

Unmasking New Promises: Expanding the Antigen Landscape For Antibody–Drug Conjugates

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SUMMARY

Cross-reactivity with normal tissues is one of the key concerns for target selection for antibody–drug conjugates. Probody therapeutics are masked antibodies that can selectively be activated by proteases

in the tumor. CX-2029, is a first-in-class Probody targeting CD71 with preliminary efficacy and a tolerable safety profile.

See related article by Johnson *et al.*, p. 4521

In this issue of *Clinical Cancer Research*, Johnson and colleagues (1) report the dose-escalation results of the first-in-human phase I study of CX-2029, a novel Probody drug conjugate (PDC) targeting transferrin receptor 1 (TfR1/CD71). This work is the first to clinically validate CD71 as a new target for antibody–drug conjugates (ADC).

The promise of ADCs relies on the concept of a tumor-targeted antibody attached to a cytotoxic payload via a linker. Upon antigen binding, the ADC is internalized, the cleavage of the linker occurs and payload mediated cell death will take place. This treatment modality has the potential of improving the efficacy while limiting the toxicity of conventional chemotherapy, overall increasing the therapeutic index of conventional drugs. Despite several hundred ADCs having entered the clinic in the last decades, as of April 2021 only 10 have been approved by the FDA, five of them during the last 2 years, speaking of the high complexities of developing these agents. Multiple components must align to build a successful ADC: target antigen selection, the antibody construct, the linker stability, and the payload conjugation and potency. All of these areas are topic of important developments in the field (Fig. 1).

One important limitation so far has been antigen selection because, unlike in hematologic tumors, most solid tumor antigens are tumor associated rather than tumor specific and thus the delivery of the payload to normal tissues has limited the therapeutic index of the ADCs. An example of this was bivatuzumab, an ADC targeting CD44v6, a receptor abundantly present in head and neck squamous cell carcinomas, but also on normal keratinocytes, leading to fatal dermal toxicity during the phase I trial (2). Its noteworthy, that of the 10 ADCs approved so far, only four have been approved in solid tumors targeting exclusively three antigens, the well-established HER2 (trastuzumab emtansine and trastuzumab deruxtecan) and most recently Trop2 (sacituzumab govitecan) and Nectin4 (enfortumab vedotin). So developmental work to broaden the spectrum of antigens for ADCs are a clear need in the field.

Probody technology is a new approach to overcome the on-target off-tumor toxicity conundrum, exploiting the hallmark of protease activity dysregulation in tumors and restricting drug activity of the ADC to the tumor microenvironment (TME). A Probody therapeutic consists of a mAb, with a specifically customized masking peptide and a protease cleavable substrate linker. When the Probody reaches the TME, tumor-associated proteases cleave the linker, releasing the mask and allowing the antibody to bind to the target antigen. The technology can be applied to firstly reduce the side effects of certain mAbs such as immune checkpoint inhibitors or T-cell engagers, second to generate ADCs that are conditionally activated; expanding the possibility to target broadly shared antigens between tumor and healthy tissues and finally to increase ADC efficacy by avoiding antigen sinks in normal tissues.

The study published by Johnson and colleagues (1) represents proof of concept of this technology applied to patients and establishes the role of CD71 in cancer treatment. CD71 is a transmembrane glycoprotein that binds transferrin and is key in cellular iron uptake. It is highly expressed in normal proliferating cells and across several human cancers. Given its fundamental role in cancer cell pathology, high expression in the membrane and its efficient internalization, it is a very attractive target for cancer therapy. The field of antibodies targeting CD71 as direct anti-cancer drugs is also expanding and has recently been reviewed by Candelaria and colleagues (3). Interestingly, CD71 has been also previously used as a delivery system for gene therapy and Senzer and colleagues (4) conducted a phase I trial of SGT53, a p53-WT-cDNA-containing liposome antibody versus CD71, with good tolerability and preliminary activity of 73% disease stabilizations ($n = 11$), with no further results of phase II trials. However, due to the toxicity profile of unmasked ADC against CD71, no ADC had been successfully developed and CX-2029 is the first-in-class drug that reaches the clinic. Preclinical toxicology studies illustrate this perfectly; in cynomolgus monkeys CD71 PDC was tolerated up to 6 mg/kg, while in contrast the unmasked CD71 ADC was lethal at low doses (2 mg/kg), allowing for a human equivalent tolerable dose way below its projected biologically active dose.

