Single-cell Pediatric Cancer Atlas
Request for Applications

Application Due: September 30, 2019

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About Alex's Lemonade Stand Foundation

Alex's Lemonade Stand Foundation (ALSF) emerged from the front yard lemonade stand of 4-year-old Alexandra "Alex" Scott, who was fighting cancer and wanted to raise money to find cures for all children with cancer. Her spirit and determination inspired others to support her cause, and when she passed away at the age of 8, she had raised $1 million. Since then, the Foundation bearing her name has evolved into a national fundraising movement. Today, ALSF is one of the leading funders of pediatric cancer research in the U.S. and Canada, funding nearly 1,000 research projects and providing programs to families affected by childhood cancer. ALSF is also the only childhood cancer research organization that has been given the NCI peer-reviewed funder designation for rigorous selection of research grants. The mission of ALSF is to improve the lives of children with cancer by funding impactful research, raising awareness, and supporting families with the ultimate goal of curing all children with cancer.

The Single-cell Pediatric Cancer Atlas Program Description

Alex's Lemonade Stand Foundation seeks to create datasets that profile pediatric cancers at a single-cell resolution level. Single-cell profiling can provide insights into the heterogeneity of cells in a tumor and the surrounding tumor microenvironment, as well as variability in the states of cancer cells, all of which can influence the cancer's response to treatments. The pediatric cancer atlas will be an open resource for discovery, and a primary goal of this effort is to produce data that can be harmonized.

The Single-cell Pediatric Cancer Atlas (ScPCA) will fund the profiling of pediatric cancer samples. This award is designed to produce an atlas of gene expression profiles for pediatric cancers of different types and from different organ sites that can be harmonized to allow comparison within and across type and site.

Data sharing is a critical component of the application, and applicants are expected to share the resulting raw and processed data (see Resource and Data Sharing).

- Applications should focus on profiling of childhood and adolescent cancers (ages 0-19).
- Applicants should profile samples with the 10X platform for single-cell profiling, *unless another sequencing modality is justified for the cancer or tissue type.*
- Applicants should consider using barcoded antibodies (CITE-seq) to profile cell surface markers if there are markers that are relevant to the tumor type or microenvironment.
- Applicants should perform single-cell RNA-sequencing if feasible. If single-nucleus RNA-sequencing is the only possibility, it should be scientifically justified.

- Applicants are required to perform RNA-sequencing of the bulk tissue from which the single cells are derived.

Application Timeline and Review

- A full proposal must be submitted that meets all guideline criteria as well as all eligibility criteria.
- Proposals will be reviewed by an independent panel of experts according to the NIH recognized peer-review process.

APPLICATION TIMELINE	
PROPOSALS DUE	September 30, 2019 by 11:59PM ET
PROJECTED START DATE	January-March 2020
GRANT DURATION	12 months

Eligibility

- Applicants must have an MD, PhD, or MD/PhD or equivalent and be appointed as faculty (or equivalent) at an academic institution.
- Applicants must have a track record of publication and funding productivity that demonstrates that the project can be accomplished by the investigators.
- Applicant institutions may be based in the United States or abroad, and applicants need not be United States citizens. Funds must be granted to nonprofit institutions or organizations.

Budget

The requested budget should be in proportion to the scope of the proposed project and should be at or under $200k USD in direct costs over one year. ALSF does not pay indirect costs for this grant program.

In addition to the full requested budget, applicants may elect to use the ALSF Childhood Cancer Data Lab (CCDL) for the quantification step of single-cell and/or bulk gene expression analysis. The CCDL can also be used for the analysis portion of expression analysis, but applicants should first discuss planned applications with the CCDL and request a letter of support so that adequate support will be available. The CCDL is also available for consultations regarding the management and sharing of your research data which is a required element of this award (see Resource & Data Sharing). For applicants interested in using the CCDL for gene expression analysis, reach out to the CCDL Director, Casey Greene, PhD, at greenescientist@gmail.com to discuss the research plan in more detail. If interested, include the request as a line item on the ALSF budget template.

Resource and Data Sharing

Applicants are expected to make raw sequencing data (FASTQ files) available to the ALSF Childhood Cancer Data Lab (CCDL) within one month of profiling on a rolling basis. As part of the ScPCA, the CCDL will uniformly process the raw sequencing data through a common pipeline to estimate gene expression and where appropriate, the levels of cell surface markers. Any data transfer agreements, if required, must allow the CCDL to make gene expression and cell surface marker abundance estimates, as well as the de-identified sample-associated metadata, available without restriction no more than six months after raw sequencing data are generated. The goal of this requirement is to make sure that reusable data are released in a timely manner. The grantee is also required to deposit raw data in the appropriate repository (either NCBI SRA or EBI ENA) within six months of the conclusion of the grant.

This commitment should be specified in the Resource Sharing Plan. Patient consent information should be provided with the application and any limitations with regards to data use/sharing as per the consent should be highlighted.

Restrictions

- Grant proposals must be focused on single cell profiling of pediatric cancer samples.
- Indirect costs are not allowed.
- Funds may not be used for research utilizing human embryonic stem cells or non-human primates. Research with human induced pluripotent stem cells is permissible.

Grant Reporting Requirements

- A final report is required at the conclusion of funding. Report must state findings, expenditures, as well as publications and presentations which acknowledge ALSF funding. No cost extensions must be requested in the final report.
- Publications, presentations and posters featuring results of the experiments funded by this grant mechanism should acknowledge "Alex's Lemonade Stand Foundation". ALSF requests copies be sent via email to Grants@AlexsLemonade.org.

Application Package Instructions

- All sections described below should be combined into one PDF (max 20 MB) and uploaded to the ALSF online application form (see Application Submission Instructions).
- All templates mentioned can be found at ALSF's Information for Grant Applicants page.

