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REVIEW

Antibody prodrugs for cancer

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ABSTRACT

Introduction: The toxicity of potent new biological therapies for cancer has limited their utility. By improving tumor specificity, antibody prodrugs can widen or even create a therapeutic window for anticancer agents that are difficult or impossible to use otherwise because of poor tolerability.

Areas covered: This review will describe the current status of the field of antibody prodrugs, focusing on Probody™ therapeutics, including the principles behind their design, application to a variety of different antibody-based therapies, preclinical examples of their activity and safety, and early results of Phase 1 trials.

Expert opinion: Proof of concept for the antibody prodrug approach, which is defined as demonstration of potent antitumor activity with improved safety, has been extensively established preclinically as well as preliminarily in early clinical trials in human patients. However, experience with antibody prodrugs is limited, and important challenges remain. Principal among them are how to design the molecules to provide the most effective protection from toxicities while preserving efficacy, how to optimize clinical pharmacology, and how to determine which among the many possible clinical applications is the best use of this promising technology.

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1. Introduction

For most of the 80 years of modern drug discovery in oncology, the clear priority has been to identify compounds that are increasingly more effective in killing tumor cells. The last 2 decades have seen the advent of a variety of promising new biological therapies that can provide extraordinarily potent tumor cell killing, including immunotherapies, antibody-drug conjugates (ADCs), T cell-engaging bispecific antibodies (TCBs), and chimeric antigen receptor T cells (CAR-Ts). For example, some TCBs have in vitro half-maximal effective concentrations (EC_{50}) for tumor cell cytotoxicity reaching the sub-pM level and effective human doses that are in the microgram per kilogram range. However, in most cases high anti-tumor potency has come with high toxicity, which has limited the ability to use these drugs at their maximally effective doses, for the entire desired treatment period, or in the broad patient populations where they are needed. Arguably, then, a new priority is to find ways to widen the difference between the efficacious and toxic doses (the therapeutic window or index) for those potent approaches already identified, so that they can be more effectively used in patients.

The root cause of the narrow therapeutic index is insufficient tumor specificity. Monoclonal antibodies have been characterized as the 'magic bullets' Paul Ehrlich sought in the early 1900s, because of their exquisite ability to bind specifically to a single protein antigen. Oncology antibody-based drug development exploits this specificity to target tumor cells preferentially by creating antibodies to individual proteins that are expressed at high levels in tumors and at low levels in normal cells, the so-called 'tumor antigens.' However, cell-surface proteins that are absolutely tumor-specific and which can be found in a significant number of patients are rare. Accordingly, none of the marketed antibody therapeutics are tumor specific, and all can mediate off-tumor, on-target toxicities. The high potency of new biological therapies exacerbates this problem, and new methods for enhancing tumor targeting are needed.

One new strategy for more specific tumor targeting of biological therapies is the use of antibody prodrugs. Conventional prodrugs are pharmacologically inactive compounds that are converted into active forms in the body after administration. They can be designed to be activated at the intended site of action, thereby lowering exposure of normal tissues to active drug and minimizing toxicity. The idea of using prodrugs to improve tolerability or other drug properties originated a century ago, and today as many as 10% of all marketed small-molecule drugs can be considered to be some form of a prodrug. However, prodrug forms of biological therapies such as antibodies were not reported until about a decade ago [1–4], and were first reviewed in this journal in 2014 [5]. Since then, preclinical data and early human trials have shown efficacy with reduced toxicity for antibody prodrugs in oncology, establishing proof of concept for the approach. This review will cover the current status of development of the antibody prodrug concept, focusing on the most advanced of the antibody prodrug platforms, Probody therapeutics. Reported data on other approaches will also be reviewed.

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Article Highlights

- Antibody prodrugs are masked antibodies engineered to be pharmacologically inactive until they are activated by proteases in the diseased tissue microenvironment.
- A variety of different formats have been reported, but all are intended to widen the therapeutic window for potent therapies that are otherwise difficult to use because of poor tolerability, or to create a therapeutic window for undruggable targets.
- Preclinical proof of concept has been extensively reported, and early clinical trial results suggest that the technology performs as designed in human patients.
- There is potential for the technology to be useful in therapeutic areas beyond oncology, and for other protein therapeutics besides antibodies.
- Future work should focus on better understanding the optimal design and use of antibody prodrugs.

