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Toxicities Associated with Antibody Drug Conjugates

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Abstract

Conventional chemotherapeutic agents, in the treatment of several cancers, lack specificity, resulting in toxicities to normal tissues and poor therapeutic index. Antibody-based immunoconjugates are specifically targeted monoclonal antibodies that deliver the cytotoxic agent to the target cell. When the cytotoxic agent used, is a highly potent drug, such molecules are referred to as antibody-drug conjugates. This represents a promising approach to enhance the efficacy of unconjugated antibodies for improved therapeutics and decreased toxicities. Safety of these molecules is still a matter of concern. Novel designing techniques are required to develop molecules, having a safe toxicity profile, along with therapeutic effectiveness. This review focuses on various toxicities associated with the use of antibody-drug conjugates.

Keywords: Antibody; Conjuagtes; mABs

Introduction

Chemotherapy is one of the important modalities of treatment for many types of cancers. Toxic effects of the available chemotherapeutic agents, often limit optimal dosing of these agents. This leads to disease relapse, development of resistance and poor quality of life, of cancer patients. The major challenge in drug development for cancers is due to close resemblance of cancer cells to normal cells, which results in increased toxicities to the normal cells, while targeting cancer cells. So, newer approaches of cancer chemotherapy, aim at selective destruction of tumor cells, with minimal action on normal cells.

As we all know that our body constitutes almost 30,000 genes. Each gene is responsible for the formation of a different protein. There is a different task done by each of these proteins, in the body. The work of targeted therapy is to prevent some of the specific proteins, in helping the survival of cancer. Targeted therapy constitutes the new drug generation, which are designed to alter the target protein, necessary for the growth and progression of the tumor and is in contrast with the conventional cancer therapy. Targeted therapy uses various approaches – direct and indirect. In direct approach, they target the antigens of the tumor, in order to alter their signaling pathway. This can be done using monoclonal antibodies or other drugs, which are known to interfere with the target proteins. In indirect approach, the expressed tumor antigens, on the surface of tumors, are identified and targeted. These antigens act as the target deice for various effector molecules, present in the ligand. These approaches form the basis of active targeting, where the monoclonal antibodies, specific to the tumor, actively target them. Another approach can be passive targeting, where the tumors are targeted, using the enhanced permeability and retention effects of the macromolecules.

The concept of magic bullet, proposed by Paul Ehrlich, over

100 years ago, led to a search for therapeutic modalities that could selectively target diseased cells. This concept envisioned the use of immune system to combat disease-causing organism [1,2]. Continuous progress in this field led to the specific elimination of target-expressing cells, in several pathological conditions. A major milestone in immune mediated therapy was the production of monoclonal antibodies (mAbs). This novel development in mAbs demonstrated significant success in the treatment of cancers. Rituximab (anti CD20 mAb), the first approved unconjugated mAb for the treatment of cancer, was found very successful. In-spite of these developments, naked antibodies failed to produce a therapeutic effect in many cancers, where antibody- targetable antigens are present on the surface of cancer cells. Since then, there was a search to enhance the therapeutic effect of these antibodies.

One such approach is to— arm the antibodies, with potent cytotoxic agents, resulting in the generation of antibody conjugates that are capable of targeted delivery to cancer cell. When the cytotoxic agent is a drug, it results in antibody-drug conjugate (ADC). The distribution of these ADCs is restricted to the target-bearing cells, leading to the improvement of the therapeutic window of the cytotoxic agent. Significant progress has been made in this field in the past few years [3,4]. However, despite the advancements in this field, untoward toxicity of the ADCs is still a major concern. This issue is yet to be addressed adequately, in order to avoid unwanted side effects and achieve significant therapeutic effects, by providing optimal delivery of ADC to the target cell.

This review relates to the untoward toxicities of ADCs, includes clinically studied and approved ADCs, with main emphasis on the adverse effects (AEs), encountered during clinical trials and post Food and Drug Administration (FDA) approval.

Brief history of antibody drug conjugates:

Studies by various researchers, in 1960s-1970s in cancer therapy, were mainly related to the generation of specific humoral responses to tumor cells, and identification of common tumor markers, in the form of polyclonal, serum-derived antibodies [5].Kohler and Milstein, in 1975, were the first to produce mAbs, by hybridoma technique [6]. This revolutionary innovation, led to the isolation of specific antibodies and identification of target antigens. In the 1980s, therapeutic mAbs were focussed on identification of antigens, involved in various cancers and the effectiveness of these mAbs, in inducing immune-mediated cytotoxicity [7].

