Review

Clinical Cancer Research

Antibody–Drug Conjugates: Future Directions in Clinical and Translational Strategies to Improve the Therapeutic Index

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Abstract

Since the first approval of gemtuzumab ozogamicin (Mylotarg; Pfizer; CD33 targeted), two additional antibody–drug conjugates (ADC), brentuximab vedotin (Adcetris; Seattle Genetics, Inc.; CD30 targeted) and inotuzumab ozogamicin (Besponsa; Pfizer; CD22 targeted), have been approved for hematologic cancers and 1 ADC, trastuzumab emtansine (Kadcyla; Genentech; HER2 targeted), has been approved to treat breast cancer. Despite a clear clinical benefit being demonstrated for all 4 approved ADCs, the toxicity profiles are comparable with those of standard-of-care chemotherapeutics, with dose-limiting toxicities associated with the mechanism of activity of the cytotoxic warhead. However, the enthusiasm to develop ADCs has not been dampened; approximately 80 ADCs are in clinical development in nearly 600 clinical trials, and 2 to 3 novel ADCs are likely to be approved within the next few years. While the promise of a more targeted chemotherapy with less toxicity has not yet been realized with ADCs, improvements in technology combined with a wealth of clinical data are helping to shape the future development of ADCs. In this review, we discuss the clinical and translational strategies associated with improving the therapeutic index for ADCs.

Introduction

Antibody–drug conjugates (ADC) were initially designed to leverage the exquisite specificity of antibodies to deliver targeted potent chemotherapeutic agents with the intention of improving the therapeutic index (the ratio between the toxic dose and the dose at which the drug becomes effective; Fig. 1; refs. 1, 2). Unfortunately, the greatest challenge to date for developing ADCs is a therapeutic index far narrower than expected (3–5). Of approximately 55 traditional ADCs for which clinical development has been halted, we estimate that at least 23 have been discontinued due to a poor therapeutic index; however, this is likely a conservative estimate based on the availability of clinical data. A narrow therapeutic window limits the dose that can be achieved, often resulting in toxic effects occurring before an ADC reaches its maximally efficacious dose. Furthermore, these toxicities limit the number of dosing cycles that patients can tolerate and often result in skipped doses, dose reductions, or study discontinuations (6, 7).

In this review, we discuss clinical and translational strategies to improve the therapeutic index of ADCs that are based on the latest clinical efficacy and safety data with next-generation antibodies and warheads currently in development. While technology plays a crucial role in expanding the therapeutic index of ADCs, we refer readers to several excellent reviews that cover novel advancements in antibody, linker, and warhead technologies in significant depth (2, 3, 8, 9).

Overview of ADCs in Clinical Development

Four ADCs have been approved over the last 20 years (Fig. 2A; ref. 2). The first ADC approved for clinical use was gemtuzumab ozogamicin (Mylotarg; Pfizer; CD33 targeted) for relapsed acute myeloid leukemia in 2000 (10). In 2010, gemtuzumab ozogamicin was withdrawn from the U.S. market when a confirmatory trial showed that it was associated with a greater rate of fatal toxicities versus standard-of-care chemotherapy (5.8% vs. 0.8%; refs. 10, 11). In 2017, gemtuzumab ozogamicin was reapproved for relapsed/refractory acute myeloid leukemia after a phase III trial with a fractionated dosing schedule lowered the peak serum concentration and improved the safety profile, with a complete response rate of 26% (12). These clinical data demonstrate the importance of understanding the relationship between the exposure, safety, and efficacy of ADCs in clinical development.

Other ADCs that have been approved are brentuximab vedotin (Adcetris; Seattle Genetics, Inc.; CD30 targeted; ref. 13) and inotuzumab ozogamicin (Besponsa; Pfizer; CD22 targeted; ref. 14), which were approved for hematologic malignancies, and trastuzumab emtansine (Kadcyla; Genentech; HER2 targeted), which was approved for breast cancer (15). Across phase II and III studies, response rates were significantly higher in patients treated with ADCs than in those treated with standard intensive chemotherapy (14, 16–18).

