The Journey of Antibody–Drug Conjugates: Lessons Learned from 40 Years of Development

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ABSTRACT Antibody–drug conjugates (ADC) represent one of the most rapidly expanding treatment modalities in oncology, with 11 ADCs approved by the FDA and more than 210 currently being tested in clinical trials. Spanning over 40 years, ADC clinical development has enhanced our understanding of the multifaceted mechanisms of action for this class of therapeutics. In this article, we discuss key insights into the toxicity, efficacy, stability, distribution, and fate of ADCs. Furthermore, we highlight ongoing challenges related to their clinical optimization, the development of rational sequencing strategies, and the identification of predictive biomarkers.

Significance: The development and utilization of ADCs have allowed for relevant improvements in the prognosis of multiple cancer types. Concomitantly, the rise of ADCs in oncology has produced several challenges, including the prediction of their activity, their utilization in sequence, and minimization of their side effects, that still too often resemble those of the cytotoxic molecule that they carry. In this review, we retrace 40 years of development in the field of ADCs and delve deep into the mechanisms of action of these complex therapeutics and reasons behind the many achievements and failures observed in the field to date.

"In Medio stat Virtus." (Virtue Stands in the Middle—Aristotle)

INTRODUCTION

For nearly a century, systemic cancer treatment has relied on chemotherapies, namely, molecules capable of inhibiting cell mitosis and inducing cell death (1). Antimetabolites, alkylating agents, anthracyclines, antimicrotubules, and multiple additional cytotoxins have formed the arsenal, which have prolonged survival and improved cure rates for patients with cancer (1). However, the narrow therapeutic window between toxicity and response of these agents limits the antitumor efficacy of doses that can be safely administered (2, 3). Their side effects, including alopecia, neutropenia, nausea, vomiting, and diarrhea, are a reflection of their mechanism of action and are related to their effects on rapidly dividing cells, such as hair follicles, bone marrow, and the gastrointestinal tract (3). In the late 1970s, the quest for more effective and tolerable chemotherapies and advancements in antibody manufacturing led to the clinical testing of antibody–drug conjugates (ADC), which aims to combine tumor targeting of monoclonal antibodies with cytotoxicity of their payload drugs (4). These clinical studies were supported by preclinical work (5). The idea that ADCs could increase the maximum tolerated dose of a drug while simultaneously decreasing its minimum efficacious dose became widespread.

Fast-forwarding 40 years, ADCs represent one of the most rapidly expanding anticancer treatment modalities. More than 370 new ADCs have entered the clinic (Fig. 1), culminating in 11 approvals by the FDA to date (4, 6, 7). The therapeutic successes of ADCs in the clinic, for both solid and hematologic malignancies, are leading to an unprecedented expansion with multiple ADCs currently being tested in

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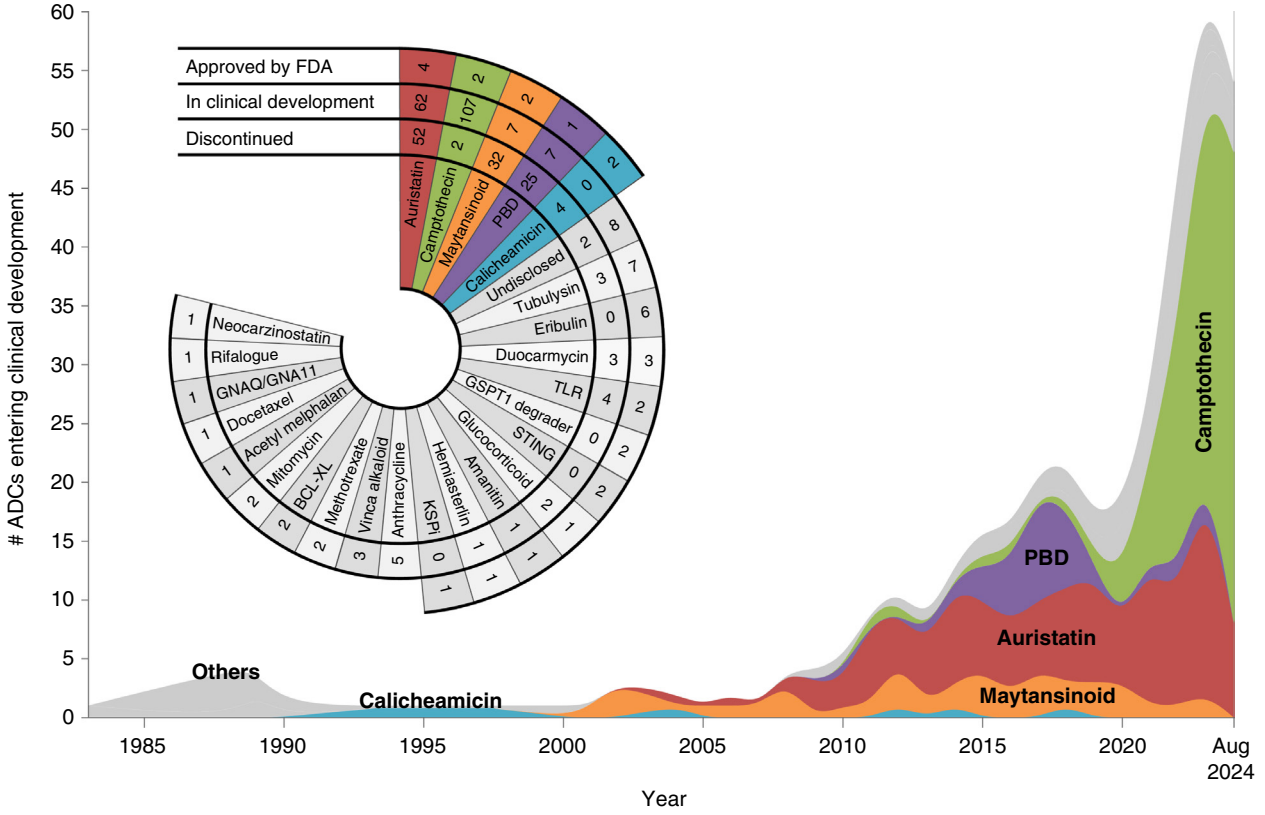

Figure 1. Trends in ADC development during four decades highlight a diversity of approaches with clearly prominent payload classes. In the 1980s, vinca alkaloids and DNA-damaging agents were predominant. The 1990s saw the advent of calicheamicin ADCs, succeeded by auristatin and maytansinoid ADCs. PBD dominated the field in 2015–2020. More recently, camptothecin ADCs represent the majority of ADCs entering clinical development. Several other ADCs with distinct payload classes have been investigated or are under investigation but have not led to approvals to date. The cumulative number of ADCs depicted is 378, of which 11 have been approved by FDA, 217 are in clinical development, and 150 have been discontinued as of August 15, 2024. PBD, pyrrolobenzodiazepine.

phase III trials, as monotherapy or in various combinations, and many more novel ADCs entering early phases of drug development (7). However, major challenges have emerged, as evident from the clinical discontinuation of more than 150 ADCs to date (Fig. 1). Despite being designed to be tumor selective, ADCs are burdened by toxicities both similar to and distinct to those of chemotherapy. Prominent and often dose-limiting toxicities are mainly tied to the drug–linker rather than to the antibody or the target (8, 9). Moreover, as most ADCs currently use payloads with the same mechanism of action [i.e., microtubule inhibitors, topoisomerase I inhibitors (TOPO1i), and DNA-acting agents], concerns have been raised about cross-resistance among ADCs with similar payloads, which may limit the use of ADCs in sequence (10). Lastly, predicting their activity has proven highly complex, with clear trends of higher efficacy correlating with higher target expression for several ADCs (11). However, subgroup analyses from other clinical trials have failed to detect a clear relationship between target expression and efficacy (11–16).

Here, we provide an in-depth review of ADCs' mechanism of action, their current and anticipated clinical role in cancer treatment, and strategies to tackle common ADC challenges to improve their development.

Mechanism of Action of ADCs: Insights from 40 Years of Development

Since the 1980s, antibody therapeutics, including ADCs, have been hailed as prototypical "magic bullet" drugs based on the concept of 1908 Nobel laureate Paul Ehrlich (17). He envisioned an ideal drug that, like a magic bullet, would hit its target without fail and without collateral damage. ADCs have been defined at times as "biological missiles," "Trojan horses," and "smart chemotherapies," with each definition highlighting different facets of their therapeutic potential. However, as our understanding of this class of therapeutics has grown, this view has increasingly been revealed as overly aspirational (6, 18–23).

The magic bullet narrative has contributed to the perception that ADCs deliver cytotoxic drugs exclusively to tumor cells while sparing normal cells, and at times, this misrepresentation has hindered their development. For decades, for example, one of the major trends for ADCs was the "pursuit of potency," focusing on novel payloads with subnanomolar to picomolar *in vitro* potency, especially in the context of drugs that were too cytotoxic to be developed as small-molecule chemotherapies (24). On the other hand, the use of payloads with insufficient potency, such as methotrexate, acetyl melphalan, docetaxel, doxorubicin, vinca alkaloids, and others, posed a significant

challenge in ADC development, especially in the 1980s and 1990s, when ADCs were still in their early stages (25, 26). During this period, the field also faced issues with suboptimal linkers and antibodies. Over time, the emphasis on increasing potency may have swung too far, resulting in ADCs with ultrapotent payloads that led to unmanageable toxicities at subefficacious doses. Currently, there is a growing emphasis on matching the payload's mechanism of action, potency, and properties with the antibody target and specific indication.

ADCs have also been impacted by toxicities at suboptimal ADC doses, in part (but not solely) related to spontaneous release and prolonged exposure of payload in the circulation. Extensive efforts have thus been dedicated to stabilize the linkage between the antibody and payload to avoid premature drug release and to improve ADC selectivity (27). Although advancements in linker technologies have contributed to the success of current ADCs, focusing on more stable linkers has faced drawbacks, including unexpected toxicities that are not observed or are not prominent in less stable ADCs, likely arising from increased normal tissue exposure to the antibody-conjugated drug. Finally, the primary disposition of ADCs stems from normal tissue uptake, an element downplayed by the "magic bullet" concept. Despite complexities, ADCs represent one of the most effective classes of chemotherapeutics. Gaining insights into their nuanced mechanisms and addressing emerging challenges is expected to promote improvements and sustain progress in the evolving and dynamic ADC field.

