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14. ABSTRACT  
We have developed a novel strategy that combines tumor immunotherapy targeting PAP and targeted immune modulation of CTLA4 and have generated a lead cellular therapy that will safely enhance vaccine-mediated immunity. This lead cellular therapy, called DC-PAPvac-C, consists of dendritic cells (DCs) co-transfected with prostate tumor antigen, PAP RNA and anti-CTLA4 RNA. In this study we will establish the preclinical efficacy and safety of our cellular therapy product, DCs transfected with RNA that encodes PAP and anti-CTLA4 and generate data required for an Investigational New Drug (IND) application. Importantly and relevant to our planned clinical trial implementation, we will develop a biomarker of therapeutic efficacy and demonstrate the feasibility of measuring these biomarkers.

In this report we have demonstrated that local CTLA4 modulation in combination with PAP-specific immunization using RNA-transfected DCs elicits robust and superior functional T cell responses in TRAMP mice. We have also demonstrated that post-transcriptional modification of mRNAs (to generate Cap1 mRNA) enhances protein production by human DCs and human DCs transfected with Cap1 PAP mRNA without the signal sequence stimulate highest frequencies of PAP-specific T cells in an in vitro human immunotherapy assay.

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dendritic cell vaccine, dendritic cells electroporated with RNA, immune checkpoint blockade, local CTLA-4 modulation, prostate cancer immunotherapy, prostatic acid phosphatase (PAP), RNA-based vaccines

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# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>4</td>
</tr>
<tr>
<td>2. Keywords</td>
<td>4</td>
</tr>
<tr>
<td>3. Overall Project Summary</td>
<td>4</td>
</tr>
<tr>
<td>4. Key Research Accomplishments</td>
<td>12</td>
</tr>
<tr>
<td>5. Conclusion</td>
<td>13</td>
</tr>
<tr>
<td>7. Inventions, Patents and Licenses</td>
<td>13</td>
</tr>
<tr>
<td>8. Reportable Outcomes</td>
<td>13</td>
</tr>
<tr>
<td>9. Other Achievements</td>
<td>13</td>
</tr>
<tr>
<td>10. References</td>
<td>13</td>
</tr>
<tr>
<td>11. Appendices</td>
<td>14</td>
</tr>
</tbody>
</table>
**Novel immune modulating cellular vaccine for prostate cancer immunotherapy (PC121288)**
**Principal Investigator: Smita Nair, PhD**

**INTRODUCTION:**
The goal of immunotherapy is to stimulate T cells that recognize and destroy tumor cells; however, a major challenge to greater vaccine efficacy is immune suppression mediated by inhibitory receptors on activated T cells, specifically cytotoxic T lymphocyte antigen 4 (CTLA4). Systemic administration of anti-CTLA4 blocking antibodies has demonstrated clinical effectiveness in melanoma patients, but, consistent with its mode of action, anti-CTLA4 antibody causes significant immune-related adverse events. Strategies for delivering anti-CTLA4 to the site of T cell activation while limiting systemic exposure are needed. Therefore, our objective is to design a prostate cancer immunotherapy strategy that will 1] Enhance the function of tumor antigen-specific T cells by targeted modulation of immune receptor function and 2] Lead to the development of a clinically effective prostate cancer immunotherapy, without inducing severe autoimmunity. We have designed an innovative approach for targeted delivery of antibodies to sites where anti-tumor T cells are induced, using dendritic cell (DCs) transfected with antibody-encoding RNA. When we immunized mice with DCs transfected with tumor antigen RNA and anti-CTLA4 RNA, we observed enhanced anti-tumor immunity, without autoimmunity. Specific to the prostate cancer antigen PAP, we activated more potent anti-PAP cytotoxic T lymphocyte (CTL) responses *in vitro* using DCs modified to express PAP and secrete the anti-CTLA4 antibody. We hypothesize that a vaccine consisting of DCs modified with RNA encoding PAP and anti-CTLA4 will result in increased immunogenicity toward PAP over PAP alone. As described in our proposal, we will conduct preclinical studies with our lead cellular therapy - DCs modified with RNAs encoding PAP and anti-CTLA4 antibody - DC-PAPvac-C. Our intention is to advance this product into clinical trials.

