Phase I/II Study of Dendritic Cell Therapy Delivered Intratumorally after Cryoablation and Anti-PD-1 Antibody (Pembrolizumab) for Patients with Non-Hodgkin Lymphoma

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†Study contributor(s) not responsible for patient care

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Mayo Clinic: Autologous dendritic cells (IND# 14322)
Merck: Pembrolizumab (Anti-PD-1 antibody, MK-3475)
Supplied Commercial Agent: Prevnar13

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IMPACT Lab	[redacted]
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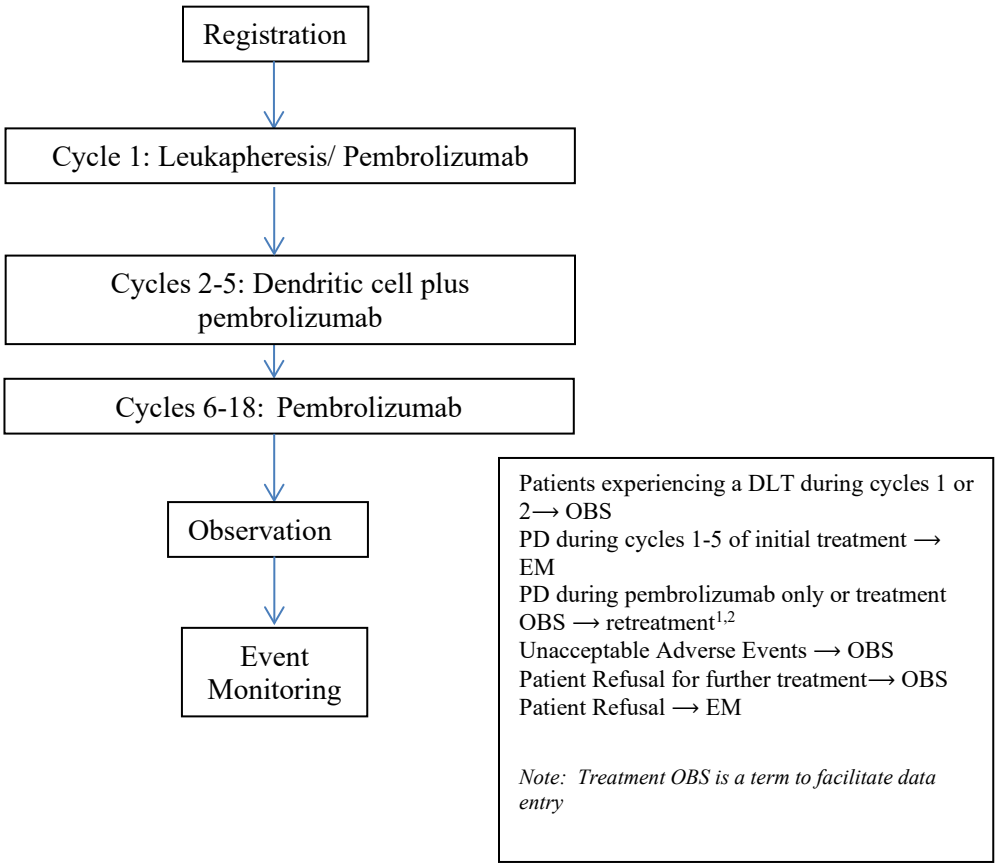
Schema Phase I

(Closed to Accrual July 9, 2018)

Prior to discussing this protocol with a patient, call the Registration Office (507-284-2753) to ensure there is a place on the study for the patient.



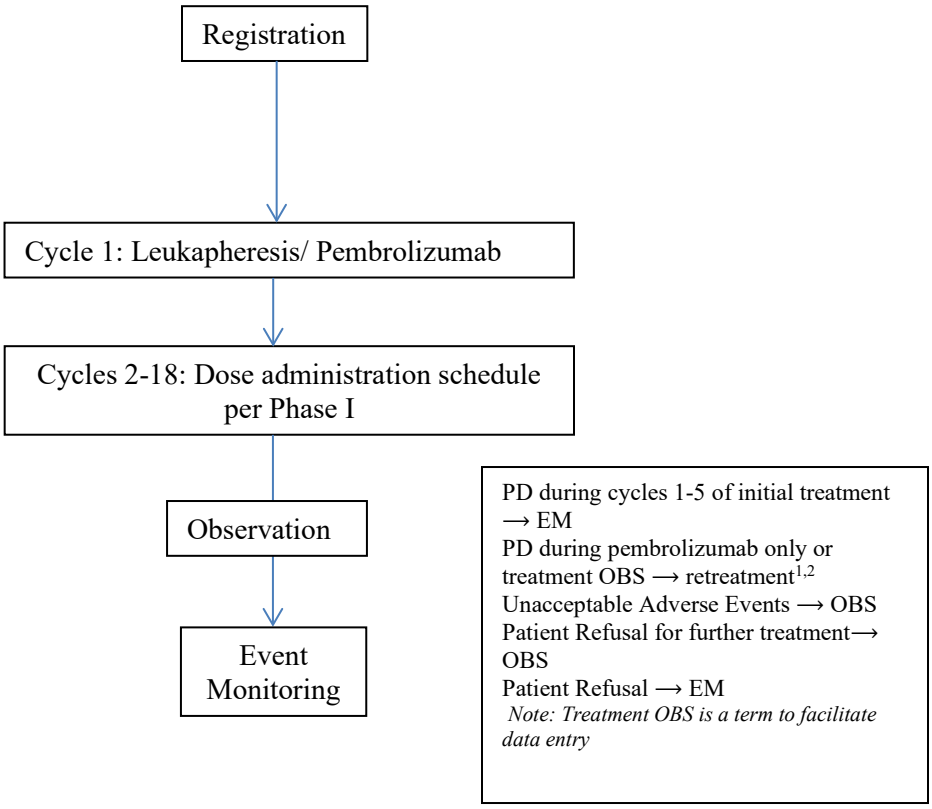
1. When retreating, new baseline disease measurement will be established. The new baseline disease measurement will be the disease measurement at the end of the cycle prior to retreatment (see Section 7.4).
2. Retreatment can be considered at any time when deemed clinically safe.

See Patient Map for more detail (located with the CRFs)

Cycle Length = 21 days

Schema Phase II

(Opened to accrual on July 9, 2018)



1. When retreating, new baseline disease measurement will be established. The new baseline disease measurement will be the disease measurement at the end of the cycle prior to retreatment (see Section 7.4).

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Generic name: Dendritic cell Brand name: Dendritic cell Mayo abbreviation: DC DCVACN Availability: Provided through Mayo Clinic	Generic name: Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein) Brand name: Prevnar13 Mayo abbreviation: PREVNAR13 Availability: Provided through Mayo Clinic	Generic name: Pembrolizumab, MK-3475 (anti-PD-1 antibody) Brand name: Keytruda® Mayo abbreviation: MK-3475 Availability: Mayo Clinic Cancer Center Pharmacy

1.0 Background

1.1 Overview of non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) is the seventh most common type of cancer in both men and women [redacted] In 2014, there were an estimated 71,000 new cases of lymphoma in the US and 19,000 deaths. Lymphoma remains one of the top ten causes of cancer deaths in the United States in both sexes.

The introduction of the anti-CD20 monoclonal antibody rituximab in 1999 has improved the survival of the most common NHL in the US – diffuse large B-cell lymphoma (DLBCL) (Habermann, Weller et al. 2006). Unfortunately, 40% of patients relapse or do not respond to first-line treatment with RCHOP and require further treatment. Some are salvaged with high-dose chemotherapy and stem cell support but many die of disease. There is an unmet need for novel therapies for patients with any type of NHL that relapse after induction therapy (Ansell and Armitage 2005).

Low grade NHL accounts for approximately one third of all cases of NHL. Follicular lymphoma (FL) is the second most common NHL. First line treatment option at the time of initial diagnosis can range among watchful waiting, localized radiation, immune-therapy and systemic chemoimmunotherapy depending on disease burden and patient symptoms. While this disease can be very responsive to chemoimmunotherapy initially, for example with 90% overall response to bendamustine and rituximab or RCHOP (Rummel, Niederle et al. 2013), it can become progressively refractory to treatments with subsequent relapses. There is no consensus regarding the choice of salvage, second and subsequent chemotherapies in treatment of these lymphomas. Ultimately it remains an incurable disease. As such, quality of life is an equally important consideration in selecting effective treatment while minimizing toxicity, making immunotherapy a potentially attractive candidate to delay chemotherapy.

1.2 Rationale for targeting immune suppression in NHL

Systemic host immune suppression is often seen in cancer patients and is thought to contribute to patient morbidity via a tumor-mediated immune evasion mechanism. Although many investigators have examined tumor cell genetics we have also focused on the role of the host immune system. Indeed, polymorphisms in host germ line immune genes have been correlated with survival in NHL patients (Habermann, Wang et al. 2008). The Mayo group has also demonstrated that patients who are immune-compromised, as measured by low absolute lymphocyte count (ALC) or high absolute monocyte count (AMC) have a poorer treatment response compared to those with normal ALC or AMC (Siddiqui, Ristow et al. 2006, Behl, Ristow et al. 2007, Porrata, Ristow et al. 2007, Wilcox, Ristow et al. 2011, Wilcox, Ristow et al. 2012). Immunologic reconstitution such as the rate of lymphocyte reconstitution (Kim, Sohn et al. 2004, Porrata and Markovic 2004) and presence of lymphocytes, natural killer cells and dendritic cells (DC) in transplanted grafts (Porrata, Gastineau et al. 2003, Gazitt, Akay et al. 2006, Porrata, Inwards et al. 2015) have also been associated with improved outcome in NHL patients who have received autologous stem cell transplants. These studies underline the importance of the patient's immune competence in determining outcome and provide rationale for immune-augmentation in therapy.

1.3 Rationale for PD-1 blockade therapy

1.31 Overview of PD-1 pathway

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) (Zhang, Schwartz et al. 2004, Francisco, Sage et al. 2010). PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade (Galon, Costes et al. 2006, Talmadge, Donkor et al. 2007). The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B- cells, T regs and Natural Killer cells (Kloor 2009), as well as subsets of macrophages and dendritic cells (Hillen, Baeten et al. 2008). The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non- hematopoietic tissues as well as in various tumors (Hiraoka 2010). Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues (Hiraoka 2010). Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

1.32 PD-1 is a marker of exhaustion in NHL T cells

We have shown that PD-1 expression is detected in FL T cells and its diffuse pattern of staining is associated with worse survival (Smeltzer, Jones et al. 2014). PD-1 expression in FL T cells is increased when FL transformed into DLBCL. In FL T cells, dim PD-1 surface expression represents an exhausted T cell phenotype and is associated with worse progression free survival (Yang, Grote et al. 2015). PD-1 expression is also detected in T cells from almost all types of NHL (Xerri, Chetaille et al. 2008, Muenst, Hoeller et al. 2010) and is likely a

marker of exhaustion in other types of NHL.

1.33 Expression of PD-1 ligands in lymph node and bone marrow in NHL

In addition to the evidence of PD-1 expression in NHL, expressions of PD-1 ligands are detected in lymphoma tumors. PD-L1 expression is increased in tumor B cells in FL lymph nodes compared to its counterpart in reactive nodes (Andorsky, Yamada et al. 2011, Myklebust, Irish et al. 2013). Additionally, soluble PD-L1 levels in the peripheral blood in diffuse large B cell lymphoma is associated with decreased overall survival (Rossille, Gressier et al. 2014).

1.34 PD-L1 signaling and dendritic cell priming of T cell

The exact mechanism of action and cells targeted by PD-1 blockade is unknown. Intriguingly while PD-L1 expression is seen in both Hodgkin lymphoma (HL) and NHL, the overall response rate is vastly different between the two diseases, 87% and 28% respectively (Ansell, Lesokhin et al. 2015); (Lesokhin, Ansell et al. ASH Annual Meeting, Blood Abstract #291). In addition to the role of PD-1/PD-L1 signaling in T cell exhaustion, we have also found that activated DC can have increased PD-L1 expression, which interferes with CD8 T cell priming and limits differentiation of effector T cell response (Pulko, Liu et al. 2009, Gibbons, Liu et al. 2014). Blockade of PD-1/PD-L1 signaling enhanced CD8 T cell response and cleared tumors in an animal model (Pulko, Liu et al. 2009, Gibbons, Liu et al. 2014). We have found that monocytes and dendritic cells response are significantly inhibited in NHL (Wilcox, Wada et al. 2009, Lin, Gustafson et al. 2011). We hypothesize that PD-1 blockade could increase the anti-tumor efficacy of dendritic cell vaccines by enhancing T cell priming.

1.35 Preliminary clinical activity of PD-1 blockade in NHL

PD-1 blockade as a single agent in relapsed/refractory NHL is approximately 28% (Lesokhin, Ansell et al. ASH Annual Meeting, Blood Abstract #291). In relapsed DLBCL, PD-1 blockade after autologous stem cell transplant achieved ~50% overall response rate in patients who had residual disease after transplant (Armand, Nagler et al. 2013). In relapsed FL, 66% ORR and 50% CR were observed with the combination therapy of rituximab and anti-PD-1 antibody (Westin, Chu et al. 2014). In this study, we will examine the tolerability and efficacy of PD-1 blockade with dendritic cell vaccine in NHL.

1.36 Clinical Trial Data

Pembrolizumab (MK-3475, previously known as SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

As of 18-Oct-2013, 1,000 patients have been treated with Pembrolizumab at several dose schedules, including 10 mg/kg every 2 weeks. MK-3475 has been generally well tolerated, as expected based on preclinical findings and other anti-PD-1 monoclonal antibodies. As of 18-Oct-2013 no serious infusions reactions have been reported in the first human clinical trial (PN001). Less than 1% of patients thus far assayed had confirmed positive anti-drug antibody (ADA) samples and among these, no or no clear impact on exposure has been observed. There is no contraindication to further clinical investigation with Pembrolizumab.

Pharmacokinetics were as expected, based on Pembrolizumab being an IgG mAb

and based on preclinical data, which support dosing once every 2 or 3 weeks. Pembrolizumab monotherapy induces an ORR of 25%/27% in patients with ipilimumab exposed melanoma by central independent RECIST and oncology review/investigator assessed irRC, respectively. Pembrolizumab monotherapy induces an ORR of 39%/43% in patients with ipilimumab-naive melanoma by central independent RECIST and oncology review/investigator assessed irRC, respectively. These responses are remarkably durable.

The preliminary 1-year survival rate for patients, many of whom have had multiple therapies, including ipilimumab, who receive Pembrolizumab is 81%. Pembrolizumab monotherapy induces an ORR of 21%/24% in patients with previously- treated NSCLC by central independent RECIST/investigator assessed irRC, respectively, with these responses also remarkably durable. Preliminary data suggest higher levels of PD-L1 expression in tumors of NSCLC are associated with increased activity (ORR 67% by investigator assessed irRC/57% by central independent RECIST); additional data are required to define the optimal PD-L1 cut point. The most commonly reported treatment emergent AEs experienced are fatigue (43.8%), nausea (26.7%), cough (25.3%), pruritus (24.6%), diarrhoea (22.3%) and rash (21.5%). Immune-related adverse events were reported in 21.4% of melanoma patients; most of these events (15.8%) were considered drug-related by the investigator. The most commonly reported, immune-related adverse events across the dose-schedules are rash (3.2%), pruritus (2.9%), vitiligo (2.9%), hypothyroidism (2.7%), arthralgia (2.2%), diarrhea (2.2%), and pneumonitis (1.9%). Review of the overall benefit:risk ratio of Pembrolizumab favors enrollment of eligible patients into clinical trials of MK-3475. The preliminary data suggest that a dose of Pembrolizumab at 2 mg/kg Q3W is appropriate for patients with melanoma.

