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***In vivo* efficacy of the novel anti-microtubule inhibitor E7130 in preclinical models of early T-cell precursor acute lymphoblastic leukemia**

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MAIN TEXT

Five-year overall survival rates for pediatric acute lymphoblastic leukemia (ALL) patients now exceed 90%, attributed to minimal/measurable residual disease-guided stratification and optimized chemotherapy¹. These protocols combine anti-microtubule agent vincristine with glucocorticoids and asparaginases¹, and more recently CD19-targeted immunotherapy in B-ALL². Approximately 15% of pediatric ALL is T-lineage (T-ALL), with a subset classified as early T-cell precursor (ETP) ALL³. ETP ALL is a particularly aggressive T-ALL defined by its distinct immunophenotype and genomic profile^{4, 5}. This subtype is often resistant to conventional induction chemotherapy and patients frequently experience early relapse⁶.

Expression of ATP-binding cassette subfamily B member 1 (*ABCB1*), encoding the P-glycoprotein (P-gp) transporter, is associated with chemoresistance and poor overall survival in ALL through efflux of chemotherapeutic drugs like vincristine^{7, 8}. E7130 is a novel next-generation anti-microtubule agent and potential alternative to vincristine as it is not a P-gp substrate⁹. E7130 has demonstrated encouraging activity against preclinical models of adult head and neck squamous cell carcinoma (HNSCC), breast, ovarian and colorectal cancer⁹. Furthermore, E7130 was recently evaluated in a phase 1 study in patients with advanced solid tumors, including HNSCC and urothelial carcinoma (ClinicalTrials.gov ID: NCT03444701)¹⁰. To date, E7130 has not been evaluated for the treatment of ALL, and therefore we sought to characterize *ABCB1* expression in ALL patients and patient-derived xenograft (PDX) models to identify candidates who may benefit most from E7130 chemotherapy. We then carried out an *in vivo* study to evaluate the therapeutic efficacy of E7130 and vincristine in pediatric ETP ALL PDX models.

To investigate if specific pediatric ALL subtypes are characterized by high *ABCB1* expression, we interrogated RNA-sequencing (RNA-seq) data from the St. Jude Cloud¹¹ and Polonen *et al* study¹² (**Supplementary Figure S1A, B**). This analysis revealed a significant

difference in *ABCB1* expression ($\log_2[\text{TPM}+1]$) between ALL subtypes (one-way ANOVA: $F = 8.74, p < 0.001$, **Figure S1A**), highest in T-ALL, with most variation within subtypes ($\omega^2 = 0.065$). When distinguishing ETP from other T-ALLs (Non-ETP), *ABCB1* expression was significantly higher in both clinical ($p < 0.001$, **Figure 1A**) and genomic ($p < 0.001$, **Supplementary Figure S1B**) ETP classifications. Consistent with these findings, *ABCB1* expression was also highest in the ETP subtype in ALL PDXs (**Supplementary Figure S1C**, methodology described previously)¹³. However, this expression had a dichotomous profile (**Figure 1B**), with 3 PDXs (ETP-2, ETP-3 and ETP-6) expressing higher levels of *ABCB1* ($3.6\text{--}4.6 \log_2[\text{TPM}+1]$) compared to the remaining 3 PDXs ($0\text{--}0.3 \log_2[\text{TPM}+1]$; ETP-1, ETP-4, ETP-5). *ABCB1* mRNA expression in the ETP ALL PDXs was validated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR, **Figure 1C**) and correlated with RNA-seq ($R = 0.93, p < 0.001$, **Figure 1D**). P-gp protein expression was measured using Western blotting (**Supplementary Figure S1D, E**) and correlated with *ABCB1* expression measured via RT-qPCR ($R = 0.66, p = 0.018$, **Supplementary Figure S1F**). High *ABCB1* expression in ETP ALL PDXs tracked with promoter hypomethylation (**Supplementary Figure S1G**).

To assess whether high expression of *ABCB1* correlated with P-gp transporter activity, we used the P-gp substrate and fluorescent dye Rhodamine-123 (Rh123, **Figure 1E**). Two *ABCB1*-high ETP (ETP-2 and ETP-3; $3.6\text{--}4.6 \log_2[\text{TPM}+1]$) and two *ABCB1*-low Non-ETP PDXs (ALL-8 and ALL-30; both $0 \log_2[\text{TPM}+1]$) were incubated with Rh123 (500 ng/mL) in the presence or absence of the non-competitive P-gp inhibitor tariquidar (TAR, 100 nM). A low temperature (4°C) incubation with Rh123, to suppress P-gp activity, served as a negative control. Rh123 efflux was assessed by flow cytometry. One-way ANOVAs with Tukey's HSD (Holm-Bonferroni adjusted) were conducted for each PDX. Both ETP PDXs had significantly lower Rh123 RFI at 37°C

compared to 4°C (ETP-2, $p = 0.009$; ETP-3, $p = 0.009$), indicating increased efflux at a physiologically relevant temperature. Median RFI values at 37°C were 73.1 (ETP-2) and 9.8 (ETP-3), compared to 117.9 and 80.1 at 4°C. TAR co-incubation partially reversed efflux at 37°C, approaching significance in both PDXs (ETP-2, $p = 0.068$; ETP-3, $p = 0.174$). For T-ALL PDXs, there was a negligible difference in RFI (all pairwise, $p = 1$) despite presence of cell surface P-gp (**Figure 1F, Supplementary Figure S1H**).

All mouse experiments were approved by the UNSW Sydney Animal Care & Ethics Committee (ACEC23/82B/iRECS5540). A tolerability study was conducted to determine the maximum tolerated dose (MTD) for E7130 in naïve immune-deficient NOD/SCID, IL-2 receptor γ -negative (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJAusb, NSG) mice¹⁴. E7130 was administered intravenously (IV), once weekly for 3 weeks at 0.045 mg/kg, 0.09 mg/kg, 0.135 mg/kg and 0.18 mg/kg, in 3-4 NSG mice per group. E7130 was well tolerated in mice dosed up to 0.135 mg/kg with minor changes in weight (**Supplementary Figure S2A, B**) and blood hematology/biochemistry parameters (**Supplementary Figure S2C, D**) across all treatment groups, compared to vehicle treated mice. Minor side effects from E7130 were most evident in the higher dose groups (0.135 mg/kg and 0.18 mg/kg), suggesting a dose-response effect. Indications included eye inflammation and local tissue damage at the injection site (including tail scabbing and swelling) not seen in the control group. Due to the toxicity observed at 0.18 mg/kg, doses of 0.09 mg/kg and 0.135 mg/kg E7130 were selected for the efficacy study. The MTD of vincristine in naïve NSG mice (1 mg/kg) was previously determined in another study by our lab¹⁴.

