Identification of antibody-drug conjugate payloads which are substrates of ATP-binding cassette drug efflux transporters

Jacob S. Roth[1], Hui Guo[1], Lu Chen[1], Min Shen[1], Omotola Gbadegesin[2], Robert W. Robey[2], Michael M. Gottesman[2], Matthew D. Hall[1]

[1]National Center for Advancing Translational Sciences, National Institutes of Health, Rockville MD and [2]Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, U.S.A.

Corresponding Author:

Matthew D. Hall, PhD

National Center for Advancing Translational Sciences

National Institutes of Health

9800 Medical Center Drive, Building B

Rockville, MD 20850

matt@nih.gov

Abstract

Aim: Antibody-drug conjugates (ADCs) are a form of targeted chemotherapy featuring an antibody recognizing a specific protein on cancer cells joined to a potent toxic payload. Numerous antibody-drug conjugates have received FDA approval; however, clinical resistance arises commonly in tumors. Resistance mechanisms include decreased expression or mutation of the antibody target, failure to release the payload from the ADC, or increased expression of ATP-binding cassette (ABC) efflux transporters associated with multidrug resistance. We therefore sought to characterize the interactions of ABC multidrug transporters with ADC payloads.

Methods: We performed a high-throughput screen with 27 common ADC payloads using cells lines expressing ABC transporters P-glycoprotein (P-gp, encoded by *ABCB1*) or ABCG2 (encoded by *ABCG2*). Confirmatory assays were also performed using cells transfected to express P-gp, ABCG2, or MRP1 (encoded by *ABCC1*).

Results: Several commonly used ADC payloads were found to be avid substrates of P-gp, including calicheamicin gamma1, monomethyl auristatin E, DM1, and DM4. All the pyrrolobenzodiazepines tested - SJG136, SGD-1882, SG2057, and SG3199 - were substrates of P-gp, ABCG2, and MRP1. The modified anthracyclines nemorubicin and its metabolite PNU-159682 were poorly transported by both ABCB1 and ABCG2 and displayed nanomolar to picomolar toxicity. Further, we found that the efficacy of the recently FDA-approved ADC mirvetuximab soravtansine, which has DM4 as the toxic payload, was decreased in cell lines with overexpression of P-gp.

Conclusion: Several commonly used ADC payloads can be effluxed from cells by ABC transporters which may lead to transporter-mediated drug resistance in patients. Future ADCs should be developed using payloads that are not substrates of ABC transporters.

Introduction

Antibody-drug conjugates (ADCs) have received significant attention as a modality for selectively targeting tumor cells. ADCs are composed of an antibody targeting a specific cell surface antigen conjugated to a small-molecule toxin (payload) via a cleavable linker[1]. Following systemic distribution and binding to the antigen, the ADC is internalized and processed within the target cell lysosomes, liberating the toxin to exert its cytotoxic effects. Thus, provided the antibody is targeted to a protein/epitope that is selectively expressed on the extracellular surface of cancer cells and minimally in other cell-types in the body, the ADC should selectively kill tumor cells[2]. The first ADC was granted FDA approval in 2000 for the treatment of acute myeloid leukemia (AML); gemtuzumab ozogamicin targets CD33 and carries N-acetyl-gamma-calicheamicin as the cytotoxic payload[3]. Since then, several other ADCs have been approved for the treatment of cancer including brentuximab vedotin for Hodgkin lymphoma[4], trastuzumab emtansine for breast cancer[5], trastuzumab deruxtecan for breast cancer[6] (recently approved for any HER2-positive solid cancer[7]), loncastuximab tesirine for diffuse large B-cell lymphoma[8], enfortumab vedotin for urothelial cancers[9] and mirvetuximab soravtansine for cisplatin-resistant ovarian cancers[10]. FDA-approved ADCs, and ADCs in clinical trials, have employed highly cytotoxic natural product-derived payloads which cause cell death via various modalities including induction of DNA damage, disruption of microtubules, and inhibition of topoisomerase[11]. Many of these agents originate from molecules that were explored as small molecule (untargeted) chemotherapeutics, but potent cytotoxic side-effects led to their abandonment. A partial listing of approved ADCs and ADCs currently in clinical trials is included in Table 1.

Despite some responses of cancers to ADCs, resistance to treatment is observed in the clinic. Clinically observed mechanisms of resistance include

downregulation or mutation of the ADC antibody target, failure of lysosomes to release the payload, activation of alternative survival pathways, and the overexpression of efflux transporters[12, 13]. Patients whose leukemic blasts had lower levels of CD33 were found to respond more poorly to treatment with gemtuzumab ozogamicin versus patients with blasts expressing higher levels[14]. Similarly, patients whose tumors were found to express a truncated form of HER2 that does not bind to trastuzumab, p95HER2, were found to be largely resistant to trastuzumab emtansine[15]. Decreased payload release in patient-derived samples has been linked to drug accumulation in the lysosomes due to increased lysosomal pH[16]. Finally, overexpression of the *ABCB1* gene which encodes the ATP-binding cassette (ABC) transporter P-glycoprotein (P-gp) has been inversely correlated with response to gemtuzumab ozogamicin in patients with AML[14, 17]. While *ad hoc* reports of ABC-mediated resistance of individual ADCs exist[18, 19], no systematic examination of the relationship between toxin sensitivity and ABC transporter expression has been reported.

