Abstract #4010

# A Single Mouse Trial Platform for Evaluation of Novel Agents in Acute Lymphoblastic Leukemia by the Pediatric Preclinical Testing Consortium

Richard B. Lock[1*], Kathryn Evans[1], Tara Pritchard[1], Cara Toscan[1], Chelsea Mayoh[1], Beverly Teicher[2], Raushan T. Kurmasheva[3], Peter J. Houghton[3] and Malcolm Smith[2].
[1]Children's Cancer Institute, Sydney, Australia; [2]National Cancer Institute, Bethesda, MD; [3]Greehey Children's Cancer Research Institute, San Antonio, TX.

NCI Pediatric Preclinical Testing Consortium
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## 1. Introduction

- The outcome for several high-risk subtypes of pediatric acute lymphoblastic leukemia (ALL) is extremely poor.
- Selecting the most active agents for clinical evaluation is critical as there are relatively few patients eligible for clinical trials.
- Conventional preclinical testing of novel agents is not sufficiently resourced to be able to encompass the vast heterogeneity between and within pediatric ALL subtypes.
- New approaches to preclinical testing in pediatric ALL are required.
- **A single mouse trial (SMT) platform using a large panel of pediatric ALL patient-derived xenografts (PDXs) allows:**
  - **preclinical assessment of novel agents on an almost clinical trial scale;**
  - **the broad heterogeneity of pediatric ALL to be approximated within a single experiment;**
  - **biomarker discovery and validation by using molecularly annotated PDXs.**

## 2. Study Methods

**Agent administration**:

Topotecan (Tpt), 0.6mg/kg IP daily × 5 × 2 weeks, repeated at 21 days.

Birinapant (Bpt), 15mg/kg IP every 3 days × 5.

**Study design and analysis:**

- 80 pediatric ALL PDXs broadly representative of all pediatric ALL subtypes were molecularly annotated by RNA-seq, exome-seq and DNA copy number analysis.
- 2 NSG mice/PDX were inoculated via tail vein injection and treatment began when the percentage of human CD45⁺ cells (%huCD45⁺) in the murine peripheral blood (PB) reached ≥ 1%.
- An event was defined as ≥ 25% huCD45⁺ cells in PB, or when the mouse exhibited leukemia-related morbidity associated with high-level leukemic infiltration (≥ 50% huCD45⁺) of at least 2 major organs.
- The Kaplan-Meier method was used to determine event-free survival (EFS) between control and treated groups.
- Treatment response was monitored using Objective Response Measures (ORM) modeled after stringent clinical criteria, which was assessed at Day 42 post treatment initiation (Houghton et al., 2007);
  - PD1 = progressive disease 1, %huCD45⁺ in PB never drops below 1% and event is reached by Day 14.
  - PD2 = progressive disease 2, %huCD45⁺ in PB never drops below 1% and event is reached after Day 14, but before Day 42.
  - SD = stable disease, %huCD45⁺ in PB never drops below 1% and event is not reached by Day 42.
  - PR = partial response, %huCD45⁺ in PB < 1% for 1 week and event is not reached by Day 42.
  - CR = complete response, %huCD45⁺ in PB < 1% for 2 consecutive weeks and event is not reached by Day 42.
  - MCR = maintained complete response, %huCD45⁺ in PB < 1% for at least 3 consecutive weeks after treatment completion and event is not reached by Day 42.
- Waterfall plots represent the ratio of the minimal %huCD45⁺ in the PB at any point after treatment initiation relative to the %huCD45⁺ at Day 0.
- PDX authenticity was verified using a 60-allele SNP array at both inoculation and at event (El-Hoss et al., 2016).

## 3. Results

### Retrospective Analysis of Single Mouse Data

- Retrospective analysis of >700 randomly selected mice from agents previously tested by our group (Jones et al., 2016) showed that the single mouse results predicted the overall group response from conventional testing 73.9% of the time (**Figure 1**).
- This increased to 85.8% if a deviation of ± one objective response measure was allowed (**Figure 1**).
- Historically, two major sources of exclusion from an experiment were bad cell source (PDX stocks contaminated with mouse thymoma) and tumor (mouse origin) (**Figure 2**).
- Elimination of bad cell source and mouse tumors by using the NSG mouse strain could increase the single mouse prediction of overall group response to > 90%.