Clear clinical benefits have been demonstrated with all 4 approved ADCs; however, each has reported toxicity profiles that are specific to its cytotoxic warhead and, therefore, they cannot be differentiated from standard-of-care chemotherapies (13–15) in terms of safety. Regardless of the obstacles, there is intense interest in developing ADCs—approximately 80 ADC candidates are

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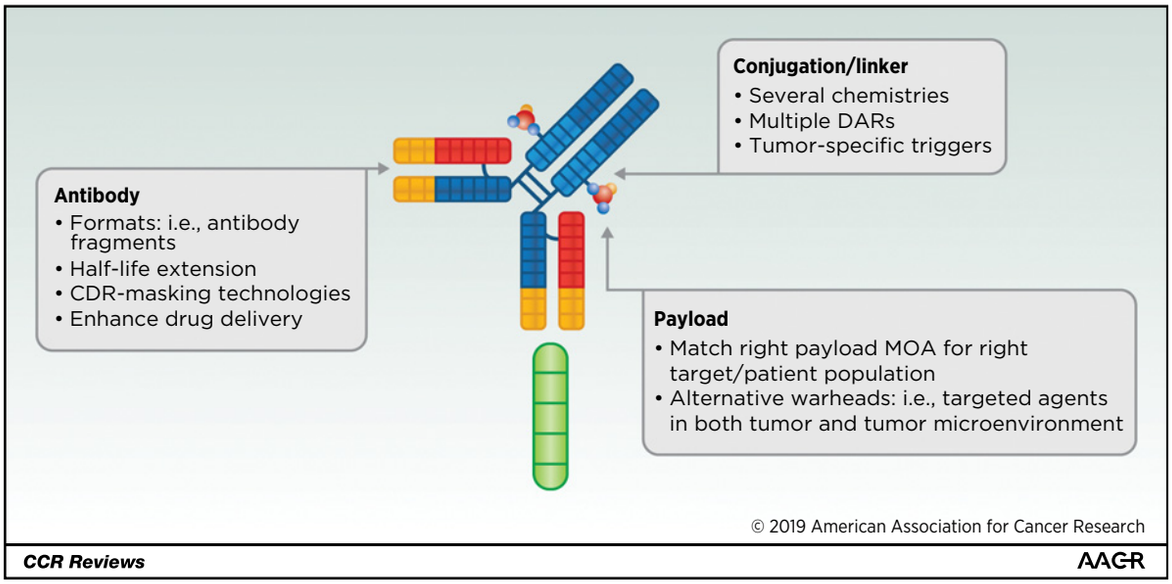

Figure 1.

ADC structure and therapeutic index optimization strategies. ADCs comprised a tumor-specific antibody, a linker, and a cytotoxic payload. Advances in chemistry of all three components are underway to potentially increase the therapeutic index. CDR, complement-determining region; DAR, drug-antibody ratio; MOA, mechanism of action.

reportedly in clinical development, with nearly 600 clinical trials ongoing—and it is likely that several new ADCs will be approved over the next few years (Fig. 2A; ref. 19) led by the recent Biologics License Application filing for polatuzumab vedotin (CD79b targeted) in relapsed/refractory diffuse large B-cell lymphoma. Although ADCs have not yet delivered on the promise of a more-targeted chemotherapy with an improved toxicity profile, new strategies may prove crucial to improving the therapeutic index of ADCs (4, 20, 21). These strategies include the use of warheads with lower potencies and alternative mechanisms of activity as described below.