The objective of this study was to determine whether several commonly used ADC payloads were substrates for transport by ABC multidrug transporters, with the goal of identifying cytoxic agents that were not subject to transport out of the cell. P-gp substrates were identified by comparing compound activity between paired sets of low-expressing (sensitive) and over-expressing (resistant) cell lines for both P-gp and ABCG2. Four paired cell sets were utilized – a drug-selected and transfected set for each transporter. Hits were confirmed in cells transfected to express P-gp, ABCG2, or MRP1 (encoded by *ABCC1*). These results should aid investigators in designing ADCs that are not subject to ABC transporter-mediated resistance.

Methods

Cell Lines

HEK-293 cells were transfected with empty vector (pcDNA) or vector containing the human *ABCB1* (MDR-19), *ABCG2* (R-5), or *ABCC1* gene (MRP1) and were maintained in EMEM with 2 mg/mL G418 added to maintain transporter expression[20]. The HeLa derivative KB-3-1 and the P-gp overexpressing KB-8-5-11 subline were maintained in DMEM; KB-8-5-11 additionally received 100 ng/mL colchicine[21]. OVCAR8, NCI-ADR-RES, and H460 parental cells were obtained from the Division of Cancer Treatment and Diagnosis Tumor Repository, National Cancer Institute at Frederick, MD. The ABCG2 expressing subline H460 MX20 was generated by selecting H460 cells in 20 nM mitoxantrone[22]. Media in all cases was supplemented with 10% FBS and 1% penicillin-streptomycin.

High-throughput screen

We explored 27 common ADC payloads, listed in Table 2, in two orthogonal, paired cell set—a drug selected and transfected set for each transporter--to identify substrates of the ABC transporters P-gp and ABCG2. As noted in Table 2, compounds were sourced from Levena Biopharma (San Diego, CA), Selleck Chem (Houston, TX), ChemieTek (Indianapolis, IN), or Microsource Discovery Systems (Gaylordsville, CT). Screening was performed as previously described[23]. Briefly, cells were plated at a density of 500 cells/well in 1536 well plates with compounds added using a 1536-head pin tool (Kalypsys, San Diego CA). Cells were incubated with cytotoxic drugs and processed with CellTiter Glo reagent (Promega, Madison, WI) to determine viability after 48 hours. P-gp substrates were identified by comparing compound activity between paired sets of low-expressing (sensitive) and over-expressing (resistant) cell lines.

Synergy experiments

Known inhibitors of the P-gp and ABCG2 pumps, tariquidar (1 μM) and Ko143 (5 μM) respectively, were co-administered with the payloads. Re-sensitization of resistant cells confirms cytotoxic compounds are transporter substrates if inhibition of efflux restores intracellular accumulation. Cells were incubated with drugs at various concentrations and processed with CellTiter Glo reagent (Promega, Madison, WI) to determine viability after 48 hours.

Data analysis

To evaluate compound activity in high-throughput screening (HTS) and synergy experiments, concentration–response curves (CRCs) were generated for each sample by plotting normalized response data against compound concentration. These curves were modeled using a four-parameter logistic regression, which provided key pharmacological parameters such as the half-maximal inhibitory concentration (IC_{50}) and maximal response (efficacy)[24]. The screen typically produced hits exhibiting a broad range of potencies and diverse curve qualities. While many compounds showed well-defined, sigmoidal dose–response behavior with both upper and lower asymptotes, others displayed atypical or poor-quality CRCs, including shallow slopes, incomplete asymptotes, or responses derived from only a single active concentration point. Such cases were classified as low-confidence actives due to limited or ambiguous activity profiles. Furthermore, a compound's area-under-the-curve (AUC) calculated based on the screening data analysis and curve fittings served as an integrated measure of compound activity, capturing both potency and efficacy, and was utilized for direct comparison of activity outcome across different cell lines and experimental conditions[23].

Confirmatory experiments with screen hits

Three-day cytotoxicity assays were performed using various payloads including other members of the PBD dimer class, as well as the ADC mirvetuximab soravtansine, with pcDNA (empty vector transfected), MDR-19 (*ABCB1* transfected), R-5 (*ABCG2* transfected) and MRP1 (*ABCC1* transfected) cells. Briefly, cells were plated (5000 cells/well) in 96-well plates and allowed to attach overnight after which the desired payloads or the ADC at various concentrations were added and incubated with cells for three days. CellTiter Glo reagent (Promega) was then used according to the manufacturer's directions to assess GI_{50} values.

Results

A High throughput screen (HTS) identifies ADC payloads as substrates of P-gp and ABCG2

We evaluated 27 common ADC payloads in two orthogonal, paired cell sets to identify substrates of the ABC transporters P-gp and ABCG2 (Table 2). Cytotoxic payloads were tested against two pairs of cell lines, one parental and one transporter-expressing line. For P-gp the pairs were: the KB 3-1 human adenocarcinoma cell line and its colchicine-selected, P-gp-overexpressing sub-line KB 8-5-11; the HEK pcDNA human embryonic kidney cell line (transfected with an empty vector plasmid control) and its *ABCB1* stably transfected sub-line MDR-19. For ABCG2 the pairs were: the H460 human lung carcinoma cell line and its mitoxantrone-selected, ABCG2-overexpressing sub-line H460 MX20; the same HEK pcDNA parent cell line was used as a comparator for the *ABCG2*-transfected HEK cell line, R-5. Transporter-specific efflux was demonstrated by testing all toxins for sensitization of transporter-expressing cells in the presence of either the P-gp inhibitor tariquidar or the ABCG2 inhibitor Ko143. As such,

six cell line conditions were used to evaluate toxins as P-gp substrates, and six cell line conditions were also used for evaluation of ABCG2 substrates.

