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Antibody-based Therapeutics for the Treatment of Human B cell Malignancies

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Abstract

The dynamic expression of various phenotypic markers during B cell development not only defines the particular stage in ontogeny but also provides the necessary growth, differentiation, maturation and survival signals. When a B cell at any given stage becomes cancerous, these cell surface molecules, intracellular signaling molecules, and the over-expressed gene products become favorite targets for potential therapeutic intervention. Various adaptive and adoptive immunotherapeutic approaches induce T cell and antibody responses against cancer cells, and successful remission leading to minimal residual disease has been obtained. Nonetheless, subsequent relapse and development of resistant clones prompted further development and several novel strategies are evolving. Engineered monoclonal antibodies with high affinity and specificity to target antigens have been developed and used either alone or in combination with chemotherapeutic drugs. They are also used as vehicles to deliver cytotoxic drugs, toxins, or radio-nuclides that are either directly conjugated or encapsulated in liposomal vesicles. Likewise, genetically engineered T cells bearing chimeric antigen receptors are used to redirect cytotoxicity to antigen-positive target cells. This review describes recent advancements in some of these adoptive immunotherapeutic strategies targeting B cell malignancies.

Keywords

Antibody-based therapeutics; B cell malignancies; Immunotoxins; Liposomes; CAR T cells; Treatment

Introduction

Immunological control of cancers has been one of the favorite strategies and holds a lot of promise to deliver the best possible outcome to patients. Increasing knowledge on various components of the immune system at cellular and molecular levels, better understanding of the intricacies of signaling molecules and processes, regulatory networks that modulate the

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immune responses, and technological advances have expanded cancer treatment options. Hematological malignancies including the various types of B cell malignancies are considered particularly amenable for therapeutic intervention with cell-mediated as well as antibody-mediated immune responses. Adaptive immunotherapy includes cellular vaccines such as gene-modified autologous cancer cells, dendritic cells pulsed with tumor cell extracts, protein/peptide vaccines consisting antigens that are unique or over-expressed by the cancer cells, DNA vaccines encoding target proteins, and agents that modulate immune functions [1–6]. The goal is to enhance activation and proliferation of tumor-specific CD4+ and CD8+ T cells, and enhance production of proinflammatory cytokines (e.g., interleukin 2, IL-2; interferon gamma, IFN γ ; tumor necrosis factor alpha, TNF α) and effector cytolytic granules (e.g., perforin, granzyme B), ultimately resulting in elimination of cancer cells.

Passive administration of monoclonal antibodies (mAbs) that recognize surface proteins on malignant B cells (e.g., rituximab recognizing CD20) induced rapid elimination of malignant B cells through complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) [7]. This success revolutionized the field of cancer immunotherapy and fueled the development of several mAbs targeting different antigens on malignant B cells [8]. Some of the antigens targeted by mAbs are: CD20 in non-Hodgkin's lymphomas (NHL) including follicular lymphoma (FL), diffused large B cell lymphoma, (DLBCL), mantle cell lymphoma (MCL); CD19 in FL; CD52 in chronic lymphocytic leukemia (CLL); CD22 in Hairy cell leukemia (HCL) and acute lymphoblastic leukemia (ALL); CD37 in CLL and CD38 in multiple myeloma (MM).

In this review, we discuss the use of mAbs as vehicles to selectively deliver toxins or small molecule drugs to cancer cells with particular emphasis on the treatment of B cell malignancies. While in immunotoxin the toxin moiety is directly linked to a mAb, small molecule drugs are encapsulated in liposomes coated with a mAb. In both cases, the mAb directs the delivery of cytotoxic agents to target cancer cells in a precisely controlled and efficient manner, thus the threshold level of effector molecules required at the target site is achieved without a need to reach high systemic levels and prevents associated side effects. In a different approach, autologous or healthy donor T cells are genetically engineered to express a chimeric antigen receptor (CAR T cells) that recognizes a surface protein on cancer cells. The CAR T cells upon administration to patients expand in vivo, persist for long periods of time, efficiently eliminate cancer cells, and prevent relapse.