Two examples of ADCs in clinical development that use warheads that inhibit topoisomerase I activity include trastuzumab deruxtecan targeting HER2 in breast and gastric cancers and sacituzumab govitecan targeting Trop2 in breast and lung cancers (22, 23). A Biologics License Application has been filed for sacituzumab govitecan for metastatic triple-negative breast cancer, and trastuzumab deruxtecan is currently in multiple late-stage pivotal clinical trials. The clinical data for trastuzumab deruxtecan from an ongoing phase I study in HER2-high metastatic breast cancer (post trastuzumab emtansine) showed an objective response rate (ORR) of 55% with median progression-free survival not reached (Table 1). Updated recent data have shown a median duration of response of 20.7 months, which compares favorably with trastuzumab emtansine, which, in a pivotal study in HER2-high metastatic breast cancer, showed an ORR of 43.6%, a median progression-free survival of 9.6 months, and a median duration of response of 12.6 months (22). In a phase I trial in third-line triple-negative breast cancer, sacituzumab govitecan demonstrated an ORR of 31% and a median progression-free survival of 5.5 months (Table 1). In this trial, sacituzumab govitecan was dosed at 10 mg/kg on days 1 and 8 every 21 days and showed improved tolerability compared with other ADCs targeting Trop2 such as PF-06664178, which had a MTD of 2.4 mg/kg, showed limited efficacy, and was terminated due to high toxicity (23).

The results from this phase I trial with sacituzumab govitecan provide an example of the importance of matching the right drug to the right target for the right patient. Even when comparing ADCs that use the same antibody against the same target in a similar patient population, trastuzumab emtansine and trastuzumab deruxtecan have demonstrated clinical activity, whereas a trastuzumab tesirine conjugate (ADCT-502) was recently discontinued due to a narrow therapeutic index (24). HER2 is known to be expressed in several normal tissues such as in the lung and the gastrointestinal tract (25). This creates two potential problems for an ADC. First, the normal expression of the antigen creates a sink for the ADC that must be overcome to maximize exposure to the tumor (26, 27). Given the high potency of the tesirine payload, doses sufficient to overcome the HER2 normal tissue sink might not be achievable. Second, the normal expression of the antigen can result in on-target toxicity. In the case of trastuzumab tesirine, pulmonary edema, a known toxicity of pyrrolobenzodiazepines (28), may have been exacerbated by the expression of HER2 in lung tissues. While general characteristics of an ADC target, such as tumor-to-normal expression ratios and internalization kinetics, may be considered, both the HER2 and Trop2 examples provide evidence that achieving clinical success with an ADC may depend on matching the technology and the target.

The non–target-mediated uptake of the cytotoxic drug into normal tissues remains a challenge with ADCs, thus limiting their therapeutic index. Although the immunoglobulin G (IgG) portion of the ADC is important for maintaining a long half-life, binding to target, and internalizing drug into tumor cells, its large size presents a physical barrier to efficient extravasation across blood vessel walls and diffusion through tumors (29). This has prompted a significant effort to explore alternative formats to traditional IgGs, including antibody fragments,

