TITLE: Phase 1/2 Study of a CpG-Activated Whole Cell Vaccine Followed by Autologous "Immunotransplant" for Mantle Cell Lymphoma

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Indication:	Mantle Cell Lymphoma
Treatment Center:	Stanford University Medical Center
Coordinating Center:	Stanford University Medical Center
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STUDY SYNOPSIS

TITLE	Phase 1/2 Study of a CpG-Activated Whole Cell Vaccine Followed by Autologous "Immunotransplant" for Mantle Cell Lymphoma
STUDY PHASE	Phase 1/2
INDICATION	Newly Diagnosed Adult Mantle Cell Lymphoma
INVESTIGATIONAL PRODUCT OR PROCEDURE	CpG-MCL Vaccine Vaccine-primed T cells
PRIMARY OBJECTIVE(S)	The primary objective of the study is to evaluate freedom from molecular residual disease at one year post-autologous transplant.
SECONDARY OBJECTIVE(S)	Secondary objectives are Time To Clinical Progression (TTP), and evaluation of anti-tumor immune responses after vaccination, and after immunotransplant.
TREATMENT SUMMARY	Patients will undergo excisional tumor biopsy or apheresis (for patients with significant peripheral blood involvement), to obtain at least 1.5 x 10^9 malignant cells, which will be used to produce a patient-specific "CpG-MCL" vaccine. Patients will receive standard induction chemotherapy. Once in remission and after recovery of sufficient number of T cells, patients will receive three 'priming' CpG-MCL vaccinations at within a period of 21 days (4-7 day intervals). Within approximately four weeks thereafter, patients will receive an infusion of rituximab as an *in vivo "*purge" as per standard institutional protocol, followed by leukapheresis to harvest vaccine-primed T cells. In preparation for AHCT, patients will then undergo peripheral blood progenitor cell (PBPC) harvesting, myeloablative chemotherapy, and AHCT per standard institutional protocol. Between day 1 and day 3 post-AHCT, patients will receive an infusion of their primed T cells together with a booster vaccination of CpG-MCL. After hematopoietic recovery post transplant, patients will receive a second booster vaccination.
SAMPLE SIZE	The goal is to enroll patients sufficient to obtain a total of 59 evaluable patients over 60 months
STATISTICAL CONSIDERATIONS	<u>*Primary Endpoint*</u> The primary endpoint of the trial is freedom from molecular residual disease at the landmark of one-year post-transplant. Based on the 2-Stage Simon Optimal Design with both error rates below 0.1 for testing a null (unacceptable) rate of MRD of 70% against an alternative (acceptable) rate of 85% has 20 patients acquired in the first stage, stopping for futility with 14 or fewer successes (MRD negative patients), otherwise going on to a total of 59 patients,

choosing the experimental treatment if there are at least 46 successes (MRD negative patients) and results determined favorable to move to additional clinical investigation. If the true rate is 70%, the expected number of patients is 36, and the probability of stopping at stage 1 is 58%. This design minimizes the expected number of patients exposed to an ineffective treatment, given the error rates.

In addition, to determine if chemotherapy regimen does influence MRD rates, we will stratify MRD status by chemotherapy regimen.

Secondary Endpoints

Secondary outcome analysis will focus on TTP (from the date of autoHCT). TTP among patients evaluable will be reported including measure of centrality and variance of the outcome.

The immune response will also be reported descriptively at the completion of the trial.

Correlative Biomarker Endpoints

At the completion of the trial, we will explore new putative immune biomarkers of clinical response (molecular MRD at 1 year).

Stopping rules:

Though similar studies using PF-3512676, whole-cell cancer vaccines, and re-infusion of T cells post-transplant have shown minimal associated adverse events, we will assess the patient cohort in an ongoing manner and use the following stopping rule for the Serious Adverse Events of: non-engraftment or early death (within 100 days from transplant) from any cause.

After patient number:	8	16	24
Non-engraftment seen in:	2	4	5
Early mortality seen in:	3	5	6

This rule would stop the study if the statistics indicate the possibility with even 80% certainty that the non-engraftment rate is > 10% or the early mortality rate is > 15%.

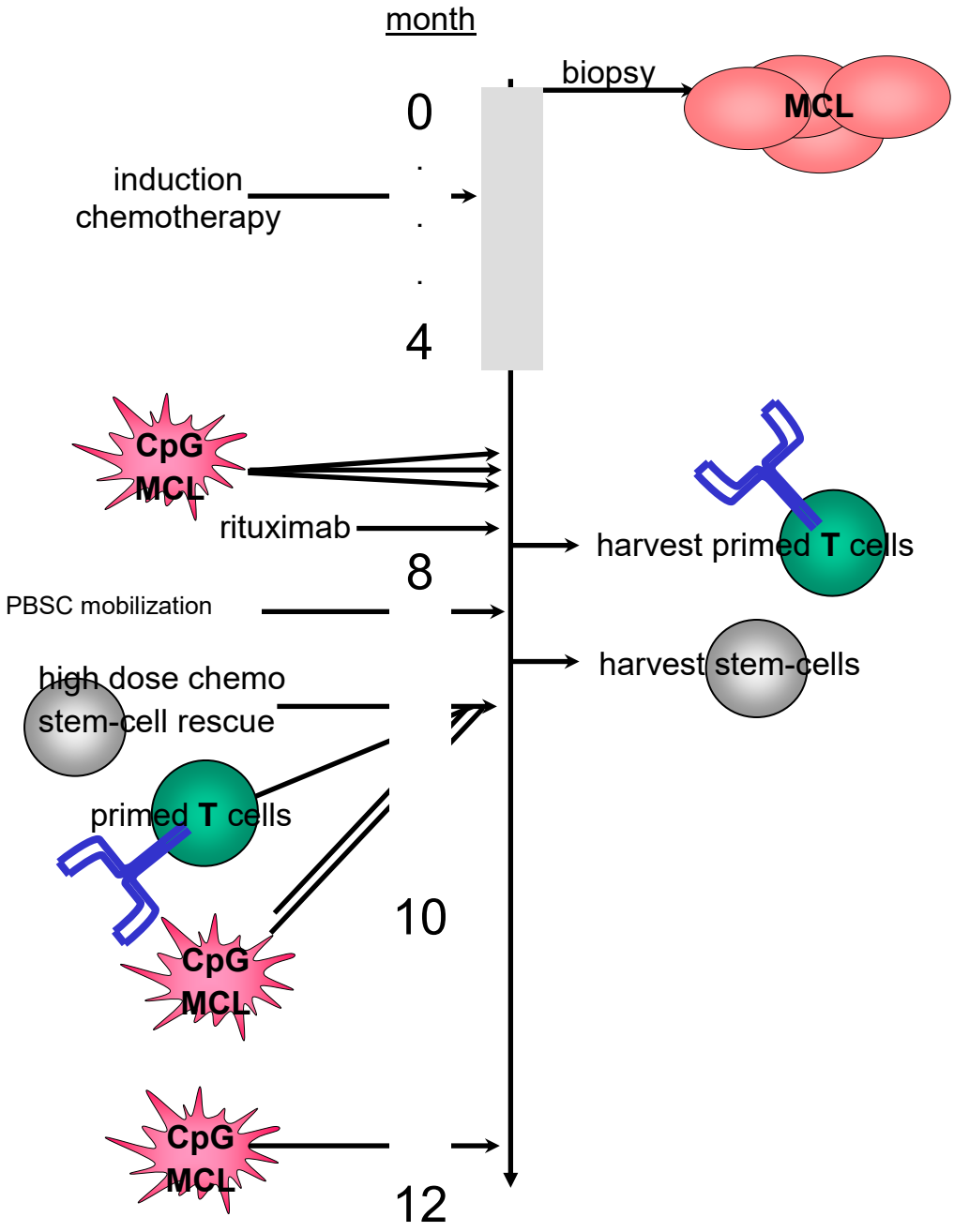

Figure 1: Treatment schema (for time interval details, see Study Calendar section 8.0)

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1. OBJECTIVES

The primary objective of the study is to evaluate freedom from molecular residual disease at one year post-autologous transplant.

Secondary endpoints are Time To Clinical Progression (TTP), and evaluation of anti-tumor immune responses after vaccination, and after immunotransplant.

An additional consideration will be to determine feasibility; specifically what proportion of enrolled patients will be able to complete the entire immunotransplant protocol.

2. BACKGROUND

2.1 RATIONALE

2.1.1 Mantle cell lymphoma (MCL): prognosis and treatment

Mantle cell lymphoma (MCL) is a distinct non-Hodgkin's lymphoma (NHL) subtype[1], representing 4-6% of all NHL[2]. The characteristic molecular feature of MCL, the (11;14) (q13;q32) translocation, places the immunoglobulin heavy chain locus upstream of the BCL1 gene, resulting in over-expression of its cyclin D1 gene product. In a series of 1361 patients diagnosed from 1988 to 1990, MCL carried the worst long-term failure-free and overall survival rate of any of the major subtypes[3]. The poor prognosis of patients with MCL makes it imperative to develop more effective treatments[4]. To date, no therapy has been shown to be curative for a significant proportion of patients, and, thus, there is no broadly recognized standard of care for MCL. Unless contraindicated by co-morbid conditions or very advanced age, most patients are currently treated with either a R-CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone)-like regimen followed by AHCT or by dose-intense versions of CHOP sometimes alternated with methotrexate and/or cytarabine. While such a hyper-CVAD+MA regimen has shown encouraging results in single-institution phase 2 studies, it was associated with significant morbidity, an 8% treatment related mortality and less satisfactory results in patients over age 60 years[5]. CHOP-like induction followed by AHCT resulted in a median progression-free survival (PFS) of 39 months, significantly longer than the 17 months for patients randomized to consolidation with interferon ($p=0.018$). Though AHCT demonstrated a prolongation in PFS, there was a continuous pattern of disease recurrence in this phase 3 trial[6]. The addition of rituximab to induction therapy has yielded significantly higher response rates in randomized and non-randomized trials[7-10]. In the context of AHCT for MCL, molecular remission as defined by quantitative PCR of clonal IgH rearrangements, was strongly predictive for outcome ($p< 0.0001$), suggesting that molecular assessment could be used to measure efficacy of other consolidative therapies[11].

