Deep Insight Section

Antibody-Drug Conjugates For the Treatment of Hematological Malignancy

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Abstract

Monoclonal antibodies raised against tumor-associated antigens have found a therapeutic niche in cancer treatment. Lately they have been modified modified either via attachment to protein toxins or highly potent, low-molecular-weight drugs. Such molecules, termed immunotoxins and antibody-drug conjugates (ADCs), respectively, represent a second revolution in antibody-mediated cancer therapy. Thus, highly toxic compounds are delivered to the interior of cancer cells based on antibody specificity for cell surface target antigens. This review discuss the processes and key considerations associated with preclinical and clinical development of ADCs, market dynamics and future projections for novel ADCs for the treatment of hematological malignancy. To date, three ADCs have been approved by the FDA: Gemtuzumab ozogamicin, Brentuximab vedotin, and ado-Trastuzumab emtansine (T-DM1). There are currently at least 11 ADCs in Phase I-III clinical trials for hematological malignancies for which targets, antibodies, linkers, and cytotoxic pay loads have been disclosed.

Key words:
Antibody-drug conjugate (ADC), Antibodies, Anticancer drugs, Hematological malignancy, Targeted therapy, ADC development and market.

Introduction

Conventional therapies, such as surgery, chemotherapy, and radiation therapy, have shown some success in the battle again cancer. However, they are often accompanied by complex and sometimes, severe side- effects due to the lack of target specificity. To circumvent this flaw and improve the efficacy and safety of cancer treatment, targeted cancer therapies, especially antibody-drug conjugates (ADCs), have been actively exploited and they are gaining a significant amount of attention during the recent years (FitzGerald et al, 2011; Beck et al, 2012; Lambert, 2013; Mullard, 2013; Leal et al, 2014; Diamantis and Banerji, 2016; Eighth world ADC Conference, 2016). The ADC technology utilizes the specificity of monoclonal antibodies with the potency of cytotoxic drug molecules, thereby taking advantage of the best characteristics of both components.

Various ADCs have been designed for use in patients with hematologic malignancies, including lymphoma, multiple myeloma, and leukemia. Lymphoma is a general term for a group of cancers that originate in the lymphatic system and is the most common type of blood cancer. There are two major categories of lymphoma: Hodgkin lymphoma, also known as Hodgkin disease, and non-Hodgkin lymphoma. Hodgkin lymphoma is a cancer that starts in white blood cells called lymphocytes, which

are part of the body's immune system. The disease is most often diagnosed in early adulthood (ages 20-40) and late adulthood (older than 55 years of age). Classical Hodgkin lymphoma is the most common type of Hodgkin lymphoma, accounting for 95% of cases. Classical Hodgkin lymphoma is distinguished from other lymphomas by the characteristic presence of CD30-positive Reed-Sternberg cells.

According to the American Cancer Society, more than 8,000 cases of Hodgkin lymphoma will be diagnosed in the United States during 2017 and approximately 1,000 will die from the disease. According to the Lymphoma Coalition, over 62,000 people worldwide are diagnosed with Hodgkin lymphoma each year and approximately 25,000 people die each year from this cancer. In the European Union, about 12,200 new cases and 2,600 deaths occurred in 2012 as a result of Hodgkin lymphoma.

One of the main challenges in the development of novel ADCs is the identification of a cell surface protein that is selectively expressed in tumors and that allows for efficient internalization of the payload drug to provide a clinical benefit (Teicher, 2009). Another challenge is to couple a highly specific monoclonal antibody to the appropriate linker–toxin combination to achieve the desired safety and efficacy profile (Polson et al, 2009).

By design, an ADC utilizes a monoclonal antibody to specifically deliver the toxic payload into target antigen expressing tumor cells, thus reducing or eliminating the payload cytotoxicity to healthy tissues. Not all tumor proteins can serve as ADC targets. To do so, they must meet the following stringent criteria:

- Cell surface localization: to allow efficient antibody/ADC binding.
- Tumour specific with decent protein expression: ideally 100,000 antigens/solid tumour cell and about 5000 antigens/hematologic cancer cell to ensure ADC specificity and efficacy.
- Higher tumour surface expression: if the identification of a tumour specific antigen is proven to be difficult, a shared cell surface antigen can also be considered under the condition that it shows a much

higher expression on cancer cell surfaces comparing to that on normal cells to reduce collateral damages.<
- Antibody to drug ratio: 4 drug molecules per antibody appears optimal because it maintains blood half-life nearly that of the naked antibody, preserves antibody binding to the target protein, and delivers sufficient number of cytotoxic molecules to the target cell to be lethal (Hamblett et al, 2004).
- Internalization: to ensure the ADC incorporation via receptor mediated endocytosis upon ADC binding.
- Desired turnover time: to facilitate maximizing ADC efficiency and efficacy.