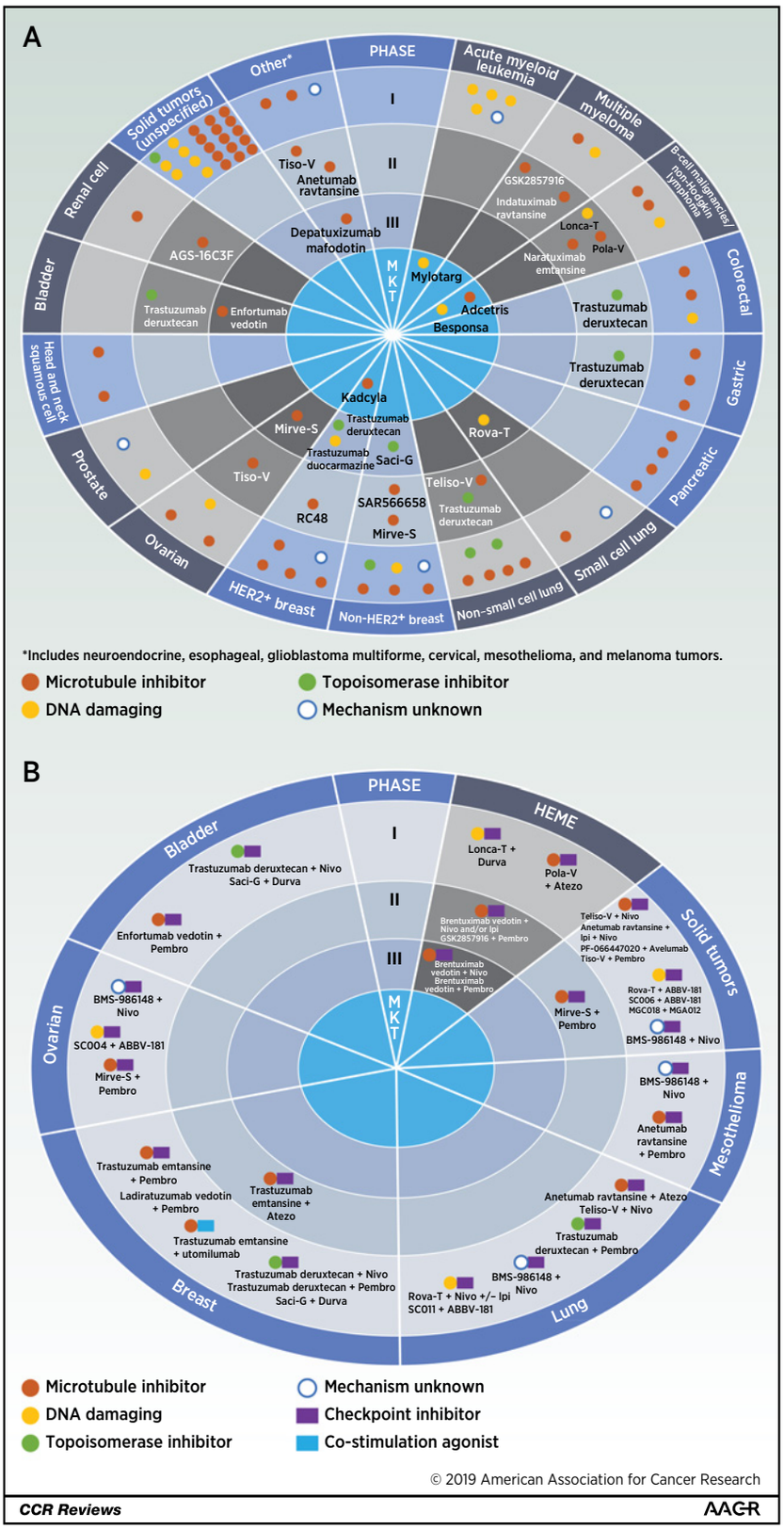

Figure 2.

ADCs in clinical development. **A,** ADCs in clinical development as of March 2019 shown by phase of development, indication, and warhead according to clinicaltrials.gov. **B,** ADCs in combination with checkpoint inhibitors in clinical development, shown by phase of development, indication, and warhead employed. Mylotarg (gemtuzumab ozogamicin) and Besponsa (inotuzumab ozogamicin) are manufactured by Pfizer; Adcetris (brentuximab vedotin) is manufactured by Seattle Genetics, Inc.; and Kadcyla (trastuzumab emtansine) is manufactured by Genentech. Atezo, atezolizumab; Durva, durvalumab; HEME, hematologic malignancies; Ipi, ipilimumab; Lonca-T, loncastuximab tesirine; Mirve-S, mirvetuximab soravtansine; MKT, marketed; Nivo, nivolumab; Pembro, pembrolizumab; Pola-V, polatuzumab vedotin; Rova-T, rovalpituzumab tesirine; Saci-G, sacituzumab govitecan; Teliso-V, telisotuzumab vedotin; Tiso-V, tisotumab vedotin.

Table 1. Topoisomerase I–targeted warheads demonstrate robust clinical efficacy

ADC	Target/warhead	Population	ORR (%)	DCR (%)	DOR (months)	PFS (months)
Sacituzumab govitecan (77–79) *Immunomedics*	TROP-2/SN-38 irinotecan metabolite (topoisomerase)	≥ 3L TNBC ($n = 110$)	31 (6 CRs)	46	7.6	5.5
		≥ 2L HR^+ BC ($n = 54$)	31 (0 CRs)	63	7.4	6.8
		≥ 2L UC ($n = 41$)	34 (2 CRs)	49	13	7.1
Trastuzumab deruxtecan (80) (DS-8201a) *Daiichi Sankyo*	HER2/exetecan topoisomerase inhibitor	≥ 3L HER2-high BC ($n = 111$)	55	94	Not reached	Not reached
		≥ 2L HER2-low BC ($n = 34$)	50	85	11	13
		≥ 3L $HER2^+$ gastric ($n = 44$)	43	80	7.0	5.6
		≥ 3L $HER2^+$ others ($n = 51$; CRC, NSCLC +)	39	84	13	12
U3-1402 (81) *Daiichi Sankyo*	HER3/exetecan	≥ 3L $HER3^+$ BC ($n = 32$)	47	94	Not reported	Not reported