Maximum Tolerated Dose of ADCs

Stemming from the original rationale behind their development, ADCs are commonly purported to increase the maximum tolerated dose (MTD) and reduce the minimum efficacious dose of their payload through selective delivery of their payload to the tumor. Preclinical data often support this concept. In tumor-bearing mice, ADCs consistently demonstrate better efficacy than unconjugated payloads, and certain ADCs exhibit better tolerability than their unconjugated drugs in toxicology studies (typically executed in rodents and nonhuman primates; ref. 28). However, clinical observations diverge from preclinical models, showing that ADCs do not significantly increase the MTD of conventional chemotherapy (29). To convert ADC doses into payload doses using a familiar unit (i.e., mg/kg), normalized cytotoxin content of a payload conjugated to an ADC was calculated as follows: $\text{Dose}_{\text{payload}} = \text{Dose}_{\text{ADC}} \times \text{DAR} \times \text{MW}_{\text{payload}}/\text{MW}_{\text{ADC}}$ (29), in which DAR is the drug–to–antibody ratio and MW is molecular weight. ADC-normalized cytotoxin content, therefore, reflects the total cytotoxic payload dose, which takes into consideration both the ADC DAR and the antibody dose. Converting ADC MTDs, recommended phase II doses, maximum administered doses, or dose-expansion doses to milligrams per kilogram of cytotoxin content (i.e., ADC dose normalized by the amount of conjugated payload) illustrates that ADCs have similar MTDs to their related unconjugated drugs that reached clinical development (Fig. 2; Supplementary Table S1; ref. 29).

From these data, it is possible to derive insights to benefit the development of future ADCs. Approved ADCs do not necessarily exhibit higher MTDs by cytotoxin content than discontinued ADCs within the same payload class. In addition, as previously highlighted in two seminal FDA analyses (8, 9), ADCs with the same payload generally achieve similar MTDs. Where on-target off-tumor toxicities arise from antibody–target engagement in normal tissues, lower MTDs may be evident than for other ADCs employing the same drug–linker (23, 30). ADCs with a lower DAR often achieve higher antibody-based doses, yet their cytotoxin doses remain comparable to ADCs with higher DAR and similar drug–linkers (29). This further emphasizes the importance of using normalized cytotoxin doses to compare ADCs with different DAR. The question of whether a higher dose of an antibody conjugated with a lower DAR is superior to a lower dose of the same antibody conjugated with a higher DAR remains unanswered. This matter is likely influenced by various factors, including the tumor type, the antibody target, the linker technology, the potency and membrane permeability of the payload, and the mechanism of action of the drug. Payload potency and drug–linker properties often correlate with target-independent side effects, referred to as "platform toxicities," which frequently dictate ADC MTDs in the clinic (23, 31, 32). For instance, camptothecin ADCs typically achieve higher normalized cytotoxin doses than auristatin or maytansinoid ADCs, which in turn achieve higher doses than highly potent pyrrolobenzodiazepine (PBD) ADCs (9). Payload potency and other properties also impact platform toxicities within payload classes. For example, in the auristatin class, side effects of monomethyl auristatin E (MMAE)–based ADCs are often neutropenia and neuropathy, whereas thrombocytopenia and ocular toxicity are common for monomethyl auristatin F (MMAF)–based ADCs. Maytansinoid ADCs may induce neuropathy and liver toxicity and also thrombocytopenia with noncleavable DM1-based ADCs or ocular toxicities with DM4-based ADCs (19, 33). In line with the inverse correlation between payload potency and MTD, ADCs incorporating more potent payloads may not achieve the same normalized cytotoxin content of other ADCs within the same payload class (Fig. 2; Supplementary Table S1). For example, clinical stage ADCs with more potent camptothecin payloads [e.g., exatecan (34)] have so far achieved lower normalized cytotoxin doses than similar ADCs with less potent payload analogs (e.g., DXd; Fig. 2; Supplementary Table S1). Whether a higher dose of an ADC with a less potent payload can improve efficacy compared with a lower dose of an ADC with a more potent payload likely depends on a number of factors. With the clinical landscape of camptothecin ADCs growing rapidly (35), the relationship between MTD and potency will become clearer, and the relevance of antibody dose for therapeutic benefit is likely to be highlighted. As discussed later in the article, altering linker and conjugation chemistry, including through site-specific conjugation and masking technologies (e.g., via proteolytically activated antibodies, antibodies with pH-dependent binding, and hydrophilic groups to mask payload hydrophobicity), have not significantly improved normalized MTDs to date (Fig. 2; Supplementary Table S1), once again reinforcing the significance of payload-related toxicities and MTDs. It should be noted that some of these approaches to mask the binding of an antibody to its target may be intended to avoid on-target off-tumor toxicities, rather than off-target, off-tumor toxicities. Finally, although ADCs bearing the same or similar payloads can achieve similar normalized cytotoxin content, the steepness of the dose–response curves commonly

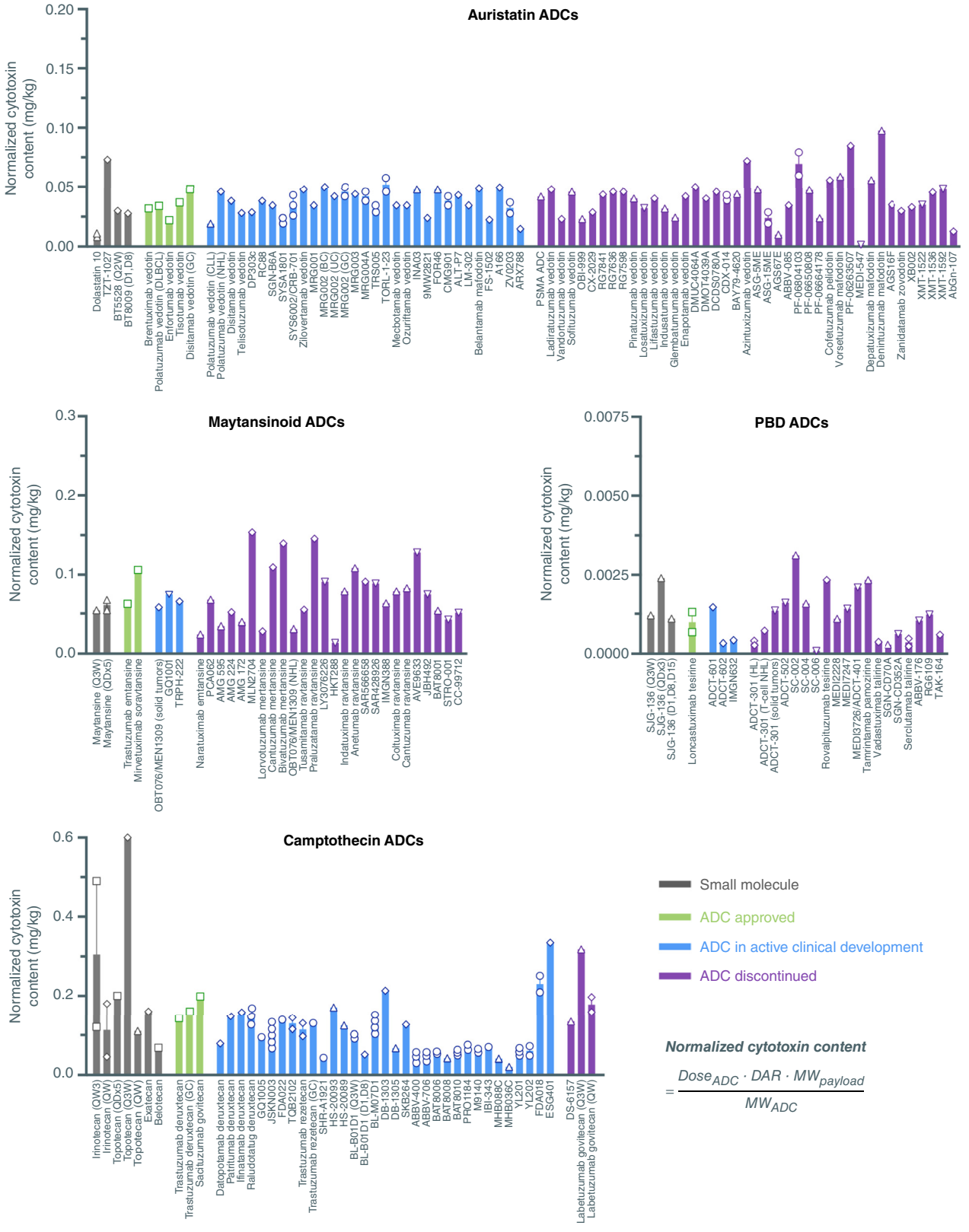

Figure 2. Human MTDs, RP2Ds, MADs, or dose-expansion doses of ADCs normalized by cytotoxic content and related small-molecule chemotherapies across the four most common ADC payload classes. Square, approved dose; up-pointing triangle, MTD; down-pointing triangle, MAD; circle, dose-expansion dose. See Supplementary Table S1 for additional details. MAD, maximum administered doses; RP2D, recommended phase II doses; PBD, pyrrolobenzodiazepine.

observed in the clinic for ADCs would generally suggest benefit from even modest increases in dose (e.g., twofold increase), if tolerated for multiple cycles of treatment.

Efficacy of ADCs Compared to Related Small-Molecule Chemotherapies

In the clinic, when dosed at or near their MTDs, multiple ADCs have demonstrated improved efficacy over related small-molecule chemotherapies, as evident from cross-trial comparisons for which data for ADCs and small molecules are available (29). More strikingly, certain ADCs showed efficacy in patients with tumor types that previously did not show significant or meaningful response rates to related small-molecule chemotherapies.

An interesting comparison involves the small-molecule irinotecan and the ADC sacituzumab govitecan (SG), both acting as prodrugs of the active TOPO1i, SN38. Irinotecan is approved for patients with colon and small cell lung cancer, and SG for patients with breast and urothelial cancers, indications in which irinotecan showed responses but not improvements over the standard of care (SOC). To date, there are no head-to-head randomized trials comparing ADCs with their direct payloads administered as small-molecule chemotherapies. The closest example is the DESTINY-Gastric01 randomized phase II trial, in which patients treated with trastuzumab deruxtecan (T-DXd), carrying the TOPO1i payload DXd, experienced a 42% overall response rate (ORR) versus 12% in those receiving physician's choice of chemotherapy (36). More than 90% of the patients received irinotecan (SN38 prodrug) as physician's choice. Like DXd, SN38 is a camptothecin derivative, although they have significant strucutural differences. In a few cases, ADCs demonstrated superior benefits to the established SOC or offered an option for unmet medical needs, redefining treatment paradigms across tumor histologies, as demonstrated by the 11 FDA-approved ADCs for >20 indications as of August 2024 (4), including the first tumor-agnostic approval of an ADC (37).