Immunoconjugates in Cancer

During the last two decades, many mAbs have been developed for the treatment of cancer, but only a few are useful as single agents in the clinic because most of them cannot efficiently kill the target cells [9]. Potentially therapeutic mAbs are conjugated to a variety of molecules including small-molecule drugs, radionuclides, peptides, and other proteins such as toxins, enzymes and cytokines to form antibody drug conjugates or immunoconjugates. With the combination of exquisite target specificity of the mAb and high potency of the cytotoxic agent, they provide sensitive discrimination between the target cancer cells and the normal tissue. In addition, because of their large molecular size, immunoconjugates enhance in vivo stability leading to prolonged effect. The mechanisms by which the cytotoxic agents induce cell death differ widely [9–11]. We focus our discussion below on the design and development of immunotoxins and their use in clinical trials for the treatment of human B cell malignancies.

Immunotoxin Structure and Mechanism of Action

The recombinant DNA technology and advances in protein engineering have enabled the generation of fusion proteins consisting of mAb or its fragment coupled to large protein

toxins from bacteria or plants. Bacterial exotoxins from *Corynebacterium diphtheriae* (DT), *Pseudomonas aeruginosa* (PE), and *Vibrio cholerae* (CET), and deglycosylated Ricin A chain (dgA) from plants have been used to generate immunotoxins targeting a variety of cell surface molecules expressed by cancer cells. These protein toxins consist of discrete functional domains, namely: a cell binding domain, a translocation domain, and an activity or death domain [9, 12]. The protein toxins upon cell surface binding are internalized and undergo several processing steps before releasing the active unit into the cytosol. While the active unit in bacterial toxins DT, PE, and CET catalyzes ADP ribosylation of elongation factor 2 (EF-2), that of plant toxin dgA inactivates ribosomes via glycosidase activity, and in both cases the end result is halting protein synthesis and apoptotic cell death [13, 14••].

Design and Production of Recombinant Immunotoxins

The first generation immunotoxins were made by chemically coupling antibodies with native toxins and were unsuitable for clinical applications due to lack of specificity, stability, and heterogeneous composition. Based on structural information, these toxins were modified to better suit intended clinical applications: (1) substitution of the (universal) cell-binding domain in the native toxin with an antibody fragment to redirect the toxin only to target cells expressing specific antigen [12], (2) removal of non-essential segments to reduce the overall size, thereby enhancing tissue (solid tumor) permeability (e.g., truncated PE38) and conferring protection from lysosomal degradation [15], and (3) silencing of immunogenic epitopes to minimize or eliminate the production of antibodies that may neutralize the toxin effect [16••]. Using recombinant DNA technology, the gene segments encoding the antigen-binding fragments of an antibody (Fab or Fv) or a cytokine/growth factor is linked to the gene encoding selected toxin domains (translocation and activity domains). The resulting plasmid(s) are expressed in bacteria to produce immunotoxins as fusion proteins.

On the antibody side, early recombinant immunotoxins were generated comprising variable regions of the heavy and light chain segments of a mAb in a single chain format (scFv) linked by a 15-aminoacid peptide [17]. This format was relatively unstable and formed aggregates with loss of activity. Later, the peptide linker was replaced with a disulfide bond between the heavy and light chain Fv fragments (dsFv) facilitated by the introduction of cysteine residues at predetermined sites. The resulting dsFv-immunotoxins showed improved activity and enhanced stability [14••, 18] (Fig. 1). In addition, affinity maturation of the Fv segments yielded immunotoxins with enhanced activity [19].

Parameters Affecting Immunotoxin Efficacy

The mAb affinity to its target antigen largely determines the stability of the antigen/antibody complex on the cell surface and directly correlates to internalization and toxin activity [20]. High density of target antigens on cell surface can enhance apoptosis induction as seen with CD22-, FCRL1-, and ROR1-immunotoxins [14••, 21, 22]. Immunotoxins generated from mAbs against different epitopes on the same antigen differ in their potency [23]. An immunotoxin binding to membrane proximal epitope induced higher cytotoxicity than those binding to distal epitope [24]. Rapid internalization of CD22 molecules, despite lower surface density compared to CD19, was responsible for superior efficacy of the CD22-immunotoxin, BL22 [25]. A combination of high antigen density and rapid internalization of CD22 resulted in higher response rates to BL22 in patients with HCL [26, 27]. Cell intrinsic factors such as anti-apoptotic proteins (e.g., BCL-2, BCL-XL, MCL-1, XIAP, and survivin) play a critical role in cell survival and toxin susceptibility [28, 29]. This is evident from: (1) the inverse correlation between BCL-2 expression and immunotoxin sensitivity [14••, 30], (2) constitutive over-expression of BCL-2 in FL and MCL [31], and (3) the ability of small-molecule inhibitors of BCL-2 (e.g., ABT-737) to increase immunotoxin sensitivity of

