AWARD NUMBER: W81XWH-20-1-0357

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

PRINCIPAL INVESTIGATOR: Dr. Kathrin Bernt

CONTRACTING ORGANIZATION: Children's Hospital of Philadelphia

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Dr. Kathrin Bernt,(submitting PI), Erin Guest (co-PI), Patrick Brown (co-PI)

E-Mail:

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

CHILDREN'S HOSPITAL OF PHILADELPHIA, THE C H O P
3401 CIVIC CENTER BLVD
PHILADELPHIA PA 19104-4319

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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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14. ABSTRACT

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 mech

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Infant acute lymphoblastic leukemia, MLL, KMT2A, lineage switch

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

	Timeline	**Site 1**	**Site 2**	**Site 3**	**% complete**
Specific Aim 1 Identify immunotherapy targets in iALL subclones	Months				
IRB approvals (with annual renewals)	-12 to -6	Dr Bernt	Dr Guest	Dr Brown	100%
HRPO review and approval	0-4				100%
Major task 1: RNA-Seq and whole genome bisulfite sequencing of bulk leukemia. - Library prep and sequencing ordered to control for batch effect - Data curation and sharing	4-18		Dr. Guest		RNA-Seq: 40% Bisulfite: 80%
Major task 2: scRNA-Seq and scATAC-Seq of leukemia - Sorting, library prep and sequencing - Data curation and sharing	4-18	Dr. Tan			90%
Major task 3: snmC sequencing - Sorting, library prep and sequencing - Data curation and sharing	4-18	Dr. Wu			60%
Major task 4: Subclone analysis and identification of antigens and epigenetic programming related to lineage switch - Data integration. Cross validation of findings between single cell and bulk techniques and ATAC/snmC data sets. - Sublcone classification and systems analysis	16-36	Dr.Tan, Dr. Bernt		Dr. Brown	100% AALL15P1 cohort 50% AALL0631 cohort
Milestones achieved: identification of iALL subclones, antigen identification, final data analysis	18-36	All	All	All	100% AALL15P1 cohort 50% AALL0631

					cohort
Specific Aim 2 Identify T cell profiles associated with CART manufacture success					
Major task 1: RNA-Seq and whole genome bisulfite sequencing of bulk T cells - Library prep and sequencing ordered to control for batch effect - Data curation and sharing	4-18		Dr. Guest		RNA-Seq: 40% Bisulfite: 80%
Major task 2: scRNA-Seq and scATAC-Seq of T cells - Sorting, library prep and sequencing - Data curation and sharing	4-18	Dr. Tan			90%
Major task 3: snmC sequencing of T cells - Sorting, library prep and sequencing - Data curation and sharing	4-18	Dr. Wu			60%
Major task 4: T cell pathway analysis, exhaustion profiling and correlation with CAR potential -Data integration and cross validation.	18-36	Dr Bernt, Dr. Tan			100% AALL15P1 cohort 50% AALL0631 cohort
Milestone(s) Achieved: identification of pathways to be targeted to improve infant T cell CAR performance, final data analysis	18-36	All	All	All	100% AALL15P1 cohort 50% AALL0631 cohort

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting

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 processing all planned samples. 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.

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 first 18 samples (mostly from AALL15P1, plus a few local samples) revealed the following 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.

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. This feature may explain why infant ALL tends to be more upfront resistant that other subtypes.

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 NK and T-cells.

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.

The manuscript was published in Blood in April of 2022: Chen et al., Blood (2022) 139 (14): 2198–2211.

Since then, we have spent much of our effort on

1. Further refining our single cell pipelines, allowing us to analyze a greater number of samples with scRNA-Seq by sample multiplexing (Lead: Kai Tan)
2. Further refining the sc5mC pipeline to allow better depth and efficiency
3. Putting in place the regulatory approvals to proceed with the AALL0631 cohort. After starting to work on this sample set, we became aware that the appropriate approvals had not been put in place by Dr Brown. Drs. Guest and Bernt worked together with the Children's Oncology Group and the NIH (CTEP) to amend the AALL0631 clinical trial and get approval for the planned studies, this is now in place, and we have resumed work.
4. We have completed the scRNA-Seq and scATAC-Seq for all samples. Bulk sequencing is in process. We have not yet completed the sc5mC-Seq, as it is more expensive and we wanted to make sure the other single cell platforms gave good results and QC first.

Opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

A first manuscript was published in the journal Blood – the most prestigious journal in hematology research: Chen et al., Blood (2022) 139 (14): 2198–2211.
https://ashpublications.org/blood/article/139/14/2198/482898/Single-cell-multiomics-reveals-increased

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We obtained all the samples of the AALL0631 clinical trial, which will form our confirmatory cohort. A substantial portion of these samples have undergone bulk 5mC sequencing already, therefore although Dr. Brown is no longer part of the consortium, we will not lose this aspect of the proposal. This was detailed in the revised SOW submitted upon Dr. Browns departure.

However, we became aware that regulatory approvals with COG and CTEP were not in place. We amended the AALL0631 clinical trial and pursued appropriate approvals for our studies from COG and CTEP. We used this hold to also improve our single cell RNA-Seq and 5mC pipelines.

While this causes a significant delay, we believe that the ability to correlate our biologic features with outcomes will make our data much more impactful. Specifically, we will use the AALL15P1 data to interrogate which of our key features (more immature phenotype, greater plasticity, steroid resistance, myeloid subclones and presence of an HSPC population) correlate with outcome in this cohort. We will then seek to confirm these outcomes measures in the AALL0631 cohort. The confirmatory cohort will comprise a total of 12 samples to be analyzed by our single cell multiomic platform, as well as bulk RNA-Seq. We will use combined data from both cohorts to deconvolute bulk RNA-Seq signatures. This can be used in future studies to interrogate the entire AALL0631 cohort using bulk RNA-Seq. This is highly meaningful as RNA-Seq is entering clinical diagnostic practice.

We received approval from COG and the NIH to proceed in June, and have already completed the planned scRNA-Seq, scATAC-Seq and acquired the cells for 5mC-Seq as initially proposed. Bulk RNA was isolated and sequencing is in progress.

The remaining months will be dedicated to data analysis. There is a possibility that quality issues on those tests where the sequencing is still in queue (5mC-Seq, bulk RNA-Seq) might require repeats. We do have enough sample material to do so if needed.

For analysis, we plan to correlate the high risk signatures we identified in the 15P1 clinical trial with outcomes data, using minimal residual disease (MRD) as a primary endpoint, and event free (EFS) and overall (OS) survival as secondary endpoints. We will use the AALL0631 samples as confirmatory cohort. We will ask whether risk signatures can be found in the bulk RNA-Seq data.

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

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.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report. However, as bulk RNA-Seq is moving into the clinical diagnostic space, our data have the potential to be used for clinical risk stratification and/or candidate CAR selection.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Dr. Brown, who was the site PI for the Johns Hopkins (bulk 5mC) team for this project, left academia to move to industry. Dr. Guest, who had worked closely with Dr. Brown on this project, and was co-PI with Dr. Brown on several similar projects for infant ALL, took over this portion of the proposal. The DOD was notified immediately, and all regulatory and re-budgeting requirements from the DOD were fulfilled.

In the last year we became aware that not all regulatory requirements from the COG and NIH side were met, and worked with COG and CTEP to correct this. All approvals are now in place.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Delays to start work with the AALL0631 samples were longer than anticipated to get all the regulatory aspects taken care of. We finally received regulatory approval from COG and CTEP to proceed in June 2023. We have since completed the scRNA-Seq and scATAC-Seq. The bulk RNA-Seq is submitted for sequencing. We are planning the completion of the 5mC sequencing for the next 3 months, after which we will spend the remainder of the runtime of the grant for data analysis. We are happy to be back on track and proceeding rapidly, and grateful for the NCE.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Due to the departure of Dr. Gao, more focus on less expensive validation experiments, and a pause in sequencing while awaiting the AALL15P1 outcomes data, the CHOP site currently still has a budget surplus. Year 1 and 2 unused funds will be applied toward sequencing of the validation cohort in Year 3.

The research technician effort and bioinformatician effort have not yet been fully utilized at Children's Mercy. The research team will allocate effort and corresponding salary support to the research technician and bioinformatician in the no cost extension. Year 1-3 unused salary funds will be applied toward the completion of the project in the no cost extension.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the

reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

All aspects of the experimental pipeline were modified and re-certified by our biosafety committee granting us permission to use COVID19+ samples should the need arise. This is not likely to be relevant given the currently targeted samples that were collected in the pre-COVID19 era.