Antibody-drug conjugates are complex bio-macromolecules combining the accurate targeting capability of a monoclonal antibody with the extremely high cytotoxicity of a payload drug via a molecular linker. As a potent new candidate for next generation cancer immunotherapy, ADCs have been demonstrated both by concept and in practice to accurately deliver the payload drugs only to the antigen bearing cells, minimizing the collateral damage to normal tissues and reducing the side-effects of otherwise highly toxic agents (Figure 1).

Bridging an antibody and a payload drug, the molecular linker is a key ingredient in the formation of an ADC. A good linker should bear a simple structure and be chemically compatible with both the antibody and the payload drug. It not only contributes to the stability and solubility of the ADC, but also dictates the release mechanism of the payload drug inside the cell. Up to this date, various linkers with high chemical complexities have been exploited but they all exert certain drawbacks that prevent their wide application in ADC developments.

FitzGerald et al, 2011 and Lambert, 2013 have summarized many of the initial pivotal studies that supported development and use of ADCs for cancer treatment. This review article will discuss issues surrounding development of ADCs for hematological malignancy, evidence from preclinical studies, current clinical trials, and future directions of research.
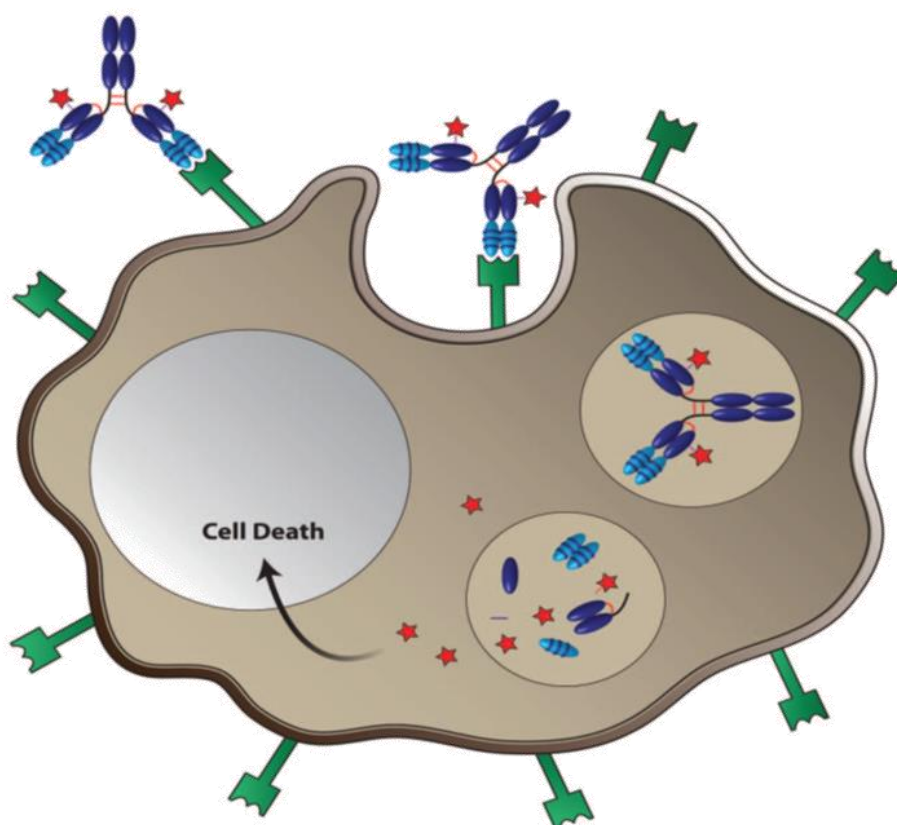
Figure 1. Schematic representation of the processes associated to the mechanism of action and biological activity of antibody drug conjugates. The monoclonal antibody component of an ADC selectively binds a cell-surface tumor antigen, resulting in internalization of the ADC-antigen complex through the process of receptor-mediated endocytosis. The ADC-antigen complex then traffics to lysosomal compartments and is degraded, releasing active cytotoxic drug inside the cell. Free drug causes cell death through either tubulin polymerization inhibition or DNA binding/damage depending on the drugs mechanism of action (taken from Panoski et al, 2014)

Preclinical Development of ADCs

In the developmental phase the ADC, it is evaluated by in vitro methods and then in animal models. The preclinical models are used to show ADC's feasibility of the concept, safety and efficacy. Following Investigational New Drug (IND) or Biologics License Agreement (BLA) regulatory submission and approval, Phase I-IV clinical trials in cancer patients are conducted for demonstrating safety and therapeutic efficacy of the ADC. After approval of New Drug Application (NDA), ADC is made available to wider population of cancer patients through large scale manufacturing, business development strategies and marketing. Different stages of ADC development are described below.

