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NCI Pediatric Preclinical Testing Consortium

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The AKR1C3-activated prodrug OBI-3424 exerts profound *in vivo* efficacy against preclinical models of T-cell acute lymphoblastic leukemia (T-ALL); a Pediatric Preclinical Testing Consortium study

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1. Introduction

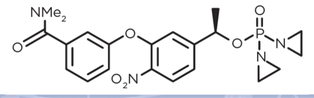
Aldo-keto reductase family 1 member C3 (AKR1C3) belongs to a superfamily of oxidoreductases that are broadly expressed in human tissues.

- Also known as 17β-hydroxysteroid dehydrogenase and prostaglandin F synthase.
- Catalyzes the reduction of aldehydes and ketones to their corresponding alcohols.
- Plays a role in the pre-receptor regulation of steroid hormones and prostaglandins.
- Expressed at high levels in several cancers including T-cell acute lymphoblastic leukemia (T-ALL).
- The discrepancy in expression of AKR1C3 between normal and tumor tissues presents an attractive strategy to develop AKR1C3-activated prodrugs as selective anticancer agents.

OBI-3424 (TH-3424) is a highly selective prodrug that is converted to a potent DNA alkylating agent in the presence of AKR1C3 and NADPH.

- OBI-3424 is a nitro-benzene prodrug of *N,N'*-bisethylenephosphoramidate (**Figure 1**).
- Our group previously showed that AKR1C3 expression is a biomarker of T-ALL sensitivity to the hypoxia-activated pre-prodrug PR-104, which is also activated under aerobic conditions by AKR1C3 (Manesh et al, 2015).
- The PPTC tested OBI-3424 to evaluate its *in vivo* efficacy against preclinical xenograft models of pediatric T-ALL.

Figure 1: Structure of OBI-3424



2. Study Methods

Drug Administration:

- OBI-3424 was tested at a dose of 2.5 mg/kg administered by intraperitoneal injection once weekly for 3 weeks. Since there is no murine equivalent of human AKR1C3, this dose of OBI-3424 was selected based on estimations to achieve exposure levels in mice that will be readily attainable in humans, and is well below the mouse maximum tolerated dose (MTD).

Study Design and Analysis:

- Pediatric acute lymphoblastic leukemia (ALL) xenografts were established from direct patient explants via tail vein injection of NOD/SCID or NSG mice, and modeled systemic disease (Suryani et al, 2014).
- Events were defined as the proportion of human CD45⁺ cells (%huCD45⁺) in the peripheral blood (PB) exceeding 25%, or the animal exhibiting 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 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 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 OBI-3424-treated animals.
- AKR1C3 mRNA expression was assessed by microarray analysis of gene expression and quantitative real-time RT-PCR. Protein levels were assessed by immunoblotting and expressed relative to actin. AKR1C3 enzymatic activity was measured by SN34037-sensitive coumberol formation in cell lysates (Jamieson et al, 2014).

3. Results

AKR1C3 expression in T-cell acute lymphoblastic leukemia

AKR1C3 mRNA and protein expression, as well as its enzymatic activity, had previously been measured in a large panel of patient-derived xenografts (PDXs) from pediatric patients presenting with B-cell precursor ALL (BCP-ALL) or T-ALL (Manesh et al, 2015).

- AKR1C3 mRNA expression was significantly more highly expressed in T-ALL compared with BCP-ALL PDXs (**Figure 2**).
- AKR1C3 enzymatic activity significantly correlated with its protein expression levels (**Figure 3**), and both were significantly higher in T-ALL compared with BCP-ALL PDXs.

