Development of a Novel Nanoparticle-based GMP Platform for the Generation of Tumor-Specific CD8+ T cells

Juan Carlos Varela[1,2], Sojung Kim[2], Lauren Suarez[2], Dan Bednárik[2], Kristi Jones[2], Scott Carmer[2], Mathias Oelke[2]

[1] Department of Hematology and Oncology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC;
[2]NexImmune, Gaithersburg, MD.

Abstract

Efficient *ex*-vivo generation of non-genetically engineered tumor-specific T cells remains a significant hurdle for the broad application of adoptive cell transfer (ACT) protocols for the treatment of cancer. Here, we describe a novel nanoparticle-based platform for generating tumor-specific T cells using artificial antigen presenting cells (aAPCs).

Our aAPCs consist of a paramagnetic nanoparticle decorated with humanized HLA-A2-Ig dimer-molecules and anti-CD28 antibodies. aAPCs are loaded with tumor derived HLA-A2 restricted peptides and are used for the enrichment and expansion of tumor-specific CD8+ T cells. Using our aAPCs we have developed a fully enclosed semi-automated, GMP T cell expansion platform that consistently generates clinically relevant numbers of tumor-specific CD8+ T cells in only 14 days, thus obviating the need for dendritic cell-based T cell expansion.

Utilizing this platform we simultaneously generated CD8+ T cells targeting the acute myeloid leukemia (AML) antigens WT1, PRAME, Survivin and Cyclin A1. During the 14 day expansion to clinically-relevant numbers of AML-specific CD8+T cells we observed fold changes in the range of 500 to >5000-fold. Importantly, the generated T cells were >90% of the memory phenotype consisting of both central memory and effector memory CD8+ T cells. In addition, we demonstrated that the AML-specific CD8+ T cells were able to mediate specific *in vitro* killing of the AML cell line THP-1.

The results reported here will be the foundation for the development of a multi-institution phase I clinical trial of adoptive T cell transfer for the treatment of relapsed AML after allogeneic hematopoietic stem cell transplant.

Artificial Antigen Presenting Cells (aAPCs)

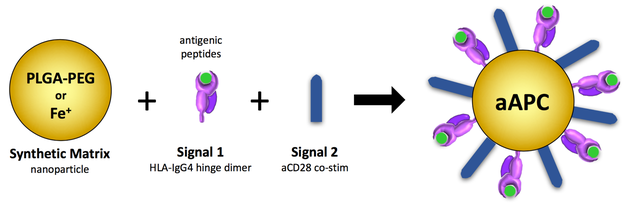


Figure 1. Humanized Artificial Antigen Presenting Cells (aAPCs). aAPCs consist of a superparamagnetic iron-oxide nanoparticle decorated with humanized HLA-IgG4-A2 hinge dimer (signal 1) and humanized anti-CD28 antibody (signal 2). aAPCs can be loaded with a HLA-A2 restricted peptide of choice and lead to the activation and proliferation of antigen-specific CD8+ T cells.

GMP, Semi-automatic T cell Expansion Platform

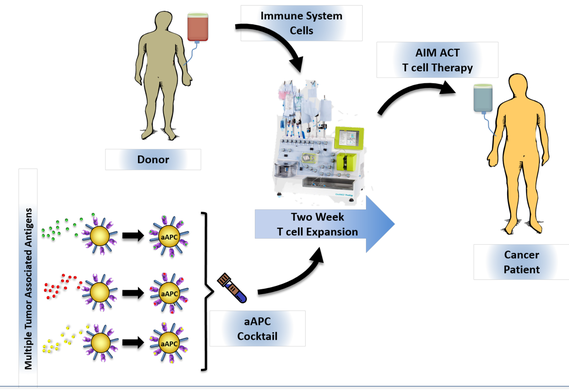


Figure 2. Erichment and Expansion of Antigen-specific CD8+ T cells. Leukopheresis product is collected from human donor and is put into a CliniMacs-Prodigy® cell expansion system (Myltenyi Biotech). Magnetic separation is used to deplete CD4+ T cells and to enrich for antigen-specific CD8+ T cells using our aAPCs. The enriched cell suspension is placed in G-REX expansion flasks in combination with a proprietary cytokine mix. Cultures are expanded for two weeks. Using this process we have consistently generated clinically-relevant numbers of CD8+ antigen-specific T cells.

