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A Genomics Driven Induced Pluripotent Stem Cell Model of Infant Acute Lymphoblastic Leukemia - Early Results

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A Genomics Driven Human Induced Pluripotent Stem Cell Model of Infant Acute Lymphoblastic Leukemia – Early Results

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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
- **In an effort 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

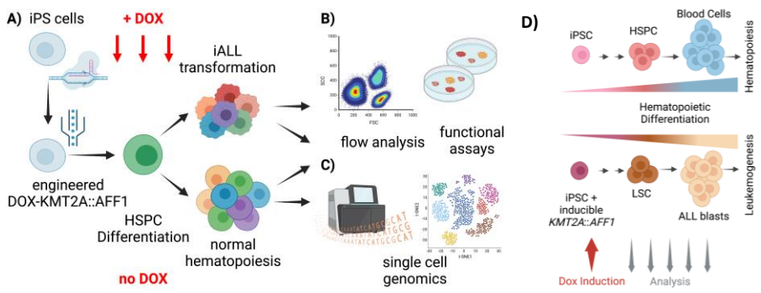

FIGURE 1. Project overview: a genomics driven iPS cell model of iALL. A) We engineered human iPS cell lines to express *KMT2A::Aff1* under doxycycline control via CRISPR gene editing technology. **B)** Directed differentiation was used to produce functional human hematopoietic stem and progenitor (HSPCs) 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. **D)** Schematic summary of differentiation and analysis

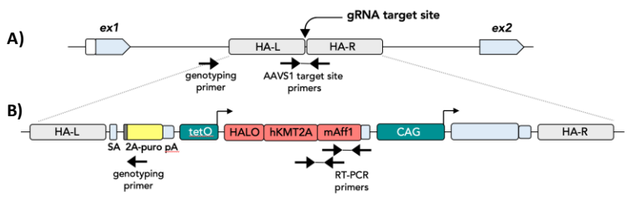

FIGURE 2. Doxycycline-regulated *KMT2A-Aff1* expression human iPS cells. A) AAVS 'safe harbor' locus used for targeting transgenes in iPS cells via CRISPR-mediated homology directed repair. **B)** Targeting vector for introduction of *KMT2A-Aff1* fusion coding sequence regulated by tet-responsive elements.

iPS Cell Differentiation

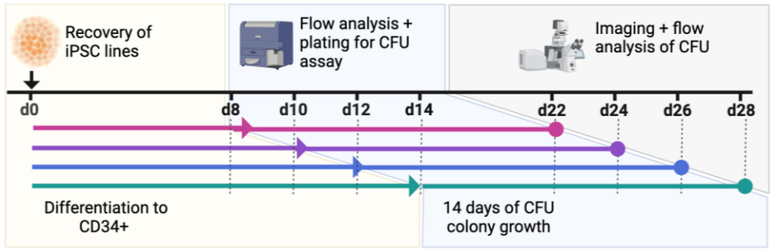

FIGURE 3. Experimental design schema

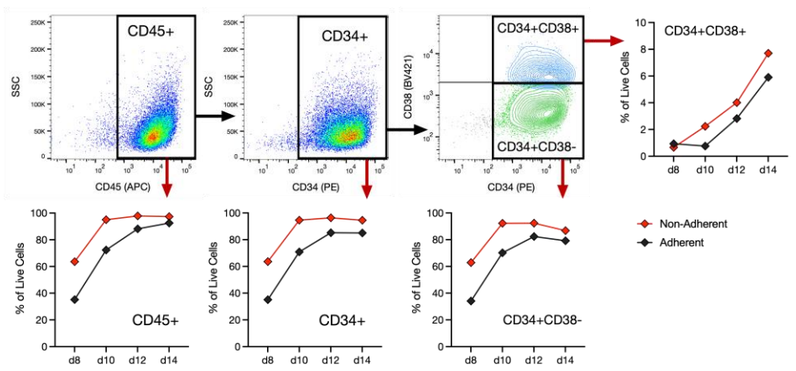

FIGURE 4. Flow cytometry analysis of directed differentiation to hematopoietic progenitors. Results demonstrate enrichment of CD45+CD34+ cells by day 10 of differentiation, especially for the non-adherent cells.

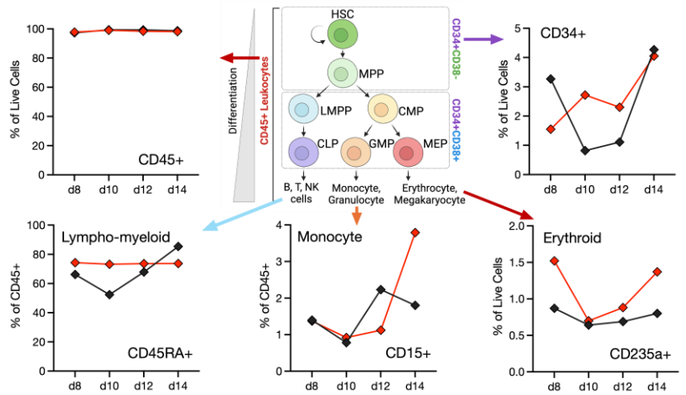

FIGURE 5. Flow cytometry analysis of colony forming unit assays. 14 days post-methylcellulose culture there was a functional enrichment in lympho-myeloid differentiated cells, as well as formation of monocyte and erythroid colonies.

Single Cell Sequencing

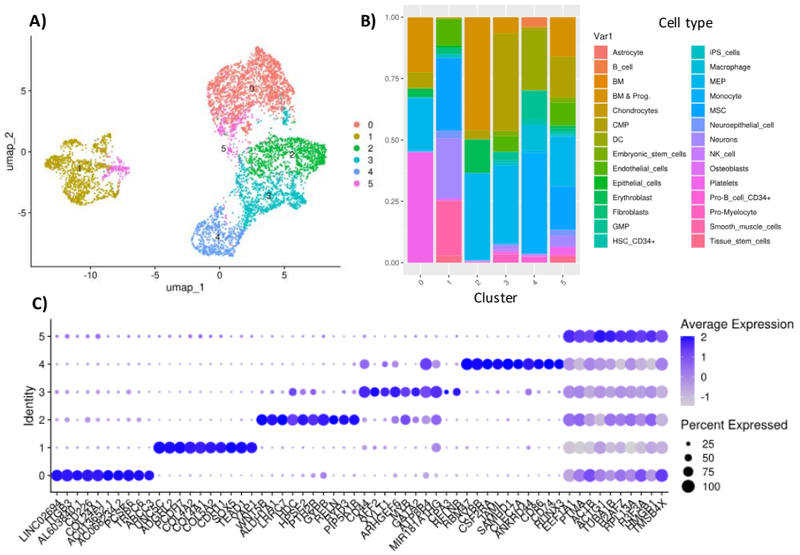

FIGURE 6. Single cell sequencing of iPS cells on day 12 of differentiation. A) UMAP clustering **B)** Classification of cell types in each cluster using the HPCA dataset in SingleR **C)** Top 10 gene markers for each cluster

Conclusions

- Utilizing directed differentiation, we have produced functional HSPCs from iPS cells
- Our cells are engineered with a doxycycline regulatable expression of *KMT2A::AFF1* fusion
- This model recapitulates hematopoietic ontogeny, with the ability to control expression of *KMT2A::AFF1* at specific developmental stages
- We are also employing CRISPR gene editing to co-introduce clinically identified variants of interest and generate additional iPS cell lines with other relevant *KMT2A* fusion oncogenes
- 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.

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