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A Genomics Driven Human Induced Pluripotent Stem Cell Model of Infant ALL – Updates on Hematopoietic Differentiation

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Introduction

- Acute lymphoblastic leukemia in infants (iALL) is a high-risk subtype of childhood leukemia, with poor survival outcomes despite intensive therapies
- Rearrangement of *KMT2A* (*KMT2A*-r) occurs in 70% of cases and is associated with refractoriness to therapy, early relapse, and rapid leukemia progression
- *KMT2A*-r generates a driver fusion oncogene, most commonly *KMT2A::AFF1* in iALL, which leads to epigenetic dysregulation of target gene transcription
- Little is known regarding how *KMT2A*-r subverts early hematopoiesis or drives the severe disease phenotype
- Research into this rare disease has been hindered by a lack of representative models
- **To understand the role of the developmental state of the cell of origin in iALL, we have created a highly controlled induced pluripotent stem (iPS) cell model system of *KMT2A::AFF1* leukemia**

Experimental Design

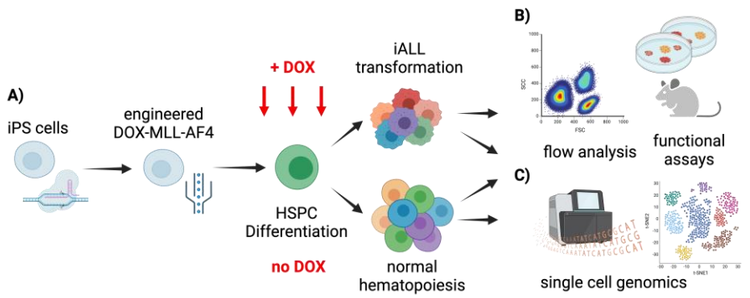

FIGURE 1. Project overview: a genomics driven iPS cell model of iALL. A) We engineered human iPS cell lines to express *KMT2A::Aff1* under doxycycline control via CRISPR gene editing technology. **B)** Directed differentiation was used to produce functional human hematopoietic stem and progenitor (HSPCs) from iPS cells confirmed by functional analysis. **C)** Single cell genomics will reveal the underlying mechanisms driving aggressive iALL based developmental stage of *KMT2A::Aff1* transformation.

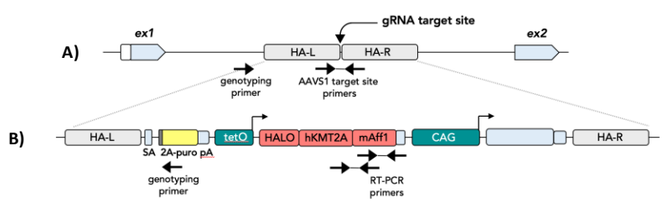

FIGURE 2. Doxycycline-regulated *KMT2A-Aff1* expression human iPS cells. A) AAVS 'safe harbor' locus used for targeting transgenes in iPS cells via CRISPR-mediated homology directed repair. **B)** Targeting vector for introduction of *KMT2A-Aff1* fusion coding sequence regulated by tet-responsive elements.

iPS Cell Differentiation

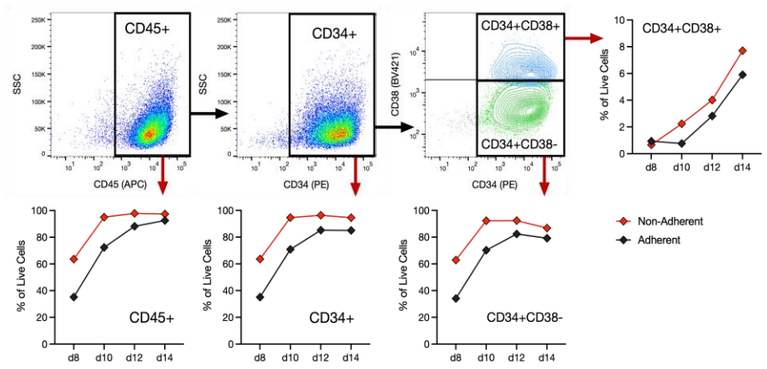

FIGURE 3. Flow cytometry analysis of directed differentiation to hematopoietic progenitors. Results demonstrate enrichment of CD45+CD34+ cells by day 10 of differentiation, especially for the non-adherent cells.

