Abstract #3187

NCI Pediatric Preclinical Testing Consortium

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Pediatric Preclinical Testing Consortium evaluation of the menin inhibitor, VTP-50469, against xenograft models of MLL-rearranged infant acute lymphoblastic leukemia

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1. Introduction

Rearrangements involving the mixed lineage leukemia (MLL, KMT2A) gene (MLL-r) occur broadly in acute leukemia, in 80% of infant acute lymphoblastic leukemia (ALL) cases, and are associated with poor outcome.

- MLL encodes a histone methyl transferase, which binds DNA directly and regulates gene expression, including the HOX genes.
- Chromosomal translocations affecting the MLL gene at 11q23 involve > 90 translocation partners.
- Oncogenic MLL fusion proteins stimulate transcription elongation which leads to dysregulated transcription (Mutean et al., 2015)

Interaction of MLL with the nuclear protein menin drives transformation of cells expressing MLL fusion proteins.

- Menin is a ubiquitously expressed tumor suppressor protein that localizes to chromatin.
- Associates with the MLL-SET1 like histone methyltransferase complex and acts as a transcriptional regulator, modulating the expression of target genes including HOXA9 and MEIS1.
- Binds directly to both wildtype MLL-1 and MLL-1 fusion protein complexes (Caslini et al.,2007).
- The menin-MLL interaction acts as a critical oncogenic cofactor of MLL fusion proteins and together drive leukemogenesis.
- Menin contains a well defined groove that serves as a MLL binding site which can accommodate small molecule inhibitors.
- Blocking the menin-MLL interaction represents an attractive therapeutic strategy (Borkin et al.,2015)

VTP-50469 is a small molecule inhibitor with a high affinity for menin with a slow dissociation rate. It is a potent inhibitor of the menin-MLL interaction.

- The PPTC tested VTP-50469 to evaluate its *in vivo* efficacy against pre-clinical models of pediatric MLL-r ALL patient-derived xenografts (PDXs).

2. Study Methods

Drug Administration:

VTP-50469 was administered at a dose of 120mg/kg by oral gavage, twice daily for 28 days.

Study design and analysis:

- Pediatric MLL-ALL xenografts were established from direct patient explants via tail vein injection of NOD/SCID mice and modeled systemic disease (Richmond et al. 2015)
- Events were defined when the proportion of human CD45⁺ cells (%huCD45⁺) in the peripheral blood (PB) exceeded 25%, or the animal exhibited leukemia-related morbidity associated with high-level leukemic infiltration (>50%) of at least 2 major organs.
- The Kaplan-Meier method compared event-free survival (EFS) between treated and control groups.
- The objective response categories are as described by Houghton et al, 2007.
  - PD = progressive disease, <50% tumor regression throughout study and >25% tumor growth at end of study
  - PD1 = when PD and the %huCD45⁺ never drops below 1% and reaches event before the end of the study, with an EFS ≤200% of median control EFS.
  - PD2 = when PD but, additionally, the %huCD45⁺ never drops below 1% and reaches event before the end of the study, with an EFS >200% of median control EFS.
  - SD = stable disease, %huCD45⁺ in PB never <1% and the mouse never reaches event during the study period (42 days from start of drug treatment).
  - PR = partial response, %huCD45⁺ in PB <1% once during the study period.
  - CR = complete response, %huCD45⁺ in PB <1% for at least 2 consecutive weekly readings during the study period.
  - MCR = maintained complete response, %huCD45⁺ in PB <1% for at least 3 consecutive weekly readings at any time after treatment has been completed.
- Waterfall plots represent the percentage ratio of the minimal %huCD45⁺ cells in the PB at any point in time after treatment initiation relative to the %huCD45⁺ at Day 0.
- Leukemia infiltration in the femoral bone marrow was also assessed prior to treatment and at Day 28 post treatment initiation or at event (whichever occurred first) in control and VTP-50469 treated animals.

