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TITLE: A Multi-Omics Approach to Overcome Resistance in Infant Leukemia by Identifying Immune Therapy Failure Mechanisms

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<b>14. ABSTRACT:</b> Most children with acute lymphoblastic leukemia can be cured with standard chemotherapy, however a very high risk group of children that do much more poorly are infants under one year of age with a particular kind of leukemia known as MLLr. Most of these small infants will die, as standard chemotherapy and even several novel specifically targeted new drugs have failed to improve outcomes. An exciting new type of therapy, known as chimeric antigen receptor (CAR) T cells, has proven very effective against leukemias that are resistant to standard drugs. CAR T cells, however, must be made from the immune cells of the patient and unfortunately we have not been successful in making CAR T cells from infants. Prior research has revealed that T cells from infants who have received therapy are very poorly suited to become CAR T cells, much more so than T cells from older children, though the reason for this is not clear. Furthermore, even when CAR T cell therapy works, older children who also have the high risk MLLr type of leukemia experience relapse more often than those who do not largely because the leukemia figures out how to hide from the CAR T cells. This proposal will focus on using new and highly detailed techniques to study both the MLLr leukemias from infants and simultaneously study the T cell function from the same patients. Our twin goals are to understand how best to target the MLLr leukemias of infants with CAR T cells (to prevent relapse) and how to make the T cells from these infants into effective CAR T cells (to maximize response). Our team is uniquely qualified to do this, representing clinicians who have run the largest clinical trials for infants with leukemia, researchers who have developed the sensitive sequencing techniques proposed and scientists who have developed CAR T cell therapy for leukemia. Collectively, our prior laboratory studies have been swiftly translated into active clinical trials testing new targeted therapies in patients with high-risk leukemias including infants. We anticipate that results from the current studies will transform the care of infants with leukemia, dramatically improving outcomes once our findings are rapidly translated to active clinical trials as we have done before.						
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## **Introduction**

Despite advances in curing children over 1 year of age with acute lymphoblastic leukemia (ALL) children under 1 year of age face grim survival rates of around 35%. Infants with ALL often experience on therapy relapses. Bone marrow transplant has persistently failed to improved outcomes. The goal of this proposal is to use a single cell multi-omic approach to better understand the biology of infant ALL, particularly with respect to immune mechanisms, as a prerequisite to develop better therapies including novel immunotherapy approaches.

## **Keywords**

Infant acute lymphoblastic leukemia, MLL, KMT2A, lineage switch

## **Accomplishments**

- **What were the major goals of the project?**

Specific Aim 1: Identify immunotherapy targets in iALL subclones;

Specific Aim 2: Identify T cell profiles associated with CART manufacture success

- **What was accomplished under these goals?**

### **Accomplishments of the entire group:**

1): major activities: we proposed to profile 30 infant ALL (iALL) samples using sc-RNA-Seq, sc-ATAC-Seq and sn-mC-Seq. We have completed a total of 18 samples at the time of Dr. Brown's departure. We also had proposed to profile several patients who underwent lineage switch under the pressure of immunotherapy. We identified two such patients, and completed the analysis of tumor evolution through serial samples. Progress was somewhat slower than anticipated due to delays in all aspects of this project due to COVID19.

2) specific objectives:

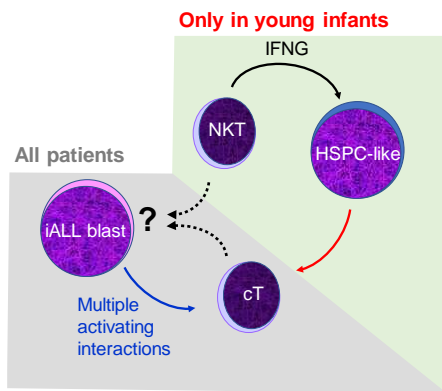
- define mechanisms of drug resistance and poor outcome
- define immune mechanisms that impact outcome
- interrogate tumor evolution through lineage switch.

3): preliminary analysis of the infant cohort revealed 3 key insights:

3.1 Infants less than 6 months of age, who have a much inferior outcome, tend to have ALL that is characterized by greater developmental and lineage plasticity. In particular, younger infants (< 6 months) often have leukemic cells that transcriptionally and phenotypically map onto hematopoietic stem and early progenitor cells.

3.2 The leukemic clone of infants less than 6 months of age contains a subpopulation that expresses reduced levels of the steroid receptor NR3C1 and steroid response genes. High dose steroids induce apoptosis in ALL cells and are a key component of ALL therapy. This feature may explain why infant ALL tends to be more upfront resistant than other subtypes of ALL.

3.3 we inferred a provocative immune interaction that is specifically found in infants less than 6 months, whereby immature NKT cells produce interferon gamma, which acts on the most immature blasts within the leukemic clone. In turn, highly immature cells in the blood of young infants (< 6 mo) are predicted to undergo immune inhibitory interactions with cytotoxic T-cells. If confirmed, this data will be critical for developing immunotherapy approaches in infants.



