Combining Gene Therapy and Nanomedicine to Enhance the Therapeutic Potential of T Cells

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ABSTRACT

To undertake adoptive immunotherapy for lung cancer, we have combined nanotechnology, bioengineering, and gene therapy to use T cells as a vehicle for the targeted delivery of nanoparticles (NPs) to the lung tumor microenvironment. In this project, we have developed a high throughput electroporator to manipulate large numbers of T cells *ex vivo* for clinical uses. A proprietary electroporator is designed to electro-transfer RNA species to redirect T-cell specificity and NPs that can be functionalized to enhance T-cell biology. By introducing both transgenes and NPs, we are generating T cells that can be rapidly modified to act as a drug-delivery system for targeted therapy of lung cancer.

Keywords: electroporation, T cell, targeted drug delivery, mRNA, high throughput

1 INTRODUCTION

Genetic modification of clinical-grade T cells to redirect specificity towards desired antigens expressed on target cells is a promising therapy for the treatment of diseases, including cancer [1-4]. However, conventional viral and non-viral methods for gene transfer often rely on integrating expression vectors and are typically laborious (taking weeks to generate clinically-significant cell doses). The genetic alteration of T-cell specificity and/or function is a tool for treating cancer and opportunistic infection. Yet, the question is "Can gene therapy using T cells be improved in regards to safety and efficacy?" The enabling technology in this application provides a new approach by leveraging the ability of a high throughput electroporation device to perform physical processes with improved efficiency beyond conventional systems and efficiently introduce desired materials such as mRNA and NPs [5-7]. The newly-fabricated device provides sample handling, mixing, and electroporation on a single device with the reduction of reagent volumes and accompanying scalability to achieve high throughput in an unbreached system for use with current good manufacturing practice (GMP).

In this research, we provide a defined pathway of using a high throughput device to rapidly electro-transfer non-integrating mRNA species [8-10] and drug-loaded NPs into clinical-grade T cells enabling adoptive immunotherapy efficacy trials with potential for infusing patients at multiple centers. Specifically we are targeting lung cancer which remains a leading cause of cancer death. It is often diagnosed at late stages and is treated systemically with cytotoxic chemotherapy, which is generally ineffective. To generate potent adoptive immunotherapy for lung cancer which expresses the c-Met antigen, we seek to use T cells as a vehicle for the targeted delivery of NPs to the tumor microenvironment. To target c-Met^{+} lung cancer, a chimeric antigen receptor (CAR) has been developed which redirects T-cell specificity to c-Met on the tumor cell surface, independent of the major histocompatibility complex (MHC).

Figure 1 shows the schematic view of the present research. The first step is numeric expansion of T cells. Peripheral blood mononuclear cells (PBMC) are collected by simple blood draw. To generate the large numbers of T cells for repeated rounds of electroporation and adoptive transfer we have numerically expanded these lymphocytes *ex vivo* by repeated stimulation on γ-irradiated K562 derived artificial antigen presenting cells (aAPC). Initially, the electroporation device introduces mRNA coding for a CAR. Then NPs are electro-transferred so that the T cells can function as a drug delivery system. The proprietary **hi**gh **t**hroughput **e**lectroporators (HiTE) have been developed to electro-transfer mRNA and NPs into T cells (> 10^9 cells). These electroporated T cells can be cryopreserved for later use or infused immediately.

