

Supplemental information

**Personalized neoantigen vaccines as early intervention in untreated patients with
lymphoplasmacytic lymphoma: a non-randomized phase 1 trial**

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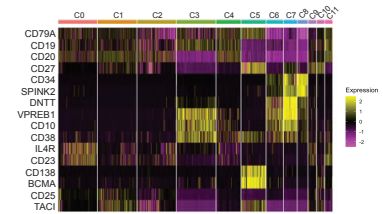
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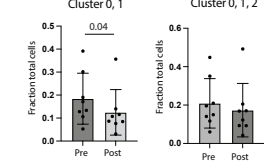
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Fig. S1. Idiotypic-neoantigen vaccine generation. Clustering and annotation of cell types identified by single cell RNA-seq analysis of bone marrow. (A) Workflow for identification, isolation, and cloning of LPL-derived Ig VH and VL sequences for LPL-001, as one representative example (top). CONSORT flow diagram of the trial (bottom). (B) UMAP of combined total microenvironment datasets of all 9 patients pre- and post-vaccine (17 bone marrow samples and 36777 cells). Twenty-five clusters were generated by Seurat analysis of gene expression. (C) UMAPs of total microenvironment dataset with depicted level of expression of selected marker genes (blue). (D) UMAPs of total microenvironment datasets split by sample. (E) UMAPs of total microenvironment dataset with highlighted cells for which corresponding single cell TCR (left) and single cell BCR (right) sequencing data was detected. (F) Heatmap of differential expression of top 20 gene markers for each cluster, compared with average expression of total input cells.

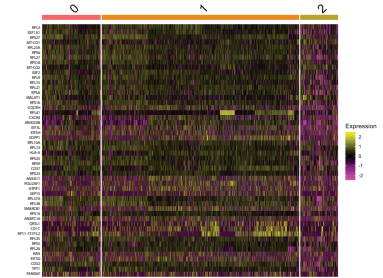
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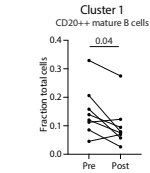
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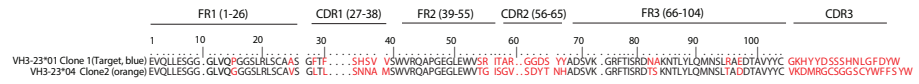
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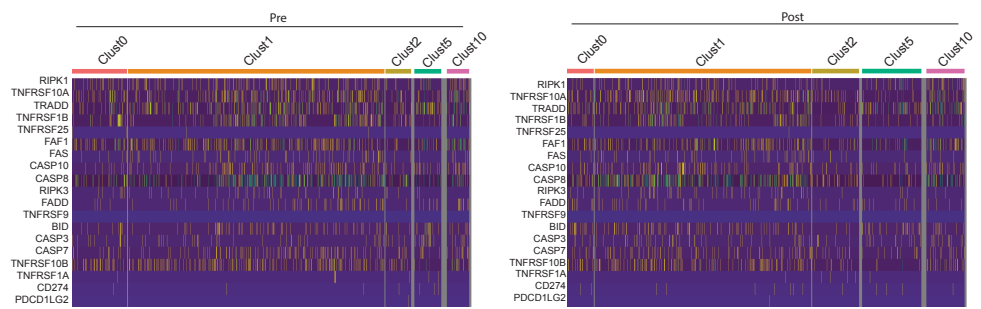
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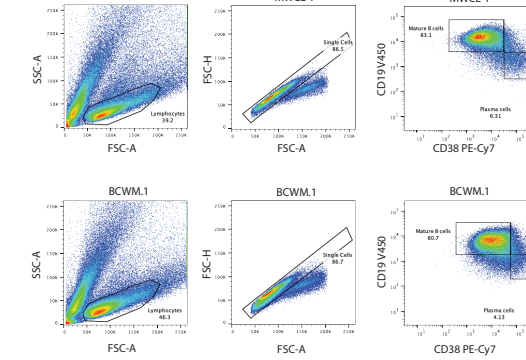


Figure S2.

Fig S2. Annotation of B-cell subpopulations by single cell RNA-seq. (A) Heatmap of differential expression of select marker genes for B cell clusters. (B) Cell frequencies of combined B-lineage clusters 0 and 1 (left; $P < 0.05$) and 0, 1 and 2 (right; $P = 0.25$), comparing aggregate values for individual patients ($n = 8$) in pre- and post-vaccine bone marrow samples. Data are presented as mean values $\pm$ SD. Two-sided Wilcoxon matched-pairs signed rank test was used. (C) Heatmap of top 50 differentially expressed genes in pre vaccination B cell clusters, showing distinct profile of cluster 2, compared with clusters 0 and 1. (D) Cluster 1 B-lineage cell frequencies for individual patients ($n = 8$) in paired pre- and post-vaccine bone marrow samples. Two-sided Wilcoxon matched-pairs signed rank test was used. (E) Amino acid sequence alignment of LPL-002 Ig heavy chain variable region gene HV3-23 of the targeted tumor BCR clone used to manufacture idiotype vaccine, compared a non-targeted minor BCR clone 2 (blue and orange respectively in Fig. 1E). (F) Heatmap of expression of select death cell receptor genes and immune checkpoint molecules in tumor cell clusters pre and post vaccination compared with average expression of total input cells. (G) Flow cytometry analysis of plasma cell-like and mature B cell subpopulations of MWCL-1 and BCWM.1 LPL cell lines. Source data are provided as a Source Data file.

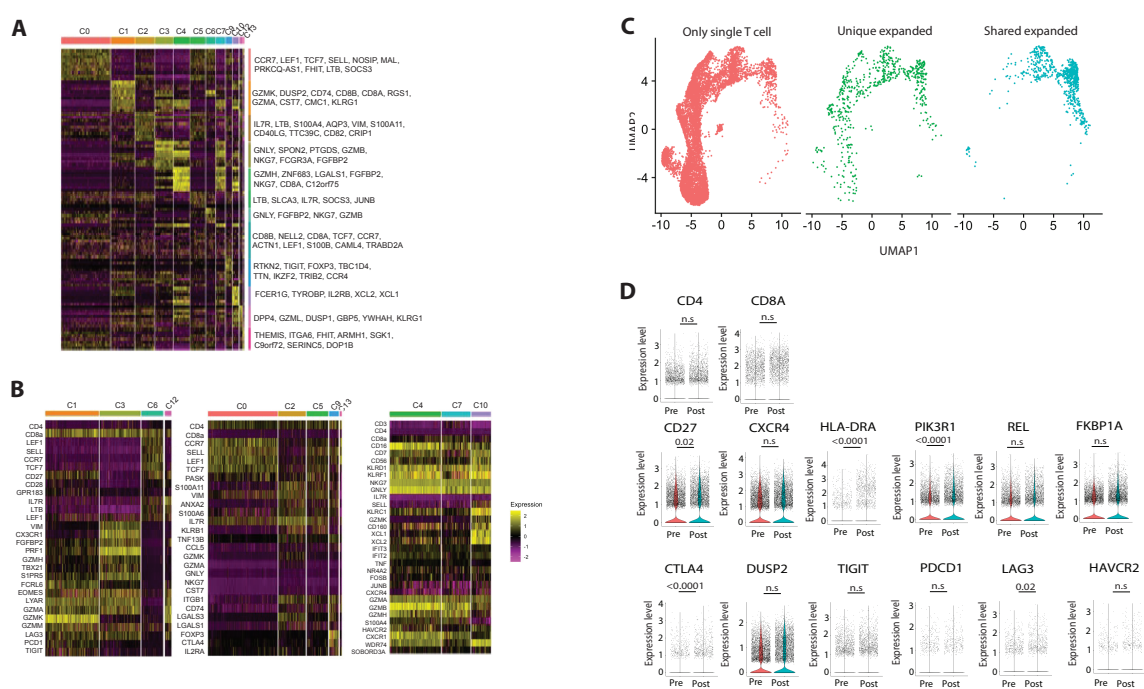


Figure S3.

Fig S3. Annotation of T-cell subpopulations by single cell RNA-seq. (A) Heatmap of differential expression of top 20 marker genes for each T- or NK-cell cluster, compared with average expression by total T-/NK- cells. (B) Heatmaps of select differentially expressed marker genes in subpopulations of CD8 (left) CD4 (center) and NK cells (right) by cell cluster. (C) UMAP plots of T cell populations with overlapped TCR clonotype data as indicated. (D) Violin plots of expression levels of selected gene markers by the total T cell population, excluding the top 20 T-cell clonotypes, pre- vs. post-vaccine. Wilcoxon rank sum test was used. n.s. not significant.

