

# Resistance to Antibody–Drug Conjugates

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## Abstract

Antibody–drug conjugates (ADC) are multicomponent molecules constituted by an antibody covalently linked to a potent cytotoxic agent. ADCs combine high target specificity provided by the antibody together with strong antitumoral properties provided by the attached cytotoxic agent. At present, four ADCs have been approved and over 60 are being explored in clinical trials. Despite their effectiveness, resistance to these

drugs unfortunately occurs. Efforts to understand the bases underlying such resistance are being carried out with the final purpose of counteracting them. In this review, we report described mechanisms of resistance to ADCs used in the clinic along with other potential ones that may contribute to resistance acquisition. We also discuss strategies to overcome resistance to ADCs. *Cancer Res*; 78(9); 2159–65. ©2018 AACR.

## Introduction

Antibody–drug conjugates (ADC) are multicomponent therapies that combine an antibody and a cytotoxic agent. Because of the characteristic high specificity of the antibodies, these antitumoral therapies offer the possibility to specifically deliver highly potent drugs to target tumor cells expressing the antigen (1). At the time of preparing this review, four ADCs have been approved, although there are currently over 60 ADCs in clinical trials (2). In 2011, brentuximab vedotin (BV) was approved by the FDA for the treatment of Hodgkin lymphoma (HL), after autologous stem cell transplant failure, and systemic anaplastic large cell lymphoma (ALCL), after multiagent chemotherapy failure (3). This compound targets CD30 and delivers the monomethyl auristatin E (MMAE) via a protease-cleavable linker. In 2013, trastuzumab emtansine (T-DM1) gained FDA approval for HER2-positive metastatic breast cancer (4). T-DM1 combines the humanized antibody against HER2 trastuzumab with a potent antimicrotubule agent, the maytansinoid DM1 (5). In 2017, two more ADCs have been approved by the FDA, both including a cytotoxic agent from the class of calicheamicins. Inotuzumab ozogamicin (IO), which targets CD22 was developed for relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL; ref. 6). Gemtuzumab ozogamicin (GO), which targets CD33, was developed for the treatment of adults with newly diagnosed CD33-positive acute myeloid leukemia (AML) and for patients ages 2 years and older with relapsed or refractory CD33-positive AML. Of note, GO originally received accelerated approval in May 2000 but was voluntarily withdrawn from the market after trials failed to verify clinical benefit and

demonstrated safety concerns. The current approval includes a lower recommended dose, a different schedule and a new patient population (7).

## Components of ADCs

### Antibody moiety

Monoclonal antibodies (mAb) constitute the backbone of ADCs, and recognize antigens present in cancer cells. The antigen should be able to internalize so that the bound ADC is transported into the cell, where the cytotoxic agent exerts its antitumor action (8). It is relevant to mention that mAbs themselves may exert antitumor actions, e.g., by decreasing signals that emanate from their targets and that stimulate tumor progression, and also through Fc-mediated effector functions. In fact, naked mAbs have been used as single agents for treating cancer, as is the case of trastuzumab in HER2<sup>+</sup> tumors or cetuximab in colorectal cancer (9–11). However, the validation of activity of the naked mAb is not a requirement for the development of an active ADC (2). In addition, it is desirable that the immune-mediated antitumor actions of the mAbs are maintained when conjugated to the cytotoxic agent. In this respect, it has been reported that T-DM1 preserves the capability to promote antibody-dependent cell mediated cytotoxicity (ADCC; ref. 12). However, some antibodies have been engineered to minimize immune system-mediated actions, in an attempt to mitigate secondary side effects such as thrombocytopenia (13). Isotype selection of the mAb influences in this aspect. Most approved mAbs belong to three human IgG isotypes: IgG1, IgG2 and IgG4 (Fig. 1). IgG1 can usually support ADCC, whereas IgG2 and IgG4 are typically inefficient or limited in this function (14). Several conjugation chemistries of the cytotoxic agent have been described (15) and they are briefly schematized in Fig. 1.