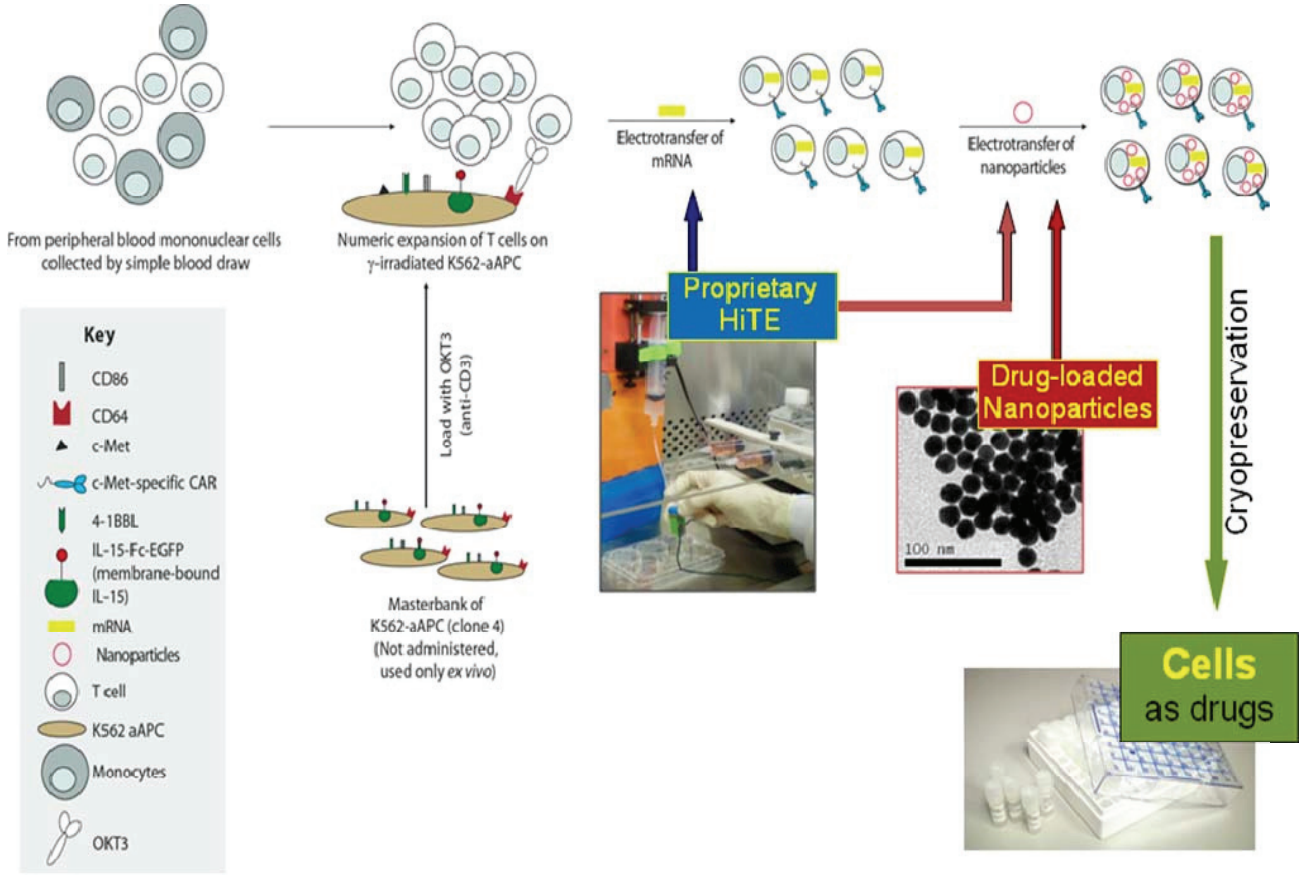

Figure 1: Outline of the experimental platform which uses electro-transfer of mRNA and nanoparticles to redirect T-cell specificity and function.

2 MATERIALS AND METHODS

We have developed HiTE for introducing mRNA and NPs into human *ex vivo* propagated T cells. It uses a continuous flow to avoid the need to use multiple cuvettes and to synchronously electroporate large numbers of T cells. The device was fabricated by sandwiching two aluminum plates with a polystyrene spacer, which was connected to two syringe pumps to deliver T cells, mRNA, NPs, and culture media (Figure 2). PDMS joint structures were developed by micro-molding technique and used to connect HiTE to syringe pumps and a cell collecting reservoir. The gap between two electrodes was fixed to 2 mm and the area of electrodes was 8 x 20 mm. The electric field strength was also optimized to 1 kV/cm. We used a commercially available pulse generator (ECM830, BTX) which has a multiple pulsing mode to deliver a user-defined electrical pulse to the HiTE. Since we can adapt our technology to an unbreached and disposal system, we can minimize the potential for cross-contamination between patient products.