Format Instructions

- PAGE HEADER: All pages of the application should be numbered; the name of the principal investigator should appear in the upper right-hand corner of each page.
- FORMAT: Follow NIH format guidelines: Arial, Helvetica, Palatino Linotype, or Georgia fonts with a font size of 11 points or larger with a minimum of ½ inch margins.
- ORDER & LENGTH: The order of the application should be followed, adhering to the maximum number of pages allowed for each subsection indicated in parentheses.

Section Descriptions

1. **Project Information**
    a. **Cover Page (1 page):** Download and complete the Cover Page Template.
    b. **Table of Contents (1 page):** Provide a Table of Contents with page numbers to the corresponding sections.
    c. **Scientific Abstract (0.5 page):** Summarize the research objectives and rationale.
    d. **Impact Statement (0.5 page):** How will this project impact childhood cancer?
2. **Budget/Justification (3 pages):**
    a. **Budget Template (1.5 pages):** Complete the ALSF budget template. The signature from an institutional representative on the cover page of this grant application specifically acknowledges and accepts this budget.
        i. The award amount is $200,000 over 1 year.
        ii. ALSF adheres to the NIH salary cap for principal investigator(s).
        iii. Indirect costs are not allowed.
        iv. If using the ALSF Childhood Cancer Data Lab for single-cell and/or bulk gene expression analysis, include the request as a line item on the ALSF budget template.
        v. If utilizing a sub-contractor, you must include their budget. No indirect costs will be paid to the sub-contractor.
        vi. Reasonable travel expenses to national/international research meetings to disseminate findings may be budgeted.
    b. **Budget Justification (1.5 pages)**
3. **Biographical Sketch(es):** Use the NIH five-page biographical sketch (SF424) format for the principal investigator and all key personnel.
4. **Research Plan**
    a. **Research Objectives (1 page):** List the goals, objectives and what the specific research proposed in this application is intended to accomplish. Specify the cancer type(s) to be assayed, the nature of the samples to be assayed, the number of cells/nuclei expected, what basic metadata is available, what

additional metadata (treatment outcome, additional profiling, etc.) is available, provide a rationale for the cell surface marker(s) selected for characterization, and provide any additional information that supports the importance of single-cell characterization of this set of samples. Investigators should describe the value of the data that they propose to generate in the context of ongoing single-cell profiling efforts under "Human Cell Atlas" and "Human Tumor Atlas" projects. Investigators who are funded to perform single-cell profiling under these or other projects must discuss how their proposed project is distinct from ongoing, funded single-cell profiling efforts.

   b. **Technical Approach (1 page):** Provide the bulk and single cell profiling technologies to be used and a justification of the choice, the depth of characterization in terms of number of cells and reads per sample, the number of samples, any quality control work that will be performed throughout the process and the metrics that will be used as thresholds.
   c. **Literature Cited (no limit):** Use Vancouver or NIH style (numbered citations within text) format.

5. **Human Subjects (1 page)**: If approved, include the IRB approval letter or equivalent. If approval is pending, indicate the expected approval date. Any funds awarded will be held until the letter is received. If IRB approval is not applicable, include a note in this section.
6. **Vertebrate Animals (1 page)**: If approved, include the IACUC approval letter or equivalent. If approval is pending indicate the expected approval date. Any funds awarded will be held until the letter is received. If IACUC approval is not applicable, include a note in this section.
7. **Letters of Support (no limit)**: Include any appropriate letters from individuals confirming their roles in the project. Institutional letters of support are not required, but can be included, especially if there are issues of feasibility that can be addressed.
8. **Resource Sharing (1 page)**: Describe the outputs from the proposed project and how they will be shared. Note that this mechanism has specific requirements, and the requirements should be addressed in this section.
9. **Appendix:** A brief appendix is allowed with the following limitations.
   a. Appendices should be included only if they are essential to the understanding of the application, including one accepted but not yet published manuscript or two pages of additional information such as a summary of the protocol if applicable and/or supplementary figures.
   b. Excessive appendices will result in the application being rejected administratively.

Application Submission Instructions

Applicants must not use the IE browser as it is not compatible with the online portal. Chrome and Firefox browsers are recommended.

1. To start an application, navigate to the portal at ALSFapps.force.com.
    a. Returning applicants: login with your username and password.
    b. First time applicants: click the "New User?" link and complete your one-time registration and then login.
2. After you've logged in, follow the directions on the dashboard to submit your application. Complete the online form with applicant contact and project information.
    a. Enter the Project Title first, even if tentative. Then Save.
    b. Applicant will be asked for basic contact information for themselves, co-PI(s) if applicable, grant manager and institution. If someone other than the PI is entering information into the portal, the "Contact Person" name entered **must** be the PI's name.
    c. In the respective sections enter the project title, budget request amount, type of childhood cancer the project focuses on, as well as a 250-word summary of the research project in lay terms. The applicant will be asked to release this summary for use at ALSF's discretion should the proposal be funded. The applicant may copy and paste information from other documents into these sections.
3. The application document must be uploaded as **one PDF** (maximum of 20 MB).
4. You may save your application to finish later**.** Go to ALSFapps.force.com and login again. You will land on your dashboard. Click "Applications" to edit your application in progress.
5. Once completed, submit the application by clicking Review & Submit. You will see error messages for any required fields that need to be completed.
6. After your application has been successfully submitted an email confirmation will be sent. You will not be able to amend the application after submission.

Contact

- If you have any questions regarding this grant mechanism, reach out to Anna Greene, Director of Science, at 866-333-1213 or by email at a.greene@alexslemonade.org.