Advancement of Antibody-Drug Conjugates in Cancer Treatment: A Review

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Declaration

It is hereby declared that

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2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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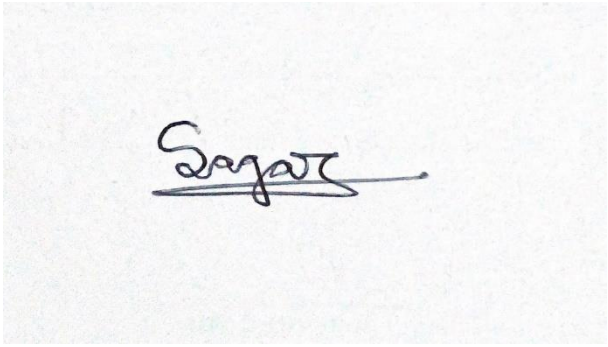

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Approval

The project titled "Advancement of Antibody-Drug Conjugates in Cancer Treatment: A Review" submitted by Baejid Hossain Sagar (18146012) of Spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 24/1/22.

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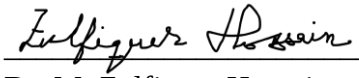

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Ethics Statement

The project does not involve any clinical trial or human participants and no animals were used or harmed.

Abstract

Antibody-drug conjugates (ADCs) are a rapidly growing class of biotherapeutic, targeted to selectively introduce cytotoxic agents into the cancer cell by means of monoclonal antibodies. USFDA has approved ten ADCs till date indicated for the treatment of breast cancer, urothelial cancer, myeloma, acute leukemia, and lymphoma. Besides, more than 80 ADCs are currently undergoing different phases of clinical trials. However, toxicity and non-specificity in treatment of solid tumors have led to challenges in the development of this novel and emerging class of anticancer treatment agents. Recent development in antibody, payload, and linker manufacturing technologies are helping to reduce the toxicity and define the future of ADCs. The current review is a compilation that reflects the recent advancements in the field of ADCs and covers the basic aspects of ADCs, emphasizing on the current development in ADCs, as well as future directions.

Keywords: Antibody-drug conjugates; cytotoxic payloads; monoclonal antibodies; linkers

Dedication

Dedicated to my faculty members, family and friends.

Acknowledgement

I would like to express my sincere gratitude to my project supervisor, Dr. Eva Rahman Kabir for providing the guidance and support in completing my project. I also sincerely thank for her precious time spent on proofreading and correcting my mistakes. I would also like to acknowledge Dr. M. Zulfiquer Hossain for his valuable suggestions and encouragement.

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List of Acronyms

MMAE	Monomethyl auristatin E
MMAF	Monomethyl auristatin F
ADC	Antibody-Drug Conjugate
MDR	Multi-drug Resistant
AML	Acute Myeloid Leukemia
ALL	Acute Lymphocytic Leukemia
DBCL	Diffuse B-cell lymphoma
DLBCL	Diffuse large B cell lymphoma
PBD	Pyrrolobenzodiazepine
DAR	Drug-Antibody Ratio

Chapter 1

Introduction

1.1 Introduction

The conventional treatment option for cancer is chemotherapy. However, the drugs incorporated in chemotherapy remain a matter of concern due to its serious adverse reactions. Moreover, the increased incidences of drug resistance have raised concern undoubtedly (Eaton et al., 2015). There have been ongoing attempts to enhance the effectiveness and reduce side effects of those cytotoxic drugs by combining various chemotherapeutic drugs targeted for cancer therapy and utilizing agents with higher potency like auristatin and maytansine. Despite such promising effects, systemic toxicity and narrow therapeutic window have restricted their clinical use of these conventional drugs/options (Francisco et al., 2003). This led to the advancement in the development of monoclonal antibodies and then antibody-drug conjugates which have shown promising results as they possess target specific drug delivering properties. The design and development of antibody-drug conjugates (ADCs) are based on the concept of conjugation of these monoclonal antibodies with cytotoxic drugs (Zhang et al., 2020). The basic component and structure of an ADC has been shown in Figure 1.

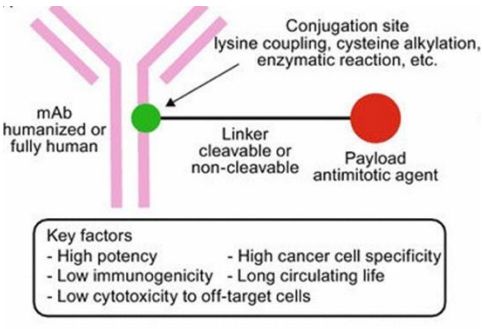

Figure 1: Antibody-drug conjugate. Adapted from (Tsuchikama & An, 2018)