### Cytotoxic agents used in ADCs

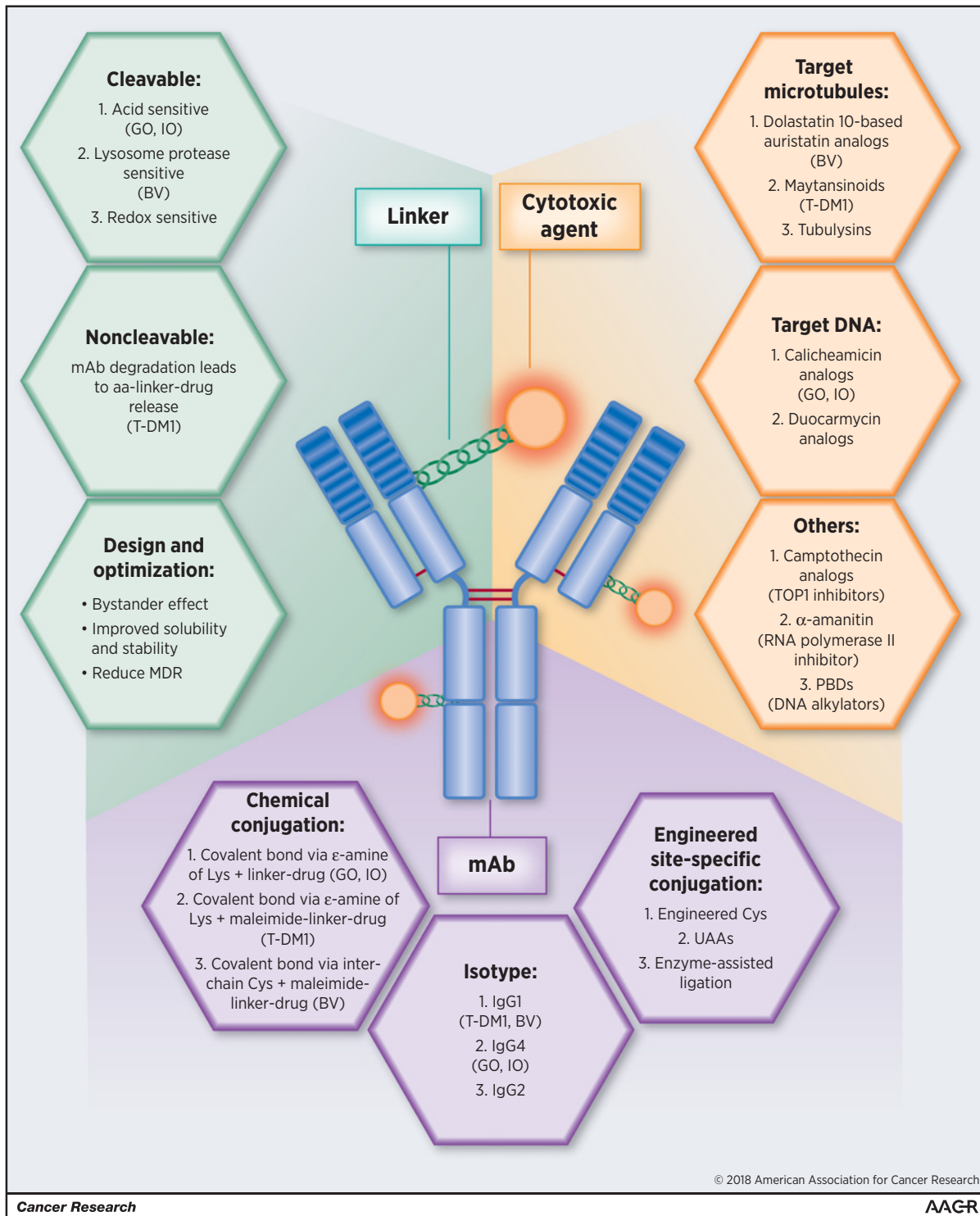
The most frequently used cytotoxic agents are DNA damaging or antimicrotubule compounds (2). The first group comprises calicheamicin analogs [used in GO (ref. 7) and IO (ref. 6)], and duocarmycin analogs (16). The second group includes dolastatin 10-based auristatin analogs (used in BV; ref. 3), maytansinoids (used in T-DM1; ref. 4) and tubulysins (13). Other drugs used in clinical-stage ADCs are topoisomerase inhibitors (camptothecin

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**Figure 1.**

ADCs design landscape. The rational design of the three main components of an ADC is crucial for its success. Two classes of linkers (green), cleavable and noncleavable, can be optimized to improve stability and solubility of ADCs, to influence bystander effect, or to circumvent multidrug resistance. The two main groups of cytotoxic agents (yellow) target either DNA or microtubule, although other cytotoxic agents used in ADCs can inhibit enzymes. mAbs (purple) are based on three isotypes, which can be conjugated to the linker-cytotoxic, both by thiosuccinimide linkage to different amino acid residues (chemical conjugation) or by site-specific conjugation. The last one can be achieved by (i) insertion of additional engineered cysteine residues, (ii) insertion of genetically encoded unnatural amino acids (UAA), and (iii) enzyme-assisted ligation by formylglycine-generating enzyme, transglutaminases, or sortases. Each section of the figure shows examples of commercially available ADCs that make use of different component options.

analogs; ref. 17) and DNA-alkylators (pyrrolobenzodiazepines, PBDs; ref. 18) as well as RNA polymerase II inhibitors (e.g.,  $\alpha$ -amanitin; ref. 19). These drugs have been selected for high cytotoxicity, so that they can destroy tumor cells at the intracellular concentrations achieved after ADC delivery.

### Linker design and optimization

The linker used to bind the cytotoxic agent to the antibody must have high stability in circulation, because unstable binding can lead to the delivery of the agent in the bloodstream causing toxicity and a decrease of efficacy (20). Yet, it should allow efficient release of the drug once the ADC is internalized. Two main classes of linkers are currently used in the clinic: cleavable and noncleavable (Fig. 1).

Cleavable linkers can be processed chemically or enzymatically. Three different types of release mechanisms have been developed: (i) Acid sensitive, such as hydrazone linkers, that are cleaved in the lysosome as a result of low pH (21); (ii) lysosomal protease sensitive, such as valine-alanine and valine-citrulline peptide linkers, that are cleaved by lysosomal enzymes (22); and (iii) redox-sensitive, such as disulfide bond-based linkers, that are reduced intracellularly (23).

Noncleavable linkers are highly stable in circulation and inside the cell, so they depend on complete proteolytic degradation of the mAb moiety in the lysosome after internalization of the ADC. The active catabolite released includes the cytotoxic agent joined to the linker still attached to an amino acid residue of the mAb (24). Examples of noncleavable linkers include the thioether linker used in T-DM1 (25) or maleimidocaproic acid linked to monomethyl auristatin F (mc-MMAF), used in some ADCs under clinical evaluation, such as depatuzumab mafodotin (26).

