

**AWARD NUMBER:** W81XWH-19-1-0196

**TITLE:** Precision Combinatorial Immunotherapeutic Targeting of Cytokine Receptor Kinase Signaling in CRLF2-Rearranged ALL

**PRINCIPAL INVESTIGATOR:** Terry Fry, MD

**CONTRACTING ORGANIZATION:**

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# REPORT DOCUMENTATION PAGE

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<b>14. ABSTRACT:</b> Ph-like ALL is a high-risk subset of B-ALL defined by an activated kinase gene expression profile similar to that of BCR-ABL1-rearranged (Ph+) ALL and driven by a diverse range of genetic alterations that activate cytokine receptor signaling pathways. Children, AYAs, and older adults with Ph-like ALL have >60% relapse risk and experience significant leukemia-associated mortality. Approximately 50% of Ph-like ALL cases harbor rearrangements in CRLF2 (CRLF2-R) and frequent concomitant JAK2 point mutations. In addition to patients with Ph-like ALL, CRLF2 rearrangements (usually P2RY8-CRLF2 fusions) with JAK2 point mutations occur in approximately 60% of children and AYAs with trisomy 21/Down Syndrome-associated ALL (DS-ALL) and also induce hyperactive JAK/STAT signaling. Children with DS-ALL have substantial toxicity with chemotherapy and inferior clinical outcomes. CD19CART immunotherapy has proven highly successful at inducing remissions in 80-90% of patients with relapsed/refractory ALL. However, emerging data indicates that up to 50% of children and AYAs will relapse, most within a year. As an alternative strategy, the Fry laboratory developed CAR constructs targeting the TSLPR (encoded by CRLF2) and demonstrated potent in vivo activity of T cells transduced with anti-TSLPR CAR constructs (TSLPRCART) in CRLF2-R Ph-like ALL PDX models generated by the Tasian laboratory. Based on our promising preclinical data, a phase 1 clinical trial of TSLPRCART for children and AYAs with relapsed CRLF2/TSLPR-overexpressing ALL will soon open at the NIH. TKIs and CART immunotherapies have the potential to act synergistically in acute leukemias via co-targeting of oncogenic pathways using two distinct approaches: one (CART) targeting a cell surface cytokine receptor protein and the other (TKI) targeting critical receptor-mediated and intracellular kinase signaling pathways. Furthermore, combining multi-targeted CAR T cells with TKIs is strategically analogous to the paradigm of non-cross-resistant cytotoxic chemotherapy regimens that is required to achieve cure in children with ALL.					
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**1. INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Ph-like ALL is a high-risk subset of B-ALL defined by an activated kinase gene expression profile similar to that of *BCR-ABL1*-rearranged (Ph+) ALL and driven by a diverse range of genetic alterations that activate cytokine receptor signaling pathways. Children, AYAs, and older adults with Ph-like ALL have >60% relapse risk and experience significant leukemia-associated mortality. Approximately 50% of Ph-like ALL cases harbor rearrangements in *CRLF2* (*CRLF2-R*) and frequent concomitant *JAK2* point mutations. In addition to patients with Ph-like ALL, *CRLF2* rearrangements (usually *P2RY8-CRLF2* fusions) with *JAK2* point mutations occur in approximately 60% of children and AYAs with trisomy 21/Down Syndrome-associated ALL (DS-ALL) and also induce hyperactive JAK/STAT signaling. Children with DS-ALL have substantial toxicity with chemotherapy and inferior clinical outcomes. CD19CAR immunotherapy has proven highly successful at inducing remissions in 80-90% of patients with relapsed/refractory ALL. However, emerging data indicates that up to 50% of children and AYAs will relapse, most within a year. As an alternative strategy, the Fry laboratory developed CAR constructs targeting the TSLPR (encoded by *CRLF2*) and demonstrated potent *in vivo* activity of T cells transduced with anti-TSLPR CAR constructs (TSLPRCART) in *CRLF2-R* Ph-like ALL PDX models generated by the Tasian laboratory. Based on our promising preclinical data, a phase 1 clinical trial of TSLPRCART for children and AYAs with relapsed *CRLF2*/TSLPR-overexpressing ALL will soon open at the NIH. TKIs and CART immunotherapies have the potential to act synergistically in acute leukemias via co-targeting of oncogenic pathways using two distinct approaches: one (CART) targeting a cell surface cytokine receptor protein and the other (TKI) targeting critical receptor-mediated and intracellular kinase signaling pathways. Furthermore, combining multi-targeted CAR T cells with TKIs is strategically analogous to the paradigm of non-cross-resistant cytotoxic chemotherapy regimens that is required to achieve cure in children with ALL. This application is directly relevant to FY18 PRCRP Topic Areas of (1) Blood Cancer, (2) Immunotherapy, and (3) Cancer in Children, Adolescents, and Young Adults. *The primary hypothesis of this proposal is that durable remissions in patients with CRLF2-R Ph-like ALL or DS-ALL can be achieved using rationally combined immune and molecular kinase therapies that target critical and necessary signaling pathways in malignant cells.* The project is divided into the following Aims:

**Aim 1.** Develop combinatorial CAR constructs targeting TSLPR plus CD19 and/or CD22 and test the anti-leukemia efficacy of multi-targeted CARTs against *CRLF2-R* ALL in children, adults, and DS patients.

**Aim 2.** Determine the preclinical efficacy of multi-specific CARTs and kinase inhibitors against *CRLF2-R* ALL.

**Aim 3.** To delineate the impact of DS-associated immunodeficiency and aging on the potency of CART generated from patients with DS-ALL and to determine functionality of autologous T cell transduction for clinical immunotherapy with CARTs.

**Study Design:** These complementary and imminently clinically-translatable studies will test and validate the potential treatment efficacy of multi-targeted CART immunotherapy and/or kinase inhibition in multiple subtypes of *CRLF2-R* ALL characterized by TSLPR overexpression and hyperactivation of cytokine signaling, as well as increase our understanding of T cell functionality and therapeutic potential in patients with trisomy 21. In this work, we will develop synergistic, non-

overlapping treatment strategies to improve leukemia remission durability and to mitigate development of immunotherapeutic resistance in three very high-risk subtypes of B-ALL.

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

Acute lymphoblastic leukemia, immunotherapy, kinase, cytokine receptor, thymic stromal lymphopoietin, chimeric antigen receptor, t cell

**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.*

**Specific Aim 1: Develop combinatorial CAR constructs targeting TSLPR plus CD19 and/or CD22 and test the antileukemia efficacy of multi-targeted CARTs against CRLF2-R ALL.**

Major task 1: design of bispecific TSLPR x 19/22 CAR constructs & T cell manufacturing

Major task 2: new ALL PDX model creation

- expand previously established childhood/adolescent *CRLF2-R* Ph-like ALL for subsequent experimental therapeutic studies
- establish 2-4 primary xenograft models of adult *CRLF2-R* Ph-like ALL and expand in secondary recipients for subsequent experimental therapeutic studies
- establish 2-4 primary xenograft models of DS-ALL and expand in secondary recipients for subsequent experimental studies

Major task 3: *in vitro* testing of TSLPRCARTs with *CRLF2-R* ALL cell lines MUTZ5 and MHH-CALL-4

- cytokine production assays (IL-2, IFN-gamma)
- cytotoxicity assays (Cell Titer Glo) with ALL cell lines + CAR T cells +/- TKI

Major task 4: *in vivo* testing of TSLPRCARTs

- preclinical testing of TSLPRCART and ruxolitinib monotherapies in bioluminescent *CRLF2-R* cell line MUTZ5 xenograft models
- preclinical testing of TSLPRCART + ruxolitinib in bioluminescent *CRLF2-R* cell line MUTZ5 xenograft models
- preclinical testing of TSLPRCARTxCD19 in bioluminescent *CRLF2-R* cell line MUTZ5 xenograft models
- preclinical testing of TSLPRCARTxCD22 in bioluminescent *CRLF2-R* cell line MUTZ5 xenograft models

Major task 5: TSLPR surface density quantification of phase 1 clinical trial ALL patient samples