1.37 Rationale for Dose Selection/Regimen/Modification

The dose regimen of 200 mg Q3W of Pembrolizumab is planned for all urothelial cancer trials. Available PK results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in PK exposures obtained at a given dose among tumor types. An open-label Phase 1 trial (PN001) in melanoma subjects is being conducted to evaluate the safety and clinical activity of single agent Pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of Pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No maximum tolerated dose (MTD) has been identified.

In KEYNOTE-001, two randomized cohort evaluations of melanoma subjects receiving Pembrolizumab at a dose of 2 mg/kg versus 10 mg/kg Q3W have been completed. The clinical efficacy and safety data demonstrate a lack of clinically important differences in efficacy response or safety profile at these doses. For example, in Cohort B2, advanced melanoma subjects who had received prior ipilimumab therapy were randomized to receive Pembrolizumab at 2 mg/kg versus 10 mg/kg Q3W. The overall response rate (ORR) was 26% (21/81) in the 2mg/kg group and 26% (25/79) in the 10 mg/kg group (full analysis set (FAS)). The proportion of subjects with drug-related adverse events (AEs), grade 3-5 drug- related AEs, serious drug-related AEs, death or discontinuation due to an

AE was comparable between groups or lower in the 10 mg/kg group.

Available pharmacokinetic results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in pharmacokinetic exposures obtained at a given dose among tumor types. Population PK analysis has been performed and has confirmed the expectation that intrinsic factors do not affect exposure to pembrolizumab to a clinically meaningful extent. Taken together, these data support the use of lower doses (with similar exposure to 2 mg/kg Q3W) in all solid tumor indications. 2 mg/kg Q3W is being evaluated in NSCLC in PN001, Cohort F30 and PN010, and 200 mg Q3W is being evaluated in head and neck cancer in PN012, which are expected to provide additional data supporting the dose selection.

Selection of 200 mg as the appropriate dose for a switch to fixed dosing is based on simulation results indicating that 200 mg will provide exposures that are reasonably consistent with those obtained with 2 mg/kg dose and importantly will maintain individual patient exposures within the exposure range established in melanoma as associated with maximal clinical response. A population PK model, which characterized the influence of body weight and other patient covariates on exposure, has been developed using available data from 476 subjects from PN001. The distribution of exposures from the 200 mg fixed dose are predicted to considerably overlap those obtained with the 2 mg/kg dose, with some tendency for individual values to range slightly higher with the 200 mg fixed dose. The slight increase in PK variability predicted for the fixed dose relative to weight-based dosing is not expected to be clinically important given that the range of individual exposures is well contained within the range of exposures shown in the melanoma studies of 2 and 10 mg/kg to provide similar efficacy and safety. The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different tumor types and indication settings.

1.4 Rationale for autologous dendritic cell vaccine therapy

1.41 Evidence for dendritic cell vaccine in cancer

Dendritic cells are the professional antigen-presenting cells that induce and regulate both adaptive and innate immune responses (Steinman 1991, Boczkowski, Nair et al. 1996, Steinman 2003). DC have been used in the past by exposing these cells to some form of tumor antigen *in vitro*, and then returning antigen loaded DC to the patient to stimulate anti-tumor immunity. Clinical trials of DC immunotherapy have suggested this approach can result in significant stimulation of the immune response against many different forms of cancer.

The presence of DC within the graft and an associated graft-versus-lymphoma response in transplant patients has suggested a role for dendritic cell-based vaccines in treating NHL (Hsu, Benike et al. 1996, Dean, Masci et al. 2005, Inoges, Rodriguez-Calvillo et al. 2006, Di Nicola, Zappasodi et al. 2009). DC immunotherapy consists of exposing dendritic cells to tumor antigen and using the antigen-loaded DC as a vaccine to stimulate anti-tumor response.

1.42 DC generation for vaccine therapy

Results of this immunotherapy method against many different forms of cancer

have been encouraging in animal models but have had mixed results in clinical trials (Rosenberg, Yang et al. 2004). These trials have been difficult to interpret as the methods of DC preparation and the resulting quality of DC used in the trials have varied significantly. Available evidence suggests that the quality of the DC preparation used in a vaccination protocol has a substantial impact on the immune response elicited (Finkelman, Lees et al. 1996, Steinman 2003, Steinman, Hawiger et al. 2003, Yamazaki, Iyoda et al. 2003). Specifically, the functional status of DCs depends on their state of activation at the time they are delivered. Completely activated DC (mature DC or mDC) are able to migrate to lymph nodes and initiate stimulation of the immune system *in vivo,* while immature DC can inhibit effector T cell function and induce tolerance (Dhodapkar, Steinman et al. 2001). Unfortunately, many clinical trials were initiated before this distinction came to light and patients received immature DC; inhibiting the immune response instead of stimulating it. Thus, the lack of potent immune stimulation and unsatisfactory clinical response demonstrated in earlier trials of DC immunotherapy may at least in part be attributable to the functional state of the DC employed.

Many published trials in NHL have utilized immature, potentially tolerizing DC (see Table 1). This is likely because lymphoma patients have a largely unrecognized yet profound deficiency in the ability to generate mature DC using conventional DC generation protocols (Lin, Gustafson et al. 2011, Laborde, Lin et al. 2014). We have developed a method that consistently generated highly pure, mature DC from NHL patients *ex vivo*. This culture method is serum-free and easily adaptable to scale-up GMP practice. This significant improvement in DC vaccines assures the potency and purity of the DC preparation. Thus, we by-pass the immune suppressive qualities we have identified in lymphoma patients by isolating the monocytes, culturing them to generate potent immune stimulatory DC, and injecting these cells back into the patient. This DC manufacturing method was used in LS1081 clinical trial to treat patients with newly diagnosed or relapsed/refractory indolent B-cell NHL (Table 1). We were able to make adequate vaccines to complete treatments for all 15 patients in the study.

1.43 *In situ* DC tumor antigen loading for vaccine therapy

Differences in loading of DC with antigen *in vitro* may induce immune tolerance rather than stimulation (Steinman 2003, Yamazaki, Iyoda et al. 2003, Marigo, Dolcetti et al. 2008). The optimal method for preparation of antigen and antigen loading to DC remains to be identified. Animal studies and small clinical trials have used whole lymphoma cells, exosomes from heat-shocked lymphoma cells, idiotype proteins, and proteoliposomes from tumor cells as antigens (Hsu, Benike et al. 1996, Timmerman, Czerwinski et al. 2002, Chen, Wang et al. 2006, Inoges, Rodriguez-Calvillo et al. 2006, Neelapu, Gause et al. 2007, Di Nicola, Zappasodi et al. 2009). While idiotype containing unique determinants of variable regions of the clonal tumor immunoglobulin molecule offer specific, definitive targets for immune monitoring of treatment, immune stimulation against tumor may also be limited. The production of idiotype protein with recombinant DNA is also labor and time intensive and likely not practical for widespread clinical use. Whole tumor cells or liposome preparations from the tumor cells are easier to generate and contain a wider range of epitopes, but are limited by the quantity of the primary tumor.

Route of DC delivery also critically impacts efficacy of DC to stimulate an anti-tumor response. Inconsistent generation of systemic anti-tumor immune responses with intravenous and subcutaneous administration has prompted evaluation of novel delivery approach (Mody, Dubey et al. 2015). Intratumor DC delivery was shown to generate anti-tumor immunity and tumor regression in animal models (Kim, Sohn et al. 2004, Song and Levy 2005, Lee, Cho et al. 2006). Clinical trials to date have shown this approach to be safe with promising response (Table 1; LS1081 preliminary results)(Kolstad, Kumari et al. 2015).

Antigen can be generated *in vivo* using conventional therapies including chemical, radiation or even cryoablation (Sabel, Arora et al. 2006, Fagnoni, Zerbini et al. 2008, Sabel 2009, Kolstad, Kumari et al. 2015). Recently Kolstad et al reported promising results in FL patients treated with radiation, and intra-tumor injection of rituximab and DC ((Kolstad, Kumari et al. 2015); Table 1). Cryoablation is an acceptable clinical option for the targeted destruction of lymphoma masses. It provides an opportunity for generation of tumor antigen *in vivo*, but currently relies on local ability to generate an active immune response. In arm A of our phase I clinical trial LS1081, we used cryoablation as a novel approach to *in situ* lymphoma tumor antigen loading. Patients underwent cryoablation of a lymphoma tumor and subsequently received up to eight doses of intra-tumor injections of autologous DC over the course of 6 months. Direct injection *in vivo* offers two advantages: delivery of mature DCs into a milieu of dying tumor cells, resulting in maximum exposure of tumor cell antigens; and avoidance of the suppressive effects of blood monocytes. Systemic response in non-cryoablated tumors was assessed. This combined approach was found to be feasible and safe. We will now examine combining this approach with pembrolizumab to enhance clinical efficacy.

1.44 Safety considerations from DC therapy

DC vaccine therapy clinical trials in B-cell NHL have thus far demonstrated no significant adverse events or autoimmunity (Table 1). DC immunotherapy has been used for many different tumors types where adverse events (such as induced autoimmunity) were monitored. Importantly, very few significant adverse effects of DC vaccination have been reported and DC immunotherapy clinical trials performed to date have shown no evidence of resultant generation of auto-immunity. To date, 86 patients have been treated with DC therapy without any serious drug related toxicity reported. In our trial LS1081, 15 patients were treated without any autoimmunity or grade 3 or higher adverse events attributable to treatments. The most common adverse event was injection site pain, more often with cryoablation, that usually resolved within 2 days of treatments (Table 2).

Table 1. Summary of clinical trials using dendritic cell vaccines for NHL

Histopath Diagnosis	Phase (N)	Ag source	DC source	Delivery	Safety	Clinical outcome
Follicular (Hsu, Benike et al. 1996)	I (4)	Idiotype	Blood *	IV	No Grade 3-4 AEs; no AI	1 CR, 1 PR, 1 molecular CR
Follicular (Timmerman, Czerwinski et al. 2002)	I (10) II (25)	Idiotype	Blood *	IV	No Grade 3-4 AEs; no AI	I: 3 CR, 1 PR, 6 PD II: 16 CR, 7 PR, 2 PD
Follicular, lympho-plasmacytoid (Di Nicola, Zappasodi et al. 2009)	I (18)	Heat-shocked autologous tumor cells	Matured from monocytes	SC	No Grade 3-4 AEs; no AI	3 CR, 3 PR, 8 SD,4 PD
Follicular (Kolstad, 2015)	I (14)	in situ loading via intra-tumor injection	Immature DC from monocytes	Intra-tumor	No Grade 3-4 AEs; No AI	2 CR, 3 PR
Indolent B-cell NHL (LS1081 preliminary results)	I (Arm A: 10; Arm B: 5)	Arm A: in situ via intra-tumor injections Arm B: tumor lysate	Matured from monocytes	Arm A: intra-tumor Arm B: intradermal	No Grade 3-4 AEs; no AI	Arm A: 5 PR Arm B: 1 CR, 2 PR

Blood-derived DC used gradient centrifugation and adhesion method. DCs were characterized by negative phenotype markers without description of maturation markers normally used in release criteria. (AI = autoimmunity. CR = complete remission. IV = intravenous. PD = progressive disease. PR = partial remission. SC = subcutaneous. SD = stable disease.)

Table 2. Summary of adverse events attributable to treatments in LS1081 study

		Grade							
		1		2		3		4	
Adverse Event		**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Type	**Arm**								
Urticaria	**A**	2	25.0	1	12.5				
Bronchospasm	**A**			1	12.5				
Injection site reaction	**A**			1	12.5				
Lymph node pain	**A**			1	12.5				
Rash maculo-papular	**A**			1	12.5				
Vasovagal reaction	**A**					1	12.5		

Grade 3 vasovagal reaction was due to line placement for leukopheresis prior to active vaccine

treatment. Reaction resolved with IV fluids and reverse Trendelenburg maneuver.

1.5 Rationale for pneumococcal conjugate vaccine (PCV) therapy

We have found that, compared to healthy donors, NHL patients have significantly decreased number of lymphocytes, particularly CD4+ cells (Lin, Gustafson et al. 2011). This combined with the *in vitro* suppressed immune function argue for T cell stimulation to augment immune response with DC vaccine. PCV (Prevnar®) is approved by the US Food and Drug Administration for vaccination against pneumococcal infections in children and works by activating helper T cells to interact with B cells for antibody production (Pletz, Maus et al. 2008). Pneumococcal vaccination has been shown to be safe and recommended for cancer patients that will or have undergone chemotherapy (Melcher 2005). Thus, the use of pneumococcal vaccines presents little if any additional risk to the patient. The use of these vaccines does allow determination of the state of the immune system and its response to the DC based vaccines. Prevnar has been used in two clinical trials for multiple myeloma patients in this manner. These trials included one with adoptive T cell transfer after stem cell transplant (Rapoport, Stadtmauer et al. 2005) and another with lenalidomide (Noonan et al. ASH Annual Meeting 2008, Blood abstract 2772). Both trials reported minimal toxicity. In addition there may be a bystander effect on immunity with enhanced lymphocyte trafficking and the response to one antigen leading to enhanced response to anti-tumor antigens (Mitchell, Batich et al. 2015). Preliminary study in our lab showed that Prevnar enhanced mature DC stimulated CD4 T cell proliferation by approximately 1.75 fold compared to mature DC stimulation alone (Figure 1). Importantly, a review of the results of DC therapy in melanoma suggest that addition of adjuvant capable of T cell help, significantly improved overall clinical response (Engell-Noerregaard, Hansen et al. 2009, Mitchell, Batich et al. 2015). We are including this vaccine to enable us to monitor the immune response prior to and after our intervention. The vaccine will also allow us to quantify any potential in vivo bystander effect due to the combination of DC and pneumococcal vaccine. In the LS1081 study, no adverse events were seen with Prevnar administrations.

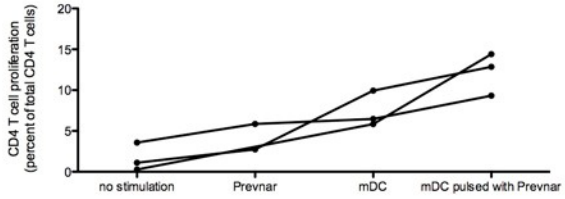

Figure 1. CD4+ T cell proliferation is stimulated with mature dendritic cells pulsed with Prevnar. Monocyte-depleted PBMNC from healthy donors were labeled with CFSE and cultured for 7 days without stimulation or with Prevnar, autologous mature dendritic cells (mDC), or autologous mDC pulsed with Prevnar. At the end of culture, cells were stained with 7-AAD, anti-CD3 and anti-CD4 and analyzed by flow cytometry. Percent of live, CD4 T cells that proliferated are shown here for 3 donors. CD4 T cell proliferation was the greatest in co-culture with Prevnar-pulsed mDC.

