A Single Mouse Trial Platform for Evaluation of Novel Agents in Acute Lymphoblastic Leukemia by the 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
- Study 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 mortality 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).
  - PDI = progressive disease 1, %huCD45+ in PB never drops below 1% and event is not reached by Day 42.
  - PDI = 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.
  - CR = complete response, %huCD45+ in PB = 1% for 2 consecutive weeks and event is not reached by Day 42.
  - MCRI = 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

3.1 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 ≥1 objective response measure was allowed (Figure 1). (A Single Mouse Trial Platform for Evaluation of Novel Agents in Acute Lymphoblastic Leukemia (ALL) is extremely poor.
- 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 high-quality NSG mouse strain could increase the single mouse prediction of overall group response to > 90%.

3.2 SMT Pilot Study with Birinapant and Topotecan
- SMT results achieved for 72 (90.0%) and 71 (88.8%) of the intended 80 mice for birinapant and topotecan, respectively.
- Waterfall plots revealed that 30/32 (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 ORMVs 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).

3.3 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).

4.4 Discussion and Conclusions
- SMTs can almost encompass the heterogeneity of pediatric ALL in 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;
  - biomarker discovery and validation by using molecularly annotated PDXs.
- 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).

5. References

More Information
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