Gold NPs (Nanopartz, Salt Lake City, UT) were used as functionalized carriers and polystyrene latex NPs (Invitrogen, Carlsbad, CA) were used to establish the maximum size of NPs that could enter T-cell pores after electroporation. Gold NPs were conjugated to 5-carboxyfluorescein (5-FAM, 492/520 nm) to determine the electroporation efficiency using flow cytometry. mRNA coding for CAR was also generated by *in vitro* transcription.

Human T cells were isolated by density gradient centrifugation over Ficoll-Paque-Plus (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) from buffy coats containing peripheral blood mononuclear cells (PBMC) obtained from Gulf Coast Regional Blood Center (Houston, TX) after consent. We developed an effective *ex vivo* culture system to rapidly expand large numbers of primary T cells based on aAPC provided in collaboration with Dr. Carl June (University of Pennsylvania). The aAPC were derived from K562 with enforced expression of T-cell costimulatory molecules, 41BBL, CD86, and membrane-bound IL-15 (mIL-15, co-expressed with EGFP). K562-aAPC could be pre-loaded with OKT3 (CD3-specific mAb, 1 mg/mL in phosphate buffered saline (PBS)) at a concentration of 1 μL/10^6cells, γ-irradiated at 100 Gy, and

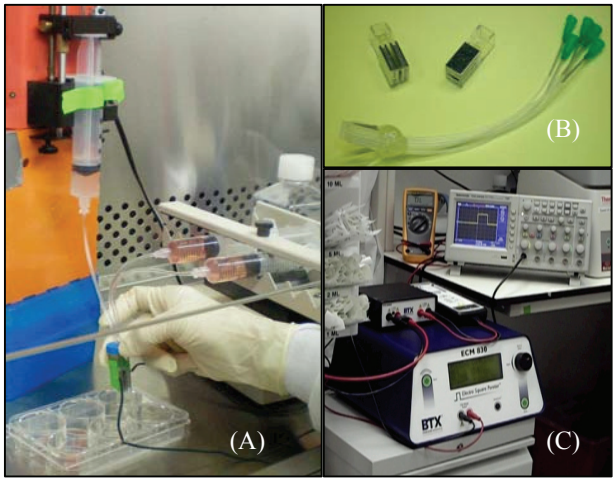

Figure 2: Description of the high throughput electroporator to electro-transfer nanoparticles into T cells in continuous flow. (A) Electroporator is connected to two syringe pumps. Extended cables deliver the electrical signal to HiTE that operates in a fume hood to maintain sterility. Sampling of electroporated cells is performed using multi-well plates. (B) Multiple inlet ports and PDMS connectors are used to fix aluminum electrodes and 2 mm gap. (C) Multiple pulsing mode (square wave) of BTX ECM830 has been used for electroporation (1 kV/cm, 6 ms).

co-cultured with T cells at a ratio of 1:1 in a culture media (RPMI 1640, GIBCO, Grand Island, NY) supplemented with 5% heat-inactivated human serum (Valley Biomedical Inc, Winchester, VA) with 50 units/mL of soluble IL-2 added every 2 days. The OKT3-loaded K562 were added to the culture every 7 days.