There is evidence that MCL may be responsive to active immunotherapy. Immunologically mediated graft-versus-lymphoma effect is evidenced after allogeneic stem cell transplantation by low relapse rates in patients already having failed AHCT[12, 13], clinical responses occurring after development of graft-versus-host disease (GVHD)[14], or after donor lymphocyte infusion[13]. In accord with the potential immunogencity of MCL, there have been several clinical trials of active immunization of MCL patients[15-17] with good evidence of immune

responses and some patients with remarkably good clinical courses. MCL tumor cells have demonstrated sensitivity in vitro to the TLR9 agonist CpG[18, 19]

2.1.2 CpG Basic Biology and Vaccines

Consistent with their B-cell lineage, MCL and other B-cell NHLs express Toll-Like Receptor 9 (TLR9). Ligation of TLR9 by its ligand oligodeoxynucleotides (usually 20-30 bases long) enriched for hypomethylated Cytosine-Guanosine repeats (CpG) activates a broad signal transduction network culminating in the upregulation of costimulatory molecules such as CD80, CD86, and CD54 as well as improved APC function with upregulation of MHC molecules[20, 21]. Additionally, TLR9 ligation can lead to upregulation of fas, which transmits a pro-apoptotic signal to the NHL cell upon exposure to fasL expressing cells such as NK- or T-cells. TLR9 ligation by CpG molecules can also induce activation of antigen presenting cells such as dendritic cells that can then more effectively present tumor antigens from nearby apoptotic tumor cells.

Hence, there are two mechanisms by which subcutaneous injection of CpG along with CpG-activated NHL can induce systemic, tumor-specific, T cell mediated immunity:

- CpG-NHL can directly present tumor antigens to T cells;
- CpG-NHL undergoing apoptosis can transfer tumor antigens to nearby dendritic cells that can, in turn, be activated by CpG and more effectively present antigens to T cells.

2.1.3 CpG in Murine Models of B-cell Malignancies

We have recently published our initial findings of an *in situ* vaccination strategy using intra-tumoral injection of CpG combined with cytotoxic therapies[22] and these data have shown the importance of co-localization of tumor antigens with the immuno-stimulant CpG. In more recent studies, we have tested lymphoma cells cultured *ex vivo* with CpG as a therapetic vaccine. Anti-tumor T cells can be generated by such a vaccine. These T cells could be transferred to syngeneic recipients in conjunction with a hematopoietic stem cell transplant and mediate the cure of large established tumors [23]. This result provides the rationale for the design of the current clinical trial.

2.1.4 CpG in Clinical Trials of B-cell Malignancies

There have been numerous studies of CpG in patients with cancer[24-29] these studies have demonstrated the safety profile of CpG in over 1500 patients. Based on our initial studies of *in situ* CpG vaccination[22], demonstrating the importance of co-localization of tumor and immuno-stimulant, we initiated a phase 1/2 trial of intra-tumoral CpG with low dose (2x200cGy) external beam irradiation for patients with recurrent, low-grade lymphoma. Therapy was extremely well tolerated in all 15 patients with none experiencing adverse reactions greater than grade 2. The only significant reactions were fever and flu-like symptoms lasting 1-4 days after injections. Notably, there was a proof of the anti-tumor efficacy with one patient achieving a complete response, two patients with partial response, and seven patients with stable disease. We have also demonstrated the induction of tumor-specific, memory CD8 T cell responses resulting from vaccination, correlating temporally with the development of clinical tumor regressions (Figure 2)[30].

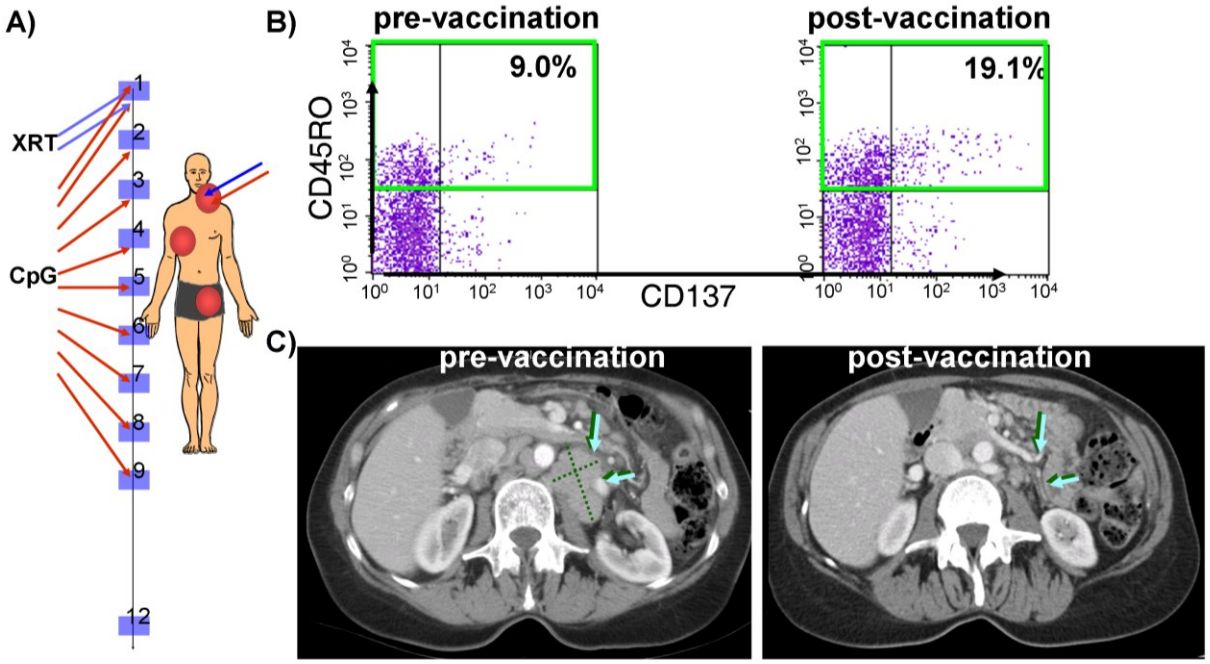

Figure 2: A phase 1/2 clinical trial of *in situ* CpG vaccination for low-grade lymphoma. A) Schema of trial, B) Memory ($CD45RO^{hi}$) CD8 tumor-specific immune response as seen by up-regulation of the activation marker CD137 upon co-culture with autologous tumor, induced by vaccination in a patient demonstrating a C) objective clinical response with resolution of retroperitoneal (and additional sites of) adenopathy.

2.15CpG in MCL

There have been several studies of *in vitro* CpG-activation of primary MCL tissues, which suggest an increase in antigen-presenting features[18, 19]. Together, these studies demonstrate up-regulation of CD40, CD54, CD80, CD86, MHC-I, and MHC-II as well as the well-described target of passive immunotherapy CD20.

We have confirmed these findings using primary tumor samples from patients with newly diagnosed or recurrent classic MCL. Culture with varying doses of CpG- oligodeoxynucleotides induced upregulation of MHC class I and II molecules as well as the co-stimulatory molecules CD80 and CD86 (Figure 3).

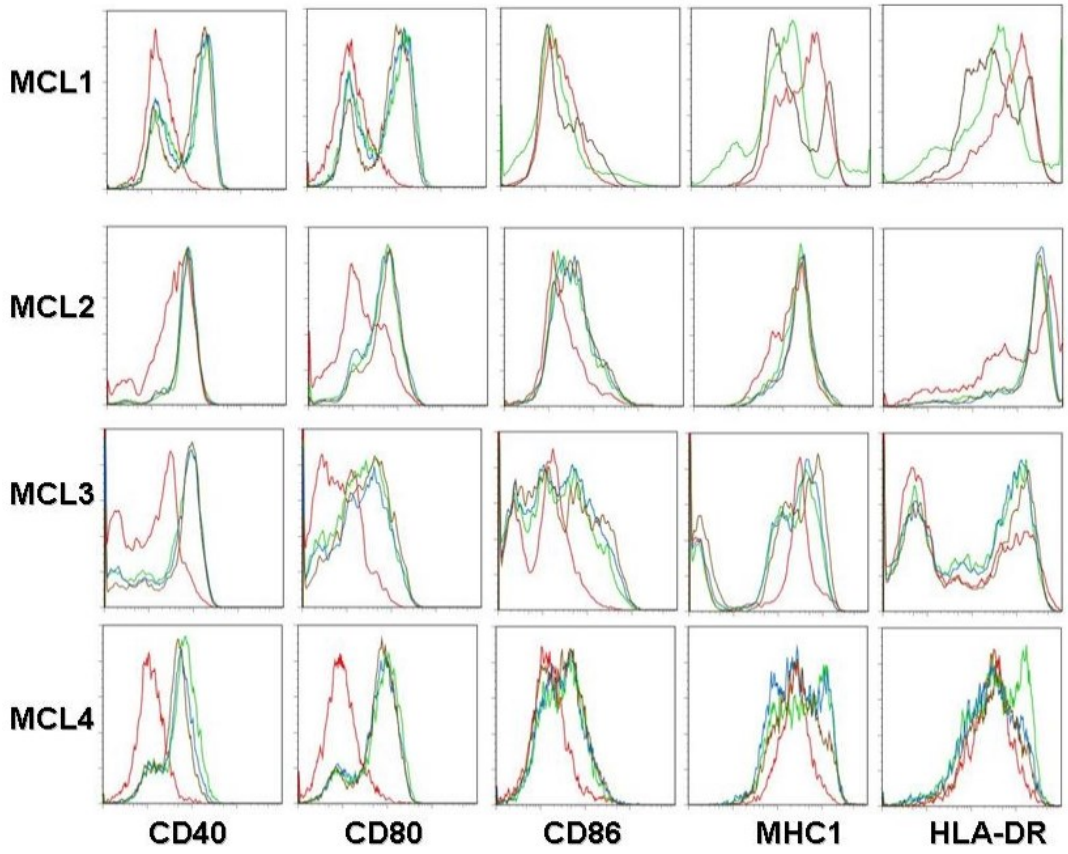

Figure 3: CpG oligodeoxynucleotides induce an immunogenic pheynotype in primary MCL cells. Single-cell suspensions of primary MCL cells from four specified patients were cultured with media alone (red line) or 2,10,or 50μg/ml of PF-3512676 (black, blue, and green lines respectively) at 37°C, 5%CO_2 for 72 hours, then flow cytometrically assessed for surface expression of the indicated antigen presentation or co-stimulatory molecules.

