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# TAG Xenograft Vaccine: Xenograft-Expanded Autologous Tumor Vaccine Genetically Modified to Express GM-CSF and Block Production of TGFβ2

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**P**reliminary studies with a variety of cell-based vaccines suggest target accessibility (potential immunogenicity) to immune-directed approaches in a variety of solid tumors.<sup>[1-7]</sup> However, four primary factors limit the generation of effective immune-mediated anticancer activity in therapeutic applications: 1) identifying and/or targeting cancer-associated immunogen(s) (target) in an individual patient<sup>[8-12]</sup>; 2) insufficient or inhibited level of antigen-presenting cell (dendritic cell, macrophage) priming and/or presentation<sup>[13-15]</sup>; 3) suboptimal T cell activation (potency) and proliferation<sup>[16-21]</sup>; and 4) cancer-induced inhibition of the anticancer immune response in both afferent and efferent limbs.<sup>[22-24]</sup>

In an effort to overcome these limitations, we have designed a novel autologous vaccine to address the inability to fully identify cancer-associated antigens, antigen recognition by the immune system (*i.e.*, antigen → immunogen), effector potency, and cancer-induced resistance.

Previous clinical investigations using two different gene vaccine approaches to

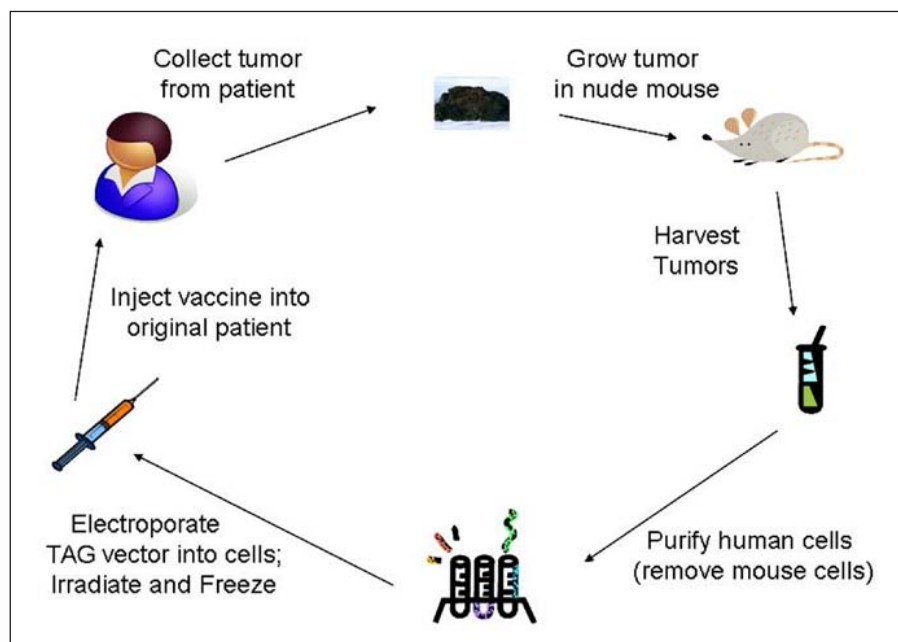


FIGURE 1. Xeno-expanded autologous vaccine strategy.

induce enhancement of tumor antigen recognition have demonstrated therapeutic efficacy.<sup>[25-27]</sup> Specifically, the use of a GM-CSF gene transduced vaccine and a TGFβ2 antisense gene vaccine, in separate trials, have demonstrated similar beneficial effects in advanced cancer patients. The GM-CSF transgene directly stimulates increased expression of tumor antigen(s) and enhances dendritic cell migration to the vaccination site. TGFβ2 blockade following intracellular TGFβ2 antisense gene expression

reduces production of immune inhibiting activity at the vaccine site. These agents have never been used in combination, but the rationale of integrating enhancement of an anticancer immune response concurrently with a reduction in cancer-induced immune suppression is conceptually sound. Both vaccines have demonstrated activity using either an allogeneic or autologous tumor cell supply for antigen.

Capacity to harvest sufficient autologous tissue necessary for appropriate

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dosing above previously defined cell concentration thresholds is limited by accessibility, size of tumor, and surgical risk. We thus, devised a novel method of expanding and extracting sufficient autologous tissue from xenografted mice.

Using a case example, xenograft-expanded autologous cancer from a single patient with advanced refractory leiomyosarcoma (LMS) was successfully harvested, expanded and extracted from xenografted mice. The LMS tumor was serially implanted in nude mice to expand the tumor mass. Xenograft tumors were harvested, transfected with a combination of TGFβ2 antisense/GM-CSF expression vector plasmid, and then dose-irradiated to prevent future tumor growth (TAG vaccine; Figure 1). An emergency protocol was approved by the FDA and Recombinant DNA Advisory Committee (RAC) to treat the patient with the xenograft-expanded autologous vaccine.<sup>[28]</sup>

### TAG Plasmid Design and Manufacturing

The TAG plasmid (Figure 2) used to transfect the autologous tumor cells was pUMVC3 (Aldevron, Fargo, ND) with a GM-CSF-2A-TGFβ2 antisense insert (derived from pCSF/human GM-CSF and pCHEK/human TGFβ2 antisense). The parent plasmid backbone contains a bacterial origin of replication and a kanamycin resistance gene; a *Cytomegalovirus* (CMV) promoter and intron A driving the hGM-CSF cDNA, followed by a 2A linker sequence and a 930 base pair fragment of the hTGFβ2 molecule in antisense orientation; followed by a rabbit hemoglobin poly-A tail.