## Mechanisms of Resistance to ADCs

Drug resistance consists in the failure or reduction of effectiveness of a treatment. Such failure/reduction may have evolved after treatment with the drug (secondary or acquired resistance) or may be present from the start of the treatment (primary or de novo resistance). In principle, mechanisms of resistance to ADCs could be similar to those raised against the individual components of the ADC, namely the mAb and the cytotoxic drug. Although future studies will define that, current available clinical data indicate that patients that become resistant to trastuzumab + a taxane still respond to T-DM1 (4), demonstrating that there is no association between T-DM1 activity and previous treatment lines, including anti-HER2 therapies or chemotherapies.

### Antigen-related resistance

ADCs are targeted therapeutics, so one predicted mechanism of resistance could consist in changes in the levels of the antigen recognized by the mAb (Fig. 2).

In a study conducted by Loganzo and colleagues (27), several breast cancer cell lines were made resistant to T-DM1 by multiple cycles of exposure to an anti-HER2 trastuzumab-maytansinoid ADC structurally similar to T-DM1. They observed that the JIMT1-TM-resistant cells showed a marked decrease in HER2 protein levels after several months from the initiation of the treatment.

BV was also used to select resistant HL and ALCL cell lines in a study carried out by Chen and colleagues (28). The resistant ALCL cell line, but not the HL one, demonstrated downregulated CD30 expression compared with the parental cell line. They also ana-

lyzed tissue biopsy samples from patients who had relapsed or progressed after BV treatment but, in this case, all of them expressed CD30. However, a recent study reported loss of CD30 expression in nodules from an ALCL patient treated with BV (29).

Paradoxically, a high antigen expression may reduce effectiveness of the ADC, likely due to a reduced drug exposure. This is the case of GO, where a high CD33 antigen load in peripheral blood is an adverse prognostic factor. High CD33 loads in blood consume GO and thereby limit its penetration in the bone marrow (30).

Truncation of the ectodomain of the antigen or its masking by extracellular matrix components have been described for HER2 as mechanisms of resistance to trastuzumab (31, 32). However, masking or truncation of the epitope has not been reported yet in preclinical models as mechanisms of resistance to ADCs.

Finally, presence of ligands of the antigens could modulate sensitivity to ADCs. Some studies have suggested that ligands such as neuregulin, that promotes heterodimerization of HER2 with HER3 and HER4, can impair the efficacy of T-DM1 (33).

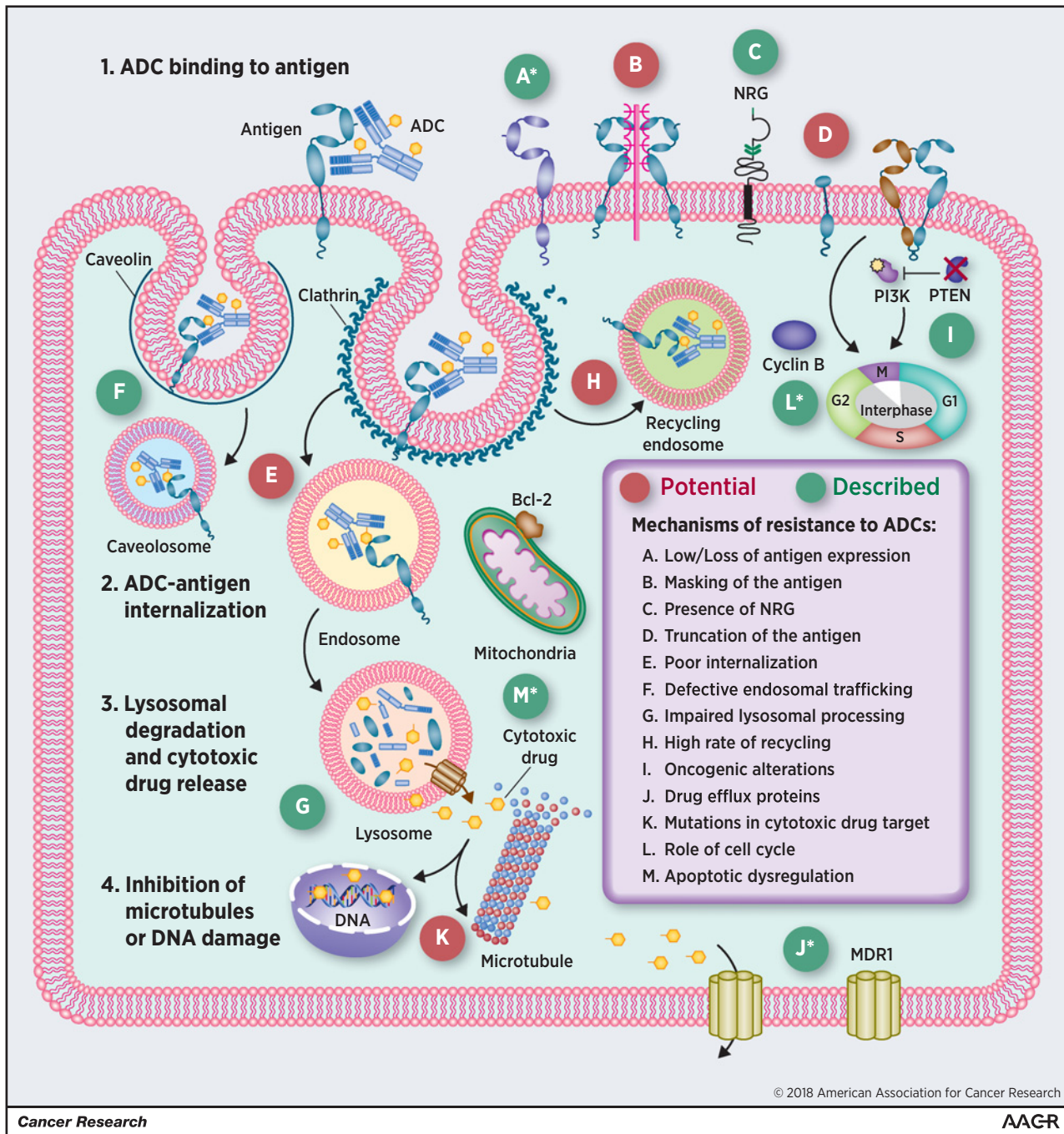
### Defects in internalization and trafficking pathways