2.1.6 CpG-MCL Vaccination Dose, Route, and Frequency

CpG-MCL vaccination will be administered within a period of 21 days (4-7 days between treatments is allowed), prior to leukapheresis for collection of primed T cells. The three 'priming' vaccinations of CpG-MCL 10^8 cells are administered subcutaneously (s.c.) together with PF-3512676 18mg s.c. The 18mg dose is well tolerated and was used in the majority of prior clinical studies (Investigator Brochure). The timing of the priming vaccinations is intended to maximize the amount of time since the immunosuppressive effects of induction chemotherapy, but minimize the delay time prior to myeloablative therapy and AHCT. There is some evidence that a cancer vaccine can induce a tumor-specific immune response in MCL patients at this time point after induction therapy[17].

Within 3 days post-transplant time-point, the vaccine primed T-cells will be administered i.v. (immunotransplant), followed by another s.c. dose of CpG-MCL vaccine with 18 mg of PF-3512676. The final, post-transplant vaccination is given s.c. along with 18 mg of PF-3512676 at ≥3 months post-AHCT, as medically feasible with resolution of interfering morbidities and medications.

The timing of the post-transplant 'boost' vaccination is to coincide with the infusion of vaccine-primed PBMCs. The timing of the final, post-transplant vaccination is intended to allow an opportunity for priming of the reconstituted immune system, as CD8 T cell levels are generally regained by this time[31].

2.1.7 Vaccination During a State of Minimal Residual Disease

Vaccination in the presence of tumor or tumor antigens specifically decreases immune response to those antigens[32-34]. Tumors secrete factors and recruit cells that impede tumor immunity[35-37]. In our recent trial of DC-Id vaccination for lymphoma, we found that 53% of patients with minimal residual disease mounted cell-mediated immune responses as opposed to 0% in those with residual disease[38]. In the European MCL phase 3 study, 81% of MCL patients were in complete remission (CR) after the completion of high dose chemotherapy and AHCT[6]. These data suggest that vaccination in a state of minimal residual disease such as the post-AHCT setting will be possible and that the absence of tumor will facilitate the development of an anti-tumor immune response.

2.1.8 Vaccination and Primed T Cell Re-Infusion post-AHCT (Immunotransplant)

The period immediately following AHCT is a setting of severe lymphodepletion. Though myeloid reconstitution occurs within weeks after AHCT, lymphoid reconstitution, particularly that of B-cells and CD4 T-cells, takes several months[31, 39]. This time period has been considered a window of opportunity for adoptive transfer of primed T-cells to be more effective in eliminating cancer[40]. Levitsky et al, have clearly shown that adoptive transfer of otherwise unstimulated T cells can cure a majority of mice in a model system of non-Hodgkin's lymphoma[41]. Dudley et al. have demonstrated the impressive clinical benefit of immunotherapy with adoptive T-cell transfer in the context of a lymphodepleted host with achievement of objective response rates as high as 50% in metastatic melanoma patients[42], including several instances of massive tumor reduction. The mechanisms suggested to explain this effect are two-fold. First, the relative reduction of T regulatory (Treg) cells with lymphodepleting chemotherapies[43, 44]) prevents inhibition of the administered vaccine. Secondly, there is decreased competition for the homeostatic proliferation cytokines (e.g. IL-7 and IL-15), which allow for expansion of the administered effector cells[41, 45]. A recent randomized study of vaccination in the post-AHCT setting demonstrated that specific immunity was induced after AHCT only with adoptive transfer of primed T-cells (collected after priming vaccinations)[46]. Our earlier pilot trial[47] was based on these advantages provided by the post-AHCT setting, and others have initiated vaccine strategies post-AHCT in AML and myeloma as well as indolent lymphoma.

Our recent pre-clinical studies have shown that the homeostatic proliferation induced by the "empty" post-transplant recipient induces *qualitative* as well as quantitative changes in the population of transferred T cells. Specifically, we have shown that there is a proportional increase in the proliferation of NK cells and CD4 or CD8 effector T cells relative to that of T_{regs} - as defined by foxP3 expression (Figure 4).

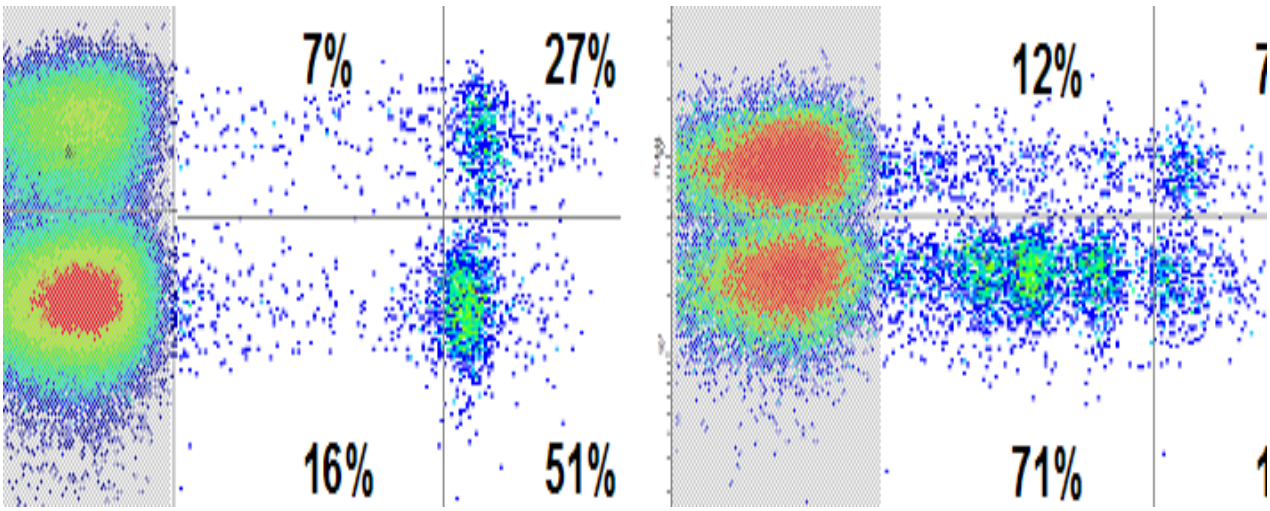

Figure 4: Homeostatic proliferation of transferred splenocytes preferentially expands $T_{effector}$ cells over $T_{regulatory}$ cells 50 x 10^6 balb/c splenocytes were labeled with CFSE and injected by tail vein along with 5 x 10^6 bone marrow cells into A) recipient mice or B) recipient mice that had received lethal (900cGy) irradiation. 14 days later spleens were harvested and stained (above results gated for CD3+, CD4+ cells).

Consistent with our finding that immunotransplant induces preferential proliferation of $T_{effectors}$ over T_{regs}, we have shown that CpG-based vaccination, such as described in Section 2.1.3. is significantly enhanced by immunotransplant. Specifically, the proportion of tumor-specific T cells induced in immunized donors is increased nearly ten-fold upon immunotransplant into lymphodepleted ('empty') recipients (Figure 5).

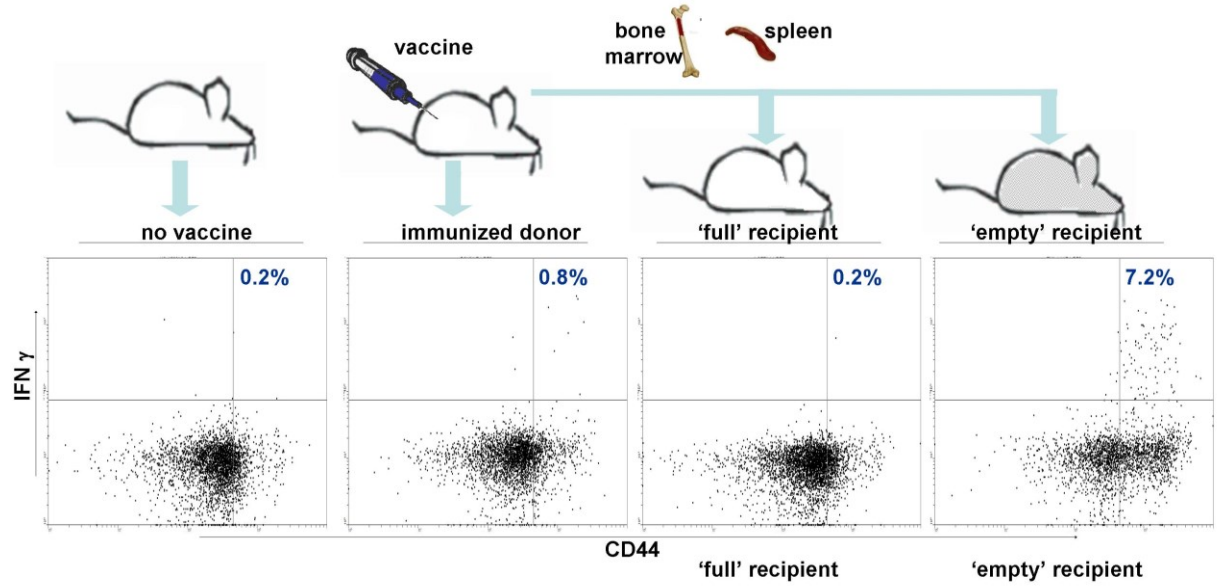

Figure 5: Immunotransplant enhances the vaccine-induced tumor-specific T-cell response. Mice received either: no vaccine, CpG-based lymphoma vaccine, or vaccinated-donor bone marrow and splenocytes after no irradiation ('full' recipients) or 900cGy TBI ('empty' recipients). On day 15 post-transplant, peripheral blood lymphocytes were tested for lymphoma-specific IFNγ production. Graphs gated for CD3(+) lymphocytes and statistics are IFNγ(+)cells as a percentage of all CD44hi cells.