Mechanism of action: ADCs are monoclonal antibodies to which cytotoxic drugs are conjugated by means of chemical linkers. The antibodies are selective for tumor cell receptors. This justifies the cancer cell specificity and potency of ADCs which cannot be achieved with conventional chemotherapy (Miller et al., 2016). In general, typical mAb for ADCs possesses strong target binding, low immunogenicity, low cross-reaction, more efficient internalization and longer plasma half-life. The mechanism of action of ADCs is such that they can bind specifically to the targeted tumor cells' receptors. The receptor-ADC complex is then internalized via clathrin- or caveolae by means of endocytosis (Jain et al., 2015). The internalization causes cell membrane's inward budding, hence developing endosome. The cleaving of ADC linkers causes the release of cytotoxic drugs from the cytoplasmic lysosomes (Zimmerman et al., 2014). Eventually, the cytotoxic drugs exert their tumor cell specific cytotoxic effects by either binding with DNA's minor groove and induce DNA breakage or by disrupting microtubules. This results in tumor cell death, mainly apoptosis as shown in Figure 2.

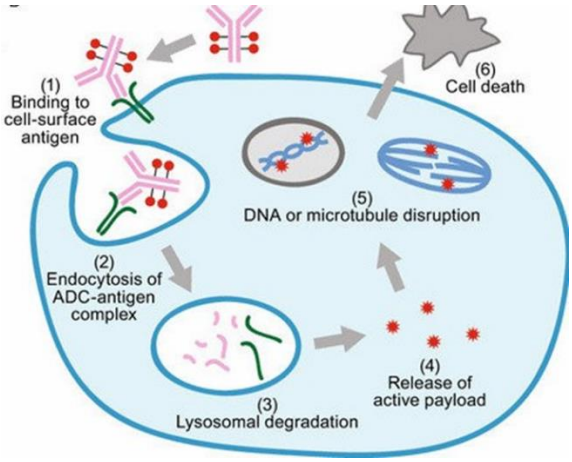

Figure 2: Mechanism of action of ADC. Adapted from (Tsuchikama & An, 2018)

The use of ADCs has spread notably along with its development over time as it has been possible to overcome the initial limitations including improved target selection, development in payloads, linker technologies and methods of conjugation. The toxic payload is mainly

passed into the tumor cells through the internalization of specific target receptors. Once the complex gets internalized by endocytosis, it undergoes specific intracellular pathways and degradation. This allows the cytotoxic drug to be released (Donaghy, 2016). It is vital to select suitable antibodies for ADC as it affects efficacy, pharmacokinetic and pharmacodynamic profiles and therapeutic window. ADCs possess poor bioavailability for oral administration, therefore are administered intravenously in order to prevent enzymatic degradation.

The first generation ADCs are mainly developed by conjugating antibodies to drugs such as doxorubicin and methotrexate using non-cleavable linker (Baron & Wang, 2018). The drawback for the first generation ADC was that it was less potent (Tai et al., 2014). Ultimately, Gemtuzumab ozogamicin was developed which had better efficacy because it included calicheamicin derivative which possesses improved potency and lowered immunogenicity by using humanized antibodies (Zammarchi et al., 2018)). However, Gemtuzumab ozogamicin has some drawbacks like unstable linker, greater proportion of unconjugated antibodies, inadequate chemistry, manufacturing and control properties, along with increased toxicity (Shao et al., 2018). This eventually led to the formulation of second generation ADCs which had better cancer-cell targeting capacity. Some of which include brentuximab vedotin, ado-trastuzumab emtansine, and inotuzumab ozogamicin (Dornan et al., 2009). The limitations of second generation ADCs are toxicity induced from off-target, rapid metabolism and competitive tendency against unconjugated antibodies (Barok et al., 2014). A newer, more target-specific ADCs are developed known as third generation ADCs. This has a better pharmacokinetic profile and balanced drug-antibody ratio (Beck et al., 2017).

ADCs can also work as bystand killers. The neighboring cells which do not express target antigen are destroyed by inducing cytotoxic payloads, which are released from cells which express ADC target antigen. The cytotoxic payloads are mainly produced from the internalization and degradation of ADC. Besides, the cytotoxic payloads are also released

without internalization within the microenvironment of tumor cells. The factors on which such bystander killing depends are ADC internalization extent, type of linker and the properties of cytotoxic agents administered. ADCs can also trigger the complement system pathways by means of exerting either antibody dependent cellular toxicity, complement dependent cytotoxicity or antibody dependent cellular phagocytosis. The immune cells then infiltrate the tumor cells (Ponte et al., 2021).

1.2 Rationale

The rationale of the review is the compilation of the progress of ADC development based on the linking of a cytotoxic drug to a tumor-targeting antibody using a chemical linker which will enable receptor-targeting selectivity towards cancer cells, leading to the elimination of tumor cells while sparing the healthy cells. This article is based on reviews of literature and does not involve any studies with human participants or animals.

Chapter 2

Methodology

The review consists of an overview of ADCs. All information for this review paper were collected from peer-reviewed research articles, collected from PubMed, Elsevier, ScienceDirect, Nature, Springer, Lancet, Taylor and Francis. Information was gathered and referenced properly, to provide better understanding of ADCs and their significance in cancer treatment.

