In vitro Support of Primary Leukemia Cells

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Background

No *in vitro* method for culturing primary leukemia cells currently exists. Thus, study of primary leukemia biology requires immunodeficient mouse models, which is costly and time consuming. Identifying a method to grow and maintain primary leukemia cells *in vitro* would allow biological and molecular assays to facilitate bench to bedside translation. Moreover, culturing primary leukemia cells will help capture the heterogeneity of these cancers, a distinct advantage over commercial cell lines.

Figure 1. Creation of PDX models using serial transplantation via intratibial injection.

Recently, Dr. Satake's industry collaborator group at Thermo Fisher developed a culture medium, using Human Plasma-like Medium (HPLM) and supplement cocktail, to support normal hematopoietic stem cells (HSCs). Because leukemia initiates from hematopoietic precursors which could mimic HSCs, we tested whether Thermo Fisher's growth medium compared with standard RPMI medium could support growth of primary B-ALL and T-ALL cells *in vitro.*

Objective

Identify media conditions to support primary B- and T-ALL cells *in vitro*.

Methods

(1) Creation of patient-derived xenograft (PDX) models (Figure 1)
Intratibial injection of 2 million patient-derived ALL cells in immunodeficient NSG mice. After mice succumbed to leukemia, ALL cells were harvested from bone marrow and spleen.

(2) Confirmation of human leukemia cells
Fresh PDX ALL cells harvested from bone marrow were stained with May-Giemsa. Phenotype (B- or T-ALL) was determined by immunocytochemistry using CD19 or CD2 surface markers, respectively.

(3) Preparation of supplemented HPLM
Human plasma-like medium (HPLM) was mixed with stem cell factor, thrombopoietin, FLT-3, IL-6, IL-3, and Thermo FIsher's proprietary supplemental cocktail according to the manufacturer's guidelines.

(4) Culture of PDX ALL cells
Plated 50,000 cells in 100uL media (HPLM or RPMI) in 96-well plate in triplicate and incubated at 37ºC with 5% CO_2. Counted live cells using trypan blue every 3 days for 15 days. Six B-ALL and 4 T-ALL samples were tested. Of these, three samples were focused on: s90 (B-ALL), s88 (T-ALL), and s116 (T-ALL). Test conditions were repeated up to 9 times.

Results

(1) Confirmation of phenotype of engrafted leukemia cells (Figure 2)
Morphology of harvested cells stained with May-Giemsa is consistent with human lymphoblasts.

Results (cont.)

Immunocytochemistry confirmed that 85-97% of these cells were human ALL.

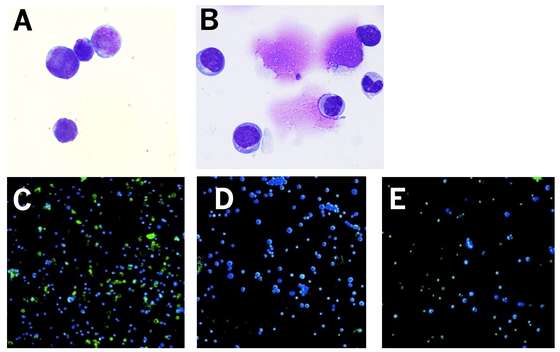

Figure 2. (A-B) B-ALL (A) and T-ALL (B) PDX lymphoblasts (May-Giemsa). (C) CD19 positive B-ALL cells, s90. (D-E) CD2 positive T-ALL cells, s88 (D) and s116 (E). CD2 and CD19 (green), DAPI (blue).

(2) Patient-derived ALL cells were successfully maintained in different growth conditions (Figure 3)

- PDX B-ALL cells from bone marrow but not spleen remained viable, suggesting enrichment of leukemia-initiating cells in this niche (**Fig 3A**).
- RPMI maintained s90 (B-ALL) with greater viability than HPLM for at least 15 days (**Fig 3A**) and supported s88 and s116 (T-ALL) for 6 days **(Fig 3B-C).**
- HPLM expanded and maintained s116 (T-ALL) for at least 15 days **(Fig 3C)** and some up to 30 days (data not shown).

Results (cont.)

- B-ALL and T-ALL are heterogeneous and require different *in vitro* growth conditions.

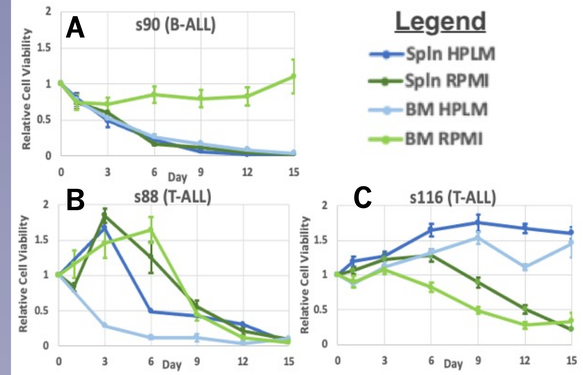

Figure 3. Cell viability relative to Day 0 cell counts. PDX B- and T-ALL samples harvested from spleen (Spln) and bone marrow (BM) were grown in supplemented HPLM or RPMI 1640. Standard error bars are shown.

Summary

Summary: Patient-derived B-ALL and T-ALL cells can be maintained *in vitro* for up to 15 days and are heterogeneous in their growth requirements.
Implications: Potential for performing *in vitro* assays on primary ALL cells, for instance lentiviral transduction, MTT/MTS assays, and drug screening for personalized treatment.

Acknowledgements

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