Monoclonal Antibodies against Hematological Malignancy

Monoclonal antibody-based treatment of cancer has been established as one of the most successful therapeutic strategies for both hematologic malignancies and solid tumors in the last 20 years. Following the development of hybridoma technology by Köhler and Milstein (1975),

combined with serological techniques and analytical tools, monoclonal antibodies were used to dissect the surface structure of human cancer cells, thus paving the way for the identification of cancer cell surface antigens suitable for targeting by antibodies. Antibody engineering has made breakthroughs over recent years making it feasible to produce humanized and fully human antibodies as the basic components of ADCs. The early generation ADCs used murine monoclonal antibodies causing significant immunogenicity, with many patients producing human anti-mouse antibodies thus reducing the efficacy of treatment. The most commonly used antibody format currently is human IgG isotypes and in particular IgG1 (Hughes, 2010; Perez et al, 2014).

Tumor-associated antigens recognized by therapeutic monoclonal antibodies against hematologic malignancies are outlined in Table 1. Hematopoietic differentiation antigens are glycoproteins usually associated with CD groupings (Van den Eynde and Scott, 1998; Weiner et al, 2010; Chan and Carter, 2010; Cheson and Leonard, 2008;

Antibody-Drug Conjugates For the Treatment of Hematological Malignancy

Leslie and Younes, 2013; Diamantis and Banerji, 2016).

Table 1. Tumor associated antigens for hematological malignancies

Antigen category	Examples of antigens	Tumor types expressing antigens
Clusters of differentiation (CD) antigens	CD19	NHL, ALL, DLBCL
	CD22	ALL, NHL, B-cell lymphoma
	CD30	NHL, ALCL, AML, HL
	CD33	AML
	CD37	NHL, CLL
	CD52	CLL
	CD74	CLL, NHL, Multiple myeloma
	CD79b	DLBCL, Follicular NHL
	CD98	AML
	CD138	Multiple myeloma

Abbreviations: NHL, non-Hodgkin lymphoma; ALL, Acute lymphocytic leukemia; DLBCL, Diffuse large B- cell lymphoma; ALCL, Anaplastic large cell lymphoma; AML, Acute myelogenous leukemia; HL, Hodgkin lymphoma; CLL, Chronic lymphocytic leukemia.

Leslie and Younes (2013) have described various CD antigens expressed by lymphoma (CD19, CD22, and CD30) and leukemia (CD22, CD33). Briefly, CD19 is expressed on the surface of B-lymphocytes throughout development with loss of expression on plasma cells. CD19 is rapidly internalized on binding with anti-CD19 antibody, MEDI-551. CD22 is involved in inhibitory B-cell receptor complex signaling and prevention of autoimmunity. Similar to CD19, it is rapidly internalized on antigen binding. CD30 is a member of the tumor-necrosis factor receptor family, expressed on activated T and B-lymphocytes, and rapidly internalized on antigen binding. CD33 a myeloid-specific transmembrane receptor is found on acute myelogenous leukemia (AML). The cell surface target Campath-1 (CD52) is expressed on chronic lymphocytic leukemia cells (Blatt et al, 2014).

Once an antigen is identified, the first step in ADC development project is extensive screening to acquire a few suitable monoclonal antibodies for specific target recognition (Zhang et al. 2010). Flow cytometry and immunohistochemistry techniques are typically used for confirming targeted protein expression on isolated cancer cell surface and on clinical tumor specimens fixed in paraffin sections.

ADC Cytotoxic payloads
The payload can be small drug molecules, radioisotopes, proteins, or bacterially/plant derived toxins. The payload drugs used in ADCs are highly toxic. They are small molecules that interact with intracellular targets and function as agents to block or disrupt crucial cellular metabolic pathways and eventually lead to cell death. Some payload drugs target tubular filaments and interfere with their dynamics to inhibit microtubule formation (e.g. Auristatins, Taxoids) or cause mitosis arrest (e.g. Maytansinoids). Other payloads inhibit crucial processes such as DNA transcription by binding to an important enzyme (Amatoxins bind to RNA polymerase) or enzyme complexes (thailanstatin A interacts with spliceosome complexes) to block transcription initiation or interfere with mRNA splicing, respectively. DNA itself is another bio-macromolecule that is frequently targeted by several payload drugs. Some payloads interact with DNA minor groove and cause DNA damage by inducing cleavages at specific sites (Calicheamicins) or by reacting with certain bases (e.g. Duocarmycins reacts with guanine). Other than the payloads mentioned above, novel chemical or biochemical entities that trigger specific downstream processes and result in cell damages, including drug carriers, proteins with toxic effects, toxic enzymes, and small molecule inhibitors targeting other crucial cellular pathways, have also been adopted as payloads (Teicher, 2009; Ducry and Stump, 2010).