Chapter 3

Components of ADCs

ADCs consist mainly of three components - monoclonal antibodies, cytotoxic payload and a cleavable or non-cleavable chemical linker. The cytotoxic payload mainly binds to the target cancer cell receptor. ADCs have a wider therapeutic index compared to the conventional anticancer drugs (Dean et al., 2021).

i. Monoclonal antibodies: Target-specific monoclonal antibodies are developed and screening is done depending on several criteria such as selectivity, tumor-perforating ability and isotype. The antibodies which are incorporated in ADCs are IgG1, IgG2, or IgG4 subclasses which have variable cross-linking properties and different complement pathways, mainly antibody dependent cellular toxicity or complement dependent cytotoxicity. IgG1 helps in the improvement of the capabilities to deliver the drug. But depending on target features and mechanism of action, IgG2 and IgG4 antibodies can also be taken into consideration. Isotype is vital for conjugation of drug and linker, especially using cysteine residues (Dean et al., 2021). The size of the antibodies is another important factor for better receptor targeting. Inappropriate size of antibodies may lead to problems in the uptake and penetrability of the solid tumors (Z. Li et al., 2019). In order to overcome this problem, smaller antibodies are developed. The drawback of such smaller antibodies is that it undergoes quicker clearance (Z. Li et al., 2019).

The antibodies undergo post-translational modifications during the synthesis and storage phase. This is done to maintain the stability, structure and functionality of the antibodies (Jefferis, 2016). Post-translational modifications include deamidation, sialylation and cleavage of c-terminal lysine (Leblanc et al., 2017). However, these modifications give rise to charged variants and heterogeneity of ADCs, which can interrupt target binding and internalization of

ADC into the cancer cells, hence reducing efficacy (Houde et al., 2010). Antibody dependent cellular toxicity and complement dependent cytotoxicity pathways may also be hampered due to these post-translational modifications. Therefore, in order to maintain consistency, any changes including ADC charge profile are analyzed frequently (Dean et al., 2021).

The design of ADC is done in such a way that it shows an efficient internalization by means of endocytosis which accelerates the entry of ADC once it is recognized (Perez et al., 2014). Internalization of the receptor is a vital factor for designing ADC because it allows the release of cytotoxic payload while exerting minimum adverse effects on the normal, cancer-free cells (Q. Li et al., 2019). The factors which are required to be assessed for developing an ADC with the ability to internalize efficiently include target penetrability, density and rate of internalization. It is difficult for ADC to reach the antigen in solid tumors compared to the hematological tumors because solid tumors are less exposed to the ADCs present in the systemic circulation after administration. Moreover, targets are introduced in the system by shedding from the surface which may facilitate hepatic clearance of ADC, hence reducing efficacy (Awuah et al., 2016). The entry of ADC into cancer cells is affected by receptor expression, rate of internalization and recycling. The minimum threshold for binding to target and affinity of mAb towards antigen can be variable. Non-internalizing ADCs have also been attempted to design which have the potential to target the structural components present in the microenvironment around the tumor (Gébleux et al., 2015). ADCs of this kind may overcome the physical obstacles related to internalization as it targets highly expressed antigen present in the stroma of the malignant tumor (Staudacher et al., 2019). Cells which have undergone apoptosis shed proteases which help in the release of cytotoxic payload for crossing the cancer cell membrane (Dal Corso et al., 2017). As non-internalizing ADCs tend to gather on the surface of the tumor cell, it is less likely to cause toxicity to the healthy cells. This suggests that non-internalizing ADCs possess better safety and efficacy (Giansanti et al., 2019).

ii. Cytotoxic payload: The primary function of cytotoxic payload is the destruction of malignant tumor cells (Tang et al., 2019). Due to low potency of the first generation ADCs, the second generation ADCs were developed which were smaller sized, yet potent drugs. However, it induced high toxicity when used as monotherapy but showed promising results between 0.01-0.1 nM when it was specifically targeted at cancer cells (Zhao et al., 2020). Despite such promising outcomes, only 2% payload of ADCs can actually approach the target site inside the cancer cell because of biodistribution, uptake, and inability to form conjugate in the systemic circulation (Teicher & Chari, 2011). As a result, a payload with a greater potency is chosen so that the ADC is still able to invade the cancer cells at smaller concentrations. It is vital to understand the mechanism of action of the cytotoxic payloads of ADCs. The ADCs being developed in recent days contain highly potent drugs which either interfere with the polymerization of tubulins or cause harm to DNA. Most ADCs being developed recently incorporate antimitotic tubulin disruptors which have the capability to selectively invade the cancer cells undergoing rapid mitotic cell division. The problem it imposes is that such drugs might not be efficacious for low-proliferating cancer cells. To overcome this problem, cytotoxic agents like topoisomerase inhibitors are used as they possess lower toxicity, therefore allow the development of ADCs with higher Drug-to-Antibody Ratio (DAR) (Dean et al., 2021). For example, Trastuzumab deruxtecan, an approved ADC which targets HER2 has a high DAR and less toxicity. Another cytotoxic agent, PBD dimers also possess greater potency at small concentrations. One such approved ADC is Loncastuximab tesirine which exhibits as a bystand cell killer but induces lower systemic toxicity because it has shorter half-lives. Immunostimulatory agents such as RNA polymerase II inhibitors, and pro-apoptotic BCL-xL inhibitors are novel cytotoxic payloads (Zhang et al., 2020). Another matter of concern needed