**KEYWORDS:**
dendritic cell vaccine, dendritic cells electroporated with RNA, immune checkpoint blockade, local CTLA4 modulation, prostate cancer immunotherapy, prostatic acid phosphatase (PAP), and RNA-based vaccines

**OVERALL PROJECT SUMMARY:**

**Protocols specific to this proposal and approval dates:**
Animal Use Regulatory Protocols (Aim 1):
Duke University IACUC Approval (Protocol A082-13-03): 03-28-2013 valid for 3 years, annual review approved on 03-27-2014
*Title: Novel Immune Modulating Cellular Vaccine for Prostate Cancer Immunotherapy*
ACURO Approval (Protocol reference number PC121288): 07-16-2013

Human Use Regulatory Protocols (Aims 2 and 3):
Duke University IRB Approval (Protocol Pro00044351): 04-11-2013 for 1 year
*Title: Healthy volunteer leukapheresis for in vitro immune assays*
HRPO Approval (HRPO A-17872.2, Proposal Number PC121288, Award Number W81XWH-13-1-0423): 08-15-2013
Duke University IRB Continuing Review Approval: 04-11-2014 for 1 year (approved on 03-14-
Duke University IRB DECLARATION OF RESEARCH NOT INVOLVING HUMAN SUBJECTS (Protocol Pro00057900): IRB Declaration is in effect from 09-25-2014 and does not expire.

Title: Tumor infiltration with immune effectors


Major Task 1: Determine the in vivo systemic and tumor-associated immune response that correlates with anti-tumor activity of the lead cellular therapy, DC-PAPvac-C (months 1-30)

Subtask 1 (1-3 months): Generate mouse PAP RNA, anti-mouse CTLA4 RNA, mouse actin RNA and control IgG RNA for murine immunotherapy studies in TRAMP mice

Status: Completed

To evaluate the lead cellular therapy (DC-murinePAPvac-murineC) in a preclinical in vivo setting we will use DCs transfected with RNA encoding mPAP and murine anti-CTLA4, cloned from hybridoma 9H10 as a vaccine in the TRAMP murine model for prostate cancer. Therefore, our first task is to clone the cDNAs of the murine analogs of prostatic acid phosphatase (mPAP) into pSP73-Sph/A64. Since, in our in vitro human studies, hPAP that was cloned without its signal sequence elicited a better CTL response we will test mPAP without the endogenous signal sequence. We will also test mPSMA (murine prostate-specific membrane antigen) without the signal sequence for its potential use as an antigen.

Anti-murine CTLA4 RNA: The anti-murine CTLA4 has already been cloned, as has murine actin (1).

Murine PAP: The cDNA encoding mPAP was purchased from TRANSomomic and was cloned into the XbaI and SacI sites of pSP73-Sph/A64 by amplifying the cDNA from the supplied plasmid with the primers:

5’-ATATATCTAGAGCCACCACATGCGAGCCGTTCTCTCTG-3’ and 5’-TATATAGAGCTCTCAGATGTTCCGATACACATCTC-3’ that included XbaI and SacI restriction sites.

Murine PSMA: The cDNA encoding mPSMA was purchased from Sino Biologicals and was cloned into the HindIII and BamHI sites of pSP73-Sph/A64 by amplifying the cDNA from the supplied plasmid with the primers:

5’-TATATAAGCTTGGCCACCAGTGGAACGCAGCAGGACAG-3’ and 5’-TATATAGATCCTAAGCTACTTCCATCACAGTCTC-3’ that included HindIII and BamHI restriction sites.