MART1 as Model Antigen

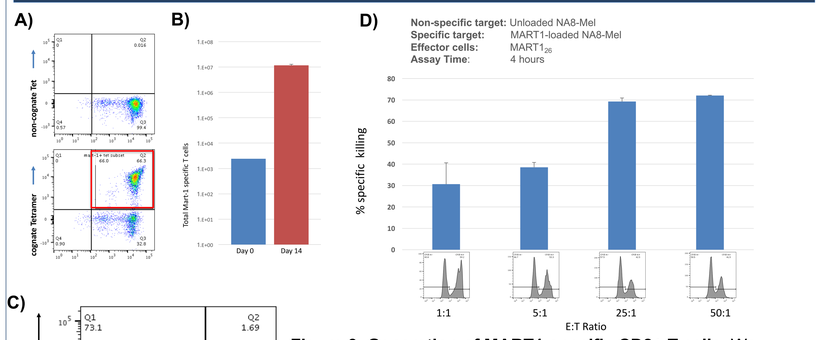
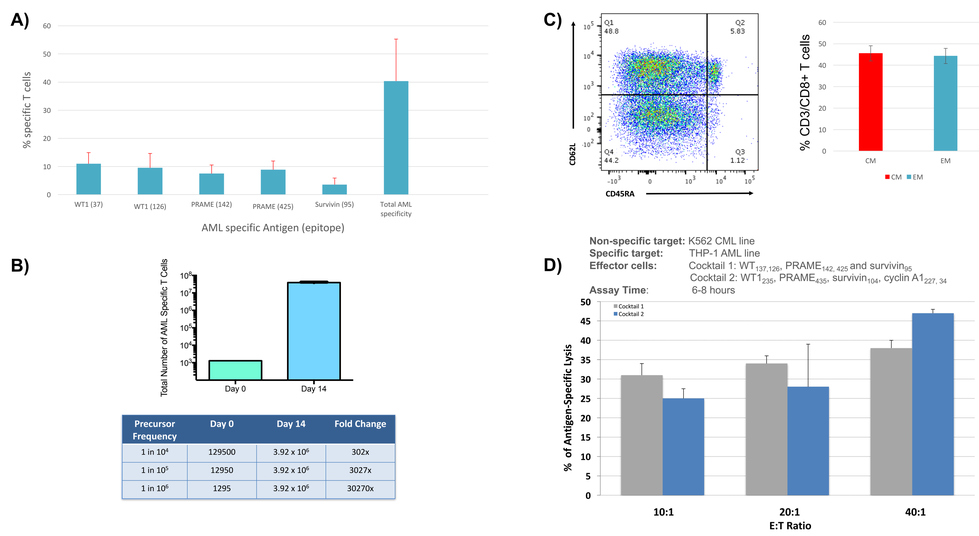


Figure 3. Generation of MART1-specific CD8+ T cells. We optimized our expansion platform by using the model tumor antigen MART1. **A)** Representative FACS plots showing MART1-specific after a 14-day expansion. **B)** Total numbers of MART1-specific CD8+ T cells on day 0 and after a 14-day expansion. **C)** Representative FACS plot showing the memory phenotype of MART1-specific CD8+ T cells on day 14 as determined by CD62L and CD45RA expression. **D)** *In vitro* cytotoxicity assay (CFSE-based) showing antigen-specific killing of MART1+ tumor cells by CD8+ MART1-specific T cells generated via our expansion platform

