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The development of payload resistant cell lines to study antibody-drug conjugate resistance in high-grade serous ovarian cancer

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Abstract

Antibody-drug conjugates (ADCs) are increasingly used to treat recurrent high grade serous ovarian cancer (HGSOC), yet their effects on tumor biology and subsequent treatment response remain poorly understood. We evaluated the short-term effects of ADCs and their respective payloads on ADC receptor expression and developed payload resistant HGSOC cell lines to determine how acquired payload resistance influences response to subsequent ADC therapy. Matched platinum sensitive and resistant HGSOC cell lines were exposed to trastuzumab deruxtecan (T-DXd) and mirvetuximab soravtansine (MIRV), or their respective payloads, DXd and DM4. DXd and DM4 resistant cell lines were generated through sequential payload dose escalation and characterized for ADC receptor expression and response to T-DXd, MIRV, and sacituzumab govitecan (SG). Short-term T-DXd and MIRV exposure reduced their respective targets in chemosensitive cells and altered several clinically relevant ADC receptors. DXd and DM4 resistant cell lines were developed and confirmed resistant. Notably, DM4 resistant cells showed increased T-DXd sensitivity, whereas DXd resistant cells exhibited reduced sensitivity to MIRV and SG. These findings demonstrate that ADC exposure dynamically alters receptor expression and that acquired payload resistance can differentially influence subsequent ADC response. Further investigation may identify biomarkers of ADC resistance and inform upon ADC sequencing strategies in HGSOC.

Introduction

Antibody-drug conjugates (ADCs) are becoming increasingly prevalent within the field of gynecologic oncology. There are currently two ADCs approved by the Federal Drug Administration (FDA) for use in high grade serous ovarian cancer (HGSOC): trastuzumab deruxtecan (TDXD) and mirvetuximab soravtansine (MIRV). TDXD targets HER2 and has a deruxtecan (DXD) topoisomerase I inhibitor payload [1], while MIRV targets FOLR1 (also referred to as FRα) and has a DM4 maytansinoid tubulin inhibitor payload [2]. While these two drugs are currently approved for use only in the recurrent, platinum-resistant setting for ovarian cancer, these – and many other – ADCs are under investigation for use in platinum-sensitive recurrence and in the upfront/first line setting [3, 4]. Despite ongoing ADC development and testing, there is a paucity of data within HGSOC regarding resistance mechanisms and subsequent drug treatment implications.

A major clinical challenge is that resistance to ADCs have the potential to arise through multiple mechanisms, including reduced expression of the target antigen on the cell surface or resistance to the cytotoxic payload-the chemotherapeutic agent conjugated to the antibody [5]. While the mechanism behind ADC resistance has yet to be systematically defined in HGSOC, accumulating clinical data in breast cancer strongly suggests that ADC resistance is largely driven by the payload [6, 7]. In the multicenter phase III DESTINY-Breat02 trial of patients with HER2-postive metastatic breast cancer, patients with refractory or resistant disease following the HER2-directed ADC trastuzumab emtansine (T-DM1) still demonstrated robust and durable responses to TDXD, another HER2-directed ADC with a distinct payload [6], suggesting that even with an ADC targeting the same receptor that resistance could

be overcome with a different payload class. Corroborating these findings, the TROPION-PanTumor01 trial in metastatic triple negative breast cancer (mTNBC) suggested that prior exposure to a topoisomerase 1 inhibitor payload influences response to the TROP2-directed ADC datopotamab deruxtecan, with durable responses observed predominantly in patients who were naïve to previous topoisomerase 1 inhibitor ADC payload treatment [7]. Finally, a real-world analyses in mTNBC patients demonstrated that treatment with TDXD following prior exposure to sacituzumab govitecan (SG), a TROP2-directed ADC carrying a similar topoisomerase 1 inhibitor payload, was associated with a shorter progression free survival (PFS) and OS compared to patients without prior SG exposure [8]. Collectively, these breast clinical studies strongly implicate the payload as the key determinant of ADC resistance and suggest that cross-resistance between ADCs depends on shared payload class. Nevertheless, whether similar mechanisms govern ADC resistance and influence optimal ADC sequencing in HGSOC remains unknown, representing a critical barrier to maximizing the therapeutic potential of ADCs for ovarian cancer patients.

Hence, the objectives of this study were twofold: first, to determine the effect of ADCs and their respective payloads on the expression of treatment receptor targets in HGSOC cell lines; second, to develop and characterize payload-resistant human HGSOC cell lines and determine the effect of payload resistance on subsequent ADC exposure.

Methods

Cell culture

Human HGSOC PEA1 and PEA2 cell lines (Sigma, Burlington, MA, 10032306 and 10032307, respectively) were cultured in RPMI 1640 media with 2mM glutamine (Gibco, Grand Island, NY, 11875-093) supplemented with 2mM sodium pyruvate (Sigma S8636), 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA A4766801), and 1% penicillin/streptomycin (Sigma P4333). All cells were kept in a monitored 37°C/5% CO2 humidified chamber.

Short-term ADC and payload exposure

Cells were treated with TDXD (0.2ug/mL for PEA1 and PEA2, MedChemExpress, Monmouth Junction, NJ, HY-138298), IgG1-DXD control (0.2ug/mL for PEA1 and PEA2, MedChemExpress HY-164152A), DXD payload (500nM for PEA1 and 50nM for PEA2, MedChemExpress HY-13631D), MIRV (8nM for PEA1 and 4nM for PEA2, MedChemExpress HY-132258A), IgG1-DM4 control (8nM for PEA1 and 4nM for PEA2, MedChemExpress HY-164669), DM4 payload (5nM for PEA1 and 2.5nM for PEA2, MedChemExpress HY-12454), and DMSO control (matched dose to ADC treatments, Sigma D8418) for 48 hours. Treatments were first tested via a cell viability assay to ensure all treatments were subtoxic (Supplementary Figure S1).

