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Preclinical evaluation of a dnTGFβR-armored GPC3 CAR-T (AZD5851) in pediatric solid tumor PDX models – A report from the Pediatric Preclinical In Vivo Testing Consortium (PIVOT)

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ABSTRACT

GPC3 is a heparan sulfate proteoglycan frequently overexpressed in pediatric malignancies, including hepatoblastoma (HBL), Wilms tumor (WT), malignant rhabdoid tumor (MRT) and rhabdomyosarcoma (RMS). AZD5851 is a GPC3-targeted CAR-T cell therapy engineered with a dominant-negative (dn) TGF-β receptor II (dnTGFβRII) to overcome TGF-β–mediated immunosuppression and enhance T-cell function. To evaluate antitumor activity, AZD5851 was tested in a panel of pediatric patient-derived xenograft (PDX) models, with analyses exploring the relationship between GPC3 expression, stromal TGF-β, and therapeutic response. (1,2,3)

STUDY METHODS

Model characterization: Nine pediatric PDX models were evaluated: 3 Wilms tumor [WT], 2 hepatoblastoma [HBL], 2 germ cell tumors [GCT], 1 malignant rhabdoid tumor [MRT], 1 rhabdomyosarcoma [RMS]). Studies were performed in NSG MHC-DKO mice (Jackson Laboratory Strain #025216). GPC3 expression was assessed by immunohistochemistry (IHC) using H-score methodology, and TGF-β staining was evaluated in tumor and stromal compartments.

Drug dosing: AZD5851 CAR-T cells (5 × 10^6 CAR-T cells/mouse; 12 × 10^6 total cells infused based on transduction efficiency) or untransduced T cells (12 × 10^6 total cells/mouse) were administered intravenously once (cells supplied by AstraZeneca).

Efficacy studies: Four to five mice per treatment group were evaluated across nine PDX models. Tumor volumes were monitored longitudinally, and event-free survival (EFS) and objective response measures (ORMs) were assessed using established PIVOT criteria (4).

Response assessment: An event was defined as a >4-fold increase in tumor volume from baseline. Because CAR-T therapies may demonstrate delayed regression, a secondary endpoint (tumor volume >1,000 mm³ or moribund status) was also analyzed. EFS distributions were estimated using Kaplan–Meier methods and compared using the log-rank test.

ORM	ORM Code	Criteria
Progressive Disease	PD	< 50% tumor regression throughout study > 25% tumor growth at end of study
Progressive Disease 1	PD1	PD the mouse's time-to-event ≤ 200% the median time-to-event in control group
Progressive Disease 2	PD2	PD the mouse's time-to-event is > 200% the median time-to-event in control group
Stable Disease	SD	< 50% tumor regression throughout study ≤ 25% tumor growth at end of study
Partial Response	PR	≥ 50% tumor regression at any point during study but measurable tumor throughout study period
Complete Response	CR	disappearance of measurable tumor mass during the study period
Maintained Complete Response	MCR	no measurable tumor mass for at least 3 consecutive weekly readings at any time after treatment has been completed

Each mouse was assigned a score from 0 to 10 based on their ORM. PD1 = 0, PD2 = 2, SD = 4, PR = 6, CR = 8, and MCR = 10. The median for the group determined the overall response.

If the median score was half-way between an ORM category, the objective response was assigned to the lower response category.

RESULTS

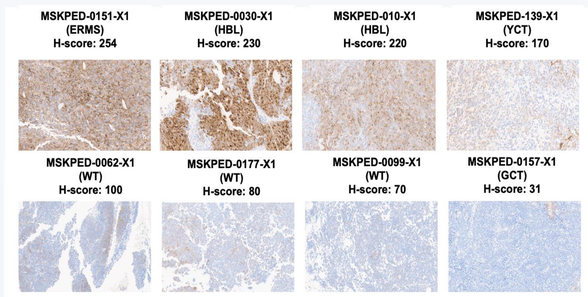

Figure 1. GPC3 immunohistochemistry (IHC) in pediatric solid tumor PDX models. Representative images show heterogeneous GPC3 expression across pediatric PDX models. ERMS, embryonal rhabdomyosarcoma; HBL, hepatoblastoma; WT, Wilms tumor; YCT, yolk sac tumor; GCT, germ cell tumor.

Table 1. Immunohistochemical assessment of GPC3 and TGF-β expression in pediatric solid tumor PDX models.

Model ID	Diagnosis	GPC3 Tumor	TGFβ * Stroma	TGFβ * Tumor
MSKPED-0151-X1	Embryonal rhabdomyosarcoma	254	40	<5
MSKPED-0030-X1	Hepatoblastoma	230	90	10
MSKPED-0010-X1	Hepatoblastoma	220	90	20
MSKPED-0139-X1	Yolk sac tumor	170	80	10
MSKPED-0062-X1	Wilms tumor	100	70	<5
MSKPED-0177-X1	Wilms tumor	80	80	<5
MSKPED-0099-X1	Wilms tumor	70	70	<5
MSKPED-0157-X1	Germ cell tumor	31	80	<5

Note: IHC staining was not performed for the malignant rhabdoid tumor (MRT) model MSKPED-0086-X1. This model was added after the initial tumor panel was submitted for staining based on prior reports of GPC3 expression in MRT.
* Values represent % positive cells ≥1+ staining

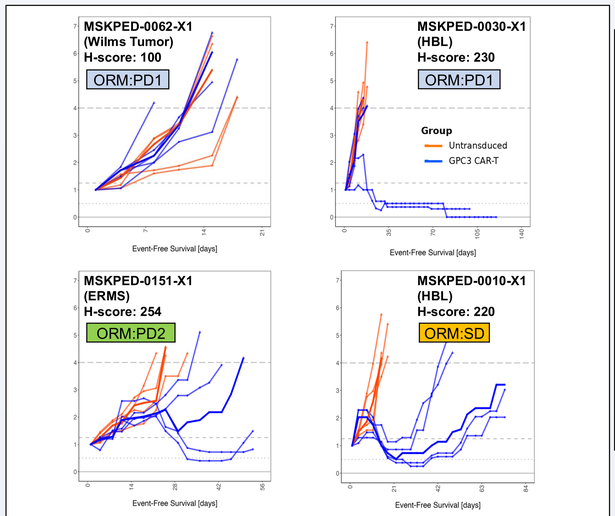

Figure 2. Tumor growth responses to AZD5851 in pediatric solid tumor PDXs. Individual tumor growth curves show relative tumor volume (RTV) over time for control (untransduced T cells, orange) and AZD5851 GPC3 CAR-T–treated mice (blue). Dashed horizontal lines denote RTV thresholds used for response assessment, including the event definition at RTV ≥4. Objective response measure (ORM) classifications are shown for each model according to PIVOT criteria.

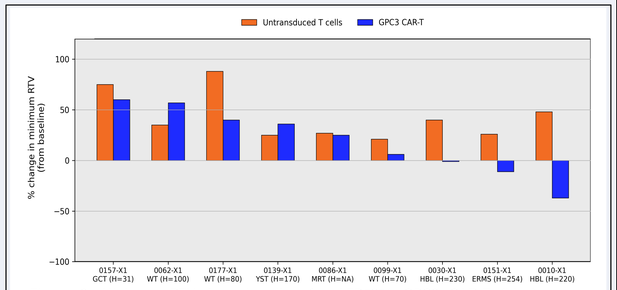

Figure 3. Minimum RTV from baseline for AZD5851 GPC3 CAR-T (blue) versus untransduced T-cell controls (orange) across PDX models.

Table 2. Summary of AZD5851 efficacy across a panel of pediatric solid tumor PDX models.

Model	Subtype	Group	N	KM med (days)	EFS T/C	Gehan-Wilcoxon p-value	minRTV mean±SD	minRTV p-value	ORM
MSKPED-0151-X1	ERMS	Untransduced T cells	5	24			1.26±0.17		PD
		GPC3 CAR-T cells	5	>49	>0.6	0.004	0.89±0.37	0.173	PD2
MSKPED-0157-X1	GCT	Untransduced T cells	5	10			1.75±0.34		PD
		GPC3 CAR-T cells	5	13	1.4	0.345	1.6±0.51	0.295	PD1
MSKPED-0010-X1	HBL	Untransduced T cells	5	15			1.48±0.09		PD
		GPC3 CAR-T cells	5	>74	>2.4	0.004	0.63±0.37	0.012	SD
MSKPED-0030-X1	HBL	Untransduced T cells	5	13			1.4±0.32		PD
		GPC3 CAR-T cells	5	17	1.4	0.138	0.99±0.84	0.600	PD1
MSKPED-0086-X1	MRT	Untransduced T cells	5	18			1.27±0.23		PD
		GPC3 CAR-T cells	5	27	1.6	0.004	1.25±0.20	0.528	PD1
MSKPED-0062-X1	WT	Untransduced T cells	5	12			1.35±0.22		PD
		GPC3 CAR-T cells	5	12	1.0	0.297	1.57±0.31	0.172	PD1
MSKPED-0099-X1	WT	Untransduced T cells	5	25			1.21±0.18		PD
		GPC3 CAR-T cells	5	29	1.2	0.918	1.06±0.08	0.230	PD1
MSKPED-0177-X1	WT	Untransduced T cells	4	10			1.88±0.37		PD
		GPC3 CAR-T cells	4	23	2.3	0.011	1.4±0.24	0.147	PD1
MSKPED-0139-X1	YST	Untransduced T cells	5	21			1.25±0.20		PD
		GPC3 CAR-T cells	5	17	0.8	0.463	1.36±0.18	0.576	PD1

Table 3. Median time to secondary endpoint* across pediatric solid tumor PDX models.

Model ID	Diagnosis	GPC3 H-score	Log-rank p-value	Median EFS (days) Untransduced T cells	Median EFS (days) GPC3 CAR-T cells
MSKPED-0151-X1	**ERMS**	**254**	**0.010**	**25**	**50**
MSKPED-0030-X1	**HBL**	**230**	**0.002**	**18**	**>99**
MSKPED-0010-X1	**HBL**	**220**	**0.003**	**25**	**74**
MSKPED-0139-X1	YCT	170	0.300	25	29
MSKPED-0062-X1	WT	100	0.300	15	15
MSKPED-0177-X1	WT	80	0.070	15	27
MSKPED-0099-X1	WT	70	0.700	29	32
MSKPED-0157-X1	GCT	31	0.200	11	18
MSKPED-0086-X1	**MRT**	**NA**	**0.003**	**25**	**29**

* Secondary endpoint defined as tumor volume ≥1000 mm³ or moribund status. EFS: Event-free survival; NA: Not available; Models with significant responses highlighted in blue.

Analysis note:
Table 2 summarizes event-free survival using the RTV ≥4 definition.
Table 3 reports median time to a secondary endpoint defined as tumor volume ≥1000 mm³ or moribund status.

SUMMARY

GPC3 and TGF-β Expression

- Pediatric PDX models demonstrated heterogeneous GPC3 expression (H-score range 31–254).
- TGF-β staining was predominantly stromal with minimal tumor cell expression.

Objective Response Assessment

- Using PIVOT objective response measures (ORM), antitumor activity was restricted to models with the highest GPC3 expression (H-score > 200), including an ERMS model demonstrating attenuated tumor progression (PD2) and an HBL model achieving stable disease (SD).

Time-to-Endpoint Analyses

- Time-to-event analyses demonstrated statistically significant prolonged tumor control exclusively in models with GPC3 H-score > 200 (among models with available IHC data), when evaluated using both standard EFS criteria and a secondary endpoint reflecting tumor burden or moribund status.

CONCLUSIONS

- AZD5851 demonstrated antitumor activity in pediatric solid tumor PDX models with high GPC3 expression (H-score >200).
- Standard event-based response criteria may underestimate CAR-T activity, particularly when responses occur after an initial period of tumor growth.
- Delayed antitumor effects are consistent with the expansion and activity kinetics of CAR-T cells in vivo.
- These findings support continued investigation of dnTGFβRII-armored GPC3 CAR-T therapy in pediatric solid tumors.

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MORE INFORMATION

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