This box summarizes key points contained in the article.

2. Antibody prodrugs

Antibody prodrugs are designed to be administered to the patient in a form that does not effectively bind antigen and to maintain this inactive form as the drug circulates and encounters target in normal tissues. However, when the drug enters a tumor, it is converted to an active antibody that can interact with its target and do what it was designed to do, such as inhibiting a T cell checkpoint or other signaling pathway, delivering a cytotoxic payload, or recruiting activated T cells, but in this case specifically within the tumor rather than outside of it. In this way, antibody prodrugs are intended to widen the therapeutic index for drugs whose systemic toxicity limits clinical utility, or even create a therapeutic window where one previously did not exist.

The most common approach to creating antibody prodrugs are protease-activated antibodies that use antigen binding site 'masks', as exemplified by Probody therapeutics (Figure 1). The mask is typically a recombinant protein extension of the light and/or heavy chain of the antibody that has been designed to block access to the antigen binding site and physically prevent binding of the antibody to the cognate antigen. A protease substrate sequence is also inserted between the mask and the antibody. When the Probody therapeutic enters the tumor microenvironment, upregulated proteases that are common in cancer tissues cleave the substrate sequence, the mask separates from the antibody, and the antibody becomes competent to bind to its target in the tumor. This doesn't happen efficiently in normal tissues because there is insufficient extracellular protease activity to remove the mask.

This strategy relies on a fundamental difference in protease biology between normal tissues and tumors. Literature published over the last 40 years describes how tumors depend on upregulated protease activity to maintain the transformed phenotype [6,7]. Proteases are required for tumors to grow locally, invade tissue, extravasate, and colonize distant sites. Proteases are also important for the regulation of signaling by growth, differentiation, and cell adhesion factors. Accordingly, protease dysregulation is a virtually universal feature of cancer.

The opposite concept applies to normal tissues at homeostasis. There are more than 500 proteases in the human genome, which play a variety of physiologic roles [7,8]. These enzymes have evolved an elaborate and redundant control system to ensure that they remain inactive until they are needed [9,10]. Many proteases are expressed as inactive precursors called *zymogens*, which require additional posttranslational steps to be activated, primarily cleavage by other proteases. There are also highly abundant and potent protease inhibitors expressed in blood and in most tissues that control proteases after activation. A commonly cited example is the blood clotting cascade. All normal humans have

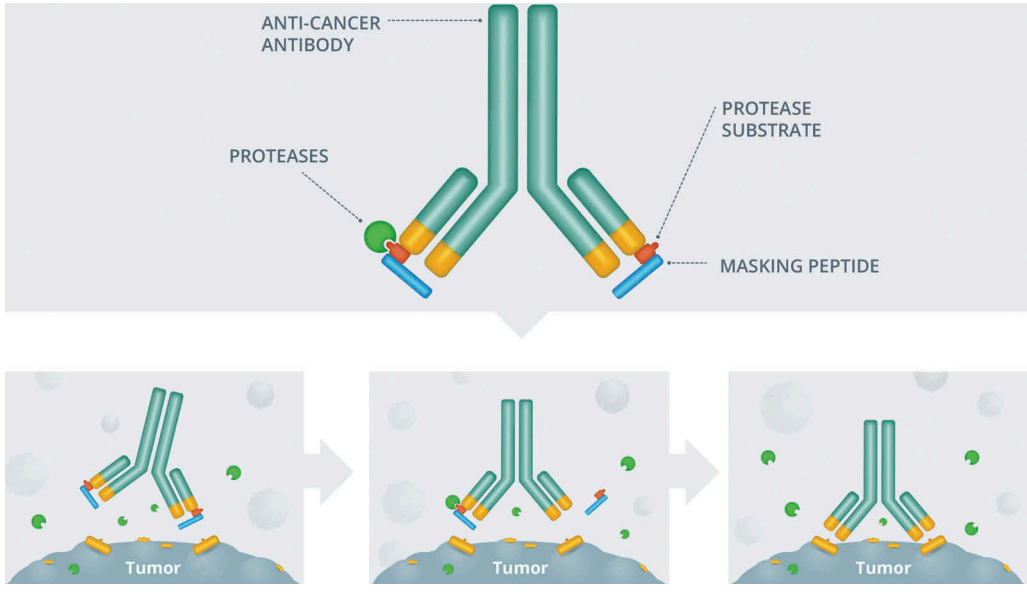

Figure 1. Design of a Probody therapeutic – a protease-activatable, masked antibody prodrug.