malignant cells in vitro and in vivo [13, 29, 32•]. Finally, epigenetic factors such as DNA hypermethylation caused resistance to anti-CD22 immunotoxin (HA22) in pediatric ALL [33]. In vitro studies demonstrated that a methylation inhibitor, 5-azacytidine, restored sensitivity to HA22 [34], suggesting that the combination of this agent and HA22 may be useful in the treatment of patients with ALL.

Immunotoxins Targeting Hematologic Malignancies

Numerous immunotoxins comprising different target antigen specificities and different toxins have been developed to treat various human cancers [9, 35]. Although solid tumors and leukemias have been targeted using immunotoxins, to date, most success is seen in hematologic malignancies. Recent reports describe in detail the various immunotoxins developed against target antigens expressed in B cell malignancies and their performance in clinical trials [35, 36]. A number of cell surface receptors for growth factors have been targeted: IL-2 receptor in Hodgkin's lymphoma, cutaneous T cell lymphoma, adult T cell leukemia, HCL and CLL [37–39], IL-4 receptor in CLL [40], and receptors for GM-CSF and IL-3 in acute myeloid leukemia (AML) [41]. Other cell surface molecules targeted by immunotoxins are: CD3 in T cell lymphoma [42], and CD19 and CD22 in BALL, HCL, MCL, CLL, and ALL [19, 30, 33, 36, 43–45]. PE-based immunotoxins targeting CD22 (BL22, HA22) induced complete remissions in some patients with drug-resistant HCL [46]. In an independent multi-center phase I study, a combination of anti-CD19 (HD37-dgRTA) and anti-CD22 (RFB4-dgRTA) immunotoxins (Combotox) produced consistent blast reduction in all 17 patients with relapsed and refractory ALL [47].

All the immunotoxins mentioned above target lineage-specific antigens on malignant cells, and, therefore, will also eliminate normal counterparts expressing the same marker (e.g., normal B cells). Therefore, there is a need to identify and develop immunotoxins directed against target antigens expressed only by malignant cells. Recent studies documented uniform and restricted expression of a receptor tyrosine kinase, ROR1, in a spectrum of B cell malignancies [14•, 48•, 49•, 50–52] and its involvement in providing survival signals [53, 54], suggesting that ROR1 could be a very useful therapeutic target. In fact, unarmed mAbs against ROR1 and a PE-based ROR1-immunotoxin (BT-1) selectively killed ROR1+ primary CLL cells and ROR1+ MCL cell lines in vitro, respectively [14•, 55]. Conceivably, such ROR1-based therapeutics could be further improved to selectively eliminate ROR1+ malignant B cells and spare the normal counterparts.

Advantages and Challenges

Immunotoxins, unlike unarmed antibodies, can directly kill target cells independent of effector components (complement proteins, NK cells, macrophages, or T cells) that may not be optimally available in a cancer patient. Immunotoxins are very potent mediators of cell death, and exquisitely target specific, requiring less than 1,000 molecules per target cell to effect cytotoxicity [56]. Unlike small-molecule cytotoxic drugs, immunotoxins when released from dying or dead target cells do not diffuse through and cause death of antigen-negative bystander cells. Use of immunotoxins in the setting of minimal residual disease (MRD) would be very encouraging, as there will not be an antigen sink, thus allowing a reduction in the number of treatment cycles/dose and nonspecific toxicity, and an overall widening of the therapeutic window.

Two major limitations in the use of recombinant immunotoxins are the production of neutralizing antibodies against the toxin and vascular leak syndrome (VLS). Bacterial proteins (toxins) can be highly immunogenic, and generation of neutralizing antibodies would hamper the efficiency of subsequent toxin administration. By the identification and silencing of mouse and human B cell epitopes, the PE-based immunotoxins became non-

immunogenic and non-antigenic, and did not induce neutralizing antibodies [16••]. Such modification allows repeated administration of toxin to the patients to achieve higher efficacy and obtain full therapeutic benefit. The other dose-limiting toxicity is the development of vascular leak syndrome (VLS) caused by damage to vascular endothelial cells. First, the unique amino acid in dgA causing VLS was identified and mutated, and the mutant toxin (RFB4 N97A) did not induce VLS and was more effective than the unmutated RFB4-RTA in killing xenografted Daudi cells in SCID mice [57]. In a second approach, a mutant chimeric immunotoxin (mcRFB4-RTA) targeting CD22 had reduced in vivo half-life and reduced VLS, but retained cytotoxicity against xenografts in mice [37, 58•].