1.51 Safety considerations for the use of 13-valent pneumococcal conjugate vaccine (PCV) therapy

PCV (Prevnar®) is approved by the US Food and Drug Administration for vaccination against pneumococcal infections in children and works by activating helper T cells to interact with B cells for antibody production (Pletz, Maus et al.

2008). Pneumococcal vaccination is considered safe for cancer patients undergoing chemotherapy with mild side effects including soreness, low-grade fever and induration at the site of vaccination lasting 1-3 days (Melcher 2005). It has been used in two clinical trials for multiple myeloma patients, one with adoptive T cell transfer after stem cell transplant (Rapoport, Stadtmauer et al. 2005) and another with lenalidomide (Noonan et al. ASH Annual Meeting 2008, Blood Abstract #2772). Both trials reported minimal toxicity and some evidence of improved immune response. As in Rapoport et al, we will use this pneumococcal vaccine to evaluate and monitor the developing immune response. The proposed Prevnar13 (Prevnar) dosing schedule in this study is similar to the Prevnar schedule in LS1081 study. No significant toxicities were observed in 15 patients who received Prevnar along with their DC vaccines. We will use the dose administration and timing as approved for use (Prevnar product insert; [redacted]

1.6 Cryoablation therapy

1.61 Cryoablation background

Percutaneous cryoablation systems are currently available from two manufacturers including Endocare, Incorporated (Irvine, CA) and Galil Medical (St. Paul, MN), each are FDA 510K cleared for treatment of soft-tissue tumors. Since 2003, investigators in this study have used cryoablation for the treatment of renal, lung, soft tissue, and bone tumors, including treatment of patients enrolled in the LS1081 study. Probes may be placed either percutaneously or intraoperatively using US or CT image guidance.

The mechanism of cryoablation is based on delivery of argon gas through a segmentally insulated probe with outer diameter ranging from 1.7 – 2.4 mm, with expansion of the gas within the probe lumen, resulting in rapid cooling (via Joule Thompson effect). With expansion of the argon gas within the probe, rapid and extreme temperature drops are produced along the distal, uninsulated probe shaft reaching –100 ºC within a few seconds. Adjacent tissues are quickly frozen, resulting in the formation of an iceball. Thawing of the iceball is achieved with active instillation of helium gas into the cryoprobes instead of argon gas.

Cell death is due to two primary causes. First, rapid freezing immediately adjacent to the probe results in intracellular ice formation, organelle disruption, and subsequent cell destruction. At a further distance from the probe, relative gradual cooling causes osmotic differences across the cell membrane with secondary cellular dehydration and death. Subsequently, further cell death occurs due to thrombosis of affected blood vessels and resultant ischemic necrosis.

The size of the iceball varies depending on the length of uninsulated tip, with commonly used cryoprobes generating an iceball of about 3 cm in diameter and 5 cm in longitudinal length along the probe shaft. The growth and size of the iceball can be controlled by the delivery system through the relative amount of argon passed through the cryoprobe. Given relative cellular tolerance to freezing temperatures, cell death occurs within about 3-5 mm internal to the iceball margin (Campbell, Krishnamurthi et al. 1998; Chosy, Nakada et al. 1998). A target lesion is typically treated with curative intent using two freezing cycles of approximately 10 minutes each, separated by a 5-minute thaw period (Figure 2).

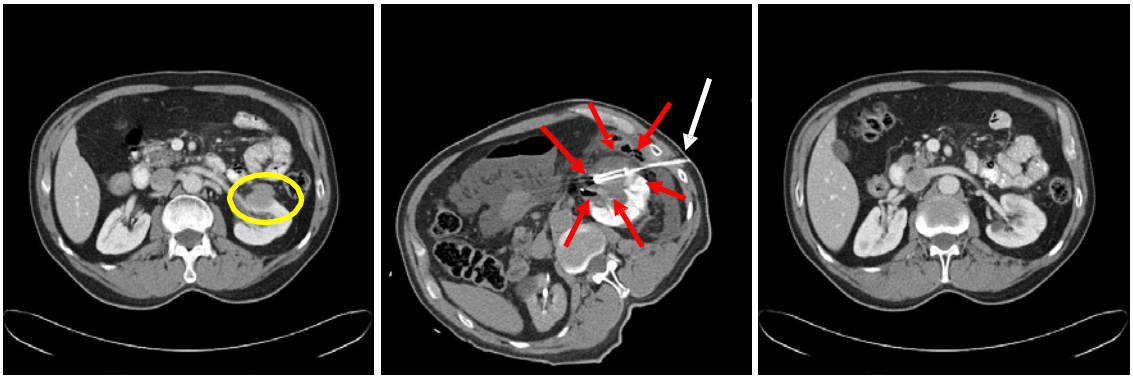

Figure 2. Example of lymphoma tumor cryoablation. Low grade follicular lymphoma lesion involving the left kidney (left panel yellow circle) can be seen before and during the course of cryoablation (middle panel). The probe used for cryoablation is indicated by the white arrow and the growing ice ball identified by the red arrows. The size and predictability of the growth of the ice ball is independent of the tumor source. Right panel shows resolution of the lesion after treatment.

1.62 Safety considerations for cryoablation

Outside of the liver, complications due to cryoablation are primarily related to inadvertent tissue injury at the ablation site. A large experience exists regarding percutaneous cryoablation of renal tumors, showing a major complication rate of 5-8% (Silverman, Tuncali et al. 2005, Littrup, Ahmed et al. 2007, Atwell, Farrell et al. 2008, Atwell, Carter et al. 2012). Similar to RFA, the majority of complications are related to local tissue trauma, including hemorrhage in about 3% of patients. Allowing for a more limited published experience, complications following bone and soft tissue ablation are even more infrequent, ranging from 0-2% (Beland, Dupuy et al. 2005, Ullrick, Hebert et al. 2008, Callstrom, Dupuy et al. 2013). Unique to cryoablation, compared to RFA, is the ability to visualize the cytotoxic ice develop using CT imaging, allow the user to precisely control the volume of tissue treated and minimize adjacent tissue injury (Figure 2).

Of particular note, a systemic phenomenon called “cryoshock” has been observed primarily in the cryoablation of liver tumors. Cryoshock is clinically manifested by severe coagulopathy, disseminated intravascular coagulation, and multiorgan failure, possibly related to a systemic inflammatory response syndrome. This syndrome has been seen in about 1% of cases of hepatic cryotherapy and only 0.04% of prostate cryoablations (Seifert and Morris 1999). We have used clinical cryoablation alone to treat a limited number of patients with lymphomatous tumor masses with no serious safety issues identified. In the LS1081 study, no cryoshock or ≥Grade 3 adverse events were observed with cryoablation treatments (Table 1).

1.7 This Study and Research Hypothesis

This study is a phase I/II design where the phase I portion will confirm the starting dose and regimen of the phase II portion (which is expected to be dose level 1) and the phase II portion will receive the same regimen as determined by the phase I portion. The patient population in the phase II portion is patients with Non-Hodgkin lymphoma. Given the evidence of PD-1 and its ligands expression in neoplastic lymphoma cells, the

tumor microenvironment, and systemically, we hypothesize that PD-1 blockade combined with DC vaccine will enhance DC priming of T cells with anti-tumor antigen and activate T cell functions. The combined immunotherapy with pembrolizumab, cryoablation followed by intra-tumor injection of DC vaccines will generate anti-tumor immunity and clinical regression of lymphoma.

2.0 Goals

2.1 Phase I

2.11 Primary

Evaluate the optimal dose schedule, safety and tolerability as measured by the incidence of significant toxicity of combination therapy with anti-PD-1 monoclonal antibody, cryoablation, and intra-tumor injection of autologous dendritic cell into the cryoablated tumor.

2.12 Secondary

2.121 Evaluate the feasibility of this combination immunotherapy.

2.122 Evaluate patient quality of life

2.2 Phase II

2.21 Primary

Test the efficacy (overall response rate) of combination therapy with anti-PD-1 monoclonal antibody, cryoablation, and intra-tumor injection of autologous dendritic cell vaccine.

2.22 Secondary

2.221 Evaluate the PR and CR rate of this combination immunotherapy.

2.222 Evaluate the progression free survival, treatment free survival, duration of response, disease-free rate at 2 years, and overall survival of this combination immunotherapy.

2.223 Evaluate the safety of this combination immunotherapy.

2.23 Correlative Studies

2.231 Assess the effect of combination immunotherapy on patients' immune status and anti-tumor immune response.

2.232 Assess the potential association between PD-1/PD-L1/PD-L2 expression in tumor and blood with clinical efficacy.

2.233 Assess the potential association between tumor antigen mutations and antigen-specific immune response with clinical efficacy.

2.234 Evaluate patient quality of life.

3.0 Patient Eligibility

3.1 Inclusion Criteria

3.11 Age ≥18 years.

3.12 Histological confirmation of biopsy-proven non-Hodgkin lymphoma, *excluding* chronic lymphocytic leukemia, primary CNS lymphoma and Burkitt's lymphoma.
Note: Small lymphocytic lymphoma (SLL) is allowed.

3.13 Patients with indolent NHL must have had ≥1 regimen of rituximab-containing regimen.
Note: This includes FL, marginal lymphoma and MALT.

3.14 Patients with aggressive NHL must have had ≥2 regimens.
Note: This includes DLBCL, MCL, PMBCL, and T cell lymphoma.

Patients with aggressive NHL must have received prior therapy – at a minimum:
- Anti-CD20 monoclonal antibody unless tumor is CD20 negative and
- An anthracycline containing regimen.
- Transformed FL must have had therapy for FL and be refractory to chemotherapy for DLBCL.

Chemotherapy refractory disease in aggressive NHL is defined as
- Stable disease of ≤12 months or progressive disease as best response to most recent chemotherapy containing regimen.
- Disease progression or recurrence ≤12 months of prior autologous SCT.

Patients with aggressive NHL must have failed autologous HSCT, or are ineligible or not consenting to autologous HSCT.

3.15 Patient must have at least 3 measurable lesions that are ≥1.5 cm in one dimension. One of the lesions must be ≥2.0 cm and is amenable to image-guided cryoablation and multiple vaccine injections as determined by Interventional Radiology and PI (including tumors that can be safely accessed using imaging guidance and treated with minimal risk to adjacent structures).

3.16 ECOG Performance Status (PS) 0 or 1(Appendix I).

3.17 The following laboratory values obtained ≤14 days prior to registration.
- Absolute neutrophil count (ANC) ≥1000/mm3
- Absolute lymphocyte count ≥200/mm3
- Platelet count ≥50,000/mm3
- Hemoglobin ≥8.0 g/dL
- Total bilirubin ≤1.5 x upper limit of normal (ULN), unless due to Gilbert's disease.
- Aspartate transaminase (AST/SGOT) and alanine transaminase (ALT/SGPT) ≤2.5 x ULN
- Creatinine ≤1.5 x ULN or calculated creatinine clearance ≥60 mL/min for subject with creatinine>1.5 x institutional ULN

3.18 Negative serum pregnancy test for women of childbearing potential ≤7 days prior to registration.
Note: A second pregnancy test may be required ≤72 hours prior to receiving the first dose of study medication.

3.19a Negative human immunodeficiency virus (HIV), hepatitis B and C, and tuberculosis (TB) test.

3.19b Provide written informed consent.

3.19c Willing to return to the enrolling institution for follow-up (during active treatment and active monitoring phase of the study).

3.19d Ability to complete questionnaire(s) by themselves or with assistance.

3.19e Willing to provide tissue and blood samples for research purposes (see Sections 6.0, 14.0 and 17.0).

3.19f Willing to use adequate contraception as defined in Section 9.0 while on the study and until 120 days after the last dose of study drug.

3.2 Exclusion Criteria

3.21 Any of the following because this study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown:
- Pregnant women
- Nursing women

3.22 Co-morbid systemic illnesses or other severe concurrent disease which, in the judgment of the investigator, would make the patient inappropriate for entry into this study or interfere significantly with the proper assessment of safety and toxicity of the prescribed regimens.

3.23 Serious non-malignant disease such as active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations or other conditions which in the opinion of the investigator would compromise protocol objectives.

3.24 Currently receiving or have received any other **investigational** agent considered as a treatment for the primary neoplasm ≤28 days or within 4 half-lives (whichever is shorter) of the agent prior to registration.

3.25 History of other primary malignancy requiring systemic treatment within 6 months of protocol enrollment.
Patients must not be receiving chemotherapy or immunotherapy for another cancer. Patients must not have another active malignancy requiring active treatment with the following acceptable EXCEPTIONS:
- Basal cell carcinoma, squamous cell carcinoma, or melanoma of the skin that has undergone or will undergo potentially curative therapy
- In situ cervical cancer that has undergone or will undergo potentially curative therapy

3.26 Prior allogeneic bone marrow or peripheral blood stem cell transplantation.

3.27 Prior autologous bone marrow or peripheral blood stem cell transplantation ≤100 days prior to registration or if recovery from the transplant is inadequate.

3.28 Major surgery other than diagnostic surgery ≤4 weeks prior to registration.

3.29a Prior chemotherapy or radiation therapy ≤2 weeks prior to registration or who has not recovered (i.e. to ≤Grade 1 or baseline) from an adverse event due to the previously administered therapy.

3.29b History of hypersensitivity and anaphylactoid reactions to pneumococcal vaccine or any component of the formulation, including diphtheria toxoid.

3.29c Active autoimmune disease such as Crohn's disease, rheumatoid arthritis, Sjögrens' disease, systemic lupus erythematosis, or similar conditions requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease/syndrome difficult to control in the past.
EXCEPTIONS:

- Vitiligo or resolved childhood asthma/atopy
- Intermittent use of bronchodilators or local steroid injections
- Hypothyroidism stable on hormone replacement,
- Diabetes stable with current management
- History of positive Coombs test but no evidence of hemolysis
- Psoriasis not requiring systemic treatment
- Conditions not expected to recur in the absence of an external trigger

3.29d Coagulopathy, including the use of Coumadin or heparin anticoagulants that cannot be discontinued for the cryoablation procedure. NOTE: Heparin for line patency without detectable lab abnormalities for coagulation will be allowed.

3.29e Corticosteroid use ≤2 weeks prior to registration.
NOTE: Patients must be off corticosteroids for at least 2 weeks prior to registration. This includes oral, IV, subcutaneous, or inhaled route of administration. Patients on chronic corticosteroid for adrenal insufficiency or other reasons may enroll if they receive less than 10 mg/day of prednisone (or equivalent).

3.29f Active CNS malignancy.

3.29g Evidence of interstitial lung disease or active, non-infectious pneumonitis.

3.29h Received a live vaccine ≤30 days prior to registration.

3.29i New York Heart Association Classification III or IV cardiovascular disease (Appendix II) or recent myocardial infarction or unstable angina pectoris or cardiac arrhythmia ≤30 days prior to registration.

