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A Genomics Driven Pluripotent Stem Cell Model of Infant Acute Lymphoblastic Leukemia

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BACKGROUND

- *KMT2A*-rearranged (*KMT2A*-r) ALL in infants (iALL) is a high-risk subtype of childhood leukemia, with poor survival outcomes despite intensive therapies.
- *KMT2A::AFF1* is the most common KMT2A-r, derived from t(4;11)(q21;23) and arises in utero.
- Infant ALL's cell of origin is thought to be a very early hematopoietic precursor, with transcriptomic studies of iALL blasts showing similarities to hematopoietic stem and progenitor cells (HSPCs), multipotent progenitors and early lymphoid progenitors (ELPs).
- Much remains unknown regarding how *KMT2A::AFF1* transforms early hematopoiesis, the cell of origin, and how it alters the severity of the disease.
- Existing iALL models fail to mimic human disease, which creates an essential, ongoing need for the development of representative model systems

EXPERIMENTAL DESIGN

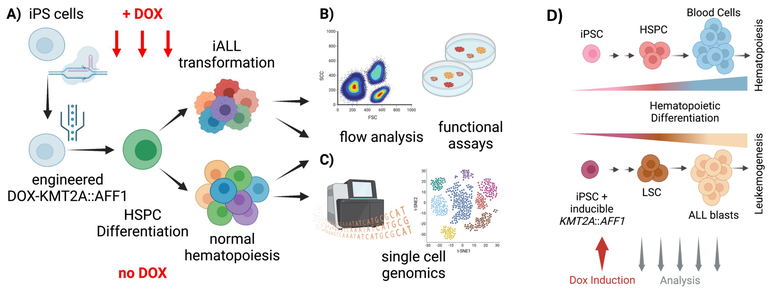

FIGURE 1. Project overview: a genomics driven iPS cell model of iALL. A) We engineered human iPS cell lines to express *KMT2A::Aff1* under doxycycline control via CRISPR gene editing technology. **B)** Directed differentiation was used to produce functional human hematopoietic stem and progenitor (HSPCs)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

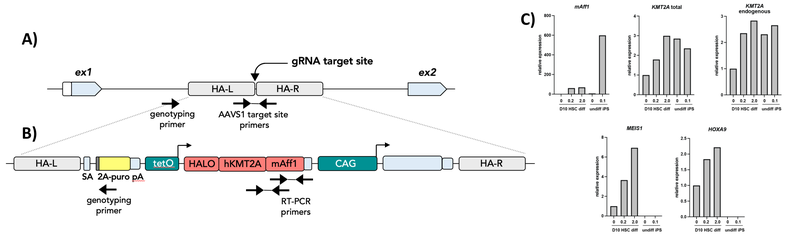

FIGURE 2. Doxycycline-regulated *KMT2A-Aff1* expression human iPS cells. A) AAVS 'safe harbor' locus used for targeting transgenes in iPS cells via CRISPR-mediated homology directed repair. **B)** Targeting vector for introduction of *KMT2A-Aff1* fusion coding sequence regulated by tet-responsive elements. **C)** q-PCR for expression of transgene and downstream targets in response to 48-hour doxycycline in undifferentiated iPS cells and cells differentiated to HSPCs.

RESULTS

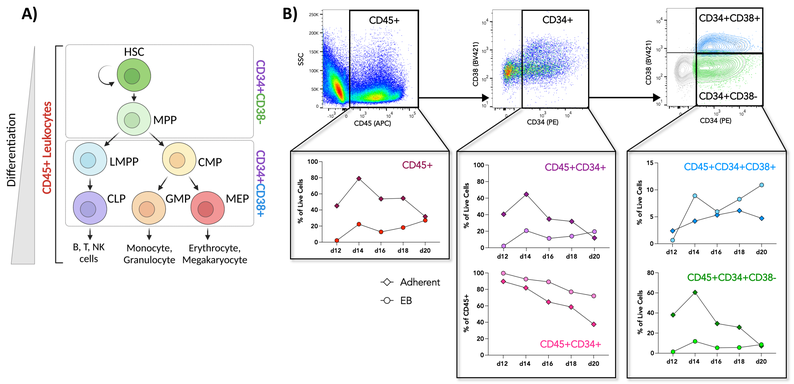

FIGURE 3. Directed differentiation of iPS cells to HSPCs. A) Differentiation hierarchy of hematopoiesis. Note color corresponding to phenotypic markers indicating differentiation state in **(B)**. **B)** Flow cytometry analysis of hematopoietic (CD45+) (CD34+) and progenitor (CD38+) cells from 2 independent iPS cell lines undergoing directed differentiation at days 12-20.