3. Results

***In vivo* efficacy of VTP-50469 against pediatric MLL-r ALL PDXs**

- VTP-50469 was well tolerated, with maximum average weight losses of 1.6-6.4% across treatment groups compared to 0-2.0% in vehicle control treated groups.
- VTP-50469 induced significant differences in EFS distribution compared to control in 6 of 8 of the evaluable MLLr-ALL PDXs (**Figure 1, Table 1**).
- VTP-50469 T-C values in MLLr-ALL PDXs ranged from 1.1 to 109.1 days (T/C 1.15-22.61), and Maintained Complete Responses (MCRs) were observed in 6 of 8 PDXs (**Table 1**).
- Two of 8 mice engrafted with an MLLr-ALL harboring the MLL-AFF1 (t4;11) translocation had not reached event 328 days following treatment initiation.
- A significant reduction ($p<0.0001$) in bone marrow infiltration at Day 28 was observed in 5 of 7 evaluable MLLr-ALL PDXs (see **Figure 2** for representative data, and **Table 1**).
- VTP-50469 at 30 mg/kg (4-fold lower than its maximum tolerated dose) also elicited MCRs in 8 of 8 mice engrafted with an MLL-AFF1 PDX (**Figure 3**).
- 52 of 60 evaluated mice treated with VTP-50469 exhibited a decrease in the proportion of leukemia cells in the peripheral blood from pre-treatment levels (**Figure 4**).
- The on-target activity of VTP-50469 was verified by its lack of efficacy against a Ph⁺-ALL PDX harbouring the *BCR-ABL1* translocation.

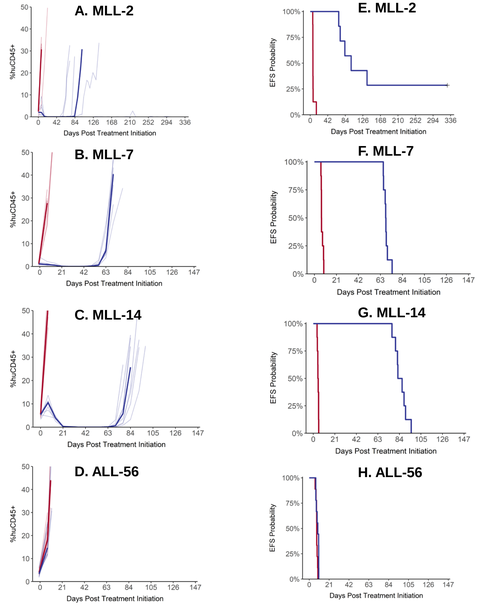


Figure 1. Responses of MLLr-ALL PDX lines to VTP-50469 *in vivo*. Engraftment plots of 3 MLL-r ALL PDXs **(A-C)** and 1 Ph⁺ ALL **(D).** Corresponding Kaplan-Meier survival curves of these 4 ALL PDXs **(E-H).** Red lines, control; blue lines, treated; bold lines, median of each group.

3. Results (continued)

Table 1. Responses of pediatric MLL-r ALL PDXs tested with VTP-50469 *in vivo*.

PDX	MLL Subtype; Translocation	N	Na	EFS T-C (Days)	EFS T/C	p-value	Min CD45	Median Response	Mean BM % huCD45+ (day*)		
									Control	VTP-50469	p-value
MLL-2	ALL t(4;11), MLL-AFF1	8	7	91.5	15.35	<0.001	0	MCR	99.58 (14)	6.28 (28)	<0.0001
MLL-3	ALL t(11;17), MLL-GAS7	8	8	59.4	8.15	0.006	10.53	MCR	95.15 (3)	56.6 (28)	ns
MLL-5	ALL t(10;11), MLL-MLLT10	6	6	1.2	1.25	0.001	29.2	PD1	99.6 (15)	81.7 (28)	0.0011
MLL-6	ALL t(11;19), MLL-ENL	8	8	109.1	22.61	<0.001	0.88	MCR	97.21 (12)	0 (28)	<0.0001
MLL-7	ALL t(4;11), MLL-AFF1	8	8	61.4	10.15	<0.001	0	MCR	98.33 (9)	0.83 (28)	<0.0001
MLL-8	ALL t(11;19), MLL-ENL	8	6	100	21.14	0.001	0	MCR	99.6 (27)	0.56 (28)	<0.0001
MLL-14	ALL t(11;19), MLL-ENL	8	8	79.9	18.19	<0.001	0.001	MCR	95.77 (12)	0.36 (28)	<0.0001
ALL-56	ALL t(9;22)(q34;q11·2), BCR-ABL1	9	9	1.1	1.15	0.196	16.82	PD1	82.5 (11)	83.2 (28)	ns

N, total number of mice entering experiment; **Na**, number of mice in analysis; **EFS T – C**, difference in median time-to-event (days) between T and 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; **Min CD45**, average minimum huCD45% for treated group; **Median response**, median response evaluation (see Methods for definitions); **BM**, bone marrow, *, days post treatment initiation on which BM samples were harvested.