Liposomes as Drug Carriers

Liposomes are spherical self-closed structures composed of lipophilic bilayers with an entrapped hydrophilic core. Their unique structure allows the encapsulation of both lipophilic and lipophobic therapeutic agents by liposomes. The bio-compatibility of liposomes if used with proper lipid composition also makes liposomes safe and effective drug carriers. Several therapeutic agents incorporated into liposomes have reached clinical use. These include liposomal doxorubicin (Doxil™) [59], daunorubicin (Daunoxome™) [60], amphotericin B (Amphotec™, Ambisome™, Abelcet™) [61], cytarabine (Depocyte™), and verteporfin (Visudyne™). Numerous liposomal formulations are in clinical trial, including vincristine, all-trans retinoid acid, topotecan, and cationic liposome-based therapeutic gene transfer vectors. Besides potential use in systemic gene delivery, cationic liposomes are routinely used as transfection reagents for plasmid DNA and oligonucleotides in the laboratory. The liposomes can be engineered to possess unique properties, such as long systemic circulation time, pH sensitivity, temperature sensitivity, and target cell specificity. These are achieved by selecting the appropriate lipid composition and surface modification of the liposomes, thus expanding the application of liposomes for drug delivery.

Liposomal delivery of anticancer drugs has been shown to greatly extend their systemic circulation time, reduce toxicity by lowering plasma-free drug concentration, and facilitate preferential localization of drugs in solid tumors based on increased endothelial permeability and reduced lymphatic drainage, or enhanced permeability and retention (EPR) effect [62]. Clearance of liposome-encapsulated drugs is mediated by phagocytic cells of the reticuloendothelial system (RES), primarily located in the liver and spleen. While free drugs are subjected to rapid renal clearance, liposomal drugs are cleared by RES, thus exhibiting prolonged systemic circulation time [63]. Coating the liposome surface with polyethyleneglycol (PEG) further reduced RES-mediated clearance of liposomes. A passive targeting effect has also been shown in liposomal drug delivery to solid tumors, where liposomes preferentially localize in these tumor tissues due to the enhanced permeation and retention (EPR) effect [64]. Liposomes and immunonanoparticles have broader implications in drug delivery, magnetic resonance imaging, and cancer treatment [65].

Targeted Liposomes

Liposomes can be targeted to specific cell populations via incorporation of a desired targeting moiety, such as a chemically conjugated ligand, antibody, or antibody fragment directed against surface molecules. Targeted delivery of these liposomes can greatly improve their selectivity to cancer cells and facilitate their cellular uptake and intracellular drug release. Examples of such targeted liposomes are CD19-targeted immunoliposomes for NHL therapy [66], folate-conjugated liposomes targeting to AML, anti-HER2 immunoliposomal doxorubicin targeting to HER2-over-expressing breast cancer cells [67], and CD20 targeted immunoliposomes consisting of antisense oligonucleotides (ODN) in CLL [68••] (Fig. 1).

