Trastuzumab Deruxtecan, Antibody–Drug Conjugate Targeting HER2, Is Effective in Pediatric Malignancies: A Report by the Pediatric Preclinical Testing Consortium

Pooja Hingorani[1], Wendong Zhang[1], Zhongting Zhang[1], Zhaohui Xu[1], Wei-Lien Wang[2], Michael E. Roth[1], Yifei Wang[1], Jonathan B. Gill[1], Douglas J. Harrison[1], Beverly A. Teicher[3], Stephen W. Erickson[4], Gregory Gatto[4], Edward A. Kolb[5], Malcolm A. Smith[3], Raushan T. Kurmasheva[6], Peter J. Houghton[6], and Richard Gorlick[1]

ABSTRACT

HER2 is expressed in many pediatric solid tumors and is a target for innovative immune therapies including CAR-T cells and antibody–drug conjugates (ADC). We evaluated the preclinical efficacy of trastuzumab deruxtecan (T-DXd, DS-8201a), a humanized monoclonal HER2-targeting antibody conjugated to a topoisomerase 1 inhibitor, DXd, in patient- and cell line–derived xenograft (PDX/CDX) models. HER2 mRNA expression was determined using RNA-seq and protein expression via IHC across multiple pediatric tumor PDX models. Osteosarcoma (OS), malignant rhabdoid tumor (MRT), and Wilms tumor (WT) models with varying HER2 expression were tested using 10 mice per group. Additional histologies such as Ewing sarcoma (EWS), rhabdomyosarcoma (RMS), neuroblastoma (NB), and brain tumors were evaluated using single mouse testing (SMT) experiments. T-DXd or vehicle control was administered intravenously to mice harboring established flank tumors at a dose of 5 mg/kg on day 1. Event-free survival (EFS) and objective response were compared between treatment and control groups. HER2 mRNA expression was observed across histologies, with the highest expression in WT (median = 22 FPKM), followed by MRT, OS, and EWS. The relationship between HER2 protein and mRNA expression was inconsistent. T-DXd significantly prolonged EFS in 6/7 OS, 2/2 MRT, and 3/3 WT PDX models. Complete response (CR) or maintained CR (MCR) were observed for 4/5 WT and MRT models, whereas stable disease was the best response among OS models. SMT experiments also demonstrated activity across multiple solid tumors. Clinical trials assessing the efficacy of a HER2-directed ADC in pediatric patients with HER2-expressing tumors should be considered.

Introduction

Relapsed or metastatic pediatric solid tumors remain a challenge to treat despite aggressive local and systemic therapies, with 5-year overall survival between 20% and 30%, predominantly due to the development of resistance to conventional chemotherapeutics. Novel approaches to therapy such as targeted therapies and immune therapies are needed and being developed to improve outcomes for these patients. One avenue of developing new therapeutics in any pediatric solid tumor is to identify strongly expressed cell surface or intracellular antigens in a majority of patients which can be used to develop immune therapy approaches such as antibody–drug conjugates (ADC) or cellular therapies such as chimeric antigen receptor T cells or adoptive cell therapies.

HER2 is one such potential target. It was first described by multiple groups in the 1980s, which has led to its multiple names in the literature (1). There are four members of the family of epidermal growth factor receptor tyrosine kinases: EGFR (ErbB-1), HER2 (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4; ref. 2). During fetal development, HER2 is widely expressed in tissues including placenta, liver, kidney, lung, and brain. Lower levels of expression are seen in adult tissues such as kidney, liver, skin, lung, jejunum, uterus, stomach, and colon (https://www.proteinatlas.org/ENSG00000141736-ERBB2/tissue). HER2 overexpression has been shown to be tumorigenic. Transfection of NIH3T3 cells with *ERBB2* transforms the cells leading to tumor formation in mice and this depends on the level of expression of HER2 within the transformed cells (3, 4). Transgenic mice expressing HER2 under the control of a mouse mammary cell-specific promoter form mammary tumors consistent with adenocarcinomas (5). HER2 is expressed and a target of interest for several pediatric cancers, including osteosarcoma (OS), Wilms tumor (WT), ependymoma, and MRT, but unlike adult cancers, *HER2* amplification is distinctly uncommon in pediatric cancers. In OS, both membranous and cytoplasmic staining of HER2 is reported. In WT, the epithelial component generally has higher expression compared with the mesenchymal and blastemal components (6–8). Targeting HER2 in pediatric cancers using monoclonal antibodies trastuzumab and small-molecule inhibitors such as lapatinib produced little evidence of clinical activity (9–12). However, HER2-based therapies continue to be studied for pediatric cancers using immunotherapy approaches such as CAR-T cells (13).

ADCs as therapeutic agents have shown success in both preclinical and clinical settings. The efficacy of ADCs depends on multiple factors, including antigen density, affinity of the antibody to the targeted surface protein, antibody internalization, and the cytotoxic payload delivered to the tumor cells. Optimization of these components can enhance the therapeutic index and permit the delivery of drug doses

[1]Division of Pediatrics, University of Texas MD Anderson Cancer Center, Houston, Texas. [2]Division of Pathology, University of Texas MD Anderson Cancer Center, Houston, Texas. [3]Cancer Therapeutics Evaluation Program, NCI, Bethesda, Maryland. [4]Global Health Technologies, RTI International, Durham, NC, USA. [5]Division of Pediatric Hematology/Oncology, Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware. [6]Greehey Children's Research Cancer Institute, San Antonio, Texas.

Corresponding Authors: Pooja Hingorani, The University of Texas MD Anderson Cancer Center, Department of Pediatrics, Houston, TX 77030. Phone: 713-792-6620; E-mail: pooja_gidwani@yahoo.com; and Richard Gorlick, RGorlick@mdanderson.org

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that would otherwise be too toxic with systemic administration (14). Trastuzumab deruxtecan (T-DXd) is one such ADC in which the humanized monoclonal HER2 antibody is linked to a topoisomerase 1 inhibitor payload called DXd via a self-immolative enzymatically cleaved linker. In preclinical studies with adult cancer cell lines, T-DXd showed activity against both low and high HER2-expressing cell lines (15), and in clinical studies it has shown activity in a broad range of HER2-expressing cancers (16–19). In this study, the *in vivo* activity of T-DXd was assessed in a panel of pediatric solid tumor PDX/CDX models with varying HER2 expression, as part of the NCI-supported Pediatric Preclinical Testing Consortium (PPTC).

Materials and Methods

Pediatric preclinical testing consortium models

PPTC is an NCI-funded collaborative initiative that includes researchers within and outside the United States that contribute to preclinical models and help evaluate new agents across a variety of pediatric cancers. All of these models are well validated, and data on their molecular and histologic characterization for most models are in the public domain at PedcBioPortal https://pedcbioportal.kidsfirstdrc.org/study/summary?id=pptc (20–23).

ERBB2/HER2 gene and protein expression analysis

PPTC xenograft RNA-seq data available at PedcBioPortal were mined for *HER2* mRNA expression across all available pediatric tumor models. Histologies with the highest mRNA expression were selected for initial *in vivo* preclinical activity evaluation of T-DXd. These included OS (7 models), WT (3 models), and MRT (2 models). A tissue microarray was created using formalin-fixed paraffin-embedded tumor models and unstained slides prepared.

HER2 protein expression was assessed in these PDX models via IHC. In WT and MRT models, IHC study was performed on 4-μm unstained slides using the Ventana PATHWAY anti-HER2 clone 4B5 assay and the Ventana Benchmark Ultra auto-stainer as specified by the manufacturer (Roche Diagnostics). In brief, following the deparaffinization and rehydration of the tissue sections, antigen retrieval was performed with Tris-EDTA buffer, pH 6.0 (Ventana Ultra CC1, mild) at 95°C for 8 minutes. Primary anti-HER2 antibody (clone 4B5, Roche Diagnostics), previously pre-titered by the manufacturer, was applied for 12 minutes at 36°C. Primary antibody detection was carried out using the manufacturer-specified polymer and staining development system, DAB (ultraView Universal DAB Detection Kit, Roche Diagnostics). As there are no established interpretation guidelines for mesenchymal tumors, staining was assessed by determining the intensity (0: none; 1: mild; 2: moderate; 3: strong) as well as the percentage of positive cells (any pattern; ref. 24). For OS PDX models and testing on human osteosarcoma samples, anti-HER2 clone CB 11 was used. For HER2 CB11, staining was performed on 4-μm unstained slides using a Leica Bond III autostainer (Leica Biosystems). Following deparaffinization and rehydration of the tissue sections, antigen retrieval was performed at 100°C for 30 minutes with Tris-EDTA buffer, pH 6.0. Endogenous peroxidase was blocked with 3% peroxide for 5 minutes. Prediluted HER2 (clone CB11, Leica Biosystems) was applied for 120 minutes. Primary antibody detection was carried out using a commercial polymer system (Bond Polymer Refine Detection, Leica Biosystems) at 30 minutes, and staining development is achieved by incubation with DAB and DAB Enhancer (Leica Biosystems) at 30 minutes. Staining was assessed by determining the intensity (0: none; 1: mild; 2: moderate; 3: strong) as well as a percentage of positive cells (any pattern).

In vivo testing

T-DXd was provided by Daiichi Sankyo Company, Limited. C.B.17SC *scid*$^{-/-}$female mice were used to propagate subcutaneous flank tumors. Ten mice were used in each control or treatment group (conventional testing) for the first set of experiments in OS, WT, CNS atypical teratoid rhabdoid tumor (ATRT), and extracranial MRT PDX models. T-DXd was administered as a single dose at 5 mg/kg intravenously. In preclinical studies, T-DXd has been used in doses ranging from 3 to 10 mg/kg intravenously. At 3 mg/kg dose, area under the curve (AUC) was reported to be 318 μg*day/mL (25). Assuming there is linearity in mouse pharmacokinetics, AUC in mice at 5 mg/kg T-DXd is estimated to be comparable with that of T-DXd in humans at clinical dosage (544 μg*day/mL @5.4 mg/kg; ref. 26), and hence this dose was selected for the current study.

The control cohort received a single dose of vehicle. Mean (± SD) tumor volumes at start of treatment were 0.103 ± 0.007 cm^3 for OS, 0.319 ± 0.04 cm^3 (ATRT), and 0.268 ± 0.028 cm^3 (MRT and WT). Tumor volumes were measured weekly as previously described (20). Subsequently, single mouse treatment (SMT) experiments were conducted across a range of pediatric solid tumor histologies to extend knowledge of the breadth of activity of T-DXd for pediatric cancers. For these studies, a single mouse was included in the treatment group and received the same dose of DXd as in conventional testing. All mice were maintained under barrier conditions, and experiments were conducted using protocols and conditions in accordance with and with the approval of the Institutional Animal Care and Use Committee at M.D. Anderson Cancer Center (ACUF study #00001656-RN00), and UT Health, San Antonio (IACUC protocol 1505X).

The *in vivo* activity of T-DXd was evaluated using standard PPTC methodology. Briefly, for solid tumor experiments, an *event* is defined as a quadrupling of tumor volume from the day treatment was initiated. The median time to event was assessed between the experimental and control cohorts. Differences in event-free survival (EFS) between experimental groups (e.g., treated vs. controls) were tested with $\alpha = 0.05$, two-sided alternative with $\rho = 1$, which is equivalent to the Peto & Peto modification of Gehan–Wilcoxon. At the conclusion of the experiment, the *minimum RTV* (minRTV) for each mouse is computed across all measurements except the initial one. The mean and standard deviation within each treatment group of minRTV is computed, and comparisons between treatment groups are performed using the Wilcoxon rank-sum test. Objective responses reported as maintained complete response (MCR), complete response (CR), partial response (PR), and stable disease were described for each model as defined previously (20).

Data availability statement

The data generated in this study are available upon request from the corresponding author.

Results

HER2 expression in pediatric solid tumor PDX models

Review of the RNA-seq data for PDX models showed that OS, WT, MRT, and ependymoma consistently demonstrated high relative mRNA expression compared with other histologies (**Fig. 1A**). HER2 protein expression was assessed in 7 OS, 3 WT, and 2 MRT PDX models that were used for drug testing. For OS PDX, IHC data were available for all seven models. Predominantly, the cytoplasmic staining of variable intensity was seen in five of seven PDX models, with four of five PDX exhibiting staining in most tumor cells. For WT PDX, weak membranous staining was seen in two of three models. For MRT PDX,

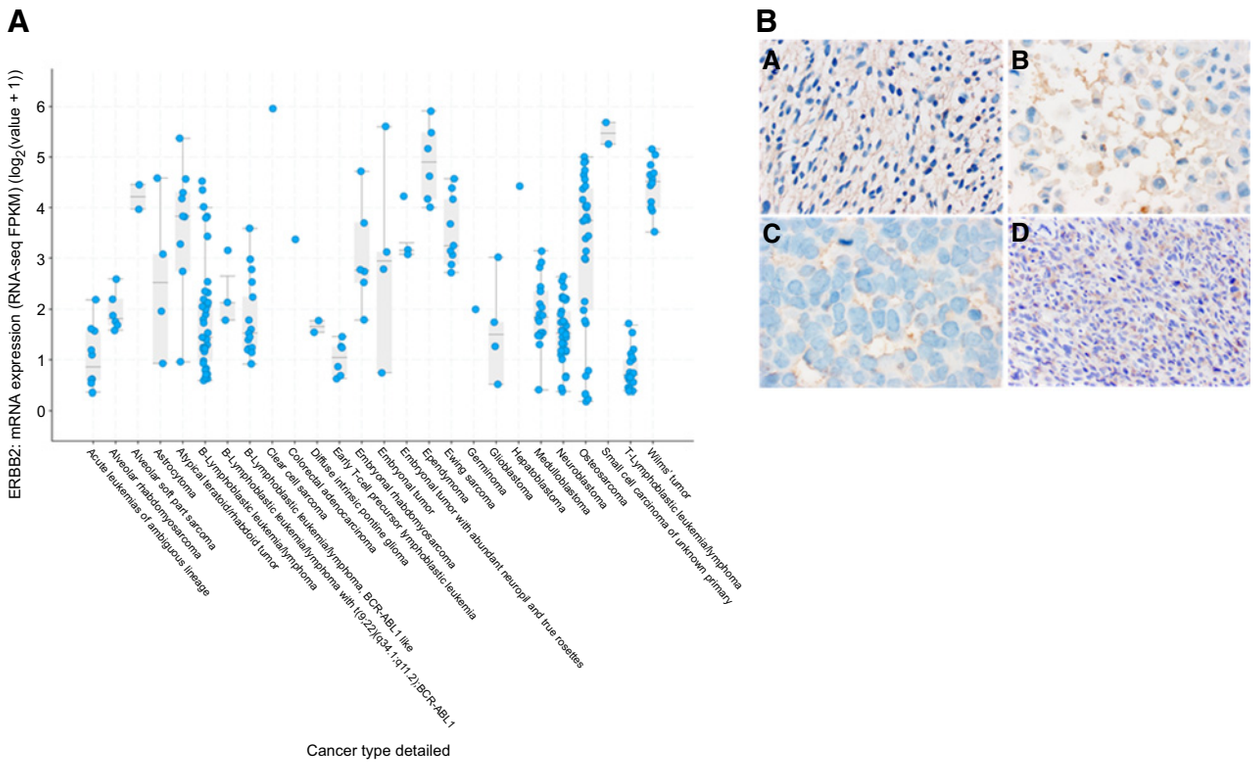

Figure 1.

HER2 expression across pediatric cancer PDX models. **A,** Relative mRNA expression of *HER2* assessed by RNA-seq in solid tumor PDX models. **B,** HER2 protein expression assessed by IHC in PDX models: **A**. RH18, rhabdomyosarcoma; **B**. RBD2, extracranial rhabdoid tumor; **C**. KT13, Wilms tumor; **D**. OS33, osteosarcoma.

RBD2 models exhibited moderate membranous labeling in up to 50% of cells, whereas no membranous or cytoplasmic staining was seen in the KT12 model (**Table 1**; **Fig. 1**).

HER2 expression in human OS tumors

Human OS tissue microarray with 41 patient formalin-fixed paraffin-embedded samples, previously decalcified with 10% formic acid, was evaluated for HER2 expression via IHC. Twenty-four of 41 samples (59%) exhibited staining for HER2, 22 of which had labeling in a majority of cells (>70%) with two cases with focal (10%) labeling. The distribution of staining intensity was 9 weak, 13 moderate, and 2 strong. The staining pattern was predominantly cytoplasmic with some concurrent membranous (**Fig. 2**).

In vivo efficacy of T-DXd

T-DXd was initially tested in 7 OS PDX models (OS1, OS2, OS9, OS17, OS31, OS33, and OS60), 3 WT models (KT10, KT11, and KT13), 2 extracranial MRT models (KT12 and RBD2), and 1 CNS ATRT model (BT29) with 10 mice per treatment and control group. Mice tolerated the treatment very well with no significant weight loss or other side effects. T-DXd induced prolonged EFS in 6/7 OS, 1/1 ATRT, 2/2 extracranial RT, and 3/3 WT models ($P < 0.05$, Gehan–Wilcoxon; **Table 1**). Tumor regression (mean minRTV < 1.0) was observed in 2/7 OS models and in all of the ATRT, WT, and extracranial MRT models studied.

Six of 7 OS models showed progressive disease as their objective response measure with one of these models (OS2) meeting criteria for a PD2 response (EFS T/C > 2.0). OS33 experienced stable disease (<50% tumor regression throughout study but ≤25% tumor growth by end of study). The extracranial MRT model RBD2 achieved MCR (no measurable tumor for >3 consecutive weeks), whereas KT12 exhibited a PR (≥50% tumor regression). The ATRT model BT29 also achieved MCR. Among the WT xenografts, KT10 and KT13 achieved MCR whereas KT11 had progressive disease with a PD2 response (EFS T/C > 2; **Table 1**; **Fig. 3A**). Kaplan–Meier curves showing EFS for conventional testing experiments are presented in **Fig. 3B**.

In SMT experiments, models that experienced CR or MCR were 1/1 ATRT, 2/9 EWS, 3/4 extracranial MRT, 2/6 fusion-negative rhabdomyosarcoma (RMS), 2/4 fusion-positive RMS, 2/2 WT, 1/1 medulloblastoma, and 1/1 glioblastoma. Seven of 31 mice remained alive at ≥ 140 days after a single dose of T-DXd, including 4 of the 5 animals engrafted with MRT (1 CNS ATRT and 3 extracranial MRT) and one animal engrafted with a WT model (**Table 2**; **Fig. 4**).

Discussion

T-DXd exhibited significant antitumor activity against several PPTC pediatric solid tumor PDX models with variable expression of HER2, demonstrated by prolonged EFS and objective responses. These data suggest that HER2 may be a relevant target across several pediatric solid tumors and provides proof of principle that HER2-targeted ADCs are an effective therapeutic strategy and worthy of clinical investigation.

Although RNA-seq data from xenograft models show variable but broad expression across tumor types, one of the challenges is precisely

Table 1. T-DXd testing results and HER2 expression in pediatric solid tumor PDX models for conventional testing ($N = 10$/agent).

Cancer type	Model	Agent	KM med (days)	EFS T - C (days)	EFS T/C	*P* value Gehan–Wilcoxon	minRTV mean ± SD	minRTV *P* value	Objective response measure	ERBB2 mRNA (FPKM)	HER2 IHC (% tumor cells, intensity)
Osteosarcoma	OS-1	control	23.9				1.249 ± 0.075				100%, 3+
		T-DXd	44.2	20.3	1.85	$P < 0.001$	1.152 ± 0.090	$P = 0.023$	PD1	31	
	OS-2	control	17.2				1.694 ± 0.405				5%, 1+
		T-DXd	47.3	30.1	2.76	$P < 0.001$	0.815 ± 0.183	$P < 0.001$	PD2	29	
	OS-9	control	29.3				1.452 ± 0.431				0
		T-DXd	33.5	4.1	1.14	$P = 0.103$	1.334 ± 0.203	$P = 1.000$	PD1	15	
	OS-17	control	35.1				1.343 ± 0.253				100%, 2+
		T-DXd	69.2	34.0	1.97	$P = 0.009$	1.172 ± 0.179	$P = 0.017$	PD1		
	OS-31	control	14.2				2.198 ± 0.331				0
		T-DXd	26.2	11.9	1.84	$P < 0.001$	1.676 ± 0.395	$P = 0.015$	PD1	24	
	OS-33	control	15.8				1.710 ± 0.221				100%, 2+
		T-DXd	77.1	61.3	4.87	$P < 0.001$	0.486 ± 0.220	$P < 0.001$	SD	0	
	OS-60	control	36.0				1.217 ± 0.121				100%, 1+
		T-DXd	39.7	3.8	1.11	$P = 0.016$	1.084 ± 0.103	$P = 0.007$	PD1	2	
CNS ATRT	BT29	control	25.6				1.340 ± 0.296				n/a
		T-DXd	>84	>58.4	>3.28	$P < 0.001$	0.019 ± 0.041	$P < 0.001$	MCR	40	
Extracranial Rhabdoid	KT-12	control	15.4				2.209 ± 0.232				0
		T-DXd	53.9	38.5	3.5	$P < 0.001$	0.272 ± 0.141	$P < 0.001$	PR	9	
	RBD2	control	7.7				3.526 ± 0.398				50%, 2+
		T-DXd	>105	>97.3	>13.57	$P < 0.001$	0.021 ± 0.066	$P < 0.001$	MCR	17	
Wilms	KT-10	control	12.9				2.000 ± 0.798				0
		T-DXd	>98	>85.1	>7.57	$P < 0.001$	0.000 ± 0.000	$P < 0.001$	MCR	23	
	KT-11	control	11.3				2.959 ± 0.789				90%, 1+
		T-DXd	29.6	18.3	2.62	$P < 0.001$	0.954 ± 0.486	$P < 0.001$	PD2	24	
	KT-13	control	26.5				2.420 ± 0.757				100%, 1+
		T-DXd	>98	>71.5	>3.69	$P < 0.001$	0.019 ± 0.060	$P < 0.001$	MCR	11	

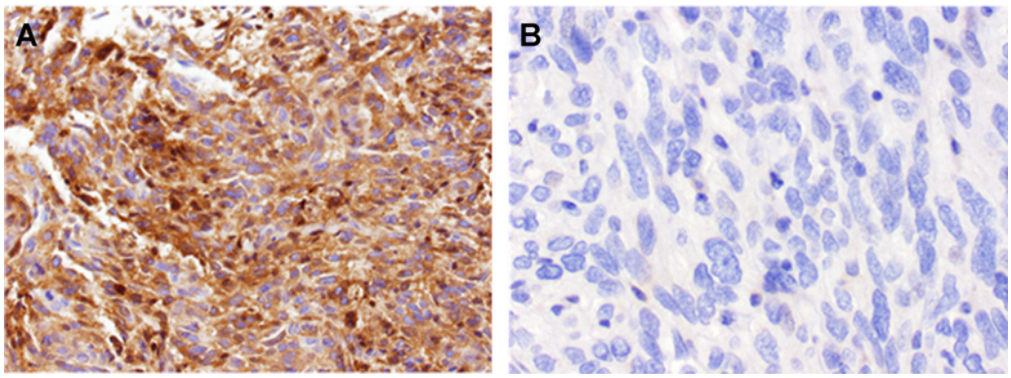

Figure 2.
Representative example of HER2 expression in human osteosarcoma tissue microarrays. **A,** Diffuse strong HER2 labeling. Staining was predominantly cytoplasmic. **B,** Negative HER2 labeling.

determining the protein expression in pediatric tumors given that *HER2* amplification is not the primary event driving protein levels unlike adult cancers such as breast cancer. In our xenograft models, some degree of protein expression was seen in the majority of models tested by IHC, but the degree of protein expression did not consistently correlate to RNA expression or response. Our results suggest that different IHC staining protocols may need to be optimized and validated for different pediatric tumors. In addition, osteosarcomas predominantly exhibited cytoplasmic staining rather than the traditional membranous pattern seen in carcinomas. The significance of this staining pattern is not certain. Given these challenges, it might not be possible to correlate response to the degree of HER2 expression beyond the presence or absence of the protein.

In this study, SMT experiments were utilized to broaden the panel of PDX models tested with T-DXd. PPTC investigators have previously described that using a single mouse for a model is equally

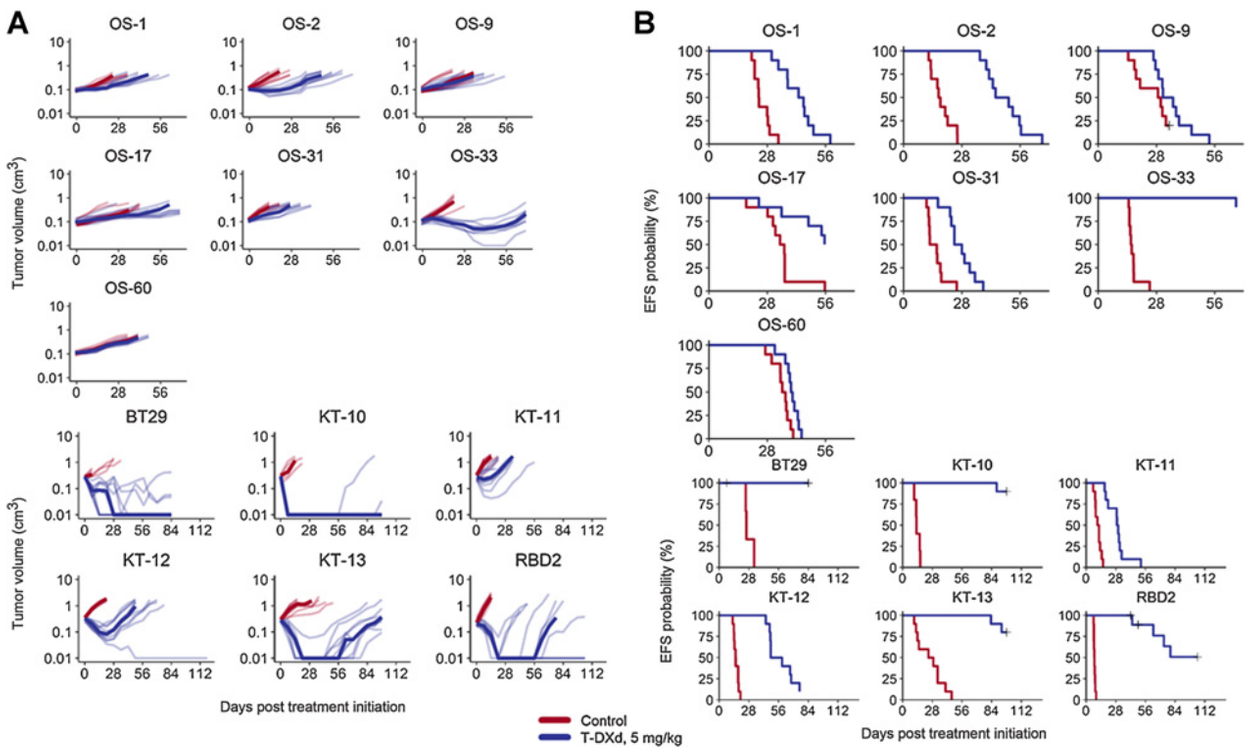

Figure 3.
Treatment effects of T-DXd in pediatric cancer models. **A,** Tumor volume changes in response to T-DXd as single agent for all osteosarcoma, rhabdoid, and WT models. Treatment groups are as described above and as shown in the legend. Bold lines represent the median tumor volume for each treatment group. Regular lines represent tumor volume change for each individual mouse tumor (spider plots). **B**, Event-free Survival in response to T-DXd as a single agent across all osteosarcoma, rhabdoid, and WT models.

Table 2. Single mouse testing experiment results across various histologies.

Cancer type	Model	Time to event	minRTV	Objective response measure^{+}	HER2 mRNA (FPKM)	Her2 IHC (% tumor cells, Intensity)
ATRT	BT-29	>147	0.000 ± NA	MCR	40	n/a
Medulloblastoma	BT-39	63.5	0.000 ± NA	CR		n/a
Glioblastoma	BT-50	>84	0.000 ± NA	MCR		n/a
Pilocytic Xanthoastrocytoma	S-1263221	79.0	0.530 ± NA	PD		n/a
Ewing sarcoma	CHLA-258	>147	0.000 ± NA	MCR	20	0
	ES-3	31.6	1.448 ± NA	PD		0
	ES-4	41.9	0.422 ± NA	PR	17	0
	ES-6	123.8	0.000 ± NA	MCR	12	0
	ES-7	40.0	0.546 ± NA	PD		0
	EW-5	53.9	0.982 ± NA	PD	8	0
	EW-8	43.2	0.729 ± NA	PD	9	0
	SK-NEP-1	32.9	0.443 ± NA	PR	23	0
	TC-71	35.1	1.174 ± NA	PD	6	10%, 1+
Extracranial rhabdoid	KT-14	>140	0.000 ± NA	MCR	19	0
	KT-16	>140	0.000 ± NA	MCR		0
	RBD-1	26.2	0.342 ± NA	PR		0
	RBD-2	>140	0.000 ± NA	MCR	17	50%, 2+
Fusion^{-} RMS	IRS-56	87.1	0.054 ± NA	PR	6	n/a
	Rh12	>49	0.000 ± NA	CR	25	n/a
	Rh18	>140	0.000 ± NA	MCR		100%, 2+
	Rh36	14.4	1.341 ± NA	PD	5	0
	JR-1	18.2	0.955 ± NA	PD		n/a
	SMSCTR	22.8	0.289 ± NA	PR		n/a
Fusion^{+} RMS	Rh30	81.2	0.000 ± NA	MCR	2	0
	Rh30R	60.1	0.000 ± NA	CR	2	0
	Rh41	28.8	0.440 ± NA	PR	3	0
	Rh65	33.5	1.450 ± NA	PD	2	n/a
Neuroblastoma	NB-1643	38.1	0.464 ± NA	PR	1	n/a
Osteosarcoma	OS-1	49.6	0.559 ± NA	PD	31	100%, 3+
Wilms	KT-10	>147	0.000 ± NA	MCR	23	0
	KT-13	133.6	0.000 ± NA	MCR	11	100%, 1+

n/a, not available.

representative of response as using a group of 8–10 mice in traditional testing (27). This SMT strategy allows for more efficient use of resources while allowing for drug response testing across more tumor models representative of tumor heterogeneity in patients. Of note, in the four models for which conventional and SMT testing was undertaken, the results were identical. Of interest also is the responsiveness of ATRT/MRT models to T-DXd for which 4 models were MCR at week 20. Whether this relates to the targeted delivery of the DXd payload, or an intrinsic sensitivity to topoisomerase 1 inhibition is unknown, as several of the MRT models were very sensitive to PLX-038A, a controlled release form of SN-38, the active moiety of irinotecan (28).

T-DXd has undergone clinical evaluation in adults with HER2-expressing tumors. T-DXd was granted accelerated approval by FDA in 2019 for the treatment of adult patients with unresectable or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2–based regimens in the metastatic setting (18). Approval was based on a phase II study that showed a confirmed objective response rate of 60.3% in the target population for this indication(16). In 2021, T-DXd was granted approval by FDA for use in adult patients with locally advanced or metastatic HER2-positive gastric or gastroesophageal (GEJ) adenocarcinoma who have received a prior trastuzumab-based regimen. This approval was based on improved overall survival for patients receiving T-DXd in a randomized study comparing T-DXd to physician's choice of either irinotecan or paclitaxel monotherapy (17). T-DXd has also shown promising activity in heavily pretreated HER2^{-} low-expressing advanced breast cancer with an objective response rate of 37% (29). Activity for T-DXd has also been observed in patients with HER2-expressing colorectal cancer (19). These data suggest that T-DXd is active in a variety of malignancies with varied levels of HER2 expression including those that have been previously treated with other HER2 based therapies as well as those with HER2 expression levels below that required for agents such as trastuzumab. Although ERBB2 gene expression and HER2 protein expression are seen at low to medium levels in renal, gastrointestinal, genitourinary, skin, and cardiac muscle as demonstrated in The Human Protein Atlas data (https://www.proteinatlas.org/ENSG00000141736-ERBB2/tissue), the adverse event profile for T-DXd to date is significant for interstitial lung disease, including pneumonitis, as well as neutropenia and anemia (16–19). Specifically, no significant cardiac toxicity has been seen in clinical trials thus far. A phase II clinical trial in patients 12–39 years with recurrent HER2-positive OS was recently activated (NCT04616560).

ADCs are a promising therapeutic approach in cancer therapy. An ideal ADC is one that can deliver large doses of the cytotoxic agent specifically to the malignant cells that express the antigen without exposure to normal tissues. Commonly used cytotoxic agents include microtubule inhibitors, topoisomerase poisons, and

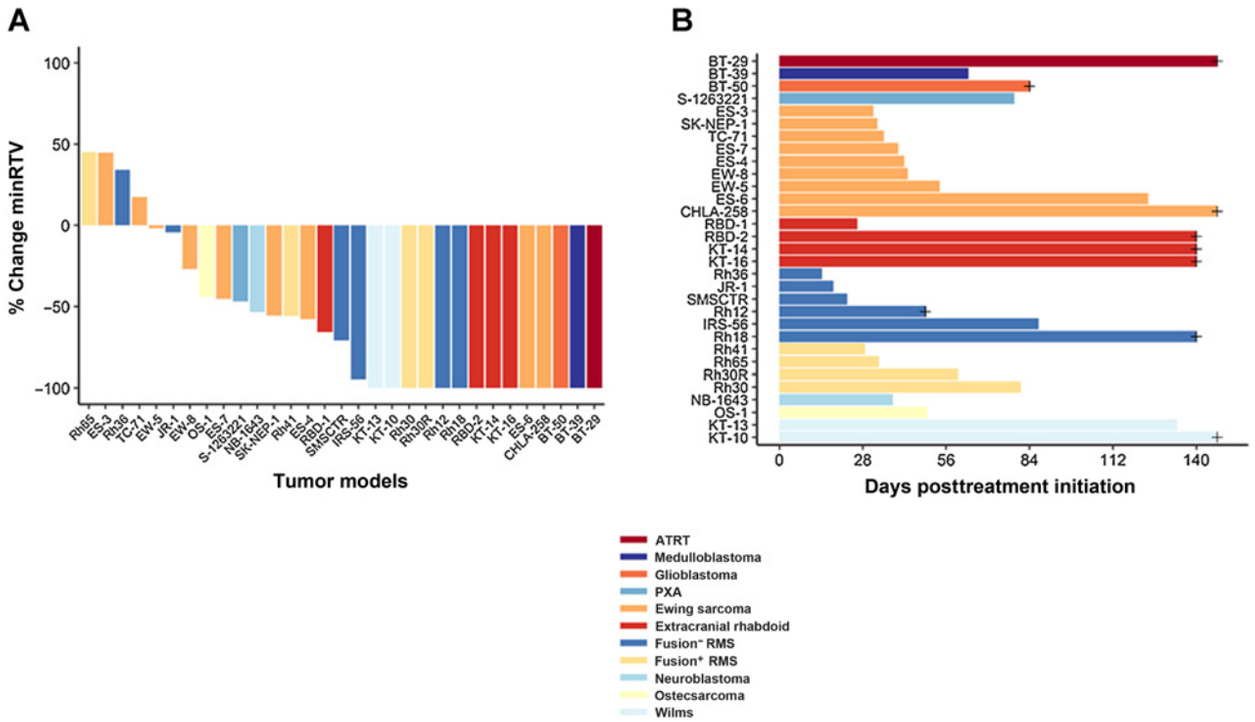

Figure 4.

Single mouse treatment experiments. **A,** Waterfall plot for tumor volume responses in SMT experiments: tumor histologies are color coded as shown in the legend; **B**, Swimmer's plot for SMT experiments: + sign shows mice without events at the end of the experiment.

other DNA-damaging agents (14). ADCs are being developed to target several cell-surface proteins that are expressed in specific pediatric tumors (e.g., LRRC15 in OS) or across pediatric tumors (e.g., B7-H3). In preclinical testing, these ADCs have shown remarkable responses in PDX models again providing proof of principle of this strategy (30, 31). Further studies need to focus on evaluating potential drug resistance mechanisms such as down-regulation of cell-surface protein on the tumor cells or resistance to the cytotoxic payloads as well as determining combination therapies of ADCs with other agents including other ADCs. In addition, comparing ADCs with the same target antigen but different payloads and vice versa would help choose the most optimal ADC for a particular tumor type.

In summary, our study highlights that HER2 may be a relevant protein to target therapeutically in several pediatric solid tumors. The HER2-targeted ADC, T-DXd, showed tumor-regressing activity against multiple pediatric solid tumor histologies, and its clinical evaluation against these histologies is supported by these results.

Authors' Disclosures

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In keeping with AACR editorial policy, a senior member of the *Molecular Cancer Therapeutics* editorial team managed the consideration process for this submission and independently rendered the final decision concerning acceptability.

Authors' Contributions

P. Hingorani: Data curation, writing–original draft, writing–review and editing. **W. Zhang:** Data curation, project administration. **Z. Zhang:** Data curation, formal analysis, methodology. **Z. Xu:** Data curation. **W. Wang:** Data curation, methodology, writing–original draft. **M.E. Roth:** Conceptualization, supervision, writing–review and editing. **Y. Wang:** Data curation, methodology. **J.B. Gill:** Methodology, writing–review and editing. **D.J. Harrison:** Writing–review and editing. **B.A. Teicher:** Resources, investigation. **S.W. Erickson:** Visualization, writing–review and editing. **G. Gatto:** Investigation, visualization, methodology. **E.A. Kolb:** Data curation, writing–review and editing. **M.A. Smith:** Conceptualization, resources, formal analysis, supervision, writing–review and editing. **R.T. Kurmasheva:** Data curation, writing–review and editing. **P.J. Houghton:** Conceptualization, formal analysis, writing–review and editing. **R. Gorlick:** Conceptualization, resources, formal analysis, funding acquisition, project administration, writing–review and editing.

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