The mask (blue) may be a peptide, protein or other moiety that is either expressed as a recombinant extension of the antibody chain(s) (green) or is conjugated such that it blocks access of the cognate antigen to the antigen binding site of the antibody (yellow). The mask is attached to the antibody via a protease cleavable linker (red). Upon entering the tumor microenvironment (lower left), proteases cleave the linker (center), the mask falls away and the antibody binds to the tumor antigen (lower right).

multiple serine proteases circulating in their blood that, if they were inappropriately activated even to a small degree, would cause catastrophic systemic clotting [11]. That this does not happen demonstrates the robustness of protease control under normal circumstances and illustrates how protease-activated antibody prodrugs could potentially remain predominantly inactive outside of tumors.

2.1. Probody therapeutics

The most advanced antibody prodrugs in development are Probody therapeutics. Constructing a Probody therapeutic entails two steps. The first step is identification of an appropriate mask through a process known as affinity masking. A synthetic peptide library displayed on the surface of bacteria is screened with the antibody to be masked, in order to identify peptides that bind specifically to the complementarity-determining regions (CDRs) of that antibody [2,3,12]. The identified peptides, when recombinantly tethered to the amino-terminus of the light or heavy chain of the antibody, effectively compete with the cognate antigen for binding to the CDRs, resulting in a shift of the concentration-binding or concentration-function curve to the right in the absence of proteases (Figure 2). The degree of shift is referred to as masking efficiency, which can be tuned for optimal in vivo performance by selecting for peptides with varying degrees of affinity for the antibody CDRs. Treatment of the Probody therapeutic with the appropriate protease(s) restores function to that of the parental antibody (Figure 2).

The second step is identification of a suitable protease substrate. To accomplish this, the proteases activated in the tumor microenvironment need to be identified, as do specific substrate sequences that are cleaved efficiently by those proteases when used in the context of a masked antibody. The tumor biology of numerous candidate proteases has been described in detail in the literature [6,7], and substrate sequences that can be cleaved by some of these proteases have been reported, although most are not specific and/or have suboptimal cleavage kinetics for this purpose. For example, substrates for matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 are attractive candidates, because both their tumor biology and their substrate preferences are well described in the literature [13]. Substrates for tumor-associated serine proteases and other proteases have also been used [12].

A critical consideration is the extent to which the candidate proteases are active in normal tissues. As described above, even though a tumor typically has significantly more protease activity than a comparable normal tissue, normal tissues are not completely proteolytically silent in the extracellular space where antibody prodrugs reside. The importance of the protease system to physiological processes, the large number and size of normal tissues compared to that of the tumor mass, and the large number of proteases in the genome mean that there can be a low amount of background extracellular protease activities in normal organs at steady state, which when combined could potentially lead to unwanted prodrug activation over time. The ideal target proteases would therefore be those that are upregulated in tumors but are quiescent in normal tissues under most circumstances, including in non-malignant disease states that may coexist in cancer patients, such as wound healing, inflammatory disease, or infection. Very little has been reported on the extracellular protease