4.0 Test Schedule

4.1 Test Schedule: Phase I Dose Level 1 and Phase II (Phase I closed to accrual)

		Active Monitoring								
	Baseline	Initial Treatment or Retreatment[18]								
		Cycle 1	Cycle 2[10] & 3[10]				Cycle 4[10] & 5[10]		Cycles 6 – 18	Optional Pembro Year Day 1,
Tests and procedures	**≤14 days prior to Registratio**	**Day 1**	**Day 1**	**Day 2**	**Day 8**	**Day 15**	**Day 1**	**Day 2**	**Day 1**	
			±2 days*	±2 days	±2 days	±2 days	±2 days	±2 days	±2 days	±2 days
Physical exam and PS	X		X				X		X[16]	X[16]
Limited exam for AE assessment (as of Add 5) • Can be done with RN/APP					X	X				
Serum pregnancy test	X[1]									
HIV, hepatitis B and C, Quantiferon TB test	X									
Pembrolizumab administration[R]		X[7]	X				X		X	X
Cryoablation[R]				X[14]						
Leukapheresis and DC manufacturing[R]		X[7]								
Cellular Vaccine (DC) administration[R]				X[2]	X[2]	X[2]		X[2]		
Prevnar administration				X[2]				X[2]		
Chemistry Profile (Creatinine, T. Bili, AST, ALT, Alk Phos)	X	X	X				X		X	X
β2microglobulin (follicular only)	X									
Thyroid function cascade (TSH, reflex to T3, T4 if abnormal)	X		X				X		X	X
LDH	X						X[15]		X[15]	X[19]
Coagulation (INR)	X									
Hematology (ALC, WBC with differential, Hgb, PLT)	X	X[12]	X				X		X	X
Adverse Event Assessment	X		X	X	x	x	X	X	X	X

Tests and procedures	Baseline	Active Monitoring								
		Initial Treatment or Retreatment[18]								
		Cycle 1	Cycle 2[10] & 3[10]				Cycle 4[10] & 5[10]		Cycles 6 – 18	Optional Pembro Year Day 1,
	≤14 days prior to Registratio	Day 1	Day 1	Day 2	Day 8	Day 15	Day 1	Day 2	Day 1	
			±2 days*	±2 days	±2 days	±2 days	±2 days	±2 days	±2 days	±2 days
Radiologic evaluations (CT Chest, abdomen and pelvis, neck if clinically indicated, or PET/CT)	X[5]	X[8]					X[9]		X[9]	X[19]
Research blood for Immune Monitoring[R]		X[7]	X[6]				X[6]		X[6]	X[19]
Tumor biopsies[3,R]		X[3,4,13]							X[3,4]	
FACT-Lym	X		X[6]				X[6]		X[6]	X[19]

NOTE: All tests, and procedures and QOL indicated to be conducted on Day 1 of any cycle should be done PRIOR to treatment on that cycle
NOTE: For this study, 1 week is equal to 7 days and 1 month is equal to 28 days. Once cycle is equal to 3 weeks or 21 days
NOTE: If a patient goes off treatment for any reason prior to cycle 18, the patient will follow schedule 4.3 or 4.4 (see Section 13.0)
NOTE: Decision for optional additional year of Pembro is determined based on clinical response and approval by PI and sponsor.

***Cycle 2 Day 1 window ±4 days**

1. For women of childbearing potential only. Must be done ≤7 days prior to registration. Pregnancy testing will be repeated if patient qualifies for re-treatment.
2. If the previously cryoablated lesion no longer appears viable by imaging, DC will be injected into the previously treated region. Prevnar will be injected in the region of the first treated lesion. Patient will get DC until supply exhausted or maximum of 8 doses whichever comes first.
3. An index tumor for biopsy is identified as accessible via needle biopsy that is not the cryoablation site.
4. An index tumor lesion that is not the cryoablated or treated lesion may be biopsied if it is easily accessible or amenable to image-guided biopsy; Day 1 Cycle 1 (may be done any time prior to Day 1 Cycle 1), Day 1 Cycle 6, and Day 1 Cycle 19 (8 weeks post last dose of treatment) and at the time of disease progression. For the time point of Day 1 Cycle 1 and at time of disease progression, if excisional biopsy is done for diagnostic purposes, then remaining tissue after pathology review can be used for research studies.
5. Up to 28 days prior to registration.
6. Blood draw for immune monitoring and FACT-Lym should be collected prior to pembrolizumab administration, DC vaccine injection and Prevnar administration. Blood draw for immune monitoring and FACT-Lym during treatment period will be collected on Day 1 of Cycles 1,

2, 4, 6, 8, 12 and 18.

7. Cycle 1 Day 1 order: blood for immune monitoring, leukapheresis, and pembrolizumab administration.
8. CT or MRI does not need to be repeated if the baseline CT whole body or PET/CT is deemed adequate by study investigator.
9. Imaging performed during active treatment period on Day 1 of Cycles 4, 8, 12, and 18. All patients who received at least 2 cycles of treatments and do not have evidence of PD should be evaluated for formal clinical response.
10. If not enough DC, then proceed to Cycle 6.
11. Patients with residual or progressive disease may be retreated if deemed clinically feasible. See Section 7.12. These patients will follow the same test schedule for retreatment, starting with Day 1 Cycle 2.
12. If the baseline CBC with differential is more than 3 days from the date of leukapheresis, then a CBC with differential needs to be drawn at or within 3 days from leukapheresis.
13. If an index tumor is amenable for excisional biopsy at Cycle 1 Day 1, then excisional biopsy may be performed at the discretion of the investigator. In this case, a separate index tumor will be identified for subsequent research biopsies.
14. Cryoablation is performed on Day 2 of Cycle 2 only. If the previously identified tumor for cryoablation is decreased in size and can no longer be cryoablated, another tumor amenable to cryoablation will be identified for treatment. If no additional tumors can be identified for cryoablation, DC will be injected into the previously identified shrinking tumor.
15. LDH performed on Day 1 of Cycles 4, 6, 8, 12, and 18.
16. After Cycle 10, responsive or stable patients can be followed every other cycle by treating physician/provider until last cycle of treatment. However, patients will be followed by nurse at each cycle of treatment for adverse event assessment. They will be seen as needed by provider.
17. If subject thyroid function tests are within normal at the end of treatment and have no clinical signs and symptoms of thyroiditis, thyroid function test does not need to be performed during active monitoring. If thyroid function tests are abnormal during treatment, these tests need to be followed until they return to normal.
18. Retreatment starts at cycle 2.
19. Every 3 months.

R Research funded.

4.2 Test Schedule: Phase 1 Dose Level -1 (Use test schedule 4.1)

	Active Monitoring								
	Baseline	Initial Treatment or Retreatment[18]							
		Cycle 1	Cycle 2[10] & 3[10]			Cycle 4[10] - 7[10]		Cycles 8 – 18	Optional Pembro Year Day 1, every cycle
Tests and procedures	**≤14 days prior to Registration**	**Day 1**	**Day 1**	**Day 8**	**Day 15**	**Day 1**	**Day 8**	**Day 1**	
Window			±2 days*	±2 days	±2 days	±2 days	±2 days	±2 days	±2 days
Physical exam and PS	X	X	X	X		X		X[16]	X[16]
Serum pregnancy test	X[1]								
Pembrolizumab administration[R]		X[7]	X			X		X	X
Cryoablation[R]				X[14]					
Leukapheresis and DC manufacturing[R]		X[7]							
Cellular Vaccine (DC) administration[R]				X[2]	X[2]		X[2]		
Prevnar administration				X[2]			X[2]		
Chemistry Profile (Creatinine, T. Bili, AST, ALT, Alk Phos)	X	X	X			X		X	X
β2 microglobulin (follicular only)	X								
Thyroid function cascade (TSH, reflex to T3, T4 if abnormal)	X		X			X		X	X
LDH	X					X[15]		X[15]	X[19]
Coagulation (INR)	X								
Hematology (ALC, WBC with differential, Hgb, PLT)	X	X[12]	X			X		X	X
Adverse Event Assessment	**X**		X	X	X	X	X	X	X
Radiologic evaluations (CT whole body, or PET/CT)	X[5]	X[8]				X[9]		X[9]	X[19]
Research blood for Immune Monitoring[R]		X[7]	X[6]			X[6]		X[6]	X[19]

Tests and procedures	Active Monitoring								
	Baseline	Initial Treatment or Retreatment[18]							
	≤14 days prior to Registration	Cycle 1	Cycle 2[10] & 3[10]			Cycle 4[10] - 7[10]		Cycles 8 – 18	Optional Pembro Year Day 1, every cycle
		Day 1	Day 1	Day 8	Day 15	Day 1	Day 8	Day 1	
Window			±2 days*	±2 days	±2 days	±2 days	±2 days	±2 days	±2 days
Tumor biopsies[3, R]		X[3, 4, 13]						X[3, 4]	
FACT-Lym		X	X[6]			X[6]		X[6]	X[19]

NOTE: All tests, and procedures and QOL indicated to be conducted on Day 1 of any cycle should be done PRIOR to treatment on that cycle

NOTE: For this study, 1 week is equal to 7 days and 1 month is equal to 28 days. Once cycle is equal to 3 weeks or 21 days

NOTE: If a patient goes off treatment for any reason prior to Cycle 18, the patient will follow Schedule 4.3 or 4.4 (see Section 13.0)

NOTE: Decision for optional additional year of pembro is determined based on clinical response and approval by PI and sponsor.

***Cycle 2 Day 1 window ±4 days**

1. For women of childbearing potential only. Must be done ≤7 days prior to registration. Pregnancy testing will be repeated if patient qualifies for re-treatment.
2. If the previously cryoablated lesion no longer appears viable by imaging, DC will be injected into the previously treated region. Prevnar will be injected in the region of the first treated lesion. Patient will get DC until supply exhausted or maximum of 8 doses whichever comes first.
3. An index tumor for biopsy is identified as accessible via needle biopsy that is not the cryoablation site.
4. An index tumor lesion that is not the cryoablated or treated lesion may be biopsied if it is easily accessible or amenable to image-guided biopsy; Day 1 Cycle 1 (may be done any time prior to Day 1 Cycle 1), Day 1 Cycle 8, and Day 1 Cycle 19 (8 weeks post last dose of treatment) and at the time of disease progression. For the time point of Day 1 Cycle 1 and at time of disease progression, if excisional biopsy is done for diagnostic purposes, then remaining tissue after pathology review can be used for research studies.
5. ≤28 days prior to registration.
6. Blood draw for immune monitoring and FACT-Lym should be collected prior to pembrolizumab DC injection and Prevnar administration. Blood draw for immune monitoring and FACT-Lym during treatment period will be collected on Day 1 of Cycles 1, 2, 4, 8, 12 and 18.
7. Cycle 1 Day 1 order: blood for immune monitoring, leukapheresis, and pembrolizumab administration.
8. CT or MRI does not need to be repeated if the baseline CT whole body or PET/CT is deemed adequate by study investigator.
9. Imaging performed during active treatment period on Day 1 of cycles 4, 8, 12, and 18. All patients who received at least 2 cycles of treatments and do not have evidence of PD should be evaluated for formal clinical response.

10. If not enough DC, then proceed to cycle 8.
11. Patients with residual or progressive disease may be retreated if deemed clinically feasible. See Section 7.12. These patients will follow the same test schedule for retreatment, starting with Day 1 Cycle 2.
12. If the baseline CBC with differential is more than 3 days from the date of leukapheresis, then a CBC with differential needs to be drawn at or within 3 days from leukapheresis.
13. If an index tumor is amenable for excisional biopsy at Cycle 1 Day 1, then excisional biopsy may be performed at the discretion of the investigator. In this case, a separate index tumor will be identified for subsequent research biopsies.
14. Cryoablation is performed on Day 8 of Cycle 2 only. If the previously identified tumor for cryoablation is decreased in size and can no longer be cryoablated, another tumor amenable to cryoablation will be identified for treatment. If no additional tumors can be identified for cryoablation, DC will be injected into the previously identified shrinking tumor.
15. LDH performed on Day 1 of Cycles 4, 8, 12, and 18.
16. After Cycle 10, responsive or stable patients can be followed every other cycle by treating physician/provider until last cycle of treatment. However, patients will be followed by nurse at each cycle of treatment for adverse event assessment. They will be seen as needed by provider.
17. If subject thyroid function tests are within normal at the end of treatment and have no clinical signs and symptoms of thyroiditis, thyroid function test does not need to be performed during active monitoring. If thyroid function tests are abnormal during treatment, these tests need to be followed until they return to normal.
18. Retreatment starts at cycle 2.
19. Every 3 months.

R Research funded.

4.3 Test Schedule for Observation for patients who do not complete initial active treatment for any reason or do not have dendritic cells for retreatment.

Tests and procedures	Active Monitoring	
	Observation[3]	
	Day 1 of each observation cycle Every 3 months for 1 year (start at Week 62 or 8 weeks after last dose whichever is earlier)	Documented PD at any time during this protocol treatment or observation, prior to entering event monitoring, at End of Observation
Physical exam and PS	X	X
Serum pregnancy test		
Pembrolizumab (MK-3475) administration[R]		
Cryoablation[R]		
Leukapheresis and DC manufacturing[R]		
Cellular Vaccine (DC) administration[R]		
Prevnar administration		
Chemistry Profile (Creatinine, TBili, AST, ALT, Alk Phos)	X	X
Thyroid function cascade	X[4]	
LDH	X	X
Coagulation (INR)		
Hematology (ALC, WBC with differential, Hgb, PLT)	X	X
Adverse Event Assessment	X	X
Radiologic evaluations (CT whole body, or PET/CT)	X	X
Research blood for Immune Monitoring[R]	X	X
Tumor biopsies[1, R]	X[1, 2]	X[1, 2]
FACT-Lym	X	X

1. An index tumor for biopsy is identified as accessible via needle biopsy that is not the cryoablation site.
2. An index tumor lesion that is not the cryoablated or treated lesion may be biopsied if it is easily accessible or amenable to image-guided biopsy; Day 1 Cycle 1 (may be done any time prior to Day 1 Cycle 1), Day 1 Cycle 8, and Day 1 Cycle 19 (8 weeks post last dose of treatment) and at the time of disease progression. For the time point of Day 1 Cycle 1 and at time of disease progression, if excisional

biopsy is done for diagnostic purposes, then remaining tissue after pathology review can be used for research studies.

3. Patients with residual or progressive disease may be retreated if deemed clinically feasible. See Section 7.12. These patients will follow the same test schedule for retreatment, starting with Day 1, Cycle 2.
4. If subject thyroid function tests are within normal at the end of treatment and have no clinical signs and symptoms of thyroiditis, thyroid function test does not need to be performed during active monitoring. If thyroid function tests are abnormal during treatment, these tests need to be followed until they return to normal.

R Research funded.

4.4 Test Schedule for Event Monitoring for patients who refuse additional treatment or observation for any reason

	Event Monitoring Phase[1]				
CRF	q. 3 months during Yr 1 post-treatment; q. 4 months during Yr 2 post- treatment; q 6 months during subsequent yrs up to 4 yrs post registration until PD	At PD[2]	After PD q. 6 months until 4 yrs post registration	Death	New Primary
Event Monitoring	X[2]	X[2]	X	X	At each occurrence

1. If a patient is still alive 4 years after registration, no further follow-up is required.
2. Submit copy of documentation of response or progression in the Supporting Documentation form in Medidata Rave.