3.4. Analyzing two patients with KMT2A (MLL) rearranged leukemia whose leukemia underwent a lineage switch (from ALL to AML) after B-cell directed immunotherapy revealed a pre-existing myeloid biased population that expanded under selective pressure.

### **Dr. Brown contribution:**

Dr Brown's panned effort entailed to "Oversee primary patient sample identification, selection, approval for use and shipment to the appropriate laboratories. Help design and supervise the bioinformatics analytics of the WGBS and other data, help integrate the data generated by the collaborating laboratories, and ensure key findings are efficiently translated into clinical trials."

#### Final report on specific task:

*Oversee primary patient sample identification and selection* – this is completed

*Oversee primary patient sample approval for use and shipment to the appropriate laboratories* – this is completed, and all samples are in the hands of Drs. Bernt and Guest.

*Help design and supervise the bioinformatic analysis of the WGBS and other data and help integrate the data by the collaborating laboratories:* Dr. Brown contributed to data analysis in monthly investigator virtual meetings, and his experience and input were helpful in the analysis of the single nuclei bisulfite sequencing data and integration with the other platforms.

*Translation of findings into clinical trials:* Dr. Brown was involved in the Children's Oncology Group's (COG) Infant Leukemia Task Force that is currently designing the next nationwide infant ALL clinical trial. Insights from this research helped shape the discussion and plan for this trial.

- **What opportunities for training and professional development has the project provided?**

Nothing to report

- **How were the results disseminated to communities of interest?**

A report of the findings from the first 18 patients was published in the journal Blood: Chen et al., Blood 2021 Dec 5; blood.2021013442. doi: 10.1182/blood.2021013442.

Findings were also presented at the Annual Meeting of the American Society of Hematology (ASH) in December of 2021 (poster presentation).

### **Impact**

- **What was the impact on the development of the principal discipline(s) of the project?**

1. **Understanding and addressing myeloid primed subclones that are present at diagnosis:** We have known for a long time that infants less than one year of age face dismal odds of survival if they are diagnosed with ALL. This is in contrast to older children, who are much more likely to be cured. Our data points to several reasons why infants face worse outcomes – some long suspected, and some unexpected and novel. Understanding why our traditional approaches fail to cure infants with

ALL is the first critical step in developing more effective therapies. Concretely, our findings provide a molecular foundation for a recent clinical observation that patients who do not respond well to initial ALL-like chemotherapy have a better outcome if they receive additional cycles of AML-like chemotherapy, while patients respond well to initial ALL-type chemotherapy have a better outcome when they continue with ALL like chemotherapy only. The small myeloid primed populations we observed in a subset of patients may explain this observation. We are currently conducting studies to explore whether the presence of myeloid primed clones is indeed associated with suboptimal response in PDX models and the second cohort that has outcomes data available. Additional efforts are aimed at developing a clinical test that will allow us to determine the presence of myeloid primed subclones to assist with treatment stratification in the future. This is particularly important as B-cell directed bispecific antibody therapy (Blinatumomab, CD19-targeted T-cell engager) will be incorporated into the next nationwide clinical trial for infant ALL.

2. **Identifying surface targets for immunotherapy:** our data suggest that a subset of infant ALL patients will be resistant to B-lymphoid immunotherapy targets. This suggest that the final cohort will be instrumental in identifying more uniform targets. This includes surface proteins such as Flt3, which already are the target of CAR-T cell strategies in development.

- **What was the impact on other disciplines?**

Our combined transcriptomic and epigenomic single cell analysis longitudinally over the course of tumor evolution is quite novel and will likely encourage other researchers to use similar approaches to understand dynamic tumor evolution.

- **What was the impact on technology transfer?** Nothing to report.
- **What was the impact on society beyond science and technology?** Nothing to report at this stage. Ultimately, loosing a young child to cancer is a highly traumatic experience, and if our efforts to improve survival for the affected babies are successful, this will have impact on the entire family and social network of the affected child.

### Products

A report of the findings from the first 18 patients was published in the journal Blood: Chen et al., Blood 2021 Dec 5; blood.2021013442. doi: 10.1182/blood.2021013442.

Findings were also presented at the Annual Meeting of the American Society of Hematology (ASH) in December of 2021 (poster presentation).

### Participants & Other Collaborating Organizations

Name:	<i>Patrick Brown</i>
Project Role:	<i>Principal Investigator</i>
Nearest person month worked:	1
Contribution to Project:	See above
Funding Support:	<i>Not applicable</i>

Name:	<i>Melissa Hynds</i>
Project Role:	<i>Research Technologist</i>

Nearest person month worked:	1
Contribution to Project:	See above
Funding Support:	<i>Not applicable</i>

**Special Reporting Requirements**

Partnering PI will submit separate progress report.

**Appendices**

Not applicable