Area under the dose response curve (AUC) values were calculated for each cell line with all payloads in the presence or absence of a specific inhibitor as previously described[23] and are displayed in the heat maps in Figure 1. In the heatmaps, red denotes high cell death and thus a more potent compound, while blue denotes less cell death. In Figure 1A, cells expressing P-gp, when compared to parental cells, were found to be less sensitive (i.e., resistant) to several ADC payloads, including calicheamicin gamma1, dolostatin 10, MMAE, tubulysin M, DM1, and DM4. When the P-gp positive lines were incubated with the inhibitor tariquidar, resistance to calicheamicin gamma1, dolostatin 10, MMAE, tubulysin M, DM1 and DM4 was reversed. In the heat map, unsupervised clustering showed that the KB 8-5-11 line treated with tariquidar clusters with the parent KB-3-1 line and the MDR-19 line with tariquidar clusters with pcDNA transfected cells, while the P-gp expressing cells cluster separately. The cytotoxicity of nemorubicin, its metabolite PNU-159682, and the duocarmycin compounds appeared to be unaffected by P-gp overexpression.

As shown in in Figure 1B, few compounds appeared to be substrates of ABCG2, with the known substrate SN-38 being the most prominent compound that was subject to transport. Addition of the ABCG2 inhibitor Ko143 did not appear to sensitize cells, as R-5 cells with Ko143 clustered with R-5 cells, and H460 MX20 cells with Ko143 clustered with H460 MX20 cells, rather than with the corresponding parental lines. In line with efflux by ABCG2, the addition of Ko143 reversed resistance to SN-38.

We also calculated the differences in AUC (deltaAUC) values between the parental and resistant lines and between the resistant lines in the absence or presence of inhibitor, as previously described[23]. Comparing deltaAUC values between the pcDNA/MDR-19 and MDR-19+tariquidar pairs and the KB 3-1/KB 8-5-11 and KB 8-5-

11+tariquidar pairs, we found good correlation, with r^2 values of 0.7207 and 0.9175, respectively (Supplementary Figure 1). The results with P-gp contrasted with those of ABCG2, as few compounds were identified as ABCG2 substrates; the deltaAUC values for the pcDNA/R-5 and R-5 Ko143/R-5 pairs as well as the H460/H460MX20 and H460 Ko143/H460MX20 pairs showed poor correlation, with r^2 values of 0.32 and 0.31 respectively (Supplementary Fig 1). Thus, more payloads appeared to be transported by P-gp than ABCG2.

Validation of HTS hits

We validated selected results in the screen, adding a cell line (MRP1) developed by transfecting HEK293 cells with a plasmid containing the *ABCC1* gene (Figure 2, Table 3). Noting that PDB dimers were substrates of P-gp in the initial screen, we included more PDB dimer payloads in our follow follow-up, and also included more camptothecin derivatives that serve as ADC payloads. Consistent with data from the screen, MMAE, DM4, calicheamicin gamma1, and DM1 were confirmed as avid substrates of P-gp and we noted a small degree of resistance conferred to these payloads by ABCG2 (Figure 2). MMAF was not a substrate of any of the transporters, although it was less potent than the related compound MMAE (Table 3). We also confirmed that nemorubicin and PNU159682 were not substrates of any of the transporters examined, making these ideal ADC payloads; PNU159682 is exquisitely toxic, with GI_{50} values in the picomolar range (Table 3).

Overexpression of ABCG2 conferred about 10-fold resistance to the camptothecin derivative exatecan, much less than the 96-fold resistance for SN-38 we previously reported for ABCG2 transfected cells[25]. This is consistent with previous reports demonstrating that ABCG2 confers less resistance to exatecan compared to other camptothecin derivatives that are ABCG2 substrates[26]. However, the exatecan

derivative Dxd (deruxtecan) was readily transported by ABCG2 and was also a substrate for P-gp (Figure 2, Table 3).

As our screen originally contained only one member of the PBD dimer family (SGD-1882), we expanded the number of PDB dimer payloads examined. We found that HEK293 cells expressing any of the three transporters conferred some resistance to all PDB dimers examined: SG3199, SGD-1882, SJG136 (also known as SG2000), and SG2057. P-gp overexpression was found to confer the highest levels of resistance, followed by ABCG2 and finally MRP1 (Figure 3, Table 3).

P-gp overexpression confers resistance to mirvetuximab soravtansine

The screening approach described here evaluated small molecule cytotoxic drugs employed as payloads in experimental and therapeutic ADCs. Examining how drug transporter-mediated susceptibility confers resistance to an ADC (where the toxin is liberated within the cell and then would be effluxed from the cell) is important to interpreting our results. We thus tested the effect of P-gp expression on the sensitivity of cultured cells to an ADC. Mirvetuximab soravtansine (MIRV) is an ADC that received FDA approval in 2022 for the treatment of folate receptor alpha-positive, platinum-resistant ovarian, fallopian tube or peritoneal cancer[10]. The toxic payload is DM4, which we found to be a P-gp substrate in our screen. Testing MIRV with the KB3-1/KB-8-5-11 pair used in our screening assay and the OVCAR8/NCI-ADR-RES cell line pair, we found that the P-gp overexpressing lines KB 8-5-11 and NCI-ADR-RES were resistant to treatment with MIRV compared to the parental lines. These results confirm that screening of payloads for susceptibility to P-gp efflux translates to resistance to and ADC carrying a P-pg substrate toxin. These data suggest that P-gp may contribute to resistance to MIRV in the clinic.

