

AWARD NUMBER: W81XWH-07-1-0250

TITLE: Development of Augmented Leukemia/Lymphoma-Specific T-Cell  
Immunotherapy for Deployment with Haploidentical Hematopoietic Progenitor-  
Cell Transplant

PRINCIPAL INVESTIGATOR: Laurence Cooper, M.D., Ph.D.

CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center  
Houston, TX 77030

REPORT DATE: May 2010

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

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# REPORT DOCUMENTATION PAGE

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<b>1. REPORT DATE</b> 1 May 2010			<b>2. REPORT TYPE</b> Annual		<b>3. DATES COVERED</b> 15 Apr 2009 – 14 Apr 2010	
<b>4. TITLE AND SUBTITLE</b>  Development of Augmented Leukemia/Lymphoma-Specific T-Cell Immunotherapy for Deployment with Haploidentical Hematopoietic Progenitor-Cell Transplant					<b>5a. CONTRACT NUMBER</b>	
					<b>5b. GRANT NUMBER</b> W81XWH-07-1-0250	
					<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b>  Laurence Cooper, M.D., Ph.D.  E-Mail: <a href="mailto:ljncooper@mdanderson.org">ljncooper@mdanderson.org</a>					<b>5d. PROJECT NUMBER</b>	
					<b>5e. TASK NUMBER</b>	
					<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  The University of Texas MD Anderson Cancer Center Houston, TX 77030					<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012						
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited					<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
					<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>13. SUPPLEMENTARY NOTES</b>						
<b>14. ABSTRACT</b> This project develops novel immune-based therapies to target childhood leukemia. By combining gene therapy with T-cell therapy we have developed new biologic agents that specifically recognize CD19 molecule on the cell surface of B-cell acute lymphoblastic leukemia (B-ALL). To translate this approach to the clinic we have adapted the Sleeping Beauty (SB) transposon/transposase system to express a CD19-specific chimeric antigen receptor (CAR). T cells that have undergone transposition express the CAR and therefore redirected specificity for CD19. To selectively propagate CD19-specific T cells to clinically-meaningful numbers we have developed artificial antigen presenting cells (aAPC) by expressing CD19 molecule and desired co-stimulatory molecules on K562 cells. Thus, by combining SB transposition and aAPC, we can harness gene therapy and T-cell therapy to develop targeted treatment for B-ALL.						
<b>15. SUBJECT TERMS</b> Leukemia, Lymphoma, Transplant, Transplantation: bone marrow and stem cell, T-Cell Immunotherapy						
<b>16. SECURITY CLASSIFICATION OF:</b>				<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC
<b>a. REPORT</b> U	<b>b. ABSTRACT</b> U	<b>c. THIS PAGE</b> U	<b>19b. TELEPHONE NUMBER</b> (include area code)			

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## INTRODUCTION

The annual report submitted May 14, 2010 was revised based on email from James Phillips on October 16, 2010 and which is reprinted below. Additional details to the prior report are provided in reference to the SOW. We apologize for any confusion.

### **Cooper, Laurence**

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**From:** Livingston, Juanita E Ms CIV USA MEDCOM USAMRMC [Juanita.Livingston@us.army.mil]  
**Sent:** Saturday, October 16, 2010 2:08 PM  
**To:** Cooper, Laurence  
**Subject:** DOD Award W81XWH-07-1-0250 Annual Report Disapproved (UNCLASSIFIED)

**Follow Up Flag:** Follow up  
**Flag Status:** Flagged

Classification: UNCLASSIFIED  
Caveats: NONE

Re: Report Disapproved  
DOD Award W81XWH-07-1-0250 Review of Annual Report Dated May 2010

Dr. Cooper:

Review of the subject report has been completed. The report is unacceptable as written. We are attaching the reviewer's comments and ask that you revise the report to incorporate the reviewer's recommendations.

Comments to PI: The report is not written in the correct format. It is not possible to determine what activities were performed this past year in accordance with the SOW. Please submit a revised progress report that better details what was accomplished this past year. You may reference publications only to support discussion in the report and cite only specific data to which you are referring and not the entire publication. The correct format for reports is in the original assistance agreement. Additionally, the Key Research Accomplishments section in an annual report should be focused solely on key findings during the reporting period, not those previously reported or on milestones achieved.