Additionally, protection from subsequent lymphoma challenge increased from 70% with vaccination to 100% with immunotransplant. Further, we have shown that the addition of a post-transplant CpG-A20 vaccine boost further enhances anti-tumor immunity insofar as the transient tumor growth otherwise seen was eliminated with the addition of the vaccine boost (Figure 6).

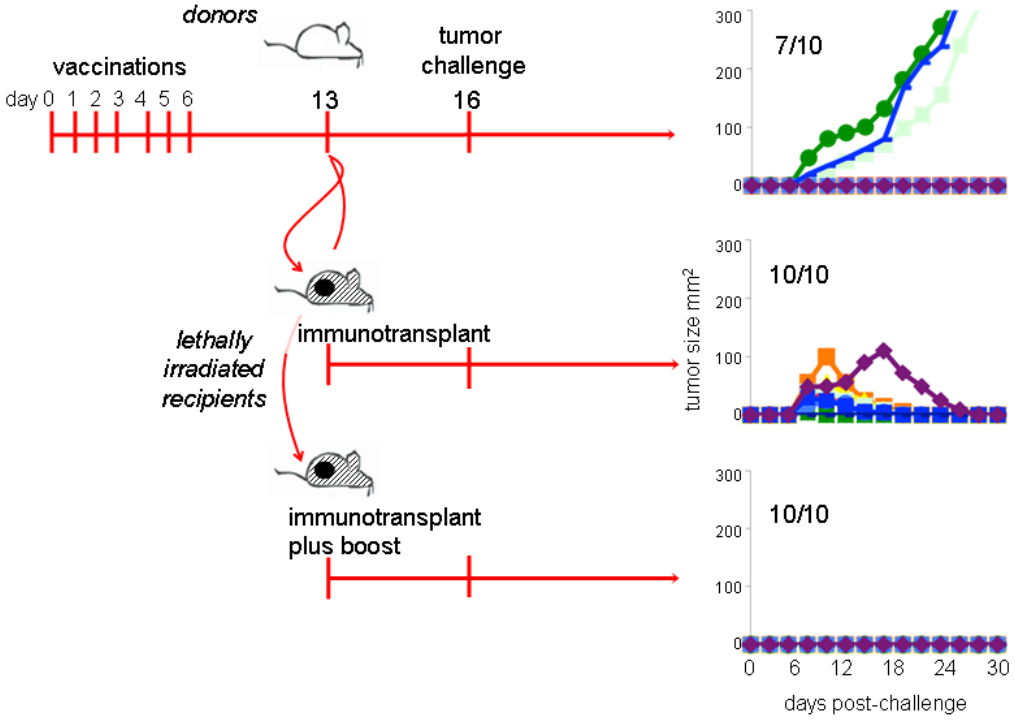

Figure 6: Immunotransplant of CpG-A20 vaccinated donors increases anti-tumor immunity. Donor mice were vaccinated with irradiated CpG-A20 cells on days 1-6. On day 13, one cohort of donors was left intact, one cohort had bone marrow and splenocytes transferred i.v. to recipients irradiated to 900cGy (immunotransplant), one cohort received the same immunotransplant *plus* an additional i.v. CpG-A20 'boost' vaccination at the time of transplant. On day 16 all cohorts were tumor-challenged s.c. and followed for tumor growth. Statistics shown are proportion of tumor-free mice at day 30.

Together, Figures 5 and 6 demonstrate that CpG-based vaccination induces a tumor-specific, memory T-cell immune response that is enhanced by immunotransplant. Our recent demonstration of a tumor-specific T-cell immune response induced by CpG-based vaccination of *low-grade* lymphoma patients (Figure 2B) suggests that immunotransplant could have the same benefit in patients.

2.19 Clinical Experience with CpG-MCL Vaccine + Immunotransplant

This trial has now been open for over 6 years with the original primary end point of anti- tumor immune response. The study presently has a total of 59 patients consented, 58 vaccines prepared, 44 vaccinated and 43 transplanted. The current projection is that it will require another year to fully accrue the trial to eventually give sufficient power to determine the end point of MRD at 1 year post transplant. So far patients are tolerating all the procedures well, with the possible exception of pneumonitis post transplant at a rate of approximately 50% that in some cases is

associated with a recovered viral organism. In the majority of cases the pneumonitis has responded to steroid therapy and is reversible. This rate may be higher than historically seen with conventional stem cell transplant. Notably, the BCNU dose in the preparative regimen was found as the major contributor and have now reduced this dose. A swimmer plot, time and event tracking, of all the patients accrued as December 2015 is shown below.

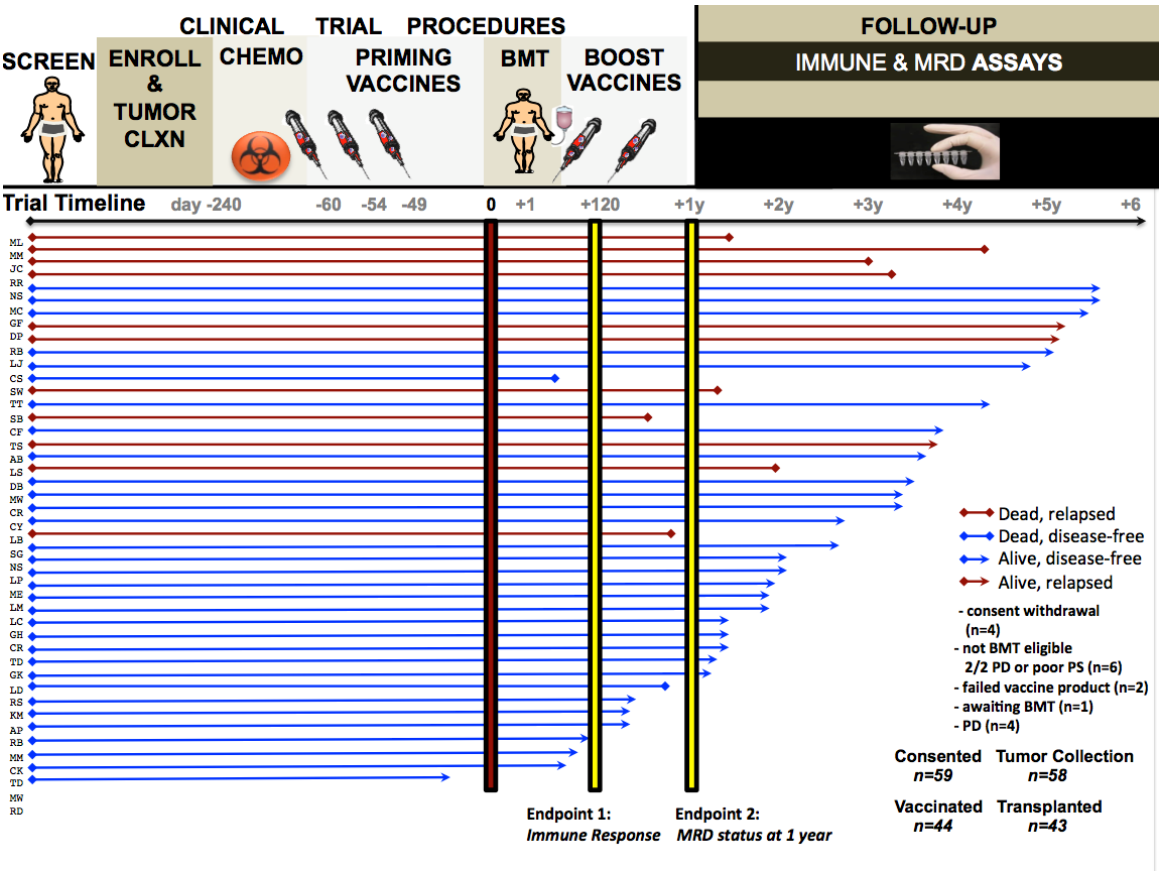

Figure 7: Clinical Experience: Summary of Results to December 2015

23 patients have been evaluated for MRD status at the landmark of 12 months post transplant by the technique of high throughput DNA sequencing on peripheral blood lymphocytes. Serial measurements on each of these patients are shown in the graph with each patient designated by the symbol/color. 19/23 patients are free of tumor DNA signal at a sensitivity of 1/100,000 (dotted line on the graph). Values below this level are of questionable significance. At least one of these patients with signals below the dotted line (blue symbol) as disappeared on further follow up. This value of 83% MRD at one year post transplant is trending to be superior to our benchmark historical rate of non vaccinated patients of 60% that we are trying to beat. But this claim will require additional cases to achieve statistical significance.