Murine PAP no signal sequence (mPAP-SS): Murine PAP minus the signal sequence was amplified from the plasmid containing the full-length cDNA with the forward primer 5’-ATATATCTAGAGCCACCACATGCGAGTTTGTGAC-3’, which contains an XbaI site for cloning and changes the AAG that serves as the first amino acid in mature PAP to the ATG start codon in PAP-SS (PAP without signal sequence) and reverse primer 5’-TATATAGAGCTCTCAGATGTTCCGATACACATCTC-3’

Murine PSMA no signal sequence (mPSMA-SS): Murine PSMA minus the signal sequence was amplified from the plasmid containing the full-length cDNA with the forward primer 5’-TATATAAGCTTGGCCACCAGTGGAACGCAGCAGGACAG-3’ and reverse primer 5’-TATATAGATCCTAAGCTACTTCCATCACAGTCTC-3’.
5’- TATATAAGCTGCCACCATGAAGAGGAGTTTTGCATG-3’, which contains a HindIII site for cloning and utilizes an ATG that lies downstream of the signal sequence as the start codon in PSMA-SS (PSMA without signal sequence) and reverse primer 5’-TATATAGGATCTTAAAGCTACTTCCATCGAGTCTC-3’ that included a BamHI restriction site.

**Subtask 2 (3-6 months):** Start first experiment with TRAMP mice, immunize mice using DC-mPAPvac-mC and controls

**Status: Completed**

TRAMP male mice were vaccinated 3 times at weekly intervals with 4 x 10^5 DCs transfected with mPAP-SS mRNA (n=3) or with mPAP-SS mRNA plus anti-CTLA4 mRNA. 7 days after the last vaccination, mice were sacrificed and prostates, spleens, and draining lymph nodes were harvested. In addition, tissues were also harvested from unvaccinated, age-matched TRAMP mice (n = 2) for analysis as a control group.

**Subtask 3 (6-12 months):** Analyze immune responses in the periphery: T cell analysis, ELISpot analysis anti-PAP antibody analysis

**Status: T cell analysis completed, anti-PAP antibody analysis ongoing**

Mice were immunized as described in Subtask 2. Untouched CD4+ T cells and CD8+ T cells were isolated from spleens using magnet-bead based techniques (Miltenyi). 50,000 T cells per well of a 96 well Multiscreen-IP plate (Millipore) were stimulated at a stimulator to effector ratio of 1:10 as indicated. In addition to TRAMP-C1 and C2 cells and B16 melanoma cells as control, the C57BL/6 syngeneic thymoma cell line, EL4, transfected with mRNA encoding either GFP (control) or mPAP-SS were used as stimulators in the ELISpot assay (Figure 1).

Plates were incubated for 24 hours at 37°C and plates were developed using an anti-mouse IFN-γ ELISpot kit (BD Biosciences) per kit instructions. Results are presented as spots per 10^5 CD8 or CD4 T cells. As expected, frequencies of PAP-specific CD4+ T cell responses were low, given that we used PAP that lacks a signal sequence that has limited access to MHC class II presentation. Clearly, the PAP + anti-CTLA4 DC vaccine was superior to the PAP only DC vaccine. Importantly, vaccine-induced cells also showed effector function against the hormone-
refractory TRAMP-C2 cell line, albeit at lower frequencies. This suggests that our vaccine has the potential to attack metastatic and advanced prostate cancer in TRAMP mice.

To assess the CTL function of vaccine-induced CD8+ T cells, splenocytes from vaccinated or unvaccinated mice were re-stimulated ex vivo for 4 days with mPAP-SS mRNA-transfected DCs at a DC to T cell ratio of 1:20. After the 4-day restimulation, untouched CD8+ T cells were isolated and analyzed using a europium release assay (1). Data is shown in Figure 2. Again, vaccine-induced CD8 cytotoxic T cells also showed effector function against the hormone-refractory TRAMP-C2 cell line, as in Figure 1 above.