A REPORT REVIEW IS NOT BEING PROVIDED FOR THIS REPORTING PERIOD DUE TO THE RECEIPT OF A MORE RECENT REPORT.

Sincerely

Phillips, James  
Grants Manager

Questions concerning the comments on your report as originally submitted may be directed to: Dr. James B. Phillips at 301-619-7522 or at James. [Phillips@amedd.army.mil](mailto:Phillips@amedd.army.mil).

To obtain a copy of the format requirements and to view a sample cover, Standard Form 298 and table of contents, click on "Research Reports" at the U.S. Army Medical Research and Materiel Command web site, <https://mrmc.amedd.army.mil/rppindex.asp>.

\*\*\*The revised report is due in this office no later than 15 NOV 2010.\*\*\*

You are to send your revised report as a PDF file to <https://ers.amedd.army.mil>.

Regards,  
Juanita Livingston  
Information Management  
Fort Detrick, MD 21702-5012

Juanita Livingston  
Technical Editor  
Information Management  
Fort Detrick, MD  
301-619-7325

Classification: UNCLASSIFIED  
Caveats: NONE

## STATEMENT OF WORK (SOW)

The SOW from the original application is reprinted below.

### Statement of Work

DOD Award W81XWH-07-1-0250

#### Title:

Development of Augmented Leukemia/Lymphoma-Specific T-Cell Immunotherapy for Deployment with Haploidentical Hematopoietic Progenitor-Cell Transplant

#### Study Site Information:

University of Texas M.D. Anderson Cancer Center  
PI: Laurence J.N. Cooper, M.D., Ph.D.  
Section Chief, Cell Therapy  
1515 Holcombe Blvd,  
Houston, TX 77030

#### Subcontract:

M. Rita I. Young, Ph.D.  
Associate Chief of Staff for Research  
Ralph H. Johnson VA Medical Center  
Charleston, SC 29401

#### **Project Tasks** (4 year time line):

For additional information refer to "Proposal" section D ("Project Milestones") and Figure 18 (Main Proposal).

#### **Task 1: Development of CD10-specific-IL2 ICK to be used in combination with CD19-specific T cells to improve persistence of infused T cells and to treat B-lineage malignancy**

- Develop CD10-specific-IL2 ICK (Mos. 1-9). Cooper laboratory and Young Laboratory.
- Combine with CD19-specific T cells *in vivo* (Mos. 10-20). Cooper laboratory
- Interim analysis (Mo. 20).

#### **Task 2: Development of CD19-specific CD4<sup>+</sup> T<sub>h</sub> cells to improve the anti-tumor response of CD19-specific CD8<sup>+</sup> T cells**

- Develop CD4<sup>+</sup> CD19-specific T cells (Mos. 16-18). Cooper laboratory and Young Laboratory.
- Combine with CD19-specific CD8<sup>+</sup> T cells *in vivo* (Mos.19-29). Cooper laboratory.
- Interim analysis (Mo. 29).

#### **Task 3: Development of chimeric antigen receptor (CAR) with CD28 co-stimulation to enhance the survival and potency of CD19-specific T cells**

- Develop CD19-specific T cells expressing second-generation chimeric antigen receptor (Mos. 28-30). Cooper laboratory and Young Laboratory.
- Evaluate immunobiology of CD19-specific T cells expressing next-generation CAR *in vivo* (Mos.31-44). Cooper laboratory.
- Interim analysis (Mo. 44).

#### **Task 4: Final analysis**

- Report writing (Mos. 45-48). Cooper laboratory.
- Preparation of second-generation clinical trial to evaluate the clinical potential of CD19-specific T-cell therapy with augmented therapeutic potential (Mos. 45-48). Cooper laboratory.

## ACTIVITIES PERFORMED IN THIS PAST YEAR IN ACCORDANCE WITH SOW

For additional details, please refer to this year's prior report.

### **Task 1: Development of CD10-specific-IL2 ICK to be used in combination with CD19-specific T cells to improve persistence of infused T cells and to treat B-lineage malignancy**

We have completed this task and no further data was gathered over this past year.