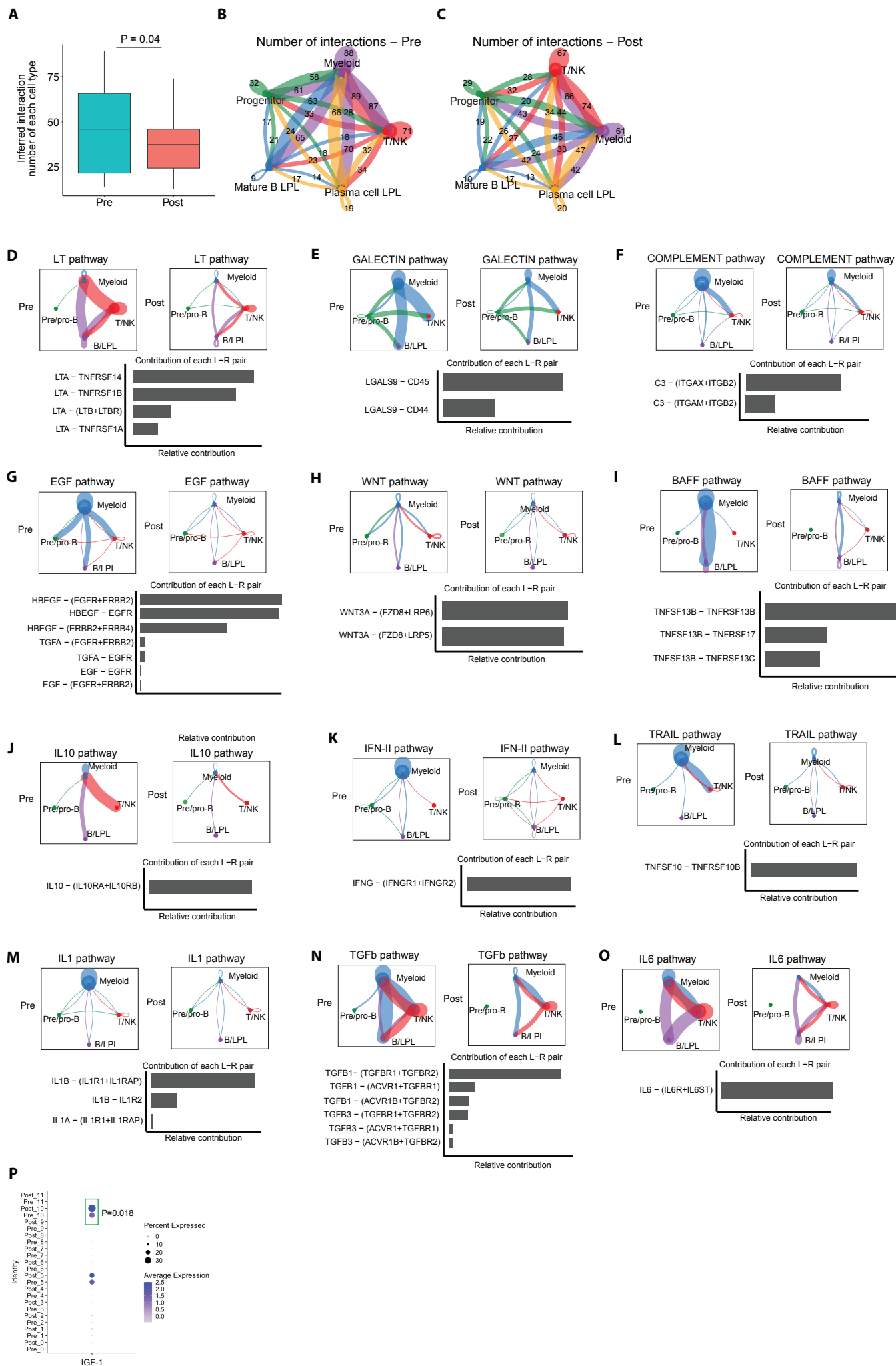


Figure S4.

Fig S4: Cell-cell communication networks in the tumor microenvironment. (A) Boxplot showing mean numbers of inferred interactions (ligand-receptor pairs) across all cell types analyzed. Aggregate data from all patients. Wilcoxon matched-pairs signed rank test was used to compare pre- and post-vaccine bone marrow samples. (B) and (C) Circle plots showing the numbers of outgoing and incoming ligand-receptor interactions between each pair of cell types, pre- and post-vaccine treatment. (D-O) Circle plots for individual signaling pathways. (P) Dot plot of expression of IGF-1 in main tumor clusters pre and post vaccination. Green rectangle indicates upregulation in post vaccine. Wilcoxon rank sum test was used.

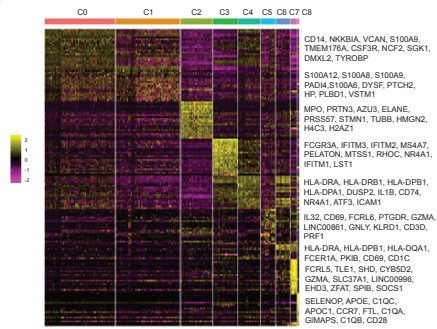
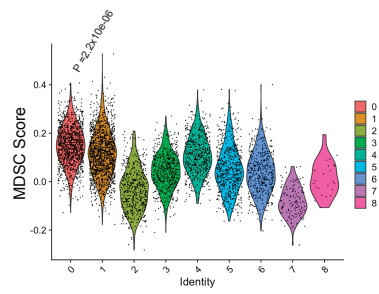
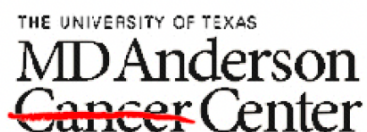
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Figure S5.

Fig S5. Annotation of myeloid cell clusters identified by scRNAseq. (A) Heatmap of differential expression of the top 20 marker genes for each myeloid cell cluster, compared with average expression by all myeloid cells. (B) Violin plot of MDSC gene signature (Wfdc17, Ifitm1, Il1b, BC100530, Srgn, Gm5483, Stfa2l1, Stfa2, Prok2, Ifitm2, Junb, Dusp1, Socs3, Btg1, 1600014C10Rik, Selp1g, Asprv1, Igfbp6, Gm5150, Pla2g7, Csf3r, Cxcr2, Tpd52, Tspo, Cyp4f18, Grina, Fabp5, Clec4d, Steap4, Map1lc3b) score in myeloid clusters calculated using AddModuleScore function in Seurat package. Wilcoxon rank sum test was used.



Protocol Page

Phase I Study of an Active Immunotherapy for Asymptomatic Phase
Lymphoplasmacytic Lymphoma with DNA Vaccines Encoding Antigen-Chemokine
Fusion
2009-0465

Core Protocol Information

<u>Short Title</u>	Immunotherapy for Asymptomatic Phase Lymphoplasmacytic Lymphoma
<u>Study Chair:</u>	Sheeba K. Thomas
<u>Additional Contact:</u>	Jasper B. Olsem Kimbra S. Harris Tundra S. Gibson
<u>Additional Memo Recipients:</u>	Recipients List OPR Recipients (for OPR use only) None Study Staff Recipients None
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<u>Full Title:</u>	Phase I Study of an Active Immunotherapy for Asymptomatic Phase Lymphoplasmacytic Lymphoma with DNA Vaccines Encoding Antigen-Chemokine Fusion
<u>Public Description:</u>	N/A
<u>Protocol Type:</u>	Standard Protocol
<u>Protocol Phase:</u>	Phase I
<u>Version Status:</u>	Activated -- Closed to new patient entry as of 09/24/2018
<u>Version:</u>	25
<u>Document Status:</u>	Saved as "Final"
<u>Submitted by:</u>	Tundra S. Gibson--9/10/2018 12:21:45 PM
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Which Committee will review this protocol?

- ☒ The Clinical Research Committee - (CRC)

**PHASE I STUDY OF AN ACTIVE IMMUNOTHERAPY FOR
ASYMPTOMATIC PHASE LYMPHOPLASMACYTIC LYMPHOMA
WITH DNA VACCINES ENCODING ANTIGEN-CHEMOKINE
FUSION**

Principal Investigator: Sheeba K. Thomas, M.D.

MD Anderson Protocol 2009-0465

Revised: August 28, 2018

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1.0 OBJECTIVES

Primary

- 1.1 To evaluate the safety, and feasibility of using a novel lymphoma DNA vaccine encoding MIP3 α -fused lymphoma idiotype in single chain format.
- 1.2 To determine the maximum tolerated dose (MTD) of the vaccine.