Publications, conference papers, and presentations

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

A first manuscript was published in the journal Blood – the most prestigious journal in hematology research: Chen et al., Blood (2022) 139 (14): 2198–2211. https://ashpublications.org/blood/article/139/14/2198/482898/Single-cell-multiomics-reveals-increased

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Conference presentation at American Society of Hematology (ASH) annual meeting 2021: https://ash.confex.com/ash/2021/webprogram/Paper146102.html

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Data from the first cohort have been deposited at the Human Tumor Atlas Network (HTAN) data portal: https://data.humantumoratlas.org/.

Analysis scripts were deposited at https://github.com/tanlabcode/KMT2Ar_Paper.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

During the second year, Dr. Brown left Johns Hopkins, and Dr. Guest assumed his role on this study. **All changes to key personnel were already executed at the time of the transition. No further changes are made or planned at the time of this report.** The already executed changes are summarized below:

Name:	**Kathrin Bernt, MD**
Project Role:	Principal Investigator
Researcher Identifier:	ORCID ID: 0000-0002-0691-356X
Nearest Person Month worked:	adjusted effort year 1: 3 calendar months 25% effort (to facilitate transition), year 2&3: 2.7 calendar months, 22.5% effort. (initially planned effort: 1.2 calendar months, 10% effort. This was adjusted when Dr. assumed the position of PI.)
Contribution to Project:	Worked with Dr. Tan on performing single cell multi-omic analysis of a first cohort of infant ALL samples. Worked with Drs. Guest (and Brown) to set up a second cohorts originating from studies AALL0631.
Funding Support:	N/A
Name:	**Kai Tan, PhD**
Project Role:	co-Investigator
Researcher Identifier:	ORCID ID: 0000-0002-9104-5567
Nearest Person Month worked:	0.6 calendar months, 5% effort Years 1-3
Contribution to Project:	Worked with Dr. Bernt on performing single cell multi-omic analysis of a first cohort of infant ALL samples. Oversaw development of analytic algorithms, data analysis and interpretation
Funding Support:	N/A

Name:	**Fatemeh Alikarami, PhD**
Project Role:	postdoctoral fellow, Bernt lab
Researcher Identifier:	ORCID ID: N/A
Nearest Person Month worked:	2.4 calendar months, 20% effort, for most of year 1 (effort was temporarily decreased to 17% 11/1/2020 – 3/30/2021). Effort was adjusted to 35% in year 2 to take over work load from Dr. Gao, who left the institution.
Contribution to Project:	Dr. Alikarami oversees the sample processing and planning of experiments for the multi-omic analysis of infant ALL cells, including coordination with collaborating groups. Dr. Alikarami is involved in data interpretation, and downstream validation experiments. Downstream validation experiments were the focus of year 2, therefore Dr. Alikarami's effort was increased in year 2. These experiments were quite extensive, provided critical validation and were instrumental to the publication of our first cohort a in April of 2022 in Blood.
Funding Support:	N/A
Name:	**Yasin Uzun, PhD**
Project Role:	research scientist, Tan lab
Researcher Identifier:	ORCID ID: 0000-0003-3478-3499
Nearest Person Month worked:	Year 1: 6 Cal Mos, 50% effort. Year 2: 32.88% effort (3.94 Cal Mos). Year 3: Dr. Uzun left the institution, his effort was picked up by Dr. Apoorva Joshi.
Contribution to Project:	Dr. Uzun conducted most of the data analysis of the first cohort. After Dr. Gao left, Dr. Uzun became the primary interface between the Bernt and Tan labs, and CHOP and CMH/JHU labs for data analysis. Year 2 focused on wet bench experimental validation from year 1, thus Dr. Uzun's effort was decreased by 12%, and Dr. Alikarami's effort was increased by 15%. Dr. Uzun left the project in year 3 and was replaced by Apoorva Joshi.
Funding Support:	N/A
Name:	**Apoorva Joshi, MS**
Project Role:	research assistant, Tan lab
Researcher Identifier:	ORCID ID: N/A
Nearest Person Month worked:	3.28% efford, 3 calendar months.
Contributions to project:	Apoorva Joshi took over from Yazin Uzun working on this project, focusing on the scRNA-Seq/ATAC-Seq.
Funding Support:	N/A
Name:	**Erin Guest, MD**
Project Role:	Co-Principal Investigator
Researcher Identifier:	ORCID ID: 0000-0003-2482-5608
Nearest Person Month worked:	1.8 calendar months, 15% effort Years 1-3
Contribution to Project:	Worked with Drs. Bernt, Brown, and Tan to analyze data from first cohort of samples originating from studies AALL0631 and AALL15P1. Prepared an application to Children's Oncology Group to obtain additional AALL15P1 samples for sequencing.
Funding Support:	N/A

