Improving Tumor Penetration of Antibodies and Antibody–Drug Conjugates: Taking Away the Barriers for Trojan Horses

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The high affinity of an antibody can result in restricted tumor penetration and heterogenous tumor distribution, with preferential binding of the antibody to tumor cells localized around tumor vasculature. This so-called "binding site barrier" effect limits the efficacy of antibody-based therapies like antibody–drug conjugates (ADC). In this issue, Bordeau and colleagues introduce an original approach to overcome this barrier through transient competitive inhibition of antibody–antigen binding. By coadministration of an anti-idiotypic anti-trastuzumab domain antibody as a competitive inhibitor, increased tumor penetration of trastuzumab as well as enhanced efficacy of the ADC ado-trastuzumab emtansine were observed in tumor-bearing mice

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Antibody-based therapy has become an integral component of cancer treatment. Several strategies have built on the initial clinical success of mAbs. Antibody–drug conjugates (ADC) are "Trojan horses" of medicinal chemistry and are among the fastest-growing classes of oncology therapeutics. To date, 10 ADCs have been approved by the FDA, and more than 80 ADCs are currently in active clinical evaluation. ADCs comprise three components: a disease selective mAb, a small-molecule therapeutic payload, and a linker that connects mAb and payload to form a conjugate. The ADC design aims to selectively deliver the payload to each tumor cell by means of the antibody, creating high efficacy combined with low toxicity, thus resulting in a wide therapeutic window. First-generation ADCs were developed after the initial clinical successes with unconjugated mAbs and comprised classical chemotherapeutic compounds such as doxorubicin as the payload. However, these conjugates showed limited therapeutic efficacy, likely due to low potency of the payload, which particularly is an issue in the case of low-level target expression. Second-generation ADCs were therefore equipped with extremely potent payloads, such as auristatins, maytansinoids, and calicheamicins. Typically, these payloads are so potent that their narrow therapeutic window prohibits their use as free drugs. The FDA approval of second-generation ADCs confirmed the clinical potential of ADCs.

Despite the clinical successes and the continuous pursuit for technical improvements, several ADCs failed very recently due to unforeseen toxicities (1). The balance between ADC potency and safety appears to be critical. Many biotech companies have put efforts in maximizing the therapeutic window by further increasing the potency of their ADCs. To this end, a trend was set to move from superpotent (usually microtubule targeting) drugs toward hyperpotent (usually DNA or RNA targeting) drugs such as pyrrolobenzodiazepines, indolinobenzodiazepines, and amanitins. Another approach has been to increase the number of drugs delivered by a single antibody by increasing the drug-to-antibody ratio (DAR). The MTD levels of these hyperpotent ADCs appear to be generally low. Administering ADCs at low MTD may make it impossible to appropriately saturate large tumor masses, leading to the effective killing of only a small fraction of cancer cells and undertreatment of the other cells.

It is obvious that the characteristics of the target antigen as well as of the antibody are of key importance for the appropriate tumor-selective delivery of mAbs and ADCs and for avoiding toxic effects in normal tissues. The suitability of a target antigen depends on its tumor specificity, absolute level and homogeneity of expression, accessibility, and internalization potential. In addition, the dose of the mAb or ADC, its size, and its affinity for the target antigen are important parameters for enabling homogenous tumor targeting and effective therapy. It is well-known from early clinical studies 20 to 30 years ago with intact murine mAbs, for which IHC assessment of distribution throughout the tumor was easy, that tumor penetration of high-affinity mAbs is limited, leading to suboptimal and heterogenous tumor exposure (2). This phenomenon has been explained by the binding site barrier (BSB) hypothesis.

In this issue, Bordeau and colleagues report on an original approach to overcome the BSB through transient competitive inhibition of mAb–antigen binding (3). They illustrated their approach for targeting HER2-positive tumors in mice using trastuzumab and the ADC trastuzumab emtansine (T-DM1), both directed against HER2. For competitive inhibition, they used an anti-idiotypic anti-trastuzumab camelid single-domain antibody (VHH; nanobody), designated 1HE. They hypothesized that transient competitive inhibition of mAb/ADC antigen binding would increase the tumor penetration and improve homogeneity of mAb/ADC distribution within solid tumors. A set of *in vivo* experiments indicated that this approach might have translational potential: (i) When administered alone, 1HE was rapidly eliminated from plasma; (ii) 1HE coadministration did not alter the plasma pharmacokinetics of radiolabeled trastuzumab or T-DM1; (iii) when coadministered with trastuzumab, elimination of radiolabeled 1HE was dramatically reduced, consistent with trastuzumab binding *in vivo*; (iv) 1HE coadministration significantly increased trastuzumab penetration into tumors; and (v) 1HE coadministration improved T-DM1 efficacy. On the basis of these results, the authors envision that the strategy may be applied to a wide range of high-affinity anticancer

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antibody therapies that are on the market and in current development, potentially providing a clinically feasible approach to enhance the efficacy of targeted therapies directed against solid tumors.