- NIH TSLPRCART trial to open in Q2 2021; estimated patient accrual over 2-3 years
- Flow cytometric immunophenotyping of bone marrow TSLPR surface antigen density at study entry (and relapse if applicable)

Milestones achieved: identification of optimal combinatorial TSLPRCART(s) in vivo in CRLF2-R ALLs, final data analysis

**Specific Aim 2: Determine the preclinical efficacy of multi-specific CARTs and JAKi against CRLF2-R ALL**

Major task 1: *in vivo* testing of TSLPRCARTs/TKIs peds ALL PDXs

- preclinical testing and efficacy comparison of TSLPRCART, CD19CART, CD22CART, TSLPRxCD19CART, TSLPRxCD22CART in 2-4 childhood CRLF2-R ALL PDX models (see tables below for cohort and total mouse numbers)
- preclinical testing and efficacy comparison of TSLPRCART + ruxolitinib in 2-4 childhood CRLF2-R ALL PDX models

Major task 2: *in vivo* testing of TSLPRCARTs/TKIs adult ALL PDXs

- preclinical testing and efficacy comparison of TSLPRCART, CD19CART, CD22CART, TSLPRxCD19CART, TSLPRxCD22CART in 2-4 adult CRLF2-R ALL PDX models
- preclinical testing and efficacy comparison of TSLPRCART + ruxolitinib in 2-4 adult ALL CRLF2-R ALL PDX models

Major task 3: *in vivo* testing of TSLPRCARTs/TKIs DS-ALL PDXs

- preclinical testing and efficacy comparison of TSLPRCART, CD19CART, CD22CART, TSLPRxCD19CART, TSLPRxCD22CART in 2-4 CRLF2-R DS-ALL PDX models
- preclinical testing and efficacy comparison of TSLPRCART + ruxolitinib in 2-4 DS-ALL CRLF2-R ALL PDX models

Major task 4: ALL TKI effects *in vitro* and *in vivo*

- Normal T cell donor flow cytometric immunophenotyping (pediatric Ph-like, adult Ph-like, children with trisomy 21/DS)
- T cell and ruxolitinib coincubation studies: T cell subset flow immunophenotyping, cell death (Annexin-V/PI flow cytometry, cell counting), cell proliferation (CSFE assays)
- TSLPRCART and ruxolitinib coincubation studies: cytokine quantification, effects on proliferation

Milestone(s) Achieved: selection of optimal TSLPRCART/TKI therapy *in vivo* in each CRLF2+ ALL subtype, final data analysis

**Specific Aim 3: To delineate the impact of immunodeficiency associated with Down Syndrome and aging upon T transduction efficiency and functionality of CARTs.**

Major task 1: B-ALL adult patient T cell functionality for CAR engineering

- Identification of patient samples at Penn (n=20-30)
- T cell flow immunophenotyping, analysis of senescence biomarkers, pre/post-treatment Treg suppression
- RNAseq to measure TCR repertoire diversity before and after T cell expansion and CAR construct transduction

Major task 2: DS-ALL T cell functionality for CAR engineering

- Identification of patient samples at CHOP and Colorado (n=6-12)
- T cell flow immunophenotyping, analysis of senescence biomarkers, pre/post-treatment Treg suppression
- RNAseq to measure TCR repertoire diversity before and after T cell expansion and CAR construct transduction

*Milestone(s) Achieved: elucidation of T cell biologic features that contribute to CAR T cell success and failure, final data analysis.*

### **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 activities to reporting accomplishments.*

Specific Aim 1 Progress to date:

\* *Note bene*: Please note that our research laboratories were closed from March to July 2020 due to institutional research restrictions during the COVID-19 pandemic.

1. Task 1: TSLPR x CD19 construct engineering done with in vitro and in vivo experiments in cell line xenograft models now ongoing.
2. Task 2: Childhood and adult ALL PDX models expansion complete. Seven new DS-ALL PDX models established in primary mice and have now completed secondary expansion.
3. Task 3: MUTZ5 in vitro cytotoxicity and cytokine production pilot studies completed.
4. Task 4: TSLPRCART and ruxolitinib in vivo testing in MUTZ5 xenograft models for dose and timing optimization. Two additional PDX model studies now completed. Some correlative biology assessments in process/results pending.
5. Task 5: Phase 1 TSLPR CAR T cell clinical trial is not yet open due to administrative delays at the NIH. Anticipated protocol submission to IRB in Q1 2021.