Secondary

- 1.3 To assess the immunogenicity of the vaccine to generate tumor-specific cellular and humoral immune responses.

2.0 BACKGROUND

Lymphoplasmacytic lymphoma (LPL) is a low grade B-cell lymphoproliferative disorder characterized by bone marrow infiltration with lymphoplasmacytic cells, together with demonstration of a monoclonal gammopathy, as defined by the Revised European-American Lymphoma and WHO classification systems.¹ Patients with a disease-related hemoglobin level of less than 10 g/dL, platelet count of less than $100 \times 10^9/L$, bulky adenopathy or organomegaly, symptomatic hyperviscosity, severe neuropathy, amyloidosis, cryoglobulinemia, cold agglutinin disease, or evidence of disease transformation should be considered for immediate therapy.² However, current recommendations are that patients who are asymptomatic from their disease should be observed, as they may have an indolent course with a long-lasting period of not requiring therapy.³

When treatment is initiated for systemic disease, overall response rates have been high. However, complete responses are infrequent, eventual relapse from disease is inevitable, and the disease remains incurable. In this study, we plan to investigate the safety and feasibility of using a novel DNA vaccine encoding a MIP3 α -fused lymphoma idiotype in single chain format in patients who are in the asymptomatic phase of their lymphoplasmacytic lymphoma. The goal is to develop a therapy that provides a well

tolerated means of controlling the disease before symptoms develop, thereby prolonging the time to initiation of treatment for symptomatic disease, or else obviating the need for initiation of such treatment altogether.

The development of a vaccine against human malignancies has been a long-sought goal, which has yet to be achieved. Many of the efforts toward this end have been frustrated by the lack of identification of a tumor-specific antigen which would allow tumor cells to be distinguished from normal cells. Conceptually, such an antigen could be used as a vaccine to induce the host's immune system to reject cells bearing that antigen.

Immunoglobulin (Ig) molecules are composed of heavy and light chains, which possess highly specific variable regions at their amino termini. The variable regions of heavy and light chains combine to form the unique antigen-recognition site of the Ig protein. These variable regions contain determinants that can themselves be recognized as antigens, or *idiotypes*. B-cell malignancies are composed of a clonal proliferation of cells synthesizing a single antibody molecule with unique variable regions in the heavy and light chains. B-cell lymphomas are neoplasms of mature resting and reactive lymphocytes, which generally express synthesized Ig on the cell surface. The idiotypic determinants of the surface Ig of a B-cell lymphoma can thus serve as a tumor-specific marker for the malignant clone.

Studies in experimental animals, as well as in man, have demonstrated the utility of the Ig idio type as a tumor-specific antigen.⁴⁻⁶ Lynch and Eisen were the first to demonstrate that active immunization against idiotypic determinants on malignant B cells could produce resistance to tumor growth, and this phenomenon of idio type tumor resistance has been reproduced subsequently in a number of syngeneic experiments.⁷⁻¹⁶ These results, taken together, provided the rationale for testing autologous tumor-derived idiotypic surface Ig (Id) as a therapeutic "vaccine" against human B-cell lymphoma.¹⁷

At the National Cancer Institute (NCI), a Phase II clinical study was performed to evaluate the ability of the idiotype vaccine to elicit tumor-specific T-cell immunity, as measured by the ability of patient T cells to specifically lyse their own tumor cells *in vitro*, and to exert antitumor effects as measured by the elimination of t(14;18)-bearing cells from the peripheral blood of uniformly treated follicular lymphoma (FL) patients in first complete remission (CR) (n = 20). Patients in this study were previously untreated and received a uniform chemotherapy regimen, PACE; Prednisone, Adriamycin, Cytosan, and Etoposide. (modified ProMACE without methotrexate). By design, therefore, they comprised a very homogeneous patient population in a minimal residual disease state. Six to 15 months after completion of chemotherapy, 20 patients in CR were treated with a series of five monthly vaccinations with autologous FL Ig protein (0.5 mg) conjugated to KLH, together with local granulocyte-macrophage colony-stimulating factor (GM-CSF, 100 or 500 microgram/m2) subcutaneously.^{[18](#)}

Of the 20 patients in CR, 11 patients had detectable translocations in their primary tumors. All 11 patients had cells from the malignant clone detectable in their blood by PCR both at diagnosis and after chemotherapy, despite being in complete remission. However, 8 of 11 patients converted to PCR negativity in their blood immediately after completion of vaccination and sustained their molecular remissions for a median of 18+ months (range: 8+ to 32+ months). The remaining 3 patients have remained continuously PCR positive.

T-cell responses specific for autologous FL or autologous FL-derived Ig protein were also observed as a result of Id vaccination. Significant production of cytokines (TNF α , GM-CSF, and IFN γ) by immune PBMC, in response to autologous tumor or autologous FL-derived Ig protein was consistently observed in 19 of the 20 (95%) patients. Additional experiments in 6 randomly selected patients revealed that the T-cells responding against autologous tumor were CD8⁺ and MHC class I restricted; whereas T-cells responding against autologous Id protein were CD4⁺ and MHC Class II restricted. Furthermore, immune T cells re-stimulated by FL *in vitro* in 6 patients, showed

significant levels of specific lysis of autologous, unmodified FL targets [range 27-44% specific lysis at effector to target (E:T) ratios of 100].

On the basis of these compelling results, a randomized double-blind placebo controlled multicenter clinical trial was designed to formally determine the clinical efficacy of therapeutic Id vaccination in FL. This Phase III trial was initiated by the National Cancer Institute, and subsequently sponsored by Biovest International Inc. Patients with previously untreated advanced stage FL were treated with PACE chemotherapy regimen until clinical remission. Patients achieving complete remission were randomized at a ratio of 2:1 to receive Id-KLH plus GM-CSF or KLH plus GM-CSF. The primary endpoint for this trial was disease free survival (DFS). Out of 234 enrolled patients, 177 achieved complete response and were subsequently randomized to receive either active or control vaccine. Of these, 117 maintained remission for the 6 month rest period and received at least one dose of vaccine. 76 patients received Id-KLH plus GM-CSF and 41 patients received KLH plus GM-CSF. Both arms were balanced for International Prognostic Index (IPI) scores, and other relevant clinical factors.¹⁹ After a median follow-up of 56.6 mo (range 12.6 - 89.3 mo), median time to relapse after randomization for the Id-KLH/GM-CSF arm was 44.2 mo, versus 30.6 mo for the control arm, suggesting benefit for this vaccine (p-value = 0.045; HR = 1.6).²⁰

The Phase III clinical study indicated that patient-specific idiotypic vaccination could be beneficial in B-cell lymphomas. The idiotypic protein required for vaccine manufacture in this study has been produced by standard hybridoma tissue culture technology. This is an expensive and time-consuming process requiring an average of 3-6 months to prepare the vaccine for each patient. Any delivery system, which does not require protein expression, holds tremendous potential for the goal of streamlining vaccine production. In our view, the most appealing aspect of DNA vaccination is its simplicity and ease of vaccine generation. *In vivo* expression of foreign genes encoding the tumor antigen by DNA vaccination requires only that the gene is cloned under regulatory elements of eukaryotic or viral elements into an expression cassette, which is then either injected in

solution via intramuscular or intradermal routes of administration or delivered into the epidermis by particle mediated bombardment of DNA-coated gold particles (gene gun). In particular, advances in antibody engineering have made it possible to readily identify and clone both murine and human Ig variable genes, including specific V-genes from B-cell malignancies.^{21,22} Once V-genes are cloned, they can be combined into single chain Fv (scFv) format, encoding a single polypeptide consisting solely of V_H and V_L genes linked together inframe with a short, 15 amino acid linker.

Preliminary studies in mice showed that the scFv is weakly immunogenic in most cases and needs to be used together with an adjuvant to render it immunogenic.²³⁻²⁶ In our laboratory, we explored a novel hypothesis, that the efficiency of DNA vaccination in vivo could be greatly increased by encoding a fusion protein consisting of idiotype (scFv) fused to a proinflammatory chemokine moiety (as a substitute for KLH). Chemokines are thought to be among the key effector molecules regulating the trafficking of professional APC, including dendritic cells (DC), selectively through peripheral tissues to reach lymph nodes.^{27,28} Several chemokines, such as monocyte chemotactic protein (MCP-3), macrophage inflammatory protein (MIP)-1 α , macrophage-derived chemokine (MDC), macrophage inflammatory protein-3 α (MIP3 α) and stromal cell-derived factor (SDF)-1 have been reported to be chemotactic for DC.^{29,30} Chemokines act by binding to a specific cell-surface heptahelical G-protein-coupled receptor, which is internalized after binding with the ligand.³¹⁻³³ DC express a variety of chemokine receptors, including CCR1, CCR2, CCR5, CCR6, CXCR1, and CXCR3.³⁴ Our hypothesis is that anti-tumor immunity can be triggered by targeting APC in vivo with a fusion protein consisting of chemokine and tumor antigen.