Name:	**Midhat Farooqi, MD, PhD**
Project Role:	Co-Investigator
Research Identifier:	ORCID ID: 0000-0002-5238-1349
Nearest Person Month worked:	0.48 calendar months, 4% effort Years 1-3Contribution to Project Worked with Drs. Bernt, Tan, and Guest to analyze data from the first cohort of samples originating from studies AALL0631 and AALL15P1. Provided input into the study design for ongoing sequencing.
Funding Support:	N/A
Name:	**Patrick Brown, MD**
Project Role:	Co-Principal Investigator
Researcher Identifier:	ORCID ID: 0000-0002-8653-1069
Nearest Person Month worked:	0.12 calendar months, 1% effort Years 1-3 planned, but Dr. Brown left the project in Year 2.
Contribution to Project:	Oversaw primary patient sample identification, selection, approval for use and shipment to the appropriate laboratories. All samples are now in the hands of Drs. Guest and Bernt, and there is a specific plan to select the confirmatory AALL0631 cohort based on available bisulfite sequencing and outcomes data. Dr. Brown consulted on the design and supervised the bioinformatics analytics of the WGBS (data with Dr. Guest now). He also helped integrate the data generated by the collaborating laboratories and contributed to the publication in Blood 2022. Dr. Brown's role in ensuring key findings are efficiently translated into clinical trials is taken over by Drs. Bernt and Guest, who are both members of the COG Infant ALL Task Force.
Funding Support:	N/A
Name:	**Rumen Kostadinov, PhD**
Project Role:	Biostatistician/Research Associate, Brown lab
Researcher Identifier:	ORCID ID: N/A
Nearest Person Month worked:	6 calendar months, 50% effort Years 1-3 planned, left in year 1.
Contribution to Project:	Create and apply data analytics for the WGBS and other sequencing data to be generated in this project. (NOTE: Dr. Kostadinov left the Brown lab at the end of year 1, his role was taken over by the groups of Drs. Guest, Wu and Tan.)
Funding Support:	N/A
Name:	**Hao Wu, PhD**
Project Role:	co-Investigator
Researcher Identifier:	ORCID ID: 0000-0003-4395-6929
Nearest Person Month worked:	0.6 calendar months, 5% effort Years 1-3
Contribution to Project:	Worked with Dr. Bernt on performing single cell DNA methylome analysis of a first cohort of infant ALL samples.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Changes to Other Support:

Kathrin M. Bernt, MD, PI:
The following awards closed in the last reporting period:

Title: Center for Developmental Mapping of Heart and Bone Tissues
Major Goals: The overarching goal of our mapping effort is to capture the molecular and cellular heterogeneity and cellular spatial organization of bone and heart.
Project Number: U54HL156090
Name of PD/PI: Kai Tan/ Kathrin Bernt/ Liming Pei
Source of Support: NIH/NHLBI
Primary Place of Performance: Children's Hospital of Philadelphia
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2020-08/2022
Total Award Amount (including Indirect Costs):
Person Months (Calendar/Academic/Summer) per budget period.

Title: Understanding mechanisms and developing therapies for MN1-driven leukemia
Major Goals: In this proposal, we will evaluate molecular mechanisms of MN1 - driven leukemic transformation and investigate new therapeutic approaches.
Project Number: N/A
Name of PD/PI: Kathrin Bernt
Source of Support: Cookies for Kids' Cancer
Primary Place of Performance: Children's Hospital of Philadelphia
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2021-12/2022
Total Award Amount (including Indirect Costs):
Person Months (Calendar/Academic/Summer) per budget period.

Title: Targeting MN1 in Hard-To-Treat Cancer
Major Goals: MN1 overexpression in AML is associated with poor prognosis. The goal of this project is to conduct translational studies identifying and testing targeted therapeutic approaches.
Project Number: N/A
Name of PD/PI: Kathrin Bernt
Source of Support: Children's Cancer Research Fund
Primary Place of Performance: Children's Hospital of Philadelphia
Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2021-03/2023

*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period.