Abbreviations: 2L, second line; 3L, third line; BC, breast cancer; CR, complete response; CRC, colorectal cancer; DCR, disease control rate; DOR, duration of response; HR, hormone receptor; NSCLC, non-small cell lung cancer; PFS, progression-free survival; TNBC, triple-negative breast cancer; UC, urothelial cancer.

alternative scaffolds, natural ligands, and small molecules (30). Three drug conjugates using smaller targeting domains have now entered the clinic. PEN-221 is a Pentarin (Tarveda Therapeutics) peptide targeting the somatostatin receptor 2 conjugated to DM1 (clinicaltrials.gov identifier: NCT02936323). PEN-866 is a small-molecule HSP90-binding ligand conjugated to SN38 (ref. 31; clinicaltrials.gov identifier: NCT03221400). BT-1718 is a bicyclic peptide targeting matrix metalloprotease 14 and is conjugated to DM1 (refs. 32, 33; clinicaltrials.gov identifier: NCT03486730). Although small formats have been shown to extravasate and diffuse through tissue faster than full-length IgG, the longer half-life of an IgG allows for greater absolute drug accumulation into tumors over time (34, 35). However, the faster clearance may improve the therapeutic index because the biodistribution is fundamentally changed, thereby altering normal tissue exposure to both intact conjugate and released drug. It remains to be seen whether these technologies will offer any improvement in the clinical therapeutic index.

Emerging Clinical and Translational Approaches

Maximizing the therapeutic index through clinical and translational strategies is central to the future success of ADCs. There are several approaches that may be considered, including but not limited to alteration of dosing regimen and use of biomarkers to optimize patient selection, capture response signals early, and inform potential combination therapies. These approaches are central to maximizing the therapeutic index and providing a personalized approach to ADC therapeutic development.

Clinical dosing schedule

One approach to overcoming a narrow therapeutic index involves changing dosing schedules through fractionated dosing. A fractionated dosing schedule may help maintain or increase dose intensity—which is considered a major driver of anticancer activity—while reducing the peak concentration. This approach has the potential to reduce the maximum serum concentration–driven toxicities and prolong exposure, thereby ensuring that a greater number of cancer cells enter the cell cycle and are exposed to drug. This has proven effective in traditional chemotherapeutics, such as in adjuvant breast cancer (36, 37). Furthermore, the success of fractionated dosing schedules with gemtuzumab ozogamicin or inotuzumab ozogamicin suggests that the same approach can be used with other ADCs. Indeed, a preclinical study of ADCs with pyrrolobenzodiazepine (PBD) warheads demonstrated that the *in vivo* efficacy and area under the concentration curve were similar regardless of whether the ADC was delivered as a single dose or as fractionated weekly doses, but that fractionated dosing reduced the plasma concentration of the drug and therefore reduced maximum serum concentration–driven toxicities (38).