In this study, we aim to translate the knowledge gained in follicular lymphoma, into a useful treatment for patients with lymphoplasmacytic lymphoma, a related indolent lymphoma. Studies performed in patients with follicular lymphoma studies targeted those patients in a minimal residual disease state following induction chemotherapy for symptomatic disease. However, patients with LPL often receive nucleoside analogs

and/or alkylating agent based therapy for treatment of symptomatic disease, which affects the function of their T cells. Since proper T cell function is indispensable to inducing a response to vaccine therapy, this study will instead enroll patients in the asymptomatic phase of LPL, who will have a similarly low tumor burden, but who should have intact T cell function.

3.0 BACKGROUND DRUG INFORMATION

3.1 VACCINE AND MECHANISM OF ACTION

scFv-CCL20 DNA vaccine: For the purpose of this clinical trial, we intend to use recombinant plasmid DNA encoding a fusion protein consisting of autologous lymphoma immunoglobulin variable regions (scFv) and a chemokine, human CCL20 (macrophage inflammatory protein-3 alpha - MIP-3 α). MIP-3 α binds to CCR6 receptor that is preferentially expressed on CD1a⁺ Langerhans cells, CD34-derived immature dendritic cells and B cells.³⁴ We hypothesize that following intradermal injection of the recombinant plasmid, the secreted fusion protein is likely to undergo an efficient uptake and internalization through the CCR6 receptor on the Langerhans cells and immature dendritic cells. The targeted delivery of this fusion protein to these professional antigen-presenting cells, and subsequent processing and presentation, can break the tolerance to generate an immune response against the idiotype. The activated idiotype-specific immunity would then serve as the main force to eradicate the antigen-expressing B-cell lymphoma cells.

3.2 ANIMAL DATA

The strategy has been tested in two different murine B-cell lymphomas, 38C-13 and A20, which express surface IgM and IgG2a, respectively. The respective scFv were cloned by RT-PCR as fusions with pro-inflammatory chemokine genes MCP-3 and IP-10, as prototypes. The scFv fusions were produced as secreted soluble proteins or as insoluble inclusion bodies in *E. coli* for purposes of characterization. Sequences encoding c-myc, FLAG or His residues as peptide tags were fused to the amino or carboxyl terminus of scFv for detection and purification purposes. We found it was efficient to express scFv fusion proteins as inclusion bodies in *E. coli* and purify by affinity chromatography,

followed by subsequent solubilization and refolding. The scFv fusion proteins produced in this way were folded in the same way as the native Ig, retaining the ability to bind specifically to polyclonal and monoclonal anti-idiotypic antibodies. Chemokine fusions retained biologic function, bound chemokine receptors, and induced chemotaxis both in vitro and in vivo.³⁵

These fusions have been tested as protein and DNA vaccines in both tumor models. Specifically, mice immunized by gene gun with plasmids encoding IP-10- (38C-13 model only) or MCP3-sFv fusions (both models), but not scFv alone, induced protective antitumor immunity against a large tumor challenge (20 times the minimum lethal dose). Moreover, T-cell subset depletion experiments revealed that DNA fusions induced effector CD4⁺ and CD8⁺ T cells, which were required for protection. Finally, the level of protection was greater than or equal to that of the prototype Id-KLH protein in both tumor models.³⁵

The precise mechanism of the “carrier” activity of IP-10 and MCP-3 remains to be elucidated. We favor the novel mechanism that the chemokine moiety targets APC for efficient receptor-mediated uptake and processing of scFv, breaking tolerance. CXC and CC chemokine receptors are known to be internalized after binding to ligand. The most compelling data supporting this hypothesis is that antitumor immunity was not observed in mice immunized with a mixture of free, unlinked chemokine and scFv proteins or different plasmids encoding free chemokine or scFv.³⁵

Furthermore, secretion of fusion proteins by transfected cells in vivo was plausible, as the DNA vaccine constructs contained sequences encoding the secretion leader (SL) from the murine IP-10 gene. We have now also tested fusion constructs without the secretion leader for their ability to elicit protective antitumor immunity. In animal models tumor protection was only observed in mice immunized with DNA vaccines encoding secreted forms of fusions between MCP3 and 38C-13 scFv; it was not seen in non-secreted forms.³⁶

In addition, a DNA vaccine encoding MCP3, containing a mutation that abrogates receptor binding, fused with scFv, was also tested. Two weeks following the last of 3 bi-weekly immunizations, all mice were challenged with a lethal dose of tumor. Among the different groups, only mice that had been immunized with the intact MCP3 fusion, containing secretion leader sequences, demonstrated prolonged survival and protection compared with PBS controls.³⁶

Thus, immunity was also abolished by disruption of the chemokine receptor binding site in the MCP3-scFv fusion, which suggested that fusions required binding to the chemokine receptors to be immunogenic. Taken together, the requirements for linkage of conformationally intact scFv, secretion of fusion protein, and functionally active chemokine strongly suggested that the mechanism underlying the triggering of antitumor immunity was the targeting of APC for chemokine receptor-mediated uptake of antigen, rather than the simple recruitment of APC by the chemokine.

In addition to targeting antigen delivery to receptors on APC, we unexpectedly found that our chemokine-antigen fusions may also work by delivering maturation signals to APC. We designed and tested constructs incorporating additional chemokine and chemokine-like molecules (defensins) that allowed us to discover this other mechanism in vivo. Defensins are a family of cationic antimicrobial proteins stored in the cytoplasmic granules of neutrophils for release into the extracellular milieu in the process of degranulation in response to microbes, including bacteria. They also serve as chemoattractants that could induce chemotaxis of DC via the CCR6 receptor.³⁷ We observed that murine bone marrow-derived immature DC (iDC) incubated with purified defensin-scFv fusion proteins (95% purity, less than 0.5 endotoxin units), but not the fusions with the functionally inactive pro-defensin form, expressed higher levels of DC maturation markers including MHC class II and B7⁺/CD40⁺ cells.³⁸ This direct effect of β -defensin 2 on DC maturation was mediated by Toll-like receptor 4, rather than CCR6.³⁸

Furthermore, we have performed a series of experiments in the A20 lymphoma model to test the efficacy of scFv-chemokine DNA vaccination in the therapeutic setting in vivo,

against minimally pre-established tumors. The therapeutic study data showed that murine DF2 β -A20 scFv fusion vaccines elicited therapeutic, T-cell dependent antitumor immunity in vivo. However, IFN- γ -deficient mice immunized with these mDF2 β -fusion constructs failed to reject tumors, which suggests that IFN- γ is required and that mDF2 β - triggers type 1-polarized adaptive immunity in vivo. Furthermore, the vaccine required fully functional mDF2 β , as fusion antigens with an inactive pro- β -defensin 2 did not elicit any antitumor immunity.³⁸ In conclusion, linkage of tumor antigen with mDF2 β likely facilitated both targeted, chemokine receptor-mediated delivery of antigen, as well as maturation of APC.

3.3 scFv-CCL20 PLASMID DNA VACCINE:

3.3.1. SOURCE: The lymphoma immunoglobulin variable regions, V_H and V_L will be cloned for each patient from the tumor cells by RT-PCR using consensus primers. After DNA sequencing and verification, the mature V region sequences are cloned as a single chain antibody gene (scFv - V_H and V_L are fused in frame with a short linker sequence) into a pUC19 plasmid with kanamycin resistance marker. After verification of the sequence of the scFv, the scFv gene is cloned in frame to 3' end of the human CCL20 (MIP-3 α) gene via a spacer sequence. The sequence of the inserted scFv-chemokine fusion gene is verified. The plasmid DNA is then amplified in E. Coli. The plasmid DNA is subsequently purified from E. Coli according to Good Manufacturing Practices standards and tested for sterility and endotoxin contamination prior to its use in any patient. The entire vaccine preparation procedure usually takes 12 months, starting from cloning V_H and V_L from tumor cells. It will be produced at Kalon Biotherapeutics, LLC (GMP) facility.