To corroborate our data shown in Figures 1 and 2 further, we demonstrate using immunofluorescence that TRAMP-C2 cells have a weak and somewhat non-homogeneous expression pattern for mPAP, which may, at least in part, explain the somewhat lower frequencies of TRAMP-C2 specific T cells when compared to TRAMP-C1 (Figure 3).

Figure 2. Analysis of CD8 CTL in TRAMP mice immunized with DC vaccines. Mice (n=3) were immunized 3 times at weekly intervals with 4 x 10^5 DCs transfected with mPAP-SS mRNA or with mPAP-SS mRNA plus anti-CTLA4 mRNA. After vaccination number 3, splenocytes were harvested and restimulated in vitro for 4 days. Unvaccinated, age-matched TRAMP mice (n = 2) were used as controls (none).

Figure 3. PAP expression in vivo and in vitro on prostate tumor cells.
Upper panel: 7 µm sections of OCT-embedded mouse prostate or in vivo TRAMP-C1 tumors were stained with DAPI (blue, nuclei) and anti-mPAP plus tetramethyl-rhodamine (red). Lower panel: Cytospins of in vitro growing TRAMP-C1 or TRAMP-C2 cells were stained as described above.
A, mouse prostate; B, in vivo TRAMP-C1 tumor; C, TRAMP-C1 in vitro cytospin; D, TRAMP-C2 in vitro cytospin.

Lymphocytes were isolated from draining lymph nodes (LN) and analyzed by flow cytometry (Figure 4).
Figure 4. Analysis of lymph node sub-populations in TRAMP mice immunized with DC vaccines. Antibodies used were anti-CD4, -CD8, -Ki67 (proliferation marker), -NK1.1 (NK cells), -CD19 (B cells), -F4/80 (macrophages), -CD25 and -FoxP3 (regulatory T cells, Tregs), -CD11b and -Grl (myeloid-derived suppressor cells, MDSCs). Cells were analyzed using a FACS caliber flow cytometer.

Subtask 4 (8-20 months): Harvest tumors from 30-week old mice to analyze tumor weight, tumor grade, tumor apoptosis and immune infiltrates and harvest mouse organs (lymph nodes, lungs, kidney, testis, colon, liver, muscle) for analysis of inflammatory infiltrates and autoimmunity

Status: Ongoing
As proposed in our specific aims, we will also determine vaccine-induced immune infiltrate changes in mouse prostate and prostate tumors. We have just optimized staining of these tissues by immunofluorescence and this analysis is ongoing.

Major Task 2: Using human prostate cancer tissue, determine the presence of immunologic markers that were identified in Aim 1 as correlated with vaccine efficacy. Develop assays to be used in subsequent human clinical trials of DC-PAPvac-C as markers of an effective immune response (months 6-30)

Subtask 1 (6-30 months): Obtain pre- and post-vaccine treatment human prostate tissue and embed them in paraffin and generate sections for immunohistochemistry.

Status: Pending
We now have a protocol that has been determined not human subjects research that has been approved by both Duke and HRPO. Data generation is pending.

Major Task 3: Perform FDA mandated validation of DC-PAPvac-C to confirm anti-CTLA4 mAb expression and PAP presentation by human DCs transfected with mRNA encoding hPAP and anti-human CTLA4 (months 1-30)

Subtask 1 (1-9 months): Characterize and optimize human PAP (hPAP) expression and anti-CTLA4 secretion by human dendritic cells (DCs)