4.5 Test Schedule for Treatment Observation for patients who have dendritic cells for retreatment

Tests and procedures	Treatment Observation		
	Day 1 of each cycle Every 3 months for 1 year post treatment(start at Week 62 or 8 weeks after last dose whichever is earlier)	**Day 1 of each cycle (Every 4 months during second year post treatment, every 6 months thereafter until 4 yr post registration)**	**Documented PD at any time during treatment or observation, prior to entering event monitoring, at End of Observation**
Physical exam and PS	X		X
Serum pregnancy test			
Pembrolizumab (MK-3475) administration[R]			
Cryoablation[R]			
Leukapheresis and DC manufacturing[R]			
Cellular Vaccine (DC) administration[R]			
Prevnar administration			
Chemistry Profile (Creatinine, TBili, AST, ALT, Alk Phos)	X		X
Thyroid function cascade	X[3]		
LDH	X		X
Coagulation (INR)			
Hematology (ALC, WBC with differential, Hgb, PLT)	X		X
Adverse Event Assessment	X		X
Radiologic evaluations (CT whole body, or PET/CT)	X		X
Research blood for Immune Monitoring[R]	X		X
Tumor biopsies [1, R]	X[1, 2]		X[1,2]
FACT-Lym	X		X

Tests and procedures	Treatment Observation		
	Day 1 of each cycle Every 3 months for 1 year post treatment(start at Week 62 or 8 weeks after last dose whichever is earlier)	**Day 1 of each cycle (Every 4 months during second year post treatment, every 6 months thereafter until 4 yr post registration)**	**Documented PD at any time during treatment or observation, prior to entering event monitoring, at End of Observation**
Event Monitoring Form*		X	

* Also complete at PD, every 6 months post PD until 4 yr post registration, death and at each new occurrence of primary up to 4 years post registration. If a patient is still alive 4 years after registration, no further follow-up is required. Submit copy of documentation of response or progression in the Supporting Documentation form in Medidata Rave.

1. An index tumor for biopsy is identified as accessible via needle biopsy that is not the cryoablation site.
2. An index tumor lesion that is not the cryoablated or treated lesion may be biopsied if it is easily accessible or amenable to image-guided biopsy; Day 1 Cycle 1 (may be done any time prior to Day 1 Cycle 1), Day 1 Cycle 8, and Day 1 Cycle 19 (8 weeks post last dose of treatment) and at the time of disease progression. For the time point of Day 1 Cycle 1 and at time of disease progression, if excisional biopsy is done for diagnostic purposes, then remaining tissue after pathology review can be used for research studies.
3. If subject thyroid function tests are within normal at the end of treatment and have no clinical signs and symptoms of thyroiditis, thyroid function test does not need to be performed during active monitoring. If thyroid function tests are abnormal during treatment, these tests need to be followed until they return to normal.

R Research funded.

4.6 Test Schedule: Phase II

Test schedule for Phase II will follow Phase I dose level 1 (See Section 4.1).

5.0 Grouping Factor:

5.1 Phase: I vs. II

5.2 Dose Level (Phase I only) *(As assigned by MCCC Registration Office*): 1 vs -1

6.0 Registration Procedures

6.1 Phase I –Closed to accrual July 9, 2018

Prior to discussing this protocol with a patient, call the Registration Office [redacted] for dose level and to ensure there is a place on the study for the patient.

Registration Procedures

To register a patient, fax [redacted] a completed eligibility checklist to the Mayo Clinic Cancer Center (MCCC) Registration Office between 8 a.m. and 4:30 p.m. central time Monday through Friday.

6.2 Phase II – Opened to accrual July 9, 2018

Registration Procedures

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the registration/randomization application. The registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for the registration/randomization application are available on the MCCC web page [redacted] and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration/randomization application can be confirmed in any of the following ways:

- Contact the MCCC Registration Office [redacted] If the patient was fully registered, the MCCC Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.3 Phase I and Phase II

6.31 Correlative Research

A mandatory correlative research component is part of this study; the patient will be automatically registered onto this component (see Sections 3.19d, 14.1, and 17.1 for required research samples).

6.32 Verification

Prior to accepting the registration, the registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.33 Documentation

Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office [redacted] If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.34 At the time of registration, the following will also be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future research of lymphoma at Mayo.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems.
- Patient has/has not given permission to give his/her sample(s) to researchers at other institutions.

6.35 Treatment on this protocol must commence at the Mayo Clinic Rochester under the supervision of a hematologist.

6.36 Treatment cannot begin prior to registration and must begin ≤21 days after registration.

6.37 Pretreatment tests / procedures must be completed within the guidelines specified on the test schedule (see Section 4.0).

6.38 All required baseline symptoms (see Section 10.6) must be documented and graded.

6.39a Study drug is available on site.

6.39b Patient questionnaire booklet is available on site; copies are not acceptable for this submission.

7.0 Protocol Treatment

7.1 Phase I Treatment Schedule (Closed to accrual July 9, 2018

The tolerated dose of this regimen in the phase I portion of this study will be used to treat patients enrolled in the phase II portion of this study. Patients will be enrolled on dose level 1 and if the regimen is intolerable, patients will be enrolled on dose level -1.

Phase II Treatment Schedule (Opened to accrual July 9, 2018)

7.11 Phase I dose level 1 and Phase II

7.111 Dose level 1 schedule

Agent	Dose Level	Number of doses	Route	Cycles[1]
Pembrolizumab	200 mg	18	IV	1-18 (Day 1)
DC	not less than 30 and no more than 60 million DC[3]	8	Injection to cryoablated tumor	2[2] (Day 2, 8, 15) 3 (Day 2, 8, 15) 4, 5 (Day 2)
Cryoablation				2 (Day 2)
Prevnar13	0.5 mL	4	Injection near cryoablated tumor	2[2] (Day 2), 3, 4, 5 (Day 2)

Cycle Definitions: 1 week = 7 days; 1 month = 28 days; 1 cycle = 21 days.
1 All timepoints are ±2 days
2 The day of cryoablation
3 Depending on availability after manufacturing

7.112 Phase I dose level 1 schedule Treatment by Cycle

Cycle	Duration	Timepoint	Day
Cycle 1	3 weeks	Week 1 to Week 3	Day 1: Leukapheresis followed by pembrolizumab (DC manufacturing in lab)
Cycle 2	3 weeks	Week 4 to Week 6	Day 1: Pembrolizumab Day 2: Cryoablation[2] of tumor followed by intra-tumor injection of DC and injection of Prevnar13 in nearby region Day 8: DC as per section 7.115[1] Day 15: DC as per section 7.115[1]
Cycle 3	3 weeks	Week 7 to Week 9	Day 1: Pembrolizumab Day 2: Prevnar13, DC per Section 7.115[1] Day 8: DC per Section 7.115[1] Day 15: DC per Section 7.115[1]

Cycle	Duration	Timepoint	Day
Cycles 4 - 5	3 weeks		Day 1: Pembrolizumab Day 2: Prevnar13, DC as per Section 7.3[1]
Cycles 6 - 18	3 weeks		Day 1: Pembrolizumab Tumor biopsy on Day 1 of Cycle 6 only

1. If supply exhausted, start Cycle 6 of pembrolizumab.
2. If the previously identified tumor for cryoablation is decreased in size and can no longer be cryoablated, another tumor amenable to cryoablation will be identified for treatment. If no additional tumors can be identified for cryoablation, DC will be injected into the previously identified shrinking tumor.

7.113 Pembrolizumab, monoclonal anti-PD1 antibody

Patients will receive pembrolizumab at 200 mg IV every 3 weeks for 18 cycles. If patient experienced adverse events requiring dosing delay (see Section 8), the new dosing interval (increased interval) can be defined as the new cycle length. Patients who achieved CR before Cycle 5 will complete treatments through cycle 6 and can then stop treatment at the discretion of the treating provider and investigators. Patients who achieved CR at or after cycle 5 could continue treatments for 2 more cycles and stop treatment at the discretion of the treating provider. The designed maximum length of treatment is 12 months (365 days). If the patient is continuing to benefit from treatment after 12 months, the patient may continue to receive treatment up to 24 months at investigator/sponsor discretion.

7.114 Pneumococcal vaccination

Patients will have four pneumococcal vaccinations (Prevnar13®) in dose 1 (phase I dose level 1schedule) and six pneumococcal vaccinations (Prevnar13®) in dose -1 (phase I dose level -1 schedule). These injections will be concurrent with the first DC vaccine administration at each cycle (Cycles 2 - 5 for phase I dose level 1 and Cycles 2 - 7 for phase I dose level -1). The will be administered near the region of DC injection.

7.115 Cellular vaccines (DC)

For DC manufacturing, patients will undergo leukapheresis from which monocytes will be collected by immunomagnetic isolation and cultured *in vitro* under GMP condition to generate mature DC.

Autologous mature dendritic cells (DC) will be prepared for administration into cryoablated tumor. DC, the generated mature DC, will be frozen and stored until ready for use. A minimum of $30x10^6$ and a maximum of $60x10^6$ mature DC will be used for each injection. Minimum DC necessary for 16 injections will be manufactured. Additional DC and lysate will be stored for immune monitoring assays and potential additional vaccination.

7.116 DC Administration

Each patient will undergo percutaneous cryoablation of a single lymphoma tumor as specified by the PI and interventional radiologist.

Our general cryoablation technique has been previously described (Atwell, Farrell et al. 2008). Due to the extended length of the procedure, requiring the patient to lie still (expected duration 45 minutes), sedation will be managed by the Department of Anesthesia. Using ultrasound and/or CT guidance, a cryoprobe will be placed in the index lymphoma node. The node will be treated using a conventional freeze-thaw-freeze cycle with the endpoint being the generation of an iceball that measures no more than 75% of the diameter of the index tumor. Specifically, incomplete treatment of the lymphoma treatment lesion will significantly minimize the risk of thermal injury to adjacent structures, yet still achieve the desired cytotoxic cell injury.

Following the freezing procedure, the cryoprobe will be actively warmed and then withdrawn. After the node has thawed, a 22 G needle will be placed under ultrasound or CT-guidance into the ablated portion of the node. The DC will be administered through this needle in 10 – 15 divided injections over different areas of the cryoablated region and then flushed with 1ml of sterile saline. Prevnar will be injected into the immediately adjacent soft tissues of the cryoablated lesion. The patient will be transferred to the radiology recovery area and observed until discharge criteria are met prior to dismissal from the hospital (minimum 2 hours).

Doses 2-8. Patients will receive seven additional doses of DC injected into the cryoablated lesion using ultrasound, CT or MRI guidance. Prevnar will be injected in the region immediately adjacent to the previously cryoablated lesion.

Patients will be monitored for 2 hours following cryoablation and DC injections or 30 minutes following only DC injections for acute toxicity. Known potential toxicity of DC injection includes dermatitis, anaphylaxis, allergic reactions such as bronchospasm or generalized urticaria, autoimmune reactions, bone pain, myalgia/arthralgia, reaction at the site of injection and renal dysfunction.

7.12 Phase I dose level -1 (De-Escalation Schedule) (closed to accrual July 9, 2018)

7.121 Phase I Dose level -1 Schedule

Agent	Dose Level	Number of doses	Route	Cycles[1]
Pembrolizumab	200 mg	18	IV	1-18, Day 1
DC	not less than 30 and no more than 60 million DC	8	Injection into Cryoablated tumor	2[2](Day 8, 15), 3 (Day 8, 15), 4, 5, 6, 7 (Day 8)
Prevnar13	0.5 mL	6	Injection near cryoablated tumor	2[2](Day 8), 3-7 (Day 8),

1 All time points are ±2 days
2 The day of cryoablation

7.122 Phase I dose level -1 Schedule Treatment by Cycle

Cycle	Duration	Timepoint	Day
Cycle 1	3 weeks	Week 1 to Week 3	Day 1: Leukapheresis followed by pembrolizumab (DC manufacturing in lab) Tumor biopsy
Cycle 2	3 weeks	Week 4 to Week 6	Day 1: Pembrolizumab Day 8: Cryoablation[2] of tumor followed by intra-tumor injection of DC vaccine and injection of Prevnar13 in nearby region Day 15: DC per Section 7.115[1]
Cycle 3	3 weeks	Week 7 to Week 9	Day 1: Pembrolizumab Day 8: Prevnar13, DC per Section 7.115[1] Day 15: DC vaccine per Section 7.115[1]
Cycle 4 - 7	3 weeks		Day 1: Pembrolizumab Day 8: Prevnar13, DC per Section 7.115[1]
Cycles 8 - 18	3 weeks		Day 1: Pembrolizumab Tumor biopsy on Day 1 of Cycle 8 only

1. If supply exhausted, start Cycle 8 of pembrolizumab.
2. If the previously identified tumor for cryoablation is decreased in size and can no longer be cryoablated, another tumor amenable to cryoablation will be identified for treatment. If no additional tumors can be identified for cryoablation, DC will be injected into the previously identified shrinking tumor.

7.13 Treatment by a local medical doctor is not allowed.

7.14 Dosing plan

Cohorts of 3 patients will be treated at a dose level at a time (see Section 16.0). The study will temporarily close between patient cohorts . Doses will not be escalated in any individual patient.

7.15 Duration of treatment

Duration of treatment is a maximum of 12 months from start of Cycle 1. If a patient remains on cycles with a length of 21 days without treatment delays, this will be 18 cycles total. If the cycle length is increased due to dosing frequency changes or dose delays, the number of cycles will be reduced to not exceed 12 months of treatment total. If a patient is continuing to benefit from treatment after 12 months, he or she may continue to receive pembrolizumab every 3 weeks for up to 24 months total from start of cycle 1 at the investigator/sponsor discretion. If a patient achieved CR before Cycle 5, the patient will continue through Cycle 6 and could stop at the discretion of the treating provider and investigator. If a patient achieved CR at or after Cycle 5, the patient could continue treatment for 2 more cycles at the discretion of treating provider.

7.16 Dose Limiting Criteria (DLT)

For this protocol, DLT will be defined as an adverse event attributed (definitely, probably, or possibly) to the study treatment during the first two cycles of treatment AND meeting the following criteria.

CTCAE System Organ Class	**DLT Definition (CTCAE v4)**
Investigations Neutrophil count decreased Platelet count decreased	≥Grade 4 ANC or PLT for ≥7 days
Infections and infestations Other, specify	Any ≥ Grade 3
Blood and lymphatic system disorders Febrile neutropenia	Defined as fever ≥38.5ₒC (38 >1 hour) with grade ≥4 neutropenia
Skin and subcutaneous tissue disorders Erythema multiforme Skin ulceration Urticaria	≥ Grade 3 erythema multiforme, ulceration, or urticaria that does not resolve to <Grade 2 within three weeks
Respiratory, thoracic and mediastinal disorders Bronchial obstruction Pneumonitis Wheezing	≥Grade 3 bronchial obstruction, pneumonitis, or wheezing
Immune system disorders Allergic reaction Autoimmune disorder	≥Grade 3 allergic reaction or autoimmunity
All Other Non-Hematologic	≥Grade 3 that does not resolve to < Grade 2 ≤ 72 hours per NCI Common Terminology Criteria for Adverse Events

Note: any adverse event that leads to a permanent discontinuation of the study agent is considered a DLT.

