysine residues, Sulfo-SPDB disulfide cleavable linker</td>
<td>n/a, 6 mg/kg, yes</td>
<td>ImmunoGen (17,107-110)</td>
</tr>
<tr>
<td>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:</td>
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<tr>
<td>RG7458, Sofituzumab Vedotin, DMUC5754A</td>
<td>Phase I, for treatment of ovarian and pancreatic cancer</td>
<td>IgG1anti-MUC16 (OC125), n/a, n/a</td>
<td>MMAE and MMAF</td>
<td>Native cysteine residues, MC-VC-PABC linker</td>
<td>n/a, 2.4 mg/kg, yes</td>
<td>Genentech, Inc. (111)</td>
</tr>
<tr>
<td>Targeting CanAg antigen, is a novel glycoform of mucin family GP:</td>
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<tr>
<td>IMGN242, HuC242-DM4, cantuzumab ravnatsine</td>
<td>Phase I, for treatment of Non-cololetal and Pancreatic Cancer</td>
<td>hu anti-CanAg (C242 or cantuzumab), n/a, n/a</td>
<td>DM4</td>
<td>Native lysine residues, SPDB disulfide cleavable linker</td>
<td>n/a, n/a, yes</td>
<td>ImmunoGen (112)</td>
</tr>
<tr>
<td>Targeting Kiat (CD117 or SCFR) antigen, a TP and RTKs having a key role in the regulation of cell differentiation and proliferation:</td>
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</tr>
<tr>
<td>LOP628, Anti c-KIT ADC</td>
<td>Phase I, for treatment of AML and solid tumors</td>
<td>hulgGlanti-(c-Kit), n/a, n/a</td>
<td>DM1</td>
<td>Native lysine residues, SMCC noncleavable thioether linker</td>
<td>n/a, n/a, no</td>
<td>Novartis (113)</td>
</tr>
<tr>
<td>Targeting EphA2 antigen, belonging to ephrin receptor subfamily of the RTKs family regulating cell migration, adhesion, proliferation and differentiation:</td>
<td></td>
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<tr>
<td>MEDI-547, MI-CP177</td>
<td>Phase I, for treatment of relapsed or refractory solid tumors associated with EphA2 expression</td>
<td>hulgGl anti-EphA2 (1C1), InM, n/a</td>
<td>MMAF</td>
<td>Native cysteine residues, MC noncleavable linker</td>
<td>4, 6.0 mg/kg, no</td>
<td>Medimmune (114,115)</td>
</tr>
<tr>
<td>Targeting Nectin 4 (PVRL4) antigen, a TP1 and member of a family of cellular adhesion molecules, involved in Ca2+ independent cellular adhesion:</td>
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<tr>
<td>ASG-22ME, AGS-22M6E, anti-nectin-4 ADC, Enfortumab vedotin</td>
<td>Phase I, for treatment of MUC</td>
<td>hulgGl anti-nectin-4 (AGS-22M6E) 0.01 nM, n/a</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a,1-3 mg/kg, yes</td>
<td>Astellas Pharma (116,117)</td>
</tr>
</tbody>
</table>

Folate receptor 1 (FOLR1), Maleimidoacrylo-valine-citrulline- (MC-VC-PABC), Carbohydrate antigen 125 (CA-125), Mucin 16 (MUC16), A high molecular weight mucin-type glycoprotein (CanAg), Erythropoietin producing hepatoma A2 receptor (EphA2 or EP HA2), Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3), Poliovirus receptor related protein 4 (PVRL4), 2 N-terminal Leucine-Rich Repeat (LRR), Human Tissue Factor (TF), Stem Cell Factor Receptor c-Kit (SCFR).