Status: Completed
We performed studies to determine the expression levels of PAP and anti-CTLA4 by human monocyte-derived DCs as a function of mRNA concentration. Furthermore, we sought to determine whether post-transcriptional modification of our mRNAs could further enhance protein production by DCs. As shown in Figure 5, we generated mRNAs by co-transcriptional incorporation of a 5'-Cap (Cap0) or by post-transcriptional capping and enzymatic methylation of the 2' hydroxy-group of the first ribose of the 5' end of the mRNA using 2'-O methyltransferase (Cap1), which should provide significant resistance to intracellular 5' exonucleases.
We transfected human DCs with increasing concentrations of anti-CTLA4 Cap0 mRNAs (1 µg + 0.5 µg, 5 µg + 2.5 µg, 10 µg + 5 µg. Heavy chain + Light chain mRNA) and quantified the secretion of functional anti-CTLA4 antibody in an indirect ELISA assay using microwell plates coated with recombinant human CTLA4 protein (extracellular domain). A standard curve was generated with commercially available anti-human CTLA4 (PharMingen). This antibody was detected using biotinylated anti-mouse IgG (Fab)_2. OD_{450nm} was measured with a spectrophotometer. As shown in Figure 6, the amount of RNA used in our previous studies (preliminary data in grant, 10 µg Heavy chain RNA and 5 µg Light chain mRNA) appeared to be within a concentration range of mRNA that allows for optimal production of anti-CTLA4 (5.8 ng /10^6 cells/ml). Cap1 mRNA did further improve antibody secretion (8.4 ng /10^6 cells/ml). Importantly, loading of DCs with both anti-CTLA4 Cap1 mRNAs and hPAP Cap1 mRNA did not significantly reduce the secretion of functional anti-CTLA4 antibody (8.2 ng /10^6 cells/ml).

Next, we performed a similar experiment to determine hPAP expression by human DCs. In this experiment, cells were transfected with increasing amounts of hPAP mRNA (1 µg, 5 µg, and 10 µg). Since we had determined in earlier experiments (preliminary data in submitted grant) that stimulation of T cells with DCs transfected with an mRNA lacking the hPAP signal sequence (necessary for secretion of the protein) led to a superior induction of cytotoxic CD8 T cell responses, we used this mRNA (PAP-SS mRNA) for all of our studies. Human monocyte-derived DCs were transfected with increasing amounts of PAP-SS mRNA as indicated (Figure 7). After 3 hours, cells were fixed, permeablized and stained with mouse anti-hPAP antibody (Proteintech Group) and analyzed by flow cytometry. As can be seen from the shift in mean fluorescence, hPAP expression was not detectable when DCs were transfected with 1 µg of hPAP mRNA. Transfection with 5 µg or 10 µg of hPAP mRNA resulted in similar expression levels of hPAP. However, transfection with 5 µg of Cap1 hPAP mRNA significantly improved the expression level of hPAP by human DCs. Anti-hPAP was detected with PE-conjugated anti-mouse IgG and cells were analyzed on a FACScalibur flow cytometer.
Subtask 2 (10-15 months): Evaluate the function of optimized DC-PAPvac-C

Status: Ongoing

So far, our studies indicate that transfection of DCs with Cap1 mRNA may lead to improved protein expression by these cells when compared to transfection with Cap0 mRNA. We therefore asked whether this improvement in protein expression could lead to an enhancement of induction of anti-hPAP T cell responses.

We chose the IFN-γ ELISpot assays for these studies for two reasons. First, ELISpot assays allow analysis of both CD4 T cell and CD8 T cell effector function and will be used for immune monitoring in future clinical trials. Second, the establishment of autologous prostate cancer cell lines that could be used as targets in cytotoxic T lymphocyte assays (CTL assays) is notoriously difficult. As such, these assays would have to be performed using the LNCaP cell line which expresses hPAP and which is HLA-A0201 positive. Hence, this assay would be limited to HLA-A0201-positive trial patients.