### **Task 2: Development of CD19-specific CD4<sup>+</sup> T<sub>h</sub> cells to improve the anti-tumor response of CD19-specific CD8<sup>+</sup> T cells**

We have developed the technology to genetically modify and propagate CD4<sup>+</sup> (and CD8<sup>+</sup>) T cells. This was published in Hum Gene Ther. 2010 Apr;21(4):427-37. The critical data are in [Figure 1](#). We have also demonstrated that the genetically modified T cells exhibit an immunophenotype consistent with memory which is predicted to improve T-cell survival and thus therapeutic potential.

### **Task 3: Development of chimeric antigen receptor (CAR) with CD28 co-stimulation to enhance the survival and potency of CD19-specific T cells**

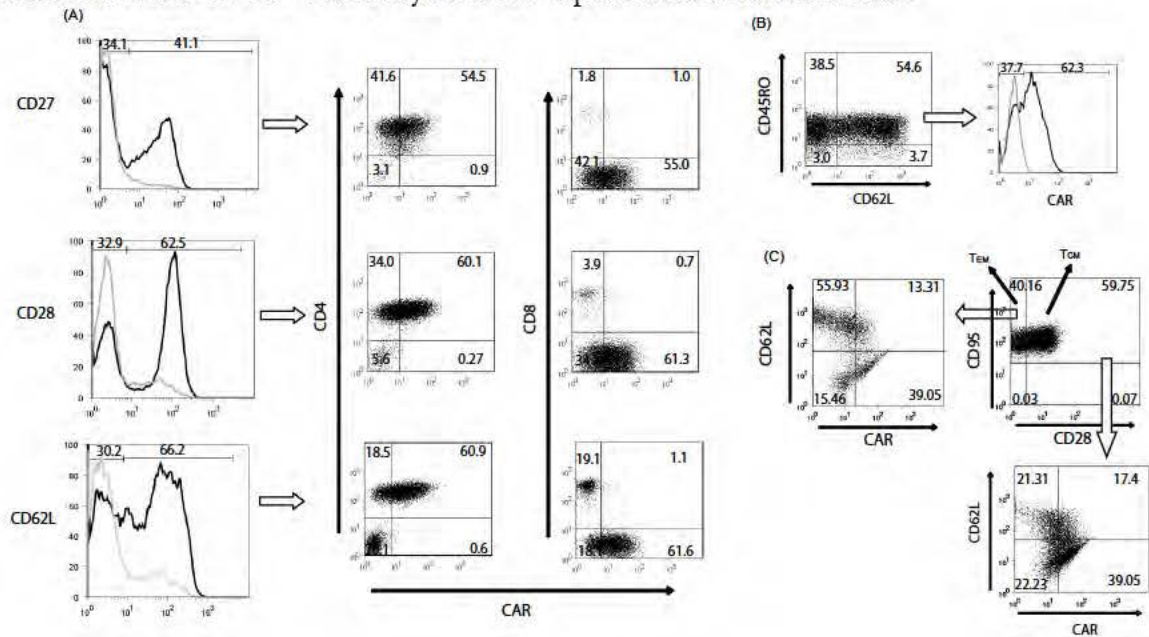
We have completed the task as initially described, as mentioned in last year's report. Therefore, we expanded our investigation to determine if T cells that express a 2<sup>nd</sup> generation CAR (that activates via chimeric CD28) can be rendered anergic to alloantigens. The rationale being that blockade of endogenous CD28 can lead to anergy when  $\alpha\beta$  T-cell receptor (TCR) engages antigen, such as disparate HLA molecules ([Figure 2](#)). In a published report (Cancer Res. 2010 May 15;70(10):3915-24) we demonstrated that CD28 blockade eliminated alloreactive CAR<sup>+</sup> T cells ([Figure 3](#)). Since the genetically modified CD4<sup>+</sup> and CD8<sup>+</sup> T cells express a CAR with its own CD28 signaling domain, the remaining T cells are able to recognize CD19 as demonstrated by cytokine production ([Figure 3](#)) and T-cell proliferation in a CAR-dependent manner ([Figure 4](#)). The successful anergization of CAR<sup>+</sup> T cells is important to be able to infuse haploidentical T cells in support of hematopoietic stem-cell transplantation.