A plot the MRD measurements on each patient, up until December 2015 is shown below.

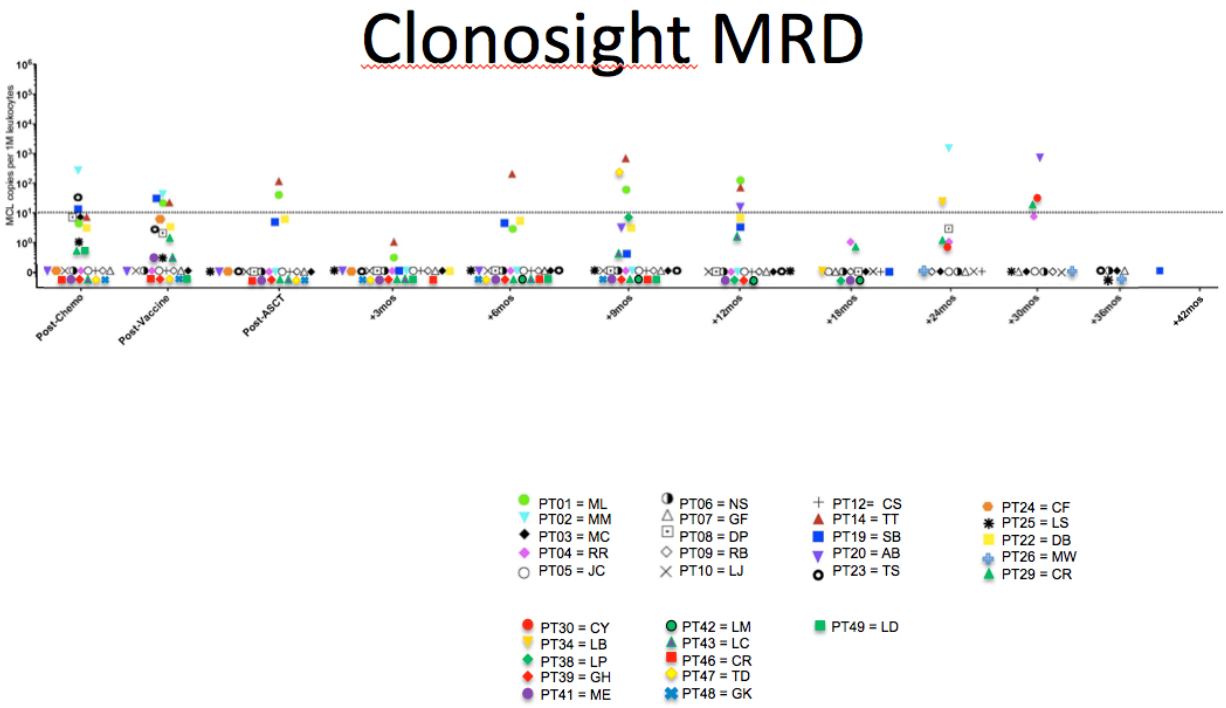

Figure 8: Molecular Residual Disease of all patients completing AHCT to Dec 2015

The full immune response testing against the autologous tumor has been completed on 21 patients. The data indicate that most, but not all, patients have made an anti-tumor immune response detected by at least one of the readouts of CD4 or CD8 T cells as detected in the PBL after recovery for stem cell transplant as shown below. A new objective of this study is to relate these immune responses to other outcomes, such as MRD and PFS. In parallel with these cellular measurements of immune response, high throughput sequencing of the T cell receptor repertoire at matching time points will be conducted. T cell clones that rise with initial immunization and rise again on subsequent boosting after transplant are observed. In some cases it has been possible to match the T cell clones that responded in vitro to those that responded in vivo. An additional objective is to validate the in vivo high throughput TCR sequencing as a substitute for the in vitro cell stimulation test.

2.2 STUDY AGENTS-Vaccine Preparation

2.2.1 Autologous CpG-Activated Lymphoma (CpG-MCL)

Patients with MCL will undergo excisional biopsy at Stanford University Medical Center prior to

initiation of induction therapy to yield at least $1.5x10^9$ cells. Alternatively, patients with significant peripheral blood involvement may undergo leukapheresis to yield the same number of MCL cells. Biopsy samples will be dissociated into single cell suspension under sterile conditions, placed directly into Culture Media as per CpG-MCL SOP and split into two fractions: $1x10^9$ cells for CpG-MCL production and the remainder will be stored in the vapor phase of liquid N2 for immune assays. An aliquot from the immune assay fraction will be used for the development of patient-specific assays of molecular residual disease (MRD).

Production of CpG-MCL

CpG-MCL production is carried out per SOP included with this product's approved IND, which is maintained on file with the Principal Investigator. Briefly, a sterile aliquot of the MCL single-cell suspension totaling $1x10^9$ cells will be suspended in medium containing 3μg/ml PF-3512676 and cultured at 37°C, 5%CO_2 for 72 hours[18] to allow for up-regulation of antigen-presenting and co-stimulatory molecules, then irradiated to 200Gy[48], split into aliquots of $1x10^8$ cells in Cryopreservation Media, composed of saline, human serum albumin, hydroxyethyl starch, 10% DMSO, and cryopreserved in the vapor-phase of liquid N_2.

Quality testing criteria of CpG-MCL is conducted as per SOP. Briefly, CpG-MCL cells and an aliquot of $5x10^6$ MCL cells cultured in parallel *without* PF-3512676 are assessed by flow cytometry for their 'identity' per non-expression of CD3, 4, 8, and co-expression of CD5 and 20 and for their 'activation' per expression of the activation markers, MHC class I, II, CD25, 40, 54, 69, 70, 80, and 86. Identity is confirmed by the phenotype of CD5(+)CD20(+)CD3(-)and effective potency is defined by a $\geq$ 2-fold up-regulation of at least 3 of the activation markers.

Lot release criteria are as follows: upon cryopreservation of at least 7 CpG-MCL cell aliquots in 1mL, a sample (of less than 10% of volume of a vial) from one vial of the cells will be set aside and tested for bacterial, fungal, and mycoplasma contamination, endotoxin content (all pre-freeze). If any of the above tests is positive, and at least 2 extra aliquots are available, each of those will be thawed and tested for all tests described. CpG-MCL product will be deemed acceptable only if initial tests or if both duplicate repeat tests are negative.

Immunization with CpG-MCL plus PF-3512676

At each vaccination time point, one aliquot of CpG-MCL $1x10^8$ cells will be thawed, aspirated into a 1ml sterile syringe, and given as a subcutaneous injection in the patient's lateral thigh. Patients receive simultaneous, adjacent injections of the adjuvant PF-3512676 18mg s.c. Patients experiencing grade $\leq$2 injection site reactions to prior vaccinations can receive subsequent vaccinations to alternate sites including the opposite lateral thigh or either deltoid.

2.2.2 Autologous Vaccine-Primed T Cells

Within approximately four weeks after the third priming CpG-MCL vaccination, patients receive standard rituximab 375mg/m^2 (used here as an *in vivo* purge) followed within 72 hours by leukapheresis to obtain *in vivo* vaccine-primed T cells. Leukapheresis is continued until sufficient PBMCs to obtain approximately 1 x 10^{10} CD3+ T cells are collected and these are immediately cryopreserved as per SOP. This number of PBMCs is comparable to that obtained

after G-CSF mobilization of PBPC as is standard of care prior to autologous transplant, though this leukapheresis will be performed separately from and in addition to the standard one.

3. STUDY DESCRIPTION

Patient Selection:
This is a single institution, single-arm, phase 1/2 study to evaluate the safety and efficacy of incorporating CpG-MCL vaccination, vaccine-primed T-cell infusion into a standard AHCT for newly diagnosed adult MCL patients. The enrollment goal is the number sufficient to obtain 59 treated patients.

3.1 Patient Inclusion criteria:

At time of enrollment:

- Patients must be newly diagnosed with mantle cell lymphoma, have an accessible disease site for excisional biopsy or have sufficient peripheral blood tumor to leukapherese at least 1.5 x 10^9 lymphoma cells in a single session.
- By standard clinical criteria, be medically appropriate to receive rituximab and standard induction chemotherapy and high-dose chemotherapy with AHCT.
- Must be between 21 to 70 years of age.
- Patients must be HIV negative.
- ECOG performance status 0, 1, or 2 or Karnofsky performance scale 50-100%.
- Patients must be capable of signing an informed consent.

3.2 Patient Exclusion criteria:

- Patients who are currently taking immunosuppressive medications.
- Patients with severe psychological or medical illness.
- Pregnant or lactating women.
- At the discretion of the principal investigator if he/she feels that the patient is unable to safely complete the study. Specifically, patients must be considered medically eligible to undergo standard high dose chemotherapy and autologous stem cell transplantation.

3.3 Inclusion of Women and Minorities

Both men and women of all ethnic groups are eligible for this trial.

3.4 Screening Evaluations

At the time of screening the following will be evaluated to verify eligibility**:**

- History and Physical Exam
- Performance Status (ECOG or Karnofsky)
- CBC with differential, comprehensive metabolic panel (electrolytes, BUN, creatinine, calcium, total protein, AST, ALT)
- Medically indicated imaging studies (e.g. PET-CT)
- Bone marrow biopsy per standard of care, but required on follow-up only if results would distinguish between partial response (PR) and complete response (CR)
- Informed Consent

3.5 Enrollment

Eligible MCL patients will undergo excisional biopsy or leukapheresis to collect circulating tumor cells to cryopreserve at least 1.5 x 10^9 malignant cells. This specimen will be used to make the patient specific CpG-MCL vaccine. The following tests will be done prior to tumor cell collection:

- Infectious Disease Testing: HBV S Ag, HBV Core Ab, HCV Ab, HCV RNA, HTLV I/II Ab, HIV 1/2 Ab, HIV-1 RNA, syphilis (RPR)

4. TREATMENT PLAN

4.1 Agent Administration

Patients will receive induction therapy by their primary oncologist with any of the accepted standard-of-care regimens for Mantle Cell Lymphoma and they must attain a PR or a CR and be eligible to proceed to AHCT.