The TAG vector was sent for GMP source manufacturing (Aldevron). The plasmid passed all quality control testing for sterility, endotoxin, bioburden, identity, residuals, etc. (data not shown). An aliquot of the purified DNA was sent for sequence analysis (Seqwright, Houston, Texas). The entire vector was sequenced in both forward and reverse orientation and the consensus sequence matched the expected vector sequence 100%

(data not shown). This GMP source plasmid DNA was used for all *in vitro* expression assays and electroporation into the patient's tumor cells for vaccine production.

### TAG Plasmid Expression

The TAG vector was electroporated into NCI-H-460 squamous cell and

NCI-H-520, non-small cell lung cancer (NSCLC) cell lines (ATCC, Manassas, VA) to test for GM-CSF expression and TGFβ2 knockdown. The cultured cell media was used for ELISA assay to quantitate the amount of secreted cytokine. Briefly, we found GM-CSF was expressed in the range of 2400–3400 pg/ml in two NSCLC cell lines, NCI-H-460 squamous cell and NCI-H-520 large cell,

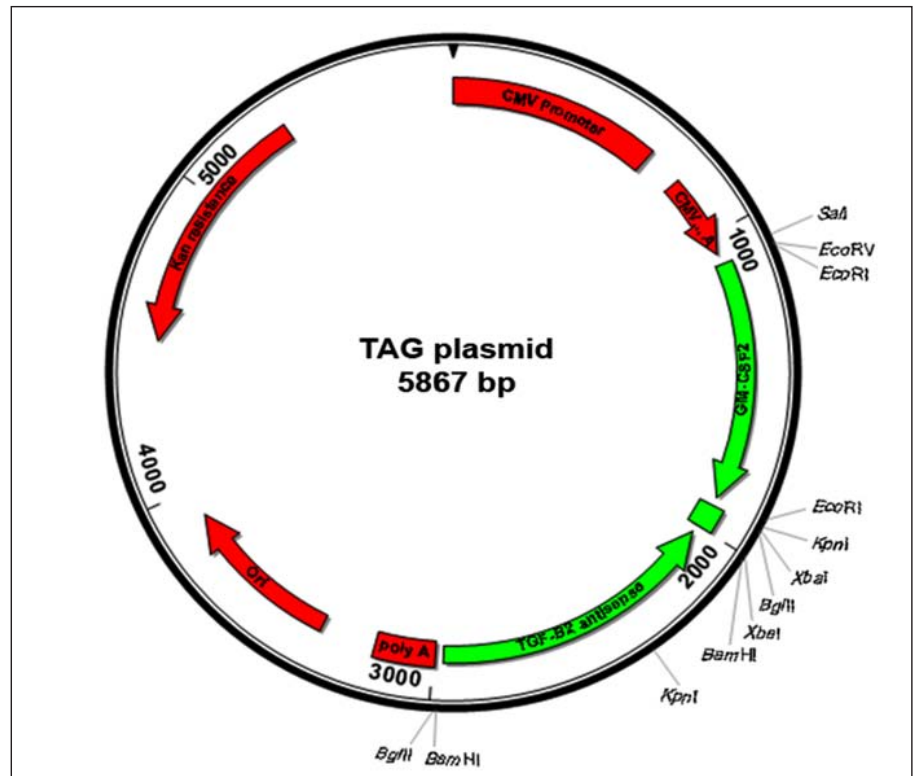


FIGURE 2. Schematic diagram of the TAG plasmid.

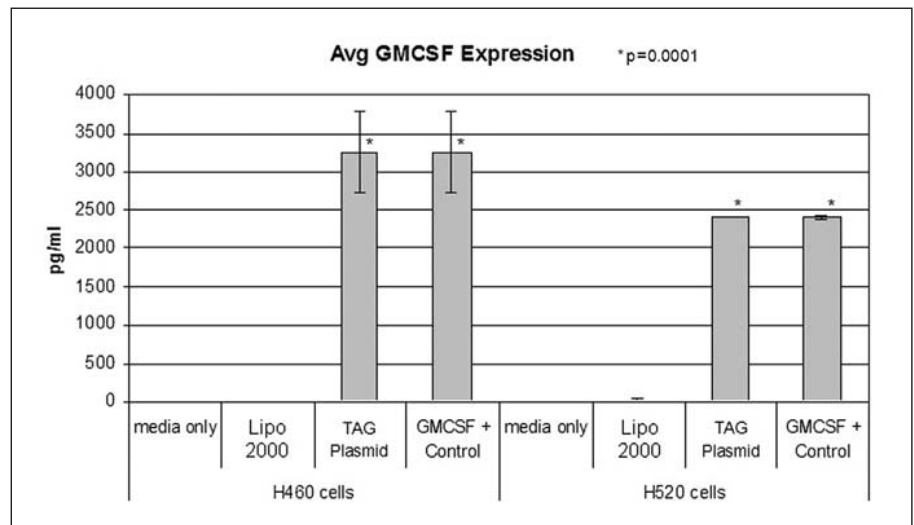


FIGURE 3. GMCSF is expressed in NCI-H-460 and NCI-H-520 cell lines 48 hours after transfection with the TAG vector. P-values are relative to media-only control.