We have continued to follow the transition plan developed per Section 14 "Supporting Documentation" of the original grant application. Thus, as mentioned in the May 2010 report we have begun assembling the data and regulatory documents for a clinical trial. As intended, this trial is the culmination of the research sponsored by U.S. Army Medical Research and Materiel Command.

## FIGURES

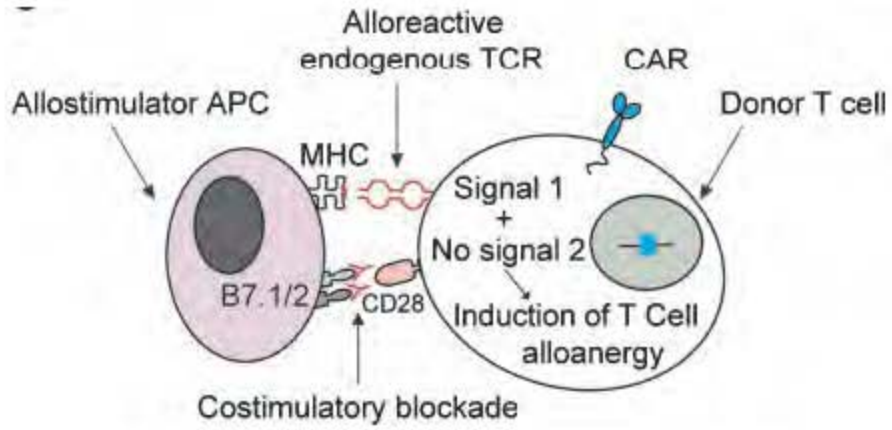
**Figure 1**

Characterization of CAR<sup>+</sup> T cells on PBMC after electro-transfer of PB vectors. A, immunophenotype of memory cell markers (CD27, CD28, CD62L) on PB modified T cells generated after 4 weeks of co-culture on aAPC. The black solid histograms reveal the percentage of T cells expressing CD27, CD28 and CD62L in the lymphocyte-gated population. T cells expressing the memory cell markers were analyzed for co-expression of CAR and CD4 or CD8. B, expression of CD45RO and CD62L on T cells generated after co-culture. CD45RO and CD62L double-positive T cells were analyzed for the expression of CAR. C, T<sub>CM</sub>, defined as CD28<sup>+</sup>CD95<sup>+</sup> and T<sub>EM</sub> defined as CD28<sup>neg</sup>CD95<sup>+</sup> were analyzed for co-expression of CD62L and CAR.