The following awards are newly active since the last reporting period:

*Title: Comprehensive analysis of non-coding mutations driving transformation in pediatric leukemias.
*Major Goals: The goal of this internal funding is to conduct a comprehensive genomic and epigenomic characterization of our internally banked patient samples from patients with leukemia, particularly AML. This will complement the FDA funded efforts to establish a comprehensive PDX platform for rare and high risk pediatric cancer.
*Status of Support: Active
*Project Number: N/A
*Name of PD/PI: Bernt
*Source of Support: The Children's Hospital of Philadelphia Research Institute
*Primary Place of Performance: Children's Hospital of Philadelphia
*Project/Proposal Start and End Date: (MM/YYYY) (if available): 1/1/2023-12/31/2023
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period.

*Title: A high-throughput single-molecule tracking pipeline to understand and treat pediatric acute myeloid leukemia
*Major Goals: Conduct pilot experiments that will provide preliminary data for a larger grant application to comprehensively interrogate the role of IDRs in leukemogenesis, and to establish a single molecule screening platform to identify novel disruptors of oncogenic IDRs.
*Status of Support: Active
*Project Number: N/A
*Name of PD/PI: Bernt
*Source of Support: The Children's Hospital of Philadelphia / Division of Oncology
*Primary Place of Performance: Children's Hospital of Philadelphia
*Project/Proposal Start and End Date: (MM/YYYY) (if available): 4/1/2023-3/30/2024
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period.

Kai Tan, PhD

The following awards closed in the last reporting period:

*Title: The goal of this project is to apply a network controllability-based systems biology approach t o identify synergistic regulators in KMT2A-R infant ALL with functional validation of targets for t herapeutic drug pairs.
Major Goals: Major Goals: The goal of this project is to apply a network controllability-based s ystems biology approach to identify synergistic regulators in KMT2A-R infant ALL with functional validation of targets for therapeutic drug pairs.
*Status of Support: Active
Project Number: 1U01CA243072-01S1
*Name of PD/PI: Kai Tan / Sarah Tasian
*Source of Support: NIH/NCI
*Primary Place of Performance: Children's Hospital of Philadelphia
Project/Proposal Start and End Date: (MM/YYYY) (if available): 06/2021-05/2023 (NCE)
*Total Award Amount (including Indirect Costs):

The following awards are newly active since the last reporting period:
Title: Center for multi-dimensional atlas of the human heart
Major Goals: Map molecular and cellular changes in heart tissues over the course of human lifespan using comprehensive multi-dimensional single-cell and imaging technologies
Status of Support: Active
Project Number: U54HL165442-01
Name of PD/PI: Kai Tan/Liming Pei/Jeffrey Moffitt
Source of Support: NIH
Primary Place of Performance: Children's Hospital of Philadelphia
Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2022-07/2026
Total Award Amount (including Indirect Costs):

Title: Comprehensive evaluation of immune function in patients receiving multimodal therapy for high risk neuroblastoma
Major Goals: We will test the novel hypothesis that markers reflective of immune cell profile and interaction with malignant cells during the course of therapy can guide treatment of children with NBL
Status of Support: Active
Project Number: CA210953P1
Name of PD/PI: Kai Tan/Steven DuBois/Trevor Pugh
Source of Support: Department of Defense
Primary Place of Performance: Children's Hospital of Philadelphia
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2022-08/2026
Total Award Amount (including Indirect Costs):

Title: Understanding Differences in Disease Biology in Childhood T-cell Acute Lymphoblastic Leukemia and Lymphoma
Major Goals: Investigate intrinsic biologic differences in T-ALL and T-LL (Aim 1) Define Microenvironmental differences in T-ALL and T-LL (Aim 2)
Status of Support: Active
Project Number: 993259
Name of PD/PI: Dave Teachey
Source of Support: Hyundai Motor America – Hope on Wheels
Primary Place of Performance: Children's Hospital of Philadelphia
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2022-12/2024
Total Award Amount (including Indirect Costs):