Specific Aim 2 Progress to date:

\* *Note bene*: Please note that our research laboratories were closed from March to July 2020 due to institutional research restrictions during the COVID-19 pandemic.

1. Task 1: PDX model studies ongoing with TSLPR CAR T cells and ruxolitinib. Bispecific TSLPRxCD19 and TSLPRxCD22 CAR constructs made. Experimental

- troubleshooting ongoing in antigen-combination Nalm-6 xenograft models for in vivo testing of bispecific CAR T cells.
2. Task 2: All adult PDX models established, no in vivo testing yet
  3. Task 3: All DS-ALL PDX models now established. In vivo testing of ruxolitinib now ongoing.
  4. Task 4: Normal T cell studies with ruxolitinib completed; in process of obtaining normal T cells from DS patients at Colorado (unable to access easily at CHOP after much investigation).

Specific Aim 3 Progress to date:

\* *Note bene*: Please note that our research laboratories were closed from March to July 2020 due to institutional research restrictions during the COVID-19 pandemic.

1. Task 1: Identified normal T cell donor sources at CHCO and CHOP; no experimental studies done yet. New single-cell/RNAseq studies pending in sorted CAR T cells from above xenograft mice studies to assess T cell expression changes in vivo over time.
2. Task 2: Identifying new collaborator at CHCO for normal DS T cell specimens. Access to DS samples at CHOP has been challenging.

**What 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.*

Three post-doctoral fellows and one junior faculty member are actively leading hands-on work for this award in our respective laboratories at Children’s Hospital Colorado and Children’s Hospital of Philadelphia. Two young research technicians (who will soon attend medical school or graduate school) are also assisting with experiments in our labs. Trainees are encouraged to present their work formally at lab meetings and scientific symposia, as well as to submit abstracts to and attend national hematology meetings. Trainees meet regularly one-on-one with their mentors Drs Fry and Tasian and regularly attend scientific seminars/workshops at their institutions to increase their knowledge and continue their career development.

**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.*

Research-in-progress and results have been shared locally with other scientific researchers at our institutions. Scientific abstracts of some of this work were submitted to the Biennial Childhood Leukemia & Lymphoma Symposium/annual iBFM 2020 meeting (postponed to 2021 due to the viral pandemic) and to the AACR 2020 annual meeting (virtual meeting poster presentation).

**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 will continue to make experimental progress to achieve the aims of the award according to the proposal timeline. We will continue to submit abstracts of our work and will plan research manuscript writing of initial results in the next year of this award.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

**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 made important discoveries about the impact of the JAK inhibitor ruxolitinib upon normal T cell and TSLPR CAR T cell function *in vitro* and *in vivo* in our leukemia studies. These preclinical results will definitively impact our design of and planning for clinical trials testing new precision medicine treatments for patients with *CRLF2*-rearranged ALL. In addition to our established Ph-like ALL models, we have also successfully developed several important new mouse models of pediatric Down Syndrome-associated ALL that provide a critical resource for this project and could also be shared with the greater scientific community in the future.

**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.*

Results from our work have broad implications for studies of other cellular immunotherapies and tyrosine kinase inhibitors in human cancer models and clinical trial development.

**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 in this reporting period. There is potential for future engagement of biotech company collaboration as we translate results from these preclinical studies to clinical trials.

**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:*

**Changes in approach and reasons for change**

*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

Nothing to report

**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.*

Please note that our research laboratories were closed from March to July 2020 due to institutional research restrictions during the COVID-19 pandemic. Both Fry and Tasian laboratories are now fully operational, albeit at reduced density.

**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 laboratory shut-downs from March to July 2020 resulting from the COVID-19 pandemic, experimental expenditures were lower than anticipated during the months March-June.

**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**

Nothing to report.

