MAR Oncology & Hematology (2024) 4:4

Review Article

Understanding Resistance Mechanisms to Antibody-Drug Conjugates

J.Chouef [1], I.Ouafki[1], H.Medyouni [1], L.Amaadour [1], K.Oualla 1, Z.Benbrahim [1], S.Arifi 1, N.Mellas [1]

1. Medical oncology department, CHU HASSAN II Fez

*Correspondence to: Jihane Chouef, MBBS, MS ENT, Associate Professor Department of ENT School of Medical Sciences and Research Greater Noida.

Received: 22 March 2024

Published: 01 April 2024

DOI: https://doi.org/10.5281/zenodo.10998929

Absract

In recent decades, the landscape of cancer treatment has undergone significant transformations, propelled by advancements in therapeutic modalities like monoclonal antibodies, immune-checkpoint inhibitors, bispecific antibodies, and innovative T-cell therapy. Among these, antibody-drug conjugates (ADCs) have emerged as a revolutionary approach in cancer therapy. Notably, several ADCs have gained approval in both hematology and clinical oncology, including trastuzumab emtansine (T-DM1), trastuzumab deruxtecan (T-DXd), and sacituzumab govitecan (SG), for managing metastatic breast cancer, along with enfortumab vedotin (EV) for urothelial carcinoma.

Despite their promise, the efficacy of ADCs is hampered by the development of resistance mechanisms, such as antigen-related resistance, internalization failure, and impaired lysosomal function, among others. In this comprehensive review, we synthesize the clinical evidence underlying the approval of T-DM1, T-DXd, SG, and EV, shedding light on their therapeutic potential. Furthermore, we delve into the intricate mechanisms of resistance to ADCs, exploring strategies to surmount these obstacles. These include the development of bispecific ADCs and the synergistic combination of ADCs with immune-checkpoint inhibitors or tyrosine-kinase inhibitors, offering promising avenues for overcoming resistance and enhancing therapeutic outcomes.

Keywords*: antibody-drug conjugate; trastuzumab emtasine; trastuzumab deruxtecan; enfortumab vedotin; sacituzumab govitecan; monoclonal antibodies; payload; linker*

Introduction

The landscape of cancer treatment has undergone profound changes over the past thirty years. Initially, chemotherapy emerged in the early 20th century, offering significant clinical advantages. However, the utilization of chemotherapeutic agents entails a perpetual balancing act between potential benefits and the systemic toxicity associated with these nonspecific drugs [1]. The introduction of monoclonal antibodies (mAbs) and targeted therapies, exemplified by agents like rituximab and trastuzumab, revolutionized clinical

oncology [2,3]. More recently, immune-checkpoint inhibitors (ICIs) and bispecific antibodies have garnered approval for treating cancer patients. Antibody-drug conjugates (ADCs), initially conceptualized by Ehrlich, represent another promising therapeutic avenue. ADCs deliver cytotoxic drugs directly to tumor cells, exhibiting a potent yet safe effect by selectively binding to tumor cells through directed antibodies, which possess exceptional affinity and specificity for particular epitopes on the target antigen [4–6].

The concept of ADCs was first mooted in the 1970s, but it wasn't until the 1990s that the first ADCs were developed and entered clinical trials. The impetus behind ADC development stemmed from the aspiration to craft more efficacious cancer treatments capable of precisely targeting cancer cells while sparing healthy tissues [4]. The exploration of ADCs in preclinical models traces back to the 1980s, with trials employing mouse immunoglobulin G (IgG) [7]. Notably, the choice of IgG isotype for ADCs hinges on considerations regarding efficacy and safety, a factor crucial in the development of approved ADCs.

ADC Composition

Antibody-drug conjugates (ADCs) comprise humanized monoclonal antibodies (mAbs) targeting tumor-specific or associated antigens, linked to cytotoxic drugs (payloads) with anti-tumor activity through either molecular cleavable or non-cleavable linkers [11]. The schematic representation of an ADC structure is depicted in Figure 1. The choice of immunoglobulin G (IgG) isotype for ADCs depends on various factors including the target antigen, the ADC's mechanism of action, and its intended clinical application. However, IgG1-based mAbs are predominantly favored due to their ease of production and superior immunogenic properties compared to other IgG2 and IgG4 subtypes [8].This transformative approach originated with promising data from BR96-doxorubicin immunoconjugate studies for treating human carcinomas in animal models. These studies revealed efficient binding of BR96-doxorubicin immunoconjugates to Lewis Y, an antigen highly expressed in cancer cells. Consequently, they demonstrated effectiveness in treating xenografted human carcinomas in mouse models, with some tumors exhibiting complete response. Importantly, the treatment was well tolerated by the animals, with no significant observed side effects [9,10].

1- Target-Antigen Selection and Antibody

The guiding signal for ADCs to locate tumor cells is the target antigen expressed on the surfaces of tumor cells [11]. The targeted antigen must be expressed exclusively or mostly in tumor cells, with minimal or negligible expression in normal tissues, in order to minimize off-target damage [12]. Antigens that are only expressed on the surfaces of tumor cells make excellent targets for ADCs. With many particular ADCs already

approved and others in development, lineage-specific antigens expressed by hematological malignancies have been thoroughly investigated as ideal candidates [13]. However, this concept does not apply to solid tumors, in which the antigens expressed are primarily "tumor-associated" rather than "tumor-specific," meaning that they are expressed on both tumor cells and normal cells [8].

The antibody consists of two antigen-binding fragments (mainly known as Fabs) and one constant fragment (Fc). The antigen recognition is mediated by Fabs, and the interaction of the antibody with effector immune cells is mediated by Fc [6]. The integrated antibody should have a high level of specificity for the tumor's target antigen. An optimal antibody molecule should have high binding affinity with the target antigen, as well as effective internalization and minimal immunogenicity, and it should feature a long plasma half-life [14]. Due to its advantage of possessing much lower immunogenicity than mouse-derived antibodies, developed antibodies are now fully humanized. Since IgG is the primary component of immunoglobulin in serum, the antibodies now employed in ADC medications are primarily IgG antibodies, which encompass four subtypes, namely IgG1, IgG2, IgG3, and IgG4 [12]. The IgG1 subtype is the most commonly used for antibody therapeutics [15]. The immune-effector functions mediated by the antibody are the activation of the complement complexes, the activation of immune cells, the induction of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and the activation of antibody-dependent cell-mediated phagocytosis (ADCP).

2- Cytotoxic Payloads

The cytotoxic payload serves as the primary effector component of ADCs, renowned for its high potency. Presently, leading cytotoxic payloads for ADCs comprise potent tubulin inhibitors (such as auristatin analogs and maytansine analogs), DNA-damaging compounds (including duocarmazine, calicheamicins, and pyrrolobenzodiazepines), the RNA polymerase II inhibitor (amanitin), and topoisomerase I inhibitors (like deruxtecan and govitecan) [1]. It has been established that the molecular and physicochemical properties of the payload significantly influence the efficacy of the ADC, in addition to its inherent potency.

3- Linkers

Linkers play a pivotal role in connecting antibodies to cytotoxic payloads while contributing to the stabilization of ADCs [16]. These linkers can be either non-cleavable, requiring extensive processing for payload release, or cleavable, often associated with tumor-specific factors such as lysosomal enzymes or alterations in pH (acidic conditions). Cleavable linkers have been shown to efficiently release the cytotoxic

payload. Conversely, non-cleavable linkers may offer more specific chemotherapy payload release, albeit necessitating complete antibody degradation within lysosomes, potentially impacting payload efficacy. Non-cleavable linkers are correlated with enhanced plasma stability and relatively prolonged half-lives [17–19]. ADCs are categorized based on a crucial parameter known as the drug-to-antibody ratio (DAR), representing the number of cytotoxic moieties attached to each antibody. ADCs with higher DARs may exhibit heightened potency but are prone to faster drug clearance and potentially increased toxicity. Conversely, ADCs with lower DARs may demonstrate reduced activity but possess higher therapeutic indices [20, 21].