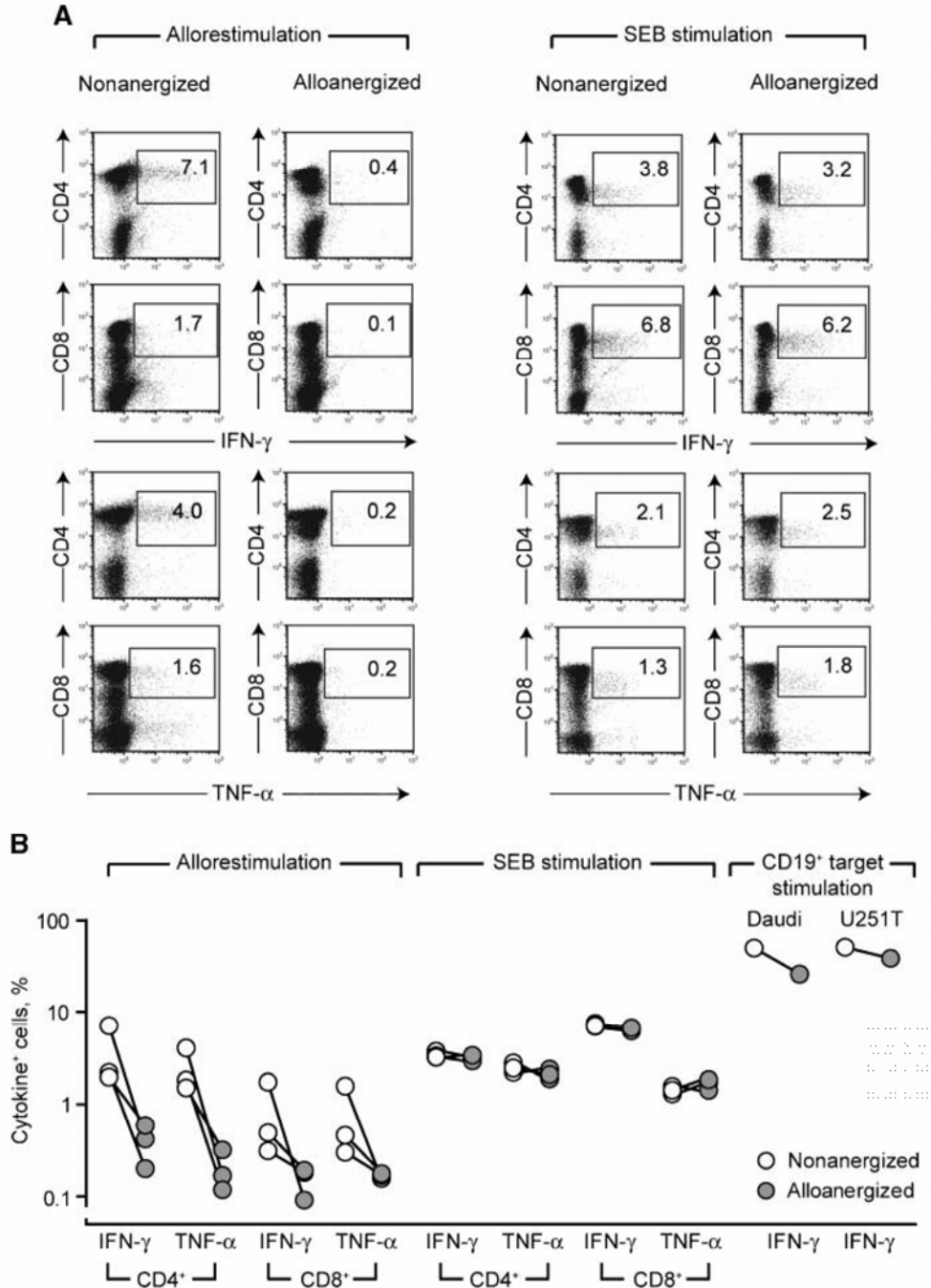
**Figure 2**

Schematic showing that preventing engagement of endogenous CD28 on CAR<sup>+</sup> T cells can be used to anergize alloreactive T cells which express a TCR that recognizes foreign MHC.



**Figure 3**

Alloantigenization of CD19-CAR cells resulted in reduced allospecific cytokine production. **(A)** Cytokine secretion by non-antigenized and alloantigenized CD19-CAR cells after restimulation with either  $\gamma$ -irradiated allostimulators or SEB. Flow cytometer dot plots are shown depicting intracellular cytokine production in CD4<sup>+</sup> and CD8<sup>+</sup> cells, gated to exclude irradiated stimulator cells and on CD3<sup>+</sup> events. Boxed regions represent cytokine<sup>+</sup> events and numbers represent frequency of cytokine<sup>+</sup> events expressed as a percentage of CD4<sup>+</sup> or CD8<sup>+</sup> cells. Results are shown for a representative experiment (of 3). **(B)** Frequencies of cytokine<sup>+</sup> cells in non-antigenized and alloantigenized CD19-CAR cells after restimulation with allostimulators, SEB or CD19<sup>+</sup> target cells.



**Figure 4**

**CD19-dependent expansion of alloenergized CD19-CAR cells** (A) Expansion of non-energized and alloenergized CD19-CAR cells on CD19<sup>+</sup> aAPC, OKT3-loaded CD19<sup>+</sup> aAPC and first-party allostimulators. (B) Expression of surface CAR on non-energized and alloenergized CD4<sup>+</sup>CD19-CAR cells after 21 days of expansion on OKT3-loaded CD19<sup>+</sup> aAPC. (C) First-party alloproliferation (<sup>3</sup>H]-thymidine incorporation) of non-energized, alloenergized and alloenergized then aAPC-expanded CD19-CAR cells after first-party allostimulation. (D) CD19-specific intracellular IFN- $\gamma$  secretion of aAPC-expanded CD19-CAR cells before and after alloenergization following incubation with CD19<sup>-</sup> and CD19<sup>+</sup> stimulator cells. Events are gated on CD3<sup>+</sup> lymphocytes. Numbers represent percentages of cells in each quadrant.