ADC Linkers
Linkers accommodate different conjugation chemistries on both antibodies and payload drugs. They are an important portion in an ADC because they not only contribute to the stability of the complex in systematic circulation but also dictate the payload release mechanisms once internalized and trafficked into designated cellular locations. Linkers are categorized based on their release mechanisms into cleavable linkers (peptide linkers, ß-glucuronide linkers, pH-sensitive linkers, and glutathione-sensitivity linkers) and non-cleavable thioether and disulfide bonds (Ducry and Stump, 2010; Jain et al., 2015). For non-cleavable linker based ADC, the release mechanism is believed to occur via internalization of the ADC followed by degradation of the monoclonal antibody component in the

lysosomes, resulting in the release of the cytotoxic drug (e.g. Maytansinoid drug) still attached via the linker to a lysine residue (Chari et al., 1992).

In Vitro Efficacy Evaluation of ADCs
Besides good specificity and binding affinity, a successful ADC antibody must also exert B tendency for internalization after antigen binding. Most of the toxic payloads function by disrupting important cellular pathways after antibody internalization via receptor mediated endocytosis (Panoski et al, 2014). Efficacy evaluation for ADC is initially performed by in vitro cytotoxicity assays for determining the surviving fractions of cell cultures by colony-forming ability and by back-extrapolation of the exponential growth curves. Chari et al (1992) synthesized maytansinoids that had 100- to 1000-fold higher cytotoxic potency than clinically used anticancer drugs. Despite high potency, maytansine was ineffective in human clinical trials (Issell et al, 1978) because of its high systemic toxicity, which resulted in low therapeutic index. However, when maytansinoids were linked to antibodies via disulfide bonds, which ensured the release of fully active drug inside the cell, the ADC conjugates showed high antigen-specific cytotoxicity for cultured human cancer cells (50% inhibiting concentration, 10 to 40 pM), low systemic toxicity in mice, and good pharmacokinetic behavior. In addition, when anti-CD19 antibody B4 was conjugated via disulfide linkers to CC-1065 potent small drug molecule (Chari et al, 1995) or to plant toxin ricin (Shah et al, 1993) proved to be extremely potent to human Burkitt's lymphoma cell line Namalwa in antigen-specific manner. These and other early pivotal studies clearly demonstrated that ADCs proved much more cytotoxic to antigen expressing tumor cells than antigen-negative control MOLT-4 cells (acute lymphoblastic leukemia) when tested at the same time.

In Vivo Efficacy Using Xenograft Animal Models for Hematological Malignancies
To verify the efficacy of ADCs against hematological malignancies and to support initiation of clinical trials, human xenograft model in immunocompromised animals have been described for variety of human cancer cell lines (Table 2). For blood malignancies, subcutaneous (Kim et al, 2015) and systemic models (Shah et al, 1993) are the most common human xenograft models available.

Table 2. Human cell lines used for xenograft studies

Target (Reference)	Cancer type	Cell line
CD19	NHL, ALL, diffuse Large B Cell lymphoma, non-B non-T ALL	Namalwa, Nalm-6, Raji, Daudi
CD22	ALL, NHL, B cell lymphoma	Reh, SEM, NSLM6, KOPN8
CD23	AML	MOLM14, KG1
CD25	NHL, HL	KARPAS299, L-540k
CD30	HL, ALCL, NHL, AML	U937, MV4-11, HL60, MOLM-14
CD33	Relapsed AML	HL60, HEL9217, TF1-alpha, U937, KG1
CD74	Multiple myeloma	BjAB, Raji
SAIL (Kim et al, 2015)	AML	OCI-AML3, THP1

Abbreviations: NHL, non-Hodgkin lymphoma; ALL, Acute lymphocytic leukemia; AML, Acute myelogenous leukemia; HL, Hodgkin lymphoma; ALCL, Anaplastic large cell lymphoma.

Following the creation of the human xenograft tumor model in immunocompromised mice (SCID) or other athymic mice (e.g. conventional nude mice, triple-deficient nude mice), clinical grading and observation are carried out following treatment of animals with the ADC regimen. Tumor growth in subcutaneous xenograft model is monitored by measuring tumor size with Vernier caliper in two dimensions (long and short axis). As for systemic xenograft model, common method is luciferase-labeled carcinomatous cells combined with bioluminescent imaging system. Both animal models are routinely monitored for signs of diseases, including weight change, development of scruffy coat, limb paralysis, and the presence of palpable tumor.