respectively (Figure 3). Conversely, the TGFβ2 levels were reduced approximately 45–60% in NCI-H-460 and NCI-H-520, respectively (Figure 4). Comparative negative controls were media alone and the transfection reagent (Lipofectamine2000, Invitrogen, Carlsbad, CA) and positive single-gene controls (GM-CSF pCSF, ATCC), or TGFβ2 pORF-mTGFβ2 (Invitrogen). Quantikine human GM-CSF and human TGFβ2 immunoassay kits (R&D Systems, Minneapolis, MN) were used to assay the secreted GM-CSF and TGFβ2 in cell culture supernatants.

Total RNA was isolated from cell pellets from the NCI-H-460 and NCI-H-520 cell lines transfected with TAG plasmid. Gene-specific primers were designed to produce a 251 base pair PCR product specific to the 2A-TGFβ2 region. Only the TGFβ2 antisense region was amplified via RT-PCR in cells transfected with the TAG vaccine (Figure 5).

### Xenograft Cell Selection and Composition

LMS tumor from the patient was expanded in a xenograft mouse system (Institute for Drug Development [IDD], San Antonio, TX). Tumors were excised and placed in a sterile container with sterile saline and gentamycin. Tumors were received at the Gradalis manufacturing facility the same day as shipped. The xenograft human tumors were minced and digested with 0.1% Collagenase NB 6, GMP grade (Crescent Chemical Co., Islandia, NY) in X-VIVO 10 (Lonza, Walkersville, MD) to generate a single cell preparation. The cell preparation was then depleted of murine cell components using the murine Ter-119 and CD45 antibody-coated microbeads followed by immunomagnetic separation (Miltenyi Biotec, Auburn, CA).

Magnetic separation of human cells from the single cell suspension was accomplished by negative selection. Following a cell count of the single cell suspension, a small aliquot of cells was retained for use as pre-depletion control. The remaining cells were

incubated with 10 μl of microbeads conjugated to monoclonal anti-mouse Ter-119 and CD45 antibodies for each 10<sup>7</sup> cells in 90 μl of buffer (0.5% bovine serum albumin [BSA] in PBS containing 2 mM EDTA) for 15 min at 4°C. The cell suspension volume and the microbeads volume were scaled up according to total number of cells that were to be purged of mouse cells.

Following incubation, the labeled cells were washed and resuspended in running buffer at 10<sup>7</sup> cells per 500 μl (MS column) or 2 x 10<sup>8</sup> cells per 1.0 ml (LS column) according to the type of separation column that would be used for the depletion of labeled cells. The cell suspension was passed through a 30 μm nylon mesh pre-separation filter to break up any clumps.

The separation column (Miltenyi Biotec) was placed in the magnetic field of the MiniMACS assembly.

The column (MS or LS) was primed by rinsing with the appropriate volume of running buffer (500 μl for MS and 1.0 ml for LS). The effluent was discarded.

The labeled cells in an appropriate column volume of buffer were applied to the column. The unlabeled cells that passed through the column were collected into a sterile 15ml centrifuge tube. The column was further washed with the appropriate volume of buffer (500 μl for MS and 1.0 ml for LS column) three times, and the effluent collected into the same tube. These became the unlabeled human tumor cells (negative fraction).

To collect the positive fraction

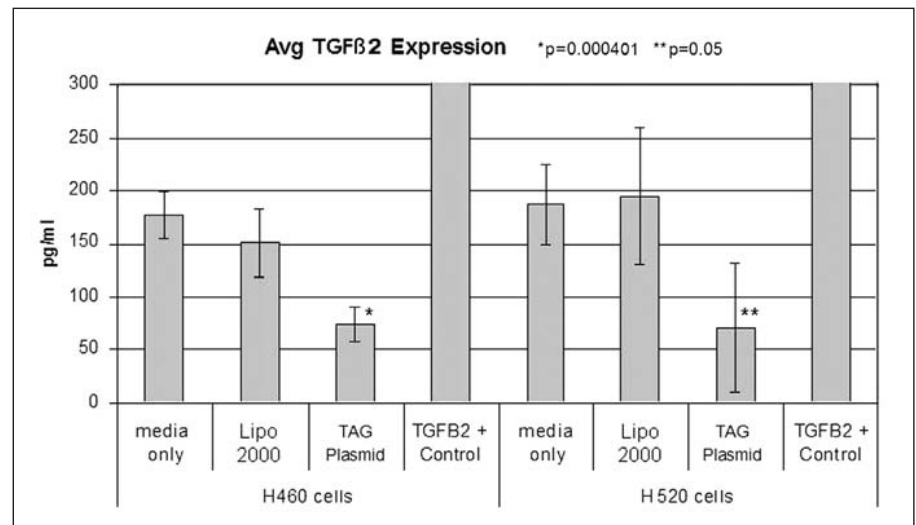


FIGURE 4. TGFβ2 levels are reduced in NCI-H-460 and NCI-H-520 cell lines 48 hours after transfection with the TAG vector. P-values are relative to media only control.