3.3.2. HOW SUPPLIED: The drug is formulated on a 0.5 ml basis with patient- specific scFv-CCL20 plasmid DNA vaccine. Each patient-specific formulation will have 500 μ g or 2500 μ g of the plasmid DNA depending on the cohort assignment of the patient. Each vial of patient-specific vaccine will be labeled to include the following information: scFv-CCL20 DNA vaccine, patient identification (last name and first initial), patient-specific lot, final volume, storage conditions, and fill date. The protocol number, drug strength,

expiration date, and the statement, “Caution: New drug – Limited by Federal law to investigational use,” will also appear on the vaccine vial label.

3.3.3 STORAGE: Prior to administration the vaccine will be stored at –20°C.

3.3.4 ADMINISTRATION: Prior to administration of the vaccine, the contents of the vial will be thawed to room temperature. After gentle agitation, the vial contents should be drawn up using a filling adaptor attached to a syringe for the Tropis™ intradermal needle free injection device (Appendix G). After the contents of the vial have been drawn up, the syringe assembly will be inserted into the needle free injector. The vial adaptor will then be removed, and the vaccine will be administered by needle-free injection device for intradermal injection in the thighs. The syringe should be refrigerated at 2°C to 8°C and the vaccine will be administered in a total volume of 0.5 mL. Due to the 0.1ml volume limit of the Tropis™ intradermal needle free injection device, the total volume of 0.5ml will be administered in 5 injections.

3.3.5. TOXICITY: The vaccine is expected to result in minimal toxicities. The most likely anticipated reactions include local erythema and induration at the sites of injection and transient flu-like symptoms. No known oncogenic or immunomodulatory sequences are detected in the plasmid.

3.3.6 DISPOSAL: M. D. Anderson Cancer Center has established a waste stream for the disposal of chemotherapy-related items and other biohazardous substances.

All empty or partially used containers of investigational agents are to be treated as toxic substances with disposal occurring immediately after use into the chemotherapy waste-stream containers. Disposal of partial or empty vials/bottles is done through a contracted third party, via incineration.

4.0 PATIENT ELIGIBILITY

4.1 INCLUSION CRITERIA

- 4.1.1 Age $\geq$ 18 years.
- 4.1.2 Tissue diagnosis of Lymphoplasmacytic Lymphoma with surface IgG, IgA or IgM phenotype with a monoclonal heavy and light chain as determined by flow cytometry. All primary diagnostic lymph node and/or bone marrow biopsies will be reviewed at the University of Texas M.D. Anderson Cancer Center (UTMDACC).
- 4.1.3 Previously untreated patients with lymphoplasmacytic lymphoma (of any subtype: IgG, IgA, IgM) in the asymptomatic phase.
- 4.1.4 Patients must provide a lymph node sample of at least 1.5cm in the long axis, or a bone marrow aspiration sample providing at least 5 million CD20 and/or CD38+ cells (approximately 10 ml).
- 4.1.5 ECOG performance status of 0 or 1.
- 4.1.6 Serum creatinine $\leq$ 1.5 mg/dl and a Creatinine clearance $\geq$ 30 ml/min.
- 4.1.7 Total Bilirubin $\leq$ 1.5 mg/dl unless believed secondary to Gilbert's disease and AST/ALT $\leq$ 2 x upper limit of normal.
- 4.1.8 Ability to provide informed consent, and to return to clinic for adequate follow-up for the period that the protocol requires.
- 4.1.9 Female subject is either post-menopausal or surgically sterilized or willing to use an acceptable method of birth control (i.e., a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study and for 30 days after the last vaccination has been administered.
- 4.1.10 Male subject agrees to use an acceptable method for contraception for the duration of the study.

4.2 EXCLUSION CRITERIA

- 4.2.1 HIV, Hepatitis B and/or Hepatitis C infection.
- 4.2.2 Pregnant or lactating females.

- 4.2.3 Patients with previous history of malignancy within the last 5 years except curatively treated squamous or basal cell carcinoma of the skin or curatively treated carcinoma in-situ of other organs.
- 4.2.4 Any medical or psychiatric condition that in the opinion of the principal investigator would compromise the patient's ability to tolerate this treatment.
- 4.2.5 Patients with New York Heart Association Class 3 or 4 disease (see Appendix F).
- 4.2.6 Patients with a history of autoimmune diseases except for Hashimoto's thyroiditis.
- 4.2.7 Patients with positive ANA and/or anti-dsDNA antibodies.

4.3 PATIENT REGISTRATION

- 4.3.1 All patients will be registered in the Clinical Oncology Research System (COrE).
- 4.3.2 All patients seen by members of the Department of Lymphoma and Myeloma, who have asymptomatic lymphoplasmacytic lymphoma, will be tracked by the research nurse/study coordinator in charge of this study. If deemed eligible for consideration of participation, participation on this protocol will be discussed with the patient. If informed consent is received, formal screening for eligibility will take place. For eligible patients not enrolled on the study, a Health Insurance Portability and Accountability Act (HIPAA) compliant pre-enrollment and enrollment log will be maintained in COrE to track reason(s) for lack of enrollment.
- 4.3.3 The patient's entry date on protocol is considered to be the day the patient is registered in COrE. The treatment start date is considered to be the day the first dose of vaccine is administered to the patient.

5.0 TREATMENT PLAN

5.1 STUDY DESIGN

Prior to vaccine production, all patients will either undergo bone marrow aspiration of 10ml or lymph node harvest, prior to starting treatment in order for their vaccine to be prepared. In addition, all patients may opt to undergo a unilateral bone marrow biopsy, for use in correlative studies. Once the vaccine for a given patient is available, he or she will receive 3 doses of DNA vaccine encoding a fusion protein consisting of autologous lymphoma immunoglobulin variable regions (scFv) and a chemokine, human CCL20 (macrophage inflammatory protein-3 alpha - MIP-3 α). The proposed study is designed to have 2 dosing cohorts. Cohort 1 patients will receive a dose of 500 μ g, and Cohort 2 patients will receive a dose of 2500 μ g.

5.2 COHORTS: Enrollment to Cohorts 1 and 2 will follow a standard 3 + 3 statistical design, and will thus require a maximum of 12 patients. Enrollment to Cohorts 1 and 2 shall be staggered. Staggering enrollment ensures that no patient is administered the vaccine until any previously exposed patient has completed a minimum of 48 hours of safety follow-up after receiving the first of three planned treatment administrations. Prior to advancing dose levels, a cohort summary will be completed and submitted to the IND office medical monitor for review and approval to proceed to the next cohort. Please see section 12.0 for criteria for discontinuing the administration of the vaccine in a given cohort.

5.3 VACCINE ADMINISTRATION: Patients will receive a series of 3 autologous lymphoma immunoglobulin derived scFV-chemokine DNA vaccinations at 4-week intervals (+/- 3 business days) according to the following schedule: 0, 4 and 8 weeks. If patients have progressive disease that requires chemotherapy or radiotherapy treatment (see section 10.0) before or while receiving vaccination, vaccination will either not begin or will be stopped, and the patient will be taken off study. Patients will be vaccinated intradermally and the injection sites will be rotated between the thighs. Patients will be observed in CTRC for two hours after vaccine administration. During the observation period, vital signs will be taken every 15 minutes during the first hour and every 30 minutes during the second hour. The use of non-steroidal anti-inflammatory drugs

(NSAIDs) and/or steroids should be avoided during the vaccination. Should NSAIDs or steroids be required for unrelated medical conditions for a course exceeding 2 weeks, the patient will be taken off of the study. A list of common aspirin containing products to avoid will be provided to the patients at the time of study entry (Appendix B). Any local skin reactions will be carefully noted and scored for erythema, induration, pain and disruption of the barrier surface.

5.4 TREATMENT MODIFICATIONS: Toxicities will be graded according to the NCI Common Toxicity Criteria v4.0. No further vaccinations will be given to patients who develop grade 3 or 4 hypersensitivity reactions or grade 3 injection site reactions. No dose modification will be made for grade 3 fever. For grade 4 fever, subsequent vaccinations will be administered at 50% of the original dose level. For all other grade 3 or 4 toxicity reactions, no further vaccinations will be given if in the opinion of the investigator the toxicity is related to the vaccine administration. Please see section 11.0 for stopping rules regarding the administration of the vaccine in a given cohort.