7.17 Dose levels

The dose level of pembrolizumab is fixed at 200 mg IV on Day 1 of every 21 days. The DLT criteria will be used to determine the timing of the subsequent administration of pembrolizumab, not for adjustment of the dose level. The dose interval may be increased by 7 days each time, the maximum dosing interval allowed is 8 weeks.

The study involves DC injection at only one dose level, but has potentially multiple injections over time. The DLT criteria will be used to determine timing of the subsequent administration of DC, and not for adjustment of DC dose level.

Note: This study will not allow dose reduction, only dose interval changes.

7.2 Patients experiencing a DLT

Investigators are to contact the Study Chair as soon as any DLT occurs. Patients who experience DLT will go to observation.

7.3 Continued pembrolizumab treatment

Patients may continue on pembrolizumab treatment for 1 year after initial treatment is terminated (please check with Study Chair).

7.4 Retreatment

Patients who qualify for retreatment (see Section 13.0) will not change dose levels and will follow the original treatment schedule starting at cycle 2. When retreating, new baseline disease measurement will be established. The new baseline disease measurement will be the disease measurement at the end of the cycle prior to the retreatment. Patients may receive all DC according to schedule as long as they do not have continued progression of disease, develop major adverse events, and have DC available.
Patients will not undergo any additional leukapheresis for the purpose of manufacturing additional DC.

7.5 Phase II

Treatment schedule for phase II will be defined as the treatment regimen from the MTD dose schedule in phase I.
NOTE: Phase II's dose schedule will follow Phase I Dose Level I (See Section 7.1)

8.0 Dosage Modification Based on Adverse Events

8.1 Treatment schedule modifications in patients based on adverse events

8.11 Patients will be evaluated by the study team prior to each dose of DC for an adverse event check. Determination will be made if the adverse event is treatment related (possible, probable or definite), i.e. a toxicity (see Section 10; Adverse event monitoring and reporting). If any adverse event has occurred, then the treatment schedule will be modified as in 8.12, 8.13, or 8.2. For cycles involving only pembrolizumab treatments (Cycles 1 and 6-18 for dose level 1 and Cycles 1 and 8-18 for dose level -1), refer to Section 8.2.

8.12 For Grade 1/2 adverse event during cycles with DC injections (Cycles 2 – 5 for dose level 1 and Cycles 2 – 7 for dose level -1): We will allow a delay in DC treatment up to four weeks for dermatological AEs ≤Grade 2; for all other Grade 1/2 adverse events not listed in Table 7.16 or 8.2, no change in procedure is required or expected.

8.13 For Grade 2/3/4 adverse events (Grade 2/3/4 described in Table 8.2 or

any grade 3/4), pembrolizumab dose schedule will be modified as described in Table 8.2. DC and Prevnar13 injections for the cycle in which pembrolizumab are delayed will also be delayed and resumed when pembrolizumab resumption criteria has been met. For a grade 2/3/4 adverse event per Table 7.16 that is attributed to treatment and does not resolve within two weeks, if the patient has not completed all treatments, he or she will not receive any further treatment on this trial. The patient will go to observation phase.

8.2 Treatment schedule modifications for pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs per Table 8.21 below. See Section 9.0 for supportive care guidelines, including use of corticosteroids.

Table 8.21 Dose Modification Guidelines for Drug-Related Adverse Events for Pembrolizumab

CTCAE System/Organ/Class (SOC)	Adverse Event	Hold Treatment for Grade	Timing for Restarting Treatment	Treatment Discontinuation
Gastrointestinal disorders	Diarrhea or Colitis	2-3	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		4	Permanently discontinue	Permanently discontinue
Investigations	AST, or ALT, or Blood bilirubin	2	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose.
		3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Metabolism and nutrition disorders	Glucose intolerance (Type 1 diabetes mellitus [if new onset]) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable.
Endocrine disorders	Endocrine disorders – Other, specify: Hypophysitis	2-4	AE resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks

CTCAE System/Organ/Class (SOC)	Adverse Event	Hold Treatment for Grade	Timing for Restarting Treatment	Treatment Discontinuation
Endocrine disorders	Hyperthyroidism	3	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		4	Permanently discontinue	Permanently discontinue
Endocrine disorders	Hypothyroidism	2	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
General disorders and administration site conditions	Infusion related reaction	2[b]	AE resolves to Grade 0-1	Permanently discontinue if AE develops despite adequate premedication
		3-4	Permanently discontinue	Permanently discontinue
Respiratory, thoracic and mediastinal disorders	Pneumonitis	2	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		3-4	Permanently discontinue	Permanently discontinue
Renal and urinary disorders	Acute kidney injury or Chronic kidney disease (e g Renal failure or Nephritis)	2	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		3-4	Permanently discontinue	Permanently discontinue
	All Other Drug-Related Adverse Events[c]	3	AE resolves to Grade 0-1	AE does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
		4	Permanently discontinue	Permanently discontinue

CTCAE System/Organ/Class (SOC)	Adverse Event	Hold Treatment for Grade	Timing for Restarting Treatment	Treatment Discontinuation
Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event. [a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued. [b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. [c] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.				

Note: The term "interruption" has the same meaning as "delay".

8.21 Other instructions for pembrolizumab

If pembrolizumab-related toxicity does not resolve to Grade 0-1 within 12 weeks after last administration of study drug, study therapy discontinuation is recommended. With Investigator agreement, patients with a laboratory adverse event still at Grade 2 may continue in the study only if asymptomatic and controlled.

In patients who continue on study therapy after experiencing an Adverse Event warranting potential dose modification, if considered drug-related by the investigator, once the patient has recovered to Grade 0-1 the dosing interval in subsequent cycles will be increased by 1 week (e.g., to 3 weeks in patients who were on an every 2-week schedule). Following each such dose delay due to toxicity, the dosing interval should increase by an additional week. For example, patients who began the study on a 3-week dosing schedule, and have stopped drug twice for due to a drug-related toxicity that meets the above criteria, should now be dosing every 5 weeks.

For patients who experience a recurrence of the same severe AEs listed above with rechallenge of pembrolizumab, a consultation with the Investigator will occur to determine whether the patient should continue in the study. A patient who experiences the same SAE of the same NCI CTCAE grade or higher with rechallenge of pembrolizumab must discontinue pembrolizumab immediately.

Reduced dose of pembrolizumab dose (ie, below 200 mg) will not be administered.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Study Chair. The reason for interruption should be documented in the patient's study record.

Pembrolizumab (MK-3475) will be withheld for drug-related Grade 4 hematologic toxicities per table in 8.3, non-hematological toxicity ≥Grade 3 including uncontrolled laboratory abnormalities, and severe or life-threatening AEs per Tables in 8.2 and 8.3.

8.3 Dose Modification for Hematological Toxicities (Platelet and Hemoglobin only) for all agents

Note: hematological toxicity is not based on CTCAE 4.0 except neutrophil, but based on lymphoma specific hematological toxicity.

Dose modification guidelines for drug-related hematological (hemoglobin and platelets) adverse events [1, 2]

Toxicity	Grade [1, 2]	Decrease from pretreatment	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject (after consultation with PI)
NHL specific Hematological Toxicity	1	11-24% decrease in HGB or PLT	No	N/A	N/A	N/A
	2	25-49% decrease in HGB or PLT				
	3	50-74% decrease in HGB or PLT	Yes	Toxicity resolves to Grade 0-2 or baseline	May increase the dosing interval by 1 week	Toxicity does not resolve within 8 weeks of last infusion *Permanent discontinuation should be considered for any severe or life-threatening event.* Go to observation if ≥2 cycles of therapy were given, otherwise go to event monitoring
	4	≥75% decrease in HGB or PLT	Yes	Toxicity resolves to Grade 0-2 or baseline	May increase the dosing interval by 1 week	

[1] If, at any level of decrease from the baseline value the platelet and/or hemoglobin counts are within normal limits or platelet count is still ≥ 100,000/μL, this will be considered a grade 0.

[2] If patient has persistent cytopenia, it is recommended to repeat bone marrow evaluation to test if there is disease progression.

9.0 Ancillary Treatment/Supportive Care

9.1 Full Supportive Care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from within 28 days before the study treatment administration until 30 days after the final dose will be recorded in the medical records. **Leukocyte reduction of all blood products for patients on protocol is required. Any blood transfusions administered must be irradiated blood products to reduce the risk of transfusion mediated graft versus host disease in patients receiving T-cell modulating therapy.**

9.11 Antiemetics

Antiemetics may be used at the discretion of the treating physician.

9.12 NSAIDS

NSAIDs use limited to standard non-prescription doses.

9.13 Blood Products and Growth Factors

Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors should follow published guidelines of the American Society of Clinical Oncology; Update of Recommendations for the Use of Hematopoietic Colony-Stimulating Factors: Evidence- Based, Clinical Practice Guidelines. J Clin Oncol 24(19): 3187-3205, 2006.

9.2 Suggested supportive care measures for the management of adverse events with potential immunologic etiology

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 8.0 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Pneumonitis**:

- For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**:

  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
  - For **T1DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:
  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:

  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs

and symptoms of thyroid disorders.

- **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
  - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
  - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.
- **Grade 3-4** hyperthyroidism
  - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Management of Infusion Reactions**:
  Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

  Table 9.51 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 9.21 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS,	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines	Subject may be premedicated 1.5h (±30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	(or equivalent dose of antihistamine) Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic)
<u>Grades 3 or 4</u> Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

9.3 Diet/Activity/Other Considerations

9.31 Diet

Subjects should maintain a normal diet unless modifications are required

to manage an AE such as diarrhea, nausea or vomiting.

9.32 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);
OR
(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;
OR
(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence[†] from heterosexual activity;
OR
(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:
Single method (one of the following is acceptable):
- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):
- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous

contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

9.33 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above.

9.34 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

10.0 Adverse Event (AE) Reporting and Monitoring

The site principal investigator is responsible for reporting any/all serious adverse events to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor/sponsor-investigator is responsible for notifying FDA and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send:
All sites	Pregnancy Reporting [redacted]	Mayo Sites – attach to MCCC Electronic SAE Reporting Form Non Mayo sites – complete and forward to [redacted]
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form [redacted]	Will automatically be sent to [redacted]

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting

Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

Unanticipated Adverse Device Event (UADE)
Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: [redacted]

a. Identify the grade and severity of the event using the CTCAE version 4.0.
b. Determine whether the event is expected or unexpected (see Section 10.2).
c. Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
e. Determine if other reporting is required (see Section 10.5).
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. Unexpected Events

Expected events - are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or

available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.
An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.
NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite - The AE *is clearly related* to the agent(s)/procedure.
Probable - The AE *is likely related* to the agent(s)/procedure.
Possible - The AE *may be related* to the agent(s)/procedure.
Unlikely - The AE *is doubtfully related* to the agent(s)/procedure.
Unrelated - The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting

For this protocol only, the following Adverse Events/Grades are expected to occur within this population and do not require Expedited Reporting. These events must still be reported via Routine Reporting (see Section 10.6).*

*Report any clinically important increase in the rate of a serious suspected adverse reaction (at your study site) over that which is listed in the protocol or investigator brochure as an expedited event.

*Report an expected event that is greater in severity or specificity than expected as an expedited event.

CTCAE Category	Adverse Event	CTCAE Grade at which the event will not be reported in an expedited manner[1]
Blood and lymphatic system disorders	Anemia	≤Grade 4
Investigations	Platelet count decreased	≤Grade 4
	Neutrophil count decreased	≤Grade 4
	Lymphocyte count decreased	≤Grade 4
	White blood cell decreased	≤Grade 4

1 These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.

Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators ONLY if they exceed the expected grade of the event.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (*i.e.*, there is no untoward medical occurrence) associated with the hospitalization:
● Hospitalizations for respite care
● Planned hospitalizations required by the protocol
● Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
● Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial
● Hospitalization for administration of study drug or insertion of access for administration of study drug
● Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
● Hospitalization, or other serious outcomes for signs and symptoms of progression of the cancer.]

10.4 Expedited Reporting Requirements for IND/IDE Agents

10.41 Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the sponsor within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour
3 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥24 hrs</td><td colspan="2">Not required</td><td>7 Calendar Days</td></tr>
</table>

Expedited AE reporting timelines are defined as: o "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report. o "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.
[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows: **Expedited 24-hour notification followed by complete report within 3 calendar days for:** • All Grade 4, and Grade 5 AEs **Expedited 7 calendar day reports for:** • Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization • Grade 3 adverse events [2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period. Effective Date: May 5, 2011

NOTE: Refer to Section 10.31 for exceptions to Expedited Reporting

10.42 General reporting instructions

The Mayo IND Coordinator will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

Contact **Merck Global Safety facsimile number:** [redacted]

Use Mayo Expedited Event Report form [redacted] for investigational agents or commercial/investigational agents on the same arm.

Note: Submit MedWatch form 3500A to Merck & Co., Inc. (Attn: [redacted] and

Biocompatibles Inc., a BTG International group company at [redacted]

10.43 Reporting of re-occurring SAEs

ALL SERIOUS adverse events that meet the criteria outlined in table10.41 MUST be immediately reported to the sponsor within the timeframes detailed in the corresponding table. This reporting includes, but is not limited to SAEs that re-occur again after resolution.

10.5 Other Required Reporting

10.51 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the Reportable Event coversheet and appropriate documentation to [redacted]
The Mayo Regulatory Affairs Office will review and process the submission to the Mayo Clinic IRB.

10.52 Death

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.

- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Per NIH OBA Appendix M, should a patient die during the study or study follow-up, no matter what the cause, the study doctor will ask the patient's family for permission to perform an autopsy. If permission is granted, a copy of the autopsy report will be sent to the sponsor after all identifying information has been removed. An autopsy will help the researchers learn more about the safety and efficacy of the treatment. Patients should advise their families about their wishes regarding autopsy.

10.53 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND/IDE will be reported. Three options are available to describe the event:
  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy
- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.

10.55 Pregnancy, Fetal Death, and Death Neonatal

If a female subject (or female partner of a male subject) taking investigational product becomes pregnant, the subject taking should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her

infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form: [redacted]

10.551 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration - Other (neonatal loss)"** under the General disorders and administration SOC.

10.6 Required Routine Reporting

10.61 Baseline and Adverse Events Evaluations

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.
Grading is per CTCAE v4.0 **unless** alternate grading is indicated in the table below:

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Investigations	Platelet count decreased	X	X
	Neutrophil count decreased	X	X
Blood and lymphatic system disorders	Anemia	X	X
Gastrointestinal disorders	# of stools	X	
	Diarrhea		X
	Nausea	X	X
	Vomiting	X	X
Respiratory, thoracic and mediastinal disorders	Cough	X	X
	Dyspnea	X	X
	Pneumonitis	X	X
Skin and subcutaneous tissue disorders	Rash maculo-papular	X	X

10.62 Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.621 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.622 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.623 Grade 5 AEs (Deaths)

10.6231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

10.8 Merck Additional Event Reporting Instructions

For the time period beginning when the consent form is signed until treatment allocation, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 10.10.1 for additional details) that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 10.10.1 for additional details), whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

All subjects with serious adverse events must be followed up for outcome.