In the trial, CX-2029 was administered in 45 patients across eight dose levels, from 0.1 to 5 mg/kg. Dose-limiting toxicity was observed in the 4 and 5 mg/kg cohorts. Recommended phase II dose was declared to be 3 mg/kg every 3 weeks. The most frequent grade 3/4 treatment-related adverse event (TRAE) was anemia (51%), followed by neutropenia (25%), with no TRAE leading to treatment discontinuation. Relevant antitumor activity was seen in 12 patients (3 partial responses and 9 prolonged stable diseases), mainly in tumors with squamous histology (1). The dose-escalation portion of this study provides preliminary evidence of the tolerability and clinical activity of CX-2029 monotherapy, expansion cohorts are currently ongoing. Work to further understand the underlying mechanism of the anemia as well

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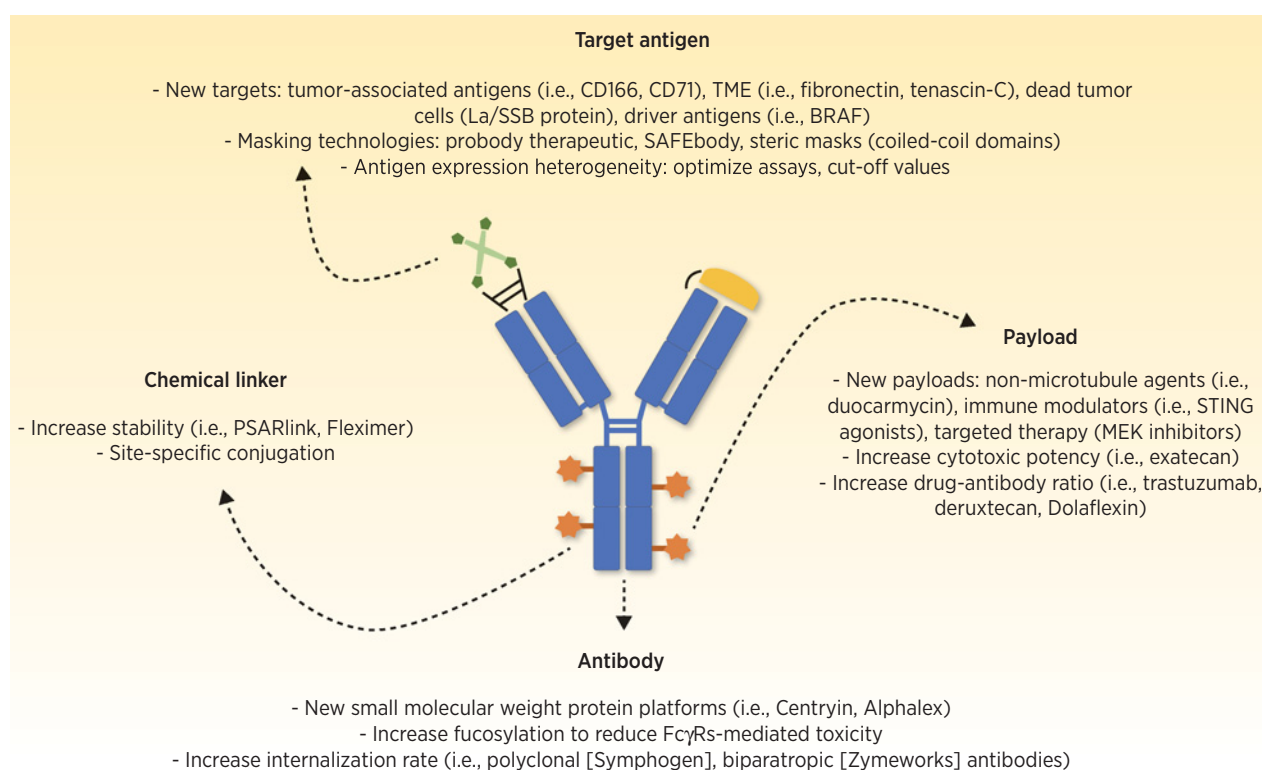