The systemic xenograft models (survival models) are considered more relevant models than subcutaneous tumor models since they may represent disseminated or metastatic cancer patients. One of the early reports of animal survival models for evaluating therapeutic

efficacy of ADC against hematological tumours was described by Shah et al (1993). Groups of 10 SCID mice were injected intravenously (i.v.) with 4 million CD19 expressing Namalwa or Nalm-6 tumor cells. After 7 days, mice bearing established systemic tumors were treated i.v. for 5 consecutive days with either ant-B4-blocked ricin ADC (100 ug/kg/day) or anti-B4 antibody as control (72 ug/kg/day). Other controls included were untreated SCID mice bearing systemically growing tumors, isotype-matched non-specific control immunotoxin, N901-bR (100 ug/kg/day i.v. for 5 days), or anti-B4 naked antibody (2 mg/kg/day i.v. for 5 days). As compared to untreated animal survival curves observed following i.v. injections of 10x fold different numbers of tumor cells, the ADC (anti-B4-blocked ricin) showed tumor specific efficacy by killing up to 3 logs of tumor cells in SCID mice as evident by significant prolongation in life of the treated animals. Only very limited or no effects on animal survival was observed in animals treated with either anti-B4 naked antibody alone or N901-blocked ricin non-specific control ADC. The difference in survival between anti-B4-bR and untreated or anti-B4 antibody-treated control animals was highly significant (p = <0.02 log-rank test; p = <0.01, Wilcoxon test). The Namalwa survival model developed by Shah et al (1993) was also successfully used to demonstrate therapeutic efficacy of another tumour specific ADC, anti-B4-DC1 (Chari et al, 1995). In this study, highly cytotoxic synthetic drug CC-1065 (DC1) was conjugated to humanized version of anti-B4 murine antibody via novel cleavable disulfide linker. Groups of SCID mice with established Namalwa tumours were treated daily for 5 day via i.v. injection with anti- B4-DC1 at DC1 dose of 80 ug/kg/day or at DC1 dose of 80 ug/kg/day or isotype-matched but non-binding conjugate, N901-DC1. The anti-B4-DC1 conjugate showed specific anti-tumour efficacy in the Namalwa aggressive B-cell lymphoma survival model in SCID mice and completely cured animals bearing large tumours. Anti-B4-DC1 was considerably more effective in this tumour model than doxorubicin, cyclophosphamide, etoposide, or vincristine chemotherapeutic drugs at their maximum tolerated doses.

Regulatory Toxicology (Safety) & Pharmacology Studies for IND Submission
Using Good Laboratory Practice (GLP) guidelines, the ADC is evaluated in rodents, canine, and/or non-human primates for pharmacokinetics, absorption-distribution-metabolism (ADME), safety, efficacy, potential mutagenicity and immunogenicity.

For determining pharmacokinetic property of the ADC, several key parameters are measured including total and conjugated antibodies, free and conjugated drugs, as well as catabolites. For ADME evaluation, pharmacokinetic analysis is performed by assessing distribution of the radioisotope labeled ADC (PET scan or postmortem radioactivity analysis), metabolism is determined using mass spectrometry (LC- MS/MS) analysis of ADC metabolite and catabolite present in tissues, and excretion is determined by measuring presence of ADC fragments in animal excreta.

Toxicological property of an ADC is evaluated following acute- and repeat-dose toxicity tests, chronic toxicity test, and tissue cross-reactivity under GLP-compliant test platforms.

Efficacy evaluation of the ADC is usually performed in human tumor xenograft models as described previously. Detailed cage-side observations and pathological analysis of tissues are done to provide a complete profile of the ADC drug safety and efficacy.

Most ADCs are generated using human monoclonal antibodies or humanized antibodies to minimize immunogenicity and the toxic payloads are usually not immunogenic. However, as a newly formed bio-macromolecule, ADCs might present unexpected immunogenicity, an undesired feature for ADC performance.

Manufacturing of ADCs
An ADC is formed by covalent biochemical conjugation of a monoclonal antibody with highly toxic payload drugs via a small molecular linker. ADCs are emerging candidates for targeted cancer therapies and due to the extreme toxicity of their payloads, ADCs are often considered as a new generation of highly hazardous and toxic pharmaceutical products. The unique nature of ADC presents challenges in its large-scale production. ADC manufacturing requires proper facilities that strictly follow the criteria and guidelines of cGMP-standard bio-macromolecule production. These manufacturing facilities are extremely stringent about aseptic production and they need to be operated under an occupational exposure limit (OEL) below 50 ng/m3. For example, a leading contract research organization, Novasep (Le Mans, France), provides fully integrated supply chain for the cGMP manufacturing of ADCs, including bio-conjugation, payload, linker and monoclonal antibodies. Novasep was inspected and approved by US FDA in May 2016.

