LAMPvax DNA Vaccines as Immunotherapy for Cancer - Three Case Studies

Cancer immunotherapy has emerged as a clinically validated tool for fighting certain kinds of cancers. These therapeutic cancer vaccines work by driving a large immune response that counters immune evasion by tumor cells, re-training the cells of the immune system to identify and remove tumor cells, or help maintain clinical remission.

As the following three case studies illustrate, LAMP Technology is a versatile platform for developing high value therapeutic vaccines. It is flexible in regards to the type of formulation, the antigen or antigens used, as well as the delivery method. Incorporation of LAMP Technology for autologous dendritic cell therapy shows a profound enhancement of the immune response. The use of ITI’s advanced LAMP Technology formulation enables therapeutic nucleic acid vaccination without resorting to direct immune system stimulation with DCs.

Case Study 1 - GRNVAC1 / hTERT DC vaccine for prostate cancer and AML

Telomerase activation is a hallmark of most cancer cells, allowing continuous replication and immortalization. It is also a potent tumor antigen and important target for the development of therapeutic cancer vaccines. In 2007, Immunomic Therapeutics issued an exclusive license for LAMP Technology to the Geron Corporation for use in GRNVAC1, a telomerase autologous DC therapy.

Pre-clinical results were first published in 2002 when Dannull and Gilboa reported that dendritic cells loaded with mRNA encoding human Telomerase reverse transcriptase (hTERT), or hTERT and the lysosomal targeting sequence of LAMP (hTERT/LAMP), generated a potent immune response in human cell lines. The inclusion of the lysosomal targeting sequence enhanced the CD4+ response, while inducing a strong cytotoxic T-cell response. The generation of a thorough and sustained anti-telomerase immune response provided a clear rationale for advancing the hTERT/LAMP DC therapy into clinical trials.

Table 1. hTERT/LAMP and hTERT DC vaccination of 20 Prostate Cancer Patients.

<table>
<thead>
<tr>
<th>Treatment Assignment</th>
<th>CD4+ fold Increase* IFN-γ Elispot</th>
<th>CD8+ fold increase* IFN-γ Elispot</th>
<th>Survival after Vac (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAMP n=9</td>
<td>43.90</td>
<td>228.12</td>
<td>21.22</td>
</tr>
<tr>
<td>TERT n=11</td>
<td>2.89</td>
<td>14.20</td>
<td>18.36</td>
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* Measured over baseline, two weeks after final vaccination.
In 2005, the Duke team reported on Phase I/II results of GRNVAC1 in a randomized clinical trial of 20 prostate cancer patients. The primary goal of the study was to enhance the CD4+ T-cell response, study how vaccination effected memory T-cell formation, and measure telomerase-specific CD8+ mediated cell killing. hTERT/LAMP treated patients exhibited higher CD4+ and CD8+ T-cell responses, associated with a trend towards memory cell formation (as determined by the secretion of the cytokine IL-2), and in generating an immune response (Table 1). As shown in Figure 4, the CD8 T-cell response was boostable up to 50 weeks after the initial vaccination.

On the basis of the prostate cancer Phase I/II study, Geron initiated a Phase II study testing GRNVAC1 for acute myelogenous leukemia in 2007. The trial completed enrollment of 25 patients and sufficient DCs were collected for 21 patients. Results from the completed trial were published in December 2010 at ASH.
At that time, 13 of 21 patients remained in extended complete remission, some up to 36 months, with 9 of 11 high risk patients in clinical remission. Median duration of follow-up was 13.2 months. Based off of ELISPOT assays, **55% of patients developed a telomerase-specific immune response** (Figure 5). All patients in clinical remission are also in molecular remission, as determined by the disease marker WT-1.

The experience with the AML patients shows that LAMP nucleic acid formulations are safe, with some patients receiving up to 30 injections of therapy, and that LAMP-mediated education of the immune system occurs, primarily through helper T-cell activation.

**Case Study 2 - Improved maturation protocol with LAMP mRNA loading of DCs**

Dendritic cells sit at the intersection of the adaptive immune response. When exposed to an antigen and danger signal, DCs co-ordinate antigen presentation with effector and cytotoxic T-cells through an exchange of activation factors and co-stimulatory molecules. Harnessing DCs for therapeutic indications is a compelling approach to combat challenging diseases like cancer. The launch of Dendreon’s Provenge in 2010 marked the first generation of DC therapies to reach the market; similar therapies are in advanced clinical trials. However, the process of
extracting a patient’s DCs and treating them to become cell therapies is prohibitively expensive due to a highly complex supply chain. The maturation protocols used may also lead to anergy and a reduction in therapeutic efficacy.

To address the manufacturing issues of autologous DCs, a group led by Dr. Kris Thielemans, Vrije Universiteit Brussel, has shown that a mixture of maturation signals and tumor antigens encoded as mRNA greatly enhances the immune stimulating ability of DCs. This mixture, referred to as TriMix, consists of CD40 ligand, constitutively active TLR4, CD70, and tumor antigen mRNA. Recognizing that CD4+ T-cell activation is necessary for effective vaccination, the lab of Dr. Thielemans has tested a variety of intracellular targeting strategies with tumor antigen mRNA, including the transmembrane / cytoplasmic domain of invariant chain (“li”), LAMP-1, and DC-LAMP (Figure 6).