Efficacy of E7130 and vincristine was evaluated against 6 ETP ALL PDXs *in vivo* (**Figure 2, Table 1**), inoculating each PDX into 6-8 NSG mice per treatment group ($2-5 \times 10^6$ cells/mouse). E7130 was administered IV and evaluated at 2 dose levels (0.09 and 0.135 mg/kg), while

vincristine was administered intraperitoneally at 1 mg/kg. Both drugs and vehicle control were administered once weekly for 3 weeks. Leukemia engraftment was measured by flow cytometry, enumerating the proportion of human CD45⁺ (%huCD45⁺) cells in the peripheral blood (PB) at weekly intervals with events defined as %huCD45⁺ exceeding 25%. Response to treatment was assessed by event-free survival (EFS), differences between the median EFS in drug treated (T) and vehicle control (C) groups (T-C and T/C), minimum %huCD45⁺ (relative to baseline following treatment initiation), and objective response measures (ORM) based on stringent clinical criteria, as previously described¹⁴.

Treatment with E7130 (0.09 mg/kg) significantly prolonged EFS in all 6 PDXs (T-C, 10.5-41.2 days; T/C, 1.8-5.9, $p \leq 0.039$; **Figure 2A-F, Table 1**) and elicited objective responses in 2/6 PDXs (ETP-4, maintained complete response [MCR]; ETP-6, complete response [CR]). At 0.135 mg/kg, E7130 significantly prolonged the EFS of all 5 evaluable PDXs (T-C 18.0-49.5 days, T/C 2.3-8.8, $p \leq 0.009$; ETP-4 excluded due to toxicity) and elicited objective responses in 4/5 PDXs (1 CR; 3 MCRs), with one PDX (ETP-3) scored as Progressive Disease 2 (PD2). Vincristine (1 mg/kg) also prolonged EFS in all 6 PDXs (T-C, 3.5-37.7 days; T/C, 1.3-6.6), reaching statistical significance in 5 PDXs ($p < 0.001$) but not in ETP-3 ($p = 0.295$), and achieving objective responses in 2/6 PDXs (ETP-4, MCR; ETP-5, CR). E7130 (0.135 mg/kg) prolonged EFS to a greater extent than E7130 (0.09 mg/kg) and vincristine for each evaluable PDX. However, 12/47 (26%) mice treated at this dose (including all mice with ETP-4) experienced toxicity or excessive weight loss leading to death or euthanasia (**Supplementary Table S1**).

There was a distinct response profile for vincristine in PDXs depending on *ABCB1* expression (**Figure 2G-I**). Vincristine treatment reduced leukemia burden in the PB (minimum %huCD45⁺) in PDXs with low *ABCB1* expression (ETP-1, -61%; ETP-4, -100%; ETP-

5, -84%) but not high expression (ETP-2, +76%; ETP-3, +100%; ETP-6, +11%; **Figure 2G**). Using a linear mixed model (PDXs nested within *ABCB1* expression groups, **Figure 2H**), the estimated marginal mean difference in EFS (Δ EMM-EFS) between E7130 (0.09 mg/kg) and vincristine was significant in *ABCB1*-high PDXs (9.0 days, $p < 0.001$), but not *ABCB1*-low (0.2 days, $p = 1$). At 0.135 mg/kg, differences were significant in both groups ($p < 0.001$), although the *ABCB1*-high Δ EMM-EFS remained larger (22.7 days vs 11.9 days). Furthermore, vincristine efficacy (T/C) negatively correlated with *ABCB1* expression ($R = -0.79$, $p = 0.06$, **Figure 2I**). These findings suggest that E7130 remains effective regardless of *ABCB1* expression, overcoming the efflux-mediated resistance to vincristine. A detailed summary of results for the efficacy study is found in **Supplementary Table S1**.

In a recent Phase 1 dose-escalation study, the MTDs of E7130 were determined to be 300 $\mu\text{g}/\text{m}^2$ (2-week cycle) and 480 $\mu\text{g}/\text{m}^2$ (3-week cycle)¹⁰. In our efficacy study, human-equivalent doses were estimated to be 271 $\mu\text{g}/\text{m}^2$ (0.09 mg/kg) and 406 $\mu\text{g}/\text{m}^2$ (0.135 mg/kg) according to FDA guidelines¹⁵, noting our study administered E7130 weekly over 3 weeks to account for pharmacokinetic differences in mice. Although 0.135 mg/kg E7130 was not well tolerated in our mice, the main symptoms (eye inflammation and scabbing at the site of injection) were not reported as dose-limiting events in patients and appear to be tolerability issues specific to these mice. Regardless, even at the lower dose of 0.09 mg/kg, E7130 performed favorably against vincristine.

In summary, we identified elevated *ABCB1* expression in ETP ALL. E7130 demonstrated robust *in vivo* single agent activity against pediatric ETP ALL PDXs, prolonging survival beyond that of vincristine in PDXs with high *ABCB1* expression. These findings establish E7130 as a promising therapy to overcome multidrug resistance in ETP ALL and warrant further evaluation.

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TABLE 1

Summary of results for the *in vivo* efficacy study of E7130 and vincristine against ETP ALL PDXs, grouped by *ABCB1* expression. The group with ETP-4 treated with E7130 (0.135 mg/kg) was excluded due to drug toxicity. KM, Kaplan-Meier; EFS, event-free survival; T-C, Treatment minus Control; T/C, Treatment divided by Control; ORM, objective response measure; PD1/2, progressive disease 1/2; CR, complete response; MCR, maintained complete response; $\log_2(\text{TPM}+1)$, $\log_2$ -transformed transcripts-per-million reads plus one.