ADC optimal efficacy requires endocytic uptake of the antibody into the cell (Fig. 2). Endocytosis can occur by different internalization routes such as clathrin-mediated (CME), caveolin-mediated and clathrin-caveolin-independent endocytosis. CME has been reported as the central route adopted by various ADCs (34). Sung and colleagues (35) have described that N87-TM cells made resistant to T-DM1 internalize trastuzumab-ADCs into caveolin-1 (CAV1)-coated vesicles. T-DM1 colocalization with CAV1 correlated with reduced response to the drug in a panel of HER2<sup>+</sup> cell lines, suggesting that caveolae-mediated endocytosis of T-DM1 may predict response. In those cells that accumulated the ADC in CAV1 vesicles, lysosomal colocalization was significantly decreased, suggesting that delivery of T-DM1 to lysosomes was inefficient. In line with this, it was found that sensitivity to an antimelanotransferrin ADC (L49-valine-citrulline-MMAF) correlated with its intracellular fate (36). In sensitive cell lines that ADC colocalized with lysosomal markers, whereas in resistant cells, with low antigen levels, the ADC colocalized with CAV1.

### Impaired lysosomal function

ADCs need to reach the lysosomes, where the cytotoxic agent is released by chemical or enzymatic cleavage. In cells made resistant to T-DM1 by prolonged exposure to the drug, lysosomal accumulation of T-DM1 has been observed (37). In these cells, the drug reached the lysosomal compartment, but the proteolytic activity was below that present in sensitive cells. Such deficiency was due to increased lysosomal pH, which in turn inhibited lysosomal proteolytic enzymes. In theory, all ADCs in which lysosomal acidic proteases play a role in the degradation of the ADC could be exposed to this mechanism of resistance. This would include both ADCs with noncleavable linkers as well as lysosomal protease sensitive cleavable linkers. Of note, lysosomal storage diseases, characterized by accumulation of undigested molecules (38), represent a clinical precedent that sustains the possibility that derangement of lysosomal function may affect ADC catabolism in patients. On the other side, it has been recently described that ADCs with the valine-citrulline linker have multiple paths to produce active catabolites and cathepsin B is dispensable for their processing (39).

Another mechanism of resistance to ADCs is related to transport of the cytotoxic agent from the lysosomal lumen to the



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**Figure 2.**

Mechanisms of resistance to ADCs. The antibody binds to its target on the plasma membrane (1); then, ADC-target complexes enter cells via receptor-mediated endocytosis (2). The internalized complexes are initially contained within endocytic vesicles that fuse to become early endosomes and eventually mature to lysosomes (3). The ADC undergoes catabolism to release the cytotoxic agent, which can then be transported from the lumen of lysosomes to the cytosol. The intracellular cytotoxic agent exerts its action, generally damaging DNA or inhibiting microtubule polymerization (4), which ultimately leads to cell death. Alterations in any of these events may lead to resistance acquisition. Circled letters indicate potential (red) or already described in the literature (green) mechanisms of resistance to ADCs. The asterisks indicate mechanisms of resistance verified using patient-derived material.

cytoplasm. This has a special relevance in ADCs with noncleavable linkers, of which catabolism releases the linker-cytotoxic agent attached to an amino acid residue. Lysosomal membranes are

impermeable to these catabolites requiring transport mechanisms to move them from the lysosomal lumen to the cytosol. To identify potential lysosomal transporters, Hamblett and

colleagues (40) performed a phenotypic shRNA screen with an anti-CD70 maytansine-based ADC. This screen identified the lysosomal membrane protein SLC46A3, whose genetic attenuation inhibited the potency of multiple noncleavable antibody-maytansine ADCs, including T-DM1.

### Drug efflux pumps

A common mechanism of resistance for chemotherapies is the elimination of the agent from the cellular cytoplasm by the ATP-binding cassette (ABC) transporters (41). These drug efflux pumps might also contribute to resistance to ADCs because many of the cytotoxic agents are substrates of ABC transporters (42, 43).

Preclinical models have reported expression of PgP/MDR1 in AML cells made resistant to GO (44). More important, clinical data suggest that MDR1 activity is significantly associated with patient outcome. It has been observed that AML blasts of responders to GO have a significantly lower MDR1 activity, assessed by efflux of a fluorescent PgP substrate, compared with nonresponders (45). Similar results were obtained with IO, which also uses calicheamicin as cytotoxic (46).

ADCs based on auristatin analogues, such as BV, may also select cell populations with MDR1 expression after chronic treatment. This was observed in HL cells lines and samples from patients that relapsed or were resistant to BV (28). In another study, Yu and colleagues (47) derived cell lines from *in vivo* xenograft tumors of non-Hodgkin lymphoma (NHL) that were made resistant to anti-CD22-valine-citrulline-MMAE and anti-CD79b-valine-citrulline-MMAE and identified MDR1 as the major driver of resistance to the valine-citrulline-MMAE-based conjugates.