***In vivo* efficacy of OBI-3424 against pediatric ALL PDXs**

- OBI-3424 was well tolerated, with only a 2.8% toxicity rate in the drug-treated groups.
- OBI-3424 was tested against a large panel of ALL PDXs, including a BCP-ALL PDX that had been lentivirally transduced to express AKR1C3 at levels equivalent to T-ALL xenografts (ALL-11/1C3, **Figure 4**, **Table 1**).
- Significant differences in EFS distribution between control and OBI-3424-treated groups were reported in 9 of 9 (100%) of the evaluable PDXs (**Table 1**, **Figure 5**), and objective responses were observed in 8 of 9 xenografts.
- T/C values in T-ALL PDXs ranged from 3.9 – 14 and were all higher than BCP-ALL PDXs (**Table 1**).
- A significant reduction in bone marrow infiltration at Day 28 was observed in 4 of 6 evaluable T-ALL PDXs (see **Figure 6** for representative data, and **Table 1**).
- Mice engrafted with lentivirally transduced ALL-11/1C3 cells exhibited greater T-C, T/C and Median Response values compared with ALL-11/EV, which highlights the importance of AKR1C3 in the *in vivo* responses of pediatric ALL PDXs to OBI-3424 (**Table 1**, **Figure 7**).
- 64 out of 67 evaluated mice treated with OBI-3424 exhibited a decrease in the proportion of leukemia cells in the peripheral blood from pre-treatment levels (**Figure 8**).

Figure 2. Expression of selected oxidoreductases in pediatric ALL PDXs. mRNA expression was quantified by Illumina microarray analysis (A), or by real-time quantitative RT-PCR analysis of AKR1C3 expression (B) (see Manesh et al, 2015).

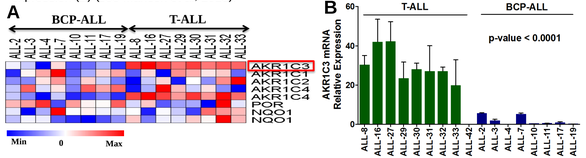


Figure 3. AKR1C3 protein expression and enzymatic activity in BCP-ALL and T-ALL PDXs. AKR1C3 protein was quantified by immunoblotting, and AKR1C3 enzymatic activity by fluorometric assay, as described previously (Jamieson et al, 2014; Manesh et al, 2015).

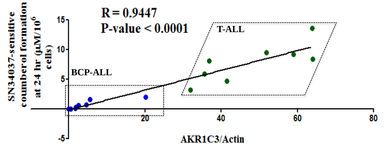
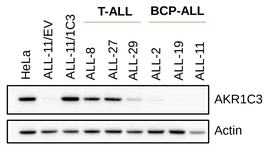


Figure 4. AKR1C3 protein expression in lentivirally transduced ALL-11 BCP-ALL PDX cells. Protein expression was assessed by immunoblotting, and compared with the HeLa cell line, as well as T-ALL and BCP-ALL xenografts. ALL-11/EV, empty vector transfected; ALL-11/1C3, AKR1C3 transfected (see Manesh et al, 2015).



3. Results (continued)

Table 1. Responses of pediatric ALL PDXs tested with OBI-3424 *in vivo*.

PDX	ALL Subtype, Status	N	Na	EFS T-C (Days)	EFS T/C	p-value	Min CD45	Median Response	Mean BM %huCD45+ (day*) Control	OBI-3424	p-value
ALL-8	T-ALL, DOD	8	8	67.3	8.74	< 0.001	0.0	MCR	97.9 (15)	0 (28)	<0.0001
ALL-27	T-ALL, DOD	8	4	70.6	11.4	0.008	0.0	MCR	49.8 (8,14)	0 (28)	NS
ALL-29	T-ALL, CR1	8	8	38.1	14.0	< 0.001	0.013	CR	92.3 (8)	10.7 (28)	<0.0001
ALL-30	T-ALL, DOD	8	7	42.1	7.52	< 0.001	0.0	MCR	95.7 (7)	0.25 (28)	<0.0001
ALL-31	T-ALL, DOD	8	8	77.8	7.50	< 0.001	0.0	MCR	98.7 (14,21)	0 (28)	<0.0001
ALL-32	T-ALL, CR2	8	8	17.1	3.92	< 0.001	1.1	SD	94.4 (8,15)	96.3 (22,28)	NS
ALL-28	B-ALL, CR1	8	8	58.2	2.46	< 0.001	0.0	MCR	69.5 (28)	0.69 (28)	<0.05
ALL-11/EV	B-ALL, CR1	8	8	21.1	2.54	< 0.001	0.13	CR	66.2 (19)	61.7 (28)	NS
ALL-11/1C3	B-ALL, CR1	8	8	47.2	3.53	< 0.001	0.0	MCR	65.8 (20, 25)	11.2 (28)	<0.0001

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; **DOD**, dead of disease; **CR1**, complete remission 1; **CR2**, complete remission 2; *, days post treatment initiation on which BM samples were harvested.