Limitations of murine mAbs are mainly, immunogenicity in humans, short serum half- life and ineffective interaction with human immune effector cells. These limitations were overcome by advances in antibody engineering techniques, which led to the generation of chimeric antibodies initially, followed by humanized and fully human mAbs [8]. MAbs are used, primarily in the treatment of cancer. Their use is increasing, in the treatment of various inflammatory and autoimmune conditions. A large number of different antigens, which are over expressed on various types of cancer cells, were identified over the time, which led to the extended research on mAbs, in oncology. Many newer mAbs have been entering clinical trials at the rate of over 40 per year, since 2007 and newer products are being approved for various conditions [9]. Inspite of the more convincing results of mAbs, in the treatment of various cancers, their success is still limited by many issues, which require further improvements in this field [10]. Lower therapeutic index of the cytotoxic drug, led to the less difference in activity against tumor cells, in comparison to normal cells, resulting in killing all the tumor cells and causing serious damage to the normal cells. In an approach to overcome this, there was the development of conjugating mAbs to the effector cells, which led to the increased activity [11]. Therefore, conjugating chemotherapeutic or other toxic agents to target specific antibodies, led to the restricted distribution of the effector molecules to target-bearing cells, resulting in the improvement of therapeutic index of the cytotoxic drug. One of the approaches of targeted delivery of the drug is mAb-based conjugates, where the cytotoxic agent is delivered to the target cell, by the antibody. This specific targeting, led to distinguishing between the target and normal cell, which resulted in less toxic effects than the conventional chemotherapeutic agents. The target, ideally, should be presented by the tumor cell, and not by the normal cell. Appropriate concentration of the payload must be delivered into cytosol, by mAb-conjugate, for effective results. These conjugates are based on the various targets expressed on the tumor cells, which enable internalization and subsequent processing of the targeted agent. Therefore, target selection is an important determinant for the safety and efficacy of antibody-based conjugate (ADC).

Characteristics of ADCs

ADC is a therapeutic approach, where a cytotoxic agent is linked to an antibody. ADC has three components: (a) an antibody, which is directed against the target antigen, on surface of the cell; (b) a cytotoxic drug; (c) linker between the antibody and the cytotoxic agent. The various obstacles, leading to decreased efficacy of ADC are: decomposition or decaying of the ADC, before reaching the target cell; altered antibody binding characteristics due to conjugation process; inappropriate stability of the linkers in the circulation, and release of drug in inactive or insufficient quantities [12]. All these factors are important, while designing an effective and safe ADC.

ADC technology involves combining mAbs that are selective for the antigen on the target cell, with potent cytotoxic agents. This must take into consideration, various factors, like target biology, linker payloads, antibody characteristics and conjugation strategies. Despite several drawbacks suffered by the early generation ADCs, clinical validation of this concept has been provided by the approval of two ADC,

for the treatment of cancer.

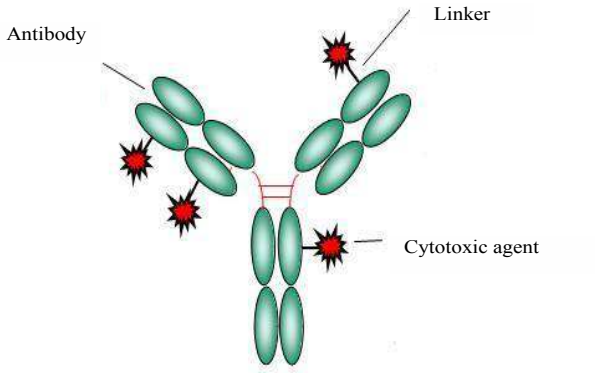

Figure 1: Components of an antibody-drug conjugate designing of ADCs

Target

For an ADC to be effective, the target cell must have a high density of targeted antigen or receptor. For example, the HER2 antigen, which is targeted by trastuzumab emtansine or T-DM1, is highly expressed to the extent of 0.5 to >2 x 106 receptors per cell, on some metastatic breast cancer cells [13]. As the mode of action of ADC is dependent on cellular uptake and internal processing, to release the active drug, effective receptor-ADC complex internalization by the tumor cell, is of paramount importance for designing an effective ADC. Another point to be considered is whether the target antigen is shed or not. This is particularly important for solid tumors, where higher concentrations of shed antigen in blood or in interstitial spaces of tumor, can limit the effectiveness of ADCs.

Linker payloads

Early ADCs were developed with the use of already approved small molecules for the treatment of various cancers, such as, doxorubicin, methotrexate, vinca alkaloids, mitomycin and 5-fluorouracil [14]. The conjugates, which resulted due to chemical modification of these molecules, lacked potency and showed only marginal activity. Consequently, the next generation ADCs considered use of more potent drugs that are too toxic for use, which include auristatins, maytansines and calicheamicins and their synthetic or semi-synthetic analogs [15-19]. These compounds exert their cytotoxic effects by cell cycle arrest, leading to apoptosis. Currently used cytotoxins used to construct ADCs, fall into two categories: drugs targeting microtubules and drugs targeting DNA. The auristatins (which are derivatives of dolastatin 10) and the maytansines, target microtubules and suppress microtubule dynamics, leading to cell cycle arrest and cell death. The calicheamicins cause cell death by targeting the minor groove of DNA and causing double-strand DNA breaks [20-23].