Maytansinoids are also substrates of drug transporters such as MDR1, so resistance to ADCs such as T-DM1 could be also linked to MDR1 activity (5). It has been recently described that in HER2<sup>+</sup> gastric cancer cells made resistant to T-DM1 (N87-TDMR), the ABC transporters ABCC2 and ABCG2 were upregulated. Furthermore, inhibition of ABCC2 and ABCG2 restored T-DM1 sensitivity (48). In other preclinical model of T-DM1 resistance (361-TM), functional induction of MRP1 was observed, and an MRP1 reversal agent or siRNA-mediated knockdown of MRP1 restored sensitivity (27).

### Alterations in the target

A potential mechanism of resistance to ADCs could be mutations in the cellular target for the cytotoxic agent. However, there are no reported ADC-resistant models with mutations in tubulin, topoisomerase I or RNA polymerase II.

### Role of cell cycle

One mechanism of resistance to T-DM1 recently proposed relates to the effect of the drug on cyclin B, a cell-cycle protein that participates in G<sub>2</sub>-M transition. In HER2<sup>+</sup> breast cancer cells sensitive to T-DM1, the drug causes an increase in cyclin B, whereas in cells made resistant to T-DM1 such increase was not observed (49). Moreover, silencing of cyclin B resulted in resistance to the drug. Interestingly, in a patient cohort of 18 HER2<sup>+</sup> breast cancer fresh explants, the antitumor action of T-DM1 paralleled cyclin B accumulation. These findings are clinically relevant, as cyclin B induction could be used to biomark T-DM1 sensitivity.

Cell-cycle dynamics may affect sensitivity to ADCs such as GO. Resting leukemic cells were not only less efficient in taking up GO but also less sensitive to the cytotoxic action of calicheamicin, whereas actively cycling cells were more sensitive to GO (50).

### Activation of signaling pathways

Activation of downstream signaling pathways may contribute to the acquisition of resistance to ADCs. Activated PI3K/AKT signaling has been associated with GO resistance *in vitro* in primary AML cells. In this study, the AKT inhibitor MK-2206 significantly sensitized resistant cells to GO or free calicheamicin (51). Although mutations in *PIK3CA* or deletions in *PTEN* represent known mechanisms of resistance to trastuzumab (52), no molecular activation of the PI3K route has been described yet as a mechanism of resistance to T-DM1. Interestingly, one clinical study is currently exploring the safety and early signs of efficacy of the combination of T-DM1 with a PI3K inhibitor (Clinical trials identifier: NCT02038010).

### Apoptotic dysregulation

Changes in apoptotic regulation may also modulate sensitivity to ADCs. A role for the pro-apoptotic proteins BAX and BAK in the regulation of GO sensitivity in AML has been described previously (53). Furthermore, the overexpression of the antiapoptotic proteins BCL-2 and BCL-X has been linked to GO resistance (54). Actually, a BCL-2 antisense (oblimersen sodium) has been combined with GO in older patients with AML in first relapse (55).

In NHL cell lines, Dorman and colleagues (56) found that the expression level of BCL-XL correlated with reduced sensitivity to anti-CD79b-valine-citrulline-MMAE. *In vivo* data showed that a BCL-2 family inhibitor, ABT-263, enhanced the activity of the ADC. These findings could be relevant for resistance to BV, because both ADCs are structurally similar.

## Strategies to Overcome Resistance and to Optimize ADCs-Based Therapies

Resistance to ADCs has been one of the factors that has limited the clinical success to these drugs. The modular structure of ADCs offers the possibility of modifying some of their components to develop new compounds capable of overcoming resistance.

One of the most frequent mechanisms of resistance to ADCs is increased expression of drug efflux pumps. A strategy to circumvent this is to change the cytotoxic agent for drugs or toxins that are poor efflux substrates. For example, vadastuximab talirine, an anti-CD33 antibody coupled to PBD, demonstrated robust activity in AML animal models, including those in which GO had minimal effect (57). Another example is DS-8201a, an anti-HER2 ADC incorporating a novel DNA topoisomerase I inhibitor, which overcame T-DM1 resistance caused by aberrant expression of ABC transporters in HER2-positive gastric cancer (48). In addition, changing auristatin-based ADCs for anthracycline-based ADCs has also been successful in NHL tumor models with acquired resistance (47). A second strategy that can be used is based on modification of the linker, increasing its hydrophilicity, which can reduce MDR due to the fact that MDR1 transports hydrophobic compounds more efficiently than hydrophilic compounds. Sulfo-SPDB (58) and mal-PEG4-N-hydroxysuccinimide are examples of polar linkers that have shown improved potency against MDR1<sup>+</sup> models (42).

The linker-cytotoxic structure can be modified to optimize ADCs (2). Heterogeneity within tumors is a main issue in cancer and this may lead to ADCs inability to kill low-antigen-expressing cells. However, ADCs may be designed to eradicate not only antigen-positive cells but also other surrounding cells, irrespective of the expression of the target antigen on their surface. This so-called bystander effect depends on the charge of the linker-cytotoxic. For

example, ADCs that incorporate MMAE as cytotoxic agent or are linked via a cleavable disulfide bond, such as the maytansinoid tubulin inhibitor DM4, release catabolites that are neutral and cross biomembranes killing neighboring cells (59, 60).