to be taken into account is drug resistance. MMAE and calicheamicin are workable substrates of P-glycoprotein but PBD dimers and topoisomerase I inhibitors have been proved to show anticancer activity in the malignant tumors which have previously shown resistance to multiple cytotoxic drugs (Takegawa et al., 2017). The cell permeability of cytotoxic payload of MMAE and PBD based ADCs allow it to act as a bystander killer by causing efflux of drug inside the adjacent antigen-negative cancer cells (Ogitani et al., 2016).

Aggregation caused by unfolding of cytotoxic payloads and exposure of hydrophobic residues raised as obstacles in the development of ADCs (Ross & Wolfe, 2016). The smaller size of cytotoxic agents exert effect on hydrophobic properties of ADCs such that the ADCs tend to aggregate more. The susceptibility is higher under thermal stress (Beckley et al., 2013). In case of unconjugated monoclonal antibodies, the activity of ADC is significantly reduced due to aggregation, hence lowering the efficacy of the drug. If hydrophobic drugs are incorporated in mAbs, then there is a chance that the drug might enter the healthy cells after getting released from linkers due to hydrophobicity and cause destruction of healthy cells. The hydrophobicity related problems can be overcome using hydrophilic spacers, linkers, or payloads (Shao et al., 2018). A novel cytotoxic payload, β-D-glucuronyl-monomethyl auristatin has been developed which possesses improved stability, higher DAR, efficient internalization, better metabolic processing, bystander killing ability and reduced toxicity without hampering the cytotoxic activity towards the malignant cells (Satomaa et al., 2018).

iii. Chemical Linker: The primary function of chemical linker is to link the monoclonal antibodies and the cytotoxic payload. The stability of ADC is maintained by linker in the systemic circulation prior to reaching the target and releasing the cytotoxic payload (Lu et al., 2016). Linkers are divided into two types: cleavable and non-cleavable (Jain et al., 2015). The cleavable linkers have the potential to undergo cleaving when required for releasing the free

cytotoxic agent into the cytoplasm (Bargh et al., 2019). The linkers which have the ability to cleave include hydrazine linkers which undergo cleaving when exposed to the acidic surrounding of endosome and lysosome. Cleaving can also be done with linkers containing proteases or agents like cathepsin B and glutathione (Bargh et al., 2019). ADCs which do not have the ability to internalize depend on such extracellular cleaving by proteases and glutathione, which shed during cancer cell lysis in order to release the cytotoxic drug (Perrino et al., 2014). The linkers which are non-cleaving, are resistant to the hydrolysis of protein by proteases, hence completely depend on lysis of antibody for releasing the cytotoxic payload which remains joined to the antibody via a linker. In this case, the payload is needed to be active and the linker needs to be bound (Jain et al., 2015). This strategy can be implemented to prevent multidrug resistance mutation 1 (MDR1) (Shefet-Carasso & Benhar, 2015). Thus, understanding the mechanism of action of ADCs is vital while selecting a suitable linker. The chemical linker is also vital to maintain hydrophobic balance between monoclonal antibodies and cytotoxic payload. This balance is necessary to prevent aggregation. The linkers, specifically the hydrophilic ones such as cyclodextrins and polyethylene glycol are found to exhibit potential to enhance circulation stability, efficacy and potency in targeting the tumor cells and conjugate pharmacokinetics (Verkade et al., 2018).

