

# Children's Hospital of Philadelphia

## Annual Progress Report: 2011 Formula Grant

### Reporting Period

July 1, 2013 – June 30, 2014

### Formula Grant Overview

The Children's Hospital of Philadelphia received \$3,521,179 in formula funds for the grant award period January 1, 2012 through December 31, 2015. Accomplishments for the reporting period are described below.

### Research Project 1: Project Title and Purpose

*Highly Active Cell Therapy of Cancer* – Our purpose is to develop engineered T cell therapies for B cell malignancies, leukemias, and certain specific solid tumors such as neuroblastoma and synovial sarcoma. Using chimeric antigen receptors (CARs) which target tumors and activate T cells (CART cells), and an efficacious clinical-grade (GMP) ex vivo cell manufacturing system, we will continue our highly promising use of CAR-engineered T cells. This grant will support preclinical studies to optimize CARs in mouse xenograft models, as well as early phase clinical trials testing a variety of CAR-mediated T cell therapy approaches.

### Anticipated Duration of Project

1/1/2012 – 12/31/2015

### Project Overview

The overall goal of this Pennsylvania Department of Health Formula Project is to develop clinically efficacious methods of treating high-risk and relapsed leukemia, lymphoma and some solid tumors with chimeric antigen receptor (CAR)-armed T cells. The long-term goal of our cell therapy group is to establish improved treatments for hematologic malignancies and other tumors by engineering, optimizing, and clinically testing these highly active anti-cancer T cells. This treatment approach could potentially obviate the need for allogeneic stem cell transplant for some patients. Our preliminary clinical data showing cures of patients with high burdens of refractory tumor provide the first clear proof of concept and proof of mechanism for an anti-cancer cell therapy, and are potentially paradigm-shifting. They suggest that the central problems of expansion and persistence of therapeutic cells in the patient after infusion are solvable using the right cell manufacturing system and the right CAR design. With successful expansion and long-term persistence in the patient, even small doses of T cells can lyse very large tumor burdens. In order to leverage the dramatic results we have seen and continue to develop these approaches for pediatric cancer patients, we are proposing a 4 year combined basic/translational cell therapy pro-

gram for CHOP. We propose 3 Aims to build on these results and establish cell therapy infrastructure at CHOP:

Aim 1. Develop novel CARs targeting antigens other than CD19, and develop RNA transfection as an alternative approach to lentiviral transduction to temporarily express CARs on T cells.

Aim 2. Perform 3 cell therapy trials testing CD19-targeted CAR+ T cell approaches in patients with B cell malignancies such as acute lymphoblastic leukemia (ALL), CLL and non-Hodgkin lymphoma (NHL).

Aim 3. Using CARs and approaches developed in Aim 1, take engineered T cell-based therapy into trials enrolling non-B cell cancers, including sarcoma (target NY-ESO-1), acute myelogenous leukemia (AML; target mesothelin), and neuroblastoma (target GD2).

### **Principal Investigator**

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### **Other Participating Researchers**

David Barrett, MD PhD – employed by The Children's Hospital of Philadelphia  
Carl H. June, MD; Bruce L. Levine, PhD; Yangbing Zhao, MD PhD – employed by the University of Pennsylvania

### **Expected Research Outcomes and Benefits**

Expected outcomes and benefits include the following:

1. Initiate a trial to test CAR-T cell therapy in children with CD19+ malignancies. CARs are chimeric antigen receptors that redirect T cells to cancer cells and activate the T cells so they kill the tumor. Any cancer target or tumor-associated antigen which is recognized by an antibody can in principle be made into a CAR.
2. Develop mRNA-based CARs to supplement permanent modification of T cell by lentivirus. We anticipate gaining a better understanding of the performance of mRNA CAR+ T cells in animal models that will guide our use of them in the clinic.
3. A better understanding of the nature and phenotype of long-term engrafting T cells and their impact on disease control in animal models.
4. Extend the CD19 CAR concept to mRNA CARs and to the allogeneic transplant setting.
5. Develop CARs that can target non B cell tumor antigens, to test CARs against AML and solid tumors.
6. Further develop clinically relevant xenograft models of pediatric cancer, translating data from these models to clinical trial design.