Biodistribution studies

Biodistribution studies can help define target density beyond tumor cells and have the potential to inform target-mediated and nontarget-mediated toxicity. Biodistribution studies in humans based on imaging analysis may be required, because target expression in animal models may not reflect distribution in humans (39, 40). Indeed, a recent study of positron emission tomography (PET) imaging with zirconium-89 labeled trastuzumab to assess HER2 status demonstrated substantial heterogeneity in HER2 expression in metastatic lesions within the same patients (41, 42). Combining the imaging analysis with fludeoxyglucose F 18–labeled PET/computed tomography imaging enabled prediction of which patients would benefit from treatment with the HER2 ADC (41). Unfortunately, preclinical models have not reflected the heterogeneity of target expression that is seen across multiple metastatic lesions in the populations of patients with relapsed and refractory disease who are frequently treated with ADCs. Imaging analysis was also used to understand tumor distribution of ADCs in a study that demonstrated significant differences in tumor uptake between an unconjugated Lewis Y mAb and the same Lewis Y mAb conjugated to calicheamicin (43, 44). However, different dose ranges were applied for naked antibody versus antibody conjugate, and nonlinear pharmacokinetics were observed, complicating data interpretation. Nevertheless, the results suggest that the process of conjugating a warhead onto an antibody may potentially alter the biophysical properties of the antibody, which could impact its biodistribution profile. These imaging examples underscore an opportunity to more fully understand the target expression profile in patients before they are treated with ADCs and to determine the potential impact on the biodistribution properties of an antibody following conjugation to a warhead.

Biomarkers to optimize patient selection

Patient-selection strategies with ADCs have previously focused primarily on target receptor expression on tumor cells; however, a more comprehensive strategy that includes markers linked to the mechanism of action of ADCs can be used to improve the likelihood of success (Fig. 3). One component of potential sensitivity to ADCs is patient response to warheads linked to the mAb. Biomarkers associated with warhead sensitivity could provide an opportunity to improve the therapeutic index by

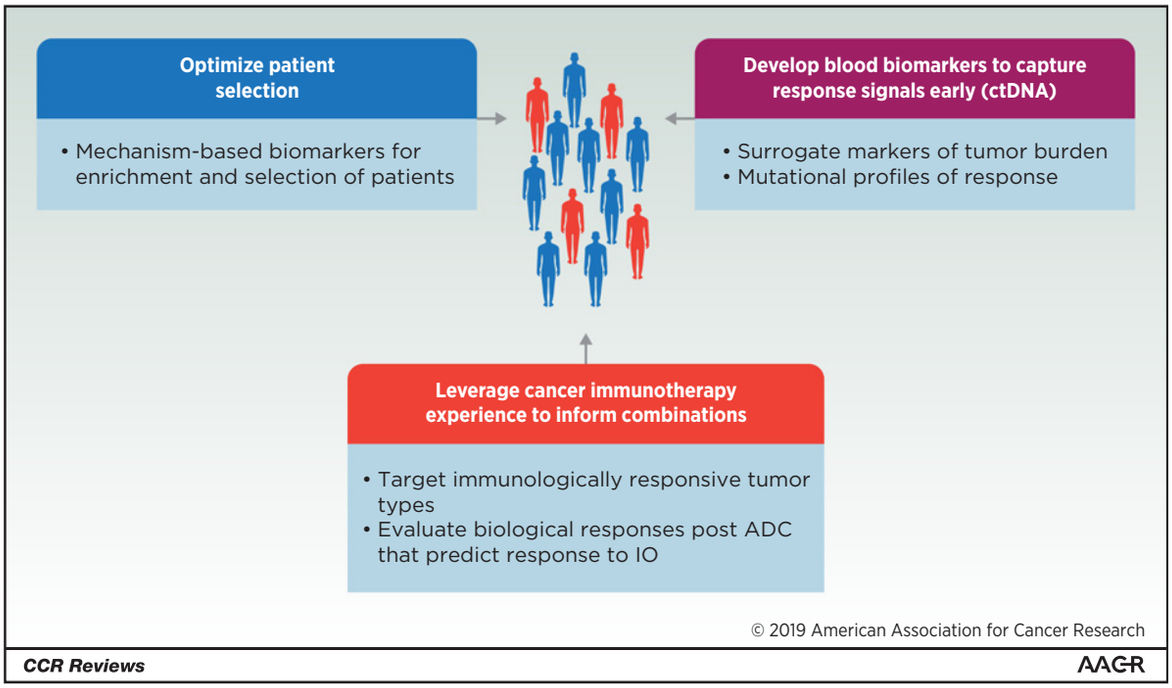

Figure 3.