While the reported transient competitive inhibition approach is an elegant concept, two aspects warrant further discussions. A first topic of discussion is whether the transient competitive inhibition strategy at its current stage of development is attractive and suitable for clinical application. A practical limitation of this approach is that for each mAb or ADC, a second pharmaceutical has to be preclinically and clinically developed. In addition, further validation of the concept seems crucial. The authors commented that mild precipitation was observed when 1HE and T-DM1 were combined in a single solution, therefore, 1HE was administered to mice at a 10-fold molar excess to T-DM1, 15 minutes after T-DM1 administration, while trastuzumab was coadministered with 1HE at a 2-fold molar excess. This indicates that transient competitive inhibition by anti-idiotypic domain antibodies needs to be controlled very well with respect to affinity, dose, and timing, otherwise, unwanted effects might occur such as reduced tumor uptake and/or sequestration in normal organs. To further establish the utility of competitive inhibition, it will be crucial to perform more extensive and accurate quantitative biodistribution/PET imaging studies with stably radiolabeled mAbs or ADCs in a variety of tumor models, as done preclinically as well as clinically with ^{89}Zr-immuno-PET (4). With respect to efficacy studies, it would be interesting to learn whether the competitive inhibition strategy is capable of widening the therapeutic window of ADCs.

A second topic of discussion is whether there are alternative approaches to overcome the BSB. Several key aspects of the BSB and antibody binding have been exploited to improve tumor targeting. First, the BSB effect is related to antibody affinity. Although there seems to be a sweet spot for intermediate-affinity mAbs, combining high tumor uptake with homogenous tumor targeting, it is challenging to define that sweet spot for each antibody–antigen combination. Second, tumor targeting becomes more homogenous at higher antibody doses (2). This strategy was elegantly explored by Cillier and colleagues (5). Using T-DM1 for therapy in tumor-bearing mice, they showed that tumor distribution and anticancer efficacy can be improved by coadministration of unconjugated trastuzumab. More recently, the same approach was exploited clinically, revealing improved tumor penetration of the anti-EGFR conjugate panitumumab–IRDye800CW when coadministered with unconjugated panitumumab (6). As an alternative to coadministration of an unconjugated mAb, the use of a conjugate with a lower DAR might be considered, which could allow a higher MTD that approaches the dose that normally is used for therapy with the parental antibody. Third, lower molecular weight antibody constructs are capable for improved tumor penetration as illustrated by Tijink and colleagues (7). They used a bivalent nanobody construct directed against EGFR, which also contained a nanobody unit directed against albumin for plasma lifetime extension. The biodistribution of this 50 kDa αEGFR–αEGFR–αAlb construct was, after radiolabeling, compared with the biodistribution of radiolabeled cetuximab (∼150 kDa). While the overall biodistribution was very similar for both constructs, the tumor distribution of the αEGFR–αEGFR–αAlb construct was much more homogenous than that of cetuximab. Inspired by these observations, a single domain–drug conjugate directed against prostate-specific membrane antigen (PSMA) as well as a nanobody–drug conjugate directed against HER2, both lifetime extended by albumin-binding units, have been presented very recently (8, 9). Both studies show homogenous tumor targeting of the construct combined with remarkable antitumor effects. Fourth, the concept of Probody therapeutics can be considered for homogenous tumor targeting, which is analogous to what Bordeau and colleagues propose. Probody therapeutics are mAb prodrugs in which the antigen-binding sites are "masked" by a covalently linked peptide that contains a protease recognition sequence. Therefore, they easily penetrate into tumors, without encountering BSB effects. Once inside the tumor, Probody therapeutics become activated, or "demasked," by proteases present in the tumor microenvironment, allowing the mAb to bind to the specific antigen. In addition to improved tumor distribution, this approach might also result in improved tumor selectivity, as enzymatic activation of the prodrug will not occur in healthy normal organs irrespective antigen expression. The capability of Probody therapeutics and Probody drug conjugates for efficient and homogenous tumor targeting has recently been demonstrated (10).

In conclusion, Bordeau and colleagues propose a very interesting solution using transient competitive inhibition to overcome the BSB effect that limits the efficacy of mAbs and ADCs. Their promising results warrant more extensive validation of the concept and comparison with other concepts under development to overcome the BSB effect.

Author's Disclosures

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