ADC manufacturing is a multistep process that can be divided into three distinct stages:

1. CGMP production of the antibody: Therapeutic antibodies are manufactured initially in pilot (5- 35 L) and then in large scale (>100 L) bioreactors using various expression systems such as hybridoma or mammalian cell lines.

2. CGMP synthesis of the drug-linker complex: Highly tailored linker molecules bearing different release mechanisms are synthesised. Pay load drugs are modified as necessary for formulating the drug-linker complex for ADC manufacturing.

3. Conjugation to form an ADC: ADC conjugation is achieved in cGMP certified reactors after small-scale conjugation protocol verification. The therapeutic impurities are removed utilizing advanced filtering devices such as ultrafiltration and tangential flow filtration (TFF) systems and characterized for:

- Structure for correct folding
- Conjugation sites for an accurate map of conjugated drug-linker locations via mass spectroscopy along with information regarding the physical attachment of the unreacted drug-linker on the antibody
- Drug to antibody molar ratio (DAR) and the distribution patter of different DAR species using advanced chromatography and mass spectroscopy analysis
- Stability for auto-fragmentation, aggregation, solubility, thermal stability, and in vitro serum stability
- Affinity to determine and compare the antigen and Fc receptor binding affinity of ADC to non- conjugated antibody using approaches such as ELISA, flow cytometry, or surface plasmon resonance (SPR)
- In vitro efficacy using conventional antigen-bearing cultured cell lines to evaluate the behaviour and cytotoxicity of an ADC
- ADC product formulation testing is usually evaluated as sterile solution and as lyophilized formulation. Sterile ADC solution is less expensive to manufacture, often frozen at -80 degrees C, and therefore needs complicated cold chain management. Lyophilized ADC may give better stability but it requires more complicated development and is therefore more expensive to manufacture.

The ADC product is finished by filling into aseptic vials via the cGMP sterile filling pipeline.

Clinical Development of ADCs

Traditional cancer chemotherapy leads to systemic toxicity in the patient. Monoclonal antibodies against antigens on cancer cells offer an alternative tumor-selective treatment. However, monoclonal antibodies on their own do not kill cancer cells. Therefore, antibody–drug conjugates uses antibodies to deliver a potent cytotoxic compound selectively to tumor cells, thus improving the therapeutic index of chemotherapeutic agents.

To date, three ADCs were approved by the FDA: Gemtuzumab ozogamicin, Brentuximab vedotin, and ado-Trastuzumab emtansine (T-DM1). Gemtuzumab ozogamicin was initially approved by FDA in 2000 for the treatment of relapsed CD33-positive acute myeloid leukemia in older patients not considered candidates for standard chemotherapy (Bross et al, 2001). However, it was withdrawn from the market after further studies which showed no real benefit (FDA press release, 2013). Brentuximab vedotin was approved in 2011 for the treatment of relapsed or refractory Hodgkin's lymphoma and relapsed or refractory systemic anaplastic large-cell lymphoma (Gopal et al, 2012; Pro et al, 2012; Senter and Sievers, 2012). Finally, the most recent ADC newcomer is T-DM1 for use in metastatic HER2-positive breast cancer (FDA Approval, 2013; Oostra and Macrae, 2014). Approval of these ADCs has spurred tremendous research interest in this field.

The FDA approved ADC for hematological malignancy, Brentuximab vedotin (Adcetris, Seattle Genetics) is composed of an anti-CD30 monoclonal antibody connected with a cleavable peptide to the highly potent tubulin inhibitor MMAE. CD30 is a member of the tumour necrosis factor (TNF) family identified on Reed–Sternberg cells of classical Hodgkin lymphoma (HL). Binding of Adcetris to the cell surface leads to internalization and lysosomal proteolytic cleavage of the linker releasing the MMAE (Senter and Sievers, 2012; Sievers and Senter, 2013). Adcetris has gained approval for the treatment of patients with relapsed or refractory CD30 HL following autologous stem cell transplant (ASCT) or patients not legible for ASCT who have failed at least two other chemotherapy treatments. Brentuximab vedotin has also been approved for patients with anaplastic large cell lymphoma (ALCL) as a second line. The accelerated approval for Hodgkin's lymphoma was based on a single-arm phase II clinical trial, where there was a 73% response rate, 32% complete remission and a median duration 20.5 months (Younes et al, 2012). The indication for ALCL was established based on the impressive results of the phase II study. Patients in this study had an 86% overall response rate and 54% complete responses (Pro et al, 2012). The most common adverse reactions were peripheral sensory neuropathy, neutropenia, fatigue, nausea and

thrombocytopenia. In USA, Brentuximab vedotin carries a black box warning for progressive multifocal leukoencephalopathy (Younes et al, 2012).