PDX	Treatment Group	KM med (days)	EFS T-C (days)	EFS T/C	p-value Gehan-Wilcoxon	ORM	<i>ABCB1</i> Expression $\log_2(\text{TPM}+1)$ [Group]
ETP-1	Vehicle control	12.3				PD1	0.3 [Low]
	E7130 (0.09 mg/kg)	29.9	17.6	2.4	< 0.001	PD2	
	E7130 (0.135 mg/kg)	43.0	30.7	3.5	< 0.001	CR	
	Vincristine (1 mg/kg)	33.6	21.3	2.7	< 0.001	PD2	
ETP-4	Vehicle control	12.1				PD1	0.0 [Low]
	E7130 (0.09 mg/kg)	53.3	41.2	4.4	< 0.001	MCR	
	E7130 (0.135 mg/kg)	-	-	-	-	-	
	Vincristine (1 mg/kg)	49.8	37.7	4.1	0.001	MCR	
ETP-5	Vehicle control	5.9				PD1	0.0 [Low]
	E7130 (0.09 mg/kg)	34.8	28.9	5.9	< 0.001	PD2	
	E7130 (0.135 mg/kg)	52.1	46.2	8.8	< 0.001	MCR	
	Vincristine (1 mg/kg)	39.1	33.2	6.6	< 0.001	CR	
ETP-2	Vehicle control	21.8				PD1	3.6 [High]
	E7130 (0.09 mg/kg)	39.3	17.5	1.8	< 0.001	PD1	
	E7130 (0.135 mg/kg)	63.7	41.9	2.9	< 0.001	MCR	
	Vincristine (1 mg/kg)	32.6	10.8	1.5	< 0.001	PD1	
ETP-3	Vehicle control	13.7				PD1	4.6 [High]
	E7130 (0.09 mg/kg)	24.2	10.5	1.8	0.039	PD1	
	E7130 (0.135 mg/kg)	31.7	18.0	2.3	0.007	PD2	
	Vincristine (1 mg/kg)	17.2	3.5	1.3	0.295	PD1	
ETP-6	Vehicle control	16.4				PD1	4.4 [High]
	E7130 (0.09 mg/kg)	48.4	32.0	3.0	< 0.001	CR	
	E7130 (0.135 mg/kg)	65.9	49.5	4.0	0.009	MCR	
	Vincristine (1 mg/kg)	34.4	18.0	2.1	0.001	PD2	