A variety of techniques have been described to incorporate targeting moieties to liposomes. First, ligands such as folate can be conjugated to a lipid composition such as polyethylene glycol–distearoyl phosphatidyl ethanolamine (PEG-DSPE) or polyethylene glycol–cholesterol (PEG-Chol). This amphiphilic lipid composition can be constructed into the lipid bilayer during the formation of membrane leaving the ligand outside the particles [69–71]. The second method involves inclusion of a reactive lipid cross-linker exposed outside the liposomes after the formation of bilayer membrane [67, 72–79]. Thus, the exposed cross-linker can react with activated targeting moieties such as thiolated proteins (antibody, ScFv, transferrin, etc.). The most useful thiolation reagent is 2-iminothiolane, also known as Traut's reagent. Based on the antibody coupling strategy, the immunoliposomes can be broadly classified into two types. The first type involves direct attachment of antibodies to lipids [73, 78], and the second type involves linking antibodies to the terminal ends of reactive PEG derivatives [67, 72, 76, 78, 80]. Most of these coupling techniques do not have control over the orientation of the antibody and hence result in a random antibody orientation at the liposome surface. A significant contribution to the development of the second type of immunoliposome, recently being adopted for formulation of HER2-targeted immunoliposomes, is an antibody coupling method called the “post-insertion” method [67]. This method involves first the formation of micelles of lipid-derivatized antibodies, which is then followed by transferring the antibody-coupled PEG-lipid micelles to the preformed liposomes, thus converting the conventional liposomes to immunoliposome through a simple one-step incubation method. Maleimide-terminated PEG-DSPE is a coupling lipid that has been validated to successfully convert commercially available liposomal doxorubicin (Doxil/Caelyx) to targeted, sterically stabilized liposomal doxorubicin.

The feasibility of using immunoliposomes as drug delivery vesicles is a result of parallel advances both in the monoclonal antibody (mAb) and liposome technology. This tumor-specific high payload delivery vesicle, combines the tumor-targeting properties of mAbs and drug delivery advantages of liposomes, therefore representing one of the most advanced forms of targeted drug delivery system. A number of studies have reported successful targeting and enhanced therapeutic efficacy as exemplified in CD19-targeted immunoliposome for the delivery of doxorubicin and antisense oligodeoxynucleotides [66, 72, 75, 78, 81, 82]. Harata et al. designed imatinib-CD19-liposome and demonstrated specific and efficient killing of Philadelphia chromosome-positive ALL (Ph+ ALL) cells [72]. In another study, Huwyler et al. used OX26 mAb-mediated liposomal targeting of transferrin receptor to overcome the blood–brain barrier [76]. Park et al. in UCSF moved anti-HER2 immunoliposomal doxorubicin to Phase II clinical trial for breast cancer therapy [67, 77], and recently, Matsumura et al. also conducted clinical trials using MCC-465, a GAH-targeted immunoliposomal doxorubicin formulation, in human stomach cancer [83].

Various cell surface receptors and antigens have been targeted through different strategies. Among these, folate receptor (FR) has been one of the targets extensively investigated. FR is selectively amplified on human malignant cells, and involved in uptake of folate or its analogs through receptor-mediated endocytosis [84, 85]. FR-targeted liposomes can be utilized to deliver therapeutic agents and antisense ODN to FR-positive cells [86, 87]. ODNs, like gene constructs, are negatively charged molecules of a much smaller size and, therefore, present a different set of challenges for their effective delivery. To address the issue of serum stability, ODNs containing modified backbones, such as phosphorothioates, were developed and showed promise in clinical trials. The role of NF- κ B in oncogenesis and tumor metastasis has been widely discussed [88], and, therefore, NF- κ B targeted anti-sense ODNs liposomal formulations could potentially be used in treatment of diverse cancers.

The advantages of liposome-based targeted delivery system reside in (1) selective delivery of high payload of cytotoxic agents towards malignant but not normal cells; (2) avoidance of

compound structure modification, because drugs are encapsulated into particles instead of through covalent bond conjugation; and (3) pharmacokinetic advantages, such as prolonged systemic circulation, optimized tissue distribution, and reduced toxicity.

Chimeric Antigen Receptor Bearing (CAR) T Cells

Allogeneic hematopoietic stem cell transplantation (HSCT), consisting of conventional T cells, has been shown to establish a long-term remission in patients with relapse or refractory B cell malignancies. However, the majority of the patients developed chronic or acute graft versus host disease (GVHD) affecting multiple organs with varying severity. Therefore, alternate modalities have been sought with an aim to preserve the graft versus leukemia (GVL) activity and minimize or eliminate the undesirable GVHD.