Chapter 4

Methods of Conjugation

Conjugation processes can vary the quality of the ADC, and have significant effect on the safety and efficacy of the ADC. There are various types of processes through which the conjugation between the monoclonal antibody and cytotoxic payload takes place, which is vital for the potency and efficacy of ADC (Sochaj et al., 2015). This mainly includes- through cysteines of reduced interchain disulfide bonds, surface-exposed lysines, and site-specific methods. The conventional techniques for conjugation are based on the conjugation of cytotoxic drugs and antibodies using lysine and interchain cysteines. The problem associated with the conventional methods is that it causes heterogeneity because the availability of lysine capable of conjugation was greater than the cysteine conjugation (Jain et al., 2015). This exhibits a higher risk of cytotoxicity and reduced binding efficiency because it is difficult to control the conjugation site and quantity, hence affecting binding of lysines proximal to Fc by means of drug conjugation (Acchione et al., 2012). ADCs which are in use today follow newer method where conjugation takes place on interchain disulfide cysteines by reducing 4 or 6 interchain disulfide bonds using reducing agent in excess such as tris(2-carboxyethyl) phosphine or dithiothreitol (Wiggins et al., 2015). As a result, the interchain disulfide bonds are not disrupted. Moreover, the sulfhydryl groups are released from cysteine residues present in the interchain disulfide bonds. Natural amino acids used for such purposes include p-acetylphenylalanine and p-azidomethylL-phenylalanine (Zimmerman et al., 2014). In addition, another method is known as SMARTag™ where chemoenzymatic reactions are used to add an aldehyde tag for site-specific conjugation (Jain et al., 2015). Formyl glycine residue is the site for conjugation which is synthesized when a cysteine undergoes enzymatic oxidation (Drake et al., 2018). Another method is the incorporation of natural and synthetic carbohydrates to the

glycan as drug conjugate target (Zhu et al., 2014). The advantages of this technique are homogeneity and consistent loading (Thompson et al., 2016). The ADCs produced by glycoengineering possess potent killing ability of the tumor cells. It can enzymatically change the glycan profile by adding carbohydrate groups in the conjugation of drug, therefore enhancing homogeneity and potency. The homogeneity of ADCs can also be improved by using enzymes to concede the engineered amino acid sequences so that the drug can be cleaved and attached by covalent bond. There is another site-specific technique for conjugation called disulfide rebridging (Schumacher et al., 2014). This technique has the controlling potential of DAR and DLD even without reengineered mAb. It uses cysteine coupling for the conjugation of bifunctional cytotoxic payload (Badescu et al., 2014). This technique has been proved to exhibit better homogeneity. The disadvantage of this process is the utilization of extra chemicals which need further purification.

Chapter 5

Factors which affect ADC activity

i. Amount of conjugated cytotoxic payload is an important factor. The potency might be low of ADCs having fewer conjugated cytotoxic payloads, along with enhanced risk of off-target toxicity, poor selectivity, faster metabolism and clearance rate (Hamblett et al., 2004).

ii. Drug-Antibody ratio is another vital factor as it has the potential to affect administered ADC dose to be uptaken by cancer cells. Depending on the cytotoxic drug potency, an increased dose of low DAR ADC may be given, therefore increasing the ADC penetrability into the solid tumor cells. On the other hand, a high DAR ADC is likely to be given at low doses, hence leading to a low concentration of antibody affecting the uptake by the cancer cells (Ponte et al., 2021).

Chapter 6

Approved ADCs

Ten ADCs have got the approval by FDA so far and the payload, linker, antibody as well as indication have been given in Table 1. Besides, as many as 80 ADCs are undergoing clinical trials at different phases.

i. Gemtuzumab ozogamicin: This is an anti-CD33 ADC containing 2/3 cytotoxic payloads of calicheamicin kind which are in conjugation with the antibody. However, it raised some concerns as it caused some fatalities rather than exerting potential therapeutic effects. The major adverse effects, including fatalities, were caused by infection as well as hemorrhage. In 2010, this resulted in the removal of the application which was submitted by Pfizer. Some changes were done with the dosing which reduced cases of liver toxicity and death rate. In 2017, Gemtuzumab ozogamicin was approved for the treatment purpose of acute myeloid leukemia. The ADC includes a monoclonal antibody known as CD33 conjugated to calicheamicin cytotoxic payload using a cleavable hydrazone linker. The success rate was found to be 26-30%. Adverse effects included hepatic disorders along with retarded recovery of hematopoiesis (Sievers et al., 2001). Other side effects included liver toxicity and pulmonary disorders.

ii. Brentuximab vedotin: An ADC which has been developed by Seattle Genetics. The MMAE molecules are linked to the anti-CD30 antibody using protease based linker which is cleavable. The conjugation is done through cysteine produced by the process of disulfide bonds interchain reduction (Francisco et al., 2003). The ADC was approved for the treatment of Hodgkin's lymphoma, systemic anaplastic large cell lymphoma, and peripheral T-cell lymphoma. The success rate was 75% for Hodgkin's lymphoma (Younes et al., 2012) and 86% for systemic

anaplastic large cell lymphoma (Younes et al., 2012). Side effects included anemia, neuropathy, thrombocytopenia and neutropenia.

iii. Trastuzumab emtansine: It has been produced by the conjugation between the antibody, anti-HER2 and maytansinoid DM1 using lysine (Lewis Phillips et al., 2008). Genentech developed the ADC. This ADC is indicated for the treatment of HER-2 positive metastatic breast cancer and early breast cancer. The drug mainly works by secreting cytotoxic payload hence inhibits ADCC mechanism, which in turn resists the signaling of HER2 activity and causes cell death. The success rate of the ADC was found to be 43.6% (Verma et al., 2012). The occurrences of side effects and serious side effects were reduced to 40.8% and 15.5% respectively.