10.81 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.
If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."
All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. [redacted]

10.82 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. [redacted]

10.83 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. [redacted]

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 10.91 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

***Note:** These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of

abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

10.84 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 10.1.- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g. transportation issues etc.) will not be considered a SAE.

10.85 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

10.9 Biocompatibles Inc., a BTG International group company Additional Event Reporting Instructions

10.91 Product malfunction

A product malfunction is defined as a failure of the device to meet its performance specifications, essential function or otherwise perform as intended. Performance specifications include all claims made in the labelling for the device. The essential function of a device refers not only to the device's labelled use, but for any use widely prescribed within the practice of medicine.

10.92 Reporting of Adverse Events, Serious Adverse Events and Device Malfunctions

The Institution and/or the Sponsor-Investigator shall report all and any serious adverse events, product malfunctions or quality complaints (regardless of causality) that they become aware of in relation to the Product and/or the investigation to BTG.

All reports will be exchanged in English and Sponsor-Investigator will also provide BTG with such information and reasonable assistance as may be requested by BTG to allow BTG to comply with their obligations.

The Institution and/or the Sponsor-Investigator shall report all SAEs and incidents impacting patient safety to [redacted] (Galil product related).

10.93 SAEs

The Sponsor-Investigator will report SAEs within **one business day** to enable BTG to comply with their obligations as the device manufacturer, under applicable laws and regulations.
All reports will contain the following, if available:

- Study title and name of the Sponsor-Investigator.
- Patient number
- Adverse event number
- Date of event occurrence/date notified
- Product details
  - Product name/ part number
  - Size / dose
  - Batch / lot number
- Adverse event details along with comprehensive event description
- Action(s) taken to treat or resolve the event
- Outcome
- Investigators opinion of causality of event i.e. related to
  - Drug
  - Device
  - Procedure
- Product returned to BTG/Galil if applicable

10.94 Device Malfunctions

The Institution and/or the Sponsor-Investigator shall report all device malfunctions and/or quality complaints [redacted] (Galil) within one business day of becoming aware of the issue.

11.0 Treatment Evaluation

NOTE: For assessment of index (target) and non-target lesions on whole body scan performed after completion of DC treatment, the baseline whole body scan will be used to assess overall treatment response of index (target) and non-target lesions. Include up to 6 index (target) lesions as measurable disease when assessing overall clinical response. The treatment lesion(s) is the lesion(s) that is cryoablated. This treatment lesion will be measured only at baseline and will not be included in assessment of overall clinical response, and will not be followed or measured.

NOTE: Subject(s) with progressive disease on anti-PD-1 monoclonal antibody alone prior to receiving cryoablation and intra-tumor injection of autologous DC will be continued on treatment to assess clinical response with the addition of these therapy as long as the provider and the investigator deemed clinically safe and reasonable.

11.1 Response Considerations

Schedule of Evaluations: PET/CT or whole body CT scans are required at baseline for all patients. For the purposes of this study, patients should be reevaluated for disease progression at the end of each cycle. In addition to a baseline PET/CT scan, confirmatory scans should also be obtained at Day 1 of Cycles 4, 8, 12 and 18 (Section 4). During observation, CT scan should be obtained every 3 months during the first year to evaluate for disease progression.

Definitions for clinical response for patients with lymphoma are from the recently revised Cheson's et al criteria published in 2014 (Cheson, Fisher et al. 2014), derived from the original criteria published in 2007 (Cheson 2007). Lymph node measurements should be taken from the CT portion of the PET/CT, or other dedicated CT scans where applicable. Measurement of lymphadenopathy for purposes of assessing for PR will be determined by adding the sum of the products of the maximal perpendicular diameters of measured lesions (SPD). The PPD of a single node is sufficient to evaluate for PD (see Table 11.2). Measurable extranodal disease should be assessed in a manner similar to that for nodal disease. For these recommendations, the spleen is considered nodal disease. Disease that is only assessable (eg, pleural effusions, bone lesions) will be recorded as present or absent only, unless, while an abnormality is still noted by imaging studies or physical examination, it is found to be histologically and pathologically negative.

Response is based on PET/CT based on the revised 2014 Lugano Classification (Cheson, Fisher et al. 2014).

11.2 Lugano Classification Response criteria (Cheson, Fisher et al. 2014)

	PET-CT Based Response	CT-Based Response
Complete Response	**Complete metabolic response (CMR)**	**Complete radiologic response (CR) (all of the following)**
Lymph nodes and extralymphatic sites	Score 1, 2, or 3* with or without a residual mass on 5PS† It is recognized that in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation	Target nodes/nodal masses must regress to ≤1.5 cm in LDi No extralymphatic sites of disease

	PET-CT Based Response	CT-Based Response
	within spleen or marrow (eg, with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic uptake	
Nonmeasured lesion	Not applicable	Absent
Organ enlargement	Not applicable	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminate, IHC negative
Partial Response	**Partial metabolic response (PMR)**	**Partial remission (PR) (all of the following)**
Lymph nodes and extralymphatic sites	Score 4 or 5† with reduced uptake compared with baseline and residual mass(es) of any size At interim, these findings suggest responding disease At end of treatment, these findings indicate residual disease	≥50% decrease in SPD of up to 6 target measurable nodes and extranodal sites When a lesion is too small to measure on CT, assign 0.5 cm X 0.5 cm as the default value When no longer visible, 0.0 X 0.0 cm For a node >0.5 cm X 0.5 cm, but smaller than normal, use actual measurement for calculation
Nonmeasured lesions	Not applicable	Absent/normal, regressed, but no increase
Organ enlargement	Not applicable	Spleen must have regressed by >50% in length beyond normal
New lesions	None	None
Bone marrow	Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan	Not Applicable
No Response or Stable Disease	**No metabolic response (NMR)**	**Stable disease (SD)**
Target nodes/nodal masses, extranodal lesions	Score 4 or 5 with no significant change in FDG uptake from baseline at interim or end of treatment	<50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
Nonmeasured	Not applicable	No increase consistent with

	PET-CT Based Response	CT-Based Response
lesions		progression
Organ enlargement	Not applicable	No increase consistent with progression
New lesions	None	None
Bone marrow	No change from baseline	Not Applicable
Progressive disease	**Progressive metabolic disease (PMD)**	**Progressive disease (PD) requires at least 1 of the following**
Individual target nodes/nodal masses	Score 4 or 5 with an increase in intensity of uptake from baseline and/or New FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment	PPD progression: An individual node/lesion must be abnormal with: LDi >1.5 cm and Increase by ≥50% from PPD nadir and An increase in LDi or SDi from nadir 0.5 cm for lesions ≤2 cm 1.0 cm for lesions >2 cm In the setting of splenomegaly, the splenic length must increase by >50% of the extent of its prior increase beyond baseline (eg, a 15-cm spleen must increase to >16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline New or recurrent splenomegaly
Extranodal lesions		
Nonmeasured lesions	None	New or clear progression of preexisting nonmeasured lesions
New lesions	New FDG-avid foci consistent with lymphoma rather than another etiology (eg, infection, inflammation). If uncertain regarding etiology of new lesions, biopsy or interval scan may be considered	Regrowth of previously resolved lesions A new node >1.5 cm in any axis A new extranodal site >1.0 cm in any axis; if <1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma Assessable disease of any size unequivocally attributable to lymphoma
Bone marrow	New or recurrent FDG-avid foci	New or recurrent involvement
Abbreviations: 5PS, 5-point scale; CT, computed tomography; FDG, fluorodeoxyglucose; IHC, immunohistochemistry; LDi, longest transverse diameter of a lesion; MRI, magnetic resonance imaging; PET, positron emission tomography; PPD, cross product of the LDi and perpendicular diameter; SDi, shortest axis perpendicular to the LDi; SPD, sum of the product of the perpendicular diameters for		

	PET-CT Based Response	CT-Based Response
multiple lesions. *A score of 3 in many patients indicates a good prognosis with standard treatment, especially if at the time of an interim scan. However, in trials involving PET where de-escalation is investigated, it may be preferable to consider a score of 3 as inadequate response (to avoid undertreatment). Measured dominant lesions: Up to six of the largest dominant nodes, nodal masses, and extranodal lesions selected to be clearly measurable in two diameters. Nodes should preferably be from disparate regions of the body and should include, where applicable, mediastinal and retroperitoneal areas. Non-nodal lesions include those in solid organs (eg, liver, spleen, kidneys, lungs), GI involvement, cutaneous lesions, or those noted on palpation. Nonmeasured lesions: Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant or measurable or that do not meet the requirements for measurability but are still considered abnormal, as well as truly assessable disease, which is any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed and followed by imaging. In Waldeyer's ring or in extranodal sites (eg, GI tract, liver, bone marrow), FDG uptake may be greater than in the mediastinum with complete metabolic response, but should be no higher than surrounding normal physiologic uptake (eg, with marrow activation as a result of chemotherapy or myeloid growth factors). †PET Deauville 5PS: 1, no uptake above background; 2, uptake≤mediastinum; 3, uptake > mediastinum but ≤ liver; 4, uptake moderately > liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.		

Note: Transformation to a more aggressive histology that requires a change in active treatment will be considered progressive disease.

11.21 Complete Response (CR).

The designation of CR requires all of the following:

11.211 Complete disappearance of all detectable clinical evidence of disease and definitely disease-related symptoms if present before therapy.

11.212 All lymph nodes and nodal masses must have regressed on CT to normal size (≤1.5 cm in their greatest transverse diameter for nodes >1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to ≤1.0 cm in their short axis after treatment.

11.213 The spleen and/or liver, if considered enlarged before therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies, and nodules related to lymphoma should disappear. However, determination of splenic involvement is not always reliable because a spleen considered normal in size may still contain lymphoma, whereas an enlarged spleen may reflect variations in anatomy, blood volume, the use of hematopoietic growth factors, or causes other than lymphoma. Similarly, other organs considered to be enlarged before therapy due to involvement by lymphoma, such as liver and kidneys, must have decreased in size.

11.214 If the bone marrow was involved by lymphoma before treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of >20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is negative by immunohistochemistry but that demonstrates a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in patient outcome.

11.22 Criteria for Partial Response (PR)

The designation of PR requires all of the following:

11.221 At least a 50% decrease in sum of the product of the diameters (SPD) of up to six of the largest dominant nodes or nodal masses. These nodes or masses should be selected according to all of the following:

- they should be clearly measurable in at least 2 perpendicular dimensions
- if possible they should be from disparate regions of the body
- they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved

11.222 No increase should be observed in the size of other nodes, liver, or spleen.

11.223 Splenic and hepatic nodules must regress by ≥50% in their SPD or, for single nodules, in the greatest transverse diameter.

11.224 With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable and no measurable disease should be present.

11.225 Bone marrow assessment is irrelevant for determination of a PR if the sample was positive before treatment. However, if positive, the cell type should be specified (eg, large-cell lymphoma or small neoplastic B cells). Patients who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders.

When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered partial responders.

11.226 No new sites of disease should be observed.

11.227 CT criteria should be used.

11.23 Criteria for Stable Disease (STAB)

11.231 A patient is considered to have SD when he or she fails to attain the criteria needed for a CR or PR (see above), but does not fulfill those for progressive disease (see below).

11.232 There must be no change in the size of the previous lesions on the post-treatment CT scan.

11.24 Relapsed Disease (after CR)/Progressive Disease (after PR, SD)

Lymph nodes should be considered abnormal if the long axis is more than 1.5 cm regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if its short axis is more than 1.0. Lymph nodes ≤1.0 x ≤1.0 cm will not be considered as abnormal for relapse or progressive disease.

11.241 Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.

11.242 At least a 50% increase from nadir in the SPD of any previously involved nodes, or in a single involved node, or the size of other lesions (eg, splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by 50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.

11.243 At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.

11.244 Transformation to a more aggressive histology that requires a change in active treatment will be considered progressive disease.

11.3 Other Response Criteria:

11.31 Cutaneous Response Criteria:

Lesions will be measured by physical exam and photographed to evaluate for response. Photographs of the skin lesions, marked with a ruler, will be saved/uploaded in QREADS for comparison purposes.

If all target lesions are cutaneous, then the following modified Lugano criteria will be utilized. However, if at least one target lesion is not cutaneous, then the cutaneous measurements will be grouped with the target nodal/extranodal lesions when calculating the SPD and the response criteria per section 11.2 will be utilized with reference to the table below for cutaneous definitions whenever applicable.

Modified Lugano Criteria for Cutaneous Lesions	
Response	**Definition**
Complete response	100% clearance of all skin lesions
Partial response	≥50% decrease in SPD of up to 6 target measurable cutaneous lesions and not meeting Complete response definition
Stable disease	<50% decrease from baseline in SPD of up to 6 target measurable cutaneous lesions; no criteria for progressive disease are met

Modified Lugano Criteria for Cutaneous Lesions	
Response	**Definition**
Progressive disease	≥50% increase from nadir in SPD of up to 6 target measurable cutaneous lesions; New cutaneous lesions

11.4 Patient Reported Outcomes: Quality of Life

11.41 Patients will complete quality of life assessments at times documented in Section 4. The assessments will allow the patients to evaluate themselves regarding quality of life, concerns and symptoms. Patient reported outcome results do not impact any treatment related decisions.

11.42 Patients will complete the Functional Assessment of Cancer Therapy – Lymphoma (FACT-Lym). This assessment consists of the FACT-General (FACT-G) and the 15-item Lymphoma specific subscale.

The Functional Assessment of Cancer Therapy – General (FACT-G) was developed to measure quality of life in cancer patients receiving therapy. It was initially validated using classical test theory (CTT) methodology in a heterogeneous sample of 545 patients with cancer, 8% of whom had either leukemia or lymphoma. The FACT-G is comprised of four subscales: physical well-being (PWB; 7-items, score range 0-28), social/family well-being (SWB; 7-items, score range 0-28), emotional well-being (EWB; 6-items, score range 0-24), and functional well-being (FWB; 7-items, score range 0-28). Users of the FACT-G are able to generate an overall score and four subscale scores with ranges and distributions that are sample-specific. All questions in the FACT-G use a 5-point rating scale (0 = Not at all; 1 = A little bit; 2 = Somewhat; 3 = Quite a bit; and 4 = Very much). Provided more than 50% of the items comprising a subscale are answered, a subscale score is computed as the prorated sum of the item responses for that subscale. Prorating, which replaces missing values with the mean of the completed items for that subscale, has been shown to be an acceptable method of imputing missing data in the FACT instruments when more than 50% of the items are answered. The FACT-G total score is computed as the sum of the four subscale scores, provided the overall item response is at least 80% (i.e., at least 22 of the 27 items were answered) and has a possible range of 0-108 points. Negatively worded items are reverse scored prior to summing so that higher subscale and total scores indicate better Quality of Life (Cella, Tulsky et al. 1993).