Using gene transfer technology, the antigen-binding fragments of a mAb in scFv format is fused with an intracellular signaling moiety of T cell receptor complex, CD3 ζ , and expressed as chimeric antigen receptors (CAR) on the surface of T cells. The resulting CAR T cells exhibit advantages over conventional TCR-mediated antigen recognition as they: (1) acquire novel and increased antigen specificity with high avidity interactions, (2) recognize target antigen-bearing malignant cells in major histocompatibility complex (MHC)-independent manner, and (3) overcome the immune tolerance established during development. While the antibody domain facilitated the recruitment and binding of CAR T cells to cancer cells, the signaling domain initiated proliferation, cytokine secretion and activation into effector T cells leading to specific and robust killing of target cancer cells. Thus, CAR T cells obviate the need for antigen processing and presentation by target cancer cells, and can also recognize non-classical T cell targets such as carbohydrates. The potential use of allogeneic CAR T cells across the MHC barrier without risking the development of GVHD is certainly very encouraging, not only from the feasibility and clinical utility but also from the product development and quality control stand points.

The first generation CAR T cells were able to recognize and kill target cancer cells in vitro, but had limited in vivo anti-tumor activity presumably due to limited expansion and persistence [89]. By including a costimulatory domain from CD28, CD134 (OX40), or CD137 (4-1BB) molecules together with the signaling domain CD3 ζ , the next generation CAR T cells exhibited enhanced activation and production of proinflammatory cytokines (IL-2 and IFN γ), were resistant to T regulatory cells in vitro, had elevated BCL-2 levels facilitating long-term persistence, and showed expansion in vivo with enhanced anti-leukemic cytotoxicity [90, 91]. A combination of two costimulatory domains produced a synergistic effect in anti-tumor activity in vitro and in vivo [92, 93] (Fig. 1). In a different approach, long-term persistence and enhanced anti-tumor activity was achieved by redirecting virus-specific CTLs transduced to express CAR against different tumor-associated antigens such as GD2, CD19, and HER2 [94–96].

Autologous CAR T Cells

T cells from patients with chronic diseases like cancer have been known to be functionally compromised on multiple levels [97]. However, CAR T cells generated from the patient's peripheral blood mononuclear cells (PBMC) can overcome the deficiencies because the recognition and signaling units are constituted quite differently from that of conventional T cells. Adoptive transfer of autologous CAR T cells specific for tumor-associated antigens resulted in anti-tumor response in early clinical trials for CD20+ B-cell lymphomas [98, 99]. Extensively pretreated patients with refractory CLL received autologous CD19-specific CAR T cells (CART19) that expanded >1,000-fold in vivo, trafficked to bone marrow, and retained high levels of CAR expression for >6 months. Each CART19 cell was estimated to eradicate at least 1,000 CLL cells, resulting in clinical antitumor response in blood and bone

marrow accompanied by complete remission in 2/3 patients [100••]. The choice of CD19 as a target antigen for the development of CAR T cells is valuable, as this protein is expressed uniformly throughout the ontogeny of normal B cells and constitutively expressed in the majority of chronic and acute B cell malignancies. By the same token, the redirected cytotoxicity exerted by CART19 cells eliminates not only malignant B cells but also normal B cells in the recipient. This leads to hypogammaglobulinemia, requiring infusion of human Ig to restore normal Ig levels. Other toxicities/side effects were clinically manageable.

On the other hand, CAR T cells specific for antigens expressed only by malignant B cells would be preferable because they would spare the normal B cells and help maintain the B cell repertoire for immune surveillance. In line with this hypothesis, the clonotypic expression of surface Ig, and the restricted expression of ROR1 or CD23 by malignant B cells could serve as specific targets for CAR T cells. CAR T cells specific for kappa light chain were shown to specifically kill kappa+ Daudi cells in a murine xenograft model [101]. ROR1-specific CAR T cells derived from both autologous and allogenic PBMCs were able to induce cytotoxicity in ROR1-positive cell lines and primary CLL cells in vitro, but not in normal B cells and other blood cells that lack ROR1 [49••]. Likewise, CD23.CAR T cells induced cytotoxicity in CD23+ tumor cell lines in vitro and primary CLL cells in vivo in an adoptive transfer model while sparing normal B cells [102]. Further studies are required to determine whether CAR T cells reactive against tumor-specific antigens provide significant clinical benefit over the CAR T cells targeting pan B cell antigens.

Advances in CAR Construction

The generation of CAR T cells relies on expression vectors that stably integrate the CAR into T cell chromosomes. The viral promotor and regulatory sequences present to ensure optimal CAR expression could potentially cause genotoxicity in recipients. To eliminate this risk, a non-viral gene transfer strategy, the Sleeping Beauty (SB) transposon/transposase system, was used to generate CD19CAR T cells, and killing of CD19+ B cell leukemia and lymphoma cells was demonstrated [99]. In another non-viral gene transfer approach, primary T cells from healthy donors were electroporated with mRNA to express CD19 CAR without any genotoxicity [103]. A high throughput microelectroporation system has been developed for rapid generation of large numbers of CD19CAR T cells to meet clinical needs [104•].