Understanding ADC Key Components and Their Mechanisms of Action

Stability

Linker instability in circulation (also referred to as premature payload released) is often cited as a significant factor limiting the therapeutic index of ADCs. Stabilizing technologies are prominently featured among purported strategies for improving the therapeutic window (24). Clearly, if the drug is not attached to the ADC, it cannot be delivered to tumor cells directly by the antibody. Furthermore, the unconjugated drug contributes to systemic toxicity. However, this view is an oversimplification that overlooks ADC disposition in normal tissues (see "ADC Fate"), in which only a small fraction of the injected dose (ID) reaches the tumor site.

Types of Linkers

The conventional ADC mechanism of action involves target binding, internalization, and catabolism, releasing a drug that exerts an intracellular effect. Linkers designed to release unmodified drugs are designated as *cleavable*, whereas those that require full catabolism of the ADC to release a modified payload are considered *noncleavable*. Most payloads lose activity when structurally modified, leading to a greater focus on the former strategy. Cleavable linkers are usually divided on the basis of the catabolic pathway used to release the payload (proteolysis, reduction, pH, glycoside hydrolase, etc.). Evidence for tumor-selective cleavable linkers is limited to *in vitro* selected cases (38–40). All FDA-approved ADCs feature cleavable linkers except trastuzumab emtansine.

Types of Instability

Linker instability is subdividable into *linker–drug instability*, wherein the drug is released over time prior to its intended site of metabolism, and *antibody–linker instability*, wherein the whole linker–drug is released from the antibody instead. Some levels of linker–drug instability are evident for most cleavable linkers because they are designed for drugs to be released by chemical or enzymatic stimuli, but the extent of cleavage in circulation varies. Antibody–linker instability is a function of the conjugation chemistry used. For instance, conjugation at lysine through amide bonds is highly stable, and maleimide conjugation at cysteine residues has variable stability (41).

(In)Stability of Approved ADCs

In addition to intrinsic ADC clearance from ADC uptake, all the approved ADCs to date are considered to have certain levels of linker instabilities (antibody–linker instability, linker–drug instability, or both) in circulation (Fig. 3A). Seven approved ADCs use thiol-maleimide (cysteine) conjugation, whereas four are conjugated at lysine residues (42). For thiol-maleimide ADCs, maleimide hydrolysis and retro-Michael reaction are competing processes, either stabilizing the antibody–linker attachment (hydrolysis) or releasing the maleimide-containing linker (retro-Michael reaction), respectively (Fig. 3B; ref. 43). The balance between these two processes is influenced by the nature of the linker adjacent to the maleimide and the chemical environment of the conjugated cysteine residue. In circulation, ~50% of maleimidocaproyl (MC) linkers used in brentuximab vedotin, polatuzumab vedotin, enfortumab vedotin, tisotumab vedotin, and T-DXd deconjugate over 1 week (44). 4-Maleimidomethyl-cyclohexane-1-carbonyl (MCC) linker used in SG has a similar deconjugation rate to MC (45). The shorter maleimide-propionyl (MP) linker (used for example in loncastuximab tesirine) features less antibody–linker instability than MC or MCC linkers, losing ~20% drug–linker over 1 week (46). For most cysteine-conjugated ADCs, antibody–linker instability exceeds linker–drug instability (47). The exception is SG, in which a highly unstable carbonate decomposes rapidly in circulation ($t_{1/2}$ ~24 hours), releasing free SN38 drug (48). Maleimide drug–linker deconjugating from antibodies is transferred to other thiol-containing biomolecules, mainly human serum albumin, which has serum concentrations of 35 to 50 mg/mL (47). Although albumin has a serum half-life of ~3 weeks in humans and maleimide deconjugation leads to formation of albumin–drug conjugate, little is known about the likely important fate of albumin–drug conjugate in patients (49). Preclinical studies have extensively documented the suitability of albumin–cytotoxin conjugates for tumor delivery, suggesting that similar uptake may occur in patients (50, 51). For ADCs with linker–drug instabilities, different chemical groups are responsible for the release of free

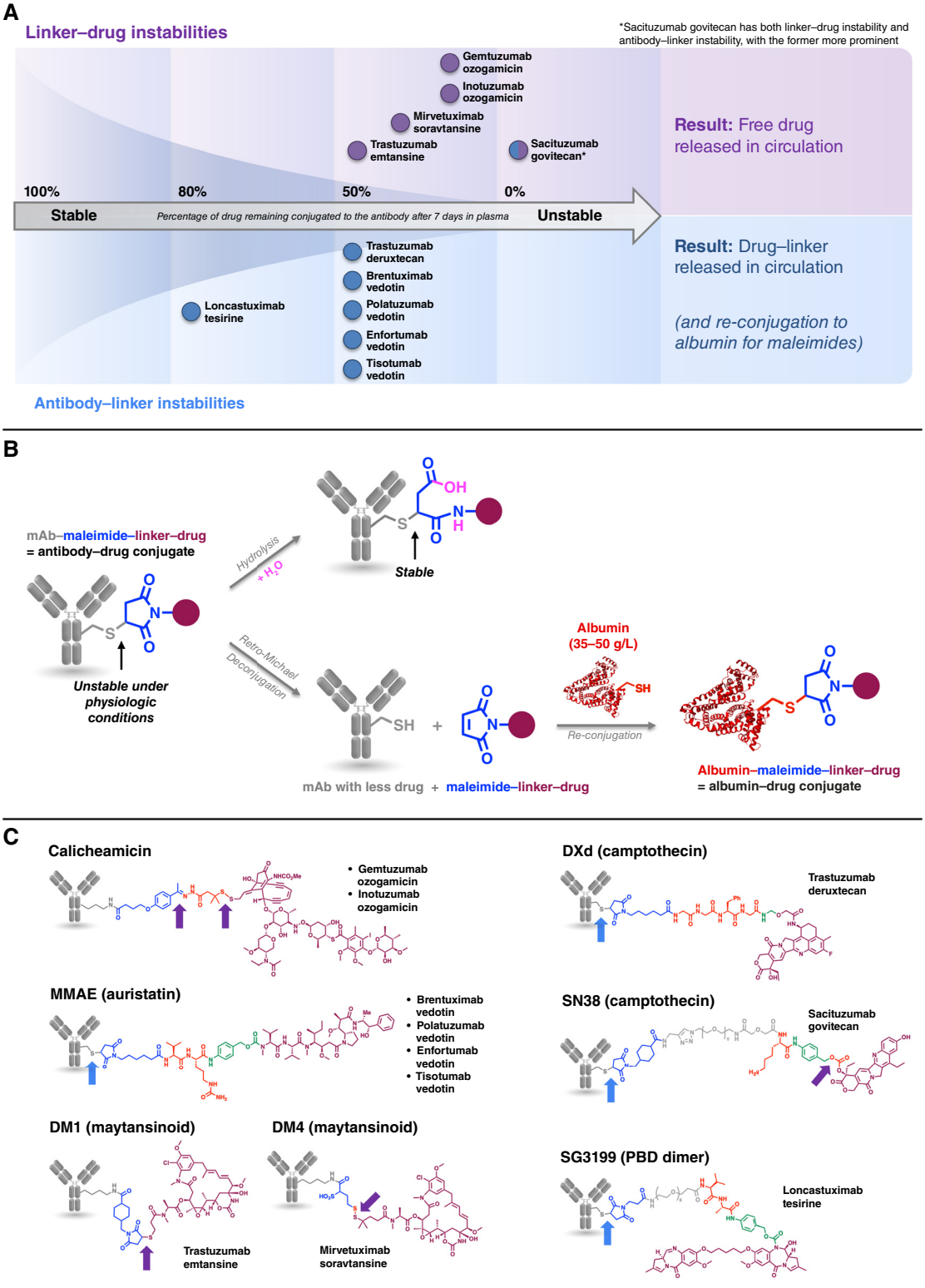

Figure 3. Stability and instabilities of ADCs. **A,** Schematic representation of approved ADC stability in circulation. Percentages denote estimations of the amount of payload remaining conjugated to the antibody after 7 days in plasma. **B,** Schematic representation of hydrolysis and retro-Michael reactions for thiol-maleimide ADCs. Hydrolysis and retro-Michael reactions are competing transformations. Thiol-maleimide stability is tunable on the basis of the linker–drug. The released drug–linker from retro-Michael reaction reconjugates to thiol-containing plasma proteins, almost exclusively albumin. **C,** Structure of approved ADCs indicating linker instabilities. Blue arrows, antibody–linker instabilities in circulation; purple arrows, linker–drug instability in circulation; blue atoms, antibody–linker conjugation; gray atoms, spacer; red atoms, cleavable linker; green atoms, self-immolative spacer; burgundy atoms, payload. MMAE, monomethyl auristatin E; PBD, pyrrolobenzodiazepine.

drug in circulation (Fig. 3C), including disulfides, (52) hydrazones (53), carbonates (54), and thiol-maleimides (55, 56). The estimated instabilities for approved ADCs (Fig. 3) are based on well-understood chemical processes, further supported by a body of preclinical literature and confirmed by limited clinical reports. Several methodologies [including comparison of conjugated drug pharmacokinetics (PK) vs. total antibody PK, and DAR over time by mass spectrometry–based techniques] are suited for a clear understanding of linker–drug instabilities and antibody–linker instabilities *in vitro* and *in vivo*. Although determination of change in DAR over time is less commonly reported in the clinical context, analysis of the widely reported PK parameters of total antibody and total conjugated drug reinforces the very clear preclinical data that show that change in DAR is both predictable and consistent.

Stabilizing ADCs

Improving antibody–linker and linker–drug stability in circulation could, in theory, reduce the unconjugated drug amount, limit toxicity, and improve efficacy. This view of the ADC therapeutic index, however, overlooks two important points. First, unconjugated free drugs can achieve pharmacologically relevant blood levels and may significantly contribute to the antitumor effect (20). Second, stabilization may increase the exposure of healthy tissues to the conjugated drug (i.e., the ADC). The tissue distribution and sites of metabolism of an ADC are of pronounced importance if each antibody carries more payload (as for ADCs with stable linkers), especially because the majority of an injected ADC dose is metabolized in normal tissues and not at the tumor site (21). ADC disposition is governed by more than stability; however, depending on the preferred sites of ADC metabolism, stabilization may result in the delivery of more payload to specific organs.