To analyze T cell responses against hPAP mRNA-transfected human monocyte derived DCs, we stimulated autologous T cells with DCs that had been transfected with mRNA encoding full-length hPAP mRNA, PAP-SS mRNA encoding hPAP lacking its signal sequence, hPAP Cap1 mRNA encoding hPAP lacking the signal sequence. T cells were negatively selected using magnetic bead-based techniques and were stimulated twice with mRNA-transfected DCs in vitro. Ten days after the second stimulation, T cells were harvested and separated into CD4 T cells and CD8 T cells with magnetic beads. Isolated cells were stimulated with PAP-SS mRNA-transfected DCs in IFN-γ ELISpot assays. Following stimulation, cells were lysed with 0.05% Tween20 and incubated with biotinylated detection antibody (Endogen). Spots were visualized with alkaline phosphatase-conjugated streptavidin (Sigma) and BCIP/NBT substrate (KPL). Spots were counted using an ELISpot reader.

As shown in Figure 8, T cells that had been stimulated with mRNAs encoding full-length hPAP protein (PAP RNA) or Cap1 mRNA encoding hPAP lacking the signal sequence (PAP RNA Cap1) stimulated similar frequencies of hPAP-specific CD4 T cells, while frequencies of hPAP-specific CD4 T cells were significantly reduced when PAP-SS mRNA-transfected DCs (PAP-SS RNA) were used to stimulate T cell responses. In contrast, stimulation of T cells with PAP-SS mRNA-transfected DCs resulted in enhanced frequencies of hPAP-specific CD8 T cells when compared to T cell responses against full-length hPAP, as had been observed in previous experiments (preliminary data in submitted grant). The highest frequencies of hPAP-specific T cells were observed when T cell responses were generated using Cap1 PAP-SS mRNA-transfected DCs (PAP RNA Cap1) as stimulators.
Figure 8. IFN-γ ELISpot analyses of T cell function. Negatively selected autologous T cells were stimulated twice with mRNA-transfected DCs, as indicated. PAP RNA (full length hPAP mRNA), PAP-SS RNA (hPAP mRNA lacking the signal sequence), and PAP RNA Cap1 (Cap1 hPAP mRNA lacking the signal sequence). For ELISpot assays, 5 x 10⁴ negatively selected CD4 or CD8 T cells were stimulated for 16 hours with DCs that had been transfected with hPAP mRNA lacking the signal sequence at a stimulator to effector ratio of 1 to 10.

Subtask 3 (8-12 months): Generate, validate, test and vial clinical-grade RNA for cGMP production of DC-PAPvac-C

**Status: Completed**

We have generated and vialled cGMP RNA and validated that the RNA is functional. Data in Figures 6-8 was generated using cGMP RNA. All cell culture regents, cytokines, and mRNA in vitro transcription kits used for Figures 6-8 were compatible with GMP manufacturing of cellular vaccines.
µm transwell inserts and allowed to migrate towards CCL-21 (Peprotech, 100 ng/ml) for 2 hours. Cells that had migrated were harvested and counted using a hemocytometer.

DCs were grown in serum and antibiotic-free media using carrier-free cytokines and immature DCs were harvested. After transfection with mRNAs (10 µg H chain mRNA, 5 µg L chain mRNA, and 5 µg PAP-SS mRNA (all Cap1)), DCs were matured in the presence of TNF-α, IL-1β, IL-6, and PGE2. First, we analyzed if these cells met the phenotypic requirements for batch release. Immature and mature cells were stained with lineage markers CD3 (T cells), CD14 (monocytes/macrophages), CD19 (B cells), and CD56 (NK cells). As can be seen in Figure 9 (left panel, top), all lineage markers were expressed by less than 10% of cells as mandated in our previous INDs. Furthermore, DCs acquired >50% expression of maturation markers CD25, CD83, CD86, and HLA-DR upon maturation as required for batch release (left panel, bottom). Last, DCs up-regulated expression of CCR-7 during maturation and acquired migratory properties toward the lymph node-derived chemokine CCL-21 (MIP-3β, Exodus) as evidenced by in vitro migration (Figure 9, right panel top and bottom).