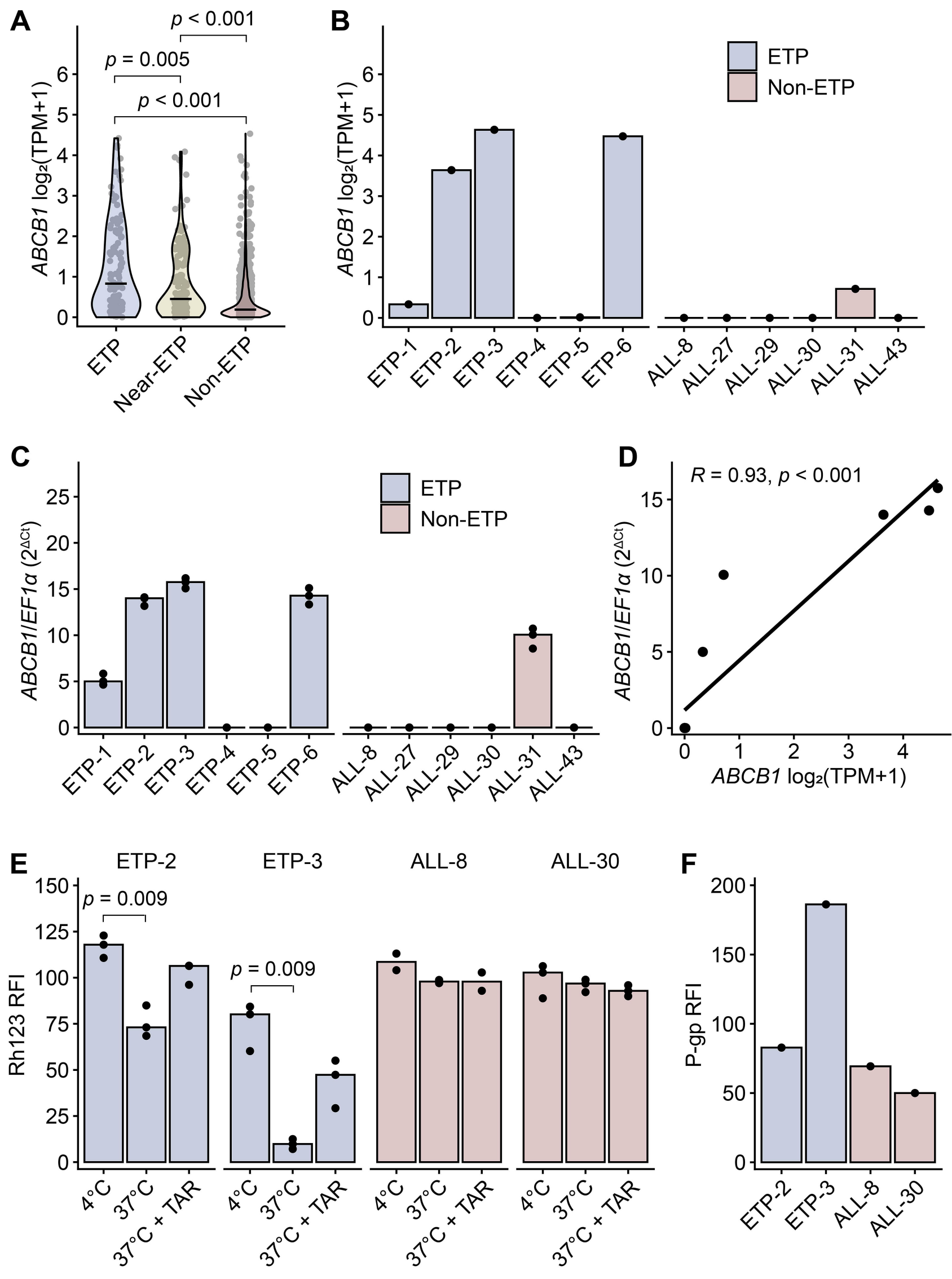
FIGURE LEGENDS

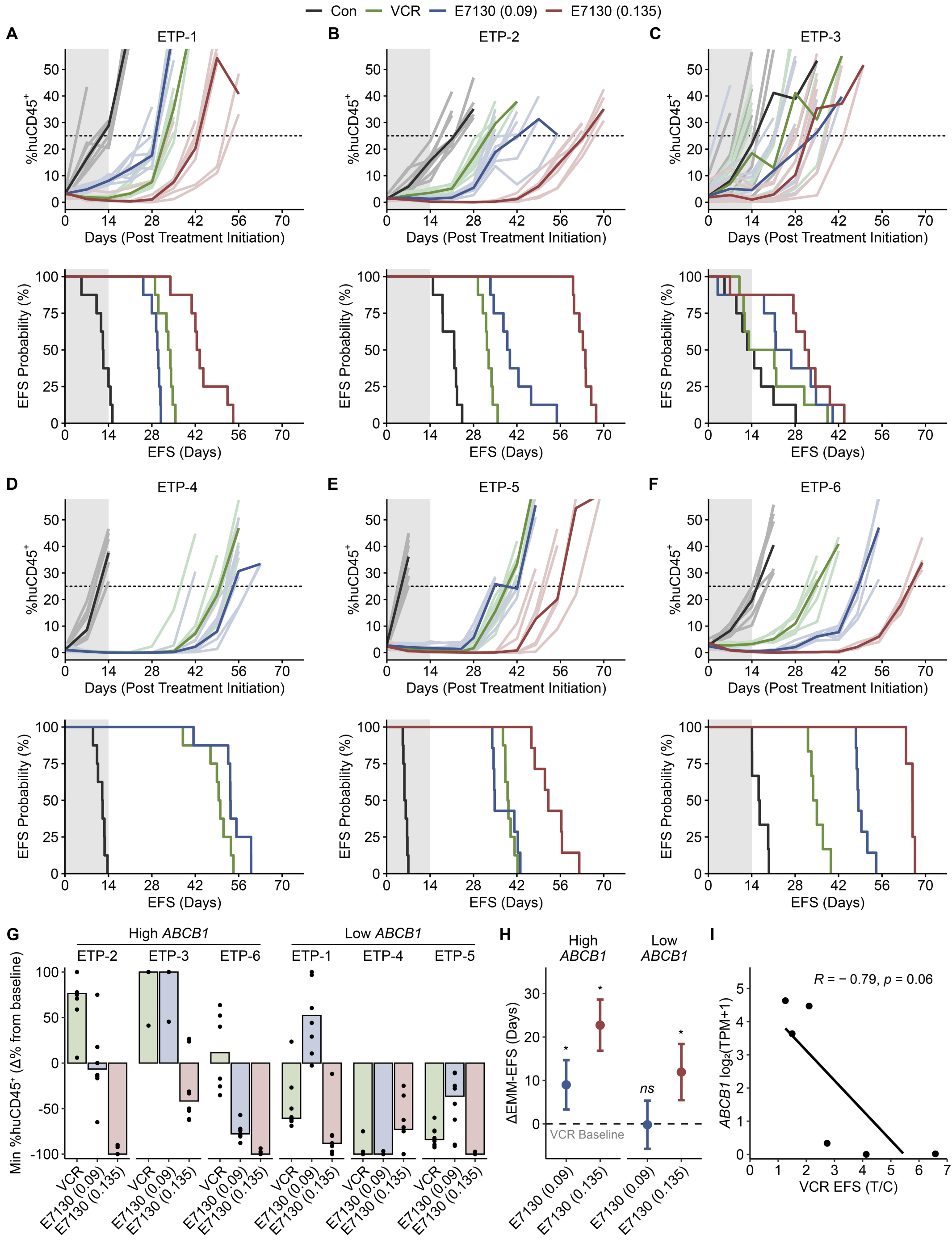
Figure 1. Expression of *ABCB1* in pediatric T-lineage ALL. (A) Violin plot of *ABCB1* expression (RNA-seq) in patient samples (Polonen study) with clinical ETP (n=110, blue), Near-ETP (n=168, yellow) and Non-ETP (n=863, red) subtypes (shown as $\log_2[\text{TPM}+1]$). Individual samples are represented as gray dots and horizontal lines show group medians. Pairwise comparisons were made using the Wilcoxon rank sum test with Holm-Bonferroni correction for multiple comparisons. (B) *ABCB1* mRNA expression in ETP (blue) and Non-ETP (red) PDXs, quantified using RNA-seq and represented as both dots and bars (n=1). (C) *ABCB1* expression in ETP (blue) and Non-ETP (red) PDXs, quantified by RT-qPCR and normalized against *EF1a*. Biological replicates are shown as black dots (n=3) and group medians are shown as bars. (D) Correlation of *ABCB1* expression using RNA-seq (x-axis) and RT-qPCR (y-axis). (E) Rh123 retention in ETP (ETP-2, ETP-3, blue) and Non-ETP (ALL-8, ALL-30, red) PDXs as measured by flow cytometry (Rh123 RFI) following low temperature incubation (4°C), biological temperature (37°C) or with the P-gp inhibitor tariquidar (37°C + TAR). All PDXs had 3 biological replicates per condition except ALL-8, where one replicate was excluded due to low cell viability. RFI was analyzed across conditions by separate one-way ANOVAs for each PDX followed by Tukey's HSD post-hoc test. P-values were subsequently corrected for multiple comparisons across models via the Holm-Bonferroni method. (F) Cell surface P-gp expression as measured by flow cytometry (RFI, n=1). R, Pearson's correlation coefficient; *p*, value.

Figure 2. Efficacy of E7130 and vincristine against a panel of ETP ALL PDXs *in vivo*.

Engrafted mice were treated with vehicle (Con, black), vincristine (VCR, green), or E7130 at 0.09 mg/kg (blue) or 0.135 mg/kg (red). Leukemia burden was measured by enumeration of human CD45⁺ cells (%huCD45⁺) in the PB over time, with treatment commencing when

the %huCD45⁺ was greater than a median of 1% for each PDX. (A-F) Engraftment (top) and Kaplan-Meier survival (bottom) plots for ETP-1 (A), ETP-2 (B), ETP-3 (C), ETP-4 (D), ETP-5 (E), ETP-6 (F). Faint lines show individual mice with bold lines indicating group median. The shaded area represents the treatment window, and the dotted horizontal line shows the threshold for an event (25% huCD45⁺ cells). (G) Waterfall plot showing the minimum %huCD45⁺ achieved by each mouse at any point post-treatment relative to baseline (shown as %), capped at +100%. Negative values indicate reduction in leukemic burden. PDXs are grouped according to high or low *ABCB1* expression. Bars show group medians and black dots show individual mice. (H) Estimated marginal mean difference in EFS ($\Delta\text{EMM-EFS}$; E7130 – VCR). Positive values indicate a survival benefit for E7130 over VCR. PDX models are grouped by high or low *ABCB1* expression. Error bars show 95% confidence interval. *, $p < 0.001$; ns, not significant. (I) Correlation of VCR EFS T/C and *ABCB1* expression ($\log_2[\text{TPM}+1]$). R, Pearson's correlation coefficient; p , p-value.