7. The overall goal is improve treatment options for patients with high-risk and relapsed cancers.

## Summary of Research Completed

### Milestones for this year:

- *Open a 3rd cell therapy trial at CHOP.* Accomplished. Current trials open at CHOP are:
  - CHP959 IRB 7706 CART19 engineered T cell trial: post stem cell transplant (SCT or allo) cohort\*
  - CHP959 IRB 7706 CART19 trial: non-SCT cohort\*  
\* combined into a single trial at FDA request
  - IRB 9915 biology trial – Lymphocyte functional capacity for immunotherapy
  - 13BT022 IRB 10763 – humanized CART19 trial
  - 11BT053 IRB 8648 – NY-ESO1 T cell receptor (TCR)-engineered T cells for synovial sarcoma
- *Continue accrual to cell therapy trials.* Accomplished. Total accrual to CHOP959 now 21 patients to SCT cohort, 11 to non-SCT cohort; 9915, 35 patients; 13BT022, 1 patient.
- *Collect biology samples on each treated/infused cell therapy patient.* Accomplished. Results reported in New England Journal of Medicine (NEJM) paper (cited #11, below). We have had 100% of patients with samples collected and >98% individual sample collection compliance on each study.
- *Report results from lab studies at a national meeting.* Accomplished – multiple abstracts at the American Society of Hematology and other national meetings. 8 abstract references from this grant period will be provided in final report.

### Report

The long-term goal of our the CHOP Cell Therapy Group (CCTG) is to establish improved treatments for leukemias and other tumors by engineering, optimizing, and clinically testing highly active anti-cancer T cells. This treatment approach could potentially replace expensive and risky bone marrow transplants for some patients. CCTG has made significant progress over the past year in both clinical trials and lab work exploring T cells engineered with chimeric antigen receptors (CARs). Our work has been directed toward testing the hypothesis that pediatric cancers can be targets for CAR-engineered T cells.

Summary of current work for Aim 1: AML CARs. In this work, we have identified CD123 expression on the majority of tested AML specimens and developed new anti-CD123 CAR T cells (CART123) for preclinical testing, advancing ahead of our initially proposed target, which was mesothelin. We demonstrated in vitro potency and specificity of CART123 against AML via degranulation assays and cytotoxicity assays using both AML cell lines and primary human AML specimens. We further demonstrated in vivo efficacy of CART123 treatment of immunocompromised mice engrafted with human AML via bioluminescent imaging (GFP+/luciferase+ MOLM14 xenograft models; Figure 1) and by quantitative flow cytometry (primary human AML xenograft models, see ref #9). The majority of MOLM14 mice treated with CART123 cleared their leukemias and were long-term survivors in comparison to saline-treated or CART19-treated mice (negative controls). Some CART123-treated primary AML xenografts

also cleared their leukemias, but many mice with high AML burdens appeared ill at 7-10 days post-CART123 treatment and died (ref #9). Subsequent analyses suggest that mice died with massive tumor lysis syndrome and/or cytokine release syndrome, highlighting the in vivo potency of these CARs. Analyses of mice engrafted with normal human hematopoietic cells that were subsequently treated with CART123 demonstrated significant myeloablation, highlighting the potential for hematologic toxicity. A manuscript detailing this work was just published in Blood (#9, below). The plan is to take CART123 forward on an RNA-transfected T cell platform if ongoing testing in non-human primates (outside the scope of this grant) show acceptable toxicity.

Aim 2 (ALL and NHL): Status of the clinical trials. We are accruing children to both allo/SCT and non-allo/SCT cohorts on CHP-959: Pilot Study of Redirected Autologous T Cells Engineered to Contain Anti-CD19 Attached to TCRz And 4-1BB Signaling Domains in Patients with Chemotherapy Resistant or Refractory CD19+ Leukemia and Lymphoma.