iv. Inotuzumab ozogamicin: This ADC was developed by Pfizer and indicated for the treatment of relapsed/refractory B-cell precursor acute lymphoblastic leukemia in adults. It mainly releases calicheamicin and targets CD22. The cytotoxic payload is linked to the antibody, anti-CD22 by a cleavable acid-labile, hydrazone linker (Lamb, 2017). The complete remission rate of the ADC was 80.7% (Kantarjian et al., 2016).

v. Polatuzumab vedotin: The ADC is developed by Genentech which is produced by conjugating MMAE using cysteines made of interchain disulfide bonds which has been reduced. The cytotoxic payload is conjugated to antibody, anti-CD79b via protease dipeptide which is a cleavable linker. The remission rate was 40% (Sehn et al., 2019). It is indicated for treating relapsed or refractory diffuse B-cell lymphoma.

vi. Sacituzumab govitecan: The ADC is prepared by Immunomedics and indicated for metastatic triple negative breast cancer (Goldenberg et al., 2015). It has a greater ratio of drug to antibody. The toxic drug, topoisomerase I inhibitor, is linked via CL2A linker using cysteine which is hydrolyzable (Goldenberg et al., 2015). The ADC includes an antibody known as

calcium signal transducer 2 conjugated to topoisomerase I inhibitor payload using an ester linker which is acid-labile in nature (Goldenberg et al., 2015). The clinical benefit rate was 45.4% (Bardia et al., 2019). The side effects of the ADC were anemia and neutropenia (Bardia et al., 2019).

vii. Belantamab mafodotin: This ADC is indicated in the treatment of R/R multiple myeloma, developed by Astellas Pharma. The cytotoxic agent, MMAF is conjugated to the antibody (fucosylated anti-BCMA) using non-cleavable linker. The ADC causes cell death by introducing the cytotoxic payload (MMAF) to the tumor cell, hence triggers antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis (Tai et al., 2014).

viii. Loncastuximab tesirine: The ADC developed by ADC Therapeutics consists of the antibody, anti-CD19 which is indicated in the treatment of R/R B-cell lymphomas (Zammarchi et al., 2018). The ADC consists of pyrrolobenzodiazepine dimer. The cytotoxic drug, tesirine is conjugated to the antibody using cathepsin-cleavable valine-alanine linker. This causes crosslinking between the interstrand of the cancer cell's DNA minor groove.

ix. Trastuzumab deruxtecan: The ADC is developed by Daiichi Sankyo and indicated for HER2-positive metastatic breast cancer. It consists of the antibody T-DM1 which is conjugated to the cytotoxic payload, Dxd (topoisomerase I inhibitor) using the tetrapeptide linker that undergoes cleaving by protease (Ogitani et al., 2016). The cytotoxic payload is released into the cancer cell, causing suppression of the tumor.

x. Enfortumab vedotin: The ADC is developed by Astellas Pharma and Seagen. This is used for the treatment of solid tumors caused by Nectin-4 positive urothelial cancer. Nectin-4 mediates Ca+-independent cell–cell adhesion through the recruitment of cadherins and modulation of cytoskeletal arrangements. The drug consists of the antibody IgG1 which is conjugated to the cytotoxic payload, MMAE using a cleavable linker.

Table 1: Approved ADCs (Zhao et al., 2020)

ADC	Brand Name	Target antigen	Antibody	Linker	Payload	Developer	Indication	Approval Information
Brentuximab vedotin	Adcetris	CD30	Chimeric IgG1	Valine-citrulline	MMAE	Seattle Genetics	Previously untreated stage III or stage IV classical Hodgkin's Lymphoma (cHL); relapsed or refractory cHL; cHL after failure of auto-HSCT or failure of at least two prior multi-agent chemotherapy regimens; systemic anaplastic large cell lymphoma, primary cutaneous anaplastic large cell lymphoma other CD30-expressing peripheral T-cell lymphomas	FDA, 2011
Trastuzumab emtansine	Kadcyla	HER2	Humanized IgG	SMCC	T-DM1	Genentech	HER2-positive, metastatic breast cancer	FDA, 2013
Inotuzumab ozogamicin	Besponsa	CD22	Humanized IgG4	ActBut	Calicheamicin	Pfizer	Monotherapy in adults with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL)	FDA, 2017
Gemtuzumab ozogamicin	Mylotarg	CD33	Humanized IgG4	ActBut	Calicheamicin	Pfizer	Single-agent and combinational therapy in newly-diagnosed CD33-positive acute myeloid leukemia (AML) in adults and relapsed or refractory CD33- positive AML	FDA, 2017