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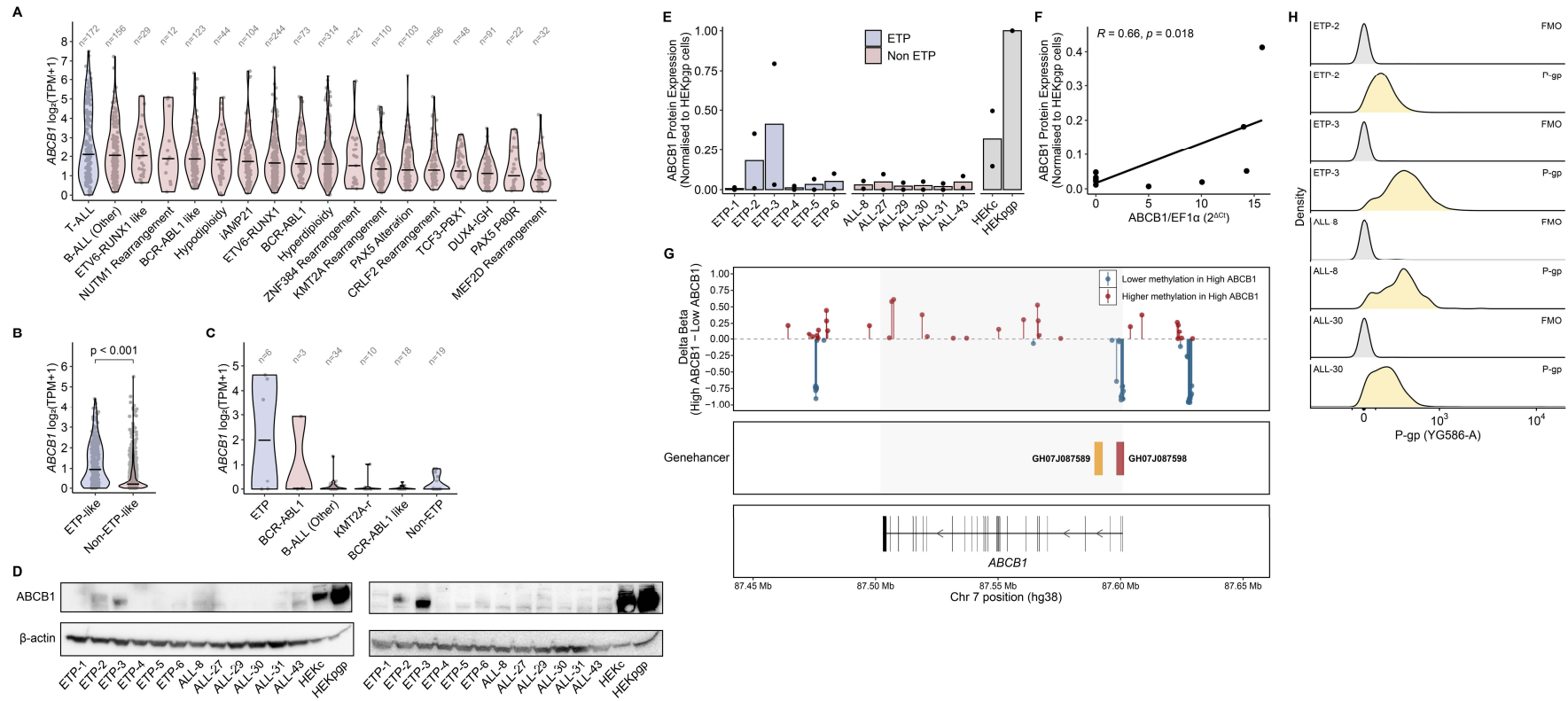
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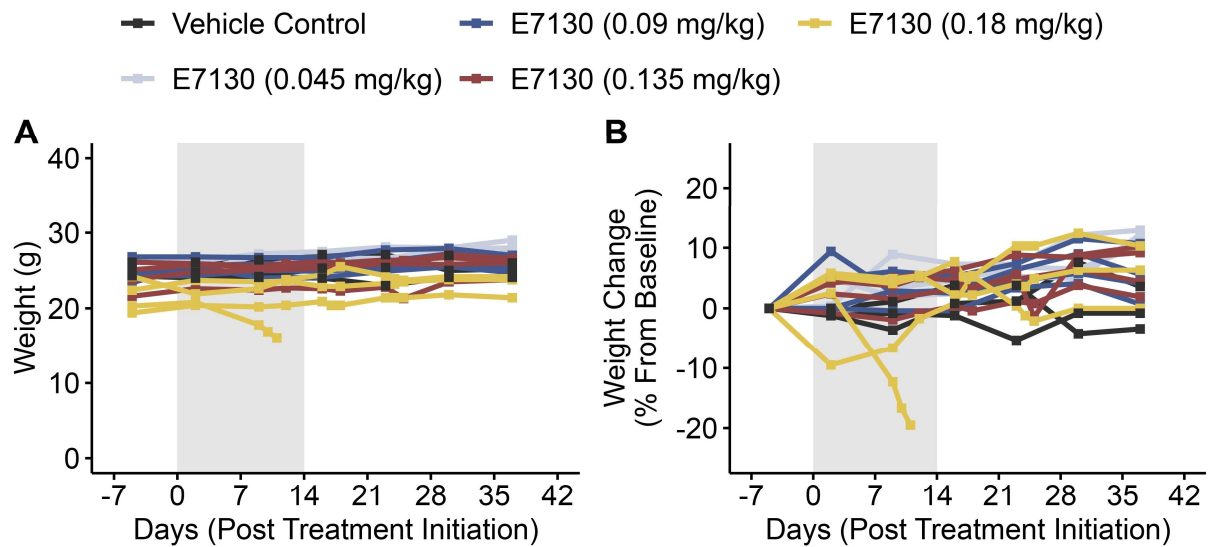
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SUPPLEMENTARY INFORMATION