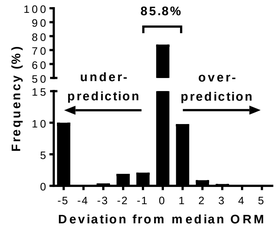


**Figure 1. Concordance within conventional testing.** The ORM of randomly selected mice was compared to the median ORM of their cohort. Single mice scored a 0 if their ORM matched their cohort median ORM. Single mice scored a +1 or -1 if their ORM was one category better or worse (respectively) than their cohort median ORM. Single mice that were excluded were assigned a -5 deviation score.

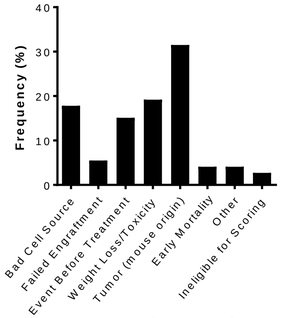


**Figure 2. Frequency and source of exclusion of single mice.** Single mice that were assigned a -5 deviation from their cohort median ORM in Figure 1.

### SMT Pilot Study with Birinapant and Topotecan

- SMT results were achieved for 72 (90.0%) and 71 (88.8%) of the intended 80 mice for birinapant and topotecan, respectively.
- Waterfall plots revealed that 30/72 (41.7%) and 60/71 (84.5%) of PDXs achieved regressions in response to birinapant and topotecan, respectively (**Figure 3**). Distinctive activity profiles were identified for each agent.
- Comparing historical ORMs from conventional drug testing performed by the PPTC with SMT results showed high concordance for both birinapant (r = 0.804, p < 0.0001, n = 17) and topotecan (r = 0.904, p = 0.0143, n = 7) (**Table 1**).

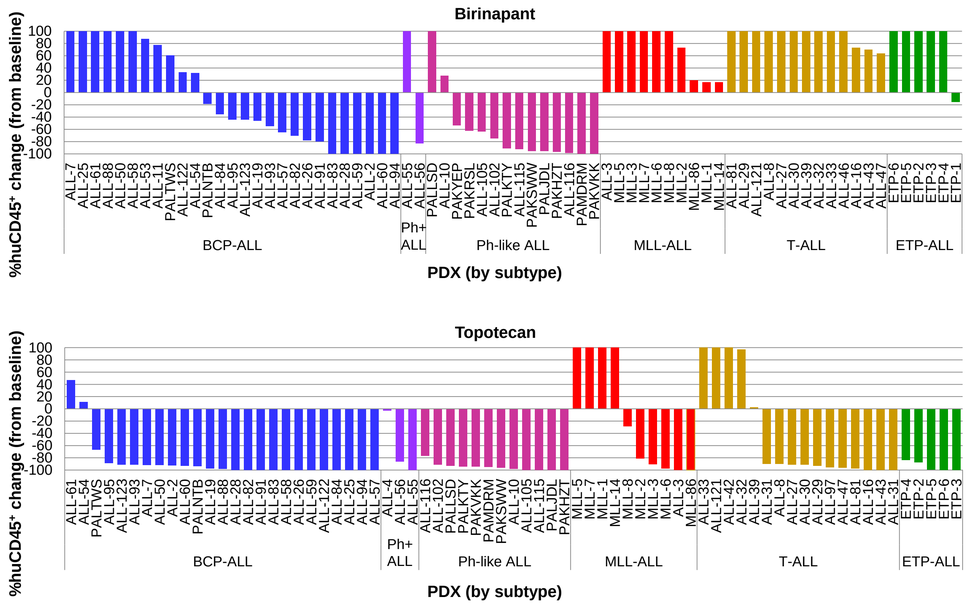


**Figure 3. Waterfall plots depicting the maximum decrease from baseline (Day 0) levels of huCD45⁺ cells in PB.** Birinapant treated (top) and topotecan treated (bottom). Each bar represents a single mouse. The graph is capped at ± 100%