Translational medicine strategies to maximize the therapeutic index. One of the key challenges for the clinical development of ADCs is the narrow index observed between safety and efficacy. The design and application of biomarkers to optimize patient selection, capture response signals early, and inform potential combination therapies are central to maximizing the therapeutic index and providing a personalized approach to ADC therapeutic development. ctDNA, circulating tumor DNA; IO, immuno-oncology.

observing responses at lower doses of ADCs, which, in turn, may broaden the therapeutic index. Although these types of sensitivity markers have been identified for some chemotherapies (45–48), they have not been used for patient selection; however, the more targeted approaches of ADCs may enable patient selection strategies based on warhead sensitivity profiles.

Biomarkers of DNA damage response have been used for patient selection for DNA damage repair inhibitors such as PARP inhibitors (49–56). Similarly, with warheads that induce DNA damage, such as topoisomerase inhibitors (TOPi) and PBD dimers, patients with aberrations in DNA damage repair pathways may have improved responses and, potentially, a broader therapeutic window. Selective knockdown and knockout of genes involved in DNA damage response (*BRCA1* and *BRCA2*) have been shown to sensitize killing by PBD dimers (57). Furthermore, an ADC conjugated to PBD demonstrated improved potency in xenografts with mutations in *BRCA* genes compared with wild-type xenografts, providing proof of concept for candidate PBD-response markers for clinical evaluation (57). Specific knockouts, knockdowns, and mutations in DNA damage response (DDR) genes and/or genes potentially involved in the regulation of DDR have been shown to confer sensitivity of tumor cells to TOPi (58–60) and PBDs (61). Interestingly, while some of the sensitivity genes are shared (such as *BRCA1*, *BRCA2*, *ATR*, and *FANCD2*), others differ, which may reflect differences in the mechanism of each specific warhead that could potentially contribute to differences in patient response.

While warhead sensitivity biomarkers have not been widely used for enrichment or preselection of patients, aberrations in DDR pathway genes can be evaluated through analysis of tissue biopsies as well as circulating tumor DNA (ctDNA) where DDR genes are included in several genomics panels qualified for clinical studies (Clinical Laboratory Improvement Amendments certified). Evaluation of ctDNA is less invasive for patients, and studies have shown concordance of genomic profiles in ctDNA and tumor tissue (62); however, similar concordance analyses will be needed in clinical studies to develop DDR genes as candidate predictive biomarkers of response. In addition to sensitivity to DDR, other factors may impact warhead sensitivity for DNA-damaging agents; for example, for topoisomerase inhibitors, expression of topoisomerases in target tumor cells may also impact clinical activity (63).

Compared with biomarkers for DNA-damaging agents, for microtubule inhibitors, tubulin isoforms and a high proliferation index may sensitize patients to response. In preclinical studies, decreases were preferentially observed in highly proliferating B cells (Ki-67$^+$ CD20$^+$ lymphocytes) compared with nonproliferating B cells (Ki-67$^-$ CD20$^+$ lymphocytes) after anti-CD22-MMAE and anti-CD79b-MMAE treatment (64).

Biomarkers to capture response signals early and monitor the duration and depth of response

Another factor central to the engineering of successful ADCs is the ability to capture response signals early and to effectively monitor the depth and duration of response. This can be especially important when attempting to optimize therapeutic index when testing new dosing regimens. ctDNA can provide a noninvasive means of monitoring both longitudinal changes in tumor

burden and patients' mutational profiles. While ctDNA levels have been shown to associate with the response to cancer immunotherapies (65), the effects of ADCs on ctDNA and associations with response have not been reported and could provide complementary information to support and better understand clinical activity. ADCs have been shown to be effective in hematology–oncology indications, including three of the four approved ADCs (gemtuzumab ozogamicin, brentuximab vedotin, and inotuzumab ozogamicin; refs. 10–14); establishing a means of monitoring the changes in tumor burden in bone marrow without invasive sampling could help make development in these indications more efficient and less burdensome for patients.