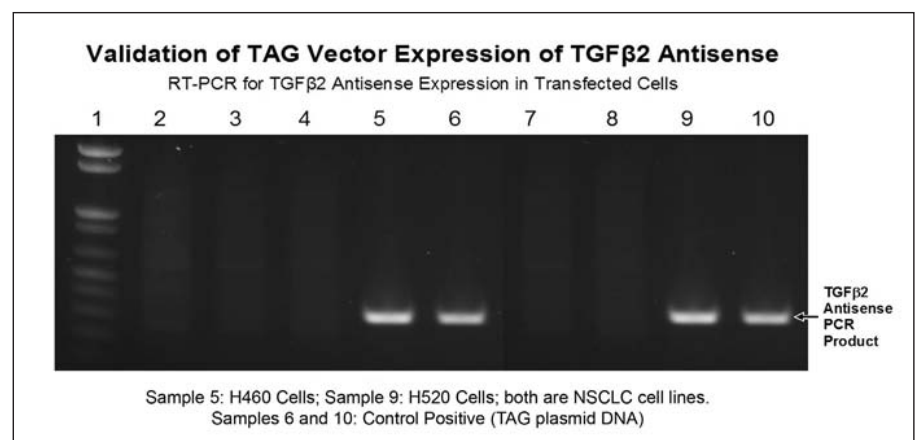


FIGURE 5. Validation of TAG vector expression of TGFβ2 antisense.

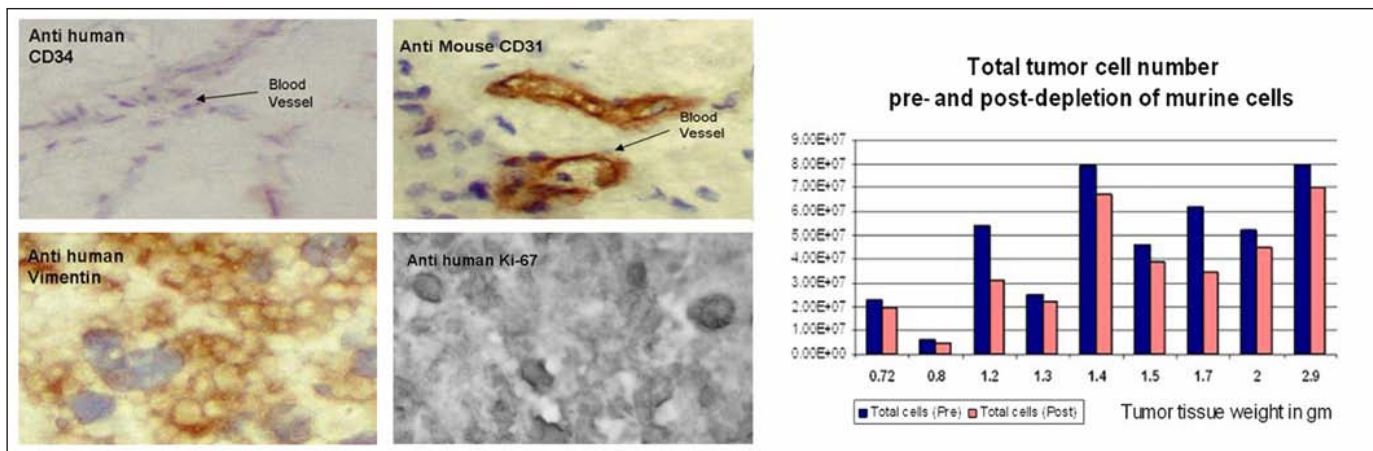


FIGURE 6. Human cells separated from mouse xenograft model.

(mouse Ter-119 and CD45 positive cells) the column was removed from the magnet and placed on top of a 15 ml sterile centrifuge tube. An appropriate volume of buffer was applied to the column. By using the plunger supplied with the column, the labeled (positive fraction) cells were collected. An aliquot of cells from both positive and negative fractions were retained for flow analysis with the prelabeled cells to calculate the depletion efficiency. The negative fraction of cells (cells of human origin) was further prepared for transfection by electroporation.