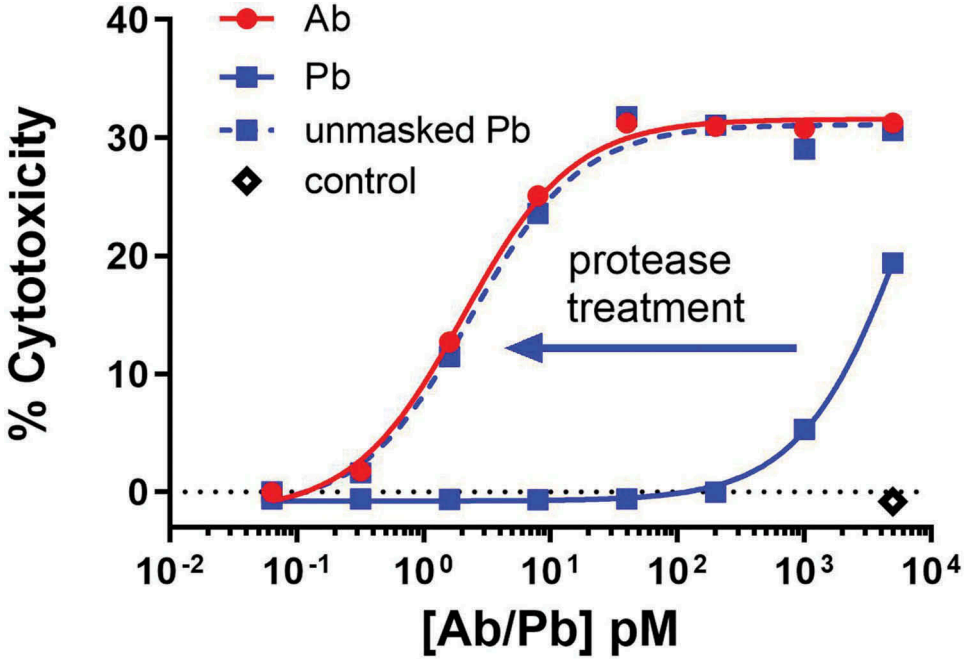

Figure 2. Probody therapeutic masking efficiency.

The mask of the Probody therapeutic inhibits binding such that the concentration-binding or concentration-function curve in the absence of protease is shifted to the right (Pb, blue curve) compared to the unmasked parental antibody (Ab, red curve). The degree of shift is called masking efficiency. Upon protease treatment and unmasking, the binding or function is restored to that of the unmasked parental antibody (dotted blue curve). Control represents a non-binding antibody control. Data depicted are derived from [26].

activity profiles of normal tissues, in part because the tools necessary to characterize them are not well developed. Protease mRNA or protein expression levels in organs are poor predictors for the presence of protease activity because of the robust posttranslational control of protease activity, as described above. A variety of zymographic methods have been developed to interrogate protease activity in biological samples [12,14], and reagent antibodies that specifically recognize the activated form of individual proteases have been developed [15,16]. However, it is still difficult to quantify multiple individual protease activities in tissues, and the complexity of the protease system means that identification of optimal substrates for antibody prodrug construction remains a significant challenge.

2.1.1. Preclinical examples of probody therapeutics

Probody therapeutics can be built in this way for virtually any antibody-based therapy, including naked antibodies such as immune checkpoint inhibitors and growth factor inhibitors, antibody-drug conjugates, bispecific and other multivalent antibody constructs, and even CAR-T cellular therapies built with single-chain antibodies. The published preclinical data and public disclosures for each of these are reviewed below.

2.1.1.1. Naked antibody-based probody therapeutics. The first antibody prodrug to be characterized in vivo was a Probody therapeutic reported by Erster et al. [2], combining an anti–vascular cell adhesion molecule 1 (anti-VCAM-1) antibody with an antigen-binding site mask identified by bacterial peptide display and a MMP protease substrate. The binding affinity of the Probody therapeutic was reduced approximately 200-fold by the tethered mask, which could be restored to that of the unmasked antibody parent by MMP-1 treatment. The Probody therapeutic was also specifically activated by tissue extracts from atherosclerotic mouse aortas where MMPs are upregulated, and when injected into apolipoprotein E (ApoE) knockout mice specifically accumulated in VCAM-1–expressing atherosclerotic lesions compared to the unmasked antibody.

Desnoyers et al. [12] constructed a Probody version of the anti-EGFR antibody cetuximab that displayed antitumor activity in mouse models similar to that of the unmasked parent antibody. The authors were the first to demonstrate that an antibody prodrug could widen the therapeutic index in animals. The pro-EGFR antibody had significantly reduced ability to bind to EGFR in human skin ex vivo and to induce rash, the principle on-target toxicity of cetuximab, in nonhuman primates. The reduced systemic toxicity was seen despite the fact that the molecule was potent in mouse tumor models.