Numerous approaches to antibody–linker stabilization have been assessed in the clinic, most notably involving site-specific conjugation methods (57). Conjugated drug exposure may be increased relative to similar unstabilized ADCs, reflecting the improved stability and biophysical characteristics, although drug-normalized MTDs hardly vary. Often, these efforts have resulted in less hematologic toxicities, whereas other tissue-specific dose-limiting toxicities emerged, which may reflect the increased burden of highly loaded ADC species in normal tissues. For example, the site-specific DAR2 CD79b vedotin ADC DCDS0780A showed higher conjugated drug exposure (about four times higher $AUC_{0\text{-}21}$) than the DAR4 CD79b–targeting vedotin: ADC polatuzumab vedotin, although similarly exposed to free monomethyl auristatin E (58, 59). The normalized cytotoxin content of DCDS0780A was similar to polatuzumab vedotin and other vedotin ADCs (site-specific and stochastically conjugated, Fig. 2). Despite the apparent preclinical advantage, target-independent ocular toxicity was prominent in the clinic for DCDS0780A and not reported in patients treated with polatuzumab vedotin. Ultimately, the less stable polatuzumab vedotin ADC was approved and DCDS0780A was discontinued. Given the complex and multivariable nature of ADC pharmacology, isolating the impacts of (in)stability is challenging. Ideally, clinical data comparing ADCs with the same target, antibody, linker, and payload would have to be available in the same patient population. Examples are scarce, thus nonideal cross-trial comparisons of ADCs with limited differences must be relied on. To date, more than 30 site-specific ADCs have been discontinued in the clinic and no site-specific ADCs have been approved. Although it is evident that site-specific technologies and/or perfectly stable linkers do not provide the ultimate solution to the challenges faced by ADCs, they serve as valuable tools in the toolbox for generating drugs with diverse profiles, which may lead to future approvals.

ADC Fate

The complex structures and catabolism of ADCs complicate our understanding of payload delivery. Over time, DAR, conjugated and unconjugated antibodies, albumin conjugate, unconjugated payload, and payload metabolites are all highly dynamic (60). ADC disposition predominantly occurs through metabolic processes, in contrast to the direct excretion pathways common to small-molecule therapeutics. The released payload and/or payload metabolite(s) are then, in turn, eliminated via canonical small-molecule routes. ADCs, like all the other antibody-based therapeutics, are mainly distributed in normal tissue, leading to predominant off-tumor uptake and clearance. In other words, the main disposition of an ADC is from target-independent uptake in normal tissues (32). Where an ADC comprises multiple DAR species, rate and location of nonspecific uptake may be DAR dependent, with a preference for more rapid clearance of higher DAR species. For certain targets widely expressed in normal tissues, on-target off-tumor uptake (in addition to nonspecific uptake) contributes to ADC disposition (21, 61). On-target (on-tumor and off-tumor) and off-target ADC catabolism generate free payload and/or payload metabolites in tissues (including a small percentage in tumor), which then redistribute in circulation (Fig. 4A).

In addition, certain linker instabilities result in the direct release of free payload in circulation or the formation of albumin conjugates via drug–linker transfer from ADC to albumin. In humans, payloads derived from both ADC disposition and linker instabilities often achieve plasma concentrations above the *in vitro* effective free drug concentration (EC_{50}) over time [Fig. 4B1 and B2 for representative PK of T-DXd (62) and polatuzumab vedotin (58) and their released payloads over 3 weeks; ref. 20]. If the payload was administered as a small-molecule chemotherapy, the exposure above the EC_{50} would have lasted minutes to hours and not days [Fig. 4B3 and B4 for representative PK of the camptothecin exatecan (63) and the auristatin dolastatin 10 (64) over 2 days], emphasizing the role of ADCs as a payload reservoir. In a sense, ADCs serve as prodrugs of their payloads, and the payload being released through a prolonged period of time, relative to the half-life of the ADC, is akin to a "continuous infusion" profile. Although it is uncertain to what extent that free payload may contribute to antitumor effects on a case-by-case basis, it is reasonable to assume that there is some contribution, in addition to direct delivery via ADC targeting (20, 65). These additional mechanisms highlight the unique role of the antibodies in enhancing the effect of their conjugated drugs, not solely relying on tumor-specific ADC uptake. In fact, the interplay of direct on-tumor uptake and the off-tumor target and nontargeted uptakes, combined with linker instabilities, contributes to a sustained drug concentration in the body and at the tumor site (Fig. 5). It is important to clarify that although only a fraction of the ADC dose reaches the tumor, the payload that is released off-tumor

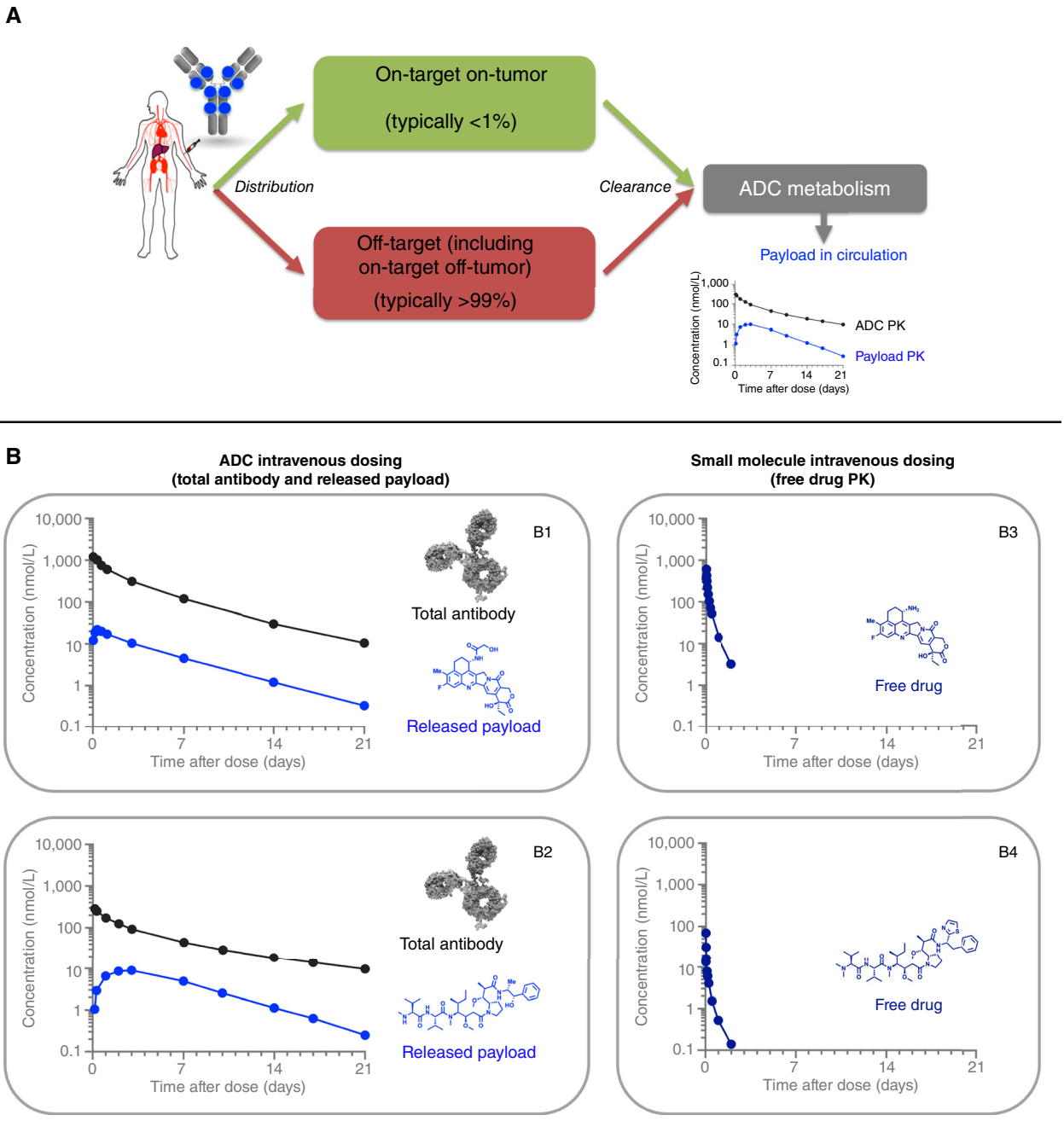

Figure 4. Distribution and clearance of ADCs after their administration. **A,** Schematic representation of ADC fate. **B,** Examples of ADC and released payload PK (typical half-life of days for both total antibody and released payload) from ADC IV dosing (**B1** and **B2**) vs. related small-molecule chemotherapy PK (typical half-life of hours) from small-molecule chemotherapy IV dosing. These representative PK are generated on the basis of the data reported for: **B1**, T-DXd dosed at 6.4 mg/kg Q3W (62); **B2**, polatuzumab vedotin dosed at 2.4 mg/kg Q3W (58); **B3**, exatecan dosed at 5 mg/m^2 Q3W (63); and **B4**, dolastatin 10 dosed at 0.4 mg/m^2 Q3W (64). These examples serve as illustrative case studies for apparent extended half-life of payloads released from ADCs compared with related unconjugated small-molecule chemotherapies.

does not necessarily accumulate in the tumor. Most of it is distributed in normal tissues and is eliminated via canonical small-molecule routes, much like conventional chemotherapy (21). Nonetheless, the plasma concentration of the released payload can reach efficacious levels during a prolonged period of time, making its contribution potentially relevant to efficacy and explaining certain payload-related systemic toxicities (20).