All investigational treatments as well as the AHCT will be administered at Stanford University Medical Center.

Investigational treatments will be administered on an outpatient basis except for those doses administered while patients are in the hospital during AHCT. Expected adverse events and appropriate dose modifications for CpG-MCL vaccine are described in Section 5. No investigational agents or therapies with the intent to treat the malignancy other than those described below may be administered while the patient is on this study.

Post Chemotherapy Assessment

Once in remission and after recovery of sufficient number of T cells, patients will be evaluated prior to receiving priming vaccinations. Study related tests, procedures and imaging must be done within 30 days before starting study agent administration including:

- History and Physical Exam
- Performance Status (ECOG or Karnofsky)
- CBC with differential; comprehensive metabolic panel (CMP) (electrolytes, BUN, creatinine, calcium, total protein, AST, ALT)
- Medically Indicated Imaging Studies (e.g. PET-CT)
- Obtain baseline research blood samples for immune assays and molecular residual disease testing. Leukapheresis [60 minute buffy coat] OR - 10 green top tubes [100 mls] of blood will be drawn for immune assays; the ClonoSEQ® MRD test (Adaptive Biotechnologies) will be used to measure molecular residual disease

Once it is considered medically feasible and any interfering morbidities and/or medications are resolved, patients will go on to receive priming vaccinations.

Priming Vaccinations
Patients will receive three priming vaccinations administered within a 21 day period (approximately 4-7 days apart). Assessments and procedures are as follows:

Within a period of 21 Days:

- Performance Status (ECOG or Karnofsky)
- Adverse event assessment
- Vital signs – prior to and 60 minutes post vaccination
- CpG-MCL vaccine s.c. injection ($1e10^8$ cells)
- PF-3512676 – s.c. injection at the same site as vaccine (18 mg in a volume of 1.2 ml)

Post-Priming Vaccinations
Within approximately four weeks after the third CpG-MCL priming vaccination, patients will receive an *in vivo* purge with rituximab 375 mg/m^2 intravenously as per institutional standard of care. Within 72 hours of rituximab purge, patients will undergo leukapheresis to obtain vaccine-primed T cells. Assessments and procedures at the time of vaccine-primed T-cell collection are as follows:

- Performance Status (ECOG or Karnofsky)
- Adverse event assessment
- CBC with differential, CMP Includes: electrolytes, BUN, Creatinine, Ca++, total protein, LFTs, within 72 hours of leukapheresis
- Within 72 hours of rituximab dose, begin leukapheresis to obtain sufficient PBMC to contain approximately $1x10^{10}$ T cells or goal identified by attending physician to ensure patient safety
- Ten percent of the total T cell product will be retained for immune assays.
- ClonoSEQ® MRD test will be drawn to assess molecular residual disease (MRD.)

Patients then proceed to PBPC mobilization/harvesting followed by high dose chemotherapy and AHCT per standard institutional protocol.

Post-AHCT Immunotransplant and CpG-MCL Vaccination
Within 72 hours following AHCT, (day +1, +2 or +3 post-AHCT) patients will receive their vaccine-primed T cells and CpG-MCL vaccine. Vaccine-primed T cells (PBMC containing approximately 0.5-1.5x10^{10} T cells) are thawed and re-infused over 15 minutes (as per standard practice of PBPC re-infusion). Approximately one hour following T-cell re-infusion, a dose of CpG-MCL is administered s.c. along with PF-3512676 18mg s.c. at the same site. During inpatient therapies, patients receive continuous monitoring as per standard of care of patients undergoing AHCT. Vaccinations and cell infusions will be initiated within one hour after cells are thawed. If no adverse reactions are observed, patients in the outpatient setting will be discharged. Assessments and procedures are as follows:

- Record Performance status (ECOG or Karnofsky)

- Draw blood for CBC with differential and comprehensive metabolic panel
- Adverse event assessment
- Infusion of vaccine-primed T cells
- Record Vital signs – prior to infusion
- CpG-MCL vaccine s.c. injection (approximately $1x10^8$ cells) - approximately one hour post infusion of T cells
- PF-3512676 – s.c. injection at the same site as vaccine (18 mg in a volume of 1.2 ml) -
- Record vital signs – approximately one hour after injections

Post-AHCT, Immunotransplant and CpG-MCL Vaccine Assessment

When considered medically feasible and any interfering morbidities and/or medications are resolved:

- Record Performance status (ECOG or Karnofsky)
- Adverse event assessment
- Leukapheresis OR 10 green top tubes (100 mls), to obtain post-immunotransplant blood samples will be collected for immune assays
- ClonoSEQ® test will be drawn to assess MRD.

If patients are medically unable to undergo these procedures within 30 days post-immunotransplant and the procedures will be delayed, the delay will be noted in the records and in the analysis of the data.

Post-AHCT Final Vaccination

Patients will receive a final vaccination with CpG-MCL s.c. along with PF-3512676 18mg s.c. at ≥3 months post-AHCT as medically feasible upon resolution of any interfering morbidities and medications. Evaluations as follows:

- Record Performance Status (ECOG or Karnofsky)
- Adverse event assessment
- Obtain blood samples for immune assays (8 green tops [80 mls])
- Vital signs – prior to injections
- CpG-MCL vaccine - s.c. injection ($1e10^8$ cells)
- PF-3512676 – s.c. injection at the same site as vaccine (18 mg in a volume of 1.2 ml)
- Vital Signs approximately one hour post injections
- ClonoSEQ® test will be drawn to assess MRD

Approximately 2 weeks following the final vaccination:

- Record Performance Status (ECOG or Karnofsky)
- Adverse event assessment
- Obtain Blood samples for immune assays (8 green tops [80] mls)
- ClonoSEQ® test will be drawn to assess MRD

Follow-up
Patients will be followed clinically for relapse with CT imaging per standard of care. Molecular residual disease will be assessed at 1 year post-AHCT using the ClonoSEQ® test, or until disease progression. Patients will be followed at the time of their standard care visits or with phone calls every 3 months the first year, and every 6 months for years 2-3 years post-AHCT or until progression.

4.2 Monitoring
A nurse and/or a physician will supervise all treatments. During inpatient therapies (primed T cell infusion and CpG-MCL vaccinations) patients receive continuous monitoring as per standard of care of patients undergoing AHCT. After all outpatient vaccine administrations ("priming" and 3 months post-transplant), patients will be monitored for approximately one hour for any acute adverse reactions. Vital signs will be monitored prior to and approximately one hour following vaccination.

4.3 Concomitant/Excluded medications
All concomitant medications administered during the study will be recorded.

4.4 Patient Follow-up and Adverse Event Assessment during Study

Before each vaccine treatment, adverse event and performance status will be assessed. A physical exam will be done within a reasonable time period prior to the first priming vaccinations, prior to the immunotransplant, and at the time of the final CpG-MCL vaccine injection. Laboratory evaluations (including CBC with differential, LFT's, creatinine, BUN) will be done within 7 days prior to the first priming vaccine, and a CBC with differential will be done within one day of the primed T-cell collection. Concomitant medications will be monitored.

Vital signs (BP, HR, RR, Temp) will be checked prior to and approximately one hour after administration of vaccine. Study nurses under the supervision of a physician will monitor patients. Adverse events will be graded using the NCI Common Toxicity Criteria for Adverse Events version 3.0.

4.5 Definition of Treatment-Limiting Adverse Event
The Investigators and the Stanford Comprehensive Cancer Center Data and Safety Monitoring Board (DSMB) will evaluate outcomes with respect to patient safety. The primary adverse events of PF-3512676 as described in the Investigator's Brochure include systemic reactions such as Grade $\leq$ 2 flu-like symptoms, fevers, myalgias, and arthralgias lasting 12-72 hours and Grade $\leq$ 2 injection site reactions including pain, erythema, and induration. The primary adverse events of whole-cell cancer vaccines include primarily injection site reactions[48-57]. The primary adverse events seen in other studies of T cell re-infusion post-transplant[46] were a less than 20% incidence of transient flu-like symptoms, but no adverse events were treatment-limiting. In this study, unacceptable adverse events, including local or systemic grade $\geq$ 3 adverse event or toxicity thought to be related to the vaccine or immunotransplant, will result in discontinuation of vaccine therapy. Grade < 3 adverse events or toxicities may prompt treatment

modifications, delay or discontinuation at the discretion of the investigators if there is evidence that unmodified continuation would increase the risk of adverse events.

4.6 Supportive Care Guidelines
Best supportive care will be administered.

4.7 Duration of Therapy
Treatment will consist of three priming CpG-MCL vaccinations, primed T cell infusion and two post-transplant CpG-MCL vaccinations unless one of the following occurs:

- Lymphoma progression
- Intercurrent illness that prevents administration of treatment
- Unacceptable adverse events including local or systemic $\geq$ grade 3 toxicity that prevents patient from continuing to participate
- Patient is no longer eligible for AHCT
- Patient decides to withdraw from the study
- Non-compliance with the protocol, defined as inability to have all treatments, follow-up appointments and tests
- General or specific changes in the patient's condition that render the patient medically unacceptable for further treatment in the judgment of the investigator.
- Patient does not engraft (ANC <500 at Day 35 post-AHCT)

Reasons for early trial discontinuation may include, but are not limited to, unacceptable adverse event or toxicity of study drug, a request to discontinue the trial from a regulatory authority, protocol violations, or poor enrollment.