Immunotherapy with antibodies that block the programmed death 1/programmed death ligand 1 (PD-1/PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) pathways (so-called immune checkpoint inhibitors) has revolutionized therapy of multiple types of cancers. These antibodies release the 'brakes' on T cell activation, which can result in robust antitumor immunity and durable remissions in some patients. However, systemic checkpoint blockade also can incite autoimmunity, because the controls of T cell activity are the same in normal tissues as they are in tumors. This is particularly problematic when attempting to use these inhibitors at their maximally effective doses and schedules, or in combination with other immunotherapies, where autoimmune toxicities are amplified. Antibody prodrug versions of immune checkpoint inhibitors could potentially induce antitumor immunity without incurring systemic autoimmunity, thus enabling the combination therapies that are needed to address patients that don't respond to individual agents when given as monotherapy.

We created a Probody version of a novel anti-PD-L1 antibody and tested it in mouse tumor and autoimmunity models [17]. At equal doses, the anti-PD-L1 Probody therapeutic and unmasked parent antibody induced the same degree of tumor growth delay in the MC38 mouse syngeneic tumor model. However, in contrast to the unmasked antibody, the Probody therapeutic bound less to circulating PD-L1-expressing T cells and did not induce autoimmune diabetes in the nonobese diabetic (NOD) mouse. Using the Zr^{89}-labeled anti-PD-L1 Probody therapeutic CX-072 and Immuno-PET imaging in mice, Giesen et al. demonstrated that the Probody therapeutic accumulated in tumors to the same degree as did the unmasked antibody but did not accumulate in PD-L1-expressing peripheral tissues [18]. Early human clinical trial data for CX-072 that demonstrate that the Probody therapeutic behaves similarly in humans as it does in mouse models are reviewed below. We have reported similar preclinical results for a Probody form of an anti-PD-1 antibody [19]. Compared to the unmasked parent antibody, Probody therapeutic versions of the anti-CTLA4 antibody ipilimumab have induced similar antitumor immunity in mice, fewer pharmacodynamic effects in peripheral tissues, and they have been less toxic in cynomolgus monkeys [20]. The ipilimumab Probody therapeutic BMS-986249 is currently being evaluated in a Phase 1/2 clinical trial.

2.1.1.2. Probody drug conjugates. The promise of antibody-drug conjugates (ADCs) has been to combine the potency of chemotherapy with the specificity of antibodies. However, as of this writing only 5 ADCs have been approved despite the hundreds under development over the past 20 years [21]. One major limitation of this approach has been the necessity to choose tumor antigen targets that are 1) expressed highly on the surface of tumor cells and are easily internalized, in order to drive uptake of the chemotherapeutic payload, but 2) are expressed at low levels or are absent on normal tissues, to avoid on-target, off-tumor toxicity. The number of targets that meet this definition is low, and most are not optimal either because of insufficient expression/internalization in tumor or too much expression on critical normal tissues. However, because antibody prodrugs are designed to avoid binding to target in normal tissues, ADC prodrugs should enable selection of the best possible tumor targets with the most desirable properties, without the limitation that they be also absent in normal tissue [22]. This should increase considerably both the number and quality of targets available, with the expectation that the resultant drugs will be more useful for treatment.

We developed a Probody Drug Conjugate (PDC) directed against CD166, an antigen that is highly expressed in many cancers and in normal tissue [23]. The PDC was efficacious in human xenograft models in the mouse and was well tolerated with extended exposure in nonhuman primates, consistent with avoiding significant binding to the normal tissue target sink. We also developed a PDC directed against CD71, the transferrin receptor, which has been an attractive but undruggable ADC target because of severe toxicity at low doses [24]. The PDC induced regressions in many different cell line-derived and patient-derived xenograft models in mice and protected against the leukopenia and severe toxicity that were induced by the unmasked ADC in nonhuman primates. Both the CD166 and CD71 PDCs are currently being studied in Phase 1 trials as described below.