Moreover, linker instabilities lead to antibody species with gradually decreasing conjugated payload over time in the blood pool, maintaining their identity as ADCs until at least one drug payload is attached. Lower DAR ADCs generally exhibit improved PK properties and different normal tissue uptake compared with the initially higher DAR ADCs, but they also carry less payload per antibody. For ADCs with fully

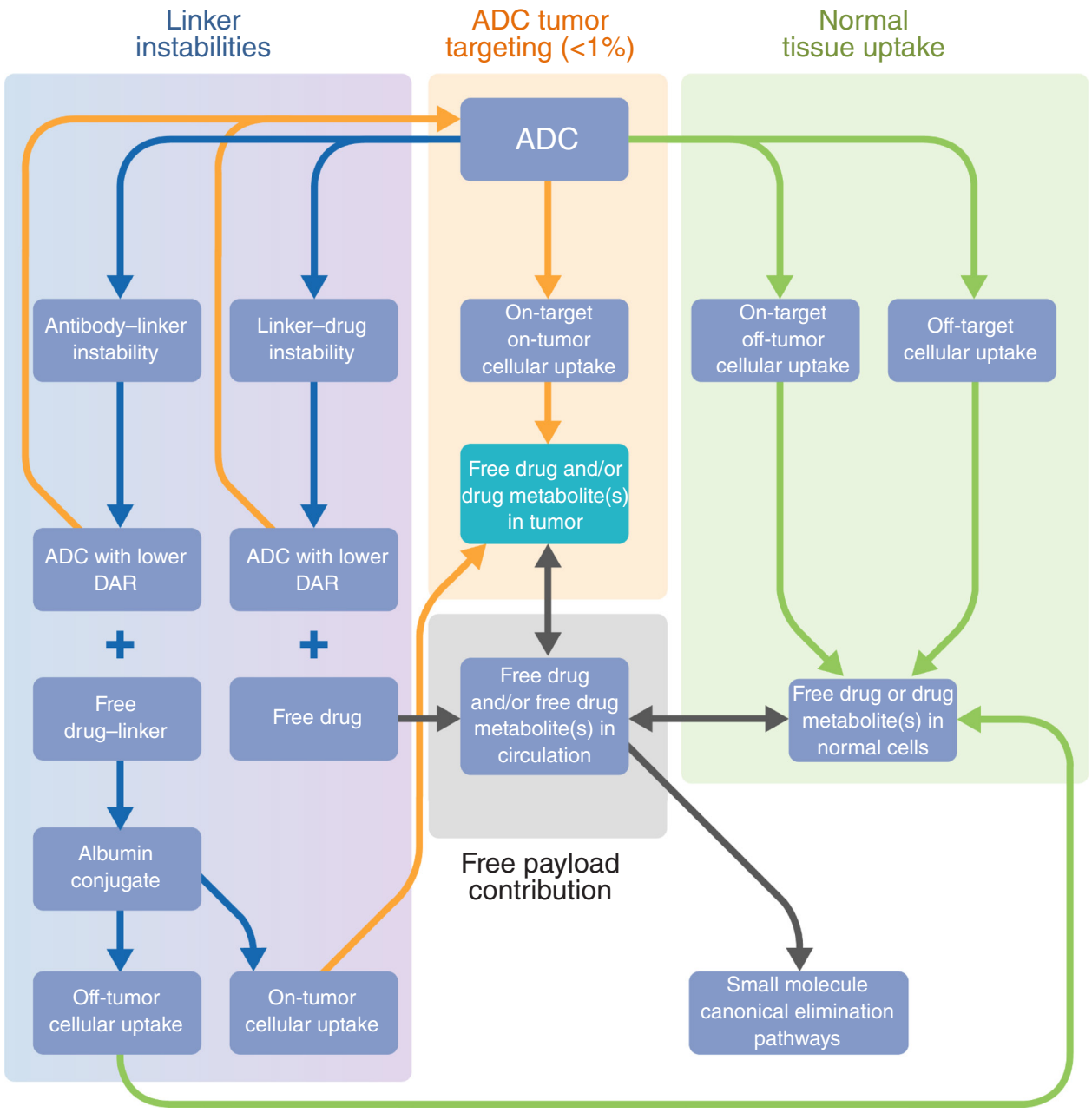

Figure 5. Representation of the fate of ADCs and their components. Normal tissue uptake is common for all ADCs and increasingly predominant for stable ADCs. Generally, direct tumor targeting accounts for less than 1% of the ID. Linker instabilities in circulation generate free drug or free drug–linker. The latter is reconjugated to thiol-containing molecules in plasma, mainly albumin. ADC tumor and normal tissue uptake generate free drug or drug metabolite(s), which also contribute to sustain payload concentration in circulation. Released payload is eliminated via canonical small molecule pathways.

stable linkers, no antibody species with less conjugated drug is present in the blood pool, and the ADC only relies on normal tissue (prevalent) or tumor site (generally <1%) uptake to release the payload. Whether these phenomena are considered positive or negative in balancing the efficacy and toxicities of ADCs still awaits clear-cut confirmation in the clinical setting and is likely contingent on multiple factors and case-by-case considerations. As discussed above, unexpected toxicities have often emerged for ADCs using stable linkers, likely because of the inevitable uptake of ADCs into normal tissues (21). With >150 novel ADCs with various technologies entering the clinic since 2022 and awaiting data, the future holds promise for better understanding correlations.

Whole-Body Distribution of ADCs: Insights from Imaging of Radiolabeled Molecules

Important insights into the body distribution of ADCs (and other antibody-based therapeutics) can be derived from PET imaging combined with radiotracers (66). Antibody imaging

studies are often done with the radionuclide zirconium-89 (^{89}Zr), given its long half-life of 78.4 hours, which matches the slow antibody clearance. From clinical PET distribution studies of the ^{89}Zr-labeled antibodies, ^{89}Zr-lumretuzumab, ^{89}Zr-MMOT0530A, ^{89}Zr-bevacizumab, and ^{89}Zr-trastuzumab, we learned that only a limited percentage of the antibody is taken up by tumors unless there is a major tumor load (67, 68). The off-tumor target and nontarget-mediated biodistribution patterns of the antibodies are largely similar for these antibodies on day 4 after tracer injection; about one third of the injected tracer dose is present in the circulation, up to 15% in the liver and 4% in the spleen and kidneys. Lower tracer concentrations are seen in the bone marrow, lungs, compact bone, muscle, adipose tissue, and brain. However, low tracer accumulation per gram of tissue can still be influenced by large-volume tissues, as is especially the case for adipose tissue. An average of 5% to 7% is expected to accumulate in adipose tissue, but this percentage can increase to as much as 19% in cases of morbid obesity. Strikingly, less than 1% of the antibody generally accumulates in tumor lesions per patient. This is consistent with studies with radiolabeled trastuzumab, in which an average tumor uptake of 0.9% ID was observed for ^{89}Zr-trastuzumab in a population with a median measurable tumor load of 99 (± 133) mL (Fig. 4). A standard human body comprises a volume of about 70 L, which shows that 0.9% ID in 99 mL tumor load is a clearly preferred tumor tissue uptake because the tumor volume comprises only 0.1%. This observation aligns with several other clinical studies conducted with radiolabeled antibodies, in which higher standardized uptake values were observed for the radioconjugate within the tumor. However, despite these higher values, the absolute amount of injected drug within the tumor was typically low (usually less than 1%), as it was predominantly distributed in normal tissues.

Tumor volume and tumor localization can also impact antibody disposition. For example, a larger tumor volume in a patient with bone metastases and a 1.2-kg liver tumor mass influenced trastuzumab PK (68). The ^{89}Zr-trastuzumab-PET scan 2 days after injection of 50 mg ^{89}Zr-trastuzumab, consisting of 1.5 mg ^{89}Zr-trastuzumab (37 MBq) replenished with 48.5 mg nonradioactive trastuzumab, showed 48% of the ^{89}Zr-trastuzumab in liver metastases and rapid trastuzumab clearance from the circulation (68). The dosing of trastuzumab and HER2-directed ADCs for metastatic breast cancer may, therefore, be heavily influenced by tumor load and may explain PK differences between patients, which can affect tumor response as shown for trastuzumab emtansine (T-DM1; ref. 69). Still, the largest part of the injected antibody is present in the body in normal tissues, explaining the occurrence of side effects. Target-mediated drug disposition is also common across antibody-based therapeutics, leading to increased clearance and nonlinear PK at doses below the amount required to overcome the antigen sink effect. At higher doses, the antigen sink can become saturated, resulting in proportional increases in antibody exposure (70). For example, margetuximab demonstrated nonlinear increases in drug exposure (AUC_{inf}) at doses of 0.1, 0.3, 1.0, and 3.0 mg/kg weekly (QW), whereas exposure was linear across doses of 10, 15, and 18 mg/kg every-three-weeks (Q3W; ref. 71). These insights into normal tissue distribution and disposition have influenced thinking about improving tumor penetration and preventing side effects.

In an early-phase clinical trial with the EGFR antibody–dye conjugate panitumumab-IRDye800CW, coadministration of the unconjugated panitumumab improved the intratumoral distribution of panitumumab-IRDye800CW. Measurement of the multiscale distribution of panitumumab-IRDye800CW, when coadministered with panitumumab, showed improved microscopic panitumumab tumor distribution with less uptake in healthy tissues (72). This made the authors suggest that a loading dose can be beneficial in reducing the binding site barrier and increasing tissue penetration of antibody–dye conjugates, which supports the possibility of applying the same dosing strategy for ADCs. It is interesting to realize that full tumor antibody saturation is difficult to achieve given the persistent uptake seen on PET scans during treatment with trastuzumab and HER3 antibody lumretuzumab for ^{89}Zr-labeled trastuzumab and lumretuzumab, respectively (73, 74). This is likely due to the constant new production and recycling of the antibody target by the tumor cells.

In hematologic malignancies, the insight into whole-body antibody distribution has influenced pretargeting approaches for both radioimmunotherapy (RIT), for which a monoclonal antibody is paired with a radioactive isotope, and bispecific antibodies. It was considered that the therapeutic index of RIT could be improved by dosing an excess of the corresponding unlabeled antibody prior to RIT (75). This preloading strategy was intended to prevent nonspecific binding to normal tissues or saturate normal cells expressing the antibody target, which would ensure a more consistent biodistribution profile of radiolabeled antibodies and extend their circulating half-life by reducing clearance rates (76–78). In the case of rituximab, a preload with unlabeled rituximab was envisioned to decrease the number of circulating B cells and improve the delivery of radiolabeled rituximab to tumor cells. However, a biodistribution study revealed that tumor uptake was higher in patients with B-cell depletion who did not receive a preload with unlabeled rituximab, indicating the need for further refinement of the preloading strategy (79).

In contrast to tumor biopsy, PET scanning with antibodies can provide information on target expression across all lesions within a patient. Numerous studies have shown major heterogeneity in antibody tracer tumor lesion uptake within and between patients (66). To learn the consequences of this heterogeneity for response to an ADC, the role of HER2-targeted molecular imaging was evaluated to identify patients who are unlikely to respond to ADC T-DM1. Patients with HER2-positive breast cancer were eligible for inclusion. Patients underwent imaging with ^{89}Zr-trastuzumab PET/CT and 2-deoxy-2-[^{18}F]fluoro-D-glucose PET-CT before T-DM1 treatment. Based on ^{89}Zr-trastuzumab uptake, lesions were visually classified as HER2 positive (visible/high uptake) or HER2 negative (background/close to background activity). Remarkably, 26 of 81 patients, despite being HER2 positive based on the biopsy, were ^{89}Zr-trastuzumab PET negative, and such patients experienced a shorter time to treatment failure (median 2.8 vs. 9.9 months) than those who were HER2 positive and underwent PET (HR 3.7, $P < 0.001$; ref. 80). When used for patient selection for T-DM1 treatment on HER2-only

imaging, 320 of the 1,000 patients would not have been treated with T-DM1 (80). More recently, it has become evident that patients with low or no HER2 expression can also respond to HER2-targeting ADCs (15, 81). Within the prospective multicenter IMPACT metastatic breast cancer trial, HER2 status was assessed with a tumor biopsy and baseline ^{89}Zr-trastuzumab PET scan in 189 newly diagnosed patients with metastatic breast cancer. Uptake in lesions was heterogeneous within and between patients for all HER2 IHC groups. ^{89}Zr-trastuzumab PET scan identified HER2-positive metastases in HER2-negative disease and HER2-negative metastases in HER2-positive disease. In addition, ^{89}Zr-trastuzumab PET scan uptake exceeded background uptake in HER2 IHC low and even negative metastases (82, 83). Potentially, ^{89}Zr-trastuzumab PET scan could refine patient selection for novel HER2-targeting strategies. Radionuclides with shorter half-lives (e.g., ^{68}Ga attached to smaller molecules such as single-domain antibody and affibody, and cyclic peptides) have also shown promising results in clinical trials for HER2 imaging (84, 85) and nectin-4 imaging (86). Finally, site-specific radiolabeled antibodies have demonstrated superior performance in preclinical studies than their stochastically modified progenitors (87). If these advantages are replicated in clinical settings, they may ultimately benefit both patients and physicians by guiding targeted therapies, including ADCs.