6.0 RESEARCH ELIGIBILITY EVALUATION

The following studies should be performed within 1 year prior to starting therapy:

- 6.1 Complete History and Physical examination (+/- 2 days).
- 6.2 Concurrent Medication Assessment, including documentation of planned method of birth control
- 6.3 CBC with differential, platelet count.
- 6.4 Serum chemistry (Na, K, Cl, Glu, CO₂, BUN, Cr, Ca²⁺, Mg²⁺, Phosphorus, Total Protein, Albumin, Alkaline Phosphatase, AST, ALT, Total Bilirubin, LDH and Creatine Kinase).
- 6.5 PT and PTT
- 6.6 Quantitative Immunoglobulin G, A and M
- 6.7 Serum and Urine Protein Electrophoresis and Immunofixation Studies
- 6.8 Serum free Kappa and Lambda light chains
- 6.9 Cryocrit

6.10 HLA typing

6.11 HIV Western Blot with reflex ELISA testing if positive Hepatitis B surface Antigen, Hepatitis C antibody, Cytomegalovirus antibody, West Nile virus by nucleic acid testing, rapid plasma reagin (RPR) screen, Human T-lymphotropic virus (HTLV) Type I/II antibodies.

6.12 ANA, ds DNA

6.13 Serum β -HCG in women of child-bearing potential. (Note: A woman of child-bearing potential (WOBCP) is a woman who has not been naturally post-menopausal for at least 12 consecutive months or who has not undergone previous surgical sterilization.)

6.14 EKG

6.15 CT scans of Neck, Chest, Abdomen and Pelvis

6.16 One red top tubes (10 ml each) for serum storage, and six green top tubes (10ml each) for PBMC isolation. For patients who have measurable serum monoclonal protein, a portion of this sample will be used to isolate idiotype M protein for immunologic assays. Specimens should be sent to the laboratory of Dr. Larry Kwak, City of Hope National Medical Center, 1500 East Duarte Road, Beckman Center, Duarte, California, 91010, Tel: 626-218-8913, Room 4350.

6.17 Bone Marrow Aspirate - prior to therapy, all patients must undergo aspiration of up to 10ml of bone marrow tissue for morphological classification, immunophenotypic characterization. A second 15ml bone marrow aspirate sample will be collected from the contralateral side for vaccine production and to bank additional tumor cells for immunologic assays. Left-over aspirate samples may be used for basic studies of lymphoma biology in vitro. Such future studies may be done without re-consenting the subjects only if the studies involve risks already outlined in the original consent form. In the event that the 15ml bone marrow aspirate sample does not provide sufficient tumor cells for the vaccine production and immunologic assays, a second procedure (bone marrow aspiration) may be performed to reach the needed yield. (a) The 10ml specimen will be used for routine histopathological and immunophenotypic

characterization. The specimen will be sent to Dr. Pei Lin in the Hematopathology Section, Department of Pathology at MDACC. (b) The 15ml specimens(s) will be sent in a sterile container to the City of Hope National Medical Center, under the direction of Dr. Larry Kwak, Tel: 626-218-8913. This portion of specimen will be used to clone the lymphoma immunoglobulin variable heavy and light chains to manufacture the vaccine and used for immune monitoring studies. Once plasmid constructs have been developed, the constructs will be shipped to the Good Manufacturing Practice Laboratory of Kalon Biotherapeutics LLC for clinical grade manufacturing of each patient-specific vaccine.

6.18 Lymph Node Harvest/Biopsy – in patients whose LPL presents primarily as lymphadenopathy, and who lack significant bone marrow involvement ($<10\%$), a safely accessible lymph node, measuring at least 1.5cm in the long axis, will be harvested in lieu of the bone marrow aspirate. One third of the specimen will be used for morphological classification and immunophenotypic characterization; it will be sent in sterile saline to Dr. Pei Lin in the Hematopathology Section, Laboratory of Pathology at UTMDACC. Two-thirds of the specimen will be used to provide starting material for manufacture of the vaccine. It will be sent in sterile saline in a sterile container to City of Hope National Medical Center, under the direction of Dr. Larry Kwak, Tel: 626-218-8913; there, it will be assigned a unique accession number. In the event that the first lymph node sample does not provide sufficient tumor cells for the vaccine production and immunologic assays, a second excisional lymph node biopsy may be performed to reach the needed yield. This second lymph node sample, if obtained, will be sent in its entirety to City of Hope National Medical Center.

7.0 PRE-TREATMENT IMAGING

If pathologic lymphadenopathy or hepatosplenomegaly were noted on CT scan imaging during the Research Eligibility Evaluation (REE) OR there is clinical concern for development of enlarged lymph nodes/organomegaly compared with scans performed for the REE, the following restaging imaging will also be performed within 1 week of receiving the 1st dose of vaccine to serve as the baseline for subsequent comparison.

Unless clinically indicated, this imaging will only be obtained if it has been ≥ 6 weeks since scans were performed for the REE.

7.1 CT scans of Neck, Chest, Abdomen and Pelvis

8.0 BASELINE EVALUATION

The following studies should be performed within 4 weeks prior to starting therapy:

- 8.1 Complete History and Physical examination (+/- 2 days).
- 8.2 Concurrent Medication Assessment, including documentation of planned method of birth control
- 8.3 CBC with differential, platelet count.
- 8.4 Serum chemistry (Na, K, Cl, Glu, CO₂, BUN, Cr, Ca²⁺, Mg²⁺, Phosphorus, Total Protein, Albumin, Alkaline Phosphatase, AST, ALT, Total Bilirubin, LDH and Creatine Kinase).
- 8.5 PT and PTT
- 8.6 Quantitative Immunoglobulin G, A and M
- 8.7 Serum and Urine Protein Electrophoresis and Immunofixation Studies
- 8.8 Serum free Kappa and Lambda light chains
- 8.9 Cryocrit (only if the patient was cryocrit positive at Research Eligibility Evaluation)

9.0 EVALUATION DURING STUDY

On the day of each vaccination, prior to its administration:

- 9.1 CLINIC VISIT WITH PHYSICAL EXAMINATION.
- 9.2 CONCURRENT MEDICATION ASSESSMENT, including documentation of planned method of birth control
- 9.3 TOXICITIES: Patients will be evaluated in the clinic for toxicities at the time of each vaccination during the clinic visit. Toxicities due to the vaccine will be graded according to the NCI CTC v4.0 during these visits. Toxicities documented on the day of the first vaccination (prior to receipt of the vaccine) will serve as the baseline assessment.

9.4 IMMUNE RESPONSE ANALYSIS: One red top tube (10ml) and 6 green top tubes of peripheral blood (10ml each) will be collected within 2 days prior to day 1 of each vaccination, for immunological studies. Send to Dr. Larry Kwak's laboratory at City of Hope National Medical Center, Tel: 626-218-8913, Beckman Center Room 4350

Within 48 hours prior to each vaccination the following studies will be performed:

9.5 LABS: CBC with differential count, platelets, PT, PTT, serum chemistry panel (Na, K, Cl, Glu, CO₂, BUN, Cr, Ca²⁺, Mg²⁺, Phosphorus, Total Protein, Albumin, Alkaline Phosphatase, AST, ALT, Total Bilirubin, LDH and Creatinine Kinase), serum and urine protein electrophoresis, quantitative IgG, IgA and IgM, serum free kappa and lambda light chains, and cryocrit (only if the patient was cryocrit positive at screening) will be drawn. Serum and Urine immunofixation studies will also be performed, if no quantifiable paraproteins are documented on both serum and urine protein electrophoresis studies.

9.6 AUTOIMMUNE EVALUATION: ANA and anti-dsDNA titers will be obtained.

10.0 POST-VACCINE THERAPY EVALUATION

The studies in 10.1-10.12 should be performed 4 weeks (+/- 5 business days) after the last vaccination and every 2 months (+/- 5 business days) thereafter for a year.

10.1 Complete History and Physical examination.

10.2 Concurrent Medication Assessment

10.3 TOXICITIES: Patients will be evaluated in the clinic for toxicities at the time of each vaccination by history and physical examination. Toxicities due to the vaccine will be graded according to the NCI CTC v4.0 during these visits.

10.4 CBC with differential and platelet count.

10.5 Serum chemistry (Na, K, Cl, Glu, CO₂, BUN, Cr, Ca²⁺, Mg²⁺, Phosphorus, Total Protein, Albumin, Alkaline Phosphatase, AST, ALT, Total Bilirubin and LDH).

10.6 Quantitative IgG, IgA and IgM

10.7 Serum and Urine Protein Electrophoresis; serum and urine immunofixation studies will also be performed if the serum and urine protein electrophoresis studies do not show quantifiable paraproteins.

10.8 Serum free Kappa and Lambda light chains

10.9 ANA and dsDNA titers will be obtained as screening tests for possible autoimmune diseases.

10.10 Re-staging with CT scans of the neck, chest, abdomen and pelvis 4 weeks (+/- 5 business days) after the final vaccination and every 6 months (+/- 5 business days) thereafter for a year.