From the immunohistochemistry evaluation in Figure 6, the murine tissue contamination was shown to be limited to the blood vessels supplying the tumor as shown by the anti-mouse CD31 staining. All the tumor cells and the surrounding tissue stained positive with antibody to human vimentin and KI-67. The blood vessels were negative for human CD34 that stains the human endothelium. Monoclonal anti-human CD34; HLA-A, B, C; monoclonal anti-mouse CD31; and H-2Kq MHC class I alloantigen were purchased from BD Biosciences (San Jose, CA). MAb vimentin and polyclonal Ki-67 were from Santa Cruz Biotechnology (Santa Cruz, CA).

Murine blood cell contaminants were removed using murine antibodies Ter-119 and CD45 that recognize mouse mature erythrocytes and erythroid and leukocytes from lymphoid and

non-lymphoid tissues, respectively. These mouse contaminants were removed and the human cells were enriched (bar graph, Figure 6). The tumor cells pre- and post-depletion dot plots are shown in Figure 7.

### Tumor Cell Transfection

To optimize the electroporation conditions for effective transfection of cells, the purified human tumor cells were electroporated with either green fluorescent protein (GFP) or beta-galactosidase ( $\beta$ -Gal) gWiz reporter gene plasmids (Aldevron). These expression plasmids have similar CMV promoter/enhancer, intron A structure to our TAG plasmid. The conditions were optimized to get maximum expression of the reporter tested by flow cytometry. Optimal conditions were 300 V, 1000  $\mu$ F using a 4 mm gap cuvette and an exponential decay waveform on the Gene Pulsar (Bio-Rad Laboratories, Hercules, CA).

A transfection efficiency of 77% (GFP) and greater than 80% expression

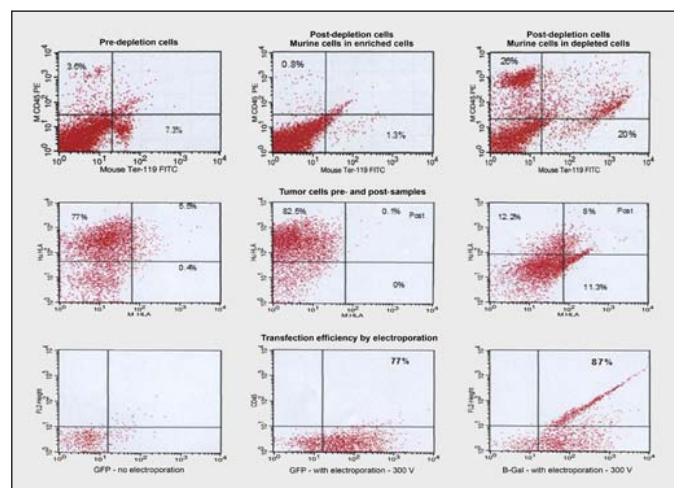


FIGURE 7.

Please refer to page 34 for full-sized image with detailed description.

of  $\beta$ -Gal were achieved (as shown in Figure 7). This was our expected transfection efficiency with the TAG vector which was electroporated into the purified human tumor cells using the same optimized conditions. The TAG vector transfected cells were irradiated and frozen for the vaccine.

The TAG vector was electroporated into the purified tumor cells *ex vivo*. Therefore, only the cells present at the time of electroporation incorporated the transfected DNA. The vector being utilized is expected to remain extra-chromosomal. Amplification of the insert by PCR suggests that the vector is non-rearranged in the cells. Previous human and animal vaccina-

tion studies with TGFβ2 antisense and GVAX (GM-CSF) vaccines have not demonstrated deleterious effects due to unwanted gene expression. It is theoretically possible that the structure or expression of a gene near an integration site (if the vector was incorporated into a chromosome) may be affected by insertion of the added DNA. However, tumor cells used for vaccinations are being irradiated with 10,000 cGy to block their growth potential. Irradiation occurs prior to final fill and freezing. Furthermore, gene modified irradiated tumor cells have a limited half-life following intradermal injection.

### Patient Vaccine Manufacturing

LMS tumor from the patient was expanded in a xenograft nude mouse system (IDD). Two manufacturing processes were performed approximately two weeks apart. The patient's xenograft expanded, excised tumors were placed in sterile saline containing gentamycin and packaged for transport to the manufacturing facility. The package containing the tumors was received, inspected and brought into the manufacturing area. All manufacturing processes occurred within one of the biosafety Level 7 (Class 10,000) vaccine manufacturing suites

and all open manipulations were performed in a biosafety Level 5 (Class 100) biosafety cabinet. The xenograft human tumors were minced and digested with 0.1% collagenase in X-VIVO 10 to generate a single cell preparation. Cells were then depleted of murine cell components using the murine Ter-119 and CD45 antibody coated microbeads followed by immunomagnetic separation. Flow cytometry results are summarized in Table 1. The flow cytometry data indicated that the residual murine cell contamination in the negative fraction was approximately 2–3%, comprised primarily of Ter-119 positive (erythroid) cells.