2.1.1.3. T cell-engaging bispecific probody therapeutics.

T cell–engaging bispecific antibodies (TCBs) are particularly attractive molecules for application of the antibody prodrug strategy. TCBs typically contain 2 different binding specificities: one arm is designed to bind and activate T cells, generally by binding CD3, and the other binds to a tumor antigen. In this way TCBs can bring tumor cells and activated T cells together and take advantage of the potency and efficiency of T cell killing without requiring antigen presentation and the induction of an endogenous immune response. These molecules are both extremely potent and toxic, typically causing cytokine release syndrome (CRS), which limits dosing and requires schedule adjustments to manage. They are also unforgiving for even a small amount of target expression in normal tissues and therefore have been difficult to use on solid-tumor targets. Finally, because T cells are present in circulation and throughout the body, TCBs can suffer from rapid clearance and poor exposure due to target-mediated drug disposition (TMDD). Potentially all of these shortcomings could be addressed by using TCB prodrugs. Multiple different masking strategies have been reported, by either blocking CD3 binding or tumor antigen binding or both. By preventing binding except in the tumor microenvironment, CRS can be reduced, more broadly expressed solid tumor targets can be selected, and serum half-life can be greatly extended.

Previous studies had shown that an EGFR-CD3 bispecific was highly efficacious in mouse tumor models but was tolerated only at very low doses in nonhuman primate toxicity studies [25]. We produced a bispecific antibody based on an intact IgG, with 2 EGFR binding arms and 2 CD3 binding scFvs [26]. To create a Probody therapeutic, all four arms were masked with peptides connected via protease cleavable linkers. Cytotoxicity to tumor cells in culture in the absence of proteases was inhibited more than 300,000-fold by masking, but the potency of the original bispecific antibody was completely recovered after protease treatment. In mouse tumor models, the Probody bispecific induced regressions at doses as low as 0.3 mg/kg. In nonhuman primates, the safety window for CRS was extended approximately 60-fold and the tolerated exposure was markedly higher compared to the unmasked parental molecule.

2.1.2. Clinical trial experience with probody therapeutics

Four antibody prodrugs, all Probody therapeutics developed by CytomX Therapeutics, are being tested in human clinical trials as of this writing. The first and most advanced is CX-072, a Probody therapeutic directed against PD-L1, a T cell checkpoint target. To construct CX-072, a novel anti-PD-L1 antibody was recombinantly masked with a peptide connected to a protease substrate cleavable by multiple different proteases. Preclinical studies demonstrated preserved antitumor activity with improved ability to avoid induction of autoimmunity, as described above [17]. In Phase 1 human studies, CX-072 behaves as an antibody prodrug [27]. First, the drug circulates in blood in the predominantly masked form and does not show evidence of significant TMDD, demonstrating that the mask effectively prevents binding to the antigen sink. Further, the mask is removed and active CX-072 is generated within the tumor microenvironment as determined by biophysical analysis of the drug in on-treatment biopsies from patients' tumors. CD8-positive T cells expanded in patient tumors during treatment and correlated with antitumor response, consistent with the intended pharmacodynamic effect and expected mechanism of action of a PD-1 pathway inhibitor. More importantly, CX-072 demonstrated antitumor activity in a variety of different cancers. Durable clinical activity as monotherapy was observed in heavily pretreated patients, with a pattern of efficacy consistent with that of the PD-pathway inhibitor class in the cancer types treated [28]. Preliminary evidence of clinical activity was also observed in combination with ipilimumab [29]. The safety profile in both studies was improved relative to historical data sets generated with unmasked PD-1 pathway inhibitors [28,29]. These data provide preliminary proof of concept for the Probody technology in human patients. ADAs were detected by a sensitive assay, but the desired systemic exposure was achieved, and no clinically significant safety events related to the ADAs were observed.

CX-2009 is a Probody drug conjugate directed to CD166 and conjugated to the linker-cytotoxic payload SPDB-DM4. As described above, CX-2009 induces regressions in a variety of mouse tumor models and is well tolerated in nonhuman primates. CX-2009 is currently being studied in a Phase 1 clinical study in CD166-expressing cancers. CX-2009 has clinical antitumor activity against several different tumors, and its safety profile is consistent with the expected nonspecific, off-target toxicities mediated by disassociation of the DM4 payload from the antibody [30]. Importantly, the safety profile is not consistent with on-target toxicity to CD166-expressing normal tissues, suggesting again that the Probody design is performing as intended.

CX-2029 is a Probody drug conjugate directed against CD71, the transferrin receptor, and is in Phase 1 studies as of this writing. BMS 986249 is a Probody therapeutic version of the anti-CTLA4 antibody ipilimumab that is also currently

in Phase 1 studies. The preclinical studies of these molecules are described above; results from the clinical trials have not yet been reported.