Clinical trials for ADCs against hematological malignancies have increased significantly in recent years. There are currently at least 11 ADCs in Phase I-II clinical trials for hematological malignancies for which targets, antibodies, linkers, and cytotoxic pay loads have been disclosed (Table 3). Most ADCs (8/11) in clinical development utilize humanized or fully human monoclonal antibodies. There are 2 ADCs that incorporate chimeric monoclonal antibodies, Indatuximab ravtansine (BT062), an anti-CD138 ADC, and Brentuximab vedotin, and anti-CD 30ADC. There are 5 ADCs that use monomethyl auristatin as the cytotoxic compound and 3 ADC that employ maytansine which causes mitosis arrest. Of the 11 ADCs in clinical development, 6 ADCs are in Phase II-III development and 5 ADCs are in Phase I development. This or next year, a market approval could become a reality for Inotuzumab ozogamicin. Currently, a marketing authorization application from Pfizer for acute lymphocytic leukemia (ALL) is being reviewed for Inotuzumab ozogamicin by EMA.

Table 3. Clinical development of antibody-drug conjugates for treating hematological malignancy

Antibody-Drug Conjugate	Target Antigen	Antibody	Linker	Cytotoxic Compound	Targeted Disease	Clinical Stage	Developer
Brentuximab vedotin	CD30	Ch IgG1	Valine-citrulline	MMAE	HL, ALCL	Approved marketed	Takeda/Seattle Genetics
Inotuzumab ozogamicin	CD22	Hz IgG4	Hydrazone	Calicheamicin	NHL, LL	Phase III	Pfizer
Coltuximab ravtansine	CD19	Hz IgG1	Hindered disulfide SPDB	Maytansine DM4	ALL, DLBCL	Phase II	ImmunoGen
Pinatuzumab vedotin (RG7593)	CD22	Human IgG1	Valine-citrulline	MMAE	DLBCL	Phase II	Genentech
DEBIO1562/ IMGN529	CD37	Hz IgG1	SMCC	Maytansine DM1	NHL	Phase II	Debiopharm/ ImmunoGen
Polatuzumab vedotin (RG7596)	CD79b	Hz IgG1	Valine-citrulline	MMAE	NHL	Phase II	Genentech/ Roche/Seattle Genetics
Indatuximab ravtansine (BT062)	CD138	Ch IgG4	SPDB-DM4	Maytansine DM4	MM	Phase II	Biotest/ ImmunoGen
SGN-CD19B	CD19	Not Identified	Not Identified	PBD	NHL	Phase I	Seattle Genetics
Brentuximab Vedotin+che-motherapy	CD30	Ch IgG1	Valine-citrulline	MMAE	Relapsed AML	Phase I	Massachusetts General Hospital
AGS67E	CD37	Human IgG2	'Protease-cleavable linker'	MMAE	NHL, CLL, AML	Phase I	Agensys
BMS-936561 (MDX-12030)	CD70	Human IgG	Valine-citrulline	Duocarmycin	NHL	Phase I	Bristol-Meyers Squibb

GSK2857916	BCMA	Hz IgG1	Maleimidocaproyl	MMAF	MM	Phase I	GlaxoSmithKline

Abbreviations: BCMA, B-cell maturation antigen; Ch, Chimeric; Hz, Humanized; SPDB, N-succinimidyl 3- (2-pyridyldithio)butyrate; SMCC, 4-(N-Maleimidomethyl) cyclohexanecarboxylic acid N- hydroxysuccinimide ester; MMAE, Monomethyl auristatin E; PBD, pyrrolobenzodiazepine; MMAF, Monomethyl auristatin F; HL, Hodgkin lymphoma; ALCL, Anaplastic large cell lymphoma; NHL, Non- Hodgkin's Lymphoma; LL, Lymphocytic Leukemia; ALL, Acute lymphocytic leukemia; DLBCL, Diffuse large B-cell lymphoma; MM, Multiple myeloma. Source: Clinicaltrials.gov: September 13, 2017; Developer company websites as of September 17, 2017; Sawas, 2017; Owonikoko et al 2016; Cohen et al, 2016; Jain et al, 2015; Pereira et al, 2015; Chari et al, 2014; Rostami et al, 2014; Trail, 2013; Leslie and Younes, 2013; Teicher and Chari, 2011.

Market Dynamics for ADCs
Since the commercialization of the first therapeutic monoclonal antibody product in 1986, as of November 2014, forty-seven naked monoclonal antibody products were approved in US and Europe for the treatment of variety of diseases. At an approval rate of 4 new monoclonal antibody products per year, approximately 70 antibody products could be on the market by 2020, and combined world-wide sales could be nearly $125 billion (Ecker et al, 2015).