Discussion

We systematically screened 27 commonly used ADC payloads (cytotoxic drugs) in two orthogonal, paired cell sets to identify substrates of the ABC transporters P-gp and ABCG2. Of the studied DNA damaging agents, calicheamicin analogs, a PDB dimer, SN-38, and doxorubicin were identified as substrates of the ABC transporter P-gp. Of the tubulin-targeting agents, vinblastine and all auristatin and maytansinoid derivatives were strong P-gp substrates, whereas MMAF was somewhat less susceptible to transport. Notably, the duocarmycin analogs, camptothecin, nemorubicin, PNU-159682, dasatinib, and α-amanitin were found to not be P-gp substrates. SN-38 was identified as a substrate of ABCG2, but no other cytotoxic payloads were observed to be transported by ABCG2. Some cytotoxic drugs exhibited decreased sensitivity to the drug-selected H460-MX20 cell line, but this sensitivity was not reversed by co-dosing with the known ABCG2 inhibitor Ko143, suggesting that the difference was not due to ABCG2 overexpression.

In vitro studies and *in vivo* mouse models have demonstrated that overexpression of ABC transporters can cause resistance to some ADCs. Early studies with gemtuzumab ozogamicin demonstrated that P-gp and possibly MRP1 (ABCC1) played a role in resistance. Naito *et al.* found that leukemia cell lines that overexpressed P-gp were resistant to treatment with gemtuzumab ozogamicin and that combining the ADC with P-gp inhibitors such as valspodar or biricodar reversed resistance[27]. In the MRP1-overexpressing cell line HL-60/ADR, addition of the MRP1 inhibitor MK-571 was found to increase sensitivity to gemtuzumab ozogamicin, suggesting that MRP1 was yet another resistance mechanism to the ADC[28]; however, overexpression of ABCG2 does not appear to cause resistance[29]. Studies using cell lines expressing P-gp, MRP1 or ABCG2 with the CD33-targeting ADC AVE9633, which has the maytansine DM4 as its

toxic payload, showed that only P-gp could confer resistance[18]. Repeated treatment with the ADC N41mab-vcMMAE, which targets nectin-4 and has MMAE as its payload, in mice xenografted with the SUM190 breast cancer cell line gave way to refractory tumors which were found to overexpress ABCB1 as the mechanism of resistance[30]. An ADC targeting the delta-like non-canonical Notch ligand 1 protein, ADCT-701, which has the PDB dimer SG3199 as the payload, was found to be less effective in adrenocortical cancer cell lines and organoids with high expression of P-gp, and the free drug was also reported to be a substrate of P-gp[31]. The findings of these studies agree with our results confirming that calicheamicin, DM4, MMAE and SG3199 are substrates of P-gp.

Overexpression of P-glycoprotein has also emerged as a marker of resistance in a subset of patients who have been treated with gemtuzumab ozogamicin and brentuximab vedotin. Early studies examining leukemic blasts from patients with resistant disease demonstrated that P-gp and MRP1 expression could lead to resistance[17, 28, 32]. P-gp expression was found to inversely correlate with clinical response to gemtuzumab ozogamicin in patients with AML[14]. Similarly, a study examining a small cohort of lymphoma patients resistant to gemtuzumab vedotin reported P-gp overexpression in a patient with Hodgkin Lymphoma[19]. A case report of a patient with bladder cancer whose disease had progressed after treatment with enfortumab vedotin, an ADC which targets nectin-4, reported high levels of P-gp in the resistant tumor[33].

These initial studies suggested that the addition of a P-gp inhibitor might be beneficial to patients whose cancer expresses P-gp, leading to some clinical trials combining P-gp inhibitors with ADC treatment. The P-gp inhibitor zosuquidar was found to reverse resistance to gemtuzumab ozogamycin in *ex vivo* studies using P-gp positive blasts obtained from patients with resistant disease[34]. In a clinical trial combining zosuquidar with gemtuzumab ozogamicin, greater overall survival was noted in patients

with P-gp-positive resistant disease[35]. A small clinical study combining cyclosporine with gemtuzumab ozogamicin in patients with resistant disease led to an increased overall and complete response rate[36]. However, the final findings from a larger trial were less positive, mostly due to toxicity from the use of cyclosporine A as the P-gp inhibitor[37]. The disappointing results from this later trial highlight the importance of P-gp inhibitor choice when designing combination trials.

Conclusion

We show that the payloads of some FDA approved ADCs are strong substrates for P-gp, suggesting that active transport and efflux of a released payload may play a role in acquired resistance to clinical ADCs. These effects may also increase exposure of the cytotoxic payload to systemic tissues and could contribute to *in vivo* off-target toxicities. We identified ADC payloads that were not substrates of either P-gp or ABCG2 – notably the duocarmycin series and PNU-159682, which were among the most cytotoxic of all the toxins studied – and suggest that these compounds be prioritizied as future ADC payloads due to the potential for reduced susceptibility to transporter-mediated acquired resistance.