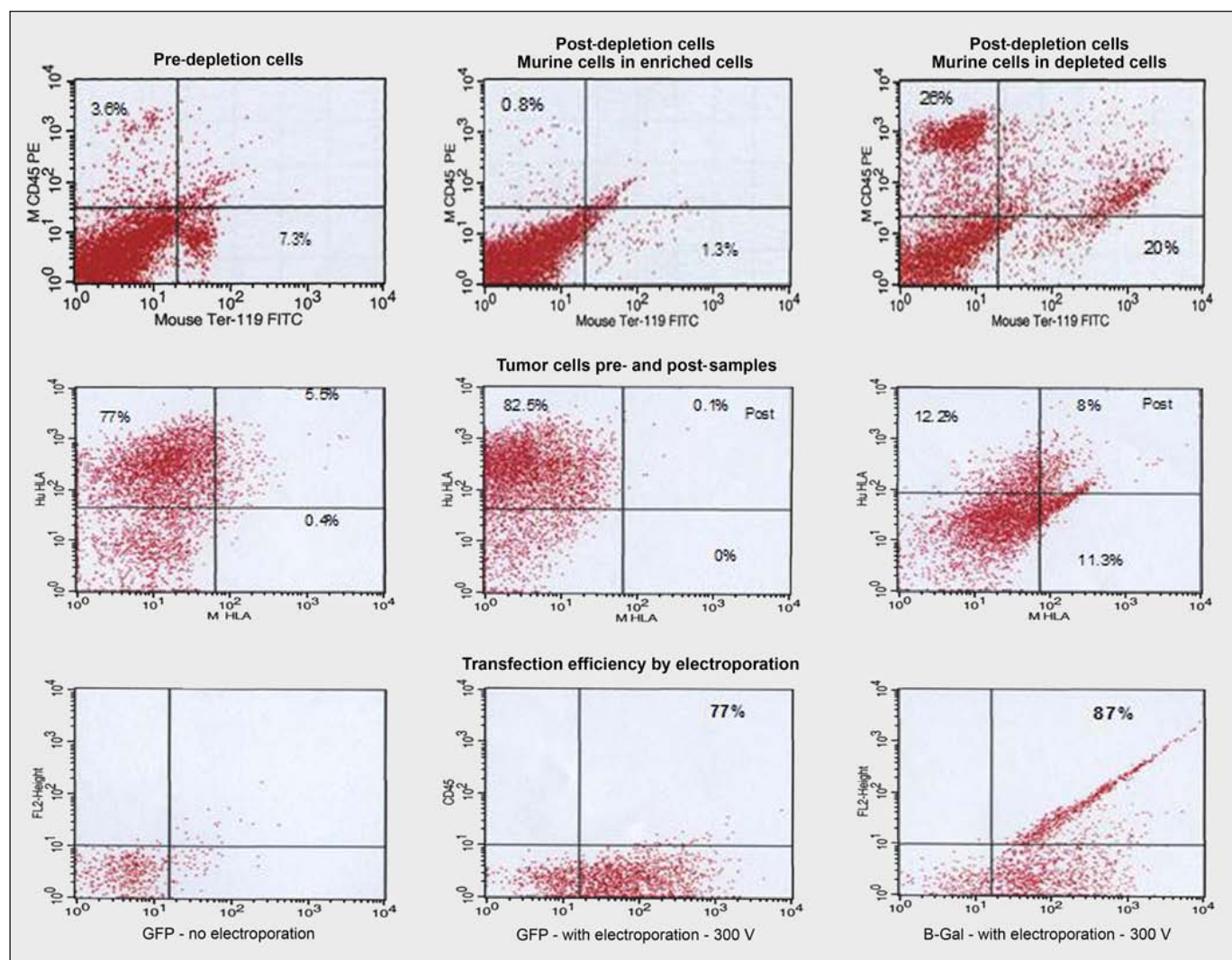


FIGURE 7. Flow cytometry analysis of human tumor cell isolation and electroporation. Top panel shows the Ter-119 and CD45 positive cells in pre-depletion sample (7.3% and 3.6% respectively), in the enriched negative human tumor cell population (1.3% and 0.8% respectively) and in the depleted positive cell population (20% and 26%). Middle panel shows the human cells in pre-depletion sample (77%), enriched negative sample (82.5%) and in the depleted positive sample (12.2%). Lower panel shows the transfection efficiency of the enriched negative cells with reporter gene eGFP 77% (enhanced GFP) and β-Gal plasmid 87% in pUMVC3 vector.

Tumor cells were transfected by electroporation with the TAG plasmid. Electroporated tumor cells were incubated overnight at 37°C to allow expression of TGFβ2 antisense and GM-CSF protein.

The following day, cells were harvested, washed and irradiated at 10,000 cGy using a local blood bank irradiator. Cells were enumerated and placed in final formulation media consisting of 10% dimethyl sulphoxide ([DMSO] Cryoserv USP; Bioniche Pharma, Galway, Ireland), 1% human serum albumin ([HSA] Flexbumin, 25% USP, Baxter, Deerfield, IL) in Plasma-Lyte A, pH 7.4 (USP grade, Baxter). Vaccine vials were frozen and stored in the vapor phase of liquid nitrogen. The results of the manufacturing processes are summarized in Table 2.