Last, we analyzed whether our cell product was free of microbial contamination and of endotoxin, as mandated by the FDA. Using Limulus Amebocyte Lysate (Pyrotell), we determined the endotoxin content of our product to be less than 0.5 E.U./ml (data not shown), which is well below the FDA accepted levels of 5 E.U./kg. We also tested our cell product for the absence of Mycoplasma in the PCR-based MycoSensor test (Agilent Technologies). As can be seen in Figure 10, our DC preparation was free of Mycoplasma contamination.

**Figure 10.** MycoSensor PCR for detection of Mycoplasma contamination. DNA was isolated from 50,000 DCs and 100 cell equivalents were analyzed by PCR according to the manual provided by the manufacturer. A DNA band at 0.32 kD indicated Mycoplasma contamination. An internal control band at 0.52 kD indicates that sample preparations do not inhibit the PCR reaction. (Pos) PCR reaction with added Mycoplasma DNA, (Int) PCR reaction with added control DNA, (Int DC) Internal control reaction in the presence of isolated DC DNA. Absence of a 0.32 kD band in PCR reactions containing DNA isolated from DCs indicates the absence of Mycoplasma contamination.

**KEY RESEARCH ACCOMPLISHMENTS:**

1. In murine studies, we have demonstrated that local CTLA4 modulation in combination with PAP-specific immunization using DCs transfected with PAP RNA and CTLA4 RNA is superior to DC PAP RNA immunization in T cell function assays.

2. Using human cells, we have demonstrated post-transcriptional modification of mRNAs enhances protein production by DCs. Post-transcriptional capping and enzymatic methylation of the 2′ hydroxy-group of the first ribose of the 5′ end of the mRNA using 2′-O methyl-transferase (Cap1) should provide significant resistance to intracellular 5′ exonucleases and will be used to generate our clinical DC vaccine.

3. Using human cells, we have demonstrated that human DCs transfected with Cap1 PAP-SS mRNA stimulated highest frequencies of PAP-specific T cells.
CONCLUSION:
Recently, two forms of immunotherapy have demonstrated clinical benefit in patients: active immunotherapy, in which subjects are immunized with antigen presenting cells activated against tumor antigens ex vivo (e.g. sipuleucel-T in prostate cancer) and treatment with systemic immune modulators, such as an antagonistic anti-CTLA4 mAb (e.g. ipilimumab in melanoma). However the use of anti-CTLA4 was associated with adverse events. We have developed a novel strategy that combines tumor immunotherapy targeting PAP and targeted immune modulation of CTLA4 and have generated a lead cellular therapy that will safely enhance vaccine-mediated immunity. This lead cellular therapy, called DC-PAPvac-C, consists of autologous monocyte-derived DCs co-transfected with prostate tumor antigen, PAP RNA and anti-CTLA4 RNA. Thus, targeted delivery of anti-CTLA4 antibody to sites where anti-tumor T cells are induced by tumor antigen-presenting DCs will potentially eliminate adverse effects associated with systemic administration of anti-CTLA4, while also enhancing vaccine-induced immune responses and expanding the potential role for immunotherapy in patients with cancer. In this study we will establish the preclinical efficacy and safety of our cellular therapy product, DCs transfected with RNA that encodes PAP and anti-CTLA4 and generate data required for an IND application. Importantly and relevant to our planned clinical trial implementation, we will develop a biomarker of therapeutic efficacy and demonstrate the feasibility of measuring these biomarkers. In this report we have demonstrated that local CTLA4 modulation in combination with PAP-specific immunization using RNA-transfected DCs elicits robust and superior functional T cell responses in TRAMP mice. We have also demonstrated that post-transcriptional modification of mRNAs (to generate Cap1 mRNA) enhances protein production by human DCs and human DCs transfected with Cap1 PAP mRNA without the signal sequence stimulate highest frequencies of PAP-specific T cells in an in vitro human immunotherapy assay.

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