10.11 10 cc of serum (one red top tube) for storage and 60 cc of peripheral blood in six green top tubes will also be collected 4 weeks (+/- 5 business days) after the last vaccination for immunological studies and every 4 months (+/- 5 business days) thereafter for one year. These samples will be delivered on the overnight to Dr. Larry Kwak at City of Hope National Medical Center, Beckman Center Room 4350, Tel: 626-218-8913.

10.12 Up to 10 - 15 ml bone marrow aspirate research sample should be obtained for immediate processing and/or storage, 4 weeks (+/- 5 business days) after the last vaccination. An additional 10 cc of serum (one red top tube) and 60 cc of peripheral blood in six green top tubes will also be collected and stored for other correlative studies (e.g. Cytokine release, reactivity against human Ig's, etc) will also be collected at this timepoint.

10.13 Optional unilateral bone marrow biopsy should be obtained at least 4 (+/- 5 business days) weeks after the last vaccination, but no later than 12 months. These samples will be utilized for correlative studies.

11.0 SPECIMEN PROCESSING ,IMMUNOLOGICAL ASSAYS AND CORRELATIVE STUDIES

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered

in the consent document. If new risks are associated with the research (e.g., analysis of germ line genetic mutations) the principal investigator must amend the protocol and obtain informed consent from all research subjects.

11.1 BONE MARROW ASPIRATION: Each patient will undergo aspiration of up to 10ml from the bone marrow on one side and 15ml from the contralateral side. In the event that the 15ml bone marrow aspirate sample does not provide sufficient tumor cells for the vaccine production and immunologic assays, a second procedure (bone marrow aspiration) may be performed to reach the needed yield (see Section 6.15). (a) The 10ml specimen will be used for routine histopathological and immunophenotypic characterization. The specimen will be sent to Dr. Pei Lin in the Hematopathology Section, Department of Pathology at MDACC. (b) The 15ml specimens(s) will be sent in a sterile container to City of Hope National Medical Center, under the direction of Dr. Larry Kwak, Tel: 626-218-8913; there, it will be assigned a unique accession number. This portion of specimen will be used to clone the lymphoma immunoglobulin variable heavy and light chains to manufacture the vaccine and used for immune monitoring studies.

11.2 LYMPH NODE HARVEST/BIOPSY: Each initial lymph node biopsy specimen will be divided as follows: (a) One-third of the specimen will be used for routine histopathological and immunophenotypic characterization. The specimen will be sent in sterile saline to Dr. Pei Lin in the Hematopathology Section, Laboratory of Pathology at UTMDACC. (b) Two-thirds of the specimen will be sent in sterile saline container to City of Hope National Cancer Center, under the direction of Dr. Larry Kwak, Tel: 626-218-8913, where it will be assigned a unique accession number. This portion of specimen will be used to clone the lymphoma immunoglobulin variable heavy and light chains to manufacture the vaccine and used for immune monitoring studies. In the event that a second lymph node specimen is obtained (see Section 6.16), it will be sent in its entirety to City of Hope National Medical Center.

11.3 BLOOD SAMPLES: All peripheral blood samples will be sent in an expedited manner to Dr. Larry Kwak at City of Hope National Cancer Center, Tel: 626-218-8913. Red top tubes will be spun down and serum divided into 1 ml aliquots for frozen storage. Peripheral blood mononuclear cells (PBMC) will be isolated prior to freezing by Ficoll-Hypaque centrifugation using standard protocols.

11.4 ASSAY FOR SERUM TUMOR-SPECIFIC ANTIBODY: Serum tumor-specific antibody will be assayed by flow cytometry. Serial dilutions of pre-immune and hyperimmune serum samples from each patient will be mixed with autologous tumor cells (or idiotype monoclonal protein when available) or isotype-matched irrelevant tumor cells (or monoclonal protein of irrelevant specificity) from a different patient. Bound anti-tumor antibody is detected with FITC labeled goat anti-human light-chain antibodies directed against the light chain not present in the immunoglobulin idiotype (Caltag Laboratories, South San Francisco). A response is considered positive when a four-fold rise in the bound antibody titer is observed as compared to the pre-vaccine serum and the isotype matched irrelevant tumor used as specificity controls.³⁸

11.5 INTERFERON GAMMA ELISPOT ASSAY TO DETERMINE THE PRECURSOR FREQUENCY OF TUMOR-SPECIFIC CYTOTOXIC T-LYMPHOCYTES: Cryopreserved pre- and post-vaccine PBMCs will be cultured for 48 hours in the presence or absence of autologous tumor cells (or idiotype monoclonal protein, when available) in human interferon gamma coated MAIP 96 well plates (Millipore). Following the incubation, plates will be developed using a secondary anti-IFN γ antibody that is biotin conjugated. Visualization of the IFN γ spots will be done by adding streptavidin alkaline phosphatase and a substrate. The plate will be scanned and the IFN γ spots will be counted by a CTL Immunospot Reader. A three-fold rise in the precursor frequency of tumor-reactive T cells in the post-vaccine PBMC sample as compared to the pre-vaccine sample will be considered positive, with a minimum precursor frequency of 1 in 80,000 cells if the precursor frequency in the pre-vaccine sample is zero.³⁹

11.6 ASSAY FOR ANTI-MIP-3 α ANTIBODIES: In a direct enzyme-linked immunosorbent assay (ELISA), pre-immune and hyperimmune serum samples from each patient will be diluted over wells of a microtiter plate that are coated with human MIP-3 α . Bound antibody is detected with horseradish peroxidase-goat antihuman IgG (Caltag Laboratories, South San Francisco). A response is considered positive when a four-fold rise in the bound antibody titer is observed in the post-vaccine serum sample as compared to the pre-vaccine serum and an irrelevant human chemokine used as specificity control.

11.7 CORRELATIVE STUDIES: Together with our collaborator at City of Hope, Dr. Peter Lee, we will analyze immune profiles that identify cellular spatial relationships within and between the tumor microenvironment from pre-vaccine and post-vaccine formalin-fixed paraffin-embedded LPL bone marrow specimens. We will use a combination of multiplexed immunohistochemistry, multispectral imaging, and automated image analysis which delivers quantitative per-cell measurements of each marker (Vectra®3 automated quantitative imaging system).

The LPL bone marrow specimens will be analyzed on two separate tissue sections; Slide 1# will be tested for CD4, CD8, kappa/lambda light chain [dependent on each patient's LPL Ig isotype], FoxP3, granzyme B, cytokeratin, and DAPI. Slide 2# will be assayed for CD4, CD8, kappa/lambda light chain, PD-1, CTLA-4, cytokeratin, and DAPI with a new serial same-species fluorescence labeling approach utilizing tyramide signal amplification (TSA, Opal™), and microwave-based antigen retrieval and antibody stripping (MWT). Samples will be imaged on a multispectral slide analysis system, and analyzed with pattern recognition software, to segment tissue into tumor and stroma, and phenotype cells using a new multinomial logistic regression learning capability, as described previously by Dr. Lee and others.

12.0 CRITERIA FOR RESPONSE AND RELAPSE/PROGRESSION⁴⁰

12.1 CRITERIA FOR RESPONSE

12.1.1 Complete Response (CR): categorized by a disappearance of serum and urine monoclonal protein determined by immunofixation, absence of malignant cells in bone marrow determined by histologic evaluation, resolution of adenopathy/ organomegaly (confirmed by computed tomography (CT) scan, and no signs or symptoms attributable to WM. Reconfirmation of the CR status is required ≥ 6 weeks later with a second immunofixation.

12.1.2 Partial Response (PR): categorized by $\geq 50\%$ reduction of serum monoclonal IgM concentration determined by protein electrophoresis, $\geq 50\%$ decrease in adenopathy/organomegaly on physical examination or on CT scan, and no new symptoms or signs of active disease

12.1.3 Minor Response (MR): categorized by a $\geq 25\%$ reduction of serum monoclonal IgM determined by protein electrophoresis and no new symptoms or signs of active disease

12.1.4 Stable Disease (SD): categorized by $<25\%$ reduction and $< 25\%$ increase of serum monoclonal IgM determined by electrophoresis without progression of adenopathy/organomegaly, cytopenias, or clinically significant symptoms caused by disease and/or signs of WM.

12.1.5 Progressive Disease (PD): categorized by $\geq 25\%$ increase in serum monoclonal IgM by protein electrophoresis confirmed by a second measurement or progression of clinically significant findings caused by disease (e.g. anemia, thrombocytopenia, leukopenia, or bulky adenopathy/organomegaly) or symptoms (e.g. unexplained recurrent fever $\geq 38.4^{\circ}\text{C}$, drenching night sweats, $\geq 10\%$ weight loss, hyperviscosity, neuropathy, or symptomatic cryoglobulinemia) attributable to WM.

12.1.6 Not Evaluable (NE)/Delayed Response (DR): in some cases, there is insufficient data and/or time for a determination of response to treatment. A delayed response might be seen, particularly after nucleoside analog or monoclonal antibody therapy. The panel recommended that patients must have been followed for ≥ 3 months after treatment initiation to be considered unresponsive to therapy. In addition, the best response is often exhibited several months after treatment discontinuation.

12.2 CRITERIA FOR RELAPSE/PROGRESSION AFTER PRIOR RESPONSE⁴⁰

12.2.1 Relapse from CR: categorized by the appearance of serum or urine monoclonal protein by immunofixation, confirmed by a second determination, recurrence of bone marrow involvement by lymphoplasmacytic cells, recurrence/development of lymphadenopathy/splenomegaly or signs/symptoms attributable to WM.

12.2.2 Progression from PR: categorized by $\geq 25\%$ increase in serum monoclonal IgM from the lowest attained response value determined by protein electrophoresis, confirmed by a second measurement or progression of clinically significant signs (e.g. unexplained recurrent fever $\geq 38.4^{\circ}\text{C}$, drenching night sweats, $\geq 10\%$ weight loss, hyperviscosity, neuropathy, or symptomatic cryoglobulinemia) attributable to WM. An absolute increase of 0.5g/dL (confirmed on a second determination) is required to define disease progression when the only criterion for progressive disease is the increase of M protein size. In a patient with a localized large mass and/or a high level of lactic dehydrogenase at relapse or progression, a biopsy should be considered to assess the possibility that transformation into a diffuse large B cell lymphoma has occurred.

13.0 CRITERIA FOR DISCONTINUING THERAPY

Patients will be removed from protocol for any of the following reasons:

13.1 Adverse event(s) occur that in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug.

13.2 The patient declines further therapy.

13.3 The patient experiences progression of Waldenström's macroglobulinemia, that requires initiation of systemic therapy to control symptoms.

13.4 Inadequate number of biopsy cells ($<1.0 \times 10^9$) for vaccine manufacture or an unforeseen vaccine manufacturing failure.

13.5 It is deemed in the best interest of the patient. In this instance, the Principal Investigator should be notified. The reasons for withdrawal should be noted in the flow sheet.

14.0 LONG TERM FOLLOW-UP PHASE

Following end of therapy on this trial, all subjects will be followed until progression to symptomatic Waldenstrom, Death, withdrawal of consent for study participation, or removal from study.

Subjects will return to the site or be contacted by telephone every 6 months (+/-4 weeks) for follow up assessments of the following:

- Second primary malignancies
- Next-line of therapy
- Progressive disease on next line of therapy
- Overall survival

Following disease progression on the next-line of therapy, subjects will only be followed for overall survival and development of 2nd primary malignancies.

15.0 STATISTICAL CONSIDERATIONS

The primary objective of the proposed phase I study is to determine the maximum tolerated dose (MTD) of a novel lymphoma DNA vaccine encoding a MIP3a-fused lymphoma idiotype. Patients will receive a series of 3 vaccinations at 4-week intervals according to the following schedule: weeks 0, 4 and 8. The standard “3+3” design is applied with 2 pre-defined dose levels. Based on the pre-clinical experience, the following 2 dose levels were chosen:

Dose level	1	2
DNA vaccine (µg)	500	2500

DLT is defined as follows: $\geq$ grade 2 allergic reaction, $\geq$ grade 2 autoimmune reaction and any grade 3 or 4 toxicity except for fever, grade 4 fever which subsequently requires 50% dose reduction. Applying the 3+3 design, the first cohort of 3 patients will be treated at dose level 1 and evaluated for DLT at the end of first cycle (4 weeks). The algorithm is as follows: (1) If 0 out of 3 patients experiences dose-limiting toxicity (DLT), the next cohort of 3 patients will be treated at the next higher dose level. (2) If 1 out of 3 patients develop a DLT, an additional 3 patients will be treated at the same dose level. If no more DLTs develop at this dose, i.e. 1 out of a total of 6 patients develops a DLT, the dose escalation continues for the next cohort of 3 patients. (3) At any given dose, if greater

than 1 out of 3 patients or 1 out of 6 patients experience DLT, the dose level exceeds the MTD and 3 more patients will be treated at the next lower dose if there are less than 6 patients already treated at that dose. Following the above scheme, MTD is defined as the highest dose level in which 6 patients have been treated with less than 2 instances of DLT. Given 2 predefined dose levels, it is anticipated that up to 12 eligible patients are required for this dose-finding phase I trial.

Note: If greater than 1 out of 3 patients or 1 out of 6 patients experience DLT at the lower dose level, this dose will be considered too toxic and the protocol will be stopped.

15.1 Analysis Plan: Toxicity type and severity will be summarized by frequency tables.

The analysis for the secondary endpoint immunogenicity will be based on intent-to-treat population. Patients receiving reduced doses will be included. The immune response is defined as at least a three-fold rise in the precursor frequency of tumor-reactive T cells in the post-vaccine PBMC sample at 12 weeks as compared to the pre-vaccine sample, with a minimum precursor frequency of 1 in 80,000 cells if the precursor frequency in the pre-vaccine sample is zero. The rate of immune response will be estimated.

16.0 DATA AND PROTOCOL MANAGEMENT

16.1 All patients must have signed an Informed Consent and an on-study confirmation of eligibility form filled out before entering on the study.

16.2 Complete records must be maintained on each patient; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on a computer database from which formal analyses will be done. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; flow sheets, specialty forms for pathology, radiation,

or surgery; and off-study summary sheet, including a final assessment by the treating physician.

16.3 REDCap (Research Electronic Data Capture) and Clinical Oncology Research System (COrE) will be used as the electronic case report form systems for this protocol.

REDCap is an electronic data capture tool hosted at MD Anderson. REDCap (www.project-redcap.org) is a secure, web-based application with controlled access designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources. In the case of multi-center studies REDCap uses Data Access Groups (DAGs) to ensure that personnel at each institution are blinded to the data from other institutions. REDCap (<https://redcap.mdanderson.org>) is hosted on a secure server by MD Anderson Cancer Center's Department of Research Information Systems & Technology Services. REDCap has undergone a Governance Risk & Compliance Assessment (May 2014) by MD Anderson's Information Security Office and found to be compliant with HIPAA, Texas Administrative Codes 202-203, University of Texas Policy 165, federal regulations outlined in 21CFR Part 11, and UTMDACC Institutional Policy #ADM0335.

Those having access to the data include the study PI and research team personnel. Users are authenticated against MDACC's Active Directory system. External collaborators are given access to the database once approved by the PI, with their access expiring in 6 months but renewable in 6 months increments at the request of the PI. The application is accessed through Secure Socket Layer (SSL). All protected health information (PHI) will be removed from the data when it is exported from REDCap for analysis. All dates for a given patient will be shifted by a randomly generated number between 0 and 364, thus preserving the distance between dates. Dates for each patient will be shifted by a different randomly generated number.

Following publication study data will be archived in REDCap. Since study data may be useful for future research studies performed under separate IRB approved protocols, study data will be archived indefinitely in REDCap. Since REDCap is a secure electronic database with controlled access, and because patient identifiers may be needed to link study data to data from other sources under future IRB approved protocols, patient identifying information will be retained in the archived database.

17.0 REPORTING REQUIREMENTS

All toxicities and adverse events will be recorded in the case report forms and appropriately graded as to severity and cause according to the NCI Common Toxicity Criteria v4.0. Signs and symptoms that are present at registration (i.e. “Baseline events”) will be recorded as adverse events during the trial if they worsen (i.e. increase in grade) according to the grading criteria of NCI CTC v4.0. The principal investigator is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial. Adverse events will be reported according to the guidelines specified below:

17.1 ADVERSE EVENT REPORTING FOR INVESTIGATIONAL AGENTS:

Serious Adverse Event Reporting (SAE)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in “The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices”. Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).
- All life-threatening or fatal events, that are unexpected, and related to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.
- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last study treatment/intervention, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.
- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

The gene therapy reporting addendum (“Additional Reporting Form for Serious Adverse Events on Gene Therapy Trials”) must be included with each SAE submitted.

It is the responsibility of the PI and the research team to ensure that serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor’s guidelines, and Institutional Review Board policy.

18.0 REFERENCES

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