Selected ADCs in Solid Tumors: Mechanism of Action

In this review, our focus centers on four ADCs: T-DM1, T-DXd (formerly known as DS-8201), SG, and EV. Table 1 provides an overview of the pivotal trials leading to the approval of these agents for cancer patient treatment.

1- Trastuzumab Emtansine

T-DM1 marked the first ADC approval for metastatic breast cancer (MBC) following the EMILIA trial results [31]. Its mechanism encompasses various actions, including the selective delivery of DM1 (a derivative of maytansine and microtubule-inhibitory agent) to HER2-positive tumor cells, trastuzumab-mediated suppression of HER2 signaling, prevention of HER2 extracellular domain shedding, and induction of ADCC [32–34]. Initially, T-DM1 selectively binds to subdomain IV of HER-2-receptor-positive cells. Subsequent to binding, the ADC-receptor complex undergoes internalization into endosomes via endocytosis [34]. Endocytic vesicles either fuse with lysosomes for degradation or are recycled to reintroduce the ADC-receptor complex to the plasma membrane [35]. The antibody component of T-DM1 undergoes lysosomal breakdown, liberating lysine-MCC-DM1 [36]. Upon release from lysosomes, MCC-DM1 effectively binds to tubulin, thereby halting microtubule polymerization and inducing cell-cycle arrest [37]. Research indicates that T-DM1 preserves trastuzumab's mechanisms of action, including suppression of HER2-ectodomain shedding, inhibition of HER2-signaling pathways, and stimulation of innate and adaptive anti-tumor immunity [32]. It operates by diminishing ligand-independent activation of downstream phosphatidylinositol 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) and RAS-mitogen-activated protein kinase (MAPK)-signaling pathways [38]. Trastuzumab's Fc domain interacts with homologous Fc receptors on natural killer cells, leading to ADCC and the destruction or phagocytosis of HER2-positive tumor cells marked by trastuzumab [39]. Trastuzumab has also been associated with additional, though less conclusively demonstrated, modes of action, such as DNA-repair inhibition, increased accumulation of the cyclin-dependent kinase inhibitor

p27Kip1, and suppression of angiogenesis [40].

3.2. Trastuzumab Deruxtecan

T-DXd represents a next-generation ADC comprising a humanized anti-HER2 antibody linked to deruxtecan, a potent topoisomerase I inhibitor (specifically, an exatecan derivative known as DXd), connected via a tetrapeptide cleavable linker. This ADC has obtained approval for patients diagnosed with metastatic breast cancer (MBC) [41]. Trastuzumab deruxtecan boasts several innovative attributes, including its effective targeting of tumors with low HER2 expression and its potent cytotoxic payload, featuring a drug-to-antibody ratio of 8:1, enabling it to impact neighboring cells [42,43].

Following encouraging outcomes and sustained efficacy observed in HER2-positive patients during phase I and II trials, T-Dxd not only superseded standard chemotherapy in the second-line treatment setting but also led to enhancements in progression-free survival (PFS) and overall survival (OS) in patients previously treated with T-DM1. Additionally, it exhibited efficacy in patients with low HER2-expressing tumors [22,44,45]. Moreover, T-DXd has received approval for treating patients with metastatic HER2-positive gastric or gastroesophageal junction adenocarcinoma who have undergone prior trastuzumab-based therapy, as well as those with previously treated HER2-mutant metastatic non-small-cell lung cancer (NSCLC), based on findings from the DESTINY-Gastric 01 and DESTINY-Lung01 phase II trials [24,25].

2- Sacituzumab Govitecan

Sacituzumab govitecan, comprising the active metabolite of irinotecan, SN-38, chemically linked to the humanized monoclonal RS7 IgG1 Trop-2 antibody, represents a pivotal component of this ADC [46]. It secured the distinction of being the first ADC approved for triple-negative breast cancer (TNBC), supported by findings from the IMMU-132-01 and ASCENT trials [26,47]. Additionally, it has gained approval for treating metastatic urothelial carcinoma (mUC) post-immune checkpoint inhibitor (ICI) progression and, more recently, for managing HR+/HER2- MBC following progression on endocrine therapy and at least two lines of chemotherapy [27,28]. Upon binding to Trop-2 receptors on cancer cells, sacituzumab govitecan undergoes internalization, facilitating intracellular delivery of SN-38 [48]. SN-38, being approximately 1000 times more potent in inducing DNA breaks, manifests a robust cytotoxic effect [49]. Characterized by a relatively high drug-to-antibody ratio (DAR) and a cleavable CL2A linker, sacituzumab govitecan leverages the intermediate stability of the CL2A linker to facilitate SN-38 release within sacituzumab-bound cells and the tumor microenvironment (TME). This mechanism likely amplifies the anti-tumor efficacy by generating therapeutic

concentrations of SN-38 in conjugate-bound cells and the TME [50, 51].

3- Enfortumab Vedotin

Enfortumab vedotin, an ADC composed of a human IgG1 anti-Nectin-4 antibody conjugated to monomethyl auristatin E (MMAE), a microtubule-disrupting agent, represents a significant advancement in cancer therapy [52]. It has garnered approval as a monotherapy for treating patients with metastatic urothelial carcinoma (mUC) following progression on immune checkpoint inhibitors (ICIs) [29]. In April 2023, enfortumab vedotin received approval for use in combination with pembrolizumab for treating mUC patients ineligible for cisplatin-containing chemotherapy, as demonstrated in the EV-103 trial [30]. Nectin-4 emerges as an optimal therapeutic target due to its overexpression across various malignancies, particularly prevalent in urothelial cancers and breast cancer [53]. Upon binding to nectin-4 and subsequent internalization, enfortumab vedotin releases MMAE intracellularly, inducing tumor cell apoptosis through microtubule disruption [54]. Additionally, enfortumab vedotin's anti-tumor effects may be mediated by other mechanisms, including inhibition of signal transduction via direct binding, as well as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [55].

Mechanisms of Resistance to ADCs

Given the intricate structures and multifaceted mechanisms of action of ADCs, resistance can arise at various stages, starting from antigen expression and recognition, through internalization and degradation, to cytotoxic drug release and apoptotic regulation. Table 2 provides an overview of resistance mechanisms and strategies to overcome them, while Figure 2 visually represents these mechanisms.

1- **Antigen-Related Resistance**

As ADCs primarily target specific antigens, one proposed mechanism of resistance revolves around the recognition of these antigens by monoclonal antibodies (mAbs). For instance, Loganzo et al. conducted a study exposing breast cancer cells to trastuzumab–maytansinoid (TM–ADC), structurally similar to T-DM1. They observed different resistance mechanisms in two cell lines: MDA MB-361-DYT2, parental cells, and JIMT1, resistant to first-line trastuzumab. Notably, the JIMT1–TM-cell models developed resistance to TM–ADC while retaining sensitivity to other chemotherapeutic agents, suggesting that diminished HER2 protein levels over prolonged treatment might lead to refractory cells and eventual drug resistance [56].

Moreover, tumor heterogeneity in antigen expression can impact ADC efficacy. This phenomenon was

elucidated in the KRISTINE trial, a phase II study of T-DM1 plus pertuzumab in the neoadjuvant setting. Tumor heterogeneity in HER2 expression, defined as HER2-negative areas by FISH in 10% of cases or ERBB2 amplification in 5-50% of tumor cells, led to suboptimal outcomes. None of the ten patients exhibiting tumor heterogeneity achieved a pathological complete response (pCR). Results indicated that patients with high levels of HER2 heterogeneity pre-treatment had inferior progression-free survival (PFS) and overall survival (OS) compared to those with low heterogeneity. This association was also observed in the ZEPHIR trial, where patients with high tumor heterogeneity on HER2-PET CT scans experienced shorter times to treatment failure [57,58].

Another potential mechanism of resistance, not yet clinically validated in ADCs, involves the accumulation of truncated forms of the antigen ectodomain. This theory was previously demonstrated with trastuzumab, where tumors expressing full-length receptors exhibited high responses to trastuzumab, whereas truncated P95HER2 showed resistance [59]. Additionally, the masking and isolation of the HER2 antigen by extracellular matrices, such as MUC4, contributed to trastuzumab refractoriness in JIMT-1, presenting another probable resistance mechanism [60].

Furthermore, sensitivity to ADCs can be influenced by the presence of ligands such as Heregulin, also known as neuregulin or NRG, a protein belonging to the epidermal growth factor (EGF) family, which impairs the efficacy of T-DM1 by promoting heterodimerization of HER2 with HER3 and HER4 [61].

Similarly, resistance to SG was evaluated by Coates et al. [62]. They reported a case study of a metastatic triple-negative breast cancer (TNBC) patient initially responding to SG therapy but eventually developing acquired resistance. The study identified multiple genomic alterations in the patient's tumor affecting both the antigen target (Trop-2) and the drug payload (SN-38). These alterations, including mutations, copy-number changes, and structural variations, resulted in reduced Trop-2 expression and increased drug efflux, leading to decreased drug exposure and resistance to SG therapy. The findings suggest that acquired resistance to SG in TNBC involves parallel genomic alterations of both the antigen and the payload targets [62].

Similarly, the association between Nectin-4 expression and resistance to EV was investigated by Klumper et al. Their study revealed that membranous Nectin-4 expression frequently decreases during the metastatic spread of urothelial carcinoma (UC), suggesting downregulation of NECTIN-4 during disease progression. Furthermore, the study demonstrated that Nectin-4 downregulation is associated with resistance to EV therapy, as absent or weak expressions correlated with shortened progression-free survival (PFS). These findings imply that monitoring Nectin-4 expression in UC patients may help predict responses to EV therapy and identify individuals who could benefit from alternative treatments [63].

2. Payload-Related Resistance

Besides antigen resistance, tumor cells may also develop resistance to the payload. This phenomenon was initially observed in NHL tumors, where replacing an auristatin-based payload with an anthracycline-based payload resulted in improved ADC response [64]. Similarly, Takegawa et al. demonstrated another instance in their study with T-DXd in T-DM1-resistant cells. Despite normal HER2 overexpression, these cells exhibited upregulated expression of ABC transporters ABCC2 and ABCG2. Notably, inhibiting these transporters restored T-DM1 efficacy, revealing a mechanism of resistance caused by increased DM1 efflux [65]. Ongoing phase I/II studies for SKB-264, a novel TROP-2-targeted ADC, further support this theory by replacing the topoisomerase-1 inhibitor's payload with a belotecan derivative [66]. Payload diversification emerges as a promising approach for enhancing ADC therapeutic effects.

Additionally, the type of payload, location of conjugations, and the average drug-to-antibody ratio (DAR) are significant factors. Payloads are conjugated to antibodies through cysteine (Cys) or lysine (Lys) residues [67]. Hamblett et al. suggested an optimal average DAR of 2–4 for cysteine-linked ADCs and 3–4 for lysine conjugates [68]. In a case study, lysine-conjugated ADCs yielded better results than cysteine-conjugated ones, despite having the same antibody and cytotoxic payload [69]. However, another study using DGN549 as a payload found that site-specific cysteine-based conjugation led to improved tolerability and efficacy. These findings underscore the importance of evaluating conjugation chemistry on a case-by-case basis, considering antibody, target, and linker payload variations [70]. Regarding payloads, DAR significantly influences ADC efficacy, although the optimal DAR remains unclear. One study evaluating ADC potency alongside DAR found that ADCs with very high DAR (~9–10) exhibited faster clearance and decreased efficacy compared to those with lower DAR [20]. However, other factors such as conjugation site and drug loading may also influence these outcomes.

3- Impairment in Internalization and Trafficking Pathways

Following binding to the target antigen, the ADC undergoes internalization into the cancer cell via endocytosis. Several pathways of endocytosis exist, including clathrin-mediated endocytosis (CME), which is the most prevalent route utilized by ADCs, as well as caveolae-mediated endocytosis and clathrin-caveolin-independent endocytosis [71]. Each endocytic pathway is governed by distinct sets of proteins, such as the adaptor protein (AP2), dynamin, epsin, and phosphatidylinositol biphosphate (PIP2), each serving specific functions in various cellular processes. In a study by Sung et al., multiple in vitro models of T-DM1 resistance were established using the same methodology. Two models displayed resistance due to reduced HER2

expression, whereas N87-TM maintained normal expression levels of transport proteins and HER2 similar to parental cells [66]. Furthermore, these resistant N87-TM cells effectively internalized trastuzumab-ADCs into caveolin-1 (CAV-1)-coated vesicles. These findings suggest the influence of the microenvironment and enzymes in antibody catabolism and delivery, favoring the cleavable over the non-cleavable linker payload of TDM-1 [72]. Additionally, in HER2-positive breast cancer, Endophilin A2 (encoded by SH3GL1) has been identified to enhance HER2 internalization and sensitize breast cancer cells to trastuzumab-based therapy. Knockdown of SH3GL1 in tumor cells resulted in diminished internalization and notably suppressed T-DM1-mediated cytotoxicity [73].

4-Dysfunction in Lysosomal Activity

Following binding to the target molecule and internalization via receptor-mediated endocytosis, ADCs enter lysosomes, where chemical or enzymatic cleavage releases the cytotoxic agents. However, several factors may impair lysosomal function. Rios-Luci et al. investigated mechanisms of T-DM1 resistance by isolating three different HER2-positive resistant clones. These clones, despite showing similar HER2 expressions to parental clones and unaltered internalization and trafficking pathways, exhibited elevated lysosomal pH levels, impaired proteolytic activity, and consequently, increased T-DM1 accumulation [74]. This compromised lysosomal function results in impaired T-DM1 processing, limiting its anti-tumor efficacy. Describing this dysfunction not only elucidates various ADC resistance pathways but also suggests potential strategies for overcoming them. Trudeau et al. published a report using UV photoactivation to create acidifying nanoparticles that manipulate intralysosomal pH, thereby restoring or enhancing the anti-tumoral properties of ADCs [75]. Another resistance mechanism to ADCs involves the transport of cytotoxic drugs from lysosomal lumens to the cytoplasm. This mechanism is particularly relevant for non-cleavable linkers, as they may necessitate specific transporters for cytoplasmic delivery. Hamblett et al. conducted a phenotypic RNA screening, identifying SLC46A3 as a direct transporter of maytansine-based catabolites to the cytoplasm. Silencing this protein leads to catabolite accumulation in lysosomes and eventual drug failure [76]. Furthermore, resistance mechanisms to T-DXd were assessed in the DAISY trial, a phase II study enrolling patients across three groups based on HER2 expression levels. Upon progression, whole-genome sequencing (WES) was performed on tumor samples to identify potential resistance mechanisms [77]. Alongside decreases in HER2 expression, mutations in the SLX4 gene were associated with resistance. SLX4 regulates DNA-damage repair and controls three endonucleases. Tumor cells with depleted SLX4 exhibited T-DXd resistance, suggesting loss-of-function mutations as additional mechanisms of T-DXd resistance [77].

The upregulation of ATP-binding cassette (ABC) transporters represents a pivotal aspect in the development of chemotherapy resistance, a phenomenon extensively observed across various cancer types [79]). These transporters, functioning as efflux pumps, facilitate the expulsion of drugs from cells, potentially culminating in the genesis of tumors exhibiting multidrug-resistant (MDR) phenotypes (Reference [80]). Notably, this mechanism holds the potential to directly engender resistance to antibody-drug conjugates (ADCs), as many of their payloads serve as substrates for ABC transporters [81,82]).MDR1-mediated efflux stands out as the predominant phenotype in this context, with maytansinoids, the payloads of ADCs like T-DM1, serving as substrates for ABC transporters such as MDR1. Hence, resistance to T-DM1 may be intricately linked to the expression of these substrates [83]). Kovtun et al. have contributed valuable insights into potential strategies for circumventing multidrug resistance. Their study involved the utilization of a hydrophilic linker, PEG4Mal, to conjugate the cytotoxic maytansinoid DM1 to the antibody. Comparisons were made with similar conjugates linked using a nonpolar linker, N-succinimidyl-4-(maleimidomethyl)cyclohexane-1-carboxylate (SMCC). The conjugates linked with PEG4Mal exhibited heightened activity and superior responses in tumors expressing MDR1 [81]. The influence of the cell cycle on tumor response to chemotherapy has long been recognized, as actively dividing leukemic cells exhibit greater sensitivity compared to their quiescent counterparts [84]. This notion underscores the significance of understanding cell-cycle dynamics in treatment efficacy. Moreover, another proposed mechanism contributing to resistance against T-DM1 involves cyclin B, a cell-cycle protein crucial for the G2–M transition. Sabbaghi et al. conducted an experiment aimed at elucidating T-DM1 resistance mechanisms by comparing cellular and molecular effects in parental and resistant cells. Despite similar HER2 expressions, binding, and intracellular uptake between the two groups, the researchers observed cyclin B1 accumulation in sensitive cells but not in resistant ones. Notably, silencing the upregulation of cyclin B1 levels partially sensitized the resistant cells, highlighting the potential of cyclin B1 levels as indicators of T-DM1 effectiveness in inducing apoptosis [85].

The activation of signaling pathways represents a significant mechanism by which malignant cells develop resistance to ADCs. Among these pathways, the PI3K/AKT/mTOR pathway stands out, regulating cell survival, growth, and metabolism. Activation of this pathway may lead to reduced sensitivity to ADCs, compromised effectiveness of the cytotoxic payload, and prolonged cell survival, as observed previously with trastuzumab in patients carrying PIK3CA mutations or PTEN deletions (Reference [84]). Notably, PTEN loss or PIK3CA hyperactivation potentially decreases sensitivity to trastuzumab via PI3K/AKT signaling activation. However, findings from an exploratory biomarker analysis in the EMILIA trial paint a different picture. In this phase III trial, T-DM1 not only prolonged overall survival (OS) and progression-free survival

(PFS) compared to lapatinib and capecitabine in metastatic breast cancer (MBC) patients previously treated with trastuzumab and taxane, but also yielded similar outcomes regardless of PIK3CA mutation status or PTEN expression levels [31]. While patients with PIK3CA mutations or decreased PTEN expression treated with lapatinib and capecitabine experienced shorter PFS and OS, those treated with T-DM1 exhibited comparable results, suggesting the potential superiority of T-DM1 over other HER2-directed therapies irrespective of tumor biomarkers [86]. Additionally, the activation of the Wnt/β-catenin pathway may contribute to resistance to ADCs. Wu et al. elucidated the role of Wnt3 in trastuzumab resistance, where Wnt3 overexpression led to increased β-catenin expression, heightened growth rates, invasiveness, and trastuzumab resistance in cells [87]. Although these mechanisms remain potential contributors to ADC resistance, they have yet to be directly linked to T-DM1 resistance. Lastly, dysregulation of apoptosis represents another pivotal factor influencing sensitivity to ADCs. Predominantly observed in hematological malignancies, dysregulation often involves the overexpression of anti-apoptotic proteins such as BCL-2 and BCL-XL, correlating with resistance to agents like gemtuzumab ozogamicin or brentuximab vedotin [88–90].

Overcoming resistance to ADCs

Despite significant advancements in ADC (Antibody-Drug Conjugate) technology, resistance remains a formidable challenge. As previously elucidated, resistance to ADCs can emerge through various mechanisms, some of which are still not comprehensively understood. Consequently, devising strategies to surmount ADC resistance constitutes a multifaceted and ongoing area of investigation, whether through innovative ADC designs or combination therapy approaches. One of the predominant mechanisms involves the upregulation of drug-efflux pumps leading to increased drug extrusion. An instance of overcoming ADC resistance was demonstrated in the aforementioned study by Takegawa et al., wherein T-DXd exhibited efficacy in overcoming T-DM1 resistance in HER2-positive gastric cancer cells. The incorporation of a novel DNA topoisomerase I inhibitor into the ADC, exhibiting aberrant expression of ABC transporters, potentially underlies this augmented anti-tumor activity [65]. These findings were recently validated in DESTINY-Breast02, a phase III trial assessing T-DXd versus chemotherapy of choice in patients with HER2+ MBC refractory to T-DM1. Notably, this trial marked the first randomized evidence showcasing the capability of one ADC to overcome resistance to another [22]. Another strategy revolves around hydrophobic compounds, which are transported more efficiently compared to hydrophilic ones. Thus, the concept involves modifying the linker to enhance hydrophilicity, thereby reducing its elimination. Examples such as Sulfo-SPDB-DM4, a highly hindered disulfide hydrophilic linker utilized in FR_-expressing tumors, and PEG4Mal, exemplify

enhanced efficacy against resistant, MDR1-positive tumors [81,91].

Another significant concern pertains to tumor heterogeneity and the limited efficacy of ADCs against tumor cells expressing low levels of antigens. Golfier et al. delved into this issue by investigating the effectiveness of anetumab ravtansine, an ADC designed to target the protein mesothelin, in eradicating tumors with varied target expressions. Their study revealed that anetumab ravtansine exhibited robust anti-tumor activity both in vitro and in vivo, selectively eliminating mesothelin-expressing tumor cells through a bystander effect. This phenomenon involves the transfer of the drug to adjacent cells lacking the target protein, thereby broadening its cytotoxic impact [92].

In a separate study, Li et al. explored the relationship between payload release rates and ADC potency. Their findings indicated that higher rates of payload release correlated with increased potency and a more pronounced bystander effect, suggesting a potential strategy for overcoming resistance and augmenting ADC efficacy [93]. However, the bystander effect's manifestation relies not only on the cytotoxic linker's characteristics but also on the mechanism of action of the cytotoxic payload and the spatial relationship between neighboring cells, rendering it a complex phenomenon necessitating further optimization through ongoing research.The bystander effect was notably identified as one of the mechanisms for overcoming resistance to T-DM1 using T-DXd. Another promising approach to address low antigen expression involves leveraging novel bispecific or biparatopic monoclonal antibodies (mAbs). This concept has already shown promise with HER2. A recently engineered biparatopic ADC exhibited distinctive attributes and demonstrated efficacy against both T-DM1-resistant tumors and those with low HER2 expression levels. Its efficacy was attributed to the simultaneous targeting of two distinct epitopes of HER2, leading to the formation of HER2-receptor clusters. This clustering enhanced receptor internalization and redirected internal trafficking from recycling to degradation pathways. Furthermore, coupling this ADC with a different payload facilitated evasion of efflux pumps [94].

Conclusions

ADCs have emerged as a crucial category of cancer therapeutics, with numerous FDA-approved ADCs currently accessible for treating various cancer types. Despite considerable progress in cancer therapy, both intrinsic and acquired drug resistance pose significant challenges to effective treatment. Various mechanisms of resistance to ADCs have been identified, including antigen-related resistance, defects in internalization, compromised lysosomal function, drug-efflux pumps, and alterations in targets. To address these challenges,

novel strategies are being pursued.Another potential mechanism of ADC resistance involves mutations in the payload target. However, there is currently insufficient data to support this hypothesis, and no reports have been made regarding mutations in tubulin, RNA polymerase II, or topoisomerase I. New-generation ADCs have demonstrated efficacy even after progression on other ADCs. For instance, the DESTINY-Breast02 trial demonstrated the superiority of T-DXd over standard chemotherapy in patients with HER2+ metastatic breast cancer refractory to T-DM1. Additionally, SHR-A1811 exhibited a high objective response rate (ORR) in patients with HER2+ metastatic breast cancer refractory to both T-DM1 and T-DXd. Another strategy involves combining ADCs with immune checkpoint inhibitors (ICIs) or tyrosine kinase inhibitors (TKIs). Numerous ongoing clinical trials are assessing the combination of T-DM1, T-DXd, sacituzumab govitecan, and enfortumab vedotin with ICIs and TKIs, aiming to improve treatment outcomes and overcome resistance mechanisms.

References

1. Diamantis, N.; Banerji, U. Antibody-Drug Conjugates—An Emerging Class of Cancer Treatment. Br. J. Cancer 2016, 114, 362–367. [CrossRef]

2. Coiffier, B.; Lepage, E.; Briere, J.; Herbrecht, R.; Tilly, H.; Bouabdallah, R.; Morel, P.; Van Den Neste, E.; Salles, G.; Gaulard, P.; et al.

CHOP Chemotherapy plus Rituximab Compared with CHOP Alone in Elderly Patients with Diffuse Large-B-Cell Lymphoma.

N. Engl. J. Med. 2002, 346, 235–242. [CrossRef]

3. Slamon, D.J.; Leyland-Jones, B.; Shak, S.; Fuchs, H.; Paton, V.; Bajamonde, A.; Fleming, T.; Eiermann, W.;Wolter, J.; Pegram, M.;

et al. Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2.

N. Engl. J. Med. 2001, 344, 783–792. [CrossRef]

4. Marei, H.E.; Cenciarelli, C.; Hasan, A. Potential of Antibody-Drug Conjugates (ADCs) for Cancer Therapy. Cancer Cell Int. 2022, 22, 255.

[CrossRef]

5. Strebhardt, K.; Ullrich, A. Paul Ehrlich's Magic Bullet Concept: 100 Years of Progress. Nat. Rev. Cancer 2008, 8, 473–480. [CrossRef]

[PubMed]

6. Hoffmann, R.M.; Coumbe, B.G.T.; Josephs, D.H.; Mele, S.; Ilieva, K.M.; Cheung, A.; Tutt, A.N.; Spicer, J.F.; Thurston, D.E.;

Crescioli, S.; et al. Antibody Structure and Engineering Considerations for the Design and Function of Antibody Drug Conjugates

(ADCs). Oncoimmunology 2018, 7, e1395127. [CrossRef] [PubMed]

7. Rowland, G.F.; O'Neill, G.J.; Davies, D.A. Suppression of Tumour Growth in Mice by a Drug-Antibody Conjugate Using a Novel

Approach to Linkage. Nature 1975, 255, 487–488. [CrossRef] [PubMed]

8. Criscitiello, C.;Morganti, S.; Curigliano, G. Antibody-Drug Conjugates in Solid Tumors: A Look into Novel Targets. J. Hematol. Oncol.

2021, 14, 20. [CrossRef]

9. Trail, P.A.; Willner, D.; Lasch, S.J.; Henderson, A.J.; Hofstead, S.; Casazza, A.M.; Firestone, R.A.; Hellström, I.; Hellström, K.E.

Cure of Xenografted Human Carcinomas by BR96-Doxorubicin Immunoconjugates. Science 1993, 261, 212–215. [CrossRef]

10. Tolcher, A.W. Antibody Drug Conjugates: Lessons from 20 Years of Clinical Experience. Ann. Oncol. 2016, 27, 2168–2172. [CrossRef]

11. Chau, C.H.; Steeg, P.S.; Figg,W.D. Antibody-Drug Conjugates for Cancer. Lancet 2019, 394, 793–804. [CrossRef] [PubMed]

12. Fu, Z.; Li, S.; Han, S.; Shi, C.; Zhang, Y. Antibody Drug Conjugate: The "Biological Missile" for Targeted Cancer Therapy.

Signal Transduct. Target. Ther. 2022, 7, 93. [CrossRef] [PubMed]

13. Damelin, M.; Zhong,W.; Myers, J.; Sapra, P. Evolving Strategies for Target Selection for Antibody-Drug Conjugates. Pharm. Res.

2015, 32, 3494–3507. [CrossRef] [PubMed]

14. De Cecco, M.; Galbraith, D.N.; McDermott, L.L.What Makes a Good Antibody-Drug Conjugate? Expert Opin. Biol. Ther. 2021, 21,

841–847. [CrossRef]

15. Carter, P.J. Potent Antibody Therapeutics by Design. Nat. Rev. Immunol. 2006, 6, 343–357. [CrossRef]

16. Saber, H.; Leighton, J.K. An FDA Oncology Analysis of Antibody-Drug Conjugates. Regul. Toxicol. Pharmacol. 2015, 71, 444–452.

[CrossRef]

17. Jain, N.; Smith, S.W.; Ghone, S.; Tomczuk, B. Current ADC Linker Chemistry. Pharm. Res. 2015, 32, 3526–3540. [CrossRef]

18. Drago, J.Z.; Modi, S.; Chandarlapaty, S. Unlocking the Potential of Antibody-Drug Conjugates for Cancer Therapy. Nat. Rev.

Clin. Oncol. 2021, 18, 327–344. [CrossRef]

19. Lu, J.; Jiang, F.; Lu, A.; Zhang, G. Linkers Having a Crucial Role in Antibody-Drug Conjugates. Int. J. Mol. Sci. 2016, 17, 561.

[CrossRef]

20. Sun, X.; Ponte, J.F.; Yoder, N.C.; Laleau, R.; Coccia, J.; Lanieri, L.; Qiu, Q.; Wu, R.; Hong, E.; Bogalhas, M.; et al. Effects of

Drug-Antibody Ratio on Pharmacokinetics, Biodistribution, Efficacy, and Tolerability of Antibody-Maytansinoid Conjugates.

Bioconjug. Chem. 2017, 28, 1371–1381. [CrossRef]

21. Abelman, R.O.; Wu, B.; Spring, L.M.; Ellisen, L.W.; Bardia, A. Mechanisms of Resistance to Antibody-Drug Conjugates. Cancers

2023, 15, 1278. [CrossRef] [PubMed]

22. André, F.; Hee Park, Y.; Kim, S.-B.; Takano, T.; Im, S.-A.; Borges, G.; Lima, J.P.; Aksoy, S.; Gavila Gregori, J.; De Laurentiis, M.;

et al. Trastuzumab Deruxtecan versus Treatment of Physician's Choice in Patients with HER2-Positive Metastatic Breast Cancer

(DESTINY-Breast02): A Randomised, Open-Label, Multicentre, Phase 3 Trial. Lancet 2023, 401, 1773–1785. [CrossRef] [PubMed]

23. Cortés, J.; Kim, S.-B.; Chung,W.-P.; Im, S.-A.; Park, Y.H.; Hegg, R.; Kim,M.H.; Tseng, L.-M.; Petry, V.; Chung, C.-F.; et al. Trastuzumab

Deruxtecan versus Trastuzumab Emtansine for Breast Cancer. N. Engl. J.Med. 2022, 386, 1143–1154. [CrossRef] [PubMed]

24. Shitara, K.; Bang, Y.-J.; Iwasa, S.; Sugimoto, N.; Ryu, M.-H.; Sakai, D.; Chung, H.-C.; Kawakami, H.; Yabusaki, H.; Lee, J.; et al.

Trastuzumab Deruxtecan in Previously Treated HER2-Positive Gastric Cancer. N. Engl. J. Med. 2020, 382, 2419–2430. [CrossRef]

25. Li, B.T.; Smit, E.F.; Goto, Y.; Nakagawa, K.; Udagawa, H.; Mazières, J.; Nagasaka, M.; Bazhenova, L.; Saltos, A.N.; Felip, E.; et al.

Trastuzumab Deruxtecan in HER2-Mutant Non-Small-Cell Lung Cancer. N. Engl. J. Med. 2022, 386, 241–251. [CrossRef]

26. Bardia, A.; Hurvitz, S.A.; Tolaney, S.M.; Loirat, D.; Punie, K.; Oliveira, M.; Brufsky, A.; Sardesai, S.D.; Kalinsky, K.; Zelnak, A.B.;

et al. Sacituzumab Govitecan in Metastatic Triple-Negative Breast Cancer. N. Engl. J. Med. 2021, 384, 1529–1541. [CrossRef]

27. Rugo, H.S.; Bardia, A.; Marmé, F.; Cortes, J.; Schmid, P.; Loirat, D.; Trédan, O.; Ciruelos, E.; Dalenc, F.; Pardo, P.G.; et al.

Sacituzumab Govitecan in Hormone Receptor-Positive/Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast

Cancer. J. Clin. Oncol. 2022, 40, 3365–3376. [CrossRef]

28. Tagawa, S.T.; Balar, A.V.; Petrylak, D.P.; Kalebasty, A.R.; Loriot, Y.; Fléchon, A.; Jain, R.K.; Agarwal, N.; Bupathi,M.; Barthelemy, P.;

et al. TROPHY-U-01: A Phase II Open-Label Study of Sacituzumab Govitecan in Patients with Metastatic Urothelial Carcinoma

Progressing After Platinum-Based Chemotherapy and Checkpoint Inhibitors. J. Clin. Oncol. 2021, 39, 2474–2485. [CrossRef]

29. Powles, T.; Rosenberg, J.E.; Sonpavde, G.P.; Loriot, Y.; Durán, I.; Lee, J.-L.; Matsubara, N.; Vulsteke, C.; Castellano, D.;Wu, C.; et al.

Enfortumab Vedotin in Previously Treated Advanced Urothelial Carcinoma. N. Engl. J. Med. 2021, 384, 1125–1135. [CrossRef]

30. Hoimes, C.J.; Flaig, T.W.; Milowsky, M.I.; Friedlander, T.W.; Bilen, M.A.; Gupta, S.; Srinivas, S.; Merchan, J.R.; McKay, R.R.;

Petrylak, D.P.; et al. Enfortumab Vedotin Plus Pembrolizumab in Previously Untreated Advanced Urothelial Cancer. J. Clin. Oncol.

2023, 41, 22–31. [CrossRef]

31. Verma, S.; Miles, D.; Gianni, L.; Krop, I.E.; Welslau, M.; Baselga, J.; Pegram, M.; Oh, D.-Y.; Diéras, V.; Guardino, E.; et al.

Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer. N. Engl. J. Med. 2012, 367, 1783–1791. [CrossRef] [PubMed]

32. Junttila, T.T.; Li, G.; Parsons, K.; Phillips, G.L.; Sliwkowski, M.X. 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,

347–356. [CrossRef] [PubMed]

33. Lewis Phillips, G.D.; Li, G.; Dugger, D.L.; Crocker, L.M.; Parsons, K.L.;Mai, E.; Blättler,W.A.; Lambert, J.M.; Chari, R.V.J.; Lutz, R.J.;

et al. Targeting HER2-Positive Breast Cancer with Trastuzumab-DM1, an Antibody-Cytotoxic Drug

Conjugate. Cancer Res. 2008,

68, 9280–9290. [CrossRef] [PubMed]

34. Hunter, F.W.; Barker, H.R.; Lipert, B.; Rothé, F.; Gebhart, G.; Piccart-Gebhart, M.J.; Sotiriou, C.; Jamieson, S.M.F. Mechanisms of

Resistance to Trastuzumab Emtansine (T-DM1) in HER2-Positive Breast Cancer. Br. J. Cancer 2020, 122, 603–612. [CrossRef] [PubMed]

35. Barok,M.; Joensuu, H.; Isola, J. Trastuzumab Emtansine: Mechanisms of Action and Drug Resistance. Breast Cancer Res. 2014, 16, 209.

[CrossRef]

36. Erickson, H.K.; Park, P.U.;Widdison,W.C.; Kovtun, Y.V.; Garrett, L.M.; Hoffman, K.; Lutz, R.J.; Goldmacher, V.S.; Blättler,W.A.

Antibody-Maytansinoid Conjugates Are Activated in Targeted Cancer Cells by Lysosomal Degradation and Linker-Dependent

Intracellular Processing. Cancer Res. 2006, 66, 4426–4433. [CrossRef]

37. Issell, B.F.; Crooke, S.T. Maytansine. Cancer Treat. Rev. 1978, 5, 199–207. [CrossRef]

38. Hudis, C.A. Trastuzumab—Mechanism of Action and Use in Clinical Practice. N. Engl. J. Med. 2007, 357, 39–51. [CrossRef]

39. Cooley, S.; Burns, L.J.; Repka, T.; Miller, J.S. Natural Killer Cell Cytotoxicity of Breast Cancer Targets Is Enhanced by Two Distinct

Mechanisms ofAntibody-DependentCellularCytotoxicity against LFA-3 andHER2/Neu. Exp. Hematol. 1999, 27, 1533–1541. [CrossRef]

40. Spector, N.L.; Blackwell, K.L. Understanding the Mechanisms behind Trastuzumab Therapy for Human Epidermal Growth

Factor Receptor 2-Positive Breast Cancer. J. Clin. Oncol. 2009, 27, 5838–5847. [CrossRef] [PubMed]

41. Ogitani, Y.; Aida, T.; Hagihara, K.; Yamaguchi, J.; Ishii, C.; Harada, N.; Soma, M.; Okamoto, H.; Oitate, M.; Arakawa, S.; et al.

DS-8201a, A Novel HER2-Targeting ADC with a Novel DNA Topoisomerase I Inhibitor, Demonstrates a Promising Antitumor

Efficacy with Differentiation from T-DM1. Clin. Cancer Res. 2016, 22, 5097–5108. [CrossRef] [PubMed]

42. Nakada, T.; Sugihara, K.; Jikoh, T.; Abe, Y.; Agatsuma, T. The Latest Research and Development into the Antibody-Drug Conjugate,

[Fam-] Trastuzumab Deruxtecan (DS-8201a), for HER2 Cancer Therapy. Chem. Pharm. Bull. 2019, 67, 173–185. [CrossRef] [PubMed]

43. Ogitani, Y.; Hagihara, K.; Oitate, M.; Naito, H.; Agatsuma, T. Bystander Killing Effect of DS-8201a, a Novel Anti-Human

Epidermal Growth Factor Receptor 2 Antibody-Drug Conjugate, in Tumors with Human Epidermal Growth Factor Receptor 2

Heterogeneity. Cancer Sci. 2016, 107, 1039–1046. [CrossRef]

44. Modi, S.; Saura, C.; Yamashita, T.; Park, Y.H.; Kim, S.-B.; Tamura, K.; Andre, F.; Iwata, H.; Ito, Y.; Tsurutani, J.; et al. Trastuzumab

Deruxtecan in Previously Treated HER2-Positive Breast Cancer. N. Engl. J. Med. 2020, 382, 610–621. [CrossRef] [PubMed]

45. Modi, S.; Jacot,W.; Yamashita, T.; Sohn, J.; Vidal, M.; Tokunaga, E.; Tsurutani, J.; Ueno, N.T.; Prat, A.; Chae, Y.S.; et al. Trastuzumab

Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N. Engl. J. Med. 2022, 387, 9–20. [CrossRef]

46. Cardillo, T.M.; Govindan, S.V.; Sharkey, R.M.; Trisal, P.; Arrojo, R.; Liu, D.; Rossi, E.A.; Chang, C.-H.; Goldenberg, D.M. Sacituzumab

Govitecan (IMMU-132), an Anti-Trop-2/SN-38 Antibody-Drug Conjugate: Characterization and Efficacy in Pancreatic,

Gastric, and Other Cancers. Bioconjug. Chem. 2015, 26, 919–931. [CrossRef]

47. Bardia, A.; Mayer, I.A.; Vahdat, L.T.; Tolaney, S.M.; Isakoff, S.J.; Diamond, J.R.; O'Shaughnessy, J.; Moroose, R.L.; Santin, A.D.;

Abramson, V.G.; et al. Sacituzumab Govitecan-Hziy in Refractory Metastatic Triple-Negative Breast Cancer. N. Engl. J. Med. 2019,

380, 741–751. [CrossRef]

48. Shih, L.B.; Xuan, H.; Aninipot, R.; Stein, R.; Goldenberg, D.M. In Vitro and in Vivo Reactivity of an Internalizing Antibody, RS7,

with Human Breast Cancer. Cancer Res. 1995, 55, 5857s–5863s.

49. Kawato, Y.; Aonuma, M.; Hirota, Y.; Kuga, H.; Sato, K. Intracellular Roles of SN-38, a Metabolite of the Camptothecin Derivative

CPT-11, in the Antitumor Effect of CPT-11. Cancer Res. 1991, 51, 4187–4191.

50. Goldenberg, D.M.; Cardillo, T.M.; Govindan, S.V.; Rossi, E.A.; Sharkey, R.M. Trop-2 Is a Novel Target for Solid Cancer Therapy

with Sacituzumab Govitecan (IMMU-132), an Antibody-Drug Conjugate (ADC). Oncotarget 2015, 6, 22496–22512. [CrossRef]

51. Weiss, J.; Glode, A.; Messersmith, W.A.; Diamond, J. Sacituzumab Govitecan: Breakthrough Targeted Therapy for Triple-Negative

Breast Cancer. Expert Rev. Anticancer Ther. 2019, 19, 673–679. [CrossRef]

52. Chang, E.;Weinstock, C.; Zhang, L.; Charlab, R.; Dorff, S.E.; Gong, Y.; Hsu, V.; Li, F.; Ricks, T.K.; Song, P.; et al. FDA Approval Summary:

Enfortumab Vedotin for Locally Advanced orMetastatic Urothelial Carcinoma. Clin. Cancer Res. 2021, 27, 922–927. [CrossRef]

53. Challita-Eid, P.M.; Satpayev, D.; Yang, P.; An, Z.; Morrison, K.; Shostak, Y.; Raitano, A.; Nadell, R.; Liu, W.; Lortie, D.R.; et al.

Enfortumab Vedotin Antibody-Drug Conjugate Targeting Nectin-4 Is a Highly Potent Therapeutic Agent in Multiple Preclinical

Cancer Models. Cancer Res. 2016, 76, 3003–3013. [CrossRef] [PubMed]

54. Alt,M.; Stecca, C.; Tobin, S.; Jiang, D.M.; Sridhar, S.S. Enfortumab Vedotin in Urothelial Cancer. Ther. Adv. Urol. 2020, 12, 1756287220980192.

[CrossRef] [PubMed]

55. Heath, E.I.; Rosenberg, J.E. The Biology and Rationale of Targeting Nectin-4 in Urothelial Carcinoma. Nat. Rev. Urol. 2021, 18, 93–103.

[CrossRef] [PubMed]

56. Loganzo, F.; Tan, X.; Sung, M.; Jin, G.; Myers, J.S.; Melamud, E.;Wang, F.; Diesl, V.; Follettie, M.T.; Musto, S.; 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, 952–963. [CrossRef]

57. Filho, O.M.; Viale, G.; Stein, S.; Trippa, L.; Yardley, D.A.; Mayer, I.A.; Abramson, V.G.; Arteaga, C.L.; Spring, L.M.;Waks, A.G.;

et al. Impact of HER2 Heterogeneity on Treatment Response of Early-Stage HER2-Positive Breast Cancer: Phase II Neoadjuvant

Clinical Trial of T-DM1 Combined with Pertuzumab. Cancer Discov. 2021, 11, 2474–2487. [CrossRef]

58. Gebhart, G.; Lamberts, L.E.;Wimana, Z.; Garcia, C.; Emonts, P.; Ameye, L.; Stroobants, S.; Huizing, M.; Aftimos, P.; Tol, J.; et al.

Molecular Imaging as a Tool to Investigate Heterogeneity of Advanced HER2-Positive Breast Cancer and to Predict Patient

Outcome under Trastuzumab Emtansine (T-DM1): The ZEPHIR Trial. Ann. Oncol. 2016, 27, 619–624. [CrossRef]

59. Scaltriti, M.; Rojo, F.; Ocaña, A.; Anido, J.; Guzman, M.; Cortes, J.; Di Cosimo, S.; Matias-Guiu, X.; Ramon y Cajal, S.; Arribas, 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, 628–638. [CrossRef]

60. Nagy, P.; Friedländer, E.; Tanner,M.; Kapanen, A.I.; Carraway, K.L.; Isola, J.; Jovin, T.M. Decreased Accessibility and Lack of Activation

of ErbB2 in JIMT-1, a Herceptin-Resistant,MUC4-Expressing Breast Cancer Cell Line. Cancer Res. 2005, 65, 473–482. [CrossRef]

61. Phillips, G.D.L.; Fields, C.T.; Li, G.; Dowbenko, D.; Schaefer, G.; Miller, K.; Andre, F.; Burris, H.A.; Albain, K.S.; Harbeck, N.; 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, 456–468. [CrossRef]

62. Coates, J.T.; Sun, S.; Leshchiner, I.; Thimmiah, N.; Martin, E.E.; McLoughlin, D.; Danysh, B.P.; Slowik, K.; Jacobs, R.A.;

Rhrissorrakrai, K.; et al. Parallel Genomic Alterations of Antigen and Payload Targets Mediate Polyclonal Acquired Clinical

Resistance to Sacituzumab Govitecan in Triple-Negative Breast Cancer. Cancer Discov. 2021, 11, 2436–2445. [CrossRef] [PubMed]

63. Klümper, N.; Ralser, D.J.; Ellinger, J.; Roghmann, F.; Albrecht, J.; Below, E.; Alajati, A.; Sikic, D.; Breyer, J.; Bolenz, C.; et al.

Membranous NECTIN-4 Expression Frequently Decreases during Metastatic Spread of Urothelial Carcinoma and Is Associated

with Enfortumab Vedotin Resistance. Clin. Cancer Res. 2023, 29, 1496–1505. [CrossRef] [PubMed]

64. Yu, S.-F.; Zheng, B.; Go, M.; Lau, J.; Spencer, S.; Raab, H.; Soriano, R.; Jhunjhunwala, S.; Cohen, R.; Caruso, M.; et al. A

Novel Anti-CD22 Anthracycline-Based Antibody-Drug Conjugate (ADC) That Overcomes Resistance to Auristatin-Based ADCs.

Clin. Cancer Res. 2015, 21, 3298–3306. [CrossRef] [PubMed]

65. Takegawa, N.; Nonagase, Y.; Yonesaka, K.; Sakai, K.; Maenishi, O.; Ogitani, Y.; Tamura, T.; Nishio, K.; Nakagawa, K.; Tsurutani, J.

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, 1682–1689. [CrossRef] [PubMed]

66. Conilh, L.; Sadilkova, L.; Viricel,W.; Dumontet, C. Payload Diversification: A Key Step in the Development of Antibody-Drug

Conjugates. J. Hematol. Oncol. 2023, 16, 3. [CrossRef]

67. Qiu, D.; Huang, Y.; Chennamsetty, N.; Miller, S.A.; Hay, M. Characterizing and Understanding the Formation of Cysteine

Conjugates and Other By-Products in a Random, Lysine-Linked Antibody Drug Conjugate. MAbs 2021, 13, 1974150. [CrossRef]

68. Hamblett, K.J.; Senter, P.D.; Chace, D.F.; Sun, M.M.C.; Lenox, J.; Cerveny, C.G.; Kissler, K.M.; Bernhardt, S.X.; Kopcha, A.K.;

Zabinski, R.F.; et al. Effects of Drug Loading on the Antitumor Activity of aMonoclonal Antibody Drug Conjugate. Clin. Cancer Res.

2004, 10, 7063–7070. [CrossRef]

69. Yoder, N.C.; Bai, C.; Tavares, D.;Widdison,W.C.;Whiteman, K.R.;Wilhelm, A.;Wilhelm, S.D.;McShea,M.A.;Maloney, E.K.; Ab, O.;

et al. A Case Study Comparing Heterogeneous Lysine- and Site-Specific Cysteine-Conjugated Maytansinoid Antibody-Drug

Conjugates (ADCs) Illustrates the Benefits of Lysine Conjugation. Mol. Pharm. 2019, 16, 3926–3937. [CrossRef]

70. Bai, C.; Reid, E.E.;Wilhelm, A.; Shizuka, M.; Maloney, E.K.; Laleau, R.; Harvey, L.; Archer, K.E.; Vitharana, D.; Adams, S.; et al.

Site-Specific Conjugation of the Indolinobenzodiazepine DGN549 to Antibodies Affords Antibody-Drug Conjugates with an

Improved Therapeutic Index as Compared with Lysine Conjugation. Bioconjug Chem. 2020, 31, 93–103. [CrossRef]

71. Kalim, M.; Chen, J.; Wang, S.; Lin, C.; Ullah, S.; Liang, K.; Ding, Q.; Chen, S.; Zhan, J. Intracellular

Trafficking of New Anticancer

Therapeutics: Antibody-Drug Conjugates. Drug Des. Dev. Ther. 2017, 11, 2265–2276. [CrossRef] [PubMed]

72. Sung, M.; Tan, X.; Lu, B.; Golas, J.; Hosselet, C.;Wang, F.; Tylaska, L.; King, L.; Zhou, D.; Dushin, R.; et al. Caveolae-Mediated

Endocytosis as a Novel Mechanism of Resistance to Trastuzumab Emtansine (T-DM1). Mol. Cancer Ther. 2018, 17, 243–253.

[CrossRef] [PubMed]

73. Baldassarre, T.; Truesdell, P.; Craig, A.W. Endophilin A2 Promotes HER2 Internalization and Sensitivity to Trastuzumab-Based

Therapy in HER2-Positive Breast Cancers. Breast Cancer Res. 2017, 19, 110. [CrossRef] [PubMed]

74. Ríos-Luci, C.; García-Alonso, S.; Díaz-Rodríguez, E.; Nadal-Serrano, M.; Arribas, J.; Ocaña, A.; Pandiella, A. Resistance to the

Antibody-Drug Conjugate T-DM1 Is Based in a Reduction in Lysosomal Proteolytic Activity. Cancer Res. 2017, 77, 4639–4651. [CrossRef]

75. Trudeau, K.M.; Colby, A.H.; Zeng, J.; Las, G.; Feng, J.H.; Grinstaff, M.W.; Shirihai, O.S. Lysosome Acidification by Photoactivated

Nanoparticles RestoRes. Autophagy under Lipotoxicity. J. Cell Biol. 2016, 214, 25–34. [CrossRef]

76. Hamblett, K.J.; Jacob, A.P.; Gurgel, J.L.; Tometsko, M.E.; Rock, B.M.; Patel, S.K.; Milburn, R.R.; Siu, S.; Ragan, S.P.; Rock, D.A.;

et al. SLC46A3 Is Required to Transport Catabolites of Noncleavable Antibody Maytansine Conjugates from the Lysosome to the

Cytoplasm. Cancer Res. 2015, 75, 5329–5340. [CrossRef]

77. Mosele, M.F.; Lusque, A.; Dieras, V.; Deluche, E.; Ducoulombier, A.; Pistilli, B.; Bachelot, T.; Viret, F.; Levy, C.; Signolle, N.; et al.

LBA1 Unraveling the Mechanism of Action and Resistance to Trastuzumab Deruxtecan (T-DXd): Biomarker Analyses from

Patients from DAISY Trial. Ann. Oncol. 2022, 33, S123. [CrossRef]

78. Shah, S.; Kim, Y.; Ostrovnaya, I.; Murali, R.; Schrader, K.A.; Lach, F.P.; Sarrel, K.; Rau-Murthy, R.; Hansen, N.; Zhang, L.; et al.

Assessment of SLX4 Mutations in Hereditary Breast Cancers. PLoS ONE 2013, 8, e66961. [CrossRef]

79. Leonard, G.D.; Fojo, T.; Bates, S.E. The Role of ABC Transporters in Clinical Practice. Oncologist 2003, 8, 411–424. [CrossRef]

80. Yu, M.; Ocana, A.; Tannock, I.F. Reversal of ATP-Binding Cassette Drug Transporter Activity to Modulate Chemoresistance:

Why Has It Failed to Provide Clinical Benefit? Cancer Metastasis Rev. 2013, 32, 211–227. [CrossRef]

81. Kovtun, Y.V.; Audette, C.A.; Mayo, M.F.; Jones, G.E.; Doherty, H.; Maloney, E.K.; Erickson, H.K.; Sun, X.; Wilhelm, S.; Ab, O.; et al.

Antibody-Maytansinoid Conjugates Designed to Bypass Multidrug Resistance. Cancer Res. 2010, 70, 2528–2537. [CrossRef]

82. 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, 150–168. [CrossRef]

83. Lambert, J.M.; Chari, R.V.J. Ado-Trastuzumab Emtansine (T-DM1): An Antibody-Drug Conjugate (ADC) for HER2-Positive

Breast Cancer. J. Med. Chem. 2014, 57, 6949–6964. [CrossRef]

84. Berns, K.; Horlings, H.M.; Hennessy, B.T.; Madiredjo, M.; Hijmans, E.M.; Beelen, K.; Linn, S.C.; Gonzalez-Angulo, A.M.;

Stemke-Hale, K.; Hauptmann, M.; 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. [CrossRef] [PubMed]

85. Sabbaghi, M.; Gil-Gómez, G.; Guardia, C.; Servitja, S.; Arpí, O.; García-Alonso, S.; Menendez, S.; Arumi-Uria, M.; Serrano, L.;

Salido, M.; et al. Defective Cyclin B1 Induction in Trastuzumab-Emtansine (T-DM1) Acquired Resistance in HER2-Positive Breast

Cancer. Clin. Cancer Res. 2017, 23, 7006–7019. [CrossRef] [PubMed]

86. Baselga, J.; Lewis Phillips, G.D.; Verma, S.; Ro, J.; Huober, J.; Guardino, A.E.; Samant, M.K.; Olsen, S.; de Haas, S.L.; Pegram, M.D.

Relationship between Tumor Biomarkers and Efficacy in EMILIA, a Phase III Study of Trastuzumab Emtansine in HER2-Positive

Metastatic Breast Cancer. Clin. Cancer Res. 2016, 22, 3755–3763. [CrossRef]

87. Wu, Y.; Ginther, C.; Kim, J.;Mosher, N.; Chung, S.; Slamon, D.; Vadgama, J.V. Expression ofWnt3 ActivatesWnt/_-Catenin Pathway

and Promotes EMT-like Phenotype in Trastuzumab-Resistant HER2-Overexpressing Breast Cancer Cells.Mol. Cancer Res. 2012, 10,

1597–1606. [CrossRef]

88. Walter, R.B.; Raden, B.W.; Cronk, M.R.; Bernstein, I.D.; Appelbaum, F.R.; Banker, D.E. The Peripheral Benzodiazepine Receptor

Ligand PK11195 Overcomes Different Resistance Mechanisms to Sensitize AML Cells to Gemtuzumab Ozogamicin. Blood 2004,

103, 4276–4284. [CrossRef]

89. Moore, J.; Seiter, K.; Kolitz, J.; Stock, W.; Giles, F.; Kalaycio, M.; Zenk, D.; Marcucci, G. 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, 777–783. [CrossRef] [PubMed]

90. Dornan, D.; Bennett, F.; Chen, Y.; Dennis, M.; Eaton, D.; Elkins, K.; French, D.; Go, M.A.T.; Jack, A.; Junutula, J.R.; 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, 2721–2729. [CrossRef]

91. Ab, O.; Whiteman, K.R.; Bartle, L.M.; Sun, X.; Singh, R.; Tavares, D.; LaBelle, A.; Payne, G.; Lutz, R.J.; Pinkas, J.; et al.

IMGN853, a Folate Receptor-_ (FR_)-Targeting Antibody-Drug Conjugate, Exhibits Potent Targeted Antitumor Activity against

FR_-Expressing Tumors. Mol. Cancer Ther. 2015, 14, 1605–1613. [CrossRef] [PubMed]

92. Golfier, S.; Kopitz, C.; Kahnert, A.; Heisler, I.; Schatz, C.A.; Stelte-Ludwig, B.; Mayer-Bartschmid, A.; Unterschemmann, K.;

Bruder, S.; Linden, L.; 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, 1537–1548. [CrossRef] [PubMed]

93. Li, F.; Emmerton, K.K.; Jonas, M.; Zhang, X.; Miyamoto, J.B.; Setter, J.R.; Nicholas, N.D.; Okeley, N.M.; Lyon, R.P.; Benjamin, D.R.;

et al. Intracellular Released Payload Influences Potency and Bystander-Killing Effects of Antibody-Drug Conjugates in Preclinical

Models. Cancer Res. 2016, 76, 2710–2719. [CrossRef]

94. Li, J.Y.; Perry, S.R.; Muniz-Medina, V.; Wang, X.; Wetzel, L.K.; Rebelatto, M.C.; Hinrichs, M.J.M.; Bezabeh, B.Z.; Fleming, R.L.;

Dimasi, N.; 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, 117–129. [CrossRef] [PubMed].