Supplementary Figure S1. Expression of *ABCB1* in pediatric ALL patients and patient-derived xenografts (PDXs). **(A)** Violin plot of *ABCB1* expression (RNA-seq) in B- (red) and T-ALL (blue) patient samples from St. Jude Cloud, separated by subtypes. Samples were from the Real-Time Clinical Genomics, Genomes for Kids, Clinical Pilot and Pediatric Cancer Genome Project datasets, batch corrected using ComBat-seq. **(B)** Violin plot of *ABCB1* expression (RNA-seq) in T-ALL patient samples from the Polonen study, separated by ETP-like (n=240, blue) and Non-ETP-like (n=1095, red) status and compared using the Wilcoxon Rank Sum test. **(C)** Violin plot of *ABCB1* expression (RNA-seq) in B- (red) and T-ALL (blue) PDXs. KMT2A-r, KMT2A rearranged. **(A-C)** Individual samples are represented as gray dots and horizontal lines show group medians. **(D)** Immunoblots (two biological replicates, left and right) of *ABCB1* (P-gp) and the β -actin loading control. **(E)** Normalized quantification of protein expression from immunoblots, calculated as the signal of *ABCB1* relative to HEKpgp (engineered to overexpress P-gp) cells. Dots represent individual replicates of immunoblots and bars show the median for each PDX or cell line. **(F)** Correlation of *ABCB1* mRNA expression, determined by RT-qPCR (x-axis), and median *ABCB1* protein expression (y-axis), for ETP and Non-ETP ALL PDXs. **(G)** Delta of mean DNA methylation (Beta) between High- and Low-*ABCB1* expressing ETP-ALL PDXs (n=3 per group), measured using MethylationEPIC array. Includes Genehancer promoter/enhancer (red) and enhancer (yellow) sites near the *ABCB1*

locus. **(H)** Cell surface P-gp expression for PDXs used in the Rhodamine-123 assay, quantified using flow cytometry. Samples were stained with a P-gp antibody (yellow) or fluorescence minus one control (FMO, grey).



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	Control				E7130 (0.045 mg/kg)			E7130 (0.09 mg/kg)			E7130 (0.135 mg/kg)				E7130 (0.18 mg/kg)				Reference range
	w1	w2	w3	w4	w1	w2	w3	w1	w2	w3	w1	w2	w3	w4	w1	w2	w3	w4	
RBC ($\times 10^6/\mu\text{L}$)	8.56	9.38	9.36	8.00	9.26	9.14	10.64	8.97	8.77	9.77	8.80	8.05	9.21	9.10	8.61	7.92	7.04	8.74	6.36-11.82
HGB (g/L)	138	204	154	132	146	190	168	143	189	158	137	175	150	151	137	326	118	150	115-192
HCT (L/L)	0.43	0.47	0.49	0.40	0.48	0.48	0.54	0.46	0.45	0.49	0.45	0.42	0.49	0.47	0.44	0.41	0.37	0.47	0.38-0.57
PLT ($\times 10^3/\mu\text{L}$)	1326	340	622	664	1644	1830	624	1458	1859	616	1704	1731	613	751	1680	1490	612	720	519-1754
WBC ($\times 10^3/\mu\text{L}$)	3	ER	3	3.8	2.6	ER	4.4	2.3	ER	4.7	3.2	ER	5.4	4.3	4.5	ER	3.6	4.8	0.3-6.7

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	Control				E7130 (0.045 mg/kg)			E7130 (0.09 mg/kg)			E7130 (0.135 mg/kg)				E7130 (0.18 mg/kg)				Reference range
	w1	w2	w3	w4	w1	w2	w3	w1	w2	w3	w1	w2	w3	w4	w1	w2	w3	w4	
ALB (g/L)	42	43	ER	45	42	42	41	41	40	42	28	41	42	46	46	41	41	44	22-60
ALP (U/L)	71	66	ER	48	63	63	67	62	73	70	48	65	75	62	5	61	71	99	19-109
ALT (U/L)	29	33	ER	43	28	29	28	30	33	32	22	31	29	34	36	29	33	35	12-38
AMY (U/L)	952	720	ER	720	995	758	743	926	766	708	640	657	697	683	817	654	676	664	529
TBIL ($\mu\text{mol/L}$)	5	5	ER	4	5	4	5	5	7	5	5	5	5	5	ER	4	5	6	2-9
BUN (mmol/L)	5.9	7.3	ER	7.6	7.2	7.6	7.3	7.2	6.7	7.1	5.1	8.0	7.3	7.2	7.7	7.8	7.5	6.6	4.2-10.1
CA (mmol/L)	2.50	2.57	ER	2.63	2.55	2.57	2.54	2.50	2.50	2.53	1.80	2.60	2.58	2.67	2.85	2.56	2.55	2.52	1.54-3.26
PHOS (mmol/L)	2.09	1.73	ER	2.06	1.96	1.84	2.07	2.49	2.27	2.12	2.12	1.91	1.94	2.09	2.37	1.94	1.93	1.96	0.83-3.42
CRE (mmol/L)	18	18	ER	18	18	32	30	34	41	20	23	39	20	31	ER	24	22	22	8-35
GLU (mmol/L)	7.9	6.7	ER	6.6	8.1	6.7	6.9	7.7	6.0	7.2	5.6	7.6	6.4	6.7	7.3	5.3	6.0	6.8	2.3-11.3
Na ⁺ (mmol/L)	149	155	ER	158	148	150	151	149	152	150	149	153	152	156	ER	151	150	150	139-166
K ⁺ (mmol/L)	7.3	7.4	ER	8.5	7.4	7.1	7.8	7.6	6.3	7.9	6.9	7.2	7.5	7.7	ER	7.1	6.9	7.3	5.8-8.8
TP (g/L)	56	58	ER	60	57	58	57	55	56	57	41	58	59	61	65	58	56	59	36-69
GLOB (g/L)	14	16	ER	15	15	16	16	14	16	14	13	18	17	16	19	17	15	15	7-19

Supplementary Figure S2. Tolerability of E7130 in naïve NSG mice. Mice were treated intravenously with either vehicle control (black) or E7130 at 0.045 mg/kg (light gray), 0.09 mg/kg (blue), 0.135 mg/kg (red) or 0.18 mg/kg (yellow) once a week, for three weeks. Plots show weight (**A**) and the % weight change from baseline (**B**). Square dots show measured timepoints and the shaded area represents the treatment window. (**C**) Weekly hematology results show the median value of each treatment group. RBC, Red Blood Cells; HGB, Hemoglobin; HCT, Hematocrit; PLT, Platelets; WBC, White Blood Cells. (**D**) Weekly biochemistry results show the median of each treatment group. ALB, Albumin; ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; AMY, Amylase; TBIL, Total Bilirubin; BUN, Blood Urea Nitrogen; CA, Calcium; PHOS, Phosphate; CRE, Creatinine; GLU, Glucose; Na⁺, Sodium; K⁺, Potassium; TP, Total Protein; GLOB, Globulin. (**C-D**) Reference ranges were determined from >300 measurements of naïve, untreated NSG mice, mean $\pm$ 2SD. Highlighted (yellow) values are outside the reference range. w, Weeks post-treatment initiation; ER, Error in sample acquisition.