These compounds are highly potent and are 100 to 1000 times more cytotoxic than the conventional anti-cancer drugs. Additionally, they lack specificity to the target cell. This can result in damage to the normal cells, resulting in untoward toxicity. Hence, in order to avoid this, a properly designed linker is required, which is stable and is efficiently cleaved on arrival, at appropriate intracellular location of the target cell. This type of controlled release is obtained by three types of linkers, which are cleaved only under certain specific conditions, which depend on location of target to which ADC is targeted: the hydrazine linkers that are susceptible to acidic conditions, the disulphide linkers to reducing equivalents and the peptide linkers to proteases [24-27]. Decrease in the therapeutic effect is observed, with the instability of the linkers, resulting in masking of tumor antigen, with mAb lacking a drug. An alternate approach to overcome this is to use linkers that are stable and uncleavable. Mechanism of drug release from ADCs, with these linkers occurs in two steps: initial internalization of ADC attached to antigen, into the cell and followed by complete degradation of the antibody in the lysosome [28].

Antibody characteristics

Immunogenicity problems, seen with the early generation ADCs, will be overcome by the use of humanized or fully human Ab fragments. Ideally, when a cytotoxic compound is attached to the mAb, it should not affect the binding specificity of the parent mAb. Apart from this, there is also preserving of biological properties of parenteral antibodies, like effector functions, modulation of signalling or receptor blockade. It is not clear, whether these payload-independent mechanisms of tumor inhibition, were contributing to the effectiveness of ADCs. In the case of unconjugated antibodies, much of the in vivo efficacy in cancer is due to Fc effector functions [29]. Accordingly, many strategies have been developed, either to increase the Fc effector function, or to intentionally reduce the effector function of unconjugated antibodies, where Ab binding or blocking is sufficient for therapeutic efficacy and effector mechanisms could lead to undesirable side effects [30, 31]. Isotypes like IgG2 and IgG4 that are devoid of Fc-mediated functions are selected for ADCs, when effector functions do not contribute to the efficacy. As of now, there are no defined rules in regard to FcγR binding that govern the selection of ADCs, with the best therapeutic index. Hence, all the three Ab isotypes (IgG1, IgG2, and IgG4) are utilized for the ADC development.

Conjugation strategies for ADCs

Type of conjugation and the sites on the Ab are important in determining the tolerability, pharmacokinetic properties and the overall effectiveness of ADC therapy. Conventional strategies depend on linker-payload conjugation, either to the lysine amines or sulfhydryl groups in cysteines. Due to the availability of multiple lysines (about 70-90 per IgG1), lysine conjugation resulted in the formation of highly heterogenous ADC mixtures. Analysis of T-DM1, by liquid chromatography/mass spectrometry, revealed the presence of drug-to-Ab ratio species, from 0 to 7, with an average of

3.5drugs/Ab [32]. Better uniformity with the small number of loaded species, resulted from cysteine conjugation.

Theoretically, each loaded species, in ADC, represents a unique conjugate and thereby, exhibits distinct properties. It was observed that, decreased drug loading from 8 to 4 or 2 drug molecules per Ab, for anti-CD30-vc MMAE, led to slower ADC clearance and improved therapeutic index [33]. Other approaches to generate ADCs, involve utilization of cysteine to serine mutations, in the hinge region of Ab. This resulted in generation of uniform ADCs, with the loading of 2 or 4 drug molecules per Ab and showed similar anti-tumor activity and pharmacokinetic properties, as compared to heterogenous ADC, with a mean loading of 4, which highlights the need for optimal drug loading, in designing safe and effective ADCs [34].

Newer strategies, due to advances in antibody engineering that allow site-specific conjugations, include: addition of C-terminal selenocysteines [35]; bacterial transglutaminase-mediated conjugation [36]; programmable CovX bodies [37]; incorporation of non-natural amino acids in cell-free expression systems [38]; aldehyde tagging [39] and N-terminal cysteine, linked to aldehyde drugs via thiazolidine linkers [40].

As of now, two ADCs have been approved. Gemtuzumab ozogamicin was approved in 2000, for the treatment of acute myelogenousleukemia (AML). However, it was voluntarily withdrawn from market, in 2010. Brentuximab vedotin (SGN-35) was recently approved in 2011, for the treatment of lymphomas. Advances in molecular biology techniques, resulted in optimization of various parameters for ADC activity, which resulted in thedevelopment of several promising ADCs that are in different stages of clinical development [41].

Toxicities associated with ADCs

Myelosuppression is the most common reported adverse effect, with the administration of ADCs. It is manifested as thrombocytopenia, anemia and leucopenia, which weakens the patient's immune system and results in increased risk of opportunistic infections. In the clinical studies, evaluating CD33-targeted gemtuzumab ozogamicin with conventional chemotherapy in relapsed AML patients, it was found that the rates of hematological toxicity, bleeding, and treatment-related mortality were similar in both the groups [42].To begin with, pluripotent stem cells are CD33-negative, as the differentiation of these cells continue, they express CD33 and are thus, targeted by gemtuzumab ozogamicin, resulting in myelosuppression. The same applies to other targets, like CD20 and CD22 in haematological malignancies. Therefore, myelosuppression is an expected complication for the drug that acts through these targets.