As indicated above, the second cell therapy trial we proposed in the grant, using allogeneic CART19 cells, is now accruing as Cohort 2 in the above trial at the FDA's suggestion. We have accrued 11 patients to Cohort 1 (no prior allo SCT) and 21 patients to Cohort 2 (s/p allo SCT). This accrual is well in excess of our anticipated accrual rate, and fortunately the Cell and Vaccine Production Facility at U Penn has been able to support the production of 3-4 cell therapy products per month for our trial. We have reported results from 25 pediatric and 5 adult patients, which is now in press in the New England Journal of Medicine (reference #11 below). This trial of chimeric antigen receptor (CAR)-modified T cells targeting CD19 was developed for patients with relapsed/refractory ALL. Autologous T cells transduced with a CD19-directed CAR (CART19/CTL019) lentiviral vector were infused into patients with relapsed/refractory ALL at doses of 0.76-20.6x10<sup>6</sup> CTL019 cells/kg. Patients were monitored for response, toxicity, expansion and persistence of circulating CTL019 T cells. 30 patients with relapsed/refractory ALL received CART19/CTL019. CRs were achieved in 27/30 patients (90%), including 2 blinatumomab-refractory patients and 15 with prior SCT. CTL019 cells proliferated in vivo and were detectable in blood, bone marrow, and cerebrospinal fluid of responding patients. Sustained remissions were achieved with 6-month event-free survival of 67% (95% confidence interval [CI], 51-88%) and overall survival of 78% (95% CI, 65-95%). Probability of 6-month CTL019 persistence was 68% (95% CI, 50-92%) and relapse-free B cell aplasia was 73% (95% CI, 57- 94%). All patients experienced cytokine release syndrome (CRS). Severe CRS, seen in 27% of patients, was associated with higher disease burden and effectively treated with the anti-IL-6 receptor antibody tocilizumab. These results from the ongoing CART19 study show that CAR-modified T-cell therapy against CD19 is effective for relapsed/refractory ALL. CART19/CTL019 achieved a high remission rate even in patients for whom previous SCT had failed, and durable remissions up to 26 months have been observed.

These study results have driven the development of a phase 2 multicenter trial in pediatric ALL, which opened on 7/16/14. The goal of this trial is to provide registration data to the Food and Drug Administration in the hope that we will be able to turn this PA DOH-supported cell therapy into an FDA approved therapy. Our compelling data underpin this hope, as does the recent decision by the FDA to award this therapy with the Breakthrough Therapy designation, the first cell therapy treatment to receive this designation and the first time an academic group has received