2.2. Other approaches to generating antibody prodrugs

A variety of alternative antibody prodrug strategies to Probody therapeutics have been reported, and the more characterized of those with available information are summarized in Table 1. Masking, for example, can also be accomplished by attaching a bulky structure that blocks antigen binding by steric hindrance. Unlike the affinity masking strategy where a unique mask that binds the CDRs is identified for each antibody, a single steric mask can potentially be reused on many different antibodies. However, the degree of antigen blockade of steric masks is not easily tuned and varies in an unpredictable fashion from antibody to antibody, which limits the flexibility of this approach. Multiple steric masking strategies have been disclosed, including using amino acid polymers [31], coiled-coiled domains [32], albumin [33], various configurations of antibody fragments [34–39], and others [36]. Trang et al. developed masked versions of a variety of naked antibodies, including rituximab (anti-CD20), trastuzumab (anti-HER2), and the murine anti-CD3 antibody 145-C11, using coiled-coiled domains as steric masks [32]. The masked antibodies showed reduced binding to antigen on cells, which was restored with protease treatment. The masked CD3 antibody induced less cytokine release and had improved circulation half-life in mice compared to the parental antibody. In another example, the anti-EGFR antibody h528Fv was masked with the latency-associated peptide derived from TGF-β and an MMP substrate [40].

A third masking approach involves using the cognate antigens themselves, or derivatives thereof, as masks tethered to the antibody [1]. For example, Donaldson et al. reported using a domain of epidermal growth factor receptor (EGFR) tethered to single-chain variable fragments (scFvs) derived from the anti-EGFR antibodies cetuximab and matuzumab through a matrix metallopeptidase 9 (MMP-9)-cleavable linker [1]. Yang et al. [41,42] masked panitumumab with a peptide that mimicked a panitumumab epitope on EGFR and combined it with the same protease substrate sequence reported by Desnoyers et al. [12] While this approach ensures that the mask will be of high affinity and will effectively block binding to the target, the affinity of these masks is more difficult to optimize, and there is the potential of antidrug antibodies (ADAs) generated to the mask cross-reacting with the native ligand and causing unintended autoimmunity.

Perhaps the most extreme example of a potent biological therapy that suffers from on-target, off-tumor toxicity are CAR-T cells. CAR-T therapy involves replacing the function of the endogenous T cell receptor with a recombinant scFv fused to intracellular signaling domains that mediate T cell activation upon scFv binding to a tumor target. While CAR-T therapies are capable of dramatic anti-tumor efficacy in patients with hematological tumors, they have been challenging to use for solid tumors, in part because of the expression of solid tumor antigens on normal tissue. Han et al. generated a CAR-T containing an EGFR-directed scFv derived from cetuximab [43]. The scFv was masked with the same peptide and protease substrate used for the EGFR Probody therapeutic reported by CytomX Therapeutics previously [12]. The masked CAR-T cells showed reduced cytotoxicity toward cancer cells in vitro that could be recovered by protease treatment, and they induced tumor growth delay comparable to the unmasked CAR-T in a mouse tumor model. Safety studies were not reported.

3. Conclusion

Antibody prodrugs are a new class of antibody-based therapeutics that have the promise of unlocking the potential of potent biological therapies whose development have been hampered by on-target toxicity. By preferentially directing antitumor activity to the tumor microenvironment, antibody prodrugs have the potential to minimize serious toxicities, improve efficacy by allowing higher doses to be given more frequently, enable wider use of combination therapies that may be more effective for more patients, and broaden the target landscape to include better targets with more desirable features.

Extensive preclinical data and early data from human clinical trials, primarily with Probody therapeutics, are encouraging, but the development of these agents is at an early stage. The ability of antibody prodrugs to widen therapeutic index is critically dependent on details of their design, especially the choice of masking strategy and protease substrate, and the optimal approach to this is not yet known. All engineered antibodies have the potential to be immunogenic because of the presence of unnatural sequences, neoepitopes, or suboptimal biophysical properties, which in extreme cases could lead to loss of drug potency or hypersensitivity reactions. Another challenge is the regulatory approval pathway. While regulators have expressed an interest in the development of better-tolerated therapies, most cancer drugs to date have been approved for marketing on the basis of efficacy rather than improved safety. This may be less of an issue for prodrugs that enable a first-in-class drug directed to a novel target, or that enable a new combination of drugs that otherwise can't be used, than it is for a prodrug whose principal advantage is improved safety (e.g. an anti-EGFR antibody prodrug).