Hepatic injury is the second most common injury, associated with ADCs, which is manifested as hepatic function abnormality (mostly up to grade 4), in the form of elevated bilirubin, alkaline phosphatase (ALP), aspartate transaminase (AST) or alanine transaminase (ALT) levels. Hepatotoxicity is dose-limiting toxicity as most of these molecules are naturally cleared from the blood, by the liver. Though transient and reversible, liver toxicity can be complicated, which may result in hepatic veno-occlusive disease. Such types of manifestations are also seen with conventional and high-dose chemotherapy [43,44].

Peripheral neuropathy is observed with the use ADCs, containing cytotoxic drugs that act by blocking the microtubules. Different groups of ADCs can be distinguished, based on the types of toxicities produced by them. ADCs that target microtubules, primarily, cause peripheral neuropathy and reversible myelosuppression [45-47]. While neurotoxicity is due to damage of microtubules, which are the key components of neurons, myelosuppression is caused due to the blockage of mitosis and proliferation of bone marrow cells. ADCs act by damaging DNA and alkylating agents target rapidly dividing cells, leading to alopecia, myelosuppression and gastrointestinal adverse effects [48]. Apart from these toxicities, adverse effects associated with the administration of mAbs, such as fever, nausea, vomiting, myalgia, diarrhoea and rash that are usually mild to moderate in intensity are also observed.

Main cause for toxicities due to ADCs include: unintentional drug release, recognition of the same antigen on normal tissues by ADC and immunogenicity, resulting in the formation of human anti-mouse antibodies (HAMA) or anti-drug-antibodies (ADAs) [49]. Presence of these antibodies, in circulation, might prevent administration of repeated cycles of therapy and also lower the levels of biologically active agent, leading to decreased efficacy. However, it was proved in many studies that all these concerns, with the use of mAbs are erroneous [50-53].

Examples of ADCs

Gemtuzumab ozogamicin is a humanized IgG4 anti-CD33 antibody. It is covalently linked to semisynthetic derivative of calicheamicin, N-acetyl-γ-calicheamicin dimethyl hydrazide, via an acid –hydrolysable 4-(4-acetylphenoxy) butanoic acid (AcBut) linker, which is stable at physiological pH [22,54]. It targets CD33, which is a cell surface antigen, present in more than 80% of the patients with AML [55]. After binding to CD33, gemtuzumab ozogamicin gets rapidly internalized into the lysosome, where the linker is hydrolysed by the acidic pH, liberating calicheamicin. Calicheamicin is then activated by glutathione to form enedyne form that eliminates the target cells [56-58].

Based on the results of phase I study [59], three phase II studies were conducted in 142 patients with AML, at first recurrence [60]. Gemtuzumab ozogamicin was administered to all these patients at a dose of 9 mg/m2 as a 2-hour intravenous infusion, at two week intervals for two doses. 30% of the patients obtained remission, of which, 16% obtained complete response. Adverse effects were delayed

infusion-related symptoms that include nausea and vomiting, sepsis, chills, fever, dyspnea, hypertension, hypotension and pneumonia. The occurrence of post-infusion symptoms was reduced significantly from 34%, after the first dose to only 12%, after the second one. Grade 3 or 4 neutropenia and thrombocytopenia was observed in 97% and 99% of the patients, respectively. Grade 3 or 4 bleeding, which included epistaxis and intracranial haemorrhage was observed in 15% of the patients. The rates of haematological toxicity and bleeding manifestations with gemtuzumab ozogamicin were similar to those reported with conventional chemotherapy [42]. Grade 3 or 4 infections of any type were observed in 28% of the patients and mucositis-related effects like stomatitis, oral ulcers and mouth pain was observed in 32% of the patients. Grade 3 or 4 bilirubin elevation was observed in 23% of the patients and grade 3 or 4, increase in AST and ALT levels were observed in 17% of the patients. In spite of the liver toxicity being transient and reversible, many patients had experienced more serious hepatic abnormalities and two deaths: one due to liver failure and another, following persistent ascites and hepatosplenomegaly. Of the 142 patients, 27 received hematopoietic stem cell transplantation (SCT), after gemtuzumab ozogamicin treatment. Among these three died after 22, 30 and 37 days following transplantation, due to hepatic veno-occlusive disease (VOD). One patient in the study, who had a history of VOD and had a relapse, after transplant, was treated with gemtuzumab ozogamicin, but died after an episode of severe liver toxicity. A total of 19 patients (13%) died during the treatment period. Causes for the death include progression of the disease, multi-organ failure, central nervous system haemorrhage and sepsis. There were no reports of development of ADAs in this study.