Two ADCs, Brentuximab vedotin (Adcetris®) and ado-Trastuzumab emtansine (Kadcyla®) have been approved by the US Food and Drug Administration. Brentuximab Vedotin (Adcetris by Seattle Genetics) was approved by the FDA in 2011 as the first new therapeutic option for patients with Hodgkin lymphoma in more than 30 years. Brentuximab Vedotin has now become the standard of care for relapsed Hodgkin lymphoma, with more than 20,000 patients treated, as noted by Jonathan Drachman, M.D., Chief Medical Officer and Executive Vice President, Research and Development of Seattle Genetics. Brentuximab Vedotin is an antibody-drug conjugate directed to CD30, a defining marker of classical Hodgkin lymphoma, which combines the targeting ability of a monoclonal antibody, attached by a protease-cleavable linker to the cell-killing microtubule disrupting agent, monomethyl auristatin E (MMAE). The drug employs a linker system that is designed to be stable in the bloodstream but to release MMAE upon internalization into CD30-expressing tumor cells. In 2014, ado-trastuzumab (Kadcyla®by Genentech) was approved by the FDA to treat Her2-positive breast cancer, representing the first ADC drug for solid tumors.

There are more than 50 ADCs at various stages of clinical development, covering a broad spectrum of oncology targets (Beck et al, 2012; Mullard, 2013). The market for ADCs was worth approximately $1.3 billion in 2016 with just two approved marketed drugs, and its potential remains very large. Total revenues, representing product sales (collaboration and royalty revenues are not considered), are expected to be $4.2 billion worldwide by 2021 at a CAGR of 25.5% from 2016 through 2021. Glyco-engineering should help with better selection of monoclonal antibodies through optimal pharmacokinetic, potency and toxicity profiles (Shah, 2014). Glyco-engineering will undoubtedly play major part in further development of better target specific monoclonal antibodies and thus on the ADC market as a whole.

Further Development of ADCs for Cancer Treatment:
The ADC space continues to develop as knowledge and innovative technologies strive to improve the therapeutic window of ADCs. Despite the clinical success of Adcetris® and Kadcyla®, the field still faces challenging tasks, such as improving targeted delivery efficiently, minimizing systemic toxicity, and tackling drug resistance (Loganzo et al, 2016). Insufficient understanding of ADCs mechanism of action, inadequate knowledge of the management and understanding of ADCs off-target toxicities, and difficulties in the selection of suitable clinical settings such as patient selection, dosing regimen are some possible explanations for the slow clinical translation of new ADCs.

As a complex entity containing three components (monoclonal antibody, linker, and chemical drug), the function of ADCs is highly sensitive to each component's attributes. Their clinical outcomes can be further improved by optimizing target selections, binding moieties (monoclonal antibody and protein scaffolds), cytotoxic drugs, linkers, conjugation sites, and conjugation chemistries. As many novel ADC technologies mature over time, we expect to see a generation of safer and more effective ADCs for clinical translation and commercialization in the future. With further developments in antigen, effector, and linker technology, the specificity and efficacy of ADCs will continue to improve, creating valuable agents for both monotherapy and combination therapy in hematologic malignancies. Additionally, further correlative biomarker studies will be crucial to improve patient selection for ADCs against hematological and other malignancies.

Summary and Conclusions:

In summary, the search for "magic bullets" that can potently eradicate cancer without damaging normal tissues continues unabated. Antibody–drug conjugates are one of the fastest growing classes of oncology therapeutics. After half a century of research, the approvals of brentuximab vedotin and trastuzumab emtansine have paved the way for ongoing clinical trials that are evaluating more than 50 further ADC candidates. The limited success of first-generation ADCs (developed in the early 2000s) informed strategies to bring second-generation ADCs to the market, which have higher levels of cytotoxic drug conjugation, lower levels of naked antibodies and more-stable linkers between the drug and the antibody. Furthermore, lessons learned during the past decade are now being used in the development of third-generation ADCs.

In this review, I have discussed the current strategies for developing ADCs against hematological malignancy and other cancers. This included selection of target antigens as well as suitable cytotoxic drugs; the design of optimized linkers; preclinical development; clinical trials and toxicity issues. The selection and engineering of antibodies for site-specific drug conjugation, which will result in higher homogeneity and increased stability, as well as the quest for new conjugation chemistries and mechanisms of action, are priorities in future ADC research and development. One important issue that may have to be addressed is the development of host resistance to ADCs, similar to mixed drug resistance (MDR) observed for chemotherapeutic drugs. Lorenzo and co-workers (2016) have suggested that the modular nature of the ADC will allow components to be switched and replaced, enabling development of second-generation ADCs that overcome acquired resistance.

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