4. Expert opinion

Investigators have become adept at identifying very potent tumor cell-killing mechanisms, but relatively little attention has been given to how to apply these discoveries in ways that can maximize their therapeutic potential while optimizing their tolerability. The tolerability of modern anticancer therapies is an increasingly important clinical problem, especially in real-world community settings where experience with novel agents is limited and sophisticated monitoring and treatment of complications are not as readily available as they are in tertiary care centers. This view is supported by the high toxicity rates seen in early access programs [44].

Table 1. Summary of major characterized antibody prodrug strategies.

Technology		Format(s)	Masking Approach(es)	Protease Substrate (s)	Disclosed Target(s)	Comments	References
AbbVie*	Protease activated Dual Variable Domain (DVD)	Bispecifics	Steric (antibody domain)	MMPs, including MMP13	CTLA4, IL10, TNFα	Published data in non-Oncology indications	36, 37
Akrevia	Aklusion™ Switchblade™	Antibodies Cytokines	• Cognate epitope • Steric masks	MMP9	EGFR, CTLA4, cytokines	Theoretical risk of cognate epitope masks inducing autoimmunity	1
Amunix	protease-triggered immune activator (ProTIA)	Bispecifics	Steric (amino acid polymer)	Undisclosed	EGFR, HER2, EpCAM, cytokines	Nonspecific universal mask	31
CytomX	Probody™ Therapeutics	Antibodies, ADCs, Bispecifics, CARs, cytokines	Affinity masking	Multi-specific	PD-L1, PD-1, CTLA4, EGFR, CD-166, CD71	• Flexible for multiple formats • Tunable for optimal therapeutic index • Multi-specific protease substrates • Clinical POC for multiple programs • Customized mask for every antibody	3–5, 12, 17–20, 23–30
Harpoon	ProTriTac™	Bispecifics	Combined steric (albumin) + affinity mask	Undisclosed	EGFR	• Combined affinity and steric masking • Enhanced clearance upon activation	33
Maverick	Conditional Bispecific Redirected Activation (COBRA™)	Bispecifics	Protease-dependent assembly	MMP2, MMP9	EGFR	• Enhanced clearance upon activation • Requires in vivo assembly	34
Seattle Genetics	Protease activated Abs	Antibodies ADCs	Steric (coiled-coil domains)	MMP2 and 9	CD19, CD20, HER2, CD3, αvβ3	Nonspecific universal mask	32

POC, proof of concept; *CTLA4 in collaboration with UCSF.

The traditional approach to managing drug toxicity is to lower the dose level incrementally or lengthen the dosing interval in order to lower exposure and lessen side effects, with the goal of administering the most drug that can be tolerated. However, because this approach does not fundamentally change the therapeutic window, it usually also lowers efficacy. The prodrug approach is intended to change the relationship between efficacy and toxicity, allowing higher doses to be given more frequently and/or for longer durations, and allowing combination therapy that may be highly effective but poorly tolerated otherwise.

The flexibility of antibody prodrug technology allows application to virtually any antibody-based therapy, or potentially to non-antibody-based therapeutic proteins. While the current focus of antibody prodrugs is on oncology applications, the general problem of how to use potent therapies with narrow therapeutic indices effectively applies equally to many other diseases. Protease-activated antibody prodrugs can be envisioned in other disease states where protease activity is upregulated, such as cardiovascular, inflammatory, and respiratory diseases [8,45–47]. For example, masked versions of anti-tumor necrosis factor-alpha (TNFα) antibodies have been reported that could, in principle, reduce the risk of infections that occur with that therapy [40]. In such cases, the proteases involved may be distinct from those in oncology, requiring different substrate identification strategies, but the fundamental prodrug concept remains the same. Finally, while the focus of this review has been on protease-activated antibody prodrugs, there are many other proposals for increasing tumor specificity of therapeutics that might provide alternative, or complementary, approaches to protease-based strategies [48–53].

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