Polatuzumab vedotin	Polivy	CD79b	Humanized IgG1	Valine-citrulline	MMAE	Genentech	Combinational use with bendamustine and a rituximab product in adult patients with relapsed or refractory diffuse B-cell lymphoma (DBCL)	FDA, 2019
Enfortumab vedotin	Padcev	Nectin-4	Human IgG1	Valine-citrulline	MMAE	Astellas Pharma	Adult patients with locally advanced or metastatic urothelial cancer	FDA, 2019
Trastuzumab deruxtecan	Enhertu	HER2	Humanized IgG1	Tetrapeptide	exatecan-derivative topoisomerase I inhibitor (DXd	Daiichi Sankyo	Adult patients with unresectable or metastatic HER2-positive breast cancer	FDA, 2019
Sacituzumab govitecan	Trodelvy	Trop-2	Humanized IgG1	Hydrolysable CL2A	SN-38 Topo I inhibitor	Immuno-medics	Adult patients with metastatic triple-negative breast cancer who have received at least two prior therapies for metastatic disease.	FDA, 2020
Belantamab mafodotin	Blenrep	BCMA	Humanized IgG1	maleimidocaproyl	MMAF	GSK	Adult patients with relapsed or refractory multiple myeloma who have received at least 4 prior selected therapies	FDA, 2020

Loncastuximab tesirine	Zynlonta	CD19	Humanized IgG1	Valine-alanine	PBD dimer	ADC Therapeutics	Adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified, DLBCL arising from low grade lymphoma, and high- grade B-cell lymphoma	FDA, 2021

Chapter 7

ADCs in Clinical trials

As many as 80 ADCs are undergoing clinical trials, mostly however remain in the Phase I and II of the clinical trials. Most of these are evaluating the safety and efficacy parameters on the tumor cells. The rest of those are performing trials on cancers related to hematology. This indicates that the focus has been shifted towards ADCs indicated for solid tumors. The receptor which has been targeted the most is HER2. The evidence to support this is that there are three ADCs targeting HER2 in Phase III of clinical trials. RC48 is a type of ADC targeting HER2 developed by RemeGen. The antibody, hertuzumab, is linked to the drug, MMAE using the protease-cleavable linker called valine-citrulline conjugated by cysteine (Yao et al., 2015). The safety studies of the drug showed promising results in the Phase I trial. This novel drug has also been indicated towards metastatic or unresectable urothelial carcinoma. Based on the Phase II trial results, the overall response rate is found to be 51.2% (Sheng et al., 2021). Most of the ADCs under clinical trials consist of the same functioning payload, which causes disruption of the tubulin and ceases mitosis, while some also causes damage to the DNA. There is a minor portion of the ADCs undergoing clinical trials that inhibits topoisomerase. There are some novel payloads as well, which target specific receptors. One such kind is BDC-1001 which consists of a payload acting as toll-like receptor agonist that is more effective against tumor cells, especially immune-mediated ones (Frega et al., 2020). Along with activating the human myeloid APCs, BDC-1001 triggers ADCC and ADCP pathways. Besides BDC-1001, there is another payload which is currently undergoing clinical trials. This novel payload targets the anti-apoptotic BCL-2 protein. The ADC using this payload is called ABBV-155 which mainly invades cancer cells that express CD276 (Zhang et al., 2020). There are also RNA polymerase II inhibitors (e.g. Amanitin derivatives) which prevents synthesis of proteins as

well as inhibits cellular transcription methods. One such ADC using amanitin derivative is HDP-101 which targets BCMA (B-cell maturation antigen) and is indicated in the treatment of R/R multiple myeloma (Figueroa-Vazquez et al., 2021). Most of the ADCs in clinical trials have been designed using conventional cysteine conjugation methods. Others are developed using lysine conjugation methods because of possibilities of heterogeneity. Moreover, there are some ADCs made by using newer conjugation technologies. One such ADC is TRPH-222, which consists of the antibody anti-CD22 to the cytotoxic payload, maytansinoid using Specific Modifiable Aldehyde Recombinant Tag (SMARTag™) technology. The technology is based on the chemoenzymatic process which is used to develop a reactive aldehyde into mAb designed for conjugation that is aldehyde-specific (Drake et al., 2018). TRPH-222 is indicated for R/R B-cell lymphoma. Another ADC in clinical trial is XMT-1592 which uses anti-Napi2b antibody to target NaPi2b-expressing cancer cells and is conjugated to the cytotoxic payload auristatin F-hydroxypropylamide (membrane-permeable) using Dolasynthen platform which is used to target glycan-remodeled Asn297 and enables site-specific conjugation. The payload undergoes further metabolism to become impermeable, thus gets locked inside the tumor and exerts a bystander effect (Yurkovetskiy et al., 2021). The permeability of ADC inside the tumor cells can be enhanced by varying the size of antibodies.