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

• **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).*

Nothing to Report

**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).*

Two abstracts of our work were submitted during the annual reporting period:

1. Bagashev *et al.* Co-Targeting of Thymic Stromal Lymphopoietin Receptor Signaling to Decrease Immunotherapeutic Resistance in *CRLF2*-Rearranged ALL. 12<sup>th</sup> Biennial Childhood Leukemia and Lymphoma Symposium abstract CLLS20-0053 (May 2020; Valencia, Spain - oral presentation; meeting delayed to March 2021 and will be held virtually due to viral pandemic)
2. Ross *et al.* Multi-Antigen Targeting of CD19, CD22 and TSLPR to Prevent Ph-like ALL Resistance. AACR 2020 annual meeting abstract #3234 (Chicago, Illinois; June 2020 - poster presentation; meeting held virtually due to viral pandemic) [https://cancerres.aacrjournals.org/content/80/16\\_Supplement/3234](https://cancerres.aacrjournals.org/content/80/16_Supplement/3234)

**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.*

Nothing to report.

- **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.*

During the course of this project, we have successfully developed several important new patient-derived xenograft (PDX) models of *CRLF2*-overexpressing Ph-like ALL and Down Syndrome-associated ALL. These PDX models are a critical resource for this project for in vivo investigation and could also be shared with the greater scientific community in the future.

- **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.*

Nothing to report.

## **7. 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”.*

Name: Terry Fry, MD (CHCO)  
Project Role: PI  
Researcher Identifier (e.g. ORCID ID): 0000-0001-8044-5226  
Nearest person month worked: 2.4 Calendar  
Contribution to Project: Dr. Fry prepared documentation necessary for initiation of research to be conducted on the proposal and oversees the entire project with Dr. Tasian.

Name: Lillie Leach, BS (CHCO)  
Project Role: research technician  
Researcher Identifier (e.g. ORCID ID): none  
Nearest person month worked: 0.6 Calendar  
Contribution to Project: Ms Leach has assisted with TSLPR CAR T cell manufacturing in the Fry laboratory following departure of Dr Ross from CHCO at completion of her post-doctoral fellowship. All animal studies at CHCO commenced after obtainment of ACURO approval.

Name: Sarah Tasian, MD (CHOP)  
Project Role: Co-PI  
Researcher Identifier (e.g. ORCID ID): 0000-0003-1327-1662  
Nearest person month worked: 2.4 Calendar  
Contribution to Project: Dr. Tasian prepared documentation necessary for initiation of research to be conducted on the proposal and oversees the entire project with Dr. Fry.

Name: Asen Bagashev, PhD (CHOP)  
Project Role: Scientist  
Researcher Identifier (e.g. ORCID ID): 0000-0003-9900-8106  
Nearest person month worked: 3 Calendar  
Contribution to Project: Dr Bagashev is responsible for *in vitro* and *in vivo* studies performed at CHOP in the Tasian laboratory. All animal studies commenced after obtainment of ACURO approval.

Name: Joseph Loftus, BS (CHOP)  
Project Role: Research technician  
Researcher Identifier (e.g. ORCID ID): none  
Nearest person month worked: 3 Calendar  
Contribution to Project: Mr Loftus is responsible creation and maintenance of the needed ALL PDX models at CHOP in the Tasian laboratory. All animal studies commenced after obtainment of ACURO approval.

**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.*

Nothing to report.

**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:* Stand Up to Cancer co-funded by the Emily F Whitehead Foundation

*Location of Organization:* New York/California and Pennsylvania

*Partner’s contribution to the project (identify one or more):* We received a two-year Phillip A Sharp Award for Innovation in Collaboration for an extension of these Department of Defense Studies (funded June 2019 to May 2021). This additional funding has primarily supported development and serial transplantation of our Down Syndrome ALL PDX models in costly immunocompromised mice with detailed genetic characterization. There is no budgetary overlap with the Department of Defense award.

## **8. 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://ers.amedd.army.mil> for each unique award.*

Please see detailed reports and allocation of research efforts for Drs Fry and Tasian detailed in our quarterly progress reports and most recent update in October 2020.

**QUAD CHARTS:** *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*