**Table 1. Comparison of SMT and historical data.** Median ORM of conventional testing versus ORM of a single mouse from SMT for available PDXs for birinapant (top) and topotecan (bottom).

| Birinapant | Conventional 30 mg/kg | SMT 15 mg/kg |
|---|---|---|
| ALL-2 | MCR | MCR |
| ALL-7 | PD1 | PD1 |
| ALL-19 | SD | PD1 |
| ALL-50 | PD2 | PD1 |
| ALL-54 | PR | PD2 |
| ALL-55 | PD2 | PD2 |
| PALNTB | CR | PD2 |
| PAKHZT | MCR | CR |
| PAKRSL | CR | PD2 |
| PALLSD | CR | PD2 |
| PAMDRM | MCR | MCR |
| PALJDL | CR | PR |
| ALL-10 | CR | PD2 |
| MLL-5 | PD2 | PD2 |
| MLL-6 | PD2 | PD2 |
| MLL-7 | PD2 | PD1 |
| MLL-14 | CR | PD2 |
| # Mice | ~304 | 17 |
| | r = 0.804 | p < 0.0001 |

| Topotecan | Conventional 0.6mg/kg | SMT 0.6mg/kg |
|---|---|---|
| ALL-2 | MCR | MCR |
| ALL-3 | CR | MCR |
| ALL-4 | PD2 | PD2 |
| ALL-7 | PR | CR |
| ALL-8 | CR | CR |
| ALL-16 | MCR | MCR |
| ALL-19 | PR | PR |
| # Mice | ~128 | 7 |
| | r = 0.904 | p = 0.0143 |

## 3. Results (continued)

### Gene Expression Signatures Associated with *In Vivo* Response

- Analysis of divergent responses observed within the BCP-ALL subtype to birinapant and the within the MLL-ALL subtype to topotecan, revealed unique gene expression signatures that distinguished between *in vivo* response (**Figure 4**).

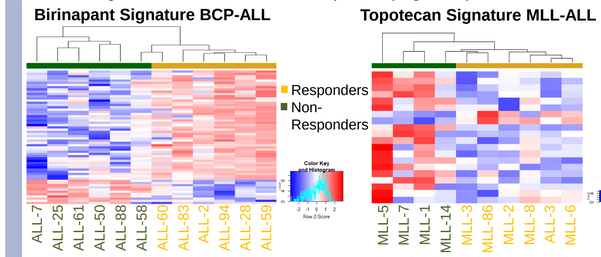


**Figure 4. Gene expression signatures associated with birinapant and topotecan sensitivity in BCP-ALL and MLL-ALL, respectively.**
(Left) A 61 gene signature (rows) separated the basal gene expression profiles of 12 BCP-ALL PDXs (columns) based on their *in vivo* response to birinapant. (Right) A 20 gene signature (rows) separated the basal gene expression profiles of 10 MLL-ALL PDXs (columns) based on their *in vivo* response to topotecan.

## 4. Discussion and Conclusions

- SMTs provide an accurate and cost-effective platform for preclinical testing of novel agents on an almost clinical trial scale.
- With the elimination of PDX stocks contaminated by thymoma and by using NSG mice instead of the historically used NOD/SCID strain (which has a propensity to develop thymoma), the success rate of SMTs is predicted to be > 90%.
- SMTs can almost encompass the heterogeneity of pediatric ALL in a single experiment.
- SMTs have the power to identify candidate molecular biomarkers or gene expression signatures associated with *in vivo* response to established and novel agents in pediatric ALL, when combined with molecularly annotated PDXs. Further work would be needed to validate these biomarkers.

## 5. References

- Houghton PJ, et al. The Pediatric Preclinical Testing Program: description of models and early testing results. Pediatr Blood Cancer; 2007;49:928-40.
- El-Hoss J, et al. A single nucleotide polymorphism genotyping platform for the authentication of patient derived xenografts. Oncotarget; 2016;7:60475-90.
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### More Information

***Corresponding author**: Richard B. Lock, PhD **Email:** rlock@ccia.org.au

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