Chapter 8

Challenges and Limitations

There are many challenges in the way of developing ADCs, mainly related to the safety and efficacy aspects of the ADCs. About clinical trials of 55 ADCs were needed to be terminated due to toxicity (Coats et al., 2019). Toxicity arises often due to the cytotoxic and chemotherapeutic agents. One of the challenges is the failure to prove efficacy of the ADCs in the clinical evaluation compared to the existing available treatments. One such example is the MM-302, an ADC where the anti-HER2 monoclonal antibody was conjugated to the payload, liposomal doxorubicin (Espelin et al., 2016). The comparison was done with standard chemotherapy and efficacy of the tested ADC was found to be inadequate (Miller et al., 2016). Another such example is rovalpituzumab tesirine, which consists of the antibody, anti-DLL3 conjugated to PBD dimers using a dipeptide linker called valine-citrulline. The ADC was indicated for the treatment of small cell lung cancer (Saunders et al., 2015). Safety and efficacy parameters raised concerns about the ADC in phase II trial because an increased level of toxicity was observed, mainly pleural effusion linked to the PBD dimers (Morgensztern et al., 2019). There are other challenges related to the components of ADCs, mainly the payloads. One such example is the payload calicheamicin, which is found to be associated with hepatotoxicity (McDonald et al., 2019). There are other causes which limited the use of some promising ADCs including neuropathy and neutropenia caused by MMAE, ocular toxicities and thrombocytopenia caused by MMAF, neutropenia, thrombocytopenia and GI disorders caused by T-DM1, ocular toxicity caused by DM4, thrombocytopenia and hepatic toxicity caused by calicheamicin, neutropenia caused by topoisomerase I inhibitors and myelosuppression, effusion and inflammation caused by PBD dimers (Saber et al., 2019). The specificity of antibodies is another limitation (Tolcher, 2016). One such limitation is associated

with the toxicity caused by BR-96 doxorubicin. This consists of doxorubicin and anti-Lewis Y antibody which causes hemorrhagic gastritis (Saleh et al., 2000). The Lewis Y antigen is mainly expressed in the gastric mucosa and is responsible for hemorrhagic gastritis. Moreover, fatal exfoliate of skin toxicity was found to be associated with bivatuzumab mertansine due to the expression of the CD44v6 antigen in the deep layers of skin (Tijink et al., 2006). The biggest limitation of preclinical models is the unpredictability of the effects of ADCs in humans (Tolcher, 2016). A large number of ADCs which have shown promising results in rodent tumor models, failed to prove safe and effective in the clinical trials.

Chapter 9

Future aspects

The easier obtainability of ADCs makes them a prospective drug delivery method for a variety of cancers. ADCs approaching clinical trials give biotech companies the confidence for transitioning away from traditional processes and toward innovative methods of developing complex biotherapeutics. This encompasses research into newer antigens of cancer cells, various forms of antibody, cytotoxic agents, linkers, and processes of conjugation, with the aim to expand the safety profile of ADCs. scFv has greater cancer cell penetrability and absorption than other developing antibody formats. ADCs that are bispecific may be able to overcome tumor heterogeneity. Off-target effects may be reduced by antibodies and other conditionally active biologics (CABs). PBD dimers, topoisomerase inhibitors, anthracyclines, and protein-specific modulators can be classified into new ADC generation, in addition to microtubule-disrupting drugs. In addition, conjugation methods which are target specific, are employed to improve stability of ADCs in the bloodstream while retaining payload efficiency. ADCs' complexity provides formidable analytical hurdles, especially when hydrophobic payloads are included. Analytical methodologies are needed for the extensive expansion of ADCs. It is critical to use the right set of analytical techniques to properly characterize product features and ensure manufacturing consistency during development and throughout the product life cycle. The growth of clinical indications for ADCs, which has shifted from hematological malignancies to tumors that are solid, demonstrates their therapeutic promise. The currently developing ADCs are being analyzed in combination with therapeutic classes, like immune checkpoint inhibitors and monoclonal antibodies that target specific antigens. The development of newer technologies facilitated the creation of new ADCs (Beck et al., 2017). Besides, it has been possible to detect antigen targets for solid as well as hematologic tumors (Alley et al.,

2010). A number of promising drugs including microtubule inhibitors, amatoxins, and anthracyclines have been discovered. In addition, development of new generation linkers has ameliorated the therapeutic window of ADCs. The development of bispecific ADCs has improved potency and selectivity (Thornlow et al., 2019). Furthermore, several clinical trials are actively investigating combination methods, such as combining with checkpoint inhibitors and standard chemotherapies. Although there are still many challenges to solve, the development of novel ADCs may open up a world of possibilities for cancer treatment in the future.

Chapter 10

Conclusion

The development of ADCs has undoubtedly given hopes to millions fighting with cancer. ADCs have not only improved safety and efficacy parameters but also developed better selectivity towards cancer cells, sparing the healthy ones. By overcoming the current challenges, it can be concluded that the relentless efforts of researchers in the way of developing novel cytotoxic payloads, newer linker technologies and modified antibodies would lead to improved ADCs, indicating an optimistic future in the treatment of cancer.

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