Supplementary Table S1. Detailed summary data for the *in vivo* E7130 and vincristine efficacy study. **N**, total number of mice entering experiment; **Nd**, number of mice experiencing toxic death; **Nx**, number of additional mice excluded from analysis; **Na**, number of mice in analysis; **Nev**, number of events; **KM med**, Kaplan-Meier estimate of median time-to-event (days); **EFS T–C**, difference in median time-to-event (days) between treatment and control groups; **EFS T/C**, relative difference in median time-to-event (days) between treatment and control groups; **minCD45**, minimum CD45; **minCD45 T/C**, relative difference in minimum CD45 between treatment and control groups; **ΔCD45/Baseline Median(IQR)**, median and interquartile range of relative CD45 change; **PD**, progressive disease, huCD45 ≥ 1% throughout study and > 25% at some point during study period (42 days); **PD1**, when PD and the mouse's time to event ≤ 200% the median time-to-event in control group; **PD2**, when PD but, additionally, time-to-event is > 200% of the median time-to-event in control group; **SD**, stable disease, huCD45 ≥ 1% but ≤ 25% throughout study period; **PR**, partial response, huCD45 < 1% at least once during the study period, but not CR; **CR**, huCD45 < 1% for at least 2 consecutive weekly readings during the study period; **MCR**, maintained complete response, huCD45 < 1% for at least 3 consecutive weekly readings at any time after treatment has been completed; **Resp rate**, response rate, percentage of mice having PR or better; **Med resp**, median response evaluation; *, All mice euthanized due to excessive weight loss (≥ 20%) before event was reached; **, One mouse euthanized due to excessive weight loss (≥ 20%) before event was reached; ***, One mouse euthanized due to excessive weight loss (≥ 20%) before event reached. Two mice were found dead from drug toxicity.

PDX Model	Grp	Animal Counts/Status					EFS Evaluation				%huCD45 evaluation					Response Evaluation								Toxicity		
		N	Nd	Nx	Na	Nev	KM med	EFS T-C	EFS T/C	Gehan-Wilcoxon (p-value)	Baseline mean±SD	Wilcoxon rank sum (p-value)	minCD45 mean±SD	minCD45 T/C	Wilcoxon rank sum (p-value)	ΔCD45/Baseline Median(IQR)	PD	PD1	PD2	SD	PR	CR	MCR	Resp rate	Med resp	Max Avg % Wgt Loss
ETP-1	Vehicle control	8	0	0	8	8	12.3				3.150±1.177		19.800±10.051			4.82(4.32,7.79)	8		0	0	0	0	0%	PD1		1.2%
	E7130 (0.09 mg/kg)	8	0	0	8	8	29.9	17.6	2.44	p < 0.001	3.062±1.006	p = 0.792	4.912±1.115	0.25	p < 0.001	0.52(0.24,1.08)	8	0	8	0	0	0	0	0%	PD2	0.0%
	E7130 (0.135 mg/kg)	8	0	0	8	8	43.0	30.7	3.50	p < 0.001	3.138±0.923	p = 0.958	0.700±1.222	0.04	p < 0.001	-0.88(-0.93,-0.80)	1	0	1	0	2	5	0	88%	CR	2.7%
	Vincristine (1 mg/kg)	8	0	0	8	8	33.6	21.3	2.74	p < 0.001	3.200±0.962	p = 1.000	1.525±0.410	0.08	p < 0.001	-0.61(-0.64,-0.44)	7	0	7	0	1	0	0	12%	PD2	6.5%
	E7130 (0.09 mg/kg) vs E7130 (0.135 mg/kg)							13.1	1.44	p < 0.001		p = 0.874		0.14	p = 0.001											
	E7130 (0.09 mg/kg) vs Vincristine (1 mg/kg)							3.7	1.12	p = 0.011		p = 1.000		0.31	p < 0.001											
	E7130 (0.135 mg/kg) vs Vincristine (1 mg/kg)							-9.3	0.78	p < 0.001		p = 0.916		2.18	p = 0.013											
ETP-2	Vehicle control	8	0	0	8	8	21.8				1.612±0.745		7.362±3.704			3.66(2.83,4.30)	8		0	0	0	0	0%	PD1		6.5%
	E7130 (0.09 mg/kg)	8	0	0	8	8	39.3	17.5	1.80	p < 0.001	1.412±0.662	p = 0.833	1.225±0.555	0.17	p < 0.001	-0.07(-0.15,0.04)	5	4	1	0	1	2	0	38%	PD1	0.0%
	E7130 (0.135 mg/kg)	8	0	0	8	8	63.7	41.9	2.92	p < 0.001	1.462±0.545	p = 0.833	0.025±0.046	0.00	p < 0.001	-1.00(-1.00,-0.98)	0	0	0	0	0	0	8	100%	MCR	17.6%
	Vincristine (1 mg/kg)	8	0	0	8	8	32.6	10.8	1.50	p < 0.001	1.325±0.618	p = 0.369	2.375±0.840	0.32	p = 0.002	0.76(0.68,0.96)	8	8	0	0	0	0	0	0%	PD1	12.7%