Clinical Development of ADCs for Treating Solid Tumors

Following the traditional paradigm of drug development, ADCs have initially been tested in patients with advanced, highly pretreated tumors, often demonstrating relevantly improved outcomes compared with the SOC, even in aggressive diseases (6). For example, T-DM1 was the first ADC to demonstrate improved overall survival (OS) in patients with HER2-positive pretreated metastatic breast cancer (88, 89). SG doubled the OS when compared with small-molecule chemotherapy among patients with highly pretreated triple-negative breast cancer (TNBC; ref. 90) and improved OS in patients with hormone receptor–positive (HR+) disease (91). SG showed activity in pretreated metastatic urothelial cancer (92), but the confirmatory phase 3 study (TROPiCS-04, SG vs. treatment of physicians' choice) did not meet its primary endpoint of OS benefit in the intention-to-treat population. Similarly, in the EVOKE-01 phase III study (SG vs. docetaxel), SG did not meet its primary endpoint of OS in patients with non–small cell lung cancer (93). In the phase 2 SACI-IO HR+ study, SG in combination with pembrolizumab did not show a statistically significant improvement in progression-free survival (PFS) compared with SG alone in patients with HR+, HER2–metastatic breast cancer unselected by PDL1 status (94). Another TROP2 ADC, sacituzumab tirumotecan, which uses the same antibody as SG, a similar pH-labile linker, but a different TOPO1i payload (KL610023, a belotecan derivative), showed improved PFS and OS compared with physicians' choice of chemotherapy (eribulin, vinorelbine, capecitabine, or gemcitabine) in patients with pretreated metastatic TNBC (phase III OptiTROP-Breast01 study; ref. 95). Enfortumab vedotin improved OS in patients with pretreated metastatic urothelial cancer, receiving approval as single agent (96) and, more recently, in combination with pembrolizumab (97). When tested in metastatic breast cancer, enfortumab vedotin showed limited efficacy (ORRs of 16% and 19% in patients with HR+ breast cancer and TNBC, respectively) and nectin-4 expression (highly expressed in both HR+ and TNBC cohorts) was similar between responders and nonresponders (98). Additional successes in pretreated patients with advanced solid tumors were achieved with mirvetuximab soravtansine targeting folate receptor alpha, approved for pretreated ovarian cancer (99), and tisotumab vedotin targeting tissue factor, approved for pretreated cervical cancer (100). A distinct path was followed by T-DXd: it was first approved as third-line treatment for patients with HER2-positive metastatic breast cancer (15%–20% of all breast cancers) and then approved as second-line treatment, showing impressive activity (fourfold improvement in PFS) in a head-to-head trial when compared with T-DM1 (101). T-DXd was also later expanded to HER2-low tumors (45%–55% of all breast cancers; ref. 15) and is currently being tested for some patients with HER2-ultralow and HER2-0 disease, for which up to 30% ORR was demonstrated in the phase II DAISY trial (81, 102). In the DESTINY-Breast06 trial, T-DXd demonstrated a statistically significant and clinically meaningful improvement in PFS compared with the SOC in patients with HR-positive, HER2-low (IHC 1+ or 2+/ISH−) and HER2-ultralow (defined as IHC 0 with membrane staining; IHC >0 <1+) metastatic breast cancer after one or more lines of endocrine therapy. A subgroup analysis showed that the PFS improvement was consistent between patients with HER2-low and HER2-ultralow expressions (103). Outside breast cancer, T-DXd received approval for the treatment of patients with HER2-positive gastric cancer (104) and HER2-mutant non–small cell lung cancer (105). More recently, relevant activity with T-DXd was observed in a multiplicity of HER2-overexpressing cancer types in the DESTINY-PanTumor02 phase II trial, culminating in accelerated approval by the FDA for the treatment of any HER2-positive (IHC 3+) solid tumor to mark the first tumor-agnostic approval of an ADC (37).

The observation of the meaningful clinical activity of certain ADCs in pretreated tumors has provided the rationale to study these agents in earlier treatment lines, as monotherapy and/or in combination with immunotherapy. The EV-302 phase III trial tested enfortumab vedotin in combination with the PD1 antibody pembrolizumab versus SOC platinum-based chemotherapy in patients with previously untreated metastatic urothelial cancer, finding a doubling in OS and reshaping the first-line treatment standard for this disease for the first time in decades (106). T-DXd is also currently being investigated with or without pertuzumab versus a taxane, trastuzumab, and pertuzumab as the first-line treatment for patients with HER2$^+$ metastatic breast cancer (DESTINY-Breast09). Similar to solid tumors, ADCs for hematologic malignancies have also first been developed as a later line of treatment, then advancing into early lines as monotherapy or in combinations with other agents. For example, brentuximab vedotin was initially approved by the FDA as a single agent for patients with classical Hodgkin lymphoma after relapse and, in 2018, received approval in combination with chemotherapy for patients with previously untreated classical Hodgkin lymphoma (107).

Notably, the relevant activity in the metastatic setting has led to attempts to use ADCs in the curative setting. T-DM1 is already established as an adjuvant "escalation" treatment for patients who are found to have residual disease at surgery after neoadjuvant treatment for HER2-positive breast cancer (108) and is also endorsed by the National Comprehensive Cancer Network (NCCN) for the adjuvant treatment of patients with stage I HER2-positive tumors (109). Given the improved efficacy that multiple ADCs have demonstrated in the metastatic setting over traditional chemotherapy, there are high expectations about their performance in the early-stage setting, and several studies are ongoing to understand if novel ADCs may be able to reduce the rates of recurrence and fully unleash their potential in the cure of cancer.

Challenges in the Development and Utilization of ADCs in the Clinic

Despite great successes, the clinical development of ADCs has also faced major challenges in the last few decades (Fig. 6). One key challenge is represented by the toxicity profile, which, as previously mentioned, often resembles the profile of traditional chemotherapy. A second challenge pertains to using ADCs in sequence, which remains controversial due to the potential development of cross-resistance mediated by alterations in the antibody or payload targets. Finally, identifying predictive biomarkers for ADCs has proven difficult, with several ADCs currently approved without a companion biomarker.

Toxicities of ADCs

ADCs have fulfilled the promise in improving antitumor activity of chemotherapy; however, in most instances, they have not yet delivered on improving the safety of anticancer treatment compared with unconjugated chemotherapy (29, 110). Most ADCs, indeed, harbor comparable or worse toxicity than unconjugated chemotherapy, with meta-analyses showing an overall incidence of treatment-related adverse events with ADCs exceeding 90%, of which 46% are grade 3 or higher (33). As mentioned, the high rate of off-target toxicities with ADCs is at least partly related to the pharmacology of these compounds, which are mainly metabolized by normal tissues. Similar to any other oncology drugs, it has been challenging to find the right balance between tolerated doses and efficacious doses for ADCs (111).

Multiple strategies are being deployed to improve the ADC toxicity profile, including optimization of dose and schedule, development of biomarkers for toxicity, implementation of remote monitoring, and education on identifying certain toxicities early on (111). ADC engineering approaches (including site-specific conjugation, stable linkers, and/or antibody masking technologies) have also been used in an attempt to decrease the incidence and severity of side effects and to increase ADC tumor specificity and uptake. So far, these engineering solutions have not yet shown significant improvements in the clinic, and the development of ADCs continues to be primarily empirical.

Sequencing of ADCs

The observation of relevant antitumor activity that can be achieved with ADCs has fueled a rapid expansion in the development of these compounds for treating all cancer types. With numerous ADCs soon expected to enter the clinic, a key question is whether cross-resistance will hamper their activity when used serially within a patient. Some insights have been derived from breast oncology. Three ADCs have gained FDA approval for treating breast cancer: T-DM1, T-DXd, and SG (110). T-DM1 and T-DXd share the exact same antibody targeting HER2 but different payloads, linkers, and DARs (112). Moreover, T-DXd and SG have different antibodies and linkers but share the same TOPO1i mechanism of action of the payload (although with significant payload structural differences between DXd and SN38 and marked differences in linker stability between T-DXd and SG; ref. 113).

The implications of overlapping features (same antibody target or same mechanism of action of the payload) across T-DM1, T-DXd, and SG can be inferred from several prospective and retrospective studies conducted in recent years. The use in sequence of two ADCs sharing the HER2 target but different payload mechanisms of action has mostly shown not to produce significant cross-resistance. For example, in the DESTINY-Breast02 trial, patients with HER2-positive metastatic breast cancer pretreated with T-DM1 were randomized to T-DXd or capecitabine plus lapatinib or trastuzumab. Despite sharing the HER2 target of an ADC that all patients had previously received, and despite virtually all patients having received previously naked trastuzumab, T-DXd was associated with meaningful advantages in PFS and OS over the comparator arm (114). Less data exist on the use of a sequence of two ADCs with different targets but with similar mechanism of action of the payload. However, early-phase data and real-world data from several multicentric experiences show a consistent finding: the use in a sequence of two ADCs carrying a TOPO1i may be associated with a degree of cross-resistance. For instance, in the TROPION-PanTumor01 phase I trial, TOPO1i ADC datopotamab deruxtecan (Dato-DXd) achieved a median PFS of 4.4 months among pretreated patients with metastatic TNBC; however, the median PFS was longer with 7.3 months for patients who were TOPO1i ADC naïve, suggesting an enhanced benefit in this group (115). Similarly, four distinct real-world studies, including a total of 331 patients with metastatic breast cancer, showed that, when using two TOPO1i ADCs in sequence, the second is associated with shorter PFS than the first (116–118). Of note, PFS in single-arm trials and retrospective studies may be confounded by several factors, including the pace of the disease and number of prior treatments, warranting caution in the interpretation of these studies. Lastly, a real-world analysis from China showed that among 79 patients (mostly HER2-positive) with metastatic breast cancer receiving ADCs in sequence, a higher ORR was achieved when the second ADC had a payload with a different mechanism of action (ORR 22.6%) compared with patients who received two ADCs with similar payloads (ORR 5.3%; ref. 119).