Based on the results of the study, gemtuzumab ozogamicin was approved, and it is the first ADC to be approved in May 2000 by the FDA, under the accelerated approval regulation. It was indicated for the treatment of relapsed CD33-positive AML patients, over 60 years of age, who are not considered for cytotoxic chemotherapy [42]. After the approval, there were several studies that associated the risk of hepatic injury and hepatic VOD to gemtuzumab ozogamicin administration [59-62]. It was reported in a study that 11 of 23 patients, who received gemtuzumab ozogamicin for relapsed AML, following SCT, developed liver injury that is characteristic of hepatic VOD. Of these 11 patients, 7 have died with persistent liver dysfunction and either multi-organ failure or sepsis. Based on the results of this study, hepatotoxicity, which was earlier attributed to nonspecific hepatocellular endocytosis was related to gemtuzumab ozogamicin that targets CD33-positive cells in hepatic sinusoids [63].

In 2004, a post approval phase III clinical trial (SWOGS0106) was started by Wyeth (now Pfizer). This study was undertaken in patients, under the age of 61, with previously untreated, de-novo, non-M3 AML, in order to compare gemtuzumab ozogamicin, combined with standard induction chemotherapy (daunomycin), as opposed to chemotherapy alone. It was observed that gemtuzumab ozogamicin failed to demonstrate clinical benefit, in comparison to standard chemotherapy. Complete response rate was similar in both the arms of the study (75% with gemtuzumab ozogamicin versus 73% with standard chemotherapy alone). More deaths were reported in the group, receiving gemtuzumab ozogamicin. 16 (5.7%) of 283 patients, treated with gemtuzumab ozogamicin, has suffered fatal toxicities, while the number of fatalities were 4 (1.4%) of the 281 patients, who received standard therapy. There were also reports of grade 4 non-hematological toxicities in 45 patients that included infection (26 patients) and hepatic VOD (1 patient) with gemtuzumab ozogamicin; while in the standard arm, toxicities were observed in 30 patients that included infections in 15 patients [64]. Significant non-specific toxicities, associated with gemtuzumab ozogamicin, is due to instability of the pH-cleavable linker in circulation, resulting in premature drug release in blood [65]. Due to these concerns, the trial was stopped, prematurely in June 2010, and Pfizer has withdrawn gemtuzumab ozogamicin from the USA market, at the request of the FDA [66].

However, gemtuzumab ozogamicin is still being evaluated in Europe. Results of two clinical trials that were conducted in France had shown survival advantage, in patients with newly diagnosed AML. In one trial, addition of gemtuzumab ozogamicin has improved the survival. Additionally, the results further suggested that a split dose regimen can lead to better patient response, compared to once every three weeks schedule. In the other trial, it was observed that addition of gemtuzumab ozogamicin did not increase toxicity. The results showed a significant overall survival benefit for older patients [67].

Brentuximab vedotin was approved in August 2011, under the FDA's accelerated approval program to treat Hodgkin's lymphoma (HL) and anaplastic large cell lymphoma (ALCL) [68]. It is a chimeric IgG1 mAb cAC10 that targets cells, with the over expression of CD30, found in HL, ALCL and a subset of non-Hodgkin's lymphoma (NHL) [69]. An enzyme-cleavable dipeptide linker, the cathepsin B-sensitive dipeptide, valine-citrulline, covalently binds the antibody cA10 to the potent anti-microtubule agent, monomethyl-auristatin E (MMAE) [17,18]. After binding to CD30 on the target cell, brentuximab vedotin is rapidly internalized in to the lysosomal compartment, where the linker is cleaved by human cathepsin B, which is a lysosomal enzyme, over-expressed in certain cancers [70,71]. The released MMAE acts on the target cells, leading to apoptotic cell death [18]. Preclinical and early clinical studies, with brentuximab vedotin, showed promising results that led to FDA approval for further studies [72-75].

A multicentre pivotal phase II trial, for evaluating the efficacy and safety of brentuximab vedotin, as a single agent in patients with relapsed or refractory HL, and a phase II study in patients with relapsed or refractory ALCL, are the two significant phase II clinical trials that led to its approval by the FDA. Both these trials are still ongoing. In the trial

for relapsed or refractory HL (ClinicalTrials.gov Identifiers: NCT ID: NCT00848926), the patients administered 1.8 mg/kg of brentuximab vedotin, every 3 weeks. Of the 102 patients enrolled in the study, 75% responded to the treatment, with a complete response rate of 34%. Median duration of response for the therapy was 6.7 months and the median duration of progression free survival was 5.6 months. 94 of the 102 enrolled patients, had drug related AEs. 56 of these patients, experienced grade 3 or greater AEs and 14 patients experienced serious drug related AEs. Treatment was discontinued in 20 patients due to AEs [75]. In the study for relapsed or refractory ALCL, in 58 patients with brentuximab vedotin, administered at 1.8 mg/kg for every 3 weeks, the objective response rate was 86%, with a complete response rate of 57%. Median duration of response for the therapy was 12.6 months and the median duration of progression free survival was 13.3 months. 53 of the 58 enrolled, had drug related AEs. 35 of these patients experienced grade 3 or greater AEs and 10 patients' experienced serious drug related AEs. Treatment was discontinued in 11 patients due to AEs [76]. However, detailed results of the two studies have not been published yet. The most common side effects, resulting from administration of brentuximab vedotin in various studies, include easy fatigability, fever, diarrhea, nausea and vomiting, anemia, neutropenia, lymphopenia, thrombocytopenia and peripheral sensory neuropathy. One patient in phase I study, after administration of brentuximab vedotin at 3.6 mg/kg, died of infectious complications following febrile neutropenia [69]. Brentuximab vedotin is presently evaluated in many clinical trials for various types of lymphomas, multiple myeloma, leukemia and solid tumors [77].