	E7130 (0.09 mg/kg) vs E7130 (0.135 mg/kg)								24.3	1.62	p < 0.001	p = 0.958			0.02	p < 0.001											
	E7130 (0.09 mg/kg) vs Vincristine (1 mg/kg)								-6.7	0.83	p = 0.002	p = 0.712			1.94	p = 0.010											
	E7130 (0.135 mg/kg) vs Vincristine (1 mg/kg)								-31.1	0.51	p < 0.001	p = 0.527			95.00	p < 0.001											
ETP-3	Vehicle control	8	0	0	8	8		13.7				3.200±3.461		10.738±10.858		2.46(2.23,3.12)	7	0	1	0	0	12%	PD1	3.4%			
	E7130 (0.09 mg/kg)	8	0	0	8	8		24.2	10.5	1.77	p = 0.039	4.062±5.284	p = 0.834	10.188±14.569	0.95	p = 0.636	1.14(1.00,1.73)	7	5	2	0	1	0	0	12%	PD1	0.0%
	E7130 (0.135 mg/kg)	8	0	0	8	8		31.7	18.0	2.32	p = 0.007	2.950±2.610	p = 1.000	2.212±2.101	0.21	p = 0.028	-0.42(-0.55,-0.18)	5	1	4	0	1	2	0	38%	PD2	5.0%
	Vincristine (1 mg/kg)	8	0	0	8	8		17.2	3.5	1.26	p = 0.295	2.413±1.985	p = 0.834	7.375±6.088	0.69	p = 0.798	2.00(1.46,2.65)	7	6	1	0	0	1	0	12%	PD1	4.9%
	E7130 (0.09 mg/kg) vs E7130 (0.135 mg/kg)								7.5	1.31	p = 0.293		p = 0.916			0.22	p = 0.141										
	E7130 (0.09 mg/kg) vs Vincristine (1 mg/kg)								-7.0	0.71	p = 0.294		p = 0.834			0.72	p = 0.916										
	E7130 (0.135 mg/kg) vs Vincristine (1 mg/kg)								-14.5	0.54	p = 0.067		p = 0.878			3.33	p = 0.115										
ETP-4	Vehicle control	8	0	0	8	8		12.1				1.100±0.504		10.325±5.135		8.53(7.05,10.12)	8	0	0	0	0	0%	PD1	0.0%			
	E7130 (0.09 mg/kg)	8	0	0	8	8		53.3	41.2	4.42	p < 0.001	1.212±0.931	p = 0.958	0.012±0.035	0.00	p < 0.001	-1.00(-1.00,-1.00)	0	0	0	0	0	1	7	100%	MCR	0.0%
	E7130 (0.135 mg/kg)*	8	8	0	0	0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13.8%	
	Vincristine (1 mg/kg)	8	0	0	8	8		49.8	37.7	4.13	p < 0.001	1.312±0.913	p = 0.875	0.028±0.070	0.00	p < 0.001	-1.00(-1.00,-0.99)	0	0	0	0	0	1	7	100%	MCR	6.6%
	E7130 (0.09 mg/kg) vs E7130 (0.135 mg/kg)								-46.3	0.13	p < 0.001		p = 1.000			29.00	p = 0.030										
	E7130 (0.09 mg/kg) vs Vincristine (1 mg/kg)								-3.6	0.93	p = 0.068		p = 0.792			2.21	p = 0.590										
	E7130 (0.135 mg/kg) vs Vincristine (1 mg/kg)								42.8	7.11	p < 0.001		p = 0.833			0.08	p = 0.056										
ETP-5	Vehicle control	8	0	0	8	8		5.9				2.900±1.269		35.812±8.049		11.93(8.55,17.07)	8	0	0	0	0	0%	PD1	2.2%			
	E7130 (0.09 mg/kg)	8	0	0	8	7		34.8	28.9	5.86	p < 0.001	2.600±1.136	p = 0.713	1.450±1.057	0.04	p < 0.001	-0.36(-0.69,-0.22)	4	0	4	1	0	3	0	38%	PD2	0.0%
	E7130 (0.135 mg/kg)**	8	1	0	7	7		52.1	46.2	8.77	p < 0.001	2.914±1.431	p = 0.954	0.014±0.038	0.00	p = 0.001	-1.00(-1.00,-1.00)	0	0	0	0	0	0	7	100%	MCR	4.9%
	Vincristine (1 mg/kg)	8	0	0	8	8		39.1	33.2	6.58	p < 0.001	2.562±1.093	p = 0.563	0.412±0.196	0.01	p < 0.001	-0.84(-0.90,-0.75)	0	0	0	0	1	7	0	100%	CR	3.2%
	E7130 (0.09 mg/kg) vs E7130 (0.135 mg/kg)								17.3	1.50	p < 0.001		p = 0.601			0.01	p = 0.001										
	E7130 (0.09 mg/kg) vs Vincristine (1 mg/kg)								4.3	1.12	p = 0.479		p = 0.958			0.28	p = 0.057										

	E7130 (0.135 mg/kg) vs Vincristine (1 mg/kg)									-13.0	0.75	p < 0.001					p = 0.779					28.87					p < 0.001				
ETP-6	Vehicle control	6	0	0	6	6	16.4				3.000±1.257				8.217±1.983				2.02(0.94,2.86)	6				0	0	0	0	0%	PD1	0.0%	
	E7130 (0.09 mg/kg)	7	0	0	7	7	48.4	32.0	2.95	p < 0.001	2.529±0.955	p = 0.615	0.557±0.127	0.07	p = 0.003	-0.78(-0.80,-0.73)	0	0	0	0	2	5	0	100%	CR	0.0%					
	E7130 (0.135 mg/kg)***	7	3	0	4	4	65.9	49.5	4.02	p = 0.009	3.325±1.170	p = 0.476	0.050±0.058	0.01	p = 0.014	-0.99(-1.00,-0.97)	0	0	0	0	0	0	4	100%	MCR	5.9%					
	Vincristine (1 mg/kg)	6	0	0	6	6	34.4	18.0	2.10	p = 0.001	2.600±1.359	p = 0.818	2.500±0.829	0.30	p = 0.002	0.11(-0.23,0.49)	6	1	5	0	0	0	0	0%	PD2	4.1%					
	E7130 (0.09 mg/kg) vs E7130 (0.135 mg/kg)							17.5	1.36	p = 0.008				p = 0.296				0.09				p = 0.010									
	E7130 (0.09 mg/kg) vs Vincristine (1 mg/kg)							-14.0	0.71	p < 0.001				p = 0.943				4.49				p = 0.003									
	E7130 (0.135 mg/kg) vs Vincristine (1 mg/kg)							-31.5	0.52	p = 0.009				p = 0.352				50.00				p = 0.014									