A prospective registry and three phase II clinical trials (SATEEN, NCT06100874; TRADE-DXd, NCT06533826; and SERIES, NCT06263543) are ongoing within the Translational Breast Cancer Research Consortium to try to answer prospectively the important question of the sequencing of ADCs with a similar payload. Confirmation of significant cross-resistance between these ADCs would reinforce the need for

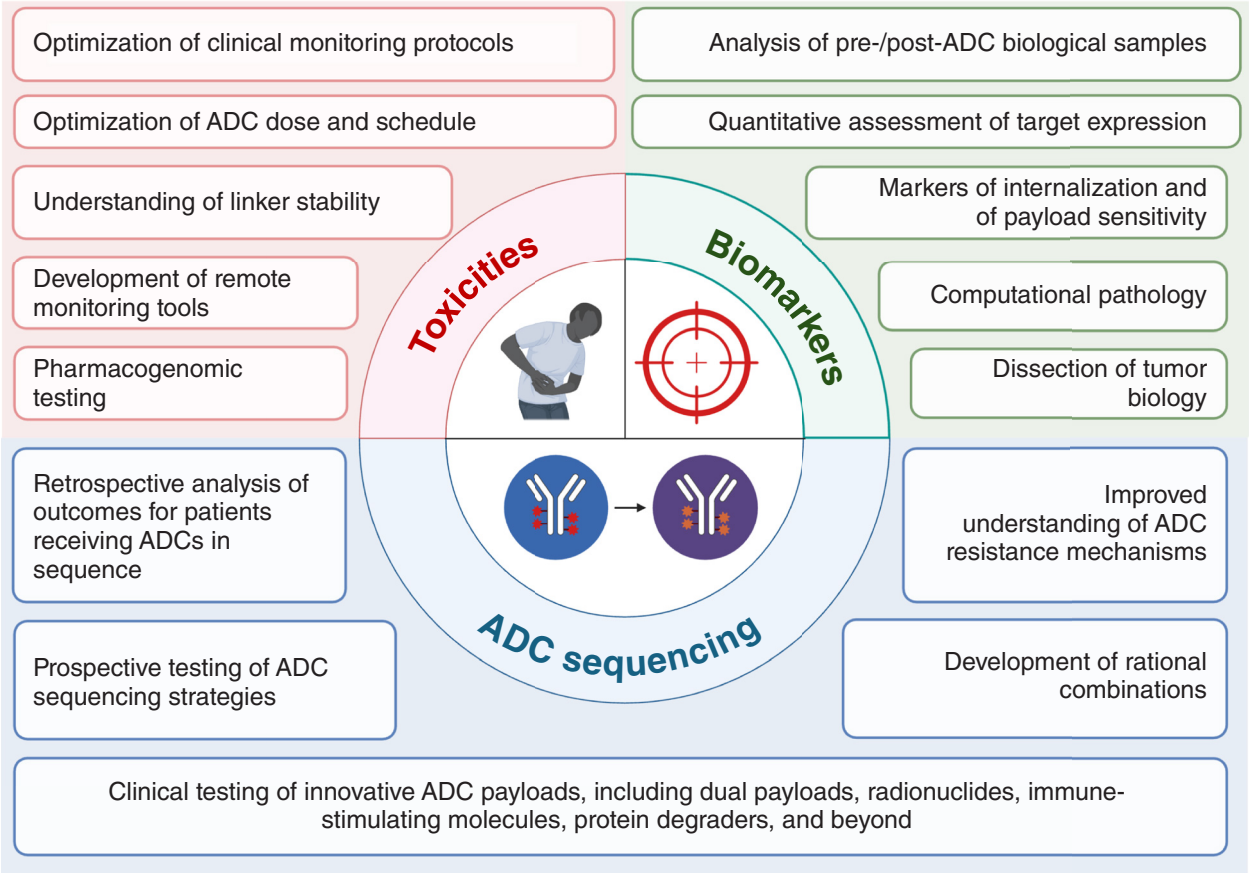

Figure 6. Key challenges in the development of ADCs and potential solutions. Three key challenges will have to be addressed in the future to enhance the clinical value of ADCs for treating patients with cancer, namely, optimizing the toxicity profile of ADCs, identifying predictive and resistance biomarkers, and investigating the impact of sequencing ADCs with overlapping targets and/or payloads with similar mechanisms of action.

payload differentiation. Indeed, despite more than 200 novel ADCs in development, most (Fig. 1) still rely on three mechanisms of action for their payloads: microtubule inhibition, TOPO1 inhibition, and DNA alkylation (7). Expanding the payload pipeline to other cytotoxic categories is expected to positively affect the activity of these compounds, albeit with potentially differing toxicity profiles, but nonetheless should be considered in future ADC development.

The Quest for Predictive Biomarkers for ADCs

The third key challenge in the field of ADCs is the identification of biomarkers that can effectively predict ADC activity and toxicity (11, 120, 121). In theory, given the targeted mechanism of action of ADCs, one would expect higher expression of the antibody target on tumor cells to predict higher activity of the compound. Indeed, certain ADCs exhibit superior efficacy, as indicated by ORR, PFS, and OS, in patients with high target expression. This held true for T-DM1, approved only for patients with HER2-positive breast cancer (122), or mirvetuximab soravtansine, approved only for patients with platinum-resistant ovarian cancer with high folate receptor alpha expression (123). T-DXd showed improved efficacy in patients who were HER2-positive (IHC 3+ or IHC 2+/FISH+) when compared with those who were HER2 low, although meaningful benefits were also observed for patients who were HER2 low or even ultralow.

For most ADCs, however, there is no clear correlation between response rates and target expression. For example, SG (TROP2 ADC) for metastatic TNBC (124), HR+/HER2− breast cancer (16), endometrial cancer (125), and urothelial cancer (126); tisotumab vedotin (tissue factor ADC) for cervical cancer (100); enfortumab vedotin (nectin-4 ADC) for urothelial cancer (127), HR+/HER2− breast cancer, and TNBC (98); brentuximab vedotin (CD30 ADC) for T-cell and B-cell non–Hodgkin lymphomas (128); polatuzumab vedotin (CD79b ADC) in diffuse large B-cell lymphoma (129); and loncastuximab tesirine (CD19 ADC) in B-cell non–Hodgkin lymphoma (130). As a consequence, most of these ADCs are approved without a requirement for prior testing of the corresponding antigen expression. Notably, a recent correlation was demonstrated between nectin-4 membranous expression and the response to enfortumab vedotin in patients with urothelial cancer (131). This finding could help identify patients with the highest likelihood of achieving a durable benefit and potentially guide the selection of therapeutic sequences and combinations for nectin-4 ADCs (132).

Clearly, we need a better understanding of why certain ADCs correlate with tumor target expression, but for other ADCs, there is no correlation (11, 120). As previously discussed in the article, the efficacy of any specific ADC is driven by a complex interrelationship of direct targeted payload delivery, free payload exposure, and tumor subtype sensitivity. Target expression and ADC properties (including linker instabilities) influence sites and rates of ADC disposition and, in turn, payload, tumor, tissue, and systemic exposures. The relationship between efficacy and ADC properties for a given target remains elusive, and several ADCs with similar linker–drug technologies to other approved ADCs failed to demonstrate clinically meaningful efficacy. Possible reasons include tumor type sensitivity to the payload class; tumor versus normal tissue target expression and heterogeneity, and intrinsic properties of the ADC (binding, tumor penetration, internalization, hydrophobicity, PK, etc.). For example, it has been shown preclinically that ADCs with lower binding-site barrier effects achieve better tumor penetration in solid tumors (via co-dosing of naked antibody or via antibody engineering strategies), which correlates with better efficacy (133, 134). In the clinic, this has yet to be fully demonstrated, but there is initial evidence that coadministration of an unconjugated antibody improved the intratumoral distribution of an antibody–dye conjugate (panitumumab-IRDye800CW) in patients (72), as previously discussed. Each of these considerations plays a role in determining the overall efficacy and success of ADCs in a given therapeutic setting. Moreover, an ADC can be pharmacologically active but may fail to demonstrate an advantage over SOC for a particular indication at a tolerated dose. Incidence, severity, and the nature of adverse events are also intricately linked to the interplay of ADC properties, and the fate of the ADC and its payload over time, mirroring the complexities described above for efficacy.

Additionally, data suggest that target expression evaluated with IHC may not be the ideal efficacy predictor, at least for some ADCs. In this setting, a multitude of strategies are being pursued to refine the prediction of efficacy with ADCs. First, novel assays promise to improve the quantitation of ADC target expression; these include quantitative immunofluorescence (135), mass spectrometry (136), reverse-phase protein array (137), and computational pathology-based analysis of IHC slides (138), among others. Target expression on circulating tumor cells and/or dynamics of circulating tumor cells have been also evaluated as predictors of ADC efficacy (139, 140). As previously mentioned, PET-based imaging with ^{89}Zr-trastuzumab is also being tested to predict the activity of trastuzumab bound ADCs, and found to correlate with the activity of T-DM1 (80). Lastly, detection of a specific genomic aberration (e.g., *ERBB2* amplifications) to guide treatment with ADCs has shown promise, with the recent HERALD trial demonstrating an ORR of 56% with T-DXd among patients with advanced solid tumors and with *HER2* amplification identified by plasma cell-free DNA (141).

Concomitantly, efforts are ongoing to investigate the relationship between tumor genomic and transcriptomic profiles with the activity of ADCs. In terms of genomic alterations, a TROP2 (*TACSTD2*) mutation was detected in a patient receiving SG (142). Another case study showed the loss of CD30 target in a patient treated with brentuximab vedotin upon relapse (143). The loss of HER2 (*ERBB2*) is a known resistance mechanism for trastuzumab and T-DM1, also linked to decreased efficacy (144, 145). Similarly, hemizygous deletions in *ERBB2* were associated with low response rates (81) and numerically shorter PFS with T-DXd (146). On the other end, activating mutations of *ERBB2* seem associated with T-DXd efficacy (14, 147), possibly due to enhanced conjugate internalization or tumor sensitivity to the payload (21, 148). Enhanced internalization has also been suggested as a mechanism leading to increased T-DM1 efficacy among patients having tumors overexpressing RAB5a, a protein involved in receptor-mediated endocytosis (149). However, acquired or *de novo* mutations in relationship with ADC efficacy are yet to be determined in larger studies, and the implications for clinical management have to be evaluated.