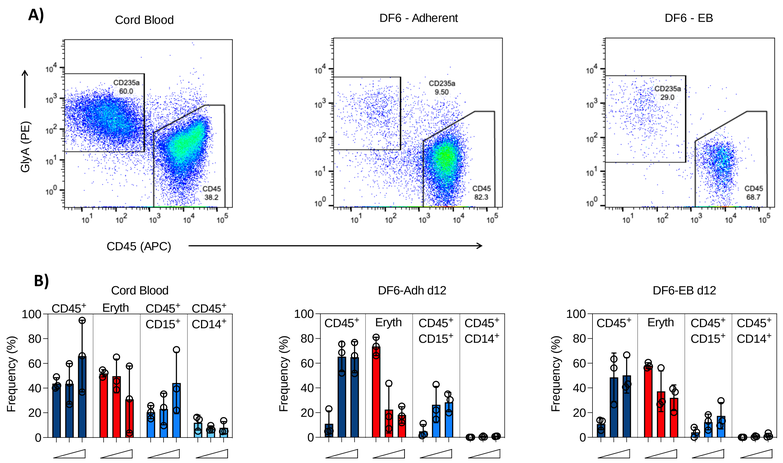

FIGURE 4. iPS cells differentiate into functional HSPCs. A) DF6 iPS cells were differentiated into HSPCs and analyzed by flow cytometry for erythroid (GlyA) and non-erythroid hematopoietic (CD45) development at days 12-20 post-differentiation. Both differentiation protocols (adherent and embryoid body (EB)) protocols yield robust frequencies of GlyA+ and CD45+ differentiated cells. **B)** CFU assays were performed at day 12 post-iPS cell differentiation and analyzed by flow cytometry 14 days later. Both differentiation protocols yield robust levels of functional HSPC differentiation similar, although not equivalent, to cord blood. We are currently using an OP9/MS5 sequential co-culture system to differentiate HSPCs to B-cell lineage.

FUTURE DIRECTIONS

- Single cell RNA sequencing will be used to determine dynamic transcriptomic changes occurring as a consequence of *KMT2A::AFF1* induction.
- We will also use single cell genomics to compare different stages of the iPS cell model to publicly available single cell RNA sequencing data (scRNA-seq) from multiple datasets encompassing fetal bone marrow and HSPC development.
- In the largest scRNA-seq study of iALL patient samples to date, we identified KMT2A-r blasts are most transcriptionally similar to early lymphoid progenitors.
- We also aim to utilize our infant scRNA-seq dataset for direct comparison of patient samples to the *KMT2A:AFF1* induced iPS cells.

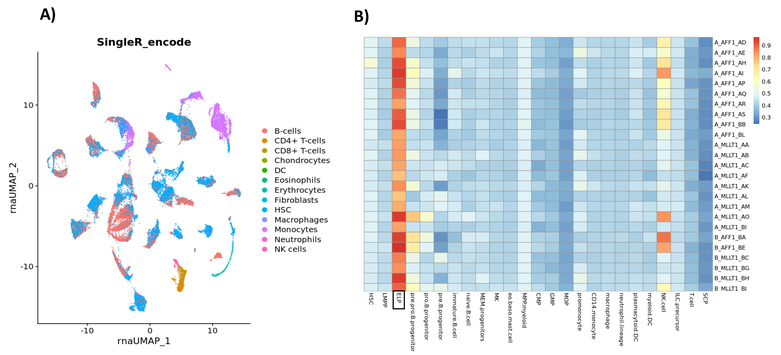

FIGURE 5. Single cell genomic analysis of iALL samples. A) UMAP dimensionality reduction and clustering of cell type assignments in iALL patient samples. **B)** Logarithmic regression model used to identify developmental cell-type similarity of *KMT2A*-r iALL cells.

CONCLUSIONS

This project is expected to produce a genetically engineered iPS model of *KMT2A:AFF1* iALL which will allow us to uncover the genomic and epigenetic landscape and cellular evolution of this unique leukemia with the long-term goal to uncover targets specific to iALL for the development of new therapies.

As one of my fellowship scholarly projects, I will be directing the single cell genomics aspect of this project. My involvement includes determining the differentiation timepoints to perform scRNA-seq, assisting in scRNA-seq library creation, performing data analysis and preparing the manuscript.

ACKNOWLEDGMENTS

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