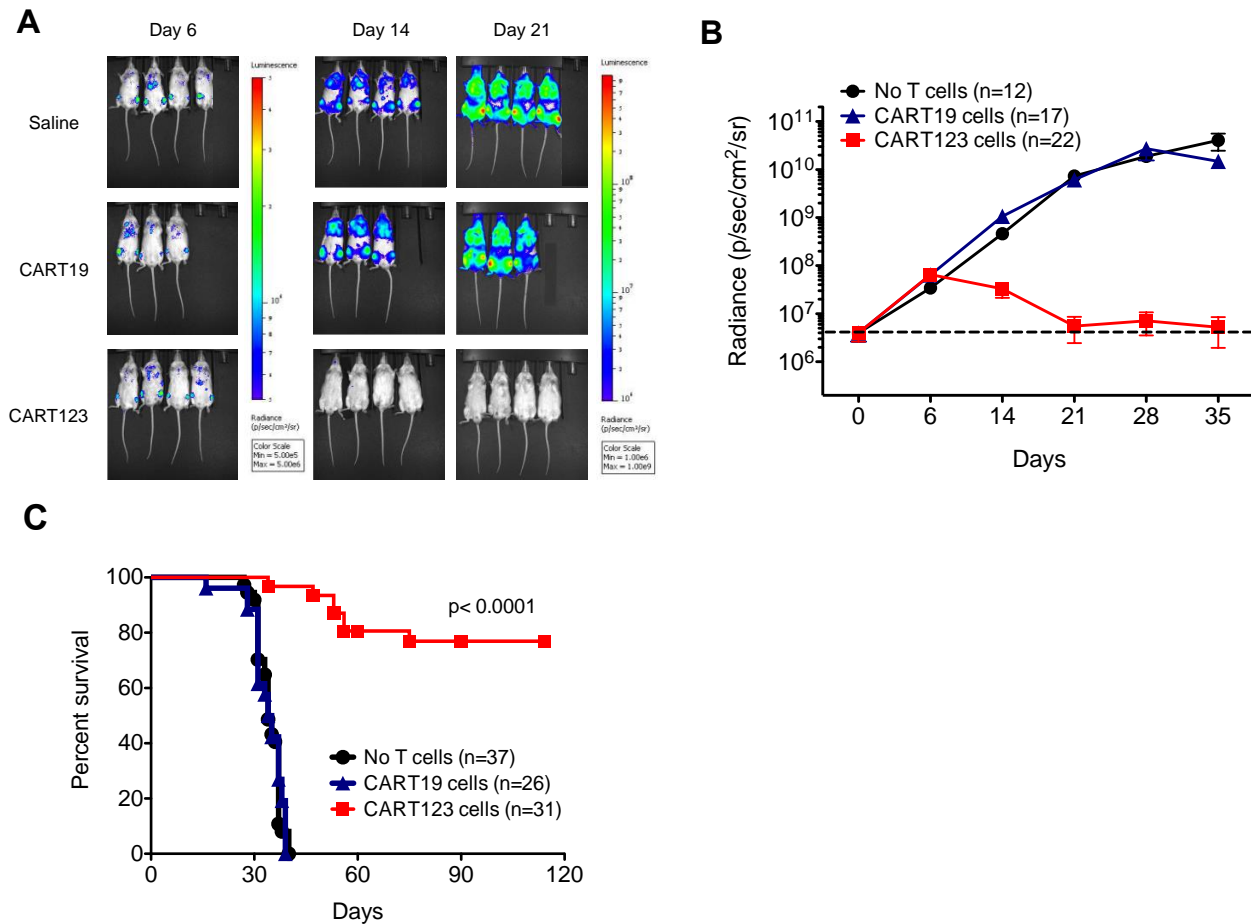
such a designation ([http://www.uphs.upenn.edu/news/News\\_Releases/2014/07/ctl019/](http://www.uphs.upenn.edu/news/News_Releases/2014/07/ctl019/)). The drug company Novartis is also investing in and has licensed this CAR T cell technology.

Aim 3 (Neuroblastoma and sarcoma): Status of the preclinical work and the clinical trials. A sarcoma study is open (NY-ESO1 TCR, see above) but has yet to accrue at CHOP. Expanding eligibility to other NY-ESO+ pediatric tumors continues to be discussed but has yet to be implemented. Preclinical studies on neuroblastoma are complete and have been submitted for publication (Singh et al., Cancer Immunology Research, revision invited). This will be reported next year when the paper is accepted. A protocol for treatment of neuroblastoma with GD2-targeted RNA CAR T cells has been written, GMP (clinical grade) RNA has been made, and the IND will be submitted next week.

Papers published with CURE grant support.

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\*equal contribution
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**Figure 1. Preclinical efficacy of CAR123 T cells in human AML xenograft models.** NOD/SCID/IL-2 receptor- $\gamma$ -chain-deficient (NSG) mice are irradiated on D0, then injected via tail vein with GFP/luciferase+ MOLM14 (human AML cell line) cells on D1. Bioluminescent imaging (BLI) is performed on Day 6 to quantify engraftment and randomize treatment groups. Vehicle (negative control), CART19 cells (negative control), or CART123 cells are injected IV on Day 7, and mice are followed with serial BLI. Quantification of BLI radiance is used as a surrogate measurement of AML burden. **(A)** Eradication of human AML occurs only in mice treated with CART123 cells, as measured by BLI radiance and displayed colorimetrically. In this false color image, blue represents low level light emission from leukemic disease burden, then green, then yellow, then red as very high disease burden. **(B)** Summary BLI data from three MOLM14 xenograft experiments demonstrate rapid leukemic progression in vehicle-treated (black) and CART19-treated (blue) mice, while AML eradication is observed in CART123-treated mice (red). Mean radiance with standard error of the mean (whiskers) are depicted at each timepoint. **(C)** Survival analysis of MOLM14 xenograft mice demonstrates significant survival of CART123-treated mice in comparison to vehicle- and CART19-treated mice. Attrition of CART123 T cell-treated mice was primarily due to BLI-detectable AML progression in facial bones and subsequent anorexia and weight loss. Summary data are from four experiments.