5. EXPECTED ADVERSE EVENTS/DOSE MODIFICATIONS

5.1 Expected Adverse Events

5.1.1 CpG-MCL (given with adjuvant PF-3512676).
Local skin reaction at injection site may occur including pain, erythema, and swelling. Low-grade transient flu-like reactions such as fevers, myalgias and arthralgias may occur. Serious side effects such as anaphylaxis and respiratory distress may occur but are unlikely.

5.1.2 Vaccine-primed T-cell Re-infusion.
Anaphylaxis, hypotension, or respiratory distress, all primarily related to the included cryopreservant (DMSO) may occur but are unlikely.

5.1.3 Excisional biopsy:
Reactions common to all surgical procedures, e.g. risk of bleeding, scarring, infection, pain, and subsequent development of lymphedema.

5.2 Dosing Delays/Dose Modifications
Once in remission and after recovery of sufficient number of T cells, patients will receive priming vaccinations; the immunotransplant and CpG-MCL vaccine within 72 hours post-AHCT, and the CpG-MCL vaccine >/= 3 months post AHCT. If a patient develops intercurrent illness or adverse event during the study, the CpG-MCL vaccinations and immunotransplant infusion may be given once it is medically feasible and any interfering morbidities and/or medications are resolved. If there is failure of lot release criteria for the first tested dose of a vaccine batch, and two additional lots are available, those will both be re-tested for all lot release criteria and if both of those additional lots pass all criteria, they may be used.

All adverse events thought to be related to the study treatment will be followed until reasonable resolution. All intercurrent illnesses and adverse events temporally associated with the vaccinations will be collected and documented.

Dose modifications of the investigational agents are not planned.

Patients experiencing local injection site reactions of grade 3 or higher will be taken off study. Patients experiencing local injection site reactions of grade 1 or 2 may receive subsequent injections in an alternate site (alternate thigh or either deltoid), or, per the discretion of the investigator, may be taken off study if additional vaccine doses are felt to be medically contra-indicated.

With evidence of grade $\leq$ 2 allergic reaction during any vaccination or cell infusion, patients will receive additional acetaminophen and diphenhydramine unless contraindicated. Should the patient develop grade 3 allergic reaction or greater, hypotension refractory to fluids, or bronchospasm, any incomplete vaccine or cell infusion will be terminated and the patient will be treated according to the standard of supportive care.

6. AGENT FORMULATIONS AND PROCUREMENT

Described under study drug section 2.2 above.

7. CORRELATIVE/SPECIAL STUDIES

CpG-MCL Phenotype and Functional Assessment: An aliquot of CpG-MCL will be set aside for flow cytometric analysis of surface markers including CD5,20,25,40,70,80,86, HLA-A,B,C, and HLA-DR as described in section 2.2.

Tumor-Specific T Cell Responses: Induction of tumor-specific CD8 T cell cytokine production

and surface activation marker up-regulation are measurements of vaccine efficacy.

An aliquot of each patient's tumor cells are thawed and activated for 3 days with PF-3512676. Patient PBMCs (5×10^5) will be cultured with either medium alone or autologous tumor cells (5×10^5) at 37°C. Afterwards, cells are stained with a panel of surface antibodies including CD4, 8, 45RO, CD137 and CD278. In other assays, brefeldin-A is added for the last 8 hours. Cells are fixed, permeabilized and stained for intra-cellular cytokines and enzymes such as IFNγ, TNFα and β, IL-2, perforin and granzyme.

Handling of Serum samples: Serum will be centrifuged at 2000 RPM for 10 minutes and stored at – 80 C for evaluation.

Handling of PBMC: PBMC are isolated by Ficoll-Hypaque centrifugation, washed and cryopreserved in: RPMI, 20% FCS, 10% DMSO and stored in liquid N_2.

MRD Testing: Tumor cells, PBMC and bone marrow cells and serum specimens will be subjected to high throughput sequencing of Ig variable region and T cell receptor genes, or alternatively real time PCR testing of Ig and T cell receptor genes with ClonoSEQ® (Adaptive Biotechnology, USA)

8. STUDY CALENDAR

Procedures	Screen	Induction Chemo	Pre-tx Visit / post-chemo	Priming Vaccine **within 21 days**	Post-Priming Vaccine **within 30 days**	Pre-AHCT **within 30-60 days**	AHCT Day 0	**Post AHCT within 3 days**	Post – AHCT **within 30 days**	Final Vaccine ≥ 3 mos Post - AHCT	Post-Final Vaccine Follow-up 2 weeks	Post-AHCT Follow-up Months to 3 years post AHCT
History & Physical	**X**		**X**					**X**		**X**		**X (standard of care)**
Adverse Event Assessment			**X**[1]	**X**[1]	**X**[1]	**X**[1]	**X**[1]	**X**[1]	**X**[1]	**X**[1]	**X**[1]	
Performance Status	**X**		**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	
Virology Labs (Standard of Care)	**X**[2]					**X**[2]						
CBC, CMP[3]	**X**		**X**		**X**		**X**					
Imaging studies review (Standard of Care)	**X**[14]		**X**[14]							**X**[14]		**X**[14]
Lymph node biopsy or apheresis for peripheral blood tumor cell collection	**X**											
BM biopsy (as needed)	**X**[4]											
Induction Chemotherapy (Standard of Care)		**X**[5, 12]										
CpG-MCL vaccine 10^8 cells				**s.c.** [12, 15]				**s.c.** [11]		**s.c.** [12]		
PF-3512676 18mg				**s.c.** [12, 15]				**s.c.** [11]		**s.c.** [12]		
Vaccine-primed T cells infusion ($0.5\text{-}1.5x10^{10}$ PBMC)								**X** [11]				
Rituximab 375mg/m (Standard of Care)					**X**[8]							
Leukapheresis			**X**[7]		**X**[8]	**X**[9]			**X**[7,]			
AHCT Preparation: mobilization, chemo (Standard of Care)						**X**[9]						
AHCT (Standard of Care)							**X**					
Immune assays			**X**[7]		**X**[8]				**X**[7,]	**X**[10]	**X**[10]	
Molecular Residual Disease (MRD) with ClonoSEQ®			**X**[13]		**X**[13]				**X**[13]	**X**[13]	**X**[13]	**X**[13] **MRD required only at 1 year post-**

												AHCT

STUDY CALENDAR FOOTNOTES

[1] Adverse event assessment..

[2] Infectious disease screening panel to include HBV S Ag, HBV Core Ab, HCV Ab, HCV RNA, HTLV I/II Ab, HIV 1/2 Ab, HIV-1 RNA, syphilis (RPR) to be done within 7 days of tumor cell collection.

[3] CBC with differential, CMP Includes: electrolytes, BUN, Creat, Ca++, total protein, LFTs, Urine pregnancy test (if applicable).

[4] BM biopsy on enrollment per standard of care, but required on follow-up only *if* results would distinguish between PR and CR.

[5] May be at Stanford or with other primary oncologist (per standard of care).

[6] Footnote #6 deleted

[7] Leukapheresis (1 hour) OR 10 green top tubes (100 mls) to obtain cells for immune assays and MRD testing, plus 10 mls peripheral blood typically in one red top tube. Post AHCT Leukapheresis will have a target date of within 30 days post-immunotransplant; if patient is medically unable to undergo the leukapheresis within 30 days post-immunotransplant and it will be delayed, the delay will be noted in the records and in the analysis of the data.

[8] A one time dose of Rituximab as per institutional standard of care, given within approximately four weeks after the third CpG-MCL priming vaccination.

Within 72 hours of rituximab purge, begin Leukapheresis to obtain approximately 1 x 10^{10} CD3+ T-cell product or a collection goal identified by attending physician to ensure patient safety. Ten percent of primed T-cell product will be retained for immune assays; CBC with differential, CMP (Includes: electroytes, BUN, Creat, Ca++, total protein, LFTs) within 72 hours of leukapheresis.

[9] AHCT preparation (mobilization, stem cell harvest and myeloablative chemotherapy) as per SUMC current protocol; (per standard of care – exact time period is not proscribed).

[10] Blood for immune assays is 8 green tops (approximately 80 mls)

[11] Post-AHCT immunotransplant and vaccination to be administered within 72 hours of AHCT.

[12] Once it is medically feasible and any interfering morbidities and/or medications are resolved, patients will receive the CpG-MCL vaccine and PF-03512676 injections.

[13]Molecular Residual Disease (MRD) – approximately 15 mls of blood be collected for the ClonoSEQ® (Adaptive Biotechnology, USA) test at the following time points:

- At the time of Leukapheresis or collection of 8GTTs for immune assays prior to vaccine administration
- At the time of Leukapheresis for T cell collection
- post-AHCT at 1 month
- post-AHCT twice at approximately 3 months: once prior to vaccine #5, and once two weeks after vaccine #5

- post-AHCT at 1 year
- at additional time points per investigator discretion.

[14]Imaging studies will be reviewed as clinically indicated and available.
[15]Priming vaccinations may be given 4-7 days apart (within a period of 21 days)

9. MEASUREMENT OF EFFECT

The primary endpoint of this study is to determine freedom from molecular residual disease at 12 months post autologous transplant, as has been previously validated to be predictive for subsequent clinical outcome in mantle cell lymphoma[6].

A Secondary endpoint will be anti tumor immune response, as measured by intracellular cytokines and/or intracellular perforin/granzyme in CD8+ T cells, and/or CD137 induction on CD4+ T cells.

Our hypothesis is that immunotransplant will significantly increase the proportion of tumor-specific T cells relative to that induced by vaccination alone.