Author contributions

Jacob S. Roth: data curation, formal analysis, investigation, visualization, writing—original draft preparation, writing—review and editing

Hui Guo: formal analysis, investigation, visualization

Lu Chen: formal analysis, investigation, visualization

Min Shen: formal analysis, investigation, visualization, writing—original draft preparation, writing—review and editing

Omotola Gbadagesin: data curation, formal analysis, investigation, writing—review and editing

Robert W. Robey: data curation, visualization, investigation, writing—original draft preparation, writing—review and editing

Michael M. Gottesman: supervision, writing—review and editing

Matthew D. Hall: conceptualization, resources, supervision, writing—original draft preparation, writing—review and editing

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Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Figure Legends

Figure 1: **Heat map of cellular response to ADC payloads**

Figure 2: **Confirmatory cytotoxicity assays with screen hits additional ADC payloads**

Three-day cytotoxicity assays were performed with the noted compounds as described in the Materials and Methods using HEK-293 cells transfected with empty vector (pcDNA, black dot), or vectors containing ABCB1 (MDR-19, blue triangle), *ABCG2* (R-5, red square) or *ABCC1* (MRP1, black box). Results from one of three independent experiments are shown. Cytotoxicity data are summarized in Table 3.

Figure 3: **P-gp overexpression confers resistance to treatment with mirvetuximab soravtansine**

Three-day cytotoxicity assays were performed with mirvetuximab soravtansine as described in the Materials and Methods using the OVCAR8/NCI/ADR-RES pair (left graph) or the KB-3-1/KB 8-5-11 pair (right graph). Results from one of three independent experiments are shown.

Table 1: Approved and clinical candidate ADC small molecule payloads.

Name	Brand Name	Payload	Linker	FDA Status
Gemtuzumab ozogamicin	Mylotarg	Calicheamicin	Cleavable	Approved
Inotuzumab ozogamicin	Besponsa	Calicheamicin	Cleavable	Approved
Trastuzumab deruxtecan	Enhertu	Dxd (exatecan derivative)	Cleavable	Approved
Datopotamab deruxtecan	Datroway	Dxd (exatecan derivative)	Cleavable	Approved
Ado-trastuzumab emtansine	Kadcyla	DM1 (Maytansinoid analog)	Non-Cleavable	Approved
Mirvetuximab soravtansine	Elahere	DM4 (Maytansinoid analog)	Cleavable	Approved
Brentuximab vedotin	Adcetris	Monomethyl auristatin E (MMAE)	Cleavable	Approved
Polatuzumab vedotin	Polivy	Monomethyl auristatin E (MMAE)	Cleavable	Approved
Enfortumab vedotin	Padcev	Monomethyl auristatin E (MMAE)	Cleavable	Approved
Tisotumab vedotin	Tivdak	Monomethyl auristatin E (MMAE)	Cleavable	Approved
Moxetumomab pasudotox	Lumoxiti	Pseudomonas exotoxin (PE38)	Non-Cleavable	Approved
Loncastuximab tesirine	Zynlonta	SG3199 (pyrrolobenzodiazepine dimer)	Cleavable	Approved
Sacituzumab govitecan	Trodelvy	SN-38 (active metabolite of irinotecan)	Cleavable	Approved
Patritumab deruxtecan	N/A	Dxd (exatecan derivative)	Cleavable	In Clinical Trials
Telisotuzumab vedotin	Teliso-V	Monomethyl auristatin E (MMAE)	Cleavable	In Clinical Trials
Disitamab vedotin	RC48	Monomethyl auristatin E (MMAE)	Cleavable	In Clinical Trials
Depatuxizumab mafodotin	Depatux-M	Monomethyl auristatin F (MMAF)	Non-Cleavable	In Clinical Trials
Vadastuximab talirine	N/A	SGD-1882 (pyrrolobenzodiazepine dimer)	Cleavable	In Clinical Trials
Camidanlumab tesirine	Cami-T	SG3199 (pyrrolobenzodiazepine dimer)	Cleavable	In Clinical Trials
Belantamab mafodotin	Blenrep	Monomethyl auristatin F (MMAF)	Non-Cleavable	Withdrawn

Table 2: ADC payloads tested in the high throughput screen

Compound	Vendor	Mechanism of action
N-Acetyl-Calicheamicin gamma1	Levena Biopharma	DNA damage
DM1	Levena Biopharma	Microtubule disruptor
PBD Dimer (SGD-1882)	Levena Biopharma	DNA damage
Monomethyl Auristatin E (MMAE)	Levena Biopharma	Microtubule disruptor
SN-38	ChemieTek	Topoisomerase inhibition
DM4	Levena Biopharma	Microtubule disruptor
alpha-Amanitin	Levena Biopharma	RNA polymerase inhibitor
Duocarmycin DM	Levena Biopharma	DNA alkylation
Monomethyl Auristatin F (MMAF)	Levena Biopharma	Microtubule disruptor
PNU 159682	Levena Biopharma	Topoisomerase inhibition
Maytansinol	Levena Biopharma	Microtubule disruptor
Duocarmycin SA	Levena Biopharma	DNA alkylation
Monomethyl Dolastatin 10	Levena Biopharma	Microtubule disruptor
Vinblastine Sulfate	Microsource Discovery	Microtubule disruptor
Ansamitocin P3	Levena Biopharma	Microtubule disruptor
Calicheamicin gamma1	Levena Biopharma	DNA damage
Nemorubicin	Levena Biopharma	Topoisomerase inhibition
Doxorubicin	Selleck Chem	Topoisomerase inhibition
Tubulysin IM-2	Levena Biopharma	Microtubule disruptor
Auristatin F	Levena Biopharma	Microtubule disruptor
Camptothecin	Selleck Chem	Topoisomerase inhibition
Dasatinib	Selleck Chem	Tyrosine kinase inhibitor
Duocarmycin MA	Levena Biopharma	DNA alkylation
Duocarmycin TA	Levena Biopharma	DNA alkylation
Tubulysin M	Levena Biopharma	Microtubule disruptor
Auristatin E	Levena Biopharma	Microtubule disruptor
Dolastatin 10	Levena Biopharma	Microtubule disruptor

Table 3: Cross-resistance profile to select ADC payloads in cells expressing P-gp, ABCG2 or MRP1

Drug	pcDNA	MDR19	RR	R5	RR	MRP1	RR
DM1 (nM)	7.8±1.4	410±120	52	35±4.1	4.5	9.5±3.7	1.2
DM4 (nM)	0.43±0.075	42±20	96	1.4±0.49	3.3	0.58±0.37	1.3
MMAE (nM)	0.90±0.23	360±110	400	5.1±1.9	5.7	1.2±0.80	1.3
MMAF (μM)	0.99±.097	2.0±1.7	2.0	0.99±.097	1.0	1.0±0.39	1.0
Calicheamicin gamma1	0.010±0.0042	0.029±0.0047	2.9	0.73±0.41	71	0.028±0.017	2.7
Exatecan (nM)	0.59±0.19	1.4±0.15	2.4	5.0±0.80	8.5	1.0±0.19	1.8
Deruxtecan (nM)	0.82±0.18	17±1.2	20	190±29	240	1.1±0.40	1.4
Nemorubicin (nM)	0.76±0.31	1.5±0.15	2.0	1.1±0.27	1.4	1.6±0.21	2.2
PNU-159682 (pM)	13±9.6	16±3.7	1.2	13±8.4	1.0	19±8.6	1.4
SJG136 (nM)	0.098±0.019	16±6.2	170	4.6±0.68	47	1.2±1.1	12
SGD-1882 (nM)	0.0066±0.0028	0.85±0.34	130	0.22±0.093	33	0.095±0.036	14
SG2057 (nM)	0.025±0.0087	5.7±1.2	230	0.73±0.34	29	0.27±0.20	11
SG3199 (nM)	0.045±0.011	5.6±1.6	120	0.90±0.31	20	0.21±0.026	4.6

Results are mean GI50 values +/- standard deviation. Relative resistance (RR) value was computed by dividing the GI50 value for the lines expressing the ABC transporters by the empty vector (pcDNA) line. Results are from 3 independent experiments.

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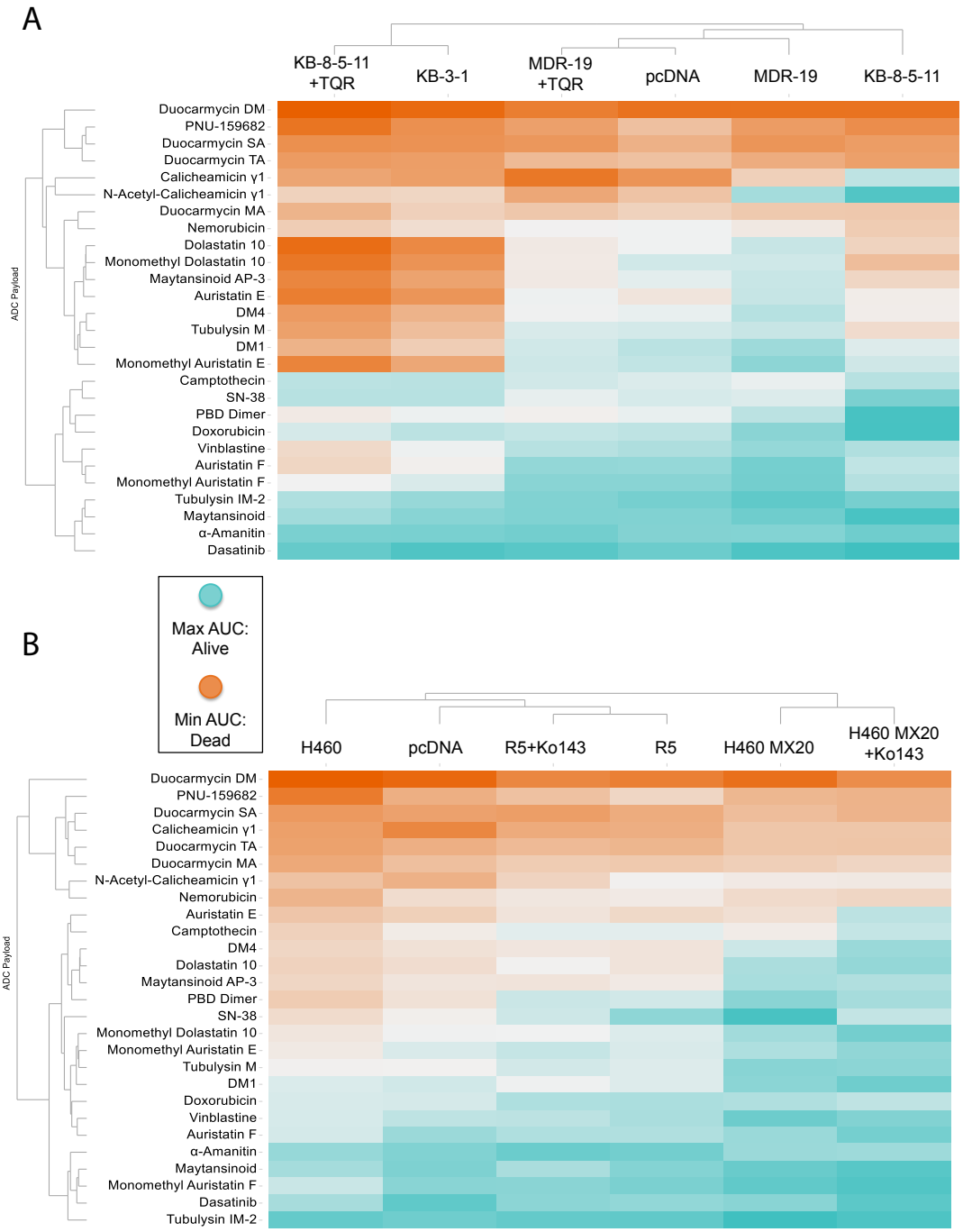
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Maytansinoid AP-3
Auristatin E
DM4
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DM1
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Maytansinoid
α-Amanitin
Dasatinib
Max AUC: Alive
Min AUC: Dead
B
H460
pcDNA
R5+Ko143
R5
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ADC Payload
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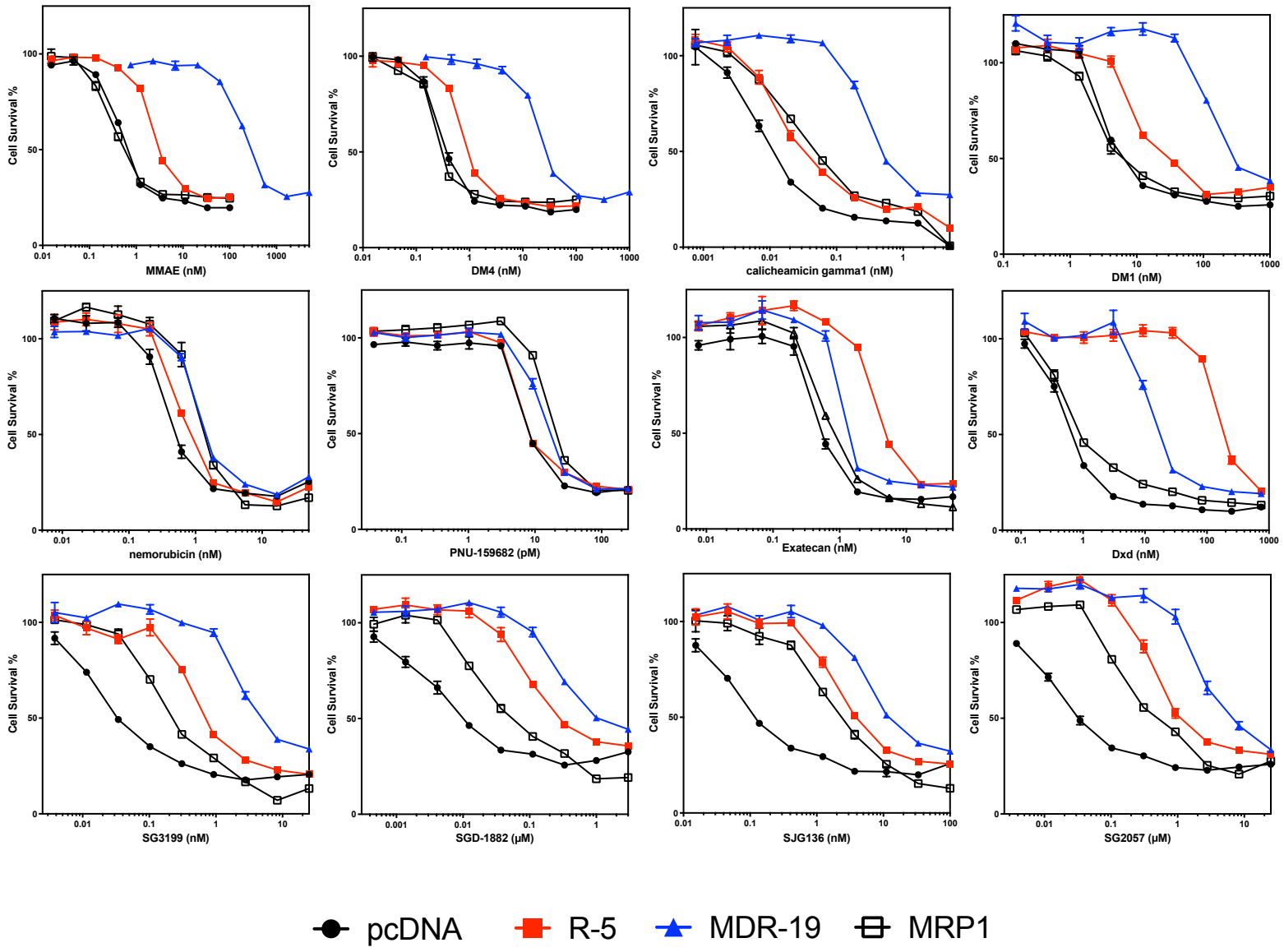
Figure 1


Cell Survival %
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DM4 (nM)
calicheamicin gamma1 (nM)
DM1 (nM)
nemorubicin (nM)
PNU-159682 (pM)
Exatecan (nM)
Dxd (nM)
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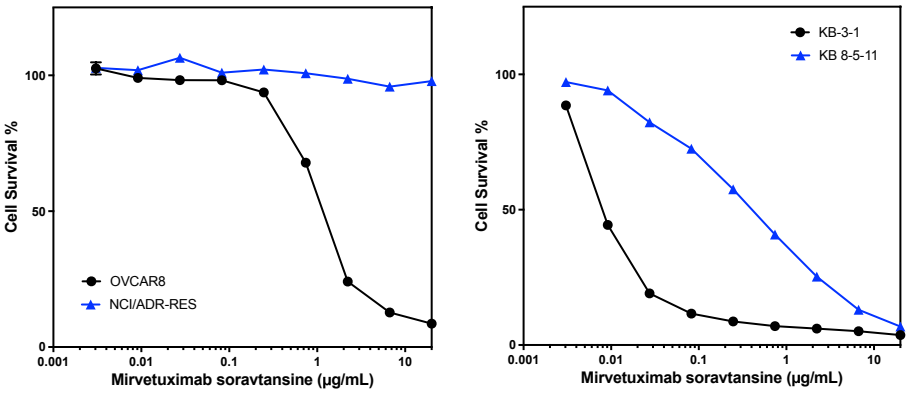
pcDNA
R-5
MDR-19
MRP1

Figure 2


Cell Survival %
100
50
0
0.001
0.01
0.1
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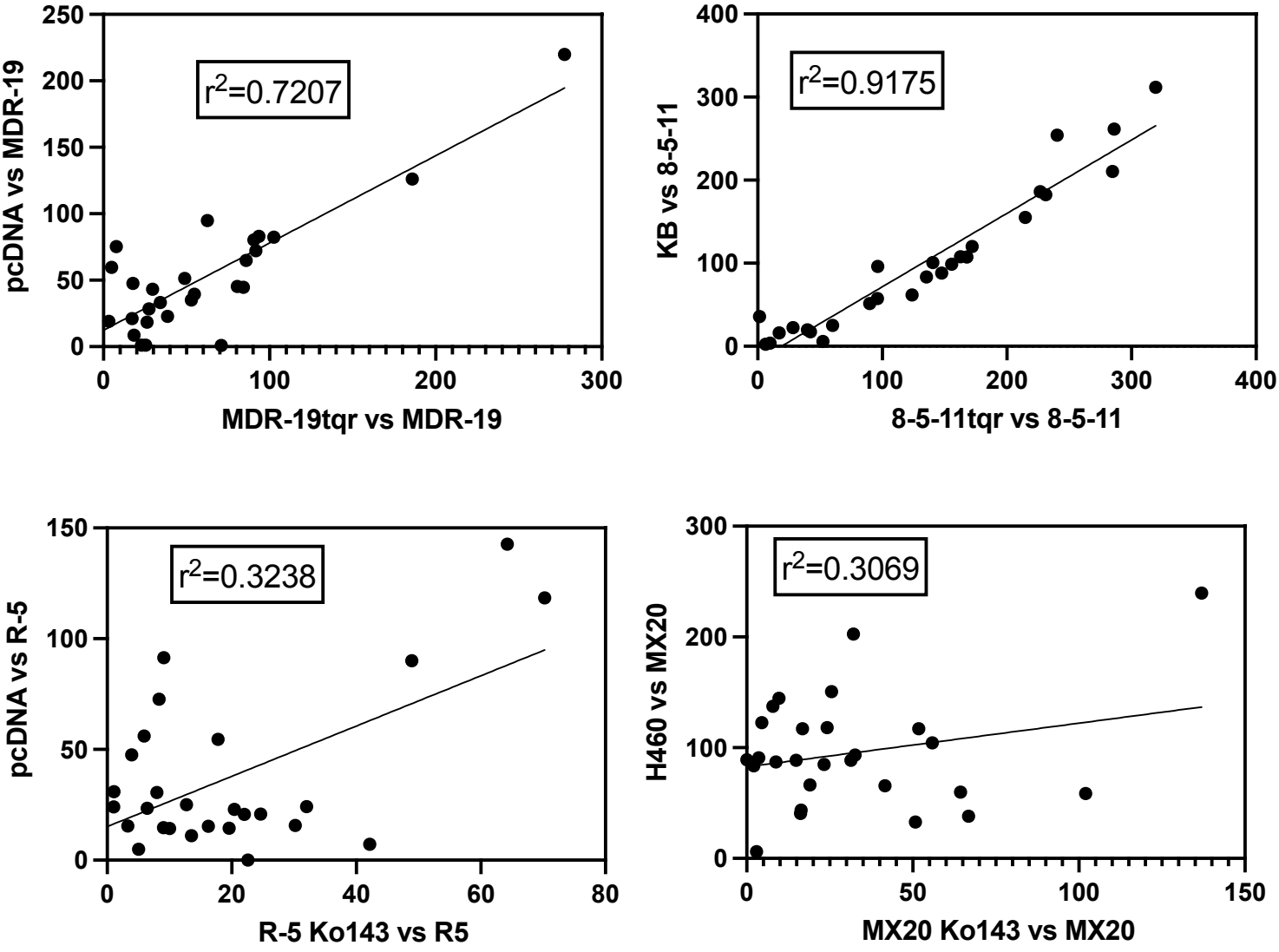
KB 8-5-11

Figure 3



Supplementary Figure 1