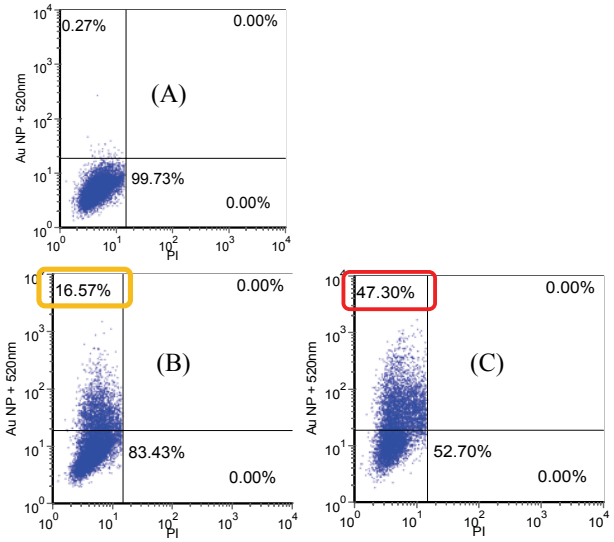

Figure 3: Flow cytometry analysis (A) Control cells, (B) 8.4 x 10^{12} NPs (20 µL) + 2 x 10^{6} cells/100µL, (C) 1.68 x 10^{13} (40 µL) + 2x10^{6} cells/100µL.

3 RESULTS AND DISCUSSION

Initially, Nucleofector Technology (Amaxa/Lonza, Walkersville, MD) was used to optimize the transfection efficiency of 7 nm size gold nanoparticles labeled FAM (Figure 3). To find the maximum size of nanoparticle transferable into T cells, several sizes of polystyrene latex nanoparticles (Invitrogen, Carlsbad, CA) were tested using Nucleofector Technology. We were able to electro-transfer up to 43nm size latex nanoparticles (Figure 4). The human T cells were activated using OKT3-loaded aAPC. After 7 days in co-culture the T cells expanded by approximately 50-fold and after 14 days the numeric expansion was almost 1,000-fold. These T cells can be electroporated to express mRNA CAR.

We have successfully generated cell expansion methods to electro-transfer NPs and mRNA using HiTE. We are currently investigating optimization of high throughput electroporation to improve uptake of mRNA coding for c-Met-specific CAR and encapsulation of chemotherapy drugs into NPs. In aggregate, this may improve the potency of clinical-grade genetically modified T cells as a vehicle for the targeted delivery of drug-loaded NPs to the lung tumor microenvironment.

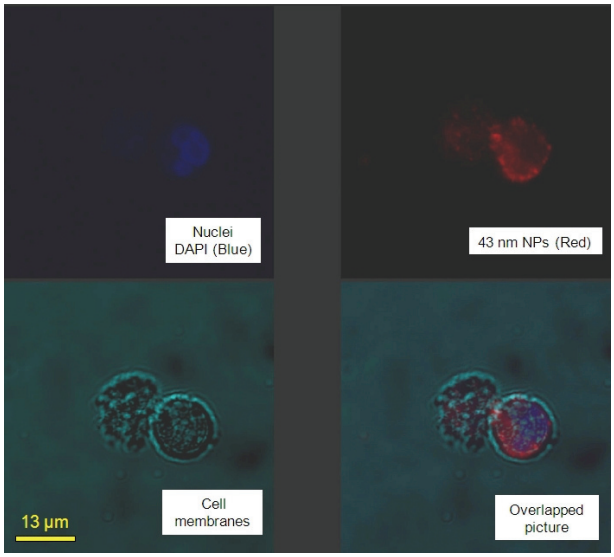

Figure 4: Confocal images of polystyrene latex nanoparticles electro-transferred into T cells. 43 nm size nanoparticles have 580 nm fluorescent color (Red) and nuclei are stained using DAPI (Blue). To assess the ability of electroporation to introduce nanoparticles into T cells, we have electro-transferred fluorescent nanoparticles that range in size up to 43 nm. We determined that nanoparticles larger than ~40 nm could not be electro-transferred into T cells, probably due to limitation in the pore size in viable cells after electroporation.

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