Another Secondary endpoint will be the clinical outcomes of progression-free and overall survival. Previous studies of 'standard' auto HCT for MCL patients have demonstrated median PFS and OS of 3.7 and 7.5 years, respectively.

Progression is defined as per 2008 modification of standard "Cheson" criteria[58]. Additional clinical endpoints will be overall survival, The initial time point for all endpoints will be the date of transplant.

Measurable disease will be detected at least by CT imaging, usually of the neck, chest, abdomen and pelvis and compared to baseline imaging. In the event that a patient has additional sites of adenopathy that are best measured by other imaging modality, those sites will also be included in baseline and follow-up imaging. All patients will require bone marrow (BM) biopsy at initial diagnosis as per standard of care. Those patients with evidence of BM involvement at any time point may require follow-up biopsy to determine extent of clinical response per standard of care.

10. REGULATORY AND REPORTING REQUIREMENTS

10.1 Adverse Event Reporting

10.1.1 Definitions:

Adverse Events (AEs): An adverse event is any untoward medical occurrence in a patient treated with the vaccine during treatment and post-treatment follow-up period regardless of causality assessment. This includes adverse clinical or laboratory findings, intercurrent illness or an exacerbation or progression of a disease/condition present at the time the study was initiated, other than signs or symptoms resulting from the disease being treated (MCL), which are considered lack-of-efficacy as opposed to an adverse event. The study will collect adverse event information during the CpG-MCLvaccine treatments and for 2 weeks following the final study related procedure. Adverse events will be followed until reasonable resolution. Adverse Events will be graded according to the NCI CTCAE v3. Adverse events occurring during the patient's induction chemotherapy will not be collected. Adverse events occurring during standard of care treatments (i.e. stem cell transplant) and that are temporally associated with study related

treatment will be collected during the study.

Serious Adverse Event (SAE): An adverse event which meets one or more the following criteria is considered serious.

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization
- Is disabling
- Is a congenital anomaly/birth defect
- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above.

Unanticipated Problem Involving Risks to Participants or Others (UPs)
Serious Adverse Events that are:

- **Unexpected**: Not in the consent form, investigator brochure, protocol, package insert, or label, or unexpected in its specificity, severity or frequency **AND**
- **Related to the research:** Caused by, or probably caused by research activity. Events caused by progression of underlying disease are NOT related. If a device is involved, caused by, or associated with the device.
- **Caused harm or increased risk of harm:** Involves harm to participants or others, or places participants or others at increased risk of harm.

Unanticipated problems involving risks to participants or others (UP), that are unexpected and related and harmful must be reported to the IRB and to the FDA.

10.2 Adverse Event Reporting Requirements

Adverse Events
Adverse events should be captured in the CRF. The research team will meet regularly to discuss AEs being experienced by the participants.

Serious Adverse Events
The Sponsor-Investigator shall promptly notify the IRB and the FDA in writing of the occurrence of any unexpected and "serious" adverse experience in accordance with GCP, regulatory agencies and institutional procedures and guidelines. All serious adverse events occurring in patients at Stanford will be reported to the Sponsor- Investigator (also the PI) within 10 days (5 days if the event is life-threatening or resulted in death). The PI will then report the events to Stanford Cancer Clinical Trials Office (CCTO) in accordance with the CCTO Standard Operating Procedures. Deaths within 30 days of a patient being treated with the study drug will be reported within 5 days, all other SAEs will be reported within 10 days of the PI becoming aware of the event. If the event qualifies as an Unanticipated Problem Involving Risks to Participants or Others (UP), the PI will report this event to the Stanford IRB as per policy and to the FDA.

SAEs which are considered suspected adverse reactions will be reported by the Investigator to Pfizer (the provider of PF-3512676) using the MedWatch 3500a form. The Medwatch Report will be faxed to Pfizer U.S. Clinical Safety (using the Pfizer provided SAE Fax Cover sheet) at fax number 866-997-8322 within 24 hours of the PI being informed of the event.

10.2.1 Forms

FDA Form 3500a (MedWatch) will be used to report serious adverse events and UPs. The form is available for download from the FDA website: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm.

10.2.2 Secondary Malignancies

Investigators will report secondary malignancies occurring on or following treatment using the appropriate form noted above. Exception: Cases of secondary AML/MDS will be reported using the NCI/CTEP Secondary AML/MDS Report Form.

10.3 Data Reporting

The data will be monitored continuously over the accrual and follow-up periods by the principal investigator (PI) and the study coordinator. The PI is responsible for maintaining the clinical protocol, reporting adverse events, assuring that consent is obtained and documented, reporting unexpected outcomes, and reporting the status of the trial to the IRB and the data monitoring committee provided by the Stanford University Cancer Center Data Safety Monitoring Board (DSMB). The DSMB will review the study data at scheduled intervals and when the data suggests safety threats to the patients. DSMB responsibilities will be to review the general process and conduct of the study, including accrual, eligibility, data completeness, data timeliness, adverse events, consent forms, and trial renewal and amendments.
There are no well-described toxicities of the CpG-MCL vaccinations in the above described studies[48, 56, 57].

11. STATISTICAL CONSIDERATIONS

Study Design/Endpoints
Primary Endpoint
Statistical evaluation of our study is based on a Simon Two Stage Optimum Design powered to the endpoint of Minimal Residual Disease at the time point of 1 year post transplant. The rate of molecular remission at the landmark of 1 year post transplant is based on the literature that has validated this endpoint for prediction of continued clinical remission after autologous stem cell transplantation for Mantle Cell lymphoma (24-26). To allow for early stopping if no clinical effect is observed, a 2-stage optimum design, following pre-specified probability of the null hypothesis (no effect on MRD rate) of 70% (p0=0.7) and alternative hypothesis of 85% (an increase in molecular MRD of 15% with vaccination; p1=0.85) with desired significance level (α) and desired power (1-β) of 0.1 and 0.9, respectively was used for sample size assessment.

Based on the 2-Stage Simon Optimal Design with both error rates below 0.1 for testing a null (unacceptable) rate of MRD of 70% against an alternative (acceptable) rate of 85% has 20 patients acquired in the first stage, stopping for futility with 14 or fewer successes (MRD negative patients), otherwise going on to a total of 59 patients, choosing the experimental treatment if there are at least 46 successes (MRD negative patients) and results determined favorable to move to additional clinical investigation. If the true rate is 70%, the expected number of patients is 36, and the probability of stopping at stage 1 is 58%. This design minimizes the expected number of patients exposed to an ineffective treatment, given the error rates.

The goal is to enroll patients sufficient to obtain a total of 59 treated patients over 60 months.

Our current rate of accrual predicts that we will equal or exceed that goal. As recent data suggests RCHOP/DHAP alternating chemotherapy is superior to RCHOP or modified RHyperCVAD. As our null, or baseline response rate is based on MRD rates using RCHOP or modified RHyperCVAD, our endpoint analysis will exclude patients who receive RCHOP/DHAP chemotherapy (currently 3 of all enrolled patients will be excluded from analysis). Finally, to determined if chemotherapy regimen does influence MRD rates, we will stratify MRD status by chemotherapy regimen.

Secondary Endpoints

Secondary outcome analysis will focus on TTP (from the date of autoHCT). In the aforementioned European MCL Network randomized trial, the median TTP was 39 months in patients consolidated with autoHCT. This is consistent with our own outcomes with MCL patients treated at Stanford with standard autoHCT. The trial is not powered to secondary endpoints. TTP among patients evaluable will be reported including measure of centrality and variance of the outcome.

The immune response will also be reported descriptively at the completion of the trial. Note that this was the original endpoint that has already been reached and therefore a clinical endpoint has now replaced immune response as the primary endpoint to which the study is powered. Our prior primary endpoint of immune response was assessed using a 2-stage optimum design allowing early stopping for immunologic futility, following pre-specified probability of the null hypothesis (no immune response) of 5% ($p0=0.05$) and alternative hypothesis of 35% (presence of an immune response with vaccination; $p1=0.35$) with desired significance level (α) and desired power ($1-\beta$) of 0.05 and 0.9, respectively. Three of the first 6 patients showed an immune response as defined by a >10% change in 1 measure of the immune response. Currently 5 of the first 10 patients showed an in immune response. Therefore we previously determined early success of stage 1 and stage 2 (stage 1 required at least 1 immune response among 6 patients, and stage 2 required at least 3 immune responses among 17 patients).

Correlative Biomarker Endpoints

At the completion of the trial, we will explore new putative immune biomarkers of clinical response (molecular MRD at 1 year). As there will be approximately 300 potential comparisons to consider, we will evaluate each based on a desired false discovery rate (FDR) of less than 20%. Putative biomarkers with a FDR of <20% will be selected for further study and validation

in subsequent clinical trials. For secondary analysis, we will model the relationship between the strength of clinical response to the vaccination and the change in the novel biomarkers with an FDR<20%. This is secondary, because it is not possible, at this point, to specify the distribution of response and whether the relationship between the response measure and the putative biomarkers will be strong or weak.

Stopping rules:
Though similar studies using PF-3512676, whole-cell cancer vaccines, and re-infusion of T cells post-transplant have shown minimal associated adverse event or toxicity, we will assess the patient cohort in an ongoing manner and use the following stopping rule for the Serious Adverse Events of: non-engraftment or early death (within 100 days from transplant) from any cause.

After patient number:	8	16	24
Non-engraftment seen in:	2	4	5
Early mortality seen in:	3	5	6

This rule would stop the study if the statistics indicate the possibility with even 80% certainty that the non-engraftment rate is > 10% or the early mortality rate is > 15%.

Sample Size/Accrual Rate
The goal is to enroll patients sufficient to obtain a total of 59 evaluable patients over 60 months.

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APPENDIX A:

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Deceased.	0	Deceased.