ADCs. Antibody with low internalization rate has no desired therapeutics index even for the tumors expressing high levels of surface antigen 99. 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 30,54,55. It has been reported that Ab with longer half-life show high elimination and rapid clearance of the ADC in plasma 136. As shown in table 1, it is not compulsory for a mAb itself to represent therapeutic activity in the ADC. However, therapeut
Targeting LIV1 (SLC39A6 or ZIP6) antigen, a member of the zinc transporter family playing a key role in tumor cell progression and metastasis:

- **AG515E, anti-LIV1** ADC
  - Clinical phase: Phase I, for treatment of MUC
  - Ab, kd, therapeutic activity: huIgG2 anti-SLITRK6, n/a, n/a
  - Payload: MMAE
  - Linkage strategy: Native cytostatic residues, VC protease-cleavable linker
  - DAR, MTD, bystander effect: n/a, n/a, yes
  - Sponsor, Reference: Agenys (119)

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

- **ABBV-399, Telisotuzumab vedotin** ADC
  - Clinical phase: Phase I, for treatment of c-Met-expressing NSCLC
  - Ab, kd, therapeutic activity: Engineered huIgG1 without the agonist activity associated with c-Met (ABT-700), 0.2 to 1.5 nM, ADC and c-Met inhibition & downstream signaling molecules
  - Payload: MMAE
  - Linkage strategy: Native cytostatic residues, VC protease-cleavable linker
  - DAR, MTD, bystander effect: ~3, 3 mg/kg, n/a
  - Sponsor, Reference: Abbvie (120-123)

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

- **BAY1187982, anti-FGFR2 ADC, Apatuzumab vedotin** ADC
  - Clinical phase: Phase I, for treatment of FGFR2-positive human malignancies
  - Ab, kd, therapeutic activity: huIgG1 anti-FGFR2 isomers FGFR2-IIIb and FGFR2-IIIc (BAY 1179470), 75 nM, n/a
  - Payload: MMAE
  - Linkage strategy: Lysine side chains and a noncleavable linker
  - DAR, MTD, bystander effect: ~4, n/a, yes
  - Sponsor, Reference: Bayer (124)

Targeting C4.4a (LYPD3) and uPAR antigen, glycosylphosphatidylinositol (GPI)-anchored proteins:

- **BAY1129880, Lupartumab, anti-C4.4a ADC** ADC
  - Clinical phase: Phase I, for treatment of LSCC
  - Ab, kd, therapeutic activity: huIgG1 anti-C4.4A, 0.002 nM, n/a
  - Payload: MMAE
  - Linkage strategy: Native cytostatic residues, MC noncleavable alkyl hydrazide linker
  - DAR, MTD, bystander effect: ~4, 1.9 mg/kg, n/a
  - Sponsor, Reference: Bayer (125)

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, anti-p-Cadherin ADC** ADC
  - Clinical phase: Phase I, for treatment of TNBC; head and neck & esophageal cancers
  - Ab, kd, therapeutic activity: IgG1 anti-p-Cadherin, n/a, n/a
  - Payload: DM1
  - Linkage strategy: Native lissamine residues, SMCC noncleavable thioether linker
  - DAR, MTD, bystander effect: n/a, n/a, yes
  - Sponsor, Reference: Novartis (126)

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

- **PF-06643507, anti-ST4 ADC** ADC
  - Clinical phase: Phase I, for treatment of lung and breast cancer with ST4 expression
  - Ab, kd, therapeutic activity: huIgG1 anti-ST4
  - Payload: MMAF
  - Linkage strategy: Native cytostatic residues, MC noncleavable linker
  - DAR, MTD, bystander effect: n/a, 0.34 mg/kg, no
  - Sponsor, Reference: Pfizer (127)

Targeting Tumor Necrosis Factor Receptor (TNFR) superfamily: 4.4A (ETBR) antigen, Auristatin-Dependent Cellular Cytotoxicity (ADC) activity is usually mediated via immune-mediated effector functions such as Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody-Dependent Cell-mediated Cytotoxicity (ADCC), Complement Dependent 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.
### ADC names

<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 TENB2 antigen, a prostate cancer target associated with the progression of poorly differentiated and androgen-independent tumor types:</strong></td>
<td></td>
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<tr>
<td>Anti-TENB2 ADC</td>
<td>Phase I, for treatment of prostate cancer</td>
<td>ThioMab version of the anti-TENB2 antibody (Pr1), 2.3 nM, n/a</td>
<td>MMAE</td>
<td>Native lysine residues, protease-labile VC-PABC-linker</td>
<td>2, n/a, n/a</td>
<td>Seattle Genetics (131,139)</td>
</tr>
<tr>
<td><strong>Targeting ETBR antigen, a G-protein coupled receptor that can activate RAF/MEK signaling:</strong></td>
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<tr>
<td>RG7636, DEDN-6526A</td>
<td>Phase I, for treatment of melanoma</td>
<td>hulgG1 anti-ETBR, n/a, n/a</td>
<td>MMAE</td>
<td>Native lysine residues, protease-labile linker</td>
<td>n/a, 2.4 mg/kg, n/a</td>
<td>Genentech, Inc. (140)</td>
</tr>
<tr>
<td><strong>Targeting integrin v3 antigen:</strong></td>
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<tr>
<td>IMGN-388</td>
<td>Phase I, for treatment of NSCLC and prostate cancer</td>
<td>hulgG1 Anti-Integrin v3</td>
<td>DM4</td>
<td>Native lysine residues, SPDB disulfide cleavable linker</td>
<td>n/a, 3.5 mg/kg, n/a</td>
<td>ImmunoGen (141)</td>
</tr>
<tr>
<td><strong>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 superfamily members:</strong></td>
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<tr>
<td>BIIB-015</td>
<td>Phase I, for treatment of breast, ovary, stomach, lung, and pancreas Cytokine-expressing tumor cells</td>
<td>hulgG1 anti-Cripto (BIIB015)</td>
<td>DM4</td>
<td>Native lysine residues, SPDB disulfide cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Biogen (142)</td>
</tr>
<tr>
<td><strong>Targeting AGS-5 (SLC44A4) antigen, a sodium-dependent transmembrane transport protein:</strong></td>
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<tr>
<td>ASG-5ME</td>
<td>Phase I, for treatment of pancreatic, prostate and gastric cancers</td>
<td>hulgG2 anti-AGS-5, n/a, n/a</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Seattle Genetics/Astellas (143)</td>
</tr>
<tr>
<td><strong>Targeting LY6E antigen, an interferon (IFN) inducible glycosylphosphatidyl inositol (GPI)-linked cell membrane protein:</strong></td>
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<tr>
<td>RG7841, DLYE5953A</td>
<td>Phase I, for treatment of HER2– breast cancer and NSCLC</td>
<td>n/a, n/a, n/a</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Genentech, Inc. (144)</td>
</tr>
<tr>
<td><strong>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:</strong></td>
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<tr>
<td>HuMax-Axl-ADC</td>
<td>Phase I, for treatment of multiple solid tumors</td>
<td>hulgG1 Anti-Axl, n/a, n/a</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Genmab (145)</td>
</tr>
<tr>
<td><strong>Targeting CD205 antigen, a type I C-type lectin receptor normally expressed on various APC and some leukocyte sub-populations:</strong></td>
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<tr>
<td>MEN1309/OTB706</td>
<td>Phase I, for treatment of NHL</td>
<td>hulgG1 anti-CD205, n/a, n/a</td>
<td>DM4</td>
<td>Native lysine residues, SPDB disulfide cleavable linker</td>
<td>n/a, n/a, yes</td>
<td>Menarini Ricerche (146)</td>
</tr>
<tr>
<td><strong>Targeting CD25 (IL-2R alpha) antigen, a TP and tumor-associated antigen (TAA), expressed on certain cancer cells:</strong></td>
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<tr>
<td>ADC-301, anti-CD25-PBD ADC</td>
<td>Phase I, for treatment of AML, ALL, relapsed HL and NHL with CD25-positive HER2-negative tumor cells</td>
<td>hulgG1 against CD25, n/a, n/a</td>
<td>PBD</td>
<td>Cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>ADC Therapeutics S.A. (147)</td>
</tr>
<tr>
<td><strong>Targeting LAMP-1 antigen, playing a key role in cell-cell adhesion and migration:</strong></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>hulgG1 Anti-LAMP1(AB-1)</td>
<td>DM4</td>
<td>Lysine residues, SPDB</td>
<td>n/a, n/a, n/a</td>
<td>Sanofi (148)</td>
</tr>
<tr>
<td><strong>Targeting MN/CA IX antigen, a TGP expressed in some human carcinomas and appears to be involved in cancer cell proliferation and transformation:</strong></td>
<td></td>
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<tr>
<td>ADC BAY79-4620, MN-IC</td>
<td>n/a</td>
<td>hulgG1 anti-MN/CA IX, n/a, n/a, ADC</td>
<td>MMAE</td>
<td>Native cysteine residues, VC protease-cleavable linker</td>
<td>n/a, n/a, n/a</td>
<td>Bayer (149)</td>
</tr>
</tbody>
</table>

| **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.** | | | | | | |

**Lymphocyte antigen 6 complex locus E (Ly6E), Antigen-Presenting Cell (APC), a subunit of the interleukin-2 receptor (IL-2R alpha), Lysosome-Associated Membrane Protein 1 (LAMP1).**

In this regard, many attempts have been made to engineer mAbs with therapeutic activity. For instance, the Fc domain affinity of anti-CD19 targeting antibodies for the FcyRIII has been enhanced, either by Fc glycol-engineering approaches, e.g., MEDI-55 150 and MDX-1342 151 or amino acid substitution, e.g., XmAβ5574 152 and XmAb 5871 or MOR-208 35,153. Such modification resulted in an increase of ADCC activity in antibody. To 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.

**Antibody-Drug Conjugates: Possibilities and Challenges**

**Table 1.**
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. 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 (Table 1). 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 (Table 1). The above cytotoxic compounds are 100 to10000 folds more potent in vitro than typical chemotherapeutic agents and are chosen based on their
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) \cite{98}. Such studies help to elucidate the in vivo factors that may be contributed to the elimination/enhancement of the cytotoxic agent \cite{27,98,164}. New studies to introduce new payloads focused on agents against Tumor-Initiating Cells (TICs) \cite{27,164}. 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 \cite{1,164}.

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 \cite{83}.

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 \cite{163,165}. Extreme hydrophobicity of payload can potentially change antibodies biological properties, resulting in hydrophobic aggregation of the antibody either during conjugation process or storage \cite{163}. 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 \cite{17,26,163,165}. 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 \cite{165}. According to the reports, about 95-99% of ADC molecules are metabolized before binding to tumor cells \cite{160}. 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
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 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 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.
2. Re-engineering of mAb is able to incorporate with unnatural amino acids, e.g. selenocysteine, acetylated phenylalanine, and para-azidomethyl-1-phenylalanine.

3. Site-specific enzyme-mediated conjugation to genetically engineered antibody is as follows:

   - Incorporating a thiolated sugar analogue, 6-thiofucose, to the antibody carbohydrate that introduces new chemically active thiol groups using fucoaryltransferase.

   - Providing a ketone reactive group on antibody glycosylation site by glycotransferases.

   - Introducing an aldehyde reactive group on the antibody using sialyltransferase or formylglycine-generating enzyme.

   - Genetically introducing specific glutamine tags to antibody whereby payloads with a primary amine group can be linked to the γ-carbonyl amide group of glutamine tags. Such reaction is catalyzed by a microbial transglutaminase which is capable of recognizing glutamines tags from naturally glutamines residues.

   - 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 Sortase.

   - Conjugation of phosphopantetheine-linked payloads to the serine residues of the peptide-tagged antibodies via phosphopantetheinyl transferases.

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. 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. 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, 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. As shown in table 1, about 50% of the ADCs are using Valine-Citrulline peptidyl (VC) linker.

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. 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. 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.

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.

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 thiouether linkage.

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 thiouether linkage when they were directed to the tumor cell lines expressing a low copy number of targeted antigen.

Cleavable linkers may increase the possibility of bystander effect. Hence, it is logical to use cleavable linkers in designing ADCs directed for the antigen that is heterogeneously expressed in tumors.

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, peripheral neuropathy can be frequently observed, whereas noncleavable linkers often trigger hematological toxicity, possibly due to an increased dose and interactions with Fcγ receptors on hematopoietic cells.

The type of linker plays an important role in ADC catabolite products with regard to processing into targeted cells or metabolizing by clearance mechanisms. The type of ADC catabolites may influence some ADC features such as IC50, Maximum Tolerated Dose (MTD), and kill Multidrug Resistance (MDR) expressing cells.
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.\(^\text{1,16}\) 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 \(^\text{1,18,20,21}\). 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 \(^\text{vs.}\) 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 \(^\text{156}\), low internalization \(^\text{159}\), off-target effect due to the target expression in normal tissues \(^\text{157}\), 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 \(^\text{156,157,159}\). 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) \(^\text{195}\) to exert therapeutic activity. Having reasonable aqueous solubility, non-immunogenic, 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.\(^\text{15,173,176-187,186,197}\)

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.

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