Development of payload-resistant cell lines

Payload treatments were first tested via cell viability assays to identify subtoxic dosing for DXD and DM4 payload treatments, referred to as starting dose X, for both PEA1 and PEA2 cell lines as noted above. Each cell line received separate escalating treatments of both payloads: three treatments at dose X, followed by three at dose 2X, and lastly three at dose 4X (visualized in Fig. 1, doses listed in Supplementary Table S2). Each treatment lasted for 48 hours, and cells were recovered in regular culture media until ready for subsequent treatment. After recovery from the last 4X dose treatment, cells were maintained in maintenance media at a dose of 0.5X and used for additional testing.

Cell viability assays

Payload-resistant cell lines and controls were plated on a 96-well plate at a concentration of 20,000 cells/well in regular cell culture media for 24 hours prior to subsequent treatments. For confirmation of payload resistance, cells were treated with regular cell culture media (control), DMSO control, and payload doses X, 2X, 4X, 8X, 16X, and 32X (all doses listed in Supplementary Table S2). For subsequent ADC exposure experiments, in addition to regular cell culture media, DXD-resistant cells and controls were treated with MIRV (16nM and 32nM for PEA1, 8nM and 16nM for PEA2) and sacituzumab govetecan (SG, 400nM and 600nM for PEA1, 500nM and 1uM for PEA2); DM4-resistant cells and controls were treated with TDXD (1.6mg/mL and 3.2mg/mL for PEA1 and PEA2). After 48 hours, cells were exposed to 10µl per well of CellTiter 96® Aqueous One Solution Cell proliferation MTS Assay (Promega, Madison WI, G3580) and incubated for 1 hour at 37°C/5% CO2 prior to absorbance reading at 490nm using a Molecular Devices (San Jose, CA) SpectraMax M2 plate reader with SoftMax Pro software v7.0.3.

Imaging

Prior to protein isolation, cells grown to confluence on 6-well dishes were imaged using a Zeiss (White Plains, NY) Axiovert 40 CFL microscope with 10X objective. AmScope (Irvine, CA) MD35A eyepiece with associated IQCrew software was used for further magnification and digital image capture.

Western blot

Protein isolation, western blot, and associated analysis were performed as previously described, with the only modification being an increase in gel electrophoresis time to 90 minutes [9]. All uncropped blots are included in Supplementary Figures S3, S4, and S5. Antibodies and dilutions were as follows: B7H4 (Proteintech (Rosemont, IL, 82850-1-RR, 1:500), CLDN6 (Cell Signaling 18932S, 1:250), FOLR1 (Proteintech 60307-1-I, 1:250), GAPDH (Santa Cruz Biotechnology, Dallas, TX, 47724, 1:250), HER2/ErbB2 (Cell Signaling Technology, Danvers, MA, 4290S, 1:125), PD-L1/CD274 (Proteintech 66248-1-Ig, 1:250), TACSTD2/TROP2 (Proteintech 27360-1-AP, 1:250), VEGFA (Proteintech 81323-2-RR, 1:250), Anti-Mouse

Secondary (Cell Signaling Technology 7076S, 1:2000), and Anti-Rabbit Secondary (Cell Signaling 7074S, 1:2000). Protein bands were quantified using ImageJ 1.47t.

Statistical analyses

GraphPad Prism (Boston, MA) v11.0.2 was used for analyses and data visualization. Unpaired t-tests were used for statistical comparison unless noted otherwise. All p-values reported as (*) for $p < 0.05$, (**) for $p < 0.01$, and (***) for $p < 0.001$.

Results

Short-term treatment of PEA1 and PEA2 cell lines with TDXD ADC, DXD payload, MIRV ADC, and DM4 payload resulted in variable protein expression changes

PEA1 and PEA2 cells lines were treated with DXD and MIRV ADCs, and their respective DXD and DM4 payloads, to determine if differential protein expression changes would be observed following short-term treatments.

In the chemosensitive PEA1 cell line, treatment with both TDXD ADC and DXD payload resulted in significantly decreased HER2 expression compared to control; however, this decrease was more striking in the DXD treatment group, with a statistically significant decrease in HER2 expression after DXD treatment compared to TDXD. Additionally, treatment with DXD payload alone resulted in significantly decreased B7H4 expression compared to control and significantly decreased FOLR1 expression compared to TDXD treatment. No significant changes were found in CLDN6 or TROP2 expression between treatment groups expression (Fig. 2A). In the chemoresistant PEA2 cell line, treatment with DXD payload alone resulted in significantly decreased HER2 expression; no difference was noted in HER2 expression between TDXD treatment and control groups. Treatment with both TDXD ADC and DXD payload resulted in significantly increased TROP2 expression compared to control; however, this increase was more striking in the DXD payload treatment group, with a statistically significant increase in TROP2 expression after DXD treatment compared to TDXD. CLDN6 expression was significantly increased after TDXD treatment, but not DXD payload. There was trend towards decreased B7H4 expression with TDXD and DXD treatments, though this was not statistically significant ($p = 0.1$). No changes in FOLR1 expression were observed between treatment groups (Fig. 2B). Western blot images used for quantification are visualized in Fig. 2C.

Treatment with MIRV and DM4 payload resulted in different effects. In PEA1 cells, treatment with MIRV ADC resulted in statistically significantly lower FOLR1 expression compared to control; DM4 payload treatment also resulted in decreased FOLR1 expression, but this was not statistically significant ($p = 0.051$). There was a trend towards decreased HER2 expression after MIRV and DM4 treatments compared to control, but these were not statistically significant ($p = 0.1$). MIRV ADC treatment resulted in significantly increased B7H4 and CLDN6 expression compared to control, while no significant changes

were noted between DM4 treatment and control. DM4 treatment resulted in significantly increased TROP2 expression compared to MIRV treatment, but neither was significantly different than controls (Fig. 3A). In PEA2 cells, no significant differences in FOLR1 expression were noted between treatment groups. Both MIRV and DM4 treatments resulted in significantly decreased HER2 expression. DM4 payload, but not MIRV ADC, treatment resulted in significantly increased CLDN6 and TROP2 expression compared to control. No significant differences in B7H4 expression were noted between MIRV and DM4 treatments compared to control (Fig. 3B). Western blot images used for quantification are visualized in Fig. 3C.

Payload-resistant HGSOC cell lines remain resistant at various payload treatment doses, and demonstrate differential changes in ADC receptor target protein expression

Next, we exposed PEA1 and PEA2 cell lines to escalating doses of DXD and DM4 payloads to create payload-resistant cell lines, as outlined in Fig. 1. Both PEA1 and PEA2 DXD- and DM4-resistant cell lines were confirmed to be resistant, with statistically significant increased cell viability compared to payload-sensitive/naïve controls at a range of payload doses (Fig. 4A). All four payload-resistant cell lines demonstrated differences in morphologic appearance compared to their payload-sensitive controls (Fig. 4B).

When compared to PEA1 payload-sensitive controls, which expressed robust FOLR1, HER2, and B7H4, mild TROP2, and no CLDN6, PEA1 DXD-resistant cells expressed less B7H4 and FOLR1, more CLDN6 and TROP2, and similar HER2. In contrast, PEA1 DM4-resistant cells also did not express CLDN6, expressed less FOLR1, HER2, and B7H4, and expressed more TROP2 than PEA1 payload-sensitive controls (Fig. 5).

PEA2 payload-sensitive controls expressed robust CLDN6 and B7H4, and mild FOLR1, HER2, and TROP2. Compared to these controls, PEA2 DXD-resistant cells expressed more FOLR1 and TROP2, less B7H4 and CLDN6, and similar HER2, while PEA2 DM4-resistant cells expressed similar CLDN6 and B7H4, slightly increased FOLR1, increased TROP2, and decreased HER2 (Fig. 5).

Payload-resistant HGSOC cell lines exhibit different responses to subsequent ADC treatment

Lastly, we exposed both payload-sensitive and payload-resistant cell lines to ADC treatments containing the alternate payload, to determine if payload resistance alters subsequent susceptibly to differential ADC treatment.

In PEA1 DM4-resistant cells, which expressed less HER2 than DM4-sensitive controls (Fig. 5), there was a small, but statistically significant, increased resistance to TDXD treatment at a lower dose, and a small increase in susceptibility to TDXD treatment at higher dose (Supplementary Figure S6A). PEA2 DM4-resistant cells, which also expressed less HER2 than their DM4-sensitive controls (Fig. 5), demonstrated increased susceptibility to TDXD ADC treatment at both treatment doses (Supplementary Figure S6B).

PEA1 DXD-resistant cells, which expressed less FOLR1 than DXD-sensitive controls (Fig. 5), were more resistant to MIRV ADC treatment at both treatment doses (Fig. 6A). Though PEA2 DXD-resistant cells expressed more FOLR1 than controls (Fig. 5), a similar resistance to subsequent MIRV treatment was observed at the higher dose (Fig. 6B). Despite PEA1 and PEA2 DXD-resistant cells demonstrating increased TROP2 protein expression (Fig. 5), there was a statistically significant resistance to treatment with the TROP2-targeting ADC SG at multiple doses compared to controls (Fig. 6C-D).

Discussion

As ADC drug development in the ovarian cancer landscape continues to advance at a rapid pace, the lack of preclinical mechanistic and translational information creates a challenge in interpreting clinical data and in clinical decision making. This study aimed to begin to understand key molecular changes after short-term ADC and payload treatment, as well as molecular changes and subsequent treatment implications after long-term development of payload resistance.

In the chemosensitive PEA1 cell line, short-term treatment with TDXD and MIRV ADCs resulted in a corresponding decrease in receptor target expression (HER2 and FOLR1, respectively). A recent study compared specimens from patients with breast cancer before and after primary TDXD treatment, and identified that nearly half of cases demonstrated loss of HER2 expression, supporting target resistance as a mechanism of ADC resistance [10]. These findings are particularly important within ovarian cancer management, and sequencing as ADC treatment is actively under investigation in the front-line maintenance setting, for example, GOG 3112 utilizing TDXD (NCT06819007), and progression after this treatment may have unique implications for subsequent therapy lines. If target resistance is a primary mechanism in some cases, additional elucidation of the associated drivers are necessary; in breast cancer, data support loss of antigen [11, 12] and impaired internalization of ADC-antigen complexes [13].

In the chemoresistant PEA2 cell line, however, treatment with TDXD and MIRV ADCs did not result in any significant changes in expression of their respective HER2 or FOLR1 receptor targets. This is in contrast to a recent retrospective review of patients with recurrent HGSOC who received MIRV treatment, which demonstrated decreased FOLR1 expression in samples obtained after MIRV treatment [14]. However, these data are limited and further investigation is necessary.

Off-target ADC receptor expression changes were also noted with these short-term treatments. Notably, in PEA2 cells, TDXD treatment resulted in increased TROP2 expression. In a recent study of 193 patient HGSOC samples, only 9 (or 4.6%) demonstrated strong co-expression of both HER2 and TROP2 [15], however no treatment responses were explored. In patients with HER2-positive breast cancer, those with poor response to HER2-targeting therapy were found to have increased TROP2 expression in post-treatment samples [16]. A possible mechanism for this is explained in a gastric cancer model of trastuzumab resistance, where resistant cells expressed higher TROP2 expression than trastuzumab-sensitive controls, with restoration of trastuzumab susceptibility with transient *TROP2* knock-down [17].

Variable expression changes in B7H4 were noted, while several treatments resulted in increased CLDN6 expression. In a comparison of 24 carboplatin/paclitaxel-sensitive versus 24 resistant samples with HGSOC, B7H4 was found to be more highly expressed in the resistant samples, and high B7H4 expression was associated with worse survival outcomes [18]. A different study of 119 patients with FIGO stage III-IV HGSOC who received platinum-containing chemotherapy demonstrated a significant association between high CLDN6 expression and platinum resistance [19]. While these studies looked specifically at changes after traditional chemotherapy, they support that idea that drug resistance may lead to continued tumor genomic alterations, which warrants further study as ADCs are incorporated into treatment paradigms.

A variety of breast cancer cell lines have been used to develop ADC resistance – mostly to trastuzumab emtansine (TDM1) – with a predominance of resistant mechanisms linked to payload via increased drug transport and decreased lysosomal activity [20]. However, to the best of our knowledge, there are no previous manuscripts reporting ADC- or payload-resistant gynecologic cancer cell lines. This, in combination with our lack of on-target ADC receptor target expression differences in PEA2 cells after short-term exposure, was the rationale for creation of DXD and DM4 payload-resistant cell lines as a method to study long-term effect of drug exposure specifically in HGSOC. We demonstrated successful creation of PEA1 and PEA2 DXD and DM4-resistant cell lines, with confirmed resistance at a variety of treatment doses well above the doses used for creation of resistance itself. Drug-resistant cell lines in other cancer types exhibit similar, variable resistance curves compared to control [21], highlighting that some cell death may still occur, but less than their drug-sensitive controls.

Both PEA1 and PEA2 DM4-resistant cell lines expressed less HER2 than their DM4-suseceptible controls. However, the DM4-resistant cell lines were more susceptible to subsequent TDXD ADC treatment than controls at higher doses.

PEA1 DXD-resistant cells were resistant to MIRV ADC treatment at different doses. This may be due to the decreased FOLR1 expression noted in the DXD-resistant cell line. Interestingly, though the PEA2 DXD-resistant cells expressed more FOLR1 protein compared to DXD-sensitive controls, they were resistant to higher dose MIRV treatment, suggesting an alternate method of resistance beyond target downregulation. A study of anti-MUC16/MMAE ADC resistance in ovarian cancer demonstrated cross resistance to other ADCs despite stable ADC target expression, caused by altered lysosomal ADC processing [22]. Our results suggest that molecular testing of recurrent specimens may provide important clinical information to drive treatment decisions after progression on a particular ADC.

As a DNA topoisomerase I inhibitor, DXD is a member of the camptothecin drug class, which is the most common payload class utilized in ADC development (reviewed by [23]). We therefore treated DXD-resistant cells with an ADC with a different camptothecin payload: SG, which targets TROP2 with an SN38 (a topoisomerase 1 inhibitor) payload. Interestingly, while PEA1 and PEA2 DXD-resistant cell lines expressed increased TROP2, they were significantly resistant to SG ADC treatment at both doses tested. DESTINY-Breast02 was the first study to demonstrate clinical efficacy of sequential ADC treatment with

the same target, but different payloads (TDM1 followed by TDXD), supporting payload resistance as a key mechanistic driver of cancer recurrence [6]. Results from the TROPION-PanTumor01 study demonstrated that treatment with datopotamab deruxtecan was less efficacious in patients with prior exposure to topoisomerase I inhibition, though sample size was small [7], and recent abstract presentation from the SATEEN trial demonstrated that treatment with SG after TDXD yielded a limited objective response rate (ORR) of only 3.7% [24]. A retrospective study in China compared ORRs to treatment of sequential ADC based on whether the second ADC was of a different or similar payload as the first, and found that using an ADC with different payload improved the ORR to 22.6 from 5.3% [25]. On a cellular level, there may be variability within mechanisms of payload resistance that will require additional investigation. In breast cancer cell lines resistant to TDXD and SG, increased drug efflux was the primary mechanism of payload resistance [26], while in TDM1-resistant breast cancer cells, cyclinB1 was found to be a major driver [27]. In ovarian cancer, drug efflux proteins MDR1, MRP1, and BCRP (encoded by the respective genes *ABCB1*, *ABCC1*, and *ABCG2*) have been linked to chemoresistance [28], but have not yet been explored in ADC and/or payload resistance. Other payload-related mechanisms of resistance identified in breast cancer studies, such as decreased lysosomal degradation of ADCs [29] and impaired transport of dissociated payload from lysosome to cytoplasm [30], have not been explored in ovarian cancer, and warrant future investigation.

It is important to highlight that some cell death was still observed in our DXD-resistant cells treated with SG. Within gynecologic oncology, a single case report was found describing successful use of TDXD after SG in recurrent, platinum-resistant HGSOC [31]. Additionally, an analysis of DESTINY-PanTumor02 Part 1 demonstrated some tumor response to TDXD in patients who had prior exposure to topoisomerase inhibition, but the sample size was small and not comparative [32]. As discussed in reviews for both breast [33] and lung cancer [34], there are likely to be multiple contributing mechanisms of resistance. Furthermore, these mechanisms could vary from patient to patient: an *in vitro* study with two different TDM1-resistant breast cancer cell lines identified that DM1 resistance was mediated by different primary mechanisms in each line [35]. Additional studies within ovarian cancer models are necessary to explore these varying mechanisms of ADC resistance in greater detail. Novel combinatorial strategies are in development to combine ADCs with other targeted therapies such as immunotherapy and tyrosine kinase inhibitors [36], so additional work is needed to better understand how ADC and payload resistance alters expression of non-ADC targets and impacts efficacy of these other drugs.

In conclusion, short-term ADC receptor expression is variably altered after exposure to ADCs and their respective payloads. DXD- and DM4-resistant HGSOC cell lines demonstrate differences in ADC receptor expression and demonstrate changes in subsequent ADC treatment response. Additional work is necessary to better understand the mechanisms underlying the observed differences, and to begin to work towards implementation of these findings into meaningful clinical studies to determine optimal drug sequencing and combinatorial strategies.

Declarations

Competing Interests

Dr. James reports funding from Swim Across America and the Lura Cook Hull Trust used to in part fund research related to this manuscript. Dr. DiSilvestro has consulted for Gilead and Daichii Sankyo for which he received compensation. Dr. Mathews reports grants/contracts with AADI, Abbvie, Artios, Astellas Pharma, AstraZeneca, Avenge Bio, Context Therapeutics, Deciphera, Debio, Elucida, EMD Serono, Genentech, Genmab, GSK, Immunogen, Merck, Moderna, Novocure, NCI, Pfizer, ProfoundBio, Regeneron, Repare, Seagen, Syros, Volastra, and Zentalis. All other authors declare no competing interests.

Author Contribution

NEJ, PD, JVP, and CM conceived this study. JVP, PDLC, SB, AB, AS, and NEJ performed all experiments related to this study. JVP and NEJ performed formal analyses. JVP and NEJ wrote this manuscript, which was edited and approved by all authors. NEJ oversaw all aspects of this study

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Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

References

1. Meric-Bernstam, F. et al. Efficacy and Safety of Trastuzumab Deruxtecan in Patients With HER2-Expressing Solid Tumors: Primary Results From the DESTINY-PanTumor02 Phase II Trial. *J. Clin. Oncol.* **42** (1), 47–58 (2024).
2. Moore, K. N. et al. Mirvetuximab Soravtansine in FRalpha-Positive, Platinum-Resistant Ovarian Cancer. *N Engl. J. Med.* **389** (23), 2162–2174 (2023).
3. Chelariu-Raicu, A. et al. Integrating antibody drug conjugates in the management of gynecologic cancers. *Int. J. Gynecol. Cancer*. **33** (3), 420–429 (2023).
4. Zhao, Y. et al. A mini-overview of antibody-drug conjugates in platinum-resistant ovarian cancer: A preclinical and clinical perspective. *Int. J. Biol. Macromol.* **304** (Pt 2), 140767 (2025).
5. Li, X. et al. *Frontiers in Antibody-Drug Conjugates: Mechanisms, Design Innovations, and Clinical Applications in Targeted Cancer Therapy*. *Pharmaceuticals (Basel)*, **19**(2). (2026).

6. Andre, F. 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* **401** (10390), 1773–1785 (2023).
7. Bardia, A. et al. Datopotamab Deruxtecan in Advanced or Metastatic HR+/HER2- and Triple-Negative Breast Cancer: Results From the Phase I TROPION-PanTumor01 Study. *J. Clin. Oncol.* **42** (19), 2281–2294 (2024).
8. Tarantino, P. et al. Outcomes with trastuzumab deruxtecan by biomarker status, line of treatment and prior receipt of sacituzumab govitecan in a large real-world database of patients with metastatic breast cancer. *ESMO Open.* **10** (7), 105330 (2025).
9. Ebott, J. et al. Enhanced amphiregulin exposure promotes modulation of the high grade serous ovarian cancer tumor immune microenvironment. *Front. Pharmacol.* **15**, 1375421 (2024).
10. Chen, W. et al. Trastuzumab Deruxtecan Resistance via Loss of HER2 Expression and Binding. *Cancer Discov*. **16** (2), 235–249 (2026).
11. Coates, J. T. 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*. **11** (10), 2436–2445 (2021).
12. Gouda, M. A. et al. Human Epidermal Growth Factor Receptor 2 Loss following Treatment with Trastuzumab Deruxtecan in Patients with Metastatic Breast Cancer. *Clin. Cancer Res.* **31** (7), 1268–1274 (2025).
13. 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.* **19** (1), 110 (2017).
14. Johannet, P. et al. Patient outcomes in advanced ovarian cancer treated with an anti-FOLR1 antibody-drug conjugate. *Gynecol. Oncol.* **195**, 173–179 (2025).
15. Zhao, C. L. et al. Folate receptor alpha, TROP2 and HER2 in high-grade serous ovarian Cancer: Expression and clinicopathological correlations. *Gynecol. Oncol.* **211**, 162–170 (2026).
16. Gion, M. et al. Correlation between trophoblast cell-surface antigen-2 (Trop-2) expression and pathological complete response in patients with HER2-positive early breast cancer treated with neoadjuvant docetaxel, carboplatin, trastuzumab, and pertuzumab. *Breast Cancer Res. Treat.* **205** (3), 589–598 (2024).
17. Wang, Y. L. et al. Abstract 2972: TROP2 upregulation and interaction with HER2 mediate trastuzumab resistance. *Cancer Res.* **86** (7_Supplement), 2972–2972 (2026).
18. Niu, N. et al. Expression of B7-H4 and IDO1 is associated with drug resistance and poor prognosis in high-grade serous ovarian carcinomas. *Hum. Pathol.* **113**, 20–27 (2021).
19. Demir, T. et al. Prognostic and Predictive Significance of Claudin-6 Expression in Advanced-Stage High-Grade Serous Ovarian Carcinoma. *Diagnostics* **16** (8), 1175 (2026).
20. Gandullo-Sanchez, L., Ocana, A. & Pandiella, A. *Generation of Antibody-Drug Conjugate Resistant Models.* Cancers (Basel), 13(18). (2021).

21. Murase, Y. et al. Therapeutic Strategies to Overcome Payload Resistance of Trastuzumab Deruxtecan in HER2-Positive Cancers. *Cancer Sci.* **117** (4), 996–1009 (2026).
22. Manning, W. et al. Mechanisms of resistance to anti-MUC16/MMAE antibody-drug conjugate therapy in ovarian cancer. *Gynecol. Oncol.* **200**, 67 (2025).
23. Colombo, R. et al. The Journey of Antibody-Drug Conjugates: Lessons Learned from 40 Years of Development. *Cancer Discov*. **14** (11), 2089–2108 (2024).
24. Tarantino, P. et al. LBA4 Efficacy and safety of sacituzumab govitecan (SG) plus trastuzumab in patients with HER2 + metastatic breast cancer after prior trastuzumab deruxtecan (T-DXd): Results from the phase II SATEEN trial. *ESMO Open.* **11**, 107604 (2026).
25. Chen, M. et al. Optimal Sequential Strategies for Antibody-Drug Conjugate in Metastatic Breast Cancer: Evaluating Efficacy and Cross-Resistance. *Oncologist* **29** (8), e957–e966 (2024).
26. Rampa, D. R. et al. Payload Diversification Overcomes Resistance and Guides Sequential Antibody-Drug Conjugate Therapy in Breast Cancer. *Clin. Cancer Res.* **32** (8), 1454–1461 (2026).
27. Sabbaghi, M. et al. Defective Cyclin B1 Induction in Trastuzumab-emtansine (T-DM1) Acquired Resistance in HER2-positive Breast Cancer. *Clin. Cancer Res.* **23** (22), 7006–7019 (2017).
28. Alatise, K. L., Gardner, S. & Alexander-Bryant, A. *Mechanisms of Drug Resistance in Ovarian Cancer and Associated Gene Targets*. *Cancers (Basel)*, **14**(24). (2022).
29. Rios-Luci, C. et al. Resistance to the Antibody-Drug Conjugate T-DM1 Is Based in a Reduction in Lysosomal Proteolytic Activity. *Cancer Res.* **77** (17), 4639–4651 (2017).
30. Hamblett, K. J. et al. SLC46A3 Is Required to Transport Catabolites of Noncleavable Antibody Maytansine Conjugates from the Lysosome to the Cytoplasm. *Cancer Res.* **75** (24), 5329–5340 (2015).
31. Ettorre, V. M. et al. Successful Sequential Use of the Antibody-Drug Conjugate Trastuzumab Deruxtecan After Progression on Sacituzumab Govitecan in a Recurrent Treatment-Resistant Ovarian Cancer Patient: A Case Report. *Cureus* **17** (10), e94784 (2025).
32. Lee, J. Y. et al. Trastuzumab deruxtecan in HER2-expressing gynecologic cancers from DESTINY-PanTumor02: antitumor activity, safety, and exploratory biomarker analyses. *J. Gynecol. Oncol.* **37** (3), e65 (2026).
33. Cerci, B. et al. Mechanisms of resistance to antibody-drug conjugates in breast cancer. *Drug Resist. Updat*. **85**, 101353 (2026).
34. Nilsson, M. B. et al. Loss of Payload Sensitivity and Other Mechanisms of Resistance to T-DXd in HER2-Mutant NSCLC: Implications for Subsequent Responsiveness to HER2 TKIs. *J. Thorac. Oncol.* **21** (6), 103555 (2026).
35. Loganzo, F. 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.* **14** (4), 952–963 (2015).

36. Bili Newman, A., Damodaran, S. & Meric-Bernstam, F. *Antibody-drug conjugates in breast cancer: Progress and future directions*. *Cell. Rep. Med.*, **7**(5). (2026).

Figures

Figure 1

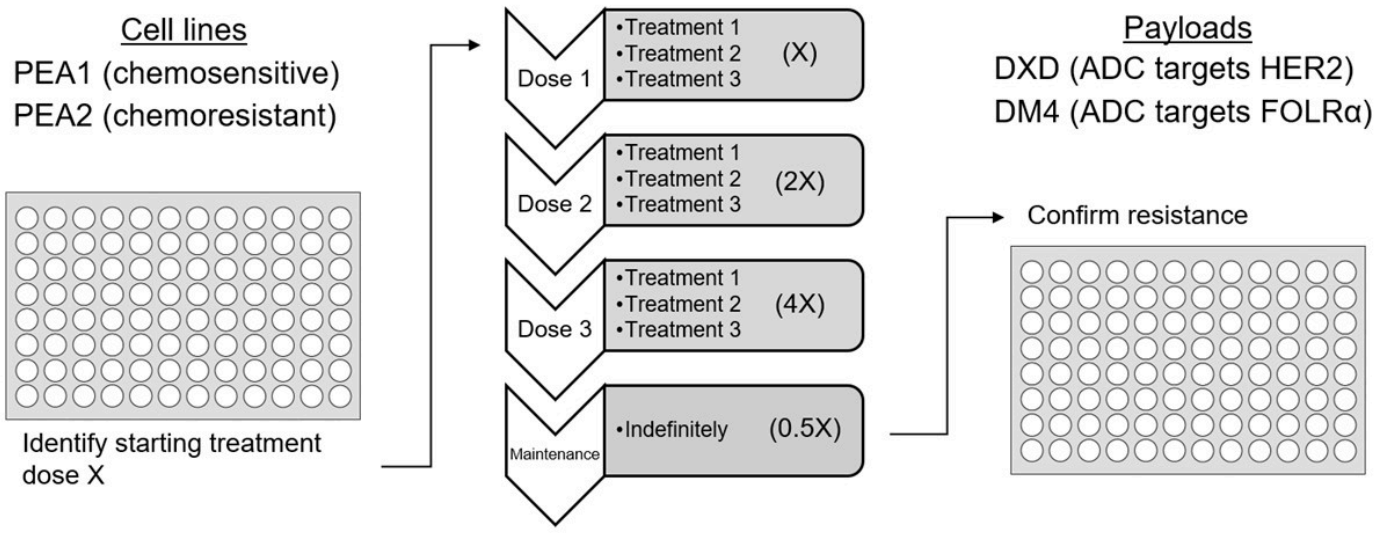


Figure 1

Treatment schema for creation of PEA1 and PEA2 DXD and DM4 payload-resistant cell lines.

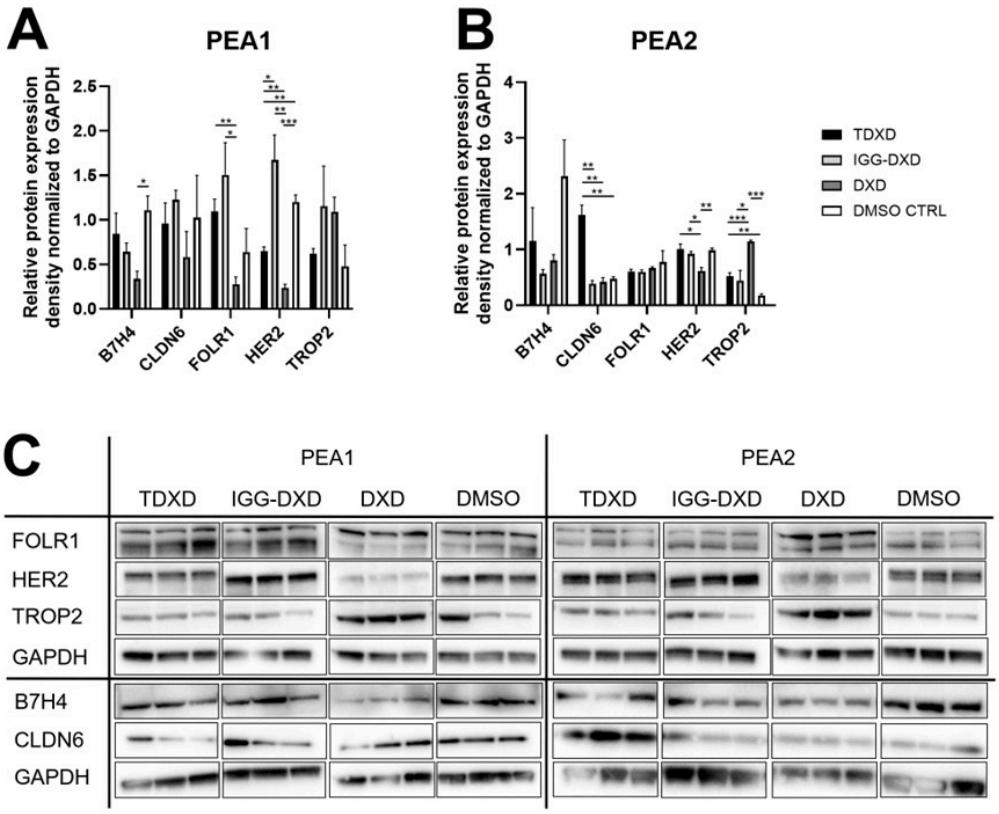


Figure 2

Short-term treatment of A) PEA1 and B) PEA2 cell lines with TDXD ADC and DXD payload results in variable protein expression changes of ADC receptor targets B7H4, CLDN6, FOLR1, HER2, and TROP2. C) Western blots with n = 3 biologic replicates used for quantification in A) and B).

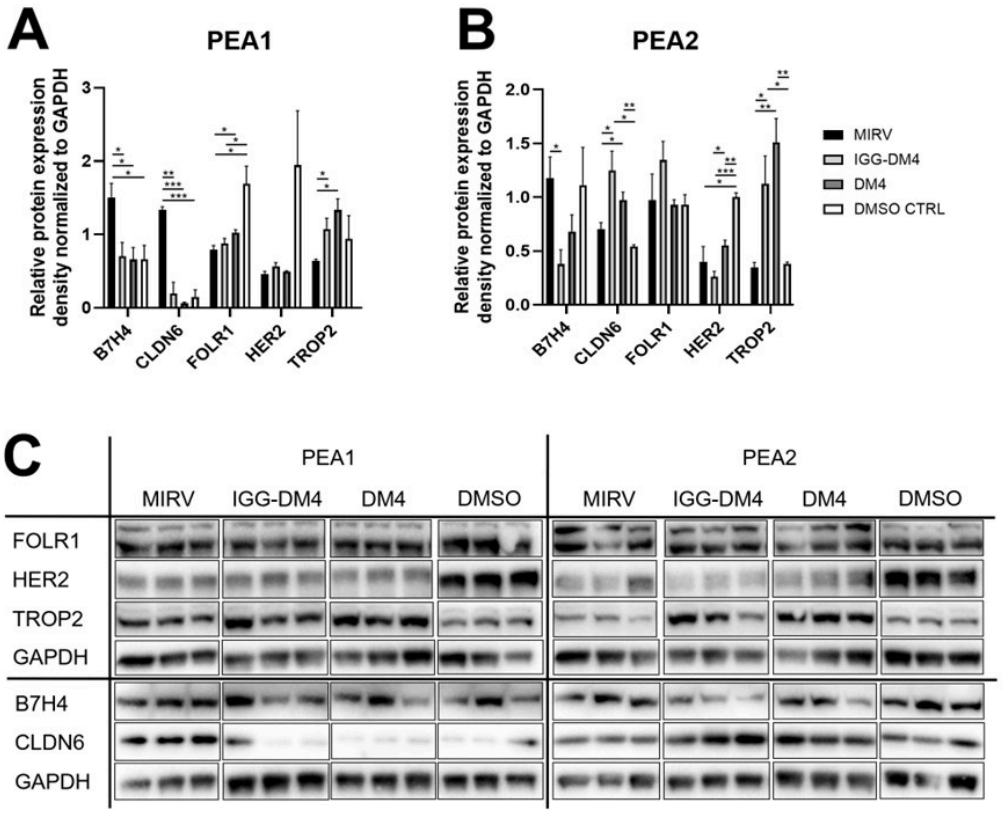


Figure 3

Short-term treatment of A) PEA1 and B) PEA2 cell lines with MIRV ADC and DM4 payload results in variable protein expression changes of ADC receptor targets B7H4, CLDN6, FOLR1, HER2, and TROP2. C) Western blots with n = 3 biologic replicates used for quantification in A) and B).

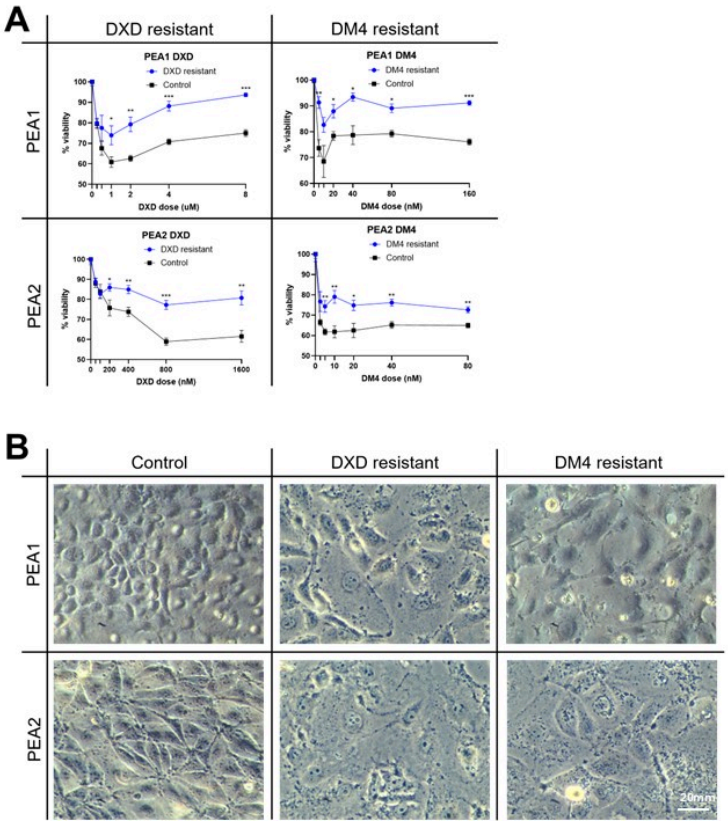


Figure 4

Development of payload PEA1 and PEA2 DXD- and DM4-resistant cell lines with A) confirmation of resistance at various payload treatment doses and B) morphologic differences between resistant and control cell lines. Mean +/- SEM graphed, with n = 3-6 biological replicates per group.

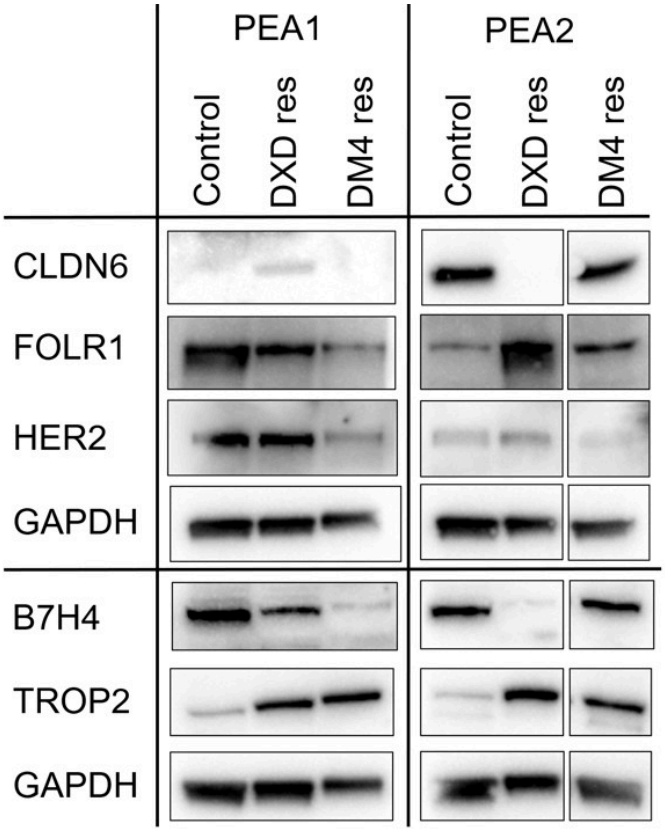


Figure 5

Figure 5

ADC receptor target protein expression in payload resistant cell lines compared to controls.

Figure 6

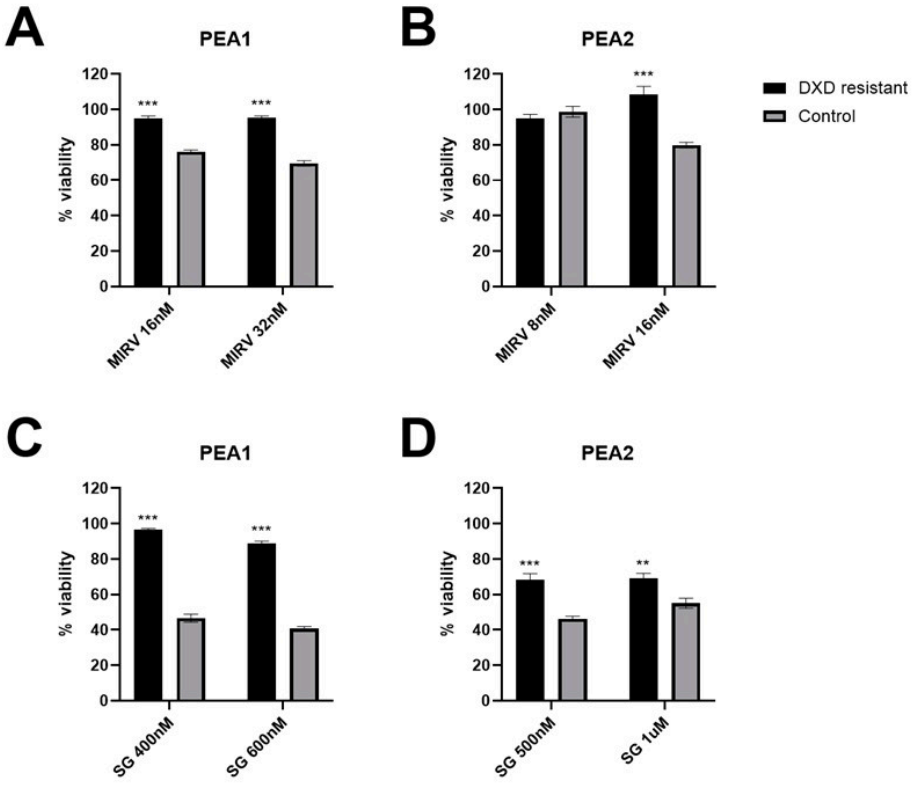


Figure 6

Responses to subsequent ADC treatment are different compared to controls in A) PEA1 DXD-resistant cells treated with MIRV, B) PEA2 DXD-resistant cells treated with MIRV, C) PEA1 DXD-resistant cells treated with SG, and D) PEA2 DXD-resistant cells treated with SG. Mean +/- SEM graphed, with n = 4-5 biological replicates per group

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supp.Fig.S1.jpg
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