Title: Improving risk allocation and developing novel therapies for children with T-ALL and T-LL
Major Goals: To understand differences in intrinsic tumor biology and the microenvironment in patients with T-cell acute lymphoblastic leukemia and T-cell acute lymphoblastic lymphoma
Status of Support: Active
Project Number: 2R01CA193776-06
Name of PD/PI: Dave Teachey
Source of Support: NIH
Primary Place of Performance: Children's Hospital of Philadelphia
Project/Proposal Start and End Date: (MM/YYYY) (if available): 02/2023-1/2028
Total Award Amount (including Indirect Costs):

Hao Wu, PhD (UPENN):
The following awards closed in the last reporting period:
Title: Charting oxygen-sensing gene regulatory network in cardiomyocytes through single-cell analysis and epigenome editing
Major Goals: The major goal of this study is to develop novel single-cell genomics and synthetic epigenome-editing methods to dissect oxygen-sensing gene regulatory mechanisms in cardiomyocytes.
Project Number: DP2 HL142044
Name of PD/PI: Hao Wu
Source of Support: NIH/NHLBI
Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/30/2017-08/30/2022

Title: Non-destructive epigenetic sequencing with DNA deaminase enyzmes.
Major Goals: his proposal advances novel genomic sequencing methods for localizing the major cytosine modifications in the genome in a non-destructive manner and for linking those changes to gene expression differences in cells. This work will help reveal how dynamic changes in cytosine modification states drive physiological or pathological processes.
Project Number: RO1 HG010646
Name of PD/PI: Hao Wu (MPI)
Source of Support: NIH/NHGRI
Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/01/2019-06/30/2023

Title: APOBEC-coupled Epigenetic Sequencing.
Major Goals: The major goal of this study is to develop a non-destructive enzymatic alternative to bisulfite-based methods for whole genome base-resolution localization of 5hmC.
Project Number: Penn Epigenetics Pilot Grant
Name of PD/PI: Hao Wu
Source of Support: University of Pennsylvania
Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 06/30/2017-06/30/2020

The following awards are newly active since the last reporting period:
*Title: Underpinnings of Corneal Innervation: Anatomical, Molecular, And Functional Studies Of Corneal Sensory Afferents In Physiologic And Pathologic States.

Major Goals: We propose to combine advanced imaging approaches, novel single-cell multi-omics, and cutting-edge mouse genetic models to perform three levels of analysis.
*Status of Support: Active
Project Number: U01EY034681
*Name of PD/PI: Hao Wu/MPI
*Source of Support: NIH/NHGRI
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/01/2022-08/30/2027
*Total Award Amount (including Indirect Costs): 6,050,000
*Person Months (Calendar/Academic/Summer) per budget period.

Erin Guest, MD (CMH):
The following awards are newly active in the last reporting period:
None

The following awards closed in the last reporting period:
*Title: Targeting Solid Tumors with Multi-antigen Specific T cells by Identifying the Genetic and Epigenetic Determinants of Therapeutic Response
*Status of Support: Completed
Project Number: N/A
Name of PD/PI: Midhat Farooqi
*Source of Support: Braden's Hope Foundation
*Primary Place of Performance: Children's Mercy Kansas City
Project/Proposal Start and End Date: 11/2019-10/2022
Total Award Amount (including Indirect Costs):
Person Months (Calendar/Academic/Summer) per budget period: 0.06 calendar months

The following awards were extended in the last reporting period:
*Title: Next Generation Sequencing to Detect Minimal Residual Disease in Infant ALL
*Status of Support: Active
Project Number: 1UG1CA233249-01 Sub-award
Name of PD/PI: Erin Guest, MD
*Source of Support: Children's Oncology Group (COG) Hematopoietic Malignancies Integrated Translational Science Center (HM-ITSC) Grant

*Primary Place of Performance: Children's Mercy Kansas City

Project/Proposal Start and End Date: 07/2020-06/2021

Total Award Amount (including Indirect Costs):
Person Months (Calendar/Academic/Summer) per budget period: 0.36 calendar months

Patrick Brown, MD (JHU):
Not applicable

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name: University of Pennsylvania
Location of Organization: (if foreign location list country): Philadelphia, PA
Partner's contribution to the project : collaboration

Beyond the 2 main sites (CHOP/Kansas Mercey), Hao Wu at the University of Pennsylvania is a collaborator for this award. Dr. Wu completed the single cell DNA-methylation analysis for this project.

6. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *N/A*

7. APPENDICES: *N/A*