New formats of mAbs, such as bispecific or biparatopic ADCs may also contribute to overcome resistance. This has been demonstrated for HER2. The first biparatopic ADC, targeting two nonoverlapping epitopes on HER2 was able to induce HER2 receptor clustering, which in turn promoted robust internalization and degradation, and also demonstrated antitumor activity in T-DM1-resistant tumor models (13). Notably, this biparatopic ADC has entered phase I trials in patients who are refractory to or ineligible for HER2-targeted therapies. Other useful approach may be coupling an ADC target to a rapidly internalizing protein using a bispecific antibody. Recently, Andreev and colleagues (61) demonstrated that a bispecific antibody that binds HER2 and the prolactin receptor at the cell surface dramatically enhanced the degradation of HER2 as well as the cell killing activity of a noncompeting HER2 ADC.

Finally, a promising approach consists of combinations of ADCs with other immunotherapies (62). Addition of ADCs to immune checkpoint inhibitors may increase the recruitment of CD8<sup>+</sup> effector T cells to tumor tissues improving the clinical response. Various clinical studies are ongoing evaluating combinations of T-DM1 or BV with PD-L1 or PD-1 inhibitors

(Clinical trials identifiers: NCT02924883, NCT03032107 and NCT01896999).

Drug refractoriness is still a major issue in oncology and little is known about the molecular basis underlying resistance, besides some well-described mechanisms, such as drug efflux pumps. Very recently, novel possible mechanisms of resistance related to the cell biology of ADCs have been described bringing light about options for therapeutic intervention. The future clinical development of ADCs could benefit from the identification of druggable mechanisms of resistance and optimal drug combinations, to maximize their therapeutic effect.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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## References

1. Vezina HE, Cotreau M, Han TH, Gupta M. Antibody-drug conjugates as cancer therapeutics: past, present, and future. *J Clin Pharmacol* 2017;57: S11–S25.
2. Beck A, Goetsch L, Dumontet C, Corvaia N. Strategies and challenges for the next generation of antibody-drug conjugates. *Nat Rev Drug Discov* 2017; 16:315–37.
3. Senter PD, Sievers EL. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. *Nat Biotechnol* 2012;30:631–7.
4. Amiri-Kordestani L, Blumenthal GM, Xu QC, Zhang L, Tang SW, Ha L, et al. FDA approval: ado-trastuzumab emtansine for the treatment of patients with HER2-positive metastatic breast cancer. *Clin Cancer Res* 2014;20: 4436–41.
5. Lambert JM, Chari RV. Ado-trastuzumab Emtansine (T-DM1): an antibody-drug conjugate (ADC) for HER2-positive breast cancer. *J Med Chem* 2014;57:6949–64.
6. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *N Engl J Med* 2016;375:740–53.
7. Appelbaum FR, Bernstein ID. Gemtuzumab ozogamicin for acute myeloid leukemia. *Blood* 2017;130:2373–76.
8. Damelin M, Zhong W, Myers J, Sapra P. Evolving strategies for target selection for antibody-drug conjugates. *Pharm Res* 2015;32:3494–507.
9. Taylor C, Hershman D, Shah N, Suci-Foca N, Petrylak DP, Taub R, et al. Augmented HER-2 specific immunity during treatment with trastuzumab and chemotherapy. *Clin Cancer Res* 2007;13:5133–43.
10. Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM, Fox JA. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin Oncol* 1999;26:60–70.
11. Esparis-Ogando A, Montero JC, Arribas J, Ocana A, Pandiella A. Targeting the EGF/HER ligand-receptor system in cancer. *Curr Pharm Des* 2016; 22:5887–98.
12. Junttila TT, Li G, Parsons K, Phillips GL, Sliwkowski MX. Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. *Breast Cancer Res Treat* 2011;128:347–56.
13. Li JY, Perry SR, Muniz-Medina V, Wang X, Wetzel LK, Rebelatto MC, et al. A Biparatopic HER2-targeting antibody-drug conjugate induces tumor regression in primary models refractory to or ineligible for HER2-targeted therapy. *Cancer Cell* 2016;29:117–29.
14. Wang X, Mathieu M, Brezski RJ. IgG Fc engineering to modulate antibody effector functions. *Protein Cell* 2018;9:63–73.
15. Tsuchikama K, An Z. Antibody-drug conjugates: recent advances in conjugation and linker chemistries. *Protein Cell* 2018;9:33–46.
16. van der Lee MM, Groothuis PG, Ubink R, van der Vleuten MA, van Achterberg TA, Loosveld EM, et al. The preclinical profile of the duocarmycin-based HER2-Targeting ADC SYD985 predicts for clinical benefit in low HER2-expressing breast cancers. *Mol Cancer Ther* 2015;14:692–703.
17. Nakada T, Masuda T, Naito H, Yoshida M, Ashida S, Morita K, et al. Novel antibody drug conjugates containing exatecan derivative-based cytotoxic payloads. *Bioorg Med Chem Lett* 2016;26:1542–45.
18. Mantaj J, Jackson PJ, Rahman KM, Thurston DE. From anthramycin to pyrrolbenzodiazepine (PBD)-containing antibody-drug conjugates (ADCs). *Angew Chem Int Ed Engl* 2017;56:462–88.
19. Liu Y, Zhang X, Han C, Wan G, Huang X, Ivan C, et al. TP53 loss creates therapeutic vulnerability in colorectal cancer. *Nature* 2015;520:697–701.
20. Thomas A, Teicher BA, Hassan R. Antibody-drug conjugates for cancer therapy. *Lancet Oncol* 2016;17:e254–e62.
21. Nolting B. Linker technologies for antibody-drug conjugates. *Methods Mol Biol* 2013;1045:71–100.
22. Dubowchik GM, Firestone RA, Padilla L, Willner D, Hofstead SJ, Mosure K, et al. Cathepsin B-labile dipeptide linkers for lysosomal release of doxorubicin from internalizing immunoconjugates: model studies of enzymatic drug release and antigen-specific in vitro anticancer activity. *Bioconjug Chem* 2002;13:855–69.
23. Erickson HK, Widdison WC, Mayo MF, Whiteman K, Audette C, Wilhelm SD, et al. Tumor delivery and in vivo processing of disulfide-linked and thioether-linked antibody-maytansinoid conjugates. *Bioconjug Chem* 2010;21:84–92.
24. Erickson HK, Park PU, Widdison WC, Kovtun YV, Garrett LM, Hoffman K, et al. Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. *Cancer Res* 2006;66:4426–33.
25. Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res* 2008;68:9280–90.