The FACT-Lym adds a lymphoma-specific subscale to the FACT-G. The content in the lymphoma subscale (e.g., “I have night sweats” and “I am bothered by itching”) is targeted towards patients on active treatment (Hlubocky, Webster et al. 2013).

12.0 Descriptive Factors

12.1 Number of prior treatment regimens (any): 0, 1, 2 >3.

12.2 Current disease status: Newly diagnosed vs. residual vs. relapsed vs. refractory.

12.3 Previous Auto SCT: Yes vs. no.

12.4 Ann Arbor stage: 1 vs. 2 vs. 3 vs. 4 (see Appendix VII).

12.5 International Prognostic Index (IPI) (Shipp, 1993): low risk (0 or 1 risk factors) vs. low intermediate risk (2 risk factors) vs. high intermediate risk (3 risk factors) vs. high risk (4 or 5 risk factors) (see Appendix VII).

12.6 Follicular Lymphoma International Prognostic Index (FLIPI) (Solal-Celigny, 2004): low risk (0 or 1 risk factor) vs. intermediate risk (2 risk factors) vs. high risk (3-5 risk factors) (see Appendix VII).

12.7 MCL International Prognostic Index (MIPI) (Hoster, 2008): 0-3 vs 4-5 vs 6-11 (see Appendix VII).

13.0 Treatment/Follow–up Decision at Evaluation of Patient

Note: For additional information regarding follow-up decision, please refer to the patient treatment schematic (Patient Map), located with the CRFs and supplementary materials.

13.1 Patients who go off protocol at any time to receive non-protocol alternate treatment will go to event monitoring (see Section 4.4).

13.2 Patients who are CR, PR, or SD not requiring non-protocol alternative treatment will continue on initial active treatment per protocol for 18 cycles (12 months).

Once treatment is completed patients may receive an additional year of optional pembrolizumab treatment (provided approval from PI and sponsor).

- If no pembrolizumab treatment will be given, patients will go to Treatment Observation (see Sections 4.5 and 13.2) if additional DC is available for retreatment or Observation (see Section 4.3) if no additional DC is available for retreatment.
- If pembrolizumab treatment will be given, patients will receive treatment for up to an additional 18 cycles (12 months) then proceed to
    - Treatment Observation (see Sections 4.5 and 13.2), if additional DC is available for retreatment.
    - Observation (see Section 4.3) if additional DC s not available for retreatment.

13.3 Treatment Observation

13.31 If the patient has achieved CR, PR, or SD in initial treatment or optional pembrolizumab cycles, not requiring non-protocol alternative treatment, the patient will be observed at the following time points: 8 weeks after the last dose of treatment and then every 3 months for 1 year; followed by monitoring every 4 months for a year, then every 6 months until 4 years post registration.

During Treatment Observation , the patient may be retreated at any time

following PD (see Section 13.8).

13.4 Patients who complete Treatment Observation without PD will have completed the study 4 years post registration.

13.5 Patients who develop PD during initial active treatment in the DC + Pembrolizumab cycles, will go to the event-monitoring phase(see Section 4.4).

13.6 Patients who develop PD during initial active treatment receiving only Pembrolizumab (cycles 6+ on dose level 1 or cycles 8+ on dose level -1) will go to observation (see Section 4.4) or retreatment per clinical judgment.

13.7 Patients who develop PD during the optional Pembrolizumab treatment will:

- Go to retreatment if DC is available
- Go to observation (see Section 4.3) if DC unavailable or if the patient refuses further treatment or if the clinician determines retreatment isn't feasible.

13.8 Patients who develop PD during the observation phase with no DC available for retreatment, will go to event monitoring (see Section 4.4).

13.9a Patients who develop PD during the treatment observation phase who have DC available will:

- Go to retreatment (see Section 13.9d).
- Complete treatment observation (see Section 4.5) if patient refuses further treatment or if clinician determines retreatment isn't feasible.

13.9b Patients who go off protocol treatment due to adverse events or DLT during initial treatment will go to observation (see Section 4.3). If PD occurs in observation, patients will go to event monitoring (see Section 4.4).

13.9c Patients who go off protocol treatment for reasons other than adverse events during initial treatment will go to the event-monitoring phase (see Section 4.4).

13.9d Retreatment

Retreatment can be considered at any time when deemed clinically safe.

- DC created from the original leukopheresis must be available
- Tumor lesion(s) must be amenable to cryoablation.

13.9e Patient who complete retreatment without PD will go to observation followed by event monitoring. Patients who complete retreatment having PD will go to event monitoring.

13.9f Ineligible

A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. The patient will go directly to the event-monitoring phase of the study (or off study, if applicable).

- If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted. Event monitoring will be required per Section 4.4 of the protocol.
- If the patient never received treatment, on-study material must be submitted and the patient will go off study.

13.9g Major violation

A patient is deemed a *major violation*, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will go directly to the event-monitoring phase of the study. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. Event monitoring will be required per Section 4.4 of the protocol.

13.9h Cancel

A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Body Fluid Biospecimens

14.1 Summary Table of Research Blood/Blood Products to Be Collected for This Protocol

Collect and process all blood/blood products according to instructions and table below. For immunological monitoring Panels1, 2, 3 and 4 will be collected.

14.11 Collection During Treatment

Correlative Study (Section for more information)	Mandatory or Optional	Blood or Body Fluid being Collected	Component being harvested	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	Day 1 of designated cycles[1, 2]	Process at site? (Yes or No)	Temperature Conditions for Storage/ Shipping[3]
Immune Monitoring Panel 1 (14.3)	Mandatory	Whole Blood	Plasma, Cells for DNA/RNA,Protein, and immunologic assays	EDTA (purple)	6 mL (1) 10 mL (2)	X	No	Room temperature
Immune Monitoring Panel 2 (14.3)	Mandatory	Whole Blood	Plasma, Cells for DNA/RNA, Protein, and immunologic assays	Heparin (green)	10 mL (4) 6 mL (1)	X	No	Room temperature
Immune Monitoring Panel 3 (14.3)	Mandatory	Whole Blood	DNA/RNA/Protein assays	Streck Cell Free DNA BCT	10 mL (1)	X	No	Room temperature
Immune Monitoring Panel 4 (14.2)	Mandatory	Whole Blood	serum	Serum Separator Tube (red tiger)	10 mL (1)	X	No	Room temperature

1. Cycle 1 order: blood for immune monitoring, leukapheresis, and MK-3475 administration. Order after Cycle 1: blood for immune monitoring, Pembrolizumab.
2. Phase I dose level 1: Cycles 1, 2, 4, 6, 8, 12, 18. Phase I dose level -1: Cycles 1, 2, 4, 8, 12, 18. Phase IIwill follow the schedule of phase I dose level 1.
3. All specimens should be sent to Ailing Xue, Human Cell Therapy Lab, Mayo Clinic, 200 First Street SW – Stabile 3, Rochester, MN 55905.

14.12 Blood Collection During Observation

Correlative Study (Section for more information)	Mandatory or Optional	Blood or Body Fluid being Collected	Component being harvested	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	Documented PD requiring active treatment at any time during treatment or observation, prior to entering event monitoring, at End of Observation	Day 1 of Cycles 19-22 Every 3 months for 1 year (start at 8 weeks after last dose)	Process at site? (Yes or No)	Temperature Conditions for Storage /Shipping[1]
Immune Monitoring Panel 1 (14.2)	Mandatory	Whole Blood	Plasma Cells for DNA/RNA, Protein, and immunologic assays	EDTA (purple)	6 mL (1) 10 mL (2)	X	X	No	Room temperature
Immune Monitoring Panel 2 (14.2)	Mandatory	Whole Blood	Plasma Cells for DNA/RNA, Protein, and immunologic assays	Heparin (green)	10 mL (4) 6 mL (1)	X	X	No	Room temperature
Immune Monitoring Panel 3 (14.3)	Mandatory	Whole Blood	DNA/RNA/Protein assays	Streck Cell Free DNA BCT	10 mL (1)	X	X	No	Room temperature
Immune Monitoring Panel 4 (14.2)	Mandatory	Whole Blood	serum	Serum Separator Tube	10 mL (1)	X	X	No	Room temperature

1. All specimens should be sent to Ailing Xue, Human Cell Therapy Lab, Mayo Clinic, 200 First Street SW – Stabile 3, Rochester, MN 55905

14.2 Background/Methodology

14.21 In vitro functional studies

In all cases, we will select CD3+ cells from the remainder of the cells found in the leukapheresis. These and T cells collected from subsequent time points can be in assessment of T cell functions including proliferation, cytokine production, response to Prevnar and DC stimulation and T cell receptor gene sequencing. In addition, we will keep samples of mDC from each patient and use these cells to stimulate peripheral blood collected as per the test schedule and assay for anti-tumor response via proliferation, ELIspot, or intracellular cytokine expression. A change above two times baseline control or more than 30% from assay prior to treatment will be considered significant response.

14.22 Immune phenotyping

We will immune phenotype blood for circulating Tregs, central memory and effector memory T cells, quantitative T, B, and NK cell panel, dendritic cells and immune suppressor cells (CD14+DRneg) and other immune modulating cells per our prior experience. PD-1, PD-L1 and PD-L2 expression on leukocyte subset will be analyzed. We will also collect and store plasma, serum and may collect white blood cells for future protein, RNA or DNA analysis. In particular, based on our preclinical studies, for patients with abnormally high percentage of CD14+HLA-DRlow/neg monocytes prior to treatment, a change greater than 10% in the percentage of these cells after treatment would be considered a statistically significant change.

15.0 Drug Information

15.1 Pembrolizumab (MK-3475, SCH 900475, Keytruda®)

15.11 Background

Pembrolizumab is a potent humanized IgG4 monoclonal antibody with high specificity of binding to the PD-1 receptor, thus inhibiting its interaction with PD-L1 and PD-L2. Based on preclinical *in vitro* data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1.

15.12 Formulation

Pembrolizumab is available as a liquid 25 mg/mL, 100 mg/vial.

15.13 Preparation and storage:

Vials should be stored in the refrigerator at temperatures between 2-8°C. Drug concentrate is further diluted with normal saline (or 5% dextrose) in the concentration range of 1 to 10 mg/mL. The infusion solution in the IV bag should be immediately administered. Diluted pembrolizumab solutions may be stored at room temperature for a cumulative period of up to 6 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion. The product can also be stored under refrigeration at 2°C to 8°C for no more than 96 hours from the time of dilution. If refrigerated, the diluted solution must be allowed to come to room temperature prior to administration. The solution must be discarded after 6 hours at room temperature or 96 hours under refrigeration.

15.14 Administration

Pembrolizumab is administered by intravenous infusion over 30 minutes via a 0.22 micron in-line filter. The final infusion volume must be between 1 and 10 mg/mL. Maximum rate of infusion should not exceed 6.7 mL/minute through a peripheral or indwelling catheter. Flush the line with 0.9% NaCL following the completion of the infusion.

15.15 Pharmacokinetic information:

a) Absorption – Because pembrolizumab is administered intravenously, it is immediately and completely bioavailable. Steady-state concentrations of pembrolizumab are reached by 16 weeks of repeated dosing with a Q3W regimen and the systemic accumulation is 2.1-fold. The peak concentration, trough concentration, and area under the plasma concentration versus time curve at steady state of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg Q3W.
b) Distribution – Pembrolizumab has a limited volume of distribution.
c) Excretion – CL is approximately 23% lower after achieving maximal change at steady state compared with the first dose. The terminal elimination half-life (t½) is estimated to be 22 days at steady state.
d) Metabolism - Pembrolizumab is catabolized through non-specific pathways; metabolism does not contribute to its CL.

15.16 Potential Drug Interactions

There are no known significant drug interactions.

15.17 Known potential toxicities

Very common known potential toxicities, ≥10%:
Gastrointestinal disorders: diarrhea, nausea, abdominal pain
Skin and subcutaneous tissue disorders: rash, pruritis
General disorders and administration site conditions: fatigue

Common known potential toxicities, ≥1% to <10%:
Blood and lymphatic system disorders: anemia
Immune system disorders: infusion related reaction
Endocrine disorders: hyperthyroidism, hypothyroidism
Metabolism and nutrition disorders: decreased appetite
Nervous system disorders: headache, dizziness, dysgeusia
Respiratory, thoracic, and mediastinal disorders: pneumonitis, dyspnea, cough
Gastrointestinal disorders: colitis, vomiting, constipation, dry mouth
Skin and subcutaneous tissue disorders: severe skin reactions, vitiligo, dry skin, erythema
Musculoskeletal and connective tissue disorders: arthralgia, myositis, musculoskeletal pain, arthritis, pain in extremity
General disorders and administration site conditions: asthenia, edema, pyrexia, influenza like illness, chills
Investigations: alanine aminotransferase increased, aspartate aminotransferase increased, blood alkaline phosphatase increased, blood creatinine increased

Uncommon known potential toxicities, ≥0.1% to <1%:
Infusion related reactions
Blood and lymphatic system disorders: neutropenia, thrombocytopenia, leukopenia, lymphopenia, eosinophilia
Endocrine disorders: hypophysitis, adrenal insufficiency, hyponatremia, Thyroiditis, hypopituitarism
Metabolism and nutrition disorders: type I diabetes mellitus, hyponatremia, hypokalemia, hypocalcemia
Psychiatric disorders: insomnia, confusional state
Nervous system disorders: epilepsy, lethargy, peripheral neuropathy
Eye disorders: uveitis, dry eye
Cardiac disorders: myocarditis, atrial fibrillation
Vascular disorders: hypertension
Gastrointestinal disorders: pancreatitis, dysphagia
Hepatobiliary disorders: hepatitis
Skin and subcutaneous tissue disorders: lichenoid keratosis, psoriasis, alopecia, dermatitis, dermatitis acneiform, eczema, hair color changes, papule
Musculoskeletal and connective tissue disorders: tenosynovitis, myelitis
Renal and urinary disorders: nephritis, acute kidney injury
Investigations: blood bilirubin increased, amylase increased, hypercalcemia
Respiratory: pneumonia aspiration

Rare known potential toxicities, <0.1% (Limited to important or life-threatening):

Blood and lymphatic system disorders: immune thrombocytopenic purpura, hemolytic anemia
Immune system disorders: sarcoidosis
Nervous system disorders: Guillain-Barre syndrome, myasthenic syndrome, exacerbation of myasthenia gravis
Gastrointestinal disorders: small intestinal perforation
Skin and subcutaneous tissue disorders: toxic epidermal necrolysis, Stevens-Johnson syndrome, erythema nodosum

The risk profile for pembrolizumab also includes two important potential risks: a) myasthenic syndrome, and b) an increased risk of severe complications (such as early severe graft versus host disease and veno-occlusive disease) of allogeneic transplant in patients with hematologic malignancies who have previously been treated with PD-1 inhibitors.

Patients with multiple myeloma who were treated with pembrolizumab in combination with either pomalidomide or lenalidomide and dexamethasone, had an increased number of serious side effects and deaths as compared to patients who received only dexamethasone and either pomalidomide or lenalidomide. The benefit-risk profile is unfavorable for the combination of pembrolizumab, pomalidomide, and dexamethasone in relapsed refractory multiple myeloma, and the combination of pembrolizumab, lenalidomide, and dexamethasