Abstract 1905

The ROR1 antibody-drug conjugate, MK-2140, enhances the efficacy of established drugs in preclinical models of pediatric acute lymphoblastic leukemia

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Pediatric Preclinical In Vivo Testing

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Introduction

- Children diagnosed with acute lymphoblastic leukemia (ALL) experience ~90% likelihood of cure. However, the outcome for high-risk ALL and children who relapse remains poor (Inaba and Pui, 2021).
- Receptor tyrosine kinase-like orphan 1 receptor (ROR1) is a cell surface antigen primarily expressed in embryonic tissues, but also overexpressed in hematological malignancies. It promotes cell proliferation and survival through the PI3K signaling pathway.
- ROR1 is not expressed on most healthy tissues, making it an attractive target for the development of anti-cancer therapeutics (Zhao Y, *et al*, 2021).
- UC-961 is a humanized IgG monoclonal antibody with high affinity for ROR1.
- MK-2140 (Zilovertamab vedotin) and VLS-211 are antibody-drug conjugates (ADCs) of UC-961 with monomethyl auristatin-E or N-propyl-N-nitrosourea payloads, respectively.
- This study evaluated the *in vivo* efficacy of MK-2140 and VLS-211 against pediatric ALL patient-derived xenografts (PDXs).

Results

ROR1 expression is significantly higher in B-lineage versus T-lineage ALL PDXs.

ROR1 mRNA and cell surface expression showed a significant correlation in the 7 PDXs used in the study.

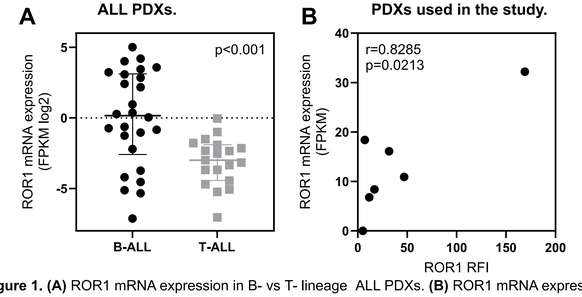

Figure 1. (A) ROR1 mRNA expression in B- vs T- lineage ALL PDXs. **(B)** ROR1 mRNA expression compared with cell surface expression by flow cytometry. RFI, Relative Fluorescence Index..

MK-2140 bound to ROR1+ ALL-7 PDX cells with near 100% occupancy within two hours of treatment, which was sustained for up to 96 h.

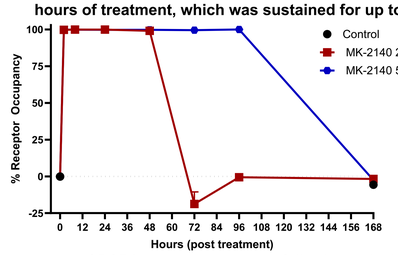

Figure 2. Assessment of ROR1 receptor occupancy following a single intravenous (IV) MK-2140 treatment of mice engrafted with ALL-7.

Plasma levels of MK-2140 were maintained at >100 nM for >96 h after a single dose (5 mg/kg) of MK-2140, consistent with the receptor occupancy data.

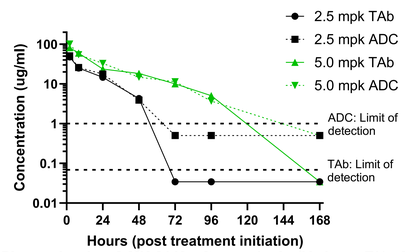

Figure 3. Plasma levels of MK-2140 after a single IV dose. TAb, Total Antibody; ADC, Antibody-drug conjugate; mpk, milligrams per kilogram.

Results (continued)

- **MK-2140 at both doses tested (2.5 and 5.0 mg/kg) significantly delayed disease progression in 4/7 PDXs, (ALL-7, ALL-25, ALL-57, and ALL-83), compared to vehicle control (T-C, 4.3 to 20.0 days).**
- **At the highest dose, (5.0 mg/kg), MK-2140 elicited objective responses (PR and CR in ALL-7 and ALL-57, respectively).**
- **VLS-211 (0.25 and 0.5 mg/kg) significantly delayed disease progression in 4/7 PDXs (ALL-7, ALL-25, ALL-57, and ALL-83; T-C, 6.1 to 43.4 days), including 3 objective responses at 0.5 mg/kg; PR, MCR and PR in ALL-25, ALL-57, and ALL-83, respectively.**

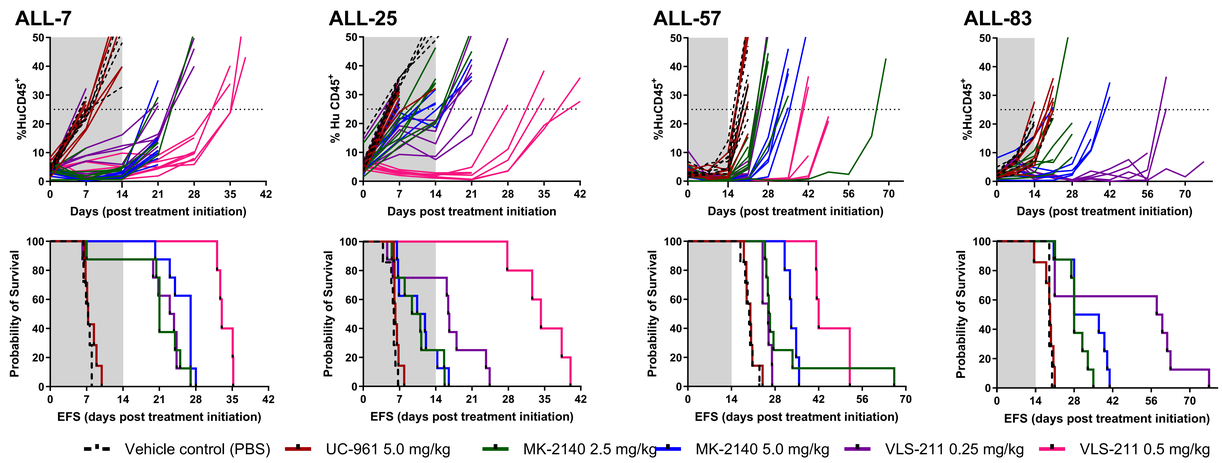

Figure 4. Engraftment and EFS of pediatric ALL PDXs in response to UC-961, MK-2140, and VLS-211 *in vivo*. Top panels represent %HuCD45+ in the PB for individual mice in each treatment group over time. Bottom panels represent EFS probability. Shaded areas indicate the treatment window. Data shown are representative of the seven selected PDXs.

PDX ID (fusion)	ROR1 mRNA expression (FPKM)	%ROR1 by flow	ROR1 RFI	Treatment Group	EFS (days)	EFS T-C (days)	EFS T/C	p-value vs. control	ORM
ALL-2 (nd)	0	49.0	5.1	Vehicle Control	14.5				
				UC-961 (ADC Control)	12.4	-2.1	0.9	>0.9999	PD1
				MK-2140 2.5 mg/kg	13.7	-0.8	0.9	0.8617	PD1
				MK-2140 5.0 mg/kg	16.1	1.6	1.1	0.5247	PD1
				VLS-211 0.25 mg/kg	12.7	-1.8	0.9	0.7952	PD1
				VLS-211 0.5 mg/kg	14.8	0.3	1.0	0.6453	PD1
ALL-4 (*BCR::ABL1*)	6.8	90.5	11.6	Vehicle Control	4.4				
				UC-961 (ADC Control)	4.1	-0.3	0.9	0.6122	PD1
				MK-2140 2.5 mg/kg	6.8	2.4	1.5	0.1997	PD1
				MK-2140 5.0 mg/kg	22.4	18.0	5.1	**0.0031**	PD2
				VLS-211 0.25 mg/kg	4.3	-0.1	1.0	0.6586	PD1
				VLS-211 0.5 mg/kg	5.0	0.6	1.1	0.1967	PD1
ALL-7 (*TCF3::HLF*)	32.2	100	169	Vehicle Control	7.3				
				UC-961 (ADC Control)	7.3	0.0	1.0	0.3720	PD1
				MK-2140 2.5 mg/kg	21.0	13.7	2.9	**0.0024**	SD
				MK-2140 5.0 mg/kg	27.0	19.7	3.7	**0.0002**	PR
				VLS-211 0.25 mg/kg	23.0	15.7	3.2	**0.0085**	PD2
				VLS-211 0.5 mg/kg	33.0	25.7	4.5	**0.0031**	PD2
ALL-25 (*TCF3::PBX1*)	10.9	99.8	47	Vehicle Control	5.9				
				UC-961 (ADC Control)	6.2	0.3	1.1	**0.0487**	PD1
				MK-2140 2.5 mg/kg	10.2	4.3	1.7	**0.0094**	PD1
				MK-2140 5.0 mg/kg	11.2	5.3	1.9	**0.0006**	PD1
				VLS-211 0.25 mg/kg	16.5	10.6	2.8	**0.0333**	PD2
				VLS-211 0.5 mg/kg	34.3	28.4	5.9	**0.0031**	PR
ALL-57 (*TCF3::PBX1*)	8.4	99.6	16.9	Vehicle Control	19.7				
				UC-961 (ADC Control)	20.1	0.3	1.0	0.7523	PD1
				MK-2140 2.5 mg/kg	26.0	6.3	1.3	**0.0005**	CR
				MK-2140 5.0 mg/kg	33.2	13.5	1.7	**0.0031**	CR
				VLS-211 0.25 mg/kg	25.8	6.1	1.3	**0.0005**	PD1
				VLS-211 0.5 mg/kg	49.0	29.3	2.5	**0.0031**	MCR
ALL-82 (*ETV6::RUNX1*)	18.4	81.0	7.1	Vehicle Control	16.4				
				UC-961 (ADC Control)	15.1	-1.3	0.9	0.3339	PD1
				MK-2140 2.5 mg/kg	13.7	-2.6	0.8	0.2333	PD1
				MK-2140 5.0 mg/kg	14.2	-2.2	0.9	0.2474	PD1
				VLS-211 0.5 mg/kg	32.9	16.6	2.0	**0.0031**	PD2
ALL-83 (*ETV6::RUNX1*)	16.1	31.7	31.7	Vehicle Control	19.0				
				UC-961 (ADC Control)	19.3	0.3	1.0	0.7897	PD1
				MK-2140 2.5 mg/kg	29.5	10.5	1.6	**0.0009**	PD1
				MK-2140 5.0 mg/kg	39.0	20.0	2.1	**0.0020**	PD2
				VLS-211 0.25 mg/kg	62.4	43.4	3.3	**0.0044**	PR

Table 1. *In vivo* efficacy of MK-2140 and VLS-211 as single agents. EFS T-C, difference in median time-to-event (days) between treated (T) and control (C) groups; EFS T/C, ratio of median time-to-event (days) between T and C groups; P-value, between C and T EFS by Gehan-Wilcoxon test; ORM, Objective Response Measure; PD1, progressive disease 1; PD2, progressive disease 2; SD, stable disease; PR, partial response; CR, complete response; MCR, maintained CR; nd, not detected. VLS-211 at 0.25 mg/kg and 0.5 mg/kg were unevaluable for ALL-82 and ALL-83, respectively. Significant P values ($P < 0.05$) in bold font.

Conclusions

- Both MK-2140 and VLS-211 exerted significant single-agent *in vivo* activity against a panel of pediatric ALL PDXs, although most models did not achieve complete responses.
- MK-2140, when used as maintenance therapy after VXL, extended time to event compared to VXL alone, and the magnitude of the extension was comparable to that observed for MK-2140 when used as a single agent.
- These results support ROR1 as a relevant immuno-oncology target for a subset of pediatric B-ALL with elevated ROR1 expression, for example those harboring *TCF3* translocations.

References

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More Information

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https://preclinicalpivot.org/

Results (continued)

Prior treatment with VXL followed by MK-2140 significantly delayed disease progression compared to VXL alone (T-C, 54.6 versus 33.7 days).

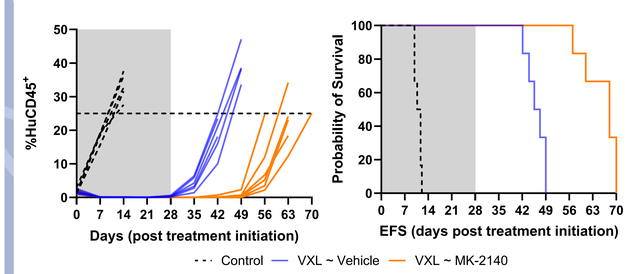

Figure 5. *In vivo* activity of MK-2140 in combination with VXL in ALL-7. Left panel, %HuCD45+ in the PB over time for individual mice. Right panel, EFS probability. Shaded area indicates the treatment window.

PDX ID	Treatment Group	EFS (days)	EFS T-C (days)	EFS T/C	p-value vs. control	ORM
ALL-7	Control	11.0				
	VXL followed by vehicle control	44.7	33.7	4.1	0.0014	CR
	VXL followed by MK-2140	65.6	54.6	6.0	0.0001	MCR

Table 2. Summary of treatment response. EFS T-C, difference in median time-to-event (days) between treated (T) and control (C) groups; EFS T/C, ratio of median time-to-event (days) between T and C groups; P-value, between C and T EFS by Gehan-Wilcoxon test; ORM, Objective Response Measure.

Study Methods

- Pediatric ALL PDXs were established in immune-deficient NSG or NOD/SCID mice from bone marrow or peripheral blood (PB) biopsies, as previously described (Lock *et al*, 2002).
- PDXs grew as orthotopic models, infiltrating the murine bone marrow, spleen and other organs and disseminating into the PB.
- Engraftment and drug responses were evaluated by flow cytometric enumeration of the proportion of human versus mouse CD45+ cells in the PB (%HuCD45+) at weekly intervals.
- ROR1 mRNA expression was quantified by RNA-seq (https://pedcbioportal.org).
- Cell surface ROR1 expression was assessed by flow cytometry and expressed as relative fluorescence index (RFI) compared to isotype control.
- PDXs were selected for *in* vivo testing based on ROR1 mRNA and cell surface expression.
- MK-2140 (2.5 mg/kg and 5.0 mg/kg) and VLS-211 (0.25 mg/kg and 0.5 mg/kg) were tested as single agents and administered IV, weekly x 3.
- The control ADC (UC-961) was administered at 5.0 mg/kg weekly x 3 weeks IV.
- For the combination study, vincristine (0.15 mg/kg once weekly), dexamethasone (5.0 mg/kg daily x 5) and *L*-asparaginase (1,250 U/kg daily x 5) (VXL) were administered intraperitoneally (IP) for 2 weeks, followed by MK-2140 (5.0 mg/kg weekly x 3 weeks, IV).
- All mice were treated with the platelet activating factor (PAF) inhibitor CV-6209 (5.0 mg/kg, IP) one hour prior to treatment with ADCs to prevent hypersensitivity reactions.
- An event was defined as >25% HuCD45+ cells in the PB, or when the mouse exhibited leukemia-related morbidity associated with high-level leukemic infiltration (>50% HuCD45+) of at least two major organs.
- Drug efficacy was assessed by event-free survival (EFS) of treated (T) and control (C) groups and by stringent objective response criteria as described by Houghton *et al*, 2007.
- The Kaplan-Meier method was used to compare time-to-event between treated and control groups
- In receptor occupancy and pharmacokinetic studies, a single dose of MK-2140 was administered and samples collected for analysis at discrete timepoints thereafter.
- UC-961, MK-2140 and VLS-211 were provided by VelosBio, Inc.