Inotuzumab ozogamicin (CMC-544) is a humanized IgG4 anti-CD22 mAb that is covalently linked to calicheamicin moiety, Calich-DMH, through an acid-labile AcBut linker, similar to Gemtuzumab ozogamicin [55,78]. CD22 is found on cell surface of mature B-cells, but it is restricted only to cytoplasm of pre- and pro-B cells, during B-cell development [79]. It is expressed on the surface of malignant B cells, in more than 90% of B-lymphoid malignancies [80]. Because of consistent expression of CD22 in B-cell cancers and lack of expression on non-hematopoietic tissues, it represents an ideal target for Ab- or ADC-based therapies. When bound to inotuzumab ozogamicin, CD22 is rapidly internalized, leading to intracellular hydrolysis and release of calicheamicin [81]. Efficacy of inotuzumab ozogamicin, as monotherapy regimen, in patients with CD22+ relapsed or recurrent NHL, was demonstrated from the results of two completed phase I studies. In the first part of the phase I trial, in 79 patients, with CD22+ relapsed or recurrent NHL, inotuzumab ozogamicin was administered intravenously, as a single agent, once every 3 or 4 weeks at escalating doses (ranging from 0.4 to 2.4 mg/m2). In the second part of the study, which included an expanded cohort of the patients (n=49), inotuzumab ozogamicin was administered at the maximum tolerated dose (MTD) of 1.8 mg/m2, every 4 weeks, as determined from the first part of the study. Common AEs, at the MTD were thrombocytopenia (89.8%), asthenia (67.3%), nausea (51%) and neutropenia (51%). During MTD with inotuzumab ozogamicin, thrombocytopenia and neutropenia were the main cause for delaying the dose and dose reductions (22 and 12 patients, respectively). 11 patients discontinued MTD, due to thrombocytopenia. Elevation of liver function tests that included increase in AST, ALT and hyperbilirubinemia in 40.8%, 26.5% and 22.4% respectively, were observed at MTD. 32 (40.5%) deaths occurred, which were mostly due to disease progression, in addition to liver insufficiency, pneumonia, sepsis and one of the undetermined causes. At the MTD, ORR in patients with follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) was 68% and 15%, respectively [80]. A second phase I trial of inotuzumab ozogamicin, which was conducted in Japanese patients with r/r, FL established an MTD of 1.8 mg/m2, at every 4 week schedule. The safety profile was similar to prior phase I study. Observed response rate was 85%, warranting further testing in FL [82]. Inotuzumab ozogamicin is currently being evaluated in several clinical trials as monotherapy, and also in combination with rituximab [83].

Apart from the treatment of hematologic malignancies, ADCs are also being developed for the treatment of solid tumors. The success rate of ADCs for solid tumors is limited. ADCs are developed in such a way that they target the cell surface antigen that is highly expressed on the tumor cells. The expression of the antigens on normal cells is at lower levels. Major obstacles are to be overcome by the ADC, on its way, to reach the target outside the vascular compartment, which limits the effectiveness of these agents [84].

Trastuzumab emtansine (T-DM1) combines the humanized IgG1 anti-HER2B, Abtrastuzumab, with maytansinoid DM1 [85,86], through a non-cleavable thioether linker, N-maleimidomethyl cyclohexane-1-carboxylate (MCC) [87]. HER2/Erb2 is an oncogenic tyrosine kinase receptor, which belongs to EGFR family, involved in the pathogenesis of various human cancers, where it shows increased expression at the protein level or amplification at the genomic level. Elevated expression of HER2 is found in 25% of all the breast cancers and in other tumors, such as ovarian, gastric, non-small-cell lung and pancreatic cancers. Increased expression of this receptor, in some of the cases is linked to shortened survival [13,88-90]. In contrast, the expression of HER2 receptor is relatively low in normal adults or in hematopoietic compartments [91]. Trastuzumab, as a naked antibody, was approved for the treatment of HER2 positive metastatic breast cancer (MBC), in 1998. Because of the stable nature of the MCC linker, upon binding of T-DM1 to HER2 receptor, it is internalized, followed by proteolytic degradation in the lysosome and release of lysine-MCC-DM1, DM1 then binds to the tips of microtubules, leading to cell death [92].