Biomarkers to inform combination studies

Combining ADCs with immune checkpoint inhibitors, T-cell agonists, and other agents that affect immunoresponse has the potential to reverse many of the evasive strategies that tumors use to circumvent immunosurveillance. Currently, approximately 36 trials with 20 individual ADCs in combination with immuno-oncology (IO) therapies are ongoing, most of which are checkpoint inhibitors (Fig. 2B). Early clinical data are available for two trials (66, 67). For mirvetuximab in combination with pembrolizumab, data indicate that responses are similar to those with monotherapy; however, firm conclusions cannot be made at this time due to limited data (66). The combination of adotrastuzumab emtansine (T-DM1) and atezolizumab was investigated in $HER2^+$ metastatic breast cancer; although no clinically significant benefit was observed with the combination in the intent-to-treat population, there was a trend toward clinical benefit in biomarker-selected subsets of patients (67).

Preclinical evidence indicates that ADCs can induce immunogenic cell death (68, 69) and provide synergistic antitumor activity when combined with IO agents (70–72). Treatment with ADCs in syngeneic mouse models has been shown to lead to increased infiltration of actively proliferating cytotoxic T lymphocytes and antigen-presenting cells in the tumor microenvironment (TME; ref. 71). Furthermore, infiltration of T cells has been observed in tumor biopsy specimens from patients after treatment with T-DM1 (70).

The rationale that combinations of ADCs and IO agents will improve clinical activity centers on the hypothesis that ADC treatment will alter the inflammatory milieu of tumor tissue, and patients with antitumor immune responses will be more likely to benefit from combination therapy. To assess the potential benefit of combining ADCs with IO agents, biomarkers can be used to evaluate the TME before and after ADC monotherapy. Monitoring changes in the TME after monotherapy can help determine whether markers predictive of response are upregulated such as infiltration of T cells (73), elevated programmed death receptor 1 ligand (PD-L1; refs. 74, 75), IFNγ, and IFNγ-inducible factors (76) involved in T-cell regulation and recruitment of immune cells into the TME. Tumor mutational burden and changes in T-cell receptor diversity and clonal expansion can also be evaluated to determine whether tumor-specific neoantigens are being released by ADC treatment. These changes could help determine whether ADCs can change "cold" TME to immunologically "warm/hot" TME. In parallel, biomarkers associated with activation of immune responses could be evaluated in peripheral blood, such as increases in proliferating ($Ki\text{-}67^+$) T cells and markers of immunogenic cell death.

Evaluation of these changes in peripheral blood and tumor tissue may provide a better understanding of the potential to improve ADC activity through combination treatment and help prioritize disease indications with the highest likelihood of success. Furthermore, these evaluations may be informative when considering dose adjustments to maximize the therapeutic index. Patients not demonstrating changes in the TME indicating a response to checkpoint inhibitors and/or markers of immunogenic cell death may be considered for dose adjustments and/or other combination strategies (e.g., T-cell agonists, oncolytic virus, or tumor vaccines).

Conclusions

With more than 80 compounds in various stages of clinical development, ADCs continue to be a cancer treatment modality with significant investment and the ambition to selectively deliver cytotoxic agents to cancer cells through specific binding of an antibody to cancer-selective targets. Although clinical gaps remain regarding the optimal application of ADCs in oncology, the study of these agents in a variety of settings is harnessing novel technologies and leveraging translational medicine to maximize the therapeutic index of these agents.

Clinical development strategies will include alternative dosing schedules and cutting-edge translational medicine to optimize patient selection, capture response signals early, match biomarkers to warhead mechanisms of action, and evaluate potential combination therapies to maximize the therapeutic index of ADCs. By incorporating these novel technologies and biomarker selection strategies, ADCs will be well positioned to provide clinical benefit to a much broader patient population.

Disclosure of Potential Conflicts of Interest

All authors are employees of AstraZeneca and hold stock/stock options in AstraZeneca. J.C. Soria is senior vice president of AstraZeneca, holds ownership interest (including patents) in Gristone and AstraZeneca, and is a consultant/advisory board member for Astex, Clovis, GlaxoSmithKline, GamaMabs, Lilly, MSD, Mission Therapeutics, Merus, Pfizer, PharmaMar, Pierre Fabre, Roche, Sanofi, Servier, Symphogen, and Takeda. No other potential conflicts of interest were disclosed.

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