The doses of vaccine manufactured in the first process were lost due to freezer failure during the overnight freezing process. The second manufacturing run was performed using smaller tumors from fewer mice. Quality control test samples were taken for the following tests:

- In-house sterility using BBL Septi-Check TSB Media (BD Biosciences) for bacterial and fungal growth;
- Flow cytometry analysis for murine cell contamination, transfection and identity;
- GLP endotoxin (US FDA “Points To Consider”);
- B/F sterility test validation;
- USP sterility;
- *Mycoplasma* detection (US FDA “Points To Consider”);
- GLP rapid *Mycoplasma* detection;
- GLP mouse antibody production; (MAP) test with lymphocytic choriomeningitis virus (LCMV) challenge;
- GLP *in vivo* assay for viral contaminants (US FDA requirements);
- GLP *in vivo* assay for viral contaminants (European addition to US FDA test); and
- GLP co-cultivation of test article cells with *Mus dunni* cells and PG4 S+L end point: 2 passes–28 days set up and first sample.

The quality control test results for manufacturing run #2 are listed in Table 3.

TABLE 1. Negative depletion results for murine cell removal.

|         | Murine Antibody | Pre-Depletion | Negative Fraction | Positive Fraction |
|---------|-----------------|---------------|-------------------|-------------------|
| Mfg. #1 | Mouse CD45      | 34.85         | 0.68              | 48.81             |
|         | Ter-119         | 3.30          | 1.45              | 20.79             |
| Mfg. #2 | Mouse CD45      | 17.59         | 0.99              | 38.34             |
|         | Ter-119         | 3.34          | 1.80              | 22.25             |

TABLE 2. Vaccine manufacturing results.

|         | Number of Tumors (Mice) | Total Weight of Tumors (g) | Vials of Vaccine (@ 2.5 x 10 <sup>7</sup> cells) |
|---------|-------------------------|----------------------------|--|
| Mfg. #1 | 21                      | 18.3                       | 14   |
| Mfg. #2 | 10                      | 4.4                        | 3  |

TABLE 3. Quality control test results for manufacturing run #2.

| Test Description   | Test Facility  | Result   |
|--|----------------|--|
| Bacterial endotoxins   | WuXi AppTec    | No inhibition or enhancement of test system shown.   |
| Environmental testing  | Gradalis, Inc. | No growth.   |
| Sterility test   | Gradalis, Inc. | No growth.   |
| Sterility test   | WuXi AppTec    | No growth.   |
| Sterility test validation (B/F)  | WuXi AppTec    | No bacteriostatic/fungistatic activity demonstrated.   |
| Detection of <i>Mycoplasma</i> DNA by polymerase chain reaction (PCR): GLP (rapid)                                 | WuXi AppTec    | No <i>Mycoplasma</i> DNA sequences detected.   |
| <i>Mycoplasma</i> detection “Points to Consider”   | WuXi AppTec    | No <i>Mycoplasma</i> contamination detected.   |
| Custom <i>in vitro</i> assays for adventitious viral contaminants  | WuXi AppTec    | No adventitious virus detected.  |
| Co-cultivation of test article cells with <i>Mus dunni</i> cells: 2 passes   | WuXi AppTec    | No xenotropic, amphotropic, or MCF MuLV retroviral contamination found. No foci observed in PG4 assay. |
| Mouse antibody production (MAP) test with LCMV challenge   | WuXi AppTec    | None of 19 murine adventitious viral contaminants detected.  |
| <i>In vivo</i> assay for viral contaminants (US FDA) – inoculation of embryonated hen eggs, adult and newborn mice | WuXi AppTec    | No viral contamination detected.   |
| <i>In vivo</i> assay for viral contaminants – animal portion   | WuXi AppTec    | No viral contamination detected.   |

All the test results indicate that the product met specifications, meaning no bacteria or viruses were detected and endotoxin was within acceptable limits.

The vaccine was conditionally approved for use by FDA and RAC under an Emergency Use protocol (BB-IND 13401). The vaccine was not used for patient treatment because the

patient became ineligible for the treatment protocol.

The vaccine manufacturing process for xenograft-expanded human tumors has been developed and approved for human use. Vaccine for a single patient IND has been made. The manufacturing process represents a new avenue for autologous tumor vaccine production

when tumor tissue is limited. Further development of the technology will focus on refining the murine purging step. The Quality Control Test Plan is feasible but extremely expensive. Each test will continue to be evaluated for relevance to overall product safety. A multi-patient Phase I clinical trial is now being pursued.