Several phase I and phase II clinical trials have shown clinical efficacy and safety of T-DM1, as a single agent and in combination with other agents, in patients with HER2 positive MBC [93]. Based on the dose limiting toxicity of grade

4 reversible thrombocytopenia, 3.6 mg/kg was established as MTD. In the phase II studies, T-DM1 was administered i.v. at this dose, for every 3 weeks. Most common AEs, seen in various studies, include fatigue (37.5-65.2% of the treated patients), nausea (25.0-50.9%), anemia (10.4-29.2%) and hypokalemia (4.2-24.1%). Reversible grade 3 or 4 thrombocytopenia was one of the most frequent laboratory abnormalities, in phase II studies [94-96]. Elevation of hepatic enzymes was also associated with T-DM1. The overall incidence of grade 3 or 4 elevations of ALP, ASP or ALT ranged between 0 and 13.4%, in various phase I and phase II studies. One death occurred, due to hepatic dysfunction. However, the relationship of the death to administration of T-DM1 was not clear [97]. Naked mAb, trastuzumab, itself was found to be associated with increased risk of cardiac dysfunction, which was greatest in patients, receiving concurrent anthracyclines [98]. Trastuzumab-induced cardiotoxicity has been attributed to blockade of HER2 signaling in cardiac myocytes, which manifested symptomatic congestive heart failure (CHF) or asymptomatic decline, in left ventricular ejection fraction (LVEF) [99]. This raised concerns about the cardiotoxicity profile of T-DM1. However, studies with T-DM1, as single agent till date, reported no cases of symptomatic CHF or grade 3 LVEF decline.

T-DM1 is also associated with peripheral neuropathy. A potent anti-microtubule agent, similar to that present in brentuximab vedotin is known to induce peripheral neuropathy [45, 46]. Many other exceptional AEs were reported in various studies. In one phase II study, occurrence of several AEs, like thrombocytopenia, peripheral neuropathy, epistaxis and back pain, resulted in dose reduction of T-DM1 for 6 out of 12 patients [94]. There were also reports of grade 3 hemorrhagic AEs that included epistaxis, hematochezia, subdural hemorrhage, hemorrhoidal hemorrhage and upper GI hemorrhage. Inspite of this, there were no discontinuation of treatment, due to hemorrhage. AEs related to eye, mostly reported as grade 1 and 2, include dry eyes, increased lacrimation, blurred vision and conjunctivitis. Despite all these events, T-DM1 is considered to be well tolerated and is currently being evaluated in various phase III trials. In a phase III trial, comparing T-DM1 with combination of lapatinib plus capecitabine, in patients with advanced breast cancer, higher incidence rates of AE of grade 3 or 4 were reported for the lapatinib plus capecitabine group than for the T- DM1 (57.0% vs 40.8%) [100].

Glembatumumab vedotin (CDX-011 or CR011-vcMMAE) consists of a human IgG2 mAb (CR011) that targets GPNMB protein and is conjugated via the proteolytically cleavable vc linker to the microtubule toxin MMAE. GPNMB, or osteoactivin, is a type 1 transmembrane glycoprotein that is highly expressed in numerous tumor types, including breast cancer, melanoma and gliomas [101-103]. Additionally, GPNMB mRNA was also found to be present at lower levels in macrophages, dendritic cells and on normal cell subsets of the skin [104]. GPNMB was shown to have a role in promoting invasion and metastasis of breast cancer, liver cancer and gliomas [105-107]. GPNMB is associated with the induction of endothelial cell migration and increased angiogenesis in breast cancers.

In clinical trials, glembatumumab vedotin was evaluated in advanced melanomas and breast cancer. A phase I/II trial (NCT00412828) was conducted in patients with advanced melanoma, using every 3 week schedule, which was later followed by the phase II expansion at the MTD (1.88 mg/kg). Promising antitumor activity was reported with ORR of 15%. The most common AEs, reported for any grade of severity, were rash, fatigue, alopecia, pruritus, diarrhoea and neuropathy [108,109]. Reported data from the phase II study (EMERGE, NCT01156753), with metastatic breast cancer patients, who are GPNMB+, receiving either glembatumumab vedotin at MTD (1.88 mg/kg) or investigator‘s choice (IC) single-agent chemotherapy suggests that glembatumumab vedotin is well tolerated and also indicates higher response rate, in patients with triple-negative (ER,PR and HER negative) breast cancer status. ORR was 32% for glembatumumab vedotin and 13% for IC. Hematological toxicity was less with glembatumumab vedotin, compared to IC. Glembatumumab vedotin related toxicity, included rash and neuropathy (overall grade for all severity of 38% and 18% respectively) [110].