Figure 5. Responses of T- ALL PDX lines to OBI-3424 *in vivo*. Red lines, control; blue lines, treated; bold lines, median of each group; arrows, days of treatment.

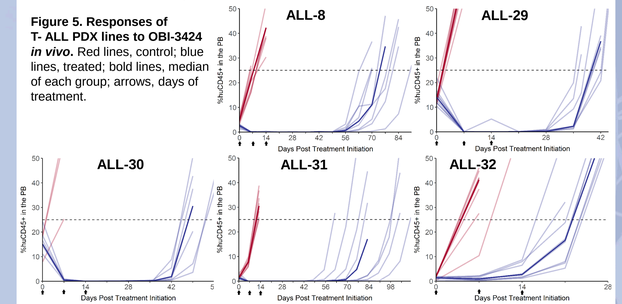


Figure 6. Effects of OBI-3424 on leukemia infiltration into the femoral BM of mice engrafted with T-ALL PDXs. The proportion of human leukemia cells in specific femoral bone marrow regions was assessed prior to treatment (Day 0, gray circles), in vehicle control mice at event (black squares), or in OBI-3424-treated mice at Day 28 post treatment initiation (red triangles). Control mice were euthanized at event on the following days: ALL-8, Day 15; ALL-29, Day 8; ALL-31, Days 14 and 21. 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.

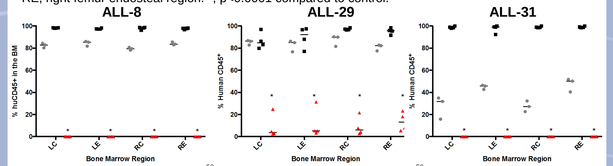
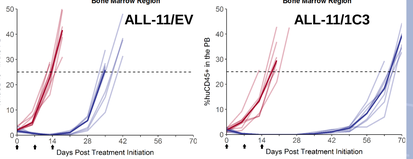
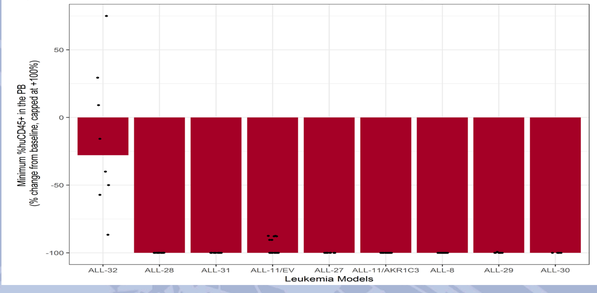


Figure 7. Responses of lentivirally transduced BCP-ALL ALL-11 PDX cells to OBI-3424 *in vivo*. Red lines, control; blue lines, treated; bold lines, median of each group; arrows, days of treatment.



3. Results (continued)

Figure 8. Waterfall plot depicting the maximum decrease from baseline levels of human leukemia cells in the murine peripheral blood in response to OBI-3424 treatment. Each symbol represents a single mouse, bars represent the median of each PDX. The graph is capped at +100%.



4. Conclusions and Discussion

- OBI-3424 exerted profound *in vivo* efficacy against a broad range of T-ALL PDXs derived predominantly from patients who experienced aggressive and fatal disease.
- A significant reduction in leukemia bone marrow infiltration was elicited by OBI-3424 in 4 of 6 evaluable T-ALL PDXs, which was apparent 14 days after completion of drug treatments.
- OBI-3424 treatment was well tolerated at a dose that is estimated to achieve exposure levels in mice that will be readily achievable in humans.
- The on-target effects of OBI-3424 were confirmed by its enhanced efficacy against a BCP-ALL PDX that had been lentivirally transduced to express AKR1C3 (ALL-11/1C3) at a level equivalent to T-ALL PDXs, compared to an empty vector control transduced sub-line (ALL-11/EV).
- OBI-3424 may represent a novel treatment for aggressive and chemoresistant T-ALL in an AKR1C3 biomarker-driven clinical trial.

5. References

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More Information

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