Figure 1.
Building the next generation of ADCs.

as the clinical management of this adverse event will be essential for the future drug development of this compound.

It is important to mention alternative strategies to generate antibody prodrugs are under investigation. For example, antibodies with an heterodimeric coiled-coil domain that sterically occlude the complementarity-determining regions and are also unmasked upon exposure to proteases are being developed. This could potentially allow for a more generalizable approach, as the same mask could be applied to different antibodies (5).

On the other hand, in the search for novel targets for ADCs, other strategies beyond targeting antigens expressed exclusively on the surface of tumors are being tested. Antigens of the TME (6), such as LRRC15, splice isoforms of fibronectin, and tenascin-C and even apoptotic cell death antigens are being evaluated. The principle underlying this new approach is based on the fact that the payload can be released extracellularly, where it diffuses inside the tumor cells provoking their death (bystander effect). Moreover, new payloads with immunomodulating agents (i.e. STING; TLR7/8), are particularly interesting in this setting given their cell trafficking capacity and potential to transform immunologic deserts into inflamed tumors.

In conclusion, decades of research have led to the recent approval of several ADCs with impressive and sustained therapeutic responses in patients. In this article, we have highlighted areas of research regarding target selection, and how unmasking strategies can increase the number of antigens for ADCs; however, it is essential to emphasize that having a good target will not necessarily ensure success (several antiHER2 ADCs have in fact failed in the clinic). Research to optimize

the different components of ADCs is ongoing and it is easy to imagine how in the near future ADCs will have a forefront role in cancer treatment.

Authors' Disclosures

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References

1. Johnson M, El-Khoueiry A, Hafez N, Lakhani N, Mamdani H, Rodon J, et al. Phase 1, first-in-human study of the probody therapeutic CX-2029 in adults with advanced solid tumor malignancies. *Clin Cancer Res* 2021;27:4521–30.
2. Tijink BM, Buter J, de Bree R, Giaccone G, Lang MS, Staab A, et al. A phase I dose escalation study with anti-CD44v6 bivatuzumab mertansine in patients with incurable squamous cell carcinoma of the head and neck or esophagus. *Clin Cancer Res* 2006;12:6064–72.
3. Candelaria PV, Leoh LS, Penichet ML, Daniels-Wells TR. Antibodies targeting the transferrin receptor 1 (TfR1) as direct anti-cancer agents. *Front Immunol* 2021;12:607692.
4. Senzer N, Nemunaitis J, Nemunaitis D, Bedell C, Edelman G, et al. Results of a phase I trial of SGT-53: a systemically administered, tumor-targeting immunoliposome nanocomplex incorporating a plasmid encoding wtp53. *Mol Ther* 2012;20:S21.
5. Trang VH, Zhang X, Yumul RC, Zeng W, Stone IJ, Wo SW, et al. A coiled-coil masking domain for selective activation of therapeutic antibodies. *Nat Biotechnol* 2019;37:761–5.
6. Dal Corso A, Cazzamalli S, Neri D. Antibody-drug conjugates: targeting the tumor microenvironment. In: Damelin M, editors. *Innovations for Next-Generation Antibody-Drug Conjugates*. Cancer Drug Discovery and Development. Cham: Humana Press; 2018. p. 299–319.