Though many ADCs have been investigated for the treatment of solid tumors, the reported results were disappointing. CMB-401, a calicheamicin antibody conjugate was initially evaluated in 19 patients with ovarian cancer [111]. Due to higher rates of incidence of AEs, like peritonitis, anemia, thrombocytopenia and allergic reactions, this ADC was withheld from further development. BR96-doxorubicin conjugate, an earlier generation ADC, was developed to target Lewis-Y (LeY) antigen that is expressed on 75% of all the breast cancers, with limited expression on normal tissues [112]. In this ADC, doxorubicin was linked to chimeric mAb, BR96, through an acid-sensitive hydrazone linkage [113]. In the randomized phase II study to evaluate BR96-doxorubicin [112], it was reported to have limited antitumor activity and also resulted in prominent GI toxicities. As a result, further studies with this ADC were discontinued.

Chemotherapy of many cancers is limited by increased incidence of untoward effects, due to resemblance of cancer cells to the normal cells. In an effort to overcome this, immune mediated therapeutic approaches was developed. Monoclonal antibodies target antigens, whose expression is either specific to or highly expressed on cancer cells, compared to normal cells. Therapeutic failure of naked antibodies, led to the development of conjugated antibodies. Monoclonal antibody-based conjugates delivered cytotoxic agent to the target cell, resulting in less toxic effects than the conventional chemotherapeutic regimens. If the cytotoxic agent conjugated to mAb is a potent drug, the molecule is referred as antibody drug conjugate. Cytotoxic drugs used in development of ADCs, include auristatins, maytansines and calicheamicins and their synthetic or semi-synthetic analogs [15-19]. These agents are bound to antibody, with a specially designed linker that is stable and efficiently

cleaved at appropriate intracellular location of the target cell.

Immunogenicity problems, which are seen with the early generation ADCs, are overcome by the use of humanized or fully human Ab fragments. Type of conjugation and the sites on the Ab, determine the tolerability, pharmacokinetic properties and the overall effectiveness of the ADC therapy. Every loaded species in ADC, represents a unique conjugate, and exhibits distinct properties. Decreased clearance and improved therapeutic index was observed, as there is a decrease in drug loading from 8 to 4 or 2 drug molecules per Ab for anti-CD30-vcMMAE.

ADCs based therapies, performed better in hematological malignancies than in solid tumors. In the hematological tumors, the expression of the antigen is homogenous in most cells and the tumor is more accessible. Additionally, liquid tumor cells are more sensitive towards cytotoxic compounds as compared to solid tumors. In the solid tumor cells, the limiting factor is the number of Ab-based molecules that are able to reach the surface of tumor cells, after extravasation of the tumor blood vessel and translocation through the tumor interstitium [114,115]. Further, heterogenous blood supply and high interstitial pressures, especially in the necrotic zones of solid tumors may limit the diffusion of drugs or ADCs to poorly perfused areas [116]. Therefore, development of novel vehicles that allow better penetration to the tumor mass in solid tumors, may improve the efficacy of therapeutic mAbs and ADCs.

The present generation ADC technology is not perfect, because despite significant progress in ADC technology, these agents still exhibit unwanted side effects in the clinical studies. Myelosuppression was the most commonly reported adverse effect, with the administration of ADCs, which manifested as thrombocytopenia, anemia and leucopenia, thereby weakening the patient‘s immune system and resulting in increased risk of opportunistic infections. Hepatic injury is the second most common injury, manifested in the form of elevated bilirubin, alkaline phosphatase (ALP), aspartate transaminase (AST) or alanine transaminase (ALT) levels. It is dose-limiting toxicity as most of these molecules are naturally cleared from the blood by the liver. Liver toxicity with ADCs, can sometimes get complicated, resulting in hepatic veno-occlusive disease as seen with the conventional and high-dose chemotherapy [43,44]. ADCs that act by targeting microtubules cause peripheral neuropathy and reversible myelosuppression [45-47], while those acting by damaging DNA, target rapidly dividing cells leading to alopecia, myelosuppression and gastrointestinal adverse effects [48]. Apart from these toxicities, fever, nausea, vomiting, myalgia, diarrhea and rash that are usually mild to moderate in intensity, were also observed.

Main cause for toxicities, due to ADCs, include: unintentional drug release, recognition of the same antigen on normal tissues by ADC or nonspecific uptake and release of the drug, within the bone marrow or liver. Advancements in linker technologies or site specific conjugation approaches may help to limit these toxicities.

Conclusion

Many targeted toxic molecules have been evaluated in the past three decades. Approval of few of these ADCs, demonstrates their ability in the future, to be a major therapeutic alternative to the standard first-line treatment. Toxicities with these agents are mainly due to unintentional drug release and recognition of the antigens on the normal cells. Myelosuppression, hepatotoxicity and peripheral neuropathy are the main toxicities with ADCs. The reported toxicities are commonly reported due to ADCs, mentioned in the review. There are also milder toxicities that are usually transient and are mild to moderate in severity, resembling those, due to unconjugated mAb administration. Newer discoveries and further knowledge, relating to tumor surface antigens, stable linkers, payloads and conjugation strategies are helpful in the development of ADCs, with safe toxic profile and more therapeutic effectiveness. Additionally, knowledge of pharmacokinetics and biodistribution of ADCs will improve the applications of targeted therapies.

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