The addition of LAMP-1 or DC-LAMP lysosomal targeting sequence to eGFP resulted in high expression in DCs electroporated both before and after maturation, while eGFP linked to the li-targeting domain resulted in much lower expression levels. When the melanoma antigen MAGE-A3 was linked to either LAMP-1 or DC-LAMP lysosomal targeting sequence, the electroporated DCs were much more efficient in stimulating the MAGE-A3 specific T-cells than when the antigen was linked to the the li-derived sorting signal.

On the basis of the intracellular targeting experiments, LAMP-1 was selected for inclusion in a clinical protocol in combination with TriMix. In a thorough study of a single melanoma patient’s immune response, the TriMix LAMP DC therapy showed: a) no major differences between CD8+ T-cells stimulated with peptide-pulsed or TAA-coelectroporated DCs, that b) T-cells stimulated with coelectroporated TriMix DCs induced more cells secreting both IFN-γ and TNF-α, and that c) TriMix DCs coelectroporated with Mage-A3, Mage-C2, tyrosinase, or gp100 mRNA generated strong, vaccine-induced responses against other Mage-A3, Mage-C2, and tyrosinase epitopes, which were not present before vaccination.

In 2011, the Thielemans group reported on a Phase I clinical trial of 11 melanoma patients treated with TriMix DC therapy and MAGE-A3, MAGE-C2, Tyrosinase and gp100 antigens linked to LAMP-1. As reported at the 2011 ASCO meeting, 8 patients were evaluated. Immune responses were documented in 6/7 patients, with 5/7 patients exhibiting a CD137+CD8+ and 4/7 patients exhibiting a CD4+ T-cell response. Regression of metastases occurred in two
patients’ lung metastases and 3 patients lymph node metastases. Four of the eight patients remain progression-free after 3.1, 3.6, 7.7 and 8.2 months of follow-up.

The Thielemans Group is initiating a Phase II trial of TriMix and lysosomal targeted antigens in melanoma patients.

**Case Study 3 - Autologous DCs targeting the tumor-associated stroma using FAP**

Tumor progression beyond a minimal size is critically dependent on normal cells such as endothelial cells, smooth muscle cells, fibroblasts, and other cell types, collectively known as the tumor stroma. Immunizing against proteins preferentially expressed by the tumor stroma could help control tumor growth. Because stromal cells, unlike tumor cells, are diploid, genetically stable, and exhibit limited proliferative capacity, targeting the stroma could substantially reduce the incidence of immune evasion.

Fibroblast associated protein (FAP) is recognized to be a potential cancer antigen for immunotherapy, due to its tightly regulated expression in the tumor stroma and structurally defined proteolytic activity; however, its function in tumors is largely unknown. FAP is expressed selectively by tumor-associated fibroblasts and pericytes in more than 90% of human epithelial cancers examined. It is also expressed during embryonic development, in tissues of healing wounds, and in chronic inflammatory and fibrotic conditions such as liver cirrhosis and idiopathic pulmonary fibrosis, as well as on bone and soft tissue sarcomas and some melanoma.

To determine whether a surface protein of the tumor stroma was antigenic, a research group led by Dr. Eli Gilboa tested autologous DCs loaded with mRNA of either MMP-9, MMP-14, or FAP and stimulated PBMCs of three human patients. A FAP-specific CTL response was induced in all three donors, while in contrast, MMP-9- and MMP-14-specific CTL could only be generated from one of three donors. Having established that the tumor stroma protein was antigenic, Gilboa and colleagues tested a vaccination strategy combining the LAMP-1-derived lysosomal targeting sequences to the COOH end of FAP. Inclusion of LAMP significantly enhanced the generation of FAP-specific IFN-gamma-secreting CD4+ T-cells (Figure 7). LAMP-modified FAP mRNA-transfected dendritic cells stimulated a more potent CTL response.

In a mouse model of aggressive tumor growth, vaccination with FAP mRNA significantly slowed tumor growth. Interestingly, vaccination of the tumor–bearing mice with FAP-LAMP was significantly more effective than vaccination with unmodified FAP antigen in slowing tumor progression (P = 0.0354) and resulted in the long-term regression of tumors in a fraction of the vaccinated animals (Figure 8, left).
To determine whether metastatic growth is also susceptible to FAP immunotherapy, FAP-immunized mice were challenged with B16/F10.9 melanoma cells i.v. and the appearance of lung metastasis was monitored. Lung metastasis was significantly inhibited in mice immunized against the FAP-LAMP antigen, which was comparable or superior to that of vaccination against the tumor cell–expressed TRP-2 antigen. Reduced metastasis also correlated with a significant extension of survival of the FAP-LAMP and TRP-2 vaccinated groups (Figure 8, right; P < 0.0001).

Compared to GRNVAC1 and TriMix DCs, which have brought elements of LAMP Technology into human clinical trials, the application of LAMP Technology to the development of a tumor stroma vaccine is interesting as a proof-of-concept: that mildly antigenic proteins, if properly presented to the immune system in a LAMP formulation, become more immunogenic than strongly antigenic proteins like Survivin and TRP-2 and help protect mice from challenge with lethal cancers.

Figure 8. FAP-LAMP constructs enhance survival on challenge with two highly aggressive cancer tumor models.