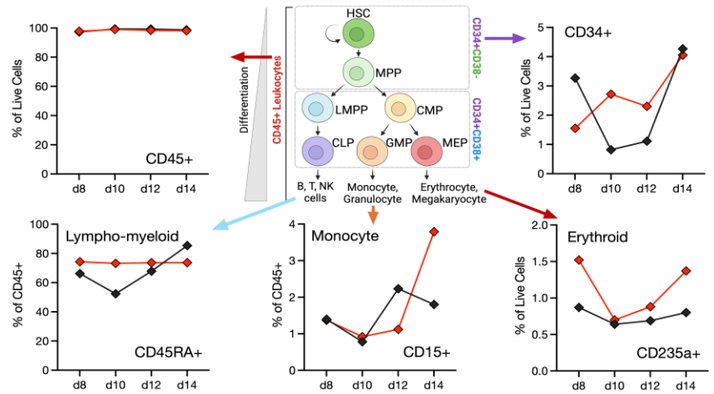

FIGURE 4. Flow cytometry analysis of colony forming unit assays. 14 days post-methylcellulose culture there was a functional enrichment in lympho-myeloid differentiated cells, as well as formation of monocyte and erythroid colonies.

Conclusions

- Utilizing directed differentiation, we have produced functional HSPCs from iPS cells
- The HPSCs produced have multilineage differentiation capacity based on flow cytometry and single cell RNA sequencing
- This model recapitulates hematopoietic ontogeny, with the ability to control expression of *KMT2A::AFF1* at specific developmental stages
- Next steps – directed differentiation of HPSCs to lymphoid lineage and doxycycline induction of differentiated cells
- Our iPS cell based iALL model system provides the opportunity to investigate a range of critical and outstanding questions of iALL disease initiation, progression, and treatment.

Single Cell Sequencing

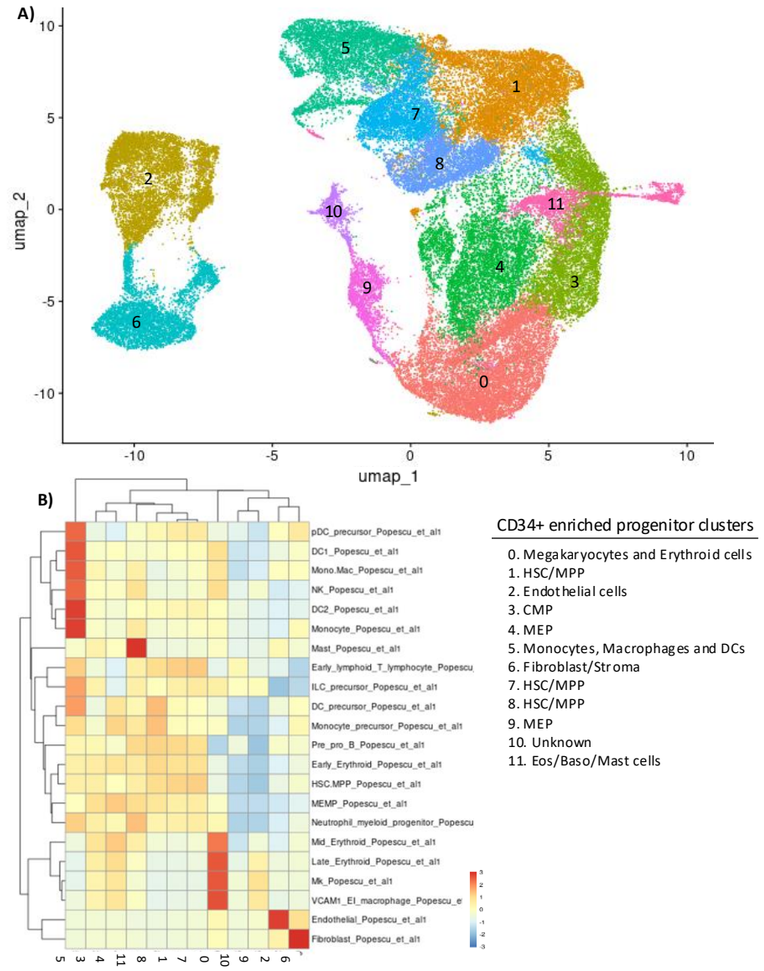

FIGURE 5. Single cell RNA sequencing of hematopoietic stem/progenitor cells from day 12 of embryoid body differentiation method. A) UMAP dimensionality reduction and clustering with corresponding cell types. **B)** Heatmap showing gene expression scores for each cluster aided in cluster identification. The x-axis represents clusters as numbered in Figure 5A. The y-axis represents a list of hematopoietic lineage-specific gene sets from Popescu et al 2019.

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