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#### REFERENCES

- [1] Kwak LW. Translational development of active immunotherapy for hematologic malignancies. *Semin Oncol*, 2003. 30(3 Suppl 8): p. 17-22.
- [2] Kaufman HL, et al. Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): a trial of the Eastern Cooperative Oncology Group. *J Clin Oncol*, 2004. 22(11): p. 2122-32.
- [3] Coppin C, et al. Immunotherapy for advanced renal cell cancer. *Cochrane Database Syst Rev*, 2005(1): p. CD001425.
- [4] Quan WD Jr, et al. Active specific immunotherapy of metastatic melanoma with an anti-idiotypic vaccine: a phase I/II trial of I-Mel-2 plus SAF-m. *J Clin Oncol*, 1997. 15(5): p. 2103-10.
- [5] Alexandroff AB, et al. BCG immunotherapy of bladder cancer: 20 years on. *Lancet*, 1999. 353(9165): p. 1689-94.
- [6] Eton O, et al. Sequential biochemotherapy versus chemotherapy for metastatic melanoma: results from a phase III randomized trial. *J Clin Oncol*, 2002. 20(8): p. 2045-52.
- [7] Walden P. Therapeutic vaccination for the treatment of malignant melanoma. *Recent Results Cancer Res*, 2007. 176: p. 219-27.
- [8] Anichini A, et al. Cytotoxic T lymphocyte clones from peripheral blood and from tumor site detect intratumor heterogeneity of melanoma cells. Analysis of specificity and mechanisms of interaction. *J Immunol*, 1989. 142(10): p. 3692-701.
- [9] Darrow TL, Slingluff CL Jr, and Seigler HF. The role of HLA class I antigens in recognition of melanoma cells by tumor-specific cytotoxic T lymphocytes. Evidence for shared tumor antigens. *J Immunol*, 1989. 142(9): p. 3329-35.
- [10] Knuth A, et al. Cytolytic T-cell clones against an autologous human melanoma: specificity study and definition of three antigens by immunoselection. *Proc Natl Acad Sci USA*, 1989. 86(8): p. 2804-8.
- [11] Topalian SL, Solomon D, and Rosenberg SA. Tumor-specific cytotoxicity by lymphocytes infiltrating human melanomas. *J Immunol*, 1989. 142(10): p. 3714-25.
- [12] Van den Eynde B, et al. Presence on a human melanoma of multiple antigens recognized by autologous CTL. *Int J Cancer*, 1989. 44(4): p. 634-40.
- [13] Hirschowitz EA, et al. Autologous dendritic cell vaccines for non-small-cell lung cancer. *J Clin Oncol*, 2004. 22(14): p. 2808-15.
- [14] Ribas A. Genetically modified dendritic cells for cancer immunotherapy. *Curr Gene Ther*, 2005. 5(6): p. 619-28.
- [15] Small EJ, et al. Granulocyte macrophage colony-stimulating factor-secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. *Clin Cancer Res*, 2007. 13(13): p. 3883-91.
- [16] Woo EY, et al. Cutting edge: regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. *J Immunol*, 2002. 168(9): p. 4272-6.
- [17] Dohadwala M, et al. Non-small cell lung cancer cyclooxygenase-2-dependent invasion is mediated by CD44. *J Biol Chem*, 2001. 276(24): p. 20809-12.
- [18] Neuner A, et al. Prognostic significance of cytokine modulation in non-small cell lung cancer. *Int J Cancer*, 2002. 101(3): p. 287-92.
- [19] Steinman RM, et al. Dendritic cell function in vivo during the steady state: a role in peripheral tolerance. *Ann NY Acad Sci*, 2003. 987: p. 15-25.
- [20] Du C and Sriram S. Mechanism of inhibition of LPS-induced IL-12p40 production by IL-10 and TGFβ in ANA-1 cells. *J Leukoc Biol*, 1998. 64(1): p. 92-7.
- [21] Takeuchi M, Alard P, and Streilein JW. TGFβ promotes immune deviation by altering accessory signals of antigen-presenting cells. *J Immunol*, 1998. 160(4): p. 1589-97.
- [22] Rook AH, et al. Effects of transforming growth factor beta on the functions of natural killer cells: depressed cytolytic activity and blunting of interferon responsiveness. *J Immunol*, 1986. 136(10): p. 3916-20.
- [23] Tsunawaki S, et al. Deactivation of macrophages by transforming growth factor-beta. *Nature*, 1988. 334(6179): p. 260-2.
- [24] Fontana A, et al. Transforming growth factor-beta inhibits the generation of cytotoxic T cells in virus-infected mice. *J Immunol*, 1989. 143(10): p. 3230-4.
- [25] Nemunaitis J, et al. Granulocyte-macrophage colony-stimulating factor gene-modified autologous tumor vaccines in non-small-cell lung cancer. *J Natl Cancer Inst*, 2004. 96(4): p. 326-31.
- [26] Nemunaitis J, et al. Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. *J Clin Oncol*, 2006. 24(29): p. 4721-30.
- [27] Nemunaitis J, et al. Phase II Trial of Belagenpumatucel-L, a TGFβ antisense gene modified allogeneic tumor vaccine in advanced non-small cell lung cancer (NSCLC) patients. *Cancer Gene Ther* (in press).
- [28] Wallraven G, Maples PB, and Nemunaitis J. Emergency use regulatory approval process for experimental gene-based therapy. *J Clin Oncol*, 2008. 26(33): p. 5488-9.