26. Phillips AC, Boghaert ER, Vaidya KS, Mitten MJ, Norvell S, Falls HD, et al. ABT-414, an antibody-drug conjugate targeting a tumor-selective EGFR epitope. *Mol Cancer Ther* 2016;15:661-9.
27. Loganzo F, Tan X, Sung M, Jin G, Myers JS, Melamud E, et al. Tumor cells chronically treated with a trastuzumab-maytansinoid antibody-drug conjugate develop varied resistance mechanisms but respond to alternate treatments. *Mol Cancer Ther* 2015;14:952-63.
28. Chen R, Hou J, Newman E, Kim Y, Donohue C, Liu X, et al. CD30 downregulation, MMAE resistance, and MDR1 upregulation are all associated with resistance to brentuximab vedotin. *Mol Cancer Ther* 2015;14:1376-84.
29. Al-Rohil RN, Torres-Cabala CA, Patel A, Tetzlaff MT, Ivan D, Nagarajan P, et al. Loss of CD30 expression after treatment with brentuximab vedotin in a patient with anaplastic large cell lymphoma: a novel finding. *J Cutan Pathol* 2016;43:1161-66.
30. van der Velden VH, Boeckx N, Jedema I, te Marvelde JG, Hoogeveen PG, Boogaerts M, et al. High CD33-antigen loads in peripheral blood limit the efficacy of gemtuzumab ozogamicin (Mylotarg) treatment in acute myeloid leukemia patients. *Leukemia* 2004;18:983-8.
31. Nagy P, Friedlander E, Tanner M, Kapanen AJ, Carraway KL, Isola J, et al. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Res* 2005;65:473-82.
32. Scaltriti M, Rojo F, Ocana A, Anido J, Guzman M, Cortes J, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst* 2007;99:628-38.
33. Phillips GD, Fields CT, Li G, Dowbenko D, Schaefer G, Miller K, et al. Dual targeting of HER2-positive cancer with trastuzumab emtansine and pertuzumab: critical role for neuregulin blockade in antitumor response to combination therapy. *Clin Cancer Res* 2014;20:456-68.
34. Kalim M, Chen J, Wang S, Lin C, Ullah S, Liang K, et al. Intracellular trafficking of new anticancer therapeutics: antibody-drug conjugates. *Drug Des Devel Ther* 2017;11:2265-76.
35. Sung M, Tan X, Lu B, Golas J, Hosselet C, Wang F, et al. Caveolae-mediated endocytosis as a novel mechanism of resistance to trastuzumab emtansine (T-DM1). *Mol Cancer Ther* 2018;17:243-53.
36. Smith LM, Nesterova A, Alley SC, Torgov MY, Carter PJ. Potent cytotoxicity of an auristatin-containing antibody-drug conjugate targeting melanoma cells expressing melanotransferrin/p97. *Mol Cancer Ther* 2006;5:1474-82.
37. Rios-Luci C, Garcia-Alonso S, Diaz-Rodriguez E, Nadal-Serrano M, Arribas J, Ocana A, et al. Resistance to the antibody-drug conjugate T-DM1 is based in a reduction in lysosomal proteolytic activity. *Cancer Res* 2017;77:4639-51.
38. Schultz ML, Tecedor L, Chang M, Davidson BL. Clarifying lysosomal storage diseases. *Trends Neurosci* 2011;34:401-10.
39. Caculitan NG, Dela Cruz Chuh J, Ma Y, Zhang D, Kozak KR, Liu Y, et al. Cathepsin B is dispensable for cellular processing of Cathepsin B-Cleavable antibody-drug conjugates. *Cancer Res* 2017;77:7027-37.
40. Hamblett KJ, Jacob AP, Gurgel JL, Tometsko ME, Rock BM, Patel SK, et al. SLC46A3 is required to transport catabolites of noncleavable antibody maytansine conjugates from the lysosome to the cytoplasm. *Cancer Res* 2015;75:5329-40.
41. Yu M, Ocana A, Tannock IF. Reversal of ATP-binding cassette drug transporter activity to modulate chemoresistance: why has it failed to provide clinical benefit? *Cancer Metastasis Rev* 2013;32:211-27.
42. Kovtun YV, Audette CA, Mayo MF, Jones GE, Doherty H, Maloney EK, et al. Antibody-maytansinoid conjugates designed to bypass multidrug resistance. *Cancer Res* 2010;70:2528-37.
43. Cianfriglia M. The biology of MDR1-P-glycoprotein (MDR1-Pgp) in designing functional antibody drug conjugates (ADCs): the experience of gemtuzumab ozogamicin. *Ann Ist Super Sanita* 2013;49:150-68.
44. Matsumoto T, Jimi S, Hara S, Takamatsu Y, Suzumiya J, Tamura K. Importance of inducible multidrug resistance 1 expression in HL-60 cells resistant to gemtuzumab ozogamicin. *Leuk Lymphoma* 2012;53:1399-405.