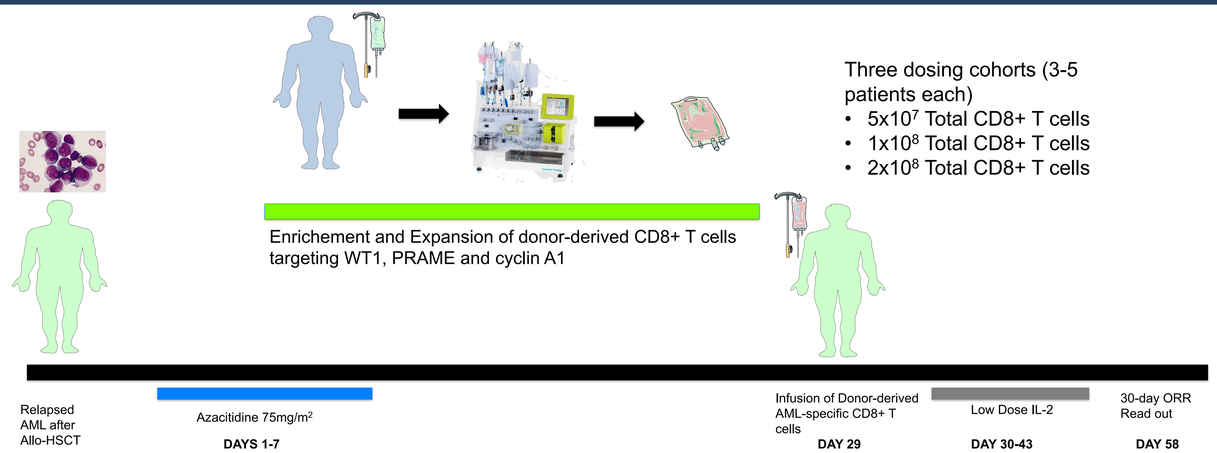
AML-specific Antigens



Precursor Frequency	Day 0	Day 14	Fold Change
1 in 10^4	129500	3.92 x 10^6	302x
1 in 10^5	12950	3.92 x 10^6	3027x
1 in 10^6	1295	3.92 x 10^6	30270x

Figure 4. Generation of AML-specific CD8+ T cellls. Using our 14-day GMP, semiautomatic T cell expansion platform, we successfully generated clinically-relevant numbers of AML-specific T cells targeting the tumor-associated antigens WT1, PRAME, Cyclin A1 and Survivin. **A)** Percentages of AML-specific of CD8+ T cells after 14 days of batched expansion. **B)** Total number of AML-specific T cells on day 0 and day 14 following expansion. Table depicts calculated fold change using different precursor frequencies **C)** Representative FACS plot and graph showing the memory phenotype of the expanded CD8+ T cells on day 14. **D)** *In vitro* cytotoxicity assay (CFSE-based) showing antigen-specific killing of the AML cell line THP1 by AML-specific CD8+ T cells generated via our expansion platform.

Phase I Clinical Trial in AML



Three dosing cohorts (3-5 patients each)

- 5x10^7 Total CD8+ T cells
- 1x10^8 Total CD8+ T cells
- 2x10^8 Total CD8+ T cells

Figure 5. Schema for a planned Phase I Clinical Trial using AML-specific CD8+ T cells for the Treatment of AML relapsed after Allogeneic Hematopoietic Stem Cell Transplant (HSCT). We are planning a multi-institutional phase I clinical trial using AML-specific CD8+ T cells generated via our expansion platform. The patient population will be AML patients who have relapsed after an allogeneic HSCT. The AML-specific CD8+ T cells will be generated from the HSCT donor. Primary objectives will be safety and dose finding. Secondary objective will be ORR at 30 days after T cell infusion.

Conclusions

- We have generated a fully humanized, GMP-grade aAPCs that can be used for the enrichment and expansion of antigen-specific CD8+ T cells
- We have developed a novel GMP, semi-automatic T cell expansion platform using our humanized aAPCS
- Using our expansion platform we have successfully generated clinically-relevant numbers of AML-specific CD8+ T cells. These AML-specific T cells are a combination of central memory and effector memory T cells and are able to effectively kill an AML cells *in vitro*.
- The data presented here will the foundation for the development and implementation of a phase I clinical trial using AML-specific CD8+ T cells for the Treatment of AML relapsed after HSCT.

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