The endogenous TCR present in CAR T cells can potentially initiate a strong allo response against host cells and tissue due to MHC disparity resulting in GVHD. To alleviate this potential problem, CD19-CAR T cells were anergized to alloantigens by coculturing with allogenic PBMC in the presence of anti-B7-1 and anti-B7-2 mAbs. Alloanergized CAR T cells lost allo reactivity, but retained their ability to recognize and kill CD19+ target malignant cells [105]. In another approach, the endogenous TCR expression in CAR T cells (CD19R28+) was irreversibly disrupted using zinc finger nucleases targeting the constant regions of TCR α or TCR β genes. As expected, the TCR^{neg}CAR+ T cells did not respond to TCR-mediated stimulation, but retained CD19 specificity and induced cytotoxicity in CD19 + leukemic cells [106••]. The high expansion capabilities and persistence in vivo of CAR T cells warrant a back-up mechanism to rapidly eliminate them when required, and this can be accomplished by including a variety of suicide genes under conditional regulation [107–109]. Overall, CAR T cells can be generated from one healthy donor and used as “off-the-self drug” for multiple patients regardless of their MHC type and without the risk of developing GVHD.

Animal Models

Natural and genetically engineered mouse models are valuable tools for in vivo evaluation of the B cell targeted and non-targeted therapies described above. The Tcl-1 mouse, over-expressing *Tcl1* oncogene, exhibit several features seen in human CLL patients [110–112] and serve as a faithful model to study not only the pathogenesis but also epigenetic and T cell modulations during the course of the disease progression and in vivo therapeutic evaluation. Interestingly, deregulated *Tcl1* has been identified to promote multiple classes of mature B cell lymphomas including Burkitt-like lymphoma and DLBCL [113–115]. Other animal models include: New Zealand Black (NZB) mice as a natural mouse model of CLL, (NZB x NZW) F1 mice engineered to express Interleukin-5, and mice over-expressing both BCL2 and a tumor necrosis factor receptor-associated factor, NOD/SCID mice, and the recently developed NOD/SCID/ γ_C^{null} and Rag2 $^{-/-}$ $\gamma_C^{-/-}$ xenograft models [116•, 117–122]. Introduction of human disease-specific target antigens such as ROR1 through transgenic and ‘knockin’ approaches will render these animal models amenable for evaluation of disease-specific targeted therapeutics. Given the multifactorial nature of the human B cell malignancies, these animal models present their individual suitability to different study designs and to investigate not only the biology of human B cell malignancies but also to evaluate in vivo efficacy of new therapeutics.

Conclusions

Currently available treatment options for human B cell malignancies include radiation, chemotherapy, and mAbs as single agent or in combination with chemotherapy. While these modalities have induced significant partial or complete responses in many patient cohorts, long-term remission is rare and disease relapse is still a major problem. Several novel strategies are under investigation, and this review describes three emerging antibody-based therapeutics: immunotoxin conjugates, immunoliposomal targeted drug delivery vehicles, and CAR T cells. All these agents selectively target and eliminate malignant B cells, and the results of preclinical studies and the ongoing clinical trials are very encouraging. The improvements of each of these agents as discussed above would further enhance their specificity, safety, and efficacy. Parallel developments in generating several relevant animal models have provided great opportunities to evaluate the novel antibody-based therapeutics and enable a faster transition to clinical application.

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Fig. 1. Schematics of mAb-based immunotherapeutics. Immunotoxin consisting the Fv domains of Ig light (*open*) and heavy (*solid red*) chains fused with the domains II and III (*yellow*) of *Pseudomonas aeruginosa* exotoxin A (dsFv-PE38), mAb-coated immunoliposome with encapsulated drug, and MHC-independent recognition of a tumor-associated antigen (TAA) on the malignant B cells (*red*) by CAR T cells expressing scFv/CD28/ 4-1BB/CD3 zeta chain receptor complex (*blue*) (a single representative interaction on the cell surface is depicted)