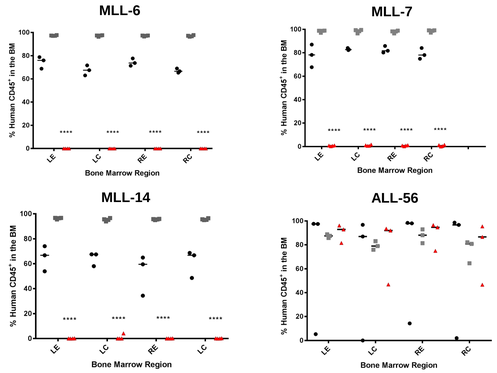


Figure 2. Effects of VTP-50469 on leukemia infiltration into the femoral BM of mice engrafted with ALL PDXs . The proportion of human leukemia cells in specific femoral bone marrow regions was assessed prior to treatment (Day 0, black circles), in vehicle control mice at event (grey squares), or in VTP-50469 -treated mice at Day 28 post treatment initiation (red triangles). Control mice were euthanized at event on the following days: MLL-6, Day 12; MLL-7, Day 9; MLL-14, Day 12; ALL-56, Day 56. Bone marrow regions: LC, left femur central region; LE, left femur endosteal region; RC, right femur central region; RE, right femur endosteal region. ****, p<0.0001 compared to control. Lines represent the median.

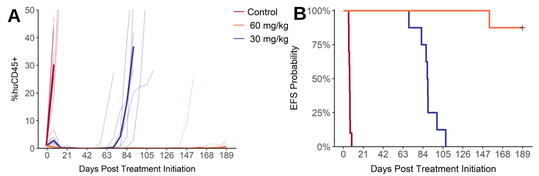
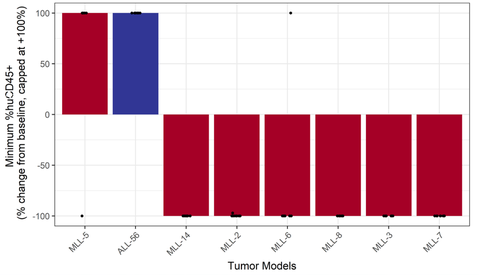


Figure 3. Dose response of MLL-2 PDX to VTP-50469 *in vivo* at 0.5 and 0.25 times the MTD. (A) Engraftment plots. **(B)** corresponding Kaplan-Meier survival curve

3. Results (continued)

Figure 4. Waterfall plot depicting the maximum decrease from baseline levels of human leukemia cells in the murine peripheral blood in response to VTP-50469 treatment. Each symbol represents a single mouse, bars represent the median of each PDX. The graph is capped at +100%.



4. Discussion and Conclusions

- VTP-50469 exerted profound *in vivo* efficacy against ALL PDXs derived from infants harboring *MLL-AFF1, MLL-GAS7,* and *MLL-ENL* translocations.
- VTP-50469 as a single agent was well tolerated by naive NSG mice up to 120 mg/kg (highest dose tested).
- A significant reduction in leukemia bone marrow infiltration was elicited by VTP-50469 in 6 of 7 evaluable MLL PDXs.
- The on-target activity of VTP-50469 was verified by its lack of efficacy against an ALL-56 PDX harbouring the BCR-ABL1 translocation
- VTP-50469 at 30 mg/kg elicited MCRs in 8 of 8 mice engrafted with an MLL-AFF1 PDX.
- VTP-50469 was also effective across a broad dose range, indicating that it may represent a novel treatment for MLL-r leukemia.

5. References

Borkin D, He S, Miao H, Kempinska K, Pollck J et al., Pharmacologic Inhibition of the Menin-MLL Interaction Blocks Progression of MLL Leukemia In Vivo, Cancer Cell 2015;27(4):589-602

Caslini C, Yang Z, El-Osta M, Milne AA, Slany RK, Hess JL. Interaction of MLL Amino Terminal Sequences with Menin Is Required for Transformation Cancer Res 2007;67(15):7275-83

Houghton PJ, Morton CL, Tucker C, Payne D, Favours E, Cole C, et al. The Pediatric Preclinical Testing Program: Description of Models and Early Testing Results. Pediatr Blood Cancer 2007;49(7):928-40.

Muntean AG and Hess JL, The Pathogenesis of Mixed-Lineage Leukemia. Annu Rev Pathol Mech Dis 2012;7:283–301

Richmond J, Evans K, High L, Mendomo A, Robbins A, Meyer C et al., Effective Targeting of the P53/MDM2 Axis in Preclinical Models of Infant *MLL*-Rearranged Acute Lymphoblastic Leukemia. Clin Cancer Res 2015;21(6):1395-1405

More Information

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