45. Walter RB, Gooley TA, van der Velden VH, Loken MR, van Dongen JJ, Flowers DA, et al. CD33 expression and P-glycoprotein-mediated drug efflux inversely correlate and predict clinical outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin monotherapy. *Blood* 2007;109:4168-70.
46. Takeshita A, Shinjo K, Yamakage N, Ono T, Hirano I, Matsui H, et al. CMC-544 (inotuzumab ozogamicin) shows less effect on multidrug resistant cells: analyses in cell lines and cells from patients with B-cell chronic lymphocytic leukaemia and lymphoma. *Br J Haematol* 2009;146:34-43.
47. Yu SF, Zheng B, Go M, Lau J, Spencer S, Raab H, et al. A novel anti-CD22 anthracycline-based antibody-drug conjugate (ADC) that overcomes resistance to auristatin-based ADCs. *Clin Cancer Res* 2015;21:3298-306.
48. Takegawa N, Nonagase Y, Yonesaka K, Sakai K, Maenishi O, Ogitani Y, et al. DS-8201a, a new HER2-targeting antibody-drug conjugate incorporating a novel DNA topoisomerase I inhibitor, overcomes HER2-positive gastric cancer T-DM1 resistance. *Int J Cancer* 2017;141:1682-89.
49. Sabbaghi M, Gil-Gomez G, Guardia C, Servitja S, Arpi O, Garcia-Alonso S, et al. Defective Cyclin B1 Induction in Trastuzumab-emtansine (T-DM1) Acquired Resistance in HER2-positive breast cancer. *Clin Cancer Res* 2017;23:7006-19.
50. Jedema I, Barge RM, van der Velden VH, Nijmeijer BA, van Dongen JJ, Willemze R, et al. Internalization and cell cycle-dependent killing of leukemic cells by Gemtuzumab Ozogamicin: rationale for efficacy in CD33-negative malignancies with endocytic capacity. *Leukemia* 2004;18:316-25.
51. Rosen DB, Harrington KH, Cordeiro JA, Leung LY, Putta S, Lacayo N, et al. AKT signaling as a novel factor associated with in vitro resistance of human AML to gemtuzumab ozogamicin. *PLoS One* 2013;8:e53518.
52. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 2007;12:395-402.
53. Haag P, Viktorsson K, Lindberg ML, Kanter L, Lewensohn R, Stenke L. Deficient activation of Bak and Bax confers resistance to gemtuzumab ozogamicin-induced apoptotic cell death in AML. *Exp Hematol* 2009;37:755-66.
54. Walter RB, Raden BW, Cronk MR, Bernstein ID, Appelbaum FR, Banker DE. The peripheral benzodiazepine receptor ligand PK11195 overcomes different resistance mechanisms to sensitize AML cells to gemtuzumab ozogamicin. *Blood* 2004;103:4276-84.
55. Moore J, Seiter K, Koltz J, Stock W, Giles F, Kalaycio M, et al. A Phase II study of Bcl-2 antisense (oblimersen sodium) combined with gemtuzumab ozogamicin in older patients with acute myeloid leukemia in first relapse. *Leuk Res* 2006;30:777-83.
56. Dornan D, Bennett F, Chen Y, Dennis M, Eaton D, Elkins K, et al. Therapeutic potential of an anti-CD79b antibody-drug conjugate, anti-CD79b-vc-MMAE, for the treatment of non-Hodgkin lymphoma. *Blood* 2009;114:2721-9.
57. Kung Sutherland MS, Walter RB, Jeffrey SC, Burke PJ, Yu C, Kostner H, et al. SGN-CD33A: a novel CD33-targeting antibody-drug conjugate using a pyrrolbenzodiazepine dimer is active in models of drug-resistant AML. *Blood* 2013;122:1455-63.
58. Ab O, Whiteman KR, Bartle LM, Sun X, Singh R, Tavares D, et al. IMGN853, a Folate Receptor-alpha (FRalpha)-targeting antibody-drug conjugate, exhibits potent targeted antitumor activity against FRalpha-Expressing Tumors. *Mol Cancer Ther* 2015;14:1605-13.
59. Golfier S, Kopitz C, Kahnert A, Heisler I, Schatz CA, Stelte-Ludwig B, et al. Anetumab ravtansine: a novel mesothelin-targeting antibody-drug conjugate cures tumors with heterogeneous target expression favored by bystander effect. *Mol Cancer Ther* 2014;13:1537-48.
60. Li F, Emmerton KK, Jonas M, Zhang X, Miyamoto JB, Setter JR, et al. Intracellular released payload influences potency and bystander-killing effects of antibody-drug conjugates in preclinical models. *Cancer Res* 2016;76:2710-9.
61. Andreev J, Thambi N, Perez Bay AE, Delfino F, Martin J, Kelly MP, et al. Bispecific antibodies and antibody-drug conjugates (ADCs) bridging her2 and prolactin receptor improve efficacy of HER2 ADCs. *Mol Cancer Ther* 2017;16:681-93.
62. Gerber HP, Sapra P, Loganzo F, May C. Combining antibody-drug conjugates and immune-mediated cancer therapy: what to expect? *Biochem Pharmacol* 2016;102:1-6.