In addition to target expression and regulation, another active field of investigation is the identification of potential biomarkers associated with sensitivity and resistance to ADC payloads (121). This effort leverages insights gained from small-molecule chemotherapeutics and involves screening for both established and novel biomarkers. For example, intrinsic or acquired high expression of ATP-binding cassette efflux transporters has been linked to chemoresistance, including ADC payload (150). The expression of SLC46A3, a lysosomal membrane transport protein, has been postulated to correlate with DM1 and pyrrolobenzodiazepine sensitivity (151). SLFN11 is a known predictor of sensitivity to DNA-damaging chemotherapy, and it has recently been shown to be a possible biomarker of TOPO1i ADC sensitivity (152, 153). Mutations of the *TOP1* gene have been shown to confer resistance to small-molecule camptothecins and, more recently, have been reported in tumors of a subset of patients treated with TOPO1i ADCs (117, 142). *TUBB3* is a taxane-resistance marker and could indicate resistance to several known microtubule inhibitor ADC payloads (154). UDP-glucuronosyltransferase (encoded by the *UGT1A1* gene) is the enzyme responsible for the glucuronidation of the SN38 payload of SG. High levels of SN38 due to impaired glucuronidation can lead to severe side effects, as have been well demonstrated for irinotecan (155). Among 495 patients enrolled in a basket trial of SG, those with *UGT1A1 *28/*28* (~10%) had an increase in the rate of most side effects, with more than double the risk of severe anemia and neutropenia (124).

Overall, the rapid expansion in the clinical development of ADCs has been paralleled by intense research on predictors of ADC efficacy, resistance, and toxicity. However, to date, most findings remain hypothesis generating, and the use of most ADCs in clinical practice is blinded to any biomarker that may guide their tailored use, with the exception of antibody target expressions for certain ADCs.

Where Do We Go Next?

The current approval pace of oncology-directed ADCs clearly demonstrates that this technology is experiencing an unprecedented expansion. It took ~20 years of research for the first ADC approval in 2000 and has taken more than 10 additional years before a second approval (4). By contrast, eight novel ADCs were approved within only the last 5 years (4), and the first agnostic approval has just been granted to an ADC (37). The expansion in interest, research, funding, and

confidence in ADC technology is fueling the introduction of more than 200 new ADCs currently in phase I testing and the initiation of multiple phase III registrational trials, which are anticipated to lead to several additional approvals in the coming years. This expansion is coinciding with the coming of age of ADC combinations, most preeminently those including immune checkpoint inhibitors (ICI) with ADCs: one ADC/ICI combination has already gained approval for the first-line treatment of metastatic urothelial cancer (97) and multiple such combinations have shown encouraging early-phase data (156). Therefore, we envision part of the future of ADCs in oncology to involve further progress on established themes: more ADCs carrying TOPO1i, microtubule inhibitors, and DNA-damaging agents; testing of approved ADCs in unexplored indications; and evaluation of combinations with ICI and other anticancer agents.

The above strategies will most likely impact practice and lead to the availability of novel, effective single agents and combination strategies for cancer therapeutics. At the same time, fulfilling Paul Erlich's dream of the "magic bullet" will also require further innovations in the design of ADCs. As previously mentioned, indeed, treatment with all the currently approved ADCs is associated with non-negligible side effects, most commonly related to the cytotoxicity of their payload (110). Combining these ADCs with additional agents commonly leads to further worsening in toxicity profile (110, 157), highlighting the importance of developing a new generation of ADCs, which may retain (or improve) the activity of established ADCs, meanwhile minimizing toxicities. The modular structure of ADCs provides a unique opportunity to achieve these tasks by fine-tuning and innovating each component of the ADC (antibody, linker, and payload).

The fine-tuning of ADCs′ molecular features is what has led, for instance, to the remarkable improvement in clinical activity observed with T-DXd over the earlier generation ADC T-DM1 (101). Although both agents comprise the same antibody and both carry cytotoxic payloads, the selection of a cytotoxic molecule with distinct potency and mechanism of action, along with different DAR and linking technology, has ultimately allowed the development of a much more active agent, with more than doubling of the response rate, and improvement in median PFS from ~7 to 29 months for HER2-positive metastatic breast cancer (101). A similar path is being followed by a large proportion of the ADCs currently in the pipeline, which attempt to improve upon the clinical profile of the approved ADCs via slight changes in their standard structure. A different strategy of fine-tuning the characteristics of ADCs involves the coadministration of additional agents, such as pretargeting with naked monoclonal antibodies or the administration of payload-binding molecules to reduce off-target toxicities, as well as the leverage of click chemistry to induce payload activation in the tumor site.

Besides the fine-tuning of ADCs, more striking changes in the ADC molecular structure are also being explored in the quest for more active and safer drugs (158). Innovation can be pursued by replacing one or more of the ADC components with novel formats. For example, through targeting more than one target epitope or protein, biparatopic or bispecific ADCs may enhance selectivity, improve cellular internalization, or expand the patient population that could benefit from the drug; peptide masks can be introduced to avoid binding of the antibody component to the target until the mask is cleaved; and the antibody can be engineered to have a pH-dependent binding or to tune its binding to Fcγ receptors, with the intention to improve selectivity, tolerability, and/or PK profile. The antibody can also be entirely replaced by a much smaller molecule, as in peptide–drug conjugates or antibody fragment–drug conjugates, which are generally cleared more rapidly but have the potential to improve tumor penetration. In terms of conjugation, site-specific technologies allow for the design of homogeneous ADCs with higher stability, dozens of which are currently in clinical testing. Finally, recognizing the major impact elicited in the field by the shift from microtubule inhibitors to TOPO1i payloads has sparked a renewed interest in payload differentiation. This involves the linking of multiple chemotherapy payloads and the exploration of completely distinct payload classes, including immunostimulatory agents, protein degraders, and radioisotopes, among others.

It should be noted that, to date, none of these innovative technologies has yet demonstrated clear signals of improvement in activity or safety compared with standard ADCs. For example, praluzatamab ravtansine (CX-2009) uses a peptide mask aimed at reducing toxicity and has demonstrated low activity (ORR <10%) and relatively common ocular toxicity (43%, 11% grade ≥3) among other associated severe adverse events (159). The doxorubicin peptide–drug conjugate AVA6000, designed to be selectively cleaved in the tumor microenvironment, was found associated with an ORR <5% and relatively high rates of alopecia (52%), fatigue (50%), and nausea (33%; ref. 160). Several site-specific ADCs featuring enhanced linker stability and homogeneity have been associated with high rates of unexpected toxicities. Examples include ARX788 (rate of any-grade keratitis 46%, interstitial lung disease 34%, hepatotoxicity up to 68%; ref. 161), A166 (rate of any-grade keratitis 84%, peripheral neuropathy 53%; ref. 162), and DP303c (any-grade keratitis ~95%; ref. 163), among others. Finally, although signals of activity have been reported with certain ADCs carrying innovative payloads, to date these have never approached the activity observed with more established ADC payloads, and relevant toxicities were observed with some (158). As an example, the immune-stimulating antibody-conjugate NJH395 was tested in a phase I trial, showing no tumor responses and high rates of cytokine release syndrome (55%), pyrexia (44%), and nausea (44%), among others (164). This brief overview of available data with innovative ADCs is far from being comprehensive and is not aimed at discouraging the pursuit of unconventional ADC structures. We believe, however, that these early results further highlight the need for a better understanding of the mechanism of action of ADCs, an understanding that is expected to benefit the clinical development of innovative ADCs as much, if not more, than traditional ADCs. In this setting, differentiating the mechanism of on-target versus off-target toxicities may help further optimize the safety of ADCs. In addition, understanding the impact of novel linkers and conjugation strategies on the whole-body distribution of the ADC and payload is critical to tackle the unexpected toxicity seen with multiple site-specific and/or more stable ADCs. Overall, we believe that embracing the complexity of pharmacology of this class of

agents represents a critical step toward the development of a new generation of ADCs that are safer, more active, and not cross-resistant with currently available ADCs.

CONCLUSION

Forty years of clinical development have turned ADCs from a hypothetical treatment modality to an established and rapidly expanding strategy to treat every type of cancer. Besides helping realize the enormous potential of this class of drugs, the last four decades have been the key to unveil the complexity of the mechanism of action of ADCs, which still remains only partly understood. In this context, the spotlight has gradually moved from the 1% of conjugate that reaches the tumor to the 99% that reaches other parts of the body. A better understanding of how this large component of the injected ADC leads to toxicity and anticancer activity is expected to inform the development of the next generation of ADCs, including those with established and/or innovative formats. The "magic bullet" concept of a drug that can selectively kill tumor cells meanwhile sparing normal cells remains the ultimate goal: to get there, we will have to embrace the failures as much as the successes in the field and keep striving to pursue the right balance in each of the intricate yet fascinating pharmacologic features of ADCs.

Authors' Disclosures

R. Colombo reports patents for WO 2023/178452, WO 2024/082055, and WO 2024/082051 pending. P. Tarantino reports grants and personal fees from AstraZeneca, as well as personal fees from Daiichi Sankyo, Gilead, Roche/Genentech, Eli Lilly and Company, Menarini/Stemline, and Novartis outside the submitted work. J.R. Rich reports patents for WO 2023/178452, WO 2024/082055, WO 2024/082051, and WO 2024/065056 pending, as well as patents for WO 2019/173911 and WO 2015/095953 issued. P.M. LoRusso reports other support from Takeda, SOTIO, Agenus, IQVIA, Pfizer, GlaxoSmithKline, QED Therapeutics, Kyowa Kirin, Kineta, Zentalis Pharmaceuticals, Molecular Templates, ABL Bio, STCube Pharmaceuticals, I-Mab, Seagen, ImCheck, Relay Therapeutics, Stemline, MEKanistic, Mersana Therapeutics, BAKX Therapeutics, Scenic Biotech, Qualigen, Roivant Sciences, NeuroTrials, Actuate Therapeutics, Atreca, Amgen CodeBreak 202, Cullinan, Dren Bio, Quanta Therapeutics, Schrodinger, Boehringer Ingelheim, Prelude, Wells Therapeutics, Zai Lab, and Modifi Bio outside the submitted work. E.G.E. de Vries reports other support from NSABP, Daiichi Sankyo, and Crescendo Biologics and grants from Amgen, Genentech, Roche, Bayer, Servier, Regeneron, and Crescendo Biologics outside the submitted work, and all paid to the institution.

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