

## Antibody-Drug Conjugates: Possibilities and Challenges

Mohammad-Reza Nejadmoghaddam<sup>1,2</sup>, Arash Minai-Tehrani<sup>2</sup>, Ramin Ghahremanzadeh<sup>2</sup>,  
Morteza Mahmoudi<sup>1</sup>, Rassoul Dinarvand<sup>1,3\*</sup>, and Amir-Hassan Zarnani<sup>4,5,6\*</sup>

1. Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

2. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

3. Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

4. Department of Immunology, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran

5. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

6. Immunology Research Center, Iran University of Medical Sciences, IUMS, Tehran, Iran

## \* Corresponding authors:

Rassoul Dinarvand, Pharm D.,  
PhD., Nanotechnology Research  
Center, Faculty of Pharmacy,  
Tehran University of Medical  
Sciences, Tehran, Iran

Amir-Hassan Zarnani, D.M.T.,  
Ph.D., Reproductive Immunology  
Research Center, Avicenna  
Research Institute, ACECR,  
Tehran, Iran

Tel: +98 21 64121014, 22432020

Fax: +98 21 66959052, 22432021

## E-mail:

dinarvand@tums.ac.ir,  
zarnania@gmail.com

Received: 29 Oct 2017

Accepted: 31 Dec 2017

## 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.

*Avicenna J Med Biotech 2019; 11(1): 3-23*

**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<sup>1</sup>. From the standpoint of nanomedicine, the antibody in ADC structure acts as a self-targeting nanoscale carrier<sup>1-3</sup>,

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<sup>4</sup>.

The first experimental design on ADC subject dates back to more than 50 years ago<sup>5</sup>. 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<sup>6,7</sup>, Trastuzumab emtansine<sup>8-11</sup>, Inotuzumab ozogamicin<sup>12</sup> and Gemtuzumab ozogamicin<sup>12,13</sup> 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<sup>14,15</sup>, whereby there are 160 ADCs undergoing preclinical trials<sup>16</sup> 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-

Antibody-Drug Conjugates: Possibilities and Challenges

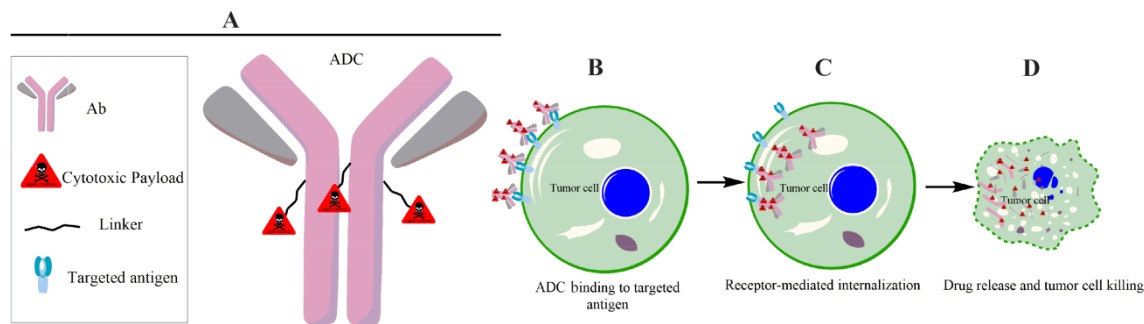


Figure 1. Schematic representation of ADC, showing the main components of an ADC and its cell cytotoxicity mechanism. Clinical efficacy of ADCs is determined by fine-tuning combination of tumor antigen, targeting antibody, cytotoxic payload and conjugation strategy (a). ADC binds to tumor target cell surface antigens (b) leading to trigger a specific receptor mediated internalization (c). The internalized ADCs are decomposed to release cytotoxic payloads inside the tumor cell either through its linkage/linker sensitivity to protease, acidic, reductive agents or by lysosomal process, leading to cell death (d).

getting, antibody, cytotoxic payload, and method of antibody linkage to the payload. 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<sup>17</sup>. 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<sup>1,18-20</sup>. 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<sup>21</sup>, melanoma<sup>22</sup>, gliomas<sup>23</sup>, and two specific breast cancer types, Basal-Like Breast Cancer (BLBC) and Triple Negative Breast Cancer (TNBC)<sup>24</sup>. 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<sup>25</sup>.

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<sup>19</sup>. However, the potential of ECD to be shed into the circulation must be con-

sidered. The shed ECDs can potentially bind to ADC and consequently reduce the targeted delivery into the tumor cells<sup>19</sup>.

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<sup>26</sup>. However, heterogeneous antigen expression can particularly be beneficial for those ADCs that possess bystander killing activity<sup>26-28</sup>. 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<sup>26-29</sup>. 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<sup>30,31</sup>.

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 exquisitely selective target that covers all types of malignancies. 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%)<sup>7,32-35</sup>. As shown in table 1, CD19 has been marked as a target to produce ADCs, including SAR3419<sup>7,34,35</sup>, SGN-CD19A<sup>32</sup>, MDX-1206<sup>36</sup>, and ADCT-402<sup>33</sup>.

**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 com-

Table 1. Current ADCs in clinical development based on targeting antigens with an overview of their properties

ADC names	Clinical phase, indication	Ab, kd, therapeutics activity	Payload	Linkage strategy	DAR, MTD, bystander effect	Sponsor, Reference
<b>Targeting HER2 antigen, a transmembrane RTKs in the growth of some cancer cells:</b>						
Kadcyla, Ado-Trastuzumab emtansine, T-DM1	Approved in 2013, for treatment of her2 positive breast cancer	huIgG1 (trastuzumab), n/a, ADCC and HER2-dependent PI3K/AKT signaling	DM1	Native lysine residues, SMCC nonreducible thioether linkage	~3.5, 3.6 mg/kg, no	Genentech, Inc. (8-11)
SYD985, Trastuzumab vc-seco-DUBA	Phase I, for treatment of USC and epithelial EOC	huIgG2 anti HER2 (Trastuzumab), n/a, no	DUO	VC-seco	~ 2.8, 1.88 mg/kg, yes	Synthon BV (37-39)
ADC XMT-1522	Phase I, for treatment of low HER+ breast, gastric and lung cancers	huIgG1anti-HER2 (HT-19), n/a, n/a	AF-HPA	Fleximer®	12, n/a, yes	Mersana Therapeutics (40)
ADC ARX788	Phase I, for treatment of low HER+ breast, ovarian, lung and gastric cancers	IgG1anti-HER2, n/a, n/a	MMAF	pAcF site-specific oxime linkage, AS269 noncleavable linker	2, n/a, n/a	Zhejiang Medicine/Ambrox (41)
ADC ADCT-502	Phase I, for treatment of low HER2+ expressing breast, NSCLC, gastroesophageal, bladder cancer	huIgG1anti-HER2 (trastuzumab)	PBD	Cysteine residues, VA-PABC	1.7, n/a, n/a	ADC Therapeutics S.A. (42)
<b>Targeting EGFR antigen, a RTKs that is essential for ductal and lobuloalveolar development:</b>						
ABT-414, Depatuzumab mafodotin	Phase II, for treatment of GBM	huIgG1 anti EGFR (ABT-806), 0.06 nM, inhibits EGFR 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	huIgG1anti-EGFRvIII, 0.61 nM, n/a	DM1	Native lysine residues, SMCC noncleavable thioether linker	~3.5, n/a, no	Amgen (44)
IMGN289, Laprituximab emtansine	Phase I, for treatment of NSCLC and HNSCC	huIgG 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	huIgG1 anti-EGFR, n/a, n/a	MMAE	VC protease-cleavable linker	n/a, n/a, n/a	Abbvie (46)
<b>Targeting CD70 ( CD27L) antigen a TP2 and member of the tumor necrosis factor family:</b>						
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, VC protease-cleavable linker	n/a, 15 mg/kg, yes	Bristol-Myers (48)
SGN-CD70A	Phase I, for treatment of RCC, MCLD, LBC, FL,	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	huIgG1, n/a, n/a	DM1	Native lysine residues, MCC noncleavable linker	n/a, n/a, no	Amgen (50)
<b>Targeting CD33 antigen, a EGP:</b>						
Mylotarg, Gemtuzumab Ozogamicin (GO)	Withdrawn 2010 and approved in 2017, for treatment of CD33 <sup>+</sup> AML	huIgG4, n/a, n/a	Calich.	Native lysine residues, (AcBut)-N-acetyl acid-labile hydrazone linker	n/a, 0.25 mg/kg, yes	Pfizer (51)
SGN-CD33A	Phase I, for treatment of AML	hu anti-CD33 with engineered cysteines, 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	Native lysine residues, 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), Calicheamicin (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 Carcinomas (HNSCC), Auristatin F-hydroxypropylamide (AF-HPA), Polyacetal-based polymer (Fleximer®), Non-natural amino acid linker para-acetyl-phenylalanine (pAcF), Amberstatin, a short polyethylene glycol (PEG) spacer terminated by an alkoxyamine (AS269).

ponent in ADCs. These prevent antibody cross reactions to other antigens, avoiding both toxicity and removal/elimination of the ADC before reaching to the tumor. The high affinity of the Ab for efficient uptake into target cells is another important factor in ADC design<sup>30,54-56</sup>. To the best of our knowledge, there is no substantial report about optimal or even minimum re-

quired binding affinity (Kd) of antibody component. In figure 4, a binding affinity less than 10 nM (Kd<10 nM) is commonly needed for the Ab component and accordingly for an effective ADC, based on frequency distribution histogram. The affinity of the antibody to its immunogen can affect the property of antibody which is termed as receptor-mediated antibody inter-

Antibody-Drug Conjugates: Possibilities and Challenges

Contd table 1.

ADC names	Clinical phase, indication	Ab, kd, therapeutics activity	Payload	Linkage strategy	DAR, MTD, bystander effect	Sponsor, Reference
<b>Targeting CD19 antigen, a TP1 on B cells as an accessory molecule for B-cell signal transduction and TAA:</b>						
SAR3419, coltuximab ravtansine	Phase II, for treatment of B-NHL and B-ALL	huIgG1 anti-CD19 (huB4), n/a, ADCC	DM4	Native lysine residues, SPDB disulfide cleavable linker	~3.5, ~4.3 mg/kg, yes	ImmunoGen (7,34,35)
SGN-CD19A	Phase I, for treatment of B-Cell Malignancies	huIgG1 anti-CD19 (hBU12), n/a, ADCC	MMAF	Native cysteine residues, MC linker, noncleavable	n/a, 6.0, no	Seattle Genetics (32)
ADCT-402	Phase I, for treatment of relapsed or refractory B-ALL	huIgG1 anti-CD19, n/a, n/a	PBD	Native cysteine residues, VA and maleimide cleavable linker	n/a, n/a, n/a	ADC Therapeutics S.A. (33)
<b>Targeting Mesothelin antigen, a glycosphosphatidyl inositol anchored protein:</b>						
BAY 94-9343, anetumab ravtansine	Phase II, for treatment of MPM	hu anti-mesothelin, n/a, n/a	DM4	Lysine residues, SPDB disulfide cleavable linker	n/a, 6.5 mg/kg, yes	Bayer (57)
BMS-986148	Phase I & II, for treatment of Mesothelin -expressing cancers	anti mesothelin	n/a	n/a	n/a, n/a, n/a	Bristol-Myers (58)
DMOT4039A	Phase I, for treatment of pancreatic and P-OC	hu anti-mesothelin (7D9.v3), n/a, n/a	MMAE	A noncleavable alkyl hydrazide linker	~ 3.5, 2.4 mg/kg, n/a	Genentech, Inc. (59,60)
<b>Targeting CD22 antigen, a transmembrane sialoglycoprotein functions as an inhibitory receptor for BCR signaling and BCR-induced cell death:</b>						
Inotuzumab, IO, Ozogamicin, CMC-544	Approved in 2017, for treatment of CD22 <sup>+</sup> ALL	huIgG4 anti-CD29(G544), n/a, no	Calich.	Native lysine residues, (AcBut)-N-acyl, Acid-labile hydrazone linker	n/a, 0.05 mg/kg, yes	Pfizer (12)
Pinatuzumab vedotin, DCDT2980S, RG7593	Phase II, for treatment of NHL and CLL	huIgG1 anti-CD22 (Epratuzumab), n/a, n/a	MMAE	Native cysteines residues, MC-VC-PAB linker	~ 2.4, 2.4 mg/kg, yes	Genentech, Inc. (61)
<b>Targeting CEACAM5 antigen, labetuzumab, CEA, CD66c, a EGP that has a role in cell adhesion and invasion:</b>						
IMMU-130, hMN14-SN38, labetuzumab govitecan, labetuzumab-SN-38	Phase II, for treatment of mCRC	huIgG1 anti-CEACAM5 (hMN14), 1.5 nM, ADCC	SN-38	Native cysteine residues, CL2A pH sensitive (Benzylcarbonate site) carbonate linker	7-8, 6-10 mg/kg, yes	Immunomedics (63-65)
SAR40870	Phase I & II, for treatment of B-Cell Malignancies	huIgG1 anti-CEACAM5, n/a, n/a	DM4	Lysine residues, SPDB disulfide cleavable linker	n/a, n/a, yes	Sanofi (66)
<b>Targeting Trop-2 (MIS1, 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:</b>						
IMMU-132, hrS7-SN-38, Sacituzumab govitecan	Phase III, for treatment of pancreatic cancers, SCLC and TNBC	huIgG1 anti-trop-2 (RS7 or Sacituzumab), 0.564 nM, ADCC	SN-38	Native cysteine residues, CL2A pH sensitive carbonate link	~7.6, 8-10 mg/kg, yes	Immunomedics (67-72)
PF-06664178, Trop-2 ADC, RN927C	Phase I, for treatment of OC, NSCLC and breast cancer	Engineered huIgG1 anti-Trop-2, 14 nM, n/a	PF06380101	Site-specific transglutaminase tag, AcLys-VC-PABC linker	2.0, n/a, n/a	Pfizer (73)
<b>Targeting PSMA antigen, a TP2 has known enzymatic activities and acts as a glutamate-preferring carboxypeptidase:</b>						
PSMA ADC	Phase I & II, for treatment of prostate cancer	hu anti-PSMA, 35.6-46.5 nM, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, 2.5 mg/kg, yes	Progenics (74,75)
MLN2704	Phase I & II, for treatment of prostate cancer	hu anti-PSMA (huJ591), n/a, n/a	DM1	Lysine residues, SPP disulfide cleavable linker	n/a, 60 mg/kg, yes	Millennium (76)

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).

nalization. Receptor-mediated antibody internalization is a key mechanism underlying antibody endocytosis that is induced through antibody binding to its specific antigen<sup>77</sup>. It has been reported that, alternative antibodies against the same immunogen can exhibit different rates of internalization<sup>19</sup>. Rapid internalization can raise both ADC efficacy and safety simultaneously,

since it reduces the opportunity of the ADC for off-target release<sup>1,98</sup>.

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<sup>99</sup>. For instance, Clathrin-coated Pit-mediated receptor internalization



Contd table 1.

ADC names	Clinical phase, indication	Ab, kd, therapeutics activity	Payload	Linkage strategy	DAR, MTD, by-stander effect	Sponsor, Reference
<b>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:</b>						
IMGN529, Naratuximab emtansine	Phase I or II, for treatment of BCL, CLL, NHL	huIgG1anti-CD37 (K7153A), n/a, ADCC and CDC,	DM1	Native lysine residues, SMCC nonreducible thioether linkage	n/a, 1.0 mg/kg, no	ImmunoGen (78,79)
AGS67E	Phase I, trial for treatment of NHL, DLBCL with high level of CD37 expression	huIgG2κ anti-CD37 (AGS67C or vCD37-9a73), n/a, n/a	MMAE	Native cysteines residues, VC protease-cleavable linker	n/a, 1.2 mg/kg, yes	Agensys (80-81)
<b>Targeting CD30 ( TNFRSF8) antigen, a tumor necrosis factor:</b>						
Adcetris, brentuximab vedotin, SGN-35	Approved in 2011, for treatment of HL and ALL.	Chimeric IgG1anti-CD30 (cAC10 or SGN30), n/a,	MMAE	Native interchain cysteine, MC-VC- PABC linker	~ 4, 1.8 mg/kg, yes	Seattle Genetics (6,7)
<b>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:</b>						
U3-1402	Phase I & II, for treatment of HER3-positive metastatic breast cancer	huIgG1anti-HER3(Patritumab)	DXd	n/a	~8, n/a, n/a	Daiichi Sankyo, Inc. (82)
<b>Targeting DLL3 antigen, scr-like kinase (Fyn3) acts as a notch ligand for cell-cell communication:</b>						
Rovalpituzumab tesirine, RoVa-T, SC16LD6.5	Phase I & II, for treatment of SCLC	huIgG1 anti-DLL3 antibody (SC-16), 2.6 nM, n/a	PBD	Native interchain cysteine, PEG8□va linker, cathepsin-B cleavable dipeptide linker	~ 2, 0.2 mg/kg, yes	Stemcentrx (83)
<b>Targeting GPNMB antigen, an EGP is involved in differentiation of osteoblasts, and cellular adhesion:</b>						
Glembatumumab Vedotin (GV), CDX-011, CR011-vcMMAE	Phase II, for treatment of GPNMB-positive breast and melanoma cancer	huIgG2 (CR011), n/a, no	MMAE	Cysteine residues, VC protease-cleavable linker	~ 4.5, 1.9 mg/kg, yes	Celldex Therapeutics (84-87)
<b>Targeting CD79b antigen, a TP1 on B cells mediates signal transduction cascade activated by BCR:</b>						
Polatuzumab vedotin, RG7596, DCDS4501A	Phase II, for treatment of NHLs and CLLs	anti-CD79b, n/a, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, 2.4 mg/kg, yes	Genentech, Inc. (88)
<b>Targeting GCC antigen, a part of calcium negative feedback system and has a role in cGMP synthesizes from GTP:</b>						
Indusatumab vedotin, MLN0264, TAK-264, 5F9-vcMMAE	Phase II, for treatment of GI malignancies	IgG1 anti- GCC (TAK-264), n/a, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, ~1.8 mg/kg, yes	Millennium (89,90)
<b>Targeting NaPi2b antigen, a sodium phosphate transporter:</b>						
Lifastuzumab vedotin, RG7599, DNIB0600A	Phase II, for treatment of NSCLC and ovarian cancer	huIgG1 anti-NaPi2b, 10.19 nM, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, 2.4 mg/kg, yes	Genentech, Inc. (91,92)
<b>Targeting CA6 antigen, a sialoglycotope of MUC-1 is over-expressed in variety of solid tumors, including breast, ovarian, cervical, lung and pancreatic tumors:</b>						
SAR566658	Phase II, for treatment of OC, breast, cervical, lung cancers	huIgG1 anti-CA6 (huDS6 IgG1), n/a, n/a	DM4	Native lysine residues, SPDB disulfide cleavable linker	6.5 mg/kg	Sanofi (93,94)
<b>Targeting CD74 antigen, a TP2 on B cells involved in the formation and transport of MHC class II protein:</b>						
Milatuzumab–doxorubicin, IMMU-110, hLL1-DOX	Phase I & II, for treatment of MM	hu anti-CD74	DOX	Native lysine residues, Acid-labile hydrazone linker	n/a, n/a, yes	Immunomedics (95)
<b>Targeting CD138 antigen, syndecan1, a type I transmembrane heparan sulfate proteoglycan participates in cell proliferation, cell migration and cell-matrix interactions:</b>						
BT-062, Indatuximab ravtansine	Phase I & II, for treatment of MM	Chimeric anti-CD138 (nBT062), n/a, n/a	DM4	Native lysine residues, SPDB disulfide cleavable linker	n/a, 2.7 mg/kg, yes	Biotest (96)
<b>Targeting BCMA antigen, a receptor for a proliferation-inducing ligand and B-cell activating factor:</b>						
GSK2857916	Phase I, for treatment of MM	Engineered afucosylated huIgG1 anti-BCMA, 1 nM, ADCC	MMAF	Native cysteine residues, MC noncleavable linker	n/a, n/a, no	GlaxoSmithKine (97)

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).

(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) <sup>118</sup>. ADC's traffic to the non-proteolytic compartments may impede its proteolytic process to release effective metabolites <sup>6</sup>. Antibody capability to induce receptor mediated internalization is somewhat a mandatory requirement in design of new generation of

Antibody-Drug Conjugates: Possibilities and Challenges

Contd table 1.

ADC names	Clinical phase, indication	Ab, kd, therapeutics activity	Payload	Linkage strategy	DAR, MTD, by-stander effect	Sponsor, Reference
<b>Targeting specific myeloma antigen:</b>						
DFRF4539A, 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)
<b>Targeting SLAMF7 (CS1) antigen:</b>						
ABBV-838	Phase I, for treatment of MM	huIgG1 anti-SLAMF7, n/a, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, n/a, n/a	Abbvie (101)
<b>Targeting CD56 antigen, associates with FGFR and stimulates RTKs to induce neurite outgrowth:</b>						
IMGN901, Lorvotuzumab mertansine, huN901-DM1/BB-10901	Phase I & II, for treatment of CD56+ MM	huIgG1 anti-CD56 (Lorvotuzumab or N901), 0.002 nM, ADCC	DM1	Lysine residues, SPP disulfide cleavable linker	3.7, 2.0 mg/kg, n/a	ImmunoGen (102)
<b>Targeting ENPP3 (CD203c) antigen, a TP2 belongs to a series of ectoenzymes, possess ATPase and ATP pyrophosphatase activities:</b>						
AGS-16C3F	Phase I & II, for treatment of RRCC	huIgG2k 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)
<b>Targeting TF (CD142) antigen, a TP and initiator of the coagulation cascade:</b>						
Humax-TF-ADC, tisetumab vedotin	Phase I & II, for treatment of Multiple solid tumours	IgG1 anti-TF	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, 1.8 mg/kg, yes	Genmab (105)
<b>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:</b>						
CDX-014	Phase I & II, for treatment of RCC	huIgG1 anti-TIM1	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, n/a, n/a	Celldex Therapeutics (106)
<b>Targeting FOLR1 antigen, a membrane-bound protein regulates transport of the vitamin B9 into cells:</b>						
IMGN853, mirvetuximab soravtansine	Phase I, for treatment of folate receptor alpha (FR $\alpha$ )-positive cancer, e.g., relapsed EOC	FR $\alpha$ -binding antibody	DM4	Native lysine residues, Sulfo- SPDB disulfide cleavable linker	n/a, 6 mg/kg, yes	ImmunoGen (17,107-110)
<b>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:</b>						
RG7458, Sofituzumab Vedotin, DMUC5754A	Phase I, for treatment of ovarian and pancreatic cancer	IgG1 anti-MUC16 (OC125), n/a, n/a	MMAE and MMAF	Native cysteine residues, MC-VC-PABC linker	n/a, 2.4 mg/kg, yes	Genentech, Inc. (111)
<b>Targeting CanAg antigen, is a novel glycoform of mucin family GP:</b>						
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)
<b>Targeting Ckit (CD117 or SCFR) antigen, a TP and RTKs having a key role in the regulation of cell differentiation and proliferation:</b>						
LOP628, Anti c-KIT ADC	Phase I, for treatment of AML and solid tumors	huIgG1 anti-(c-Kit), n/a, n/a	DM1	Native lysine residues, SMCC noncleavable thioether linker	n/a, n/a, no	Novartis (113)
<b>Targeting EphA2 antigen, belonging to ephrin receptor subfamily of the RTKs family regulating cell migration, adhesion, proliferation and differentiation:</b>						
MEDI-547, MI-CP177	Phase I, for treatment of relapsed or refractory solid tumors associated with EphA2 expression	huIgG1 anti-EphA2 (IC1), 1nM, n/a	MMAF	Native cysteines residues, MC noncleavable linker	4, 6.0 mg/kg, no	Medimmune (114,115)
<b>Targeting Nectin 4 (PVRL4) antigen, a TP1 and member of a family of cellular adhesion molecules, involved in Ca<sup>2+</sup>-independent cellular adhesion:</b>						
ASG-22ME, AGS-22M6E, anti-nectin-4 ADC, Enfortumab vedotin	Phase I, for treatment of MUC	huIgG1 anti-nectin-4 (AGS-22M6) 0.01 nM, n/a	MMAE	Native cysteines residues, VC protease-cleavable linker	n/a, 1-3 mg/kg, yes	Astellas Pharma (116,117)

Folate receptor 1(FOLR1), Maleimidocaproyl-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 EPHA2), 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<sup>99</sup>. 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<sup>30,54,55</sup>. It has been reported that Ab with longer half-life show high elimination and rapid clearance of the ADC in plasma<sup>136</sup>. As shown in table 1, it is not compulsory for a mAb itself to represent therapeutic activity in the ADC. However, thera-

Contd table 1.

ADC names	Clinical phase, indication	Ab, kd, therapeutics activity	Payload	Linkage strategy	DAR, MTD, by-stander effect	Sponsor, Reference
<b>Targeting SLITRK6 antigen, belonging to the integral TPs(SLITRK) with LRR:</b>						
AGS15E, anti-SLITRK6 ADC	Phase I, for treatment of MUC	huIgG2 $\gamma$ anti-SLITRK6, n/a, n/a	MMAE	Native cysteines residues, VC protease-cleavable linker	n/a, n/a, yes	Agensys (119)
<b>Targeting HGFR (cMet) antigen, RTKs for hepatocyte growth factor:</b>						
ABBV-399, Telisotuzumab vedotin	Phase I, for treatment of c-Met-expressing NSCLC	Engineered huIgG1 without the agonist activity associated with c-Met (ABT-700), 0.2 to 1.5 nM, ADCC and c-Met inhibition & downstream signaling molecules	MMAE	Native cysteines residues, VC protease-cleavable linker	~3.1, 3 mg/kg, n/a	Abbvie (120-123)
<b>Targeting FGFR2 antigen, type 2 RTKs with a role in both embryonic development and tissue repair:</b>						
BAY1187982, anti-FGFR2 ADC, Aprutumab ixadotin	Phase I, for treatment of FGFR2-positive human malignancies	huIgG1 anti-FGFR2 isoforms FGFR2-IIIb and FGFR2-IIIc (BAY 1179470), 75 nM, n/a	MMAE	Lysine side chains and a noncleavable linker	~4, n/a, yes	Bayer (124)
<b>Targeting C4.4a (LYPD3) and uPAR antigen, glycosylphosphatidylinositol (GPI)-anchored proteins:</b>						
BAY1129980, Lupartumab amadotin, anti-C4.4a ADC	Phase I, for treatment of LSCC	huIgG1 anti-C4.4A, 60 nM, n/a	MMAE	Native cysteine residues, noncleavable alkyl hydrazide linker	~4, 1.9 mg/kg, n/a	Bayer (125)
<b>Targeting p-Cadherin (Cadherin 3) antigen, a cell-surface protein and member of the cadherin family plays a role in cell adhesion, motility, invasion, and proliferation:</b>						
PCA062	Phase I, for treatment of TNBC; head and neck & esophageal cancers	IgG1 anti-P-cadherin, n/a, n/a	DMI	Native lysine residues, SMCC noncleavable thioether linker	n/a, n/a, n/a	Novartis (126)
<b>Targeting 5T4 (TPBG) antigen, a EGP correlated with increased invasiveness:</b>						
PF-06263507, anti-5T4 ADC	Phase I, for treatment of lung and breast cancer with 5T4 expression	huIgG1 anti-5T4	MMAF	Native cysteine residues, MC noncleavable linker	n/a, 4.34 mg/kg, no	Pfizer (127)
<b>Targeting STEAP1 antigen, cell-surface protein is predominantly expressed in prostate tissue:</b>						
RG7450, DSTP3086S, Vandortuzumab vedotin, STEAP1 ADC	Phase I, for treatment of mCRPC	huIgG1 anti-TEAP1(MSTP2109A), 2.4 nM, n/a	MMAE	Native cysteine residues, MC-vc-PAB linker	1.8-2.0, 2.4 mg/kg, yes	Genentech, Inc. (128-131)
<b>Targeting PTK7 antigen, RTKs 7 presents on TICs in the Wnt signaling pathway:</b>						
PF-06647020, h6M24-vc0101, PTK7-targeted ADC	Phase I, for treatment of NSCLC, TNBC and OC	huIgG1 anti-PTK7 (h6M24) 0.002 nM, n/a	Aur0101	Transglutaminase tag (LLQGA) located at the C-terminus of the antibody heavy chain, cleavable VC-PABC- linker	4, 1.5 mg/kg, yes	Pfizer (132,133)
<b>Targeting Ephrin-A4 (EFNA4) antigen, RTKs modulate signaling pathways that impact cell fate decisions during embryogenesis and adult tissue homeostasis:</b>						
PF-06647263	Phase I, for treatment of TNBC and OC	huIgG1 anti-Ephrin-A4 (E32), n/a, n/a	Calich.	Native lysine residues, Hydrazone-CM1(Hydrazone acetyl butyrate)	4.6, ~ 0.08 mg/kg, yes	Pfizer (113,134)
<b>Targeting LIV1(SLC39A6 or ZIP6) antigen, a member of the zinc transporter family playing a key role in tumor cell progression and metastasis:</b>						
SGN-LIV1A, anti-LIV-1	Phase I, for treatment of metastatic breast,	huIgG1 anti-LIV1(hLIV22), 4.6 nM, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	4, n/a, yes	Seattle Genetics (135)

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).

peutic activity of the mAb is a desirable property besides killing activity mediated by the cytotoxic payload<sup>137,138</sup>

Antibody therapeutic activity is usually mediated via immune-mediated effector functions such as Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody-Dependent Cellular Phagocytosis (ADCP), Com-

plement 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<sup>8-11,43,120-123</sup>. According to the obtained data in table 1, isotype 1 immunoglobulin (IgG1) seems to be prone to induce immunotherapeutic activity.

Antibody-Drug Conjugates: Possibilities and Challenges

Contd table 1.

ADC names	Clinical phase, indication	Ab, kd, therapeutics activity	Payload	Linkage strategy	DAR, MTD, by-stander effect	Sponsor, Reference
<b>Targeting TENB2 antigen, a prostate cancer target associated with the progression of poorly differentiated and androgen-independent tumor types:</b>						
Anti-TENB2 ADC	Phase I, for treatment of prostate cancer	ThioMab version of the anti-TENB2 antibody (Pr1), 2.3 nM, n/a	MMAE	Native lysine residues, protease-labile VC-PABC- linker	2, n/a, n/a	Seattle Genetics (131,139)
<b>Targeting ETBR antigen, a G-protein coupled receptor that can activate RAF/MEK signaling:</b>						
RG7636, DEDN-6526A	Phase I, for treatment of melanoma	huIgG1 anti-ETBR, n/a, n/a	MMAE	n/a	n/a, 2.4 mg/kg, n/a	Genentech, Inc. (140)
<b>Targeting integrin v3 antigen:</b>						
IMGN-388	Phase I, for treatment of NSCLC and prostate cancer	huIgG1 anti-Integrin v3	DM4	Native lysine residues, SPDB disulfide cleavable linker	n/a, 3.5 mg/kg, n/a	ImmunoGen (141)
<b>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:</b>						
BIIB-015	Phase I, for treatment of breast, ovary, stomach, lung, and pancreas Cripto-expressing tumor cells	huIgG1 anti-Cripto (BIIB015), n/a, n/a	DM4	Native lysine residues, SPDB disulfide cleavable linker	n/a, n/a, n/a	Biogen (142)
<b>Targeting AGS-5 (SLC44A4) antigen, a sodium-dependent transmembrane transport protein:</b>						
ASG-5ME	Phase I, for treatment of pancreatic, prostate and gastric cancers	huIgG2 anti-AGS-5, n/a, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, n/a, n/a	Seattle Genetics/Astellas (143)
<b>Targeting LY6E antigen, an interferon (IFN)-inducible glycosylphosphatidylinositol (GPI)-linked cell membrane protein:</b>						
RG7841, DLYE5953A	Phase I, for treatment of HER2- breast cancer and NSCLC	n/a, n/a, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, n/a, n/a	Genentech, Inc. (144)
<b>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:</b>						
HuMax-Axl-ADC	Phase I, for treatment of multiple solid tumors	huIgG1 anti-AXL, n/a, n/a	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, n/a, n/a	Genmab (145)
<b>Targeting CD205 antigen, a type I C-type lectin receptor normally expressed on various APC and some leukocyte sub-populations:</b>						
MEN1309/OBT076	Phase I, for treatment of NHL	huIgG1 anti- CD205, n/a, n/a	DM4	Native lysine residues, SPDB disulfide cleavable linker	n/a, n/a, yes	Menarini Ricerche (146)
<b>Targeting CD25 (IL-2R alpha) antigen , a TP and tumor-associated antigen (TAA), expressed on certain cancer cells:</b>						
ADCT-301, anti-CD25-PBD ADC	Phase I, for treatment of AML, ALL, relapsed HL and NHL with CD25-positive	huIgG1 against CD25, n/a, n/a	PBD	Cleavable linker	n/a, n/a, n/a	ADC Therapeutics S.A. (147)
<b>Targeting LAMP-1 antigen, playing a key role in cell-cell adhesion and migration:</b>						
SAR428926	Phase I, for treatment of HER2 negative breast expansion in LAMP-1 positive TNBC	huIgG1 anti-LAMP1(Ab-1)	DM4	Lysine residues, SPDB	n/a, n/a, n/a	Sanofi (148)
<b>Targeting MN/CA IX antigen, a TGP expressed in some human carcinomas and appears to be involved in cancer cell proliferation and transformation:</b>						
ADC BAY79-4620, MN-IC	n/a	huIgG1 anti-MN/CA IX, n/a, ADCC	MMAE	Native cysteine residues, VC protease-cleavable linker	n/a, n/a, n/a	Bayer (149)

Lymphocyte antigen 6 complex locus E (Ly6E), Antigen-Presenting Cell (APC),  $\alpha$  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 Fc $\gamma$ RIII has been enhanced, either by Fc glycol-engineering approaches, e.g. MEDI-55<sup>150</sup> and MDX-1342<sup>151</sup> or amino acid substitution, e.g. XmAb5574<sup>152</sup> and XmAb 5871 or MOR-208<sup>35,153</sup>. 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)<sup>154</sup>.

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<sup>35,155</sup>. A derivative of blinatumomab has been also constructed to induce the controlled T cell activation, named ZW38<sup>156</sup>. The ZW38 was conjugated to a microtubule cytotoxic agent for the preparation of a novel class of bsADC capable of mediating T cell cytotoxicity<sup>156</sup>. 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<sup>157,158</sup>. B10v5x225-H-vc-MMAE contains a bsAb



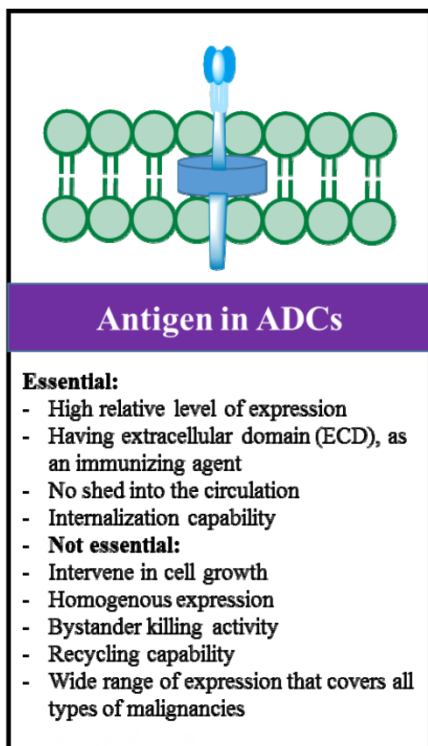


Figure 2. Main considerations in selecting tumor markers for ADC design and development.

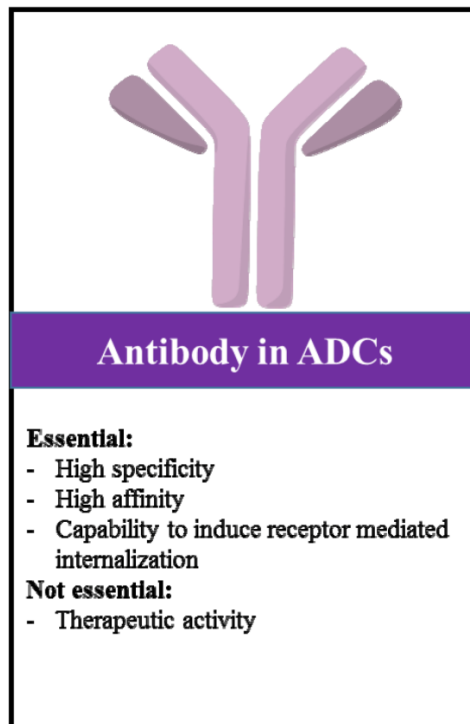


Figure 3. Main considerations in producing antibodies for ADC design and development.

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<sup>157</sup>. The side effect of B10v5x225-H-vc-MMAE can be avoided to some extent due to attenuated affinity toward EGFR receptors in healthy cells<sup>157</sup>. 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<sup>159</sup> 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<sup>160</sup>. The rate of mAb uptake by tumor cells is approximately less than 0.003-0.08% of injected dose per gram in a tumor<sup>54,55</sup>. 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 IC<sub>50</sub> of about

10 nM or less obtained from an examination with KB cells upon a 24-hr exposure time<sup>30,54,55,161</sup>. 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<sup>162,163</sup>. 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)<sup>38,98</sup>. Monomethyl auristatin derivatives which bind to tubulin and are able to inhibit microtubule assembly/polymerization (IC<sub>50</sub>=10-500 pM)<sup>32</sup> 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 to 10000 folds more potent *in vitro* than typical chemotherapeutic agents and are chosen based on their

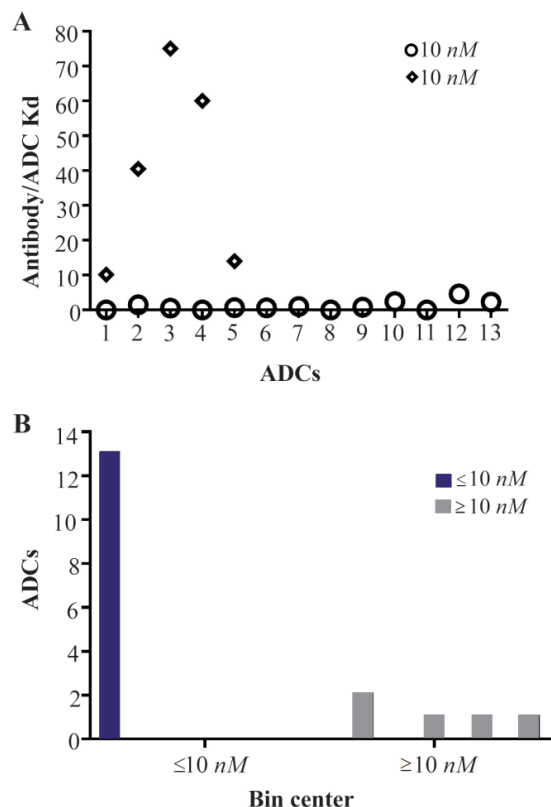



Figure 4. Kd frequency distribution (a) and histogram data (b) of current ADC in clinical development (Table S1, n=13). Antibody affinities (Kd) that have been used in current ADC in clinical development were classified into either  $\le 10 nM$  or  $\ge 10 nM$  groups. The average Kd and standard deviation of  $\le 10 nM$  group was 1.12 and 1.3 and for  $\ge 10 nM$  group was 39.9 and 28.2, respectively. Median Kd of  $\le 10 nM$  group and  $\ge 10 nM$  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  $\ge 10 nM$  groups are more than  $\le 10 nM$  groups (a).

different actions on cancer and noncancerous cells. DNA modulators have significant effects on malignant cells as they are divided more rapidly than normal cells<sup>163</sup>.

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)<sup>98</sup>. Such studies help to elucidate the *in vivo* factors that may be contributed to the elimination/enhancement of the cytotoxic agent<sup>27,98,164</sup>. New studies to introduce new payloads focused on agents against Tumor-Initiating Cells (TICs)<sup>27,164</sup>. 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,  $\alpha$ -amanitin (a bicyclic octapeptide from the fungus Amanita), and topoisomerase inhibitors including SN-38 are categorized as TIC payloads<sup>1,164</sup>.

Rovalpituzumab tesirine is one example of ADC with PBD as a payload (Table 1), that has been report-



**super cytotoxic**

Cytotoxic payload in ADCs

**Essential:**

- Highly super-toxic
- Low immunogenic
- Stable during preparation, storage and circulation
- Amenable to modification

**Not essential:**

- Effective on tumor-initiating cells
- Well-characterized metabolite profiles
- Aqueous solubility
- Bystander effect
- Compatible with standard of care
- Availability

Figure 5. Main considerations in choosing cytotoxic payloads for ADC design and development.

ed to have a potency to eliminate pulmonary neuroendocrine TICs at subpicomolar level *in vivo*<sup>83</sup>.

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<sup>163,165</sup>. Extreme hydrophobicity of payload can potentially change antibodies biological properties, resulting in hydrophobic aggregation of the antibody either during conjugation process or storage<sup>163</sup>. 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<sup>17,26,163,166</sup>. 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<sup>165</sup>. According to the reports, about 95-99% of ADC molecules are metabolized before binding to tumor cells<sup>160</sup>. 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

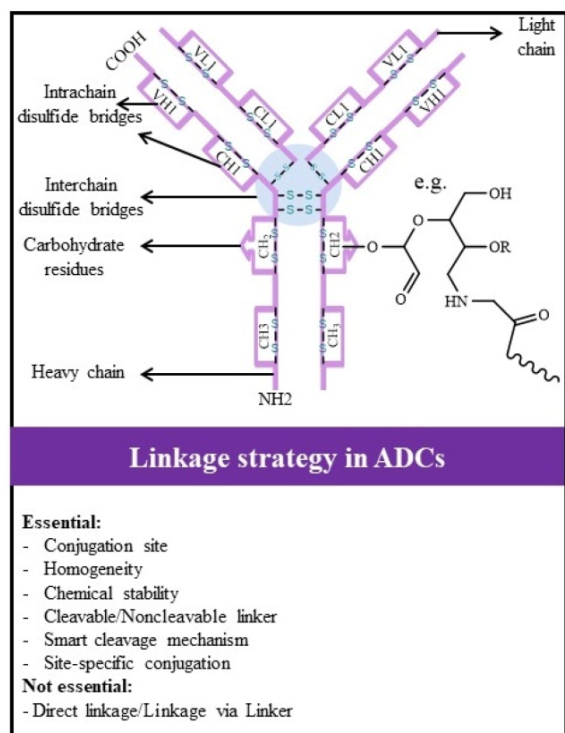


Figure 6. Main considerations for linking cytotoxic payload to antibodies in ADC design and development.

advantage to enhance ADC safety in particular <sup>1,2,167-169</sup>

Cytotoxic payload should present a dominant functional group suitable for linkage to the antibody component of ADC <sup>34</sup>. 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 <sup>170</sup>.

The copy number and heterogeneity of antigen expression are the other important issues that must be considered in the selection of cytotoxic agent <sup>30,31</sup>. 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 <sup>26</sup>.

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 <sup>54,55</sup> (Table 1). Both availability in the market and reasonable cost can be alternative rationale for choosing a cytotoxic payload in ADC design <sup>1</sup>.

### 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 an-

tibody conjugation to cytotoxic payloads, as it has a great role on balancing between ADC therapeutic efficacy and toxicity <sup>30,31,54</sup>. 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) <sup>1,171</sup>.

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 <sup>172</sup> and it results in 0-8 cytotoxic payload linkages per antibody and heterogeneity with about one million different ADC species <sup>30,173</sup>. 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) <sup>30</sup>.

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 <sup>27,30</sup>. 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 <sup>171,174,175</sup>. 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 <sup>30,173</sup>. 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 <sup>173,176</sup>. 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 <sup>173</sup>. 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<sup>177</sup>, acetyl-phenylalanine<sup>178</sup>, and para-azidomethyl-L-phenylalanine<sup>42</sup>.

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 fucosyltransferase VIII<sup>179</sup>,

Providing a ketone reactive group on antibody glycosylation site by glycotransferases<sup>180</sup>,

Introducing an aldehyde reactive group on the antibody using sialyltransferase<sup>181</sup> or formylglycine-generating enzyme<sup>182</sup>,

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 glutamines tags from naturally glutamines residues<sup>73,183-185</sup>,

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<sup>186</sup>,

Conjugation of phosphopantetheine-linked payloads to the serine residues of the peptide-tagged antibodies via phosphopantetheinyl transferases catalysis<sup>187</sup>,

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<sup>188</sup>. The azide functional groups are formed in a selective hydrolysis reaction that is mediated by an Endo-beta-N-acetylglucosaminidase (EndoS) chemoenzyme.

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<sup>98</sup>. 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<sup>1,98</sup>, 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<sup>30,31,54,189,190</sup>. 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<sup>191</sup>. 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<sup>191,192</sup>. 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<sup>163</sup>.

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<sup>163,164</sup>. 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<sup>190</sup>.

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<sup>17</sup>.

Cleavable linkers may increase the possibility of bystander effect<sup>27</sup>. Hence, it is logical to use cleavable linkers in designing ADCs directed for the antigen that is heterogeneously expressed in tumors<sup>26</sup>.

*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 $\gamma$  receptors on hematopoietic cells<sup>164</sup>.

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)<sup>192,193</sup>, and kill Multidrug Resistance (MDR) expressing cells<sup>192,194</sup>.



### 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<sup>14,15</sup>.

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<sup>1,18,20,31</sup>. 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<sup>156</sup>, low internalization<sup>159</sup>, off-target effect due to the target expression in normal tissues<sup>157</sup>, 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<sup>156,157,159</sup>. 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)<sup>195</sup> 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<sup>73,173,176-187,196,197</sup>.

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.

### Acknowledgement

This review study was supported as a Ph.D., program by a grant from Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences (TUMS) (grant no. 92-03-159-25467). We further acknowledge the numerous labs, authors, and publications that we were unable to cite in this review due to space restrictions.

### Conflict of Interest

The authors declare that they have no competing interests.

### References

1. Nejadmoghaddam MR, Zarnani AH, Ghahremanzadeh R, Ghods R, Mahmoudian J, Yousefi M, et al. Placenta-specific1 (PLAC1) is a potential target for antibody-drug conjugate-based prostate cancer immunotherapy. *Sci Rep* 2017;7(1):13373.
2. Nejadmoghaddam MR, Babamahmoodi A, Minai-Tehrani A, Zarnani AH, Dinarvand R. The use of objective oriented project planning tools for nanosafety and health concerns: a case study in nanomedicine research project. *Eur J Nanomed* 2016;8(4):225-231.
3. Venditto VJ, Szoka FC Jr. Cancer nanomedicines: so many papers and so few drugs! *Adv Drug Deliv Rev* 2013;65(1):80-88.
4. Sengupta S, Kulkarni A. Design principles for clinical efficacy of cancer nanomedicine: a look into the basics. *ACS Nano* 2013;7(4):2878-2882.
5. Decarvalho S, Rand HJ, Lewis A. Coupling of cyclic chemotherapeutic compounds to immune gamma-globulins. *Nature* 1964;202(4929):255-258.
6. Katz J, Janik JE, Younes A. Brentuximab vedotin (SGN-35). *Clin Cancer Res* 2011;17(20):6428-6436.
7. Younes A, Kim S, Romaguera J, Copeland A, Fariar Sde C, Kwak LW, et al. Phase I multidose-escalation study of the anti-CD19 maytansinoid immunoconjugate SAR3419 administered by intravenous infusion every 3 weeks to

Antibody-Drug Conjugates: Possibilities and Challenges

- patients with relapsed/refractory B-cell lymphoma. *J Clin Oncol* 2012;30(22):2776-2782.
8. Erickson HK, Park PU, Widdison WC, Kovtun YV, Garrett LM, Hoffman K, et al. Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. *Cancer Res* 2006;66(8):4426-4433.
  9. Krop IE, Beeram M, Modi S, Jones SF, Holden SN, Yu W, et al. Phase I study of trastuzumab-DM1, an HER2 antibody-drug conjugate, given every 3 weeks to patients with HER2-positive metastatic breast cancer. *J Clin Oncol* 2010;28(16):2698-2704.
  10. LoRusso PM, Weiss D, Guardino E, Girish S, Sliwkowski MX. Trastuzumab emtansine: a unique antibody-drug conjugate in development for human epidermal growth factor receptor 2-positive cancer. *Clin Cancer Res* 2011;17(20):6437-6447.
  11. Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res* 2008;68(22):9280-9290.
  12. García-Alonso S, Ocaña A, Pandiella A. Resistance to antibody-drug conjugates. *Cancer Res* 2018;78(9):2159-2165.
  13. Jen EY, Ko CW, Lee JE, Del Valle PL, Aydanian A, Jewell C, et al. FDA Approval: Gemtuzumab ozogamicin for the treatment of adults with newly-diagnosed CD33-positive acute myeloid leukemia. *Clin Cancer Res* 2018. [Epub ahead of print].
  14. Beck A, Reichert JM. Antibody-drug conjugates: present and future. *MAbs* 2014;6(1):15-17.
  15. Webb S. Pharma interest surges in antibody drug conjugates. *Nat Biotechnol* 2011;29(4):297-298.
  16. Firth D, Bell L, Squires M, Estdale S, McKee C. A rapid approach for characterization of thiol-conjugated antibody-drug conjugates and calculation of drug-antibody ratio by liquid chromatography mass spectrometry. *Anal Biochem* 2015;485:34-42.
  17. Ab O, Whiteman KR, Bartle LM, Sun X, Singh R, Tavares D, et al. IMGN853, a folate receptor alpha (FR $\alpha$ )-targeting antibody-drug conjugate, exhibits potent targeted anti-tumor activity against FR $\alpha$ -expressing tumors. *Mol Cancer Ther* 2015;14(7):1605-1613.
  18. Casi G, Neri D. Antibody-drug conjugates: basic concepts, examples and future perspectives. *J Control Release* 2012;161(2):422-428.
  19. Leipold D, Mallet WG. Case Study: An antibody-drug conjugate targeting MUC16 for ovarian cancer. In: Phillips GL, editor. *Antibody-drug conjugates and immunotoxins*. New York: Human Press; 2013. p. 221-239.
  20. Ritchie M, Tchistiakova L, Scott N. Implications of receptor-mediated endocytosis and intracellular trafficking dynamics in the development of antibody drug conjugates. *MAbs* 2013;5(1):13-21.
  21. Onaga M, Ido A, Hasuike S, Uto H, Moriuchi A, Nagata K, et al. Osteoactivin expressed during cirrhosis development in rats fed a choline-deficient, l-amino acid deficient diet, accelerates motility of hepatoma cells. *J Hepatol* 2003;39(5):779-785.
  22. Williams MD, Esmaeli B, Soheili A, Simantov R, Gombos DS, Bedikian AY, et al. GPNMB expression in uveal melanoma: a potential for targeted therapy. *Melanoma Res* 2010;20(3):184-190.
  23. Kuan CT, Wakiya K, Dowell JM, Herndon JE 2nd, Reardon DA, Graner MW, et al. Glycoprotein nonmetastatic melanoma protein B, a potential molecular therapeutic target in patients with glioblastoma multiforme. *Clin Cancer Res* 2006;12(7 Pt 1):1970-1982.
  24. Maric G, Rose AA, Annis MG, Siegel PM. Glycoprotein non-metastatic b (GPNMB): A metastatic mediator and emerging therapeutic target in cancer. *Onco Targets Ther* 2013;6:839-852.
  25. Qian X, Mills E, Torgov M, LaRochelle WJ, Jeffers M. Pharmacologically enhanced expression of GPNMB increases the sensitivity of melanoma cells to the CR011-vcMMAE antibody-drug conjugate. *Mol Oncol* 2008;2(1):81-93.
  26. Kovtun YV, Audette CA, Ye Y, Xie H, Ruberti MF, Phinney SJ, et al. Antibody-drug conjugates designed to eradicate tumors with homogeneous and heterogeneous expression of the target antigen. *Cancer Res* 2006;66(6):3214-3221.
  27. Diamantis N, Banerji U. Antibody-drug conjugates--an emerging class of cancer treatment. *Br J Cancer* 2016;114(4):362-367.
  28. Golfier S, Kopitz C, Kahnert A, Heisler I, Schatz CA, Stelte-Ludwig B, et al. Anetumab ravtansine: a novel mesothelin-targeting antibody-drug conjugate cures tumors with heterogeneous target expression favored by bystander effect. *Mol Cancer Ther* 2014;13(6):1537-1548.
  29. Shantha Kumara HM, Grieco MJ, Caballero OL, Su T, Ahmed A, Ritter E, et al. MAGE-A3 is highly expressed in a subset of colorectal cancer patients. *Cancer Immun* 2012;12:16.
  30. Panowski S, Bhakta S, Raab H, Polakis P, Junutula JR. Site-specific antibody drug conjugates for cancer therapy. *MAbs* 2014;6(1):34-45.
  31. Sievers EL, Senter PD. Antibody-drug conjugates in cancer therapy. *Annu Rev Med* 2013;64:15-29.
  32. Gerber HP, Kung-Sutherland M, Stone I, Morris-Tilden C, Miyamoto J, McCormick R, et al. Potent antitumor activity of the anti-CD19 auristatin antibody drug conjugate hBU12-vcMMAE against rituximab-sensitive and-resistant lymphomas. *Blood* 2009;113(18):4352-4361.
  33. Kahl B, Hamadani M, Caimi PF, Reid EG, Havenith K, He S, et al. First clinical results of ADCT-402, a novel pyrrolobenzodiazepine-based antibody drug conjugate (ADC), in relapsed/refractory B-cell lineage NHL. *Hematol Oncol* 2017;35(S2):49-51.
  34. Lazar AC, Wang L, Blättler WA, Amphlett G, Lambert JM, Zhang W. Analysis of the composition of immunotoxins using size-exclusion chromatography coupled to mass spectrometry. *Rapid Commun Mass Spectrom* 2005;19(13):1806-1814.

35. Naddafi F, Davami F. Anti-CD19 monoclonal antibodies: a new approach to lymphoma therapy. *Int J Mol Cell Med* 2015;4(3):143-151.
36. Rao C, Pan C, Vangipuram R, Huber M, Vemuri K, Stevens A, et al. Efficacy and toxicity of an anti-CD19 antibody drug conjugate. American Association for Cancer Research Meeting; 2010; Abstr 2452.
37. Black J, Menderes G, Bellone S, Schwab CL, Bonazzoli E, Ferrari F, et al. SYD985, a novel duocarmycin-based HER2-targeting antibody-drug conjugate, shows anti-tumor activity in uterine serous carcinoma with HER2/Neu expression. *Mol Cancer Ther* 2016;15(8):1900-1909.
38. Elgersma RC, Coumans RGE, Huijbregts T, Menge WMPB, Joosten JAF, Spijker HJ, et al. Design, synthesis, and evaluation of linker-duocarmycin payloads: toward selection of HER2-Targeting antibody-drug conjugate SYD985. *Mol Pharm* 2015;12(6):1813-1835.
39. Menderes G, Bonazzoli E, Bellone S, Black J, Altwerger G, Masserdotti A, et al. SYD985, a novel duocarmycin-based HER2-targeting antibody-drug conjugate, shows promising antitumor activity in epithelial ovarian carcinoma with HER2/Neu expression. *Gynecol Oncol* 2017; 46(1):179-186.
40. Yurkovetskiy A, Gumerov D, Ter-Ovanesyan E, Conlon P, Devit M, Bu C, et al, editors. Non-clinical pharmacokinetics of XMT-1522, a HER2 targeting auristatin-based antibody drug conjugate. American Association for Cancer Research Annual Meeting; 2017 Apr 1-5; Washington, DC. Philadelphia (PA): AACR; Cancer Res July 2017.
41. Humphreys RC, Kirtely J, Hewit A, Biroc S, Knudsen N, Skidmore L, et al, editors. Site specific conjugation of ARX-788, an antibody drug conjugate (ADC) targeting HER2, generates a potent and stable targeted therapeutic for multiple cancers. 106th Annual Meeting of the American Association for Cancer Research; 2015 April 18-22; Philadelphia, PA. Philadelphia (PA):AACR.
42. Zammarchi F, Chivers S, Williams DG, Adams L, Melinas-Gomez M, Tyrer P, et al. ADCT-502, a novel pyrrolobenzodiazepine (PBD)-based antibody-drug conjugate (ADC) targeting low HER2-expressing solid cancers. *Eur J Cancer* 2016;69:S28.
43. Gan HK, Papadopoulos KP, Fichtel L, Lassman AB, Merrell R, Van Den Bent MJ, et al. Phase I study of ABT-414 mono- or combination therapy with temozolomide (TMZ) in recurrent glioblastoma (GBM). *J Clin Oncol* 2015;33(suppl;abstr 2016).
44. Hamblett KJ, Kozlosky CJ, Siu S, Chang WS, Liu H, Foltz IN, et al. AMG 595, an anti-EGFRvIII antibody-drug conjugate, induces potent antitumor activity against EGFRvIII-Expressing glioblastoma. *Mol Cancer Ther* 2015;14(7):1614-1624.
45. Chittenden TD, Setiady YY, Park PU, Ponte JF, Dong L, Skaletskaya A, et al. IMGN289, an EGFR-targeting antibody-maytansinoid conjugate with potent activity against non-small cell lung cancer (NSCLC) regardless of dependency on EGFR pathway. Proceedings: AACR 104th Annual Meeting 2013; Apr 6-10, 2013; Washington, DC.
46. Calvo E, Cleary JM, Moreno V, et al. Preliminary results from a phase 1 study of the antibody-drug conjugate ABBV-221 in patients with solid tumors likely to express EGFR. *J Clin Oncol* 2017;35:2510.
47. Tannir NM, Forero-Torres A, Ramchandren R, Pal SK, Ansell SM, Infante JR, et al. Phase I dose-escalation study of SGN-75 in patients with CD70-positive relapsed/refractory non-Hodgkin lymphoma or metastatic renal cell carcinoma. *Invest New Drugs* 2014;32(6): 1246-1257.
48. Owonikoko TK, Hussain A, Stadler WM, Smith DC, Sznol M, Molina AM, et al. A phase 1 multicenter openlabel dose-escalation study of BMS-936561 (MDX-1203) in clear cell renal cell carcinoma (ccRCC) and Bcell non Hodgkin lymphoma (B-NHL). American Society of Clinical Oncology meeting abstracts 32;2014: 2558.
49. Sandall SL, McCormick R, Miyamoto J, Biechele T, Law C-L, Lewis TS. SGN-CD70A, a pyrrolobenzodiazepine (PBD) dimer linked ADC, mediates DNA damage pathway activation and G2 cell cycle arrest leading to cell death. Proceedings: AACR 106th Annual Meeting 2015; April 18-22, 2015; Philadelphia, PA.
50. U.S. National Library of Medicine [Internet]. Bethesda (MD): U.S. National Library of Medicine. 2000 Feb -. AMG 172 First in Human Study in Patients with Kidney Cancer; 2016 March 25 [cited 2018 June 08]; [about 4 screens]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01497821>.
51. Sievers E, Appelbaum FR, Spielberger R, Forman S, Flowers D, Smith F, et al. Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: a phase I study of an anti-CD33 calicheamicin immunoconjugate. *Blood* 1999;93(11):3678-3684.
52. Kung Sutherland MS, Walter RB, Jeffrey SC, Burke PJ, Yu C, Kostner H, et al. SGN-CD33A: a novel CD33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML. *Blood* 2013;122(8):1455-1463.
53. Lapusan S, Vidriales MB, Thomas X, De Botton S, Vekhoff A, Tang R, et al. Phase I studies of AVE9633, an anti-CD33 antibody-maytansinoid conjugate, in adult patients with relapsed/refractory acute myeloid leukemia. *Invest New Drugs* 2012;30(3):1121-1131.
54. Chari RV. Targeted cancer therapy: conferring specificity to cytotoxic drugs. *Acc Chem Res* 2008;41(1):98-107.
55. Hughes B. Antibody-drug conjugates for cancer: poised to deliver? *Nat Rev Drug Discov* 2010;9(9):665-667.
56. Rudnick SI, Lou J, Shaller CC, Tang Y, Klein-Szanto AJ, Weiner LM, et al. Influence of affinity and antigen internalization on the uptake and penetration of Anti-HER2 antibodies in solid tumors. *Cancer Res* 2011;71(6):2250-2259.
57. Bendell J, Blumenschein G, Zinner R, Hong D, Jones S, Infante J, et al. First-in-human phase 1 dose escalation study of a novel anti-mesothelin antibody drug conjugate, BAY 94-9343, patients with advanced solid tumors, American Association of Cancer Research, Washington, DC. 2013.



## Antibody-Drug Conjugates: Possibilities and Challenges

58. Zhao XY, Subramanyam B, Sarapa N, Golfier S, Dinter H. Novel antibody therapeutics targeting mesothelin in solid tumors. *Clin Cancer Drugs* 2016;3(2):76-86.
59. Weekes CD, Lamberts LE, Borad MJ, Voortman J, McWilliams RR, Diamond JR, et al. A phase I study of DMOT4039A, an antibody-drug conjugate (ADC) targeting mesothelin (MSLN), in patients (pts) with unresectable pancreatic (PC) or platinum-resistant ovarian cancer (OC). *American Society of Clinical Oncology*; 2014.
60. Weekes CD, Lamberts LE, Borad MJ, Voortman J, McWilliams RR, Diamond JR, et al. Phase I study of DMOT4039A, an antibody-drug conjugate targeting mesothelin, in patients with unresectable pancreatic or platinum-resistant ovarian cancer. *Mol Cancer Ther* 2016;15(3):439-447.
61. Advani A, Coiffier B, Czuczman MS, Dreyling M, Foran J, Gine E, et al. Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a phase I study. *J Clin Oncol* 2010;28(12):2085-2093.
62. DiJoseph JF, Armellino DC, Boghaert ER, Khandke K, Dougher MM, Sridharan L, et al. Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. *Blood* 2004;103(5):1807-1814.
63. Dotan E, Cohen SJ, Starodub AN, Lieu CH, Messersmith WA, Simpson PS, et al. Phase I/II trial of labetuzumab govitecan (Anti-CEACAM5/SN-38 antibody-drug conjugate) in patients with refractory or relapsing metastatic colorectal cancer. *J Clin Oncol* 2017;35(29):3338-3346.
64. Dotan E, Starodub A, Berlin J, Lieu CH, Guarino MJ, Marshall J, et al. A new anti-CEA-SN-38 antibody-drug conjugate (ADC), IMMU-130, is active in controlling metastatic colorectal cancer (mCRC) in patients (pts) refractory or relapsing after irinotecan-containing chemotherapies: Initial results of a phase I/II study. *American Society of Clinical Oncology*; 2015.
65. Govindan SV, Cardillo TM, Rossi EA, Trisal P, McBride WJ, Sharkey RM, et al. Improving the therapeutic index in cancer therapy by using antibody-drug conjugates designed with a moderately cytotoxic drug. *Mol Pharm* 2014;12(6):1836-1847.
66. Gazzah A, Stjepanovic N, Ryu M, Taberero J, Soria J, Bedard P, et al. First-in-human phase I trial of the anti-CEACAM5 antibody-drug conjugate SAR408701 in patients with advanced solid tumors (NCT02187848). *Europ J Cancer* 2016;69:S14-S5.
67. Bardia A, Mayer IA, Diamond JR, Moroosse RL, Isakoff SJ, Starodub AN, et al. Efficacy and safety of anti-Trop-2 antibody drug conjugate sacituzumab govitecan (IMMU-132) in heavily pretreated patients with metastatic triple-negative breast cancer. *J Clin Oncol* 2017;35(19):2141-2148.
68. Goldenberg DM, Cardillo TM, Govindan SV, Rossi EA, Sharkey RM. Trop-2 is a novel target for solid cancer therapy with sacituzumab govitecan (IMMU-132), an antibody-drug conjugate (ADC). *Oncotarget* 2015;6(26):22496-22512.
69. Heist RS, Guarino MJ, Masters G, Purcell WT, Starodub AN, Horn L, et al. Therapy of advanced nonsmall-cell lung cancer with an SN-38-anti-trop-2 drug conjugate, sacituzumab govitecan. *J Clin Oncol* 2017;35(24):2790-2797.
70. Ocean AJ, Starodub AN, Bardia A, Vahdat LT, Isakoff SJ, Guarino M, et al. Sacituzumab govitecan (IMMU-132), an anti-Trop-2-SN-38 antibody-drug conjugate for the treatment of diverse epithelial cancers: Safety and pharmacokinetics. *Cancer* 2017;123(19):3843-3854.
71. Starodub A, Camidge DR, Scheff RJ, Thomas SS, Guarino MJ, Masters GA, et al. Trop-2 as a therapeutic target for the antibody-drug conjugate (ADC), sacituzumab govitecan (IMMU-132), in patients (pts) with previously treated metastatic small-cell lung cancer (mSCLC). *American Society of Clinical Oncology meeting Abstract*. 2016;34(15 suppl):8559.
72. Starodub AN, Ocean AJ, Shah MA, Guarino MJ, Picozzi VJ, Vahdat LT, et al. First-in-human trial of a novel anti-Trop-2 antibody-SN-38 conjugate, sacituzumab govitecan, for the treatment of diverse metastatic solid tumors. *Clin Cancer Res* 2015;21(17):3870-3878.
73. Strop P, Tran TT, Dorywalska M, Delaria K, Dushin R, Wong OK, et al. RN927C, a site-specific trop-2 antibody-drug conjugate (ADC) with enhanced stability, is highly efficacious in preclinical solid tumor models. *Mol Cancer Ther* 2016;15(11):2698-2708.
74. Petrylak DP, Kantoff PW, Mega AE, Vogelzang NJ, Stephenson J, Fleming MT, et al. Prostate-specific membrane antigen antibody drug conjugate (PSMA ADC): A phase I trial in metastatic castration-resistant prostate cancer (mCRPC) previously treated with a taxane. *American Society of Clinical Oncology meeting Abstract*. 2013;31(Suppl; Abstr 5018).
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.
76. Henry MD, Wen S, Silva MD, Chandra S, Milton M, Worland PJ. A prostate-specific membrane antigen-targeted monoclonal antibody-chemotherapeutic conjugate designed for the treatment of prostate cancer. *Cancer Res* 2004;64(21):7995-8001.
77. Lammerts van Bueren JJ, Bleeker WK, Bøgh HO, Houtkamp M, Schuurman J, van de Winkel JG, et al. Effect of target dynamics on pharmacokinetics of a novel therapeutic antibody against the epidermal growth factor receptor: implications for the mechanisms of action. *Cancer Res* 2006;66(15):7630-7638.
78. Hicks SW, Lai KC, Gavrilescu LC, Yi Y, Sikka S, Shah P, et al. The antitumor activity of IMGNS29, a CD37-targeting antibody-drug conjugate, is potentiated by rituximab in non-Hodgkin lymphoma models. *Neoplasia* 2017;19(9):661-671.
79. Stathis A, Freedman AS, Flinn IW, Maddocks KJ, Weitman S, Berdeja JG, et al. A phase I study of IMGNS29, an antibody-drug conjugate (ADC) targeting CD37, in adult patients with relapsed or refractory b-cell non-hodgkin's lymphoma (NHL). *Blood* 2014;124(21):1760.



80. Doñate F, Yang P, Morrison K, Karki S, Aviña H, Lackey J, et al. Analysis of preclinical and clinical samples after treatment with a CD37 targeting antibody drug conjugate (AGS67E) support a high level of CD37 expression in NHL. *Hematol Oncol* 2017;35(S2):290-291.
81. Sawas A, Savage KJ, Perez R, Advani RH, Butturini A, Lackey J, et al. A phase I study of the anti-CD37 antibody-drug conjugate AGS67E in advanced lymphoid malignancies. Interim results. *Blood* 2015;126(23):3976.
82. Kogawa T, Yonemori K, Naito Y, Noguchi E, Shimizu C, Tamura K, et al. Phase 1/2, multicenter, nonrandomized, open-label, multiple-dose first-in-human study of U3-1402 (anti-HER3 antibody drug conjugate) in subjects with HER3-positive metastatic breast cancer. *J Clin Oncol* 2017;35:TPS1116.
83. Saunders LR, Bankovich AJ, Anderson WC, Aujay MA, Bheddah S, Black K, et al. A DLL3-targeted antibody drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. *Sci Transl Med* 2015;7(302):302ra136.
84. Ott P, Pavlick A, Johnson D, Hart L, Infante J, Luke J, et al. A phase 2 study of glembatumumab vedotin (GV), an antibody-drug conjugate (ADC) targeting gpNMB, in advanced melanoma. *Ann Oncol* 2016;27(suppl 1147: 6p).
85. Rose AA, Biondini M, Curiel R, Siegel PM. Targeting GPNMB with glembatumumab vedotin: current developments and future opportunities for the treatment of cancer. *Pharmacol Ther* 2017;179:127-141.
86. Saleh M, Bendell J, Rose A, Siegel P, Hart L, Sirpal S, et al. Correlation of GPNMB expression with outcome in breast cancer (BC) patients treated with the antibody-drug conjugate (ADC), CDX-011 (CR011-vcMMAE). *J Clin Oncol* 2010;28(15\_suppl):1095.
87. Yardley DA, Melisko ME, Forero A, Daniel BR, Montero AJ, Guthrie TH, et al. METRIC: a randomized international study of the antibody-drug conjugate glembatumumab vedotin (GV or CDX-011) in patients (pts) with metastatic gpNMB-overexpressing triple negative breast cancer (TNBC). *J Clin Oncol* 2015;33(Suppl; abstr TPS-1110).
88. Palanca-Wessels MC, Czuczman M, Salles G, Assouline S, Sehn LH, Flinn I, et al. Safety and activity of the anti-CD79B antibody-drug conjugate polatuzumab vedotin in relapsed or refractory B-cell non-Hodgkin lymphoma and chronic lymphocytic leukaemia: a phase I study. *Lancet Oncol* 2015;16(6):704-715.
89. Almhanna K, Wright D, Mercade TM, Van Laethem JL, Gracian AC, Guillen-Ponce C, et al. A phase II study of antibody-drug conjugate, TAK-264 (MLN0264) in previously treated patients with advanced or metastatic pancreatic adenocarcinoma expressing guanylyl cyclase C. *Invest New Drugs* 2017;35(5):634-641.
90. Cruz Zambrano C, Almhanna K, Messersmith WA, Rodon Ahnert J, Ryan DP, Faris JE, et al. MLN0264, an investigational antiguanlyl cyclase C (GCC) antibody-drug conjugate (ADC), in patients (pts) with advanced gastrointestinal (GI) malignancies: Phase I study. *J Clin Oncol* 2014;32(Suppl; abstr 3456).
91. Burris HA, Gordon MS, Gerber DE, Spigel DR, Mendelson DS, Schiller JH, et al. A phase I study of DNIB 0600A, an antibody-drug conjugate (ADC) targeting NaPi2b, in patients (pts) with non-small cell lung cancer (NSCLC) or platinum-resistant ovarian cancer (OC). *J Clin Oncol* 2014;32(Suppl; abstr 2504).
92. Lin K, Rubinfeld B, Zhang C, Firestein R, Harstad E, Roth L, et al. Preclinical development of an anti-NaPi2b (SLC34A2) antibody-drug conjugate as a therapeutic for non-small cell lung and ovarian cancers. *Clin Cancer Res* 2015;21(22):5139-5150.
93. Gomez-Roca CA, Boni V, Moreno V, Morris JC, Delord JP, Calvo E, et al. A phase I study of SAR566658, an anti CA6-antibody drug conjugate (ADC), in patients (Pts) with CA6-positive advanced solid tumors (STs)(NCT-01156870). *J Clin Oncol* 2016;34:2511.
94. Ilovich O, Natarajan A, Hori S, Sathirachinda A, Kimura R, Srinivasan A, et al. Development and validation of an immuno-PET tracer as a companion diagnostic agent for antibody-drug conjugate therapy to target the CA6 epitope. *Radiology* 2015;276(1):191-198.
95. Kaufman JL, Niesvizky R, Stadtmauer EA, Chanan-Khan A, Siegel D, Horne H, et al. Phase I, multicentre, dose-escalation trial of monotherapy with milatuzumab (humanized anti-CD74 monoclonal antibody) in relapsed or refractory multiple myeloma. *Br J Haematol* 2013; 163(4):478-486.
96. Kelly KR, Chanan-Khan A, Heffner LT, Somlo G, Siegel DS, Zimmerman T, et al. Indatuximab ravtansine (BT062) in combination with lenalidomide and lowdose dexamethasone in patients with relapsed and/or refractory multiple myeloma: clinical activity in patients already exposed to lenalidomide and bortezomib. *Blood* 2014; 124(21):4736.
97. Tai YT, Mayes PA, Acharya C, Zhong MY, Cea M, Cagnetta A, et al. Novel anti-B-cell maturation antigen antibody-drug conjugate (GSK2857916) selectively induces killing of multiple myeloma. *Blood* 2014;123(20): 3128-3138.
98. Khandelwal A, Saber H, Shapiro MA, Zhao H. Antibody-drug conjugate development. In: Phillips GL, editor. *Antibody-drug conjugates and immunotoxins*. New York: Human Press; 2013. p. 23-38.
99. Xu S. Internalization, trafficking, intracellular processing and actions of antibody-drug conjugates. *Pharm Res* 2015;32(11):3577-3583.
100. U.S. National Library of Medicine [Internet]. Bethesda (MD): U.S. National Library of Medicine. 2000 Feb -. A Study of DFRF4539A in Patients with Relapsed or Refractory Multiple Myeloma; 2016 November 02 [cited 2018 June 08]; [about 4 screens]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01432353>.
101. Gish K, Kim H, Power R, Fox M, Hickson J, McGonigal T, et al., editors. Preclinical evaluation of Abbv-838, a first-in-class anti-Cs1 antibody-drug conjugate for the treatment of multiple myeloma. *Haematologica* 2016; 101:253.
102. Chanan-Khan A, Wolf JL, Garcia J, Gharibo M, Jagannath S, Manfredi D, et al. Efficacy analysis from phase I

- study of lorvotuzumab mertansine (IMGN901), used as monotherapy, in patients with heavily pretreated CD56-positive multiple myeloma—a preliminary efficacy analysis. *Blood* 2010;116(21):1962.
103. Doñate F, Raitano A, Morrison K, An Z, Capo L, Aviña H, et al. AGS16F is a novel antibody drug conjugate directed against ENPP3 for the treatment of renal cell carcinoma. *Clin Cancer Res* 2016;22(8):1989-1999.
  104. Thompson JA, Motzer R, Molina AM, Choueiri TK, Heath EI, Kollmannsberger CK, et al. Phase I studies of anti-ENPP3 antibody drug conjugates (ADCs) in advanced refractory renal cell carcinomas (RRCC). *J Clin Oncol* 2015;33(Suppl 2503).
  105. Lassen U, Hong D, Diamantis N, Subbiah V, Kumar R, Sorensen M, et al. A phase I, first-in-human study to evaluate the tolerability, pharmacokinetics and preliminary efficacy of HuMax-tissue factor-ADC (TFADC) in patients with solid tumors. *J Clin Oncol* 2015;33(Suppl; Abstr 2570).
  106. Thompson JA, Motzer R, Molina AM, Choueiri TK, Heath EI, Kollmannsberger CK, et al. Phase I studies of anti-ENPP3 antibody drug conjugates (ADCs) in advanced refractory renal cell carcinomas (RRCC). *J Clin Oncol* 2015;33(Suppl 2503).
  107. Martin L, Konner J, Moore K, Seward S, Matulonis U, Perez R, et al. Characterization of folate receptor alpha (FR $\alpha$ ) expression in archival tumor and biopsy samples in a phase I study of mirvetuximab soravtansine, a FR $\alpha$ -targeting antibody-drug conjugate (ADC), in relapsed epithelial ovarian cancer patients. *Gynecol Oncol* 2017; 145:34.
  108. Moore KN, Matulonis UA, O'Malley DM, Konner JA, Martin LP, Perez RP, et al. Mirvetuximab soravtansine (IMGN853), a folate receptor alpha (FR $\alpha$ )-targeting antibody-drug conjugate (ADC), in platinum-resistant epithelial ovarian cancer (EOC) patients (pts): Activity and safety analyses in phase I pooled expansion cohorts. American Society of Clinical Oncology; 2017. *J Clin Oncol* 2017;35(no. 15):5547-5547.
  109. Moore KN, Ponte J, LoRusso P, Birrer MJ, Bauer TM, Borghaei H, et al. Relationship of pharmacokinetics (PK), toxicity, and initial evidence of clinical activity with IMGN853, a folate receptor alpha (FR $\alpha$ ) targeting antibody drug conjugate in patients (Pts) with epithelial ovarian cancer (EOC) and other FR $\alpha$ -positive solid tumors. *J Clin Oncol* 2014;32(Suppl; Abstr 5571).
  110. O'Malley DM, Moore KN, Vergote I, Martin LP, Gilbert L, Gonzalez Martin A, et al. Safety findings from FORWARD II: A Phase 1b study evaluating the folate receptor alpha (FR $\alpha$ )-targeting antibody-drug conjugate (ADC) mirvetuximab soravtansine (IMGN853) in combination with bevacizumab, carboplatin, pegylated liposomal doxorubicin (PLD), or pembrolizumab in patients (pts) with ovarian cancer. *J Clin Oncol* 2017;35:5553.
  111. Liu J, Moore K, Birrer M, Berlin S, Matulonis U, Infante J, et al. Targeting MUC16 with the antibody-drug conjugate DMUC5754A in patients with platinum resistant ovarian cancer: A phase I study of safety and pharmacokinetics. AACR Annual Meeting; April 6-10; Washington, DC: 2013.
  112. Goff LW, Papadopoulos K, Posey J, Phan A, Patnaik A, Miller J, et al. A phase II study of IMGN242 (huC242-DM4) in patients with CanAg-positive gastric or gastroesophageal (GE) junction cancer. *J Clin Oncol* 2009;27 (15S):e15625-e.
  113. Hong DS, Garrido-Laguna I, Krop IE, Subbiah V, Werner TL, Cotter CM, et al. First-in-human dose escalation, safety, and PK study of a novel EFNA4-ADC in patients with advanced solid tumors. *J Clin Oncol* 2015; 33(15\_suppl):2520.
  114. Annunziata CM, Kohn EC, LoRusso P, Houston ND, Coleman RL, Buzoianu M, et al. Phase 1, open-label study of MEDI-547 in patients with relapsed or refractory solid tumors. *Invest New Drugs* 2013;31(1):77-84.
  115. Jacobson O, Niu G, Kiesewetter DO, Li Q, Yang G, Cook K, et al. PET-guided evaluation and optimization of internalized antibody-drug conjugates targeting erythropoietin producing hepatoma A2 receptor. *J Nucl Med* 2017;58(11):1838-1844.
  116. Challita-Eid PM, Satpayev D, Yang P, An Z, Morrison K, Shostak Y, et al. Enfortumab vedotin antibody-drug conjugate targeting nectin-4 is a highly potent therapeutic agent in multiple preclinical cancer models. *Cancer Res* 2016;76(10):3003-3013.
  117. Rosenberg JE, Heath EI, Van Veldhuizen PJ, Merchan JR, Lang JM, Ruether JD, et al. Anti-tumor activity, safety and pharmacokinetics (PK) of ASG-22CE (ASG-22ME; enfortumab vedotin) in a phase I dose escalation trial in patients (Pts) with metastatic urothelial cancer (mUC). *J Clin Oncol* 2016;34(15\_suppl):4533-4533.
  118. Le PU, Nabi IR. Distinct caveolae-mediated endocytic pathways target the Golgi apparatus and the endoplasmic reticulum. *J Cell Sc* 2003;116(Pt 6):1059-1071.
  119. Petrylak D, Heath E, Sonpavde G, George S, Morgans A, Eigel B, et al. Interim analysis of a phase I dose escalation trial of the antibody drug conjugate (ADC) AGS15E (ASG-15ME) in patients (Pts) with metastatic urothelial cancer (mUC). *Ann Oncol* 2016;27(suppl 6): 780PD.
  120. Angevin E, Strickler JH, Weekes CD, et al. Phase I study of ABBV-399, a c-Met antibody-drug conjugate (ADC), as monotherapy and in combination with erlotinib in patients (pts) with non-small cell lung cancer (NSCLC). *J Clin Oncol* 2017;35:2509.
  121. Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cervený CG, Chace DF, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. *Nat Biotechnol* 2003;21(7):778-784.
  122. Wang J, Anderson MG, Oleksijew A, Vaidya KS, Boghaert ER, Tucker L, et al. ABBV-399, a c-Met antibody-drug conjugate that targets both MET-Amplified and c-Met-Overexpressing tumors, irrespective of MET pathway dependence. *Clin Cancer Res* 2017;23(4):992-1000.
  123. Wang J, Goetsch L, Tucker L, Zhang Q, Gonzalez A, Vaidya KS, et al. Anti-c-Met monoclonal antibody ABT-700 breaks oncogene addiction in tumors with MET amplification. *BMC Cancer* 2016;16:105.
  124. Sommer A, Kopitz C, Schatz CA, Nising CF, Mahlerlert C, Lerchen HG, et al. Preclinical efficacy of the

- auristatin-based antibody-drug conjugate BAY 1187982 for the treatment of FGFR2-positive solid tumors. *Cancer Res* 2016;76(21):6331-6339.
125. Willuda J, Linden L, Lerchen H-G, Kopitz C, Stelte-Ludwig B, Pena C, et al. Preclinical antitumor efficacy of BAY 1129980-a novel auristatin-based anti-C4. 4A (LYPD3) antibody-drug conjugate for the treatment of non-small cell lung cancer. *Mol Cancer Ther* 2017;16(5):893-904.
  126. Menezes D, Abrams TJ, Karim C, Tang Y, Ying C, Miller K, et al. Development and activity of a novel antibody-drug conjugate for the treatment of P-cadherin expressing cancers. *Cancer Res* 2015;75(15):1682.
  127. Shapiro GI, Vaishampayan UN, LoRusso P, Barton J, Hua S, Reich SD, et al. First-in-human trial of an anti-5T4 antibody-monomethylauristatin conjugate, PF-06263507, in patients with advanced solid tumors. *Invest New Drugs* 2017;35(3):315-323.
  128. Danila DC, Fleisher M, Carrasquillo JA, Gilbert H, Morris MJ, Bellomo LP, et al. STEAP1 as a predictive biomarker for antibody-drug conjugate (ADC) activity in metastatic castration resistant prostate cancer (mCRPC). *J Clin Oncol* 2014;32(Suppl; Abstr 5024).
  129. Danila DC, Scher HI, Szafer-Glusman E, Herkal A, Suttman R, Fleisher M, et al. Predictive biomarkers of tumor sensitivity to STEAP1 antibody-drug conjugate (ADC) in patients (pts) with metastatic castration resistant prostate cancer (mCRPC). *AACR 106th Annual Meeting* 2015; April 18-22, 2015; Philadelphia, PA.
  130. Danila DC, Szmulewitz RZ, Baron AD, Higano CS, Scher HI, Morris MJ, et al. A phase I study of DSTP-3086S, an antibody-drug conjugate (ADC) targeting STEAP-1, in patients (pts) with metastatic castration-resistant prostate cancer (CRPC). *J Clin Oncol* 2014; 32(Suppl; abstr 5024).
  131. Williams SP, Ogasawara A, Tinianow JN, Flores JE, Kan D, Lau J, et al. ImmunoPET helps predicting the efficacy of antibody-drug conjugates targeting TENB2 and STEAP1. *Oncotarget* 2016;7(18):25103-25112.
  132. Damelin M, Bankovich A, Bernstein J, Lucas J, Chen L, Williams S, et al. A PTK7-targeted antibody-drug conjugate reduces tumor-initiating cells and induces sustained tumor regressions. *Sci Transl Med* 2017;9(372). pii: eaag2611.
  133. Sachdev J, Maitland M, Sharma M, Moreno V, Boni V, Kummur S, et al. A phase I study of PF-06647020, an antibody-drug conjugate (ADC) targeting protein tyrosine kinase 7 (PTK7), in patients with advanced solid tumors including platinum resistant ovarian cancer (OVCA). *Ann Oncol* 2016;27(suppl 6):LBA35.
  134. Damelin M, Bankovich A, Park A, Aguilar J, Anderson W, Santaguada M, et al. Anti-EFNA4 calicheamicin conjugates effectively target triple-negative breast and ovarian tumor-initiating cells to result in sustained tumor regressions. *Clin Cancer Res* 2015;21(18):4165-4173.
  135. Sussman D, Smith LM, Anderson ME, Duniho S, Hunter JH, Kostner H, et al. SGN-LIV1A: a novel antibody-drug conjugate targeting LIV-1 for the treatment of metastatic breast cancer. *Mol Cancer Ther* 2014;13(12):2991-3000.
  136. Alley SC, Zhang X, Okeley NM, Anderson M, Law CL, Senter PD, et al. The pharmacologic basis for antibody-auristatin conjugate activity. *J Pharmacol Exp Ther* 2009;330(3):932-938.
  137. Deckert J, Park PU, Chicklas S, Yi Y, Li M, Lai KC, et al. A novel anti-CD37 antibody-drug conjugate with multiple anti-tumor mechanisms for the treatment of B-cell malignancies. *Blood* 2013;122(20):3500-3510.
  138. English DP, Bellone S, Schwab CL, Bortolomai I, Bonazzoli E, Cocco E, et al. T-DM1, a novel antibody-drug conjugate, is highly effective against primary HER2 overexpressing uterine serous carcinoma in vitro and in vivo. *Cancer Med* 2014;3(5):1256-1265.
  139. Boswell CA, Mundo EE, Firestein R, Zhang C, Mao W, Gill H, et al. An integrated approach to identify normal tissue expression of targets for antibody-drug conjugates: case study of TENB2. *Br J Pharmacol* 2013;168(2):445-457.
  140. The ASCO Post [Internet]. Huntington: HSP News Service, L.L.C., Phase I Trial of New Antibody-Drug Conjugate Shows Promise Against All Forms of Melanoma; 2014 August 04 [cited 2018 June 08]; [about 2 screens]. Available from: <http://www.ascopost.com/News/15102>.
  141. Bendell J, Moore K, Qin A, Johnson D, Schindler J, Papadopoulos K, et al. 472 A phase I study of IMGN-388, an antibody drug conjugate targeting av integrin, in patients with solid tumors. *Eur J Cancer Suppl* 2010;8(7):152.
  142. Kelly RK, Olson DL, Sun Y, Wen D, Wortham KA, Antognetti G, et al. An antibody-cytotoxic conjugate, BIIB015, is a new targeted therapy for Cripto positive tumours. *Eur J Cancer* 2011;47(11):1736-1746.
  143. Coveler AL, Von Hoff DD, Ko AH, Whiting NC, Zhao B, Wolpin BM. A phase I study of ASG-5ME, a novel antibody-drug conjugate, in pancreatic ductal adenocarcinoma. *J Clin Oncol* 2013;31(Suppl 4; abstr 176).
  144. Modi S, Eder JP, Lorusso P, Weekes C, Chandarlapaty S, Tolaney SM, et al. A phase I study evaluating DLYE-5953A, an antibody-drug conjugate targeting the tumor-associated antigen lymphocyte antigen 6 complex locus E (Ly6E), in patients (Pts) with solid tumors. *Ann Oncol* 2016;27(suppl\_6):3570.
  145. Lassen UN, Ramalingam SS, Lopez JS, Harvey RD, Ameratunga M, de Hoon J, et al. GCT1021-01, a first-in-human, open-label, dose-escalation trial with expansion cohorts to evaluate safety of Axl-specific antibody-drug conjugate (HuMax-Axl-ADC) in patients with solid tumors (NCT02988817). *J Clin Oncol* 2017;35:TPS2605.
  146. Canzonieri V, Gattei V, Spina M, Polizzi-Anselmo A, Attanasio N, Kaplan A, et al. CD205, a target antigen for a novel antibody drug conjugate (ADC): Evaluation of antigen expression on non-Hodgkin lymphoma (NHL). *J Clin Oncol* 2017;35(15\_suppl): published online before print.
  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 pyrroplomenzodiazapine-



## Antibody-Drug Conjugates: Possibilities and Challenges

- based antibody drug conjugate, in relapsed/refractory hodgkin/non-hodgkin lymphoma. *Hematol Oncol* 2017; 35(S2):270-271.
148. Baudat Y, Cameron B, Dabdoubi T, Lefebvre AM, Merino-Trigo A, Thomas C, et al, editors. Characterization of a novel maytansinoid-antibody-drug conjugate targeting LAMP1 expressed at the surface of tumor cells. 107th Annual Meeting of the American Association for Cancer Research; 2016 Apr 16-20; New Orleans, LA. Philadelphia: American Association for Cancer Research; 2016.
  149. Petrul HM, Schatz CA, Kopitz CC, Adnane L, McCabe TJ, Trail P, et al. Therapeutic mechanism and efficacy of the antibody-drug conjugate BAY 79-4620 targeting human carbonic anhydrase 9. *Mol Cancer Ther* 2012;11(2):340-349.
  150. Herbst R, Wang Y, Gallagher S, Mittereder N, Kuta E, Damschroder M, et al. B-cell depletion in vitro and in vivo with an afucosylated anti-CD19 antibody. *J Pharmacol Exp Ther* 2010;335(1):213-222.
  151. Cardarelli PM, Rao-Naik C, Chen S, Huang H, Pham A, Moldovan-Loomis M-C, et al. A nonfucosylated human antibody to CD19 with potent B-cell depletive activity for therapy of B-cell malignancies. *Cancer Immunol Immunother* 2010;59(2):257-265.
  152. Horton HM, Bernett MJ, Pong E, Peipp M, Karki S, Chu SY, et al. Potent in vitro and in vivo activity of an Fc-engineered anti-CD19 monoclonal antibody against lymphoma and leukemia. *Cancer Res* 2008;68(19):8049-8057.
  153. Awan FT, Lapalombella R, Trotta R, Butchar JP, Yu B, Benson DM, et al. CD19 targeting of chronic lymphocytic leukemia with a novel Fc-domain-engineered monoclonal antibody. *Blood* 2010;115(6):1204-1213.
  154. Reichert JM. Bispecific antibodies and ADCs: once and future kings? *MAbs* 2011;3(4):329-330.
  155. Golay J, D'Amico A, Borleri G, Bonzi M, Valgardsdotir R, Alzani R, et al. A novel method using blinatumomab for efficient, clinical-grade expansion of polyclonal T cells for adoptive immunotherapy. *J Immunol* 2014; 193(9):4739-4747.
  156. Ng G, Spreter T, Davies R, Wickman G. ZW38, a novel azymetric bispecific CD19-directed CD3 T cell engager antibody drug conjugate with controlled T cell activation and improved B cell cytotoxicity. *Blood* 2016;128(22):1841.
  157. Sellmann C, Doerner A, Knuehl C, Rasche N, Sood V, Krah S, et al. Balancing selectivity and efficacy of bispecific epidermal growth factor receptor (EGFR)× c-MET antibodies and antibody-drug conjugates. *J Biol Chem* 2016;291(48):25106-25119.
  158. Sierra JR, Tsao MS. c-MET as a potential therapeutic target and biomarker in cancer. *Ther Adv Med Oncol* 2011;3(1 suppl):S21-S35.
  159. Andreev J, Thambi N, Bay AEP, Delfino F, Martin J, Kelly MP, et al. Bispecific antibodies and antibody-drug conjugates (ADCs) bridging HER2 and prolactin receptor improve efficacy of HER2 ADCs. *Mol Cancer Ther* 2017;16(4):681-693.
  160. Azvolinsky A. Conjugating antibodies to cytotoxic agents: getting the best of both worlds? *J Natl Cancer Inst* 2013;105(23):1765-1766.
  161. Chari RJ, Goldmacher VS, Lambert JM, Blattler WA, inventors; Google Patents, assignee. Cytotoxic agents comprising maytansinoids and their therapeutic use. United States patent US 5,416,064 A. 1995 May 16.
  162. Sullivan R, Paré GC, Frederiksen LJ, Semenza GL, Graham CH. Hypoxia-induced resistance to anticancer drugs is associated with decreased senescence and requires hypoxia-inducible factor-1 activity. *Mol Cancer Ther* 2008;7(7):1961-1973.
  163. Widdison WC, Chari RVJ. Factors involved in the design of cytotoxic payloads for antibody-drug conjugates. In: Phillips G, editor. *Antibody-Drug Conjugates and Immunotoxins*. New York: Human Press; 2013. p. 93-115.
  164. Vankemmelbeke M, Durrant L. Third-generation antibody drug conjugates for cancer therapy--a balancing act. *Ther Deliv* 2016;7(3):141-144.
  165. Govindan SV, Goldenberg DM. Designing immunconjugates for cancer therapy. *Expert Opin Biol Ther* 2012;12(7):873-890.
  166. Maurya DK, Ayuzawa R, Doi C, Troyer D, Tamura M. Topoisomerase I inhibitor SN-38 effectively attenuates growth of human non-small cell lung cancer cell lines invitro and in vivo. *J Environ Pathol Toxicol Oncol* 2011;30(1):1-10.
  167. Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Arrojo R, Chang CH, et al. Sacituzumab govitecan (IMMU-132), an anti-Trop-2/SN-38 antibody-drug conjugate: Characterization and efficacy in pancreatic, gastric, and other cancers. *Bioconjug Chem* 2015;26(5):919-931.
  168. Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Goldenberg DM. Humanized anti-Trop-2 IgG-SN-38 conjugate for effective treatment of diverse epithelial cancers: preclinical studies in human cancer xenograft models and monkeys. *Clin Cancer Res* 2011;17(10):3157-3169.
  169. Sharkey RM, Govindan SV, Cardillo TM, Goldenberg DM. Epratuzumab-SN-38: a new antibody-drug conjugate for the therapy of hematologic malignancies. *Mol Cancer Ther* 2012;11(1):224-234.
  170. Acchione M, Kwon H, Jochheim CM, Atkins WM. Impact of linker and conjugation chemistry on antigen binding, Fc receptor binding and thermal stability of model antibody-drug conjugates. *MAbs* 2012;4(3):362-372.
  171. Sochaj AM, Świdarska KW, Otlewski J. Current methods for the synthesis of homogeneous antibody-drug conjugates. *Biotechnol Adv* 2015;33(6 Pt 1):775-784.
  172. Wang L, Amphlett G, Blättler WA, Lambert JM, Zhang W. Structural characterization of the maytansinoid monoclonal antibody immunoconjugate, huN901-DM1, by mass spectrometry. *Protein Sci* 2005;14(9):2436-2446.
  173. Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, et al. Site-specific conjugation of a cytotoxic



- drug to an antibody improves the therapeutic index. *Nat Biotechnol* 2008b;26(8):925-932.
174. Schroeder DD, Tankersky DL, Lundblad JL. A new preparation of modified immune serum globulin (human) suitable for intravenous administration. *Vox Sang* 1981;40(6):373-382.
  175. Willner D, Trail PA, Hofstead SJ, King HD, Lasch SJ, Braslawsky GR, et al. (6-Maleimidocaproyl)hydrazone of doxorubicin--a new derivative for the preparation of immunoconjugates of doxorubicin. *Bioconjug Chem* 1993;4(6):521-527.
  176. Junutula JR, Bhakta S, Raab H, Ervin KE, Eigenbrot C, Vandlen R, et al. Rapid identification of reactive cysteine residues for site-specific labeling of antibody-Fabs. *J Immunol Methods* 2008;332(1-2):41-52.
  177. Hofer T, Skeffington LR, Chapman CM, Rader C. Molecularly defined antibody conjugation through a selenocysteine interface. *Biochemistry* 2009;48(50):12047-12057.
  178. Axup JY, Bajjuri KM, Ritland M, Hutchins BM, Kim CH, Kazane SA, et al. Synthesis of site-specific antibody-drug conjugates using unnatural amino acids. *Proc Natl Acad Sci USA* 2012;109(40):16101-16106.
  179. Okeley NM, Toki BE, Zhang X, Jeffrey SC, Burke PJ, Alley SC, et al. Metabolic engineering of monoclonal antibody carbohydrates for antibody-drug conjugation. *Bioconjug Chem* 2013;24(10):1650-1655.
  180. Zhu Z, Ramakrishnan B, Li J, Wang Y, Feng Y, Prabakaran P, et al. Site-specific antibody-drug conjugation through an engineered glycotransferase and a chemically reactive sugar. *MAbs* 2014;6(5):1190-200.
  181. Zhou Q, Stefano JE, Manning C, Kyazike J, Chen B, Gianolio DA, et al. Site-specific antibody-drug conjugation through glycoengineering. *Bioconjug Chem* 2014;25(3):510-520.
  182. Drake PM, Albers AE, Baker J, Banas S, Barfield RM, Bhat AS, et al. Aldehyde tag coupled with HIPS chemistry enables the production of ADCs conjugated site-specifically to different antibody regions with distinct in vivo efficacy and PK outcomes. *Bioconjug Chem* 2014;25(7):1331-1341.
  183. Dennler P, Chiotellis A, Fischer E, Brégeon D, Belmont C, Gauthier L, et al. Transglutaminase-based chemoenzymatic conjugation approach yields homogeneous antibody-drug conjugates. *Bioconjug Chem* 2014;25(3):569-578.
  184. Spidel J, Vaessen B, Albone E, Cheng X, Verdi A, Kline JB. Site-Specific conjugation to native and engineered lysines in human immunoglobulins by microbial transglutaminase. *Bioconjug Chem* 2017;28(9):2471-2484.
  185. Strop P, Liu SH, Dorywalska M, Delaria K, Dushin RG, Tran TT, et al. Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates. *Chem Biol* 2013;20(2):161-167.
  186. Beerli RR, Hell T, Merkel AS, Grawunder U. Sortase enzyme-mediated generation of site-specifically conjugated antibody drug conjugates with high in vitro and in vivo potency. *PLoS One* 2015;10(7):e0131177.
  187. Grünewald J, Klock HE, Cellitti SE, Bursulaya B, McMullan D, Jones DH, et al. Efficient preparation of site-specific antibody-drug conjugates using phosphopantetheinyl transferases. *Bioconjug Chem* 2015;26(12):2554-2562.
  188. Tang F, Wang LX, Huang W. Chemoenzymatic synthesis of glycoengineered IgG antibodies and glycosite-specific antibody-drug conjugates. *Nat Protoc* 2017;12(8):1702-1721.
  189. McCombs JR, Owen SC. Antibody drug conjugates: design and selection of linker, payload and conjugation chemistry. *AAPS J* 2015;17(2):339-351.
  190. Ritter A. Antibody-drug conjugates: looking ahead to an emerging class of biotherapeutic. *Pharm Technol* 2012;36(1):42-47.
  191. Dyba M, Tarasova NI, Michejda CJ. Small molecule toxins targeting tumor receptors. *Curr Pharm Des* 2004;10(19):2311-2334.
  192. Goldmacher VS, Singh R, Chittenden T, Kovtun Y. Linker technology and impact of linker design on ADC properties. In: Phillips GL, editor. *Antibody-drug conjugates and immunotoxins*. New York: Human Press; 2013. p. 117-135.
  193. Erickson HK, Lambert JM. ADME of antibody-maytansinoid conjugates. *AAPS J* 2012;14(4):799-805.
  194. Kovtun YV, Audette CA, Mayo MF, Jones GE, Doherty H, Maloney EK, et al. Antibody-maytansinoid conjugates designed to bypass multidrug resistance. *Cancer Res* 2010;70(6):2528-2537.
  195. Sun X, Ponte JF, Yoder NC, Laleau R, Coccia J, Lanieri L, et al. Effects of drug-antibody ratio on pharmacokinetics, biodistribution, efficacy, and tolerability of antibody-maytansinoid conjugates. *Bioconjug Chem* 2017;28(5):1371-1381.
  196. van Geel R, Wijdeven MA, Heesbeen R, Verkade JM, Wasiel AA, van Berkel SS, et al. Chemoenzymatic conjugation of toxic payloads to the globally conserved N-glycan of native mAbs provides homogeneous and highly efficacious antibody-drug conjugates. *Bioconjug Chem* 2015;26(11):2233-2242.
  197. Zimmerman ES, Heibeck TH, Gill A, Li X, Murray CJ, Madlansacay MR, et al. Production of site-specific antibody-drug conjugates using optimized non-natural amino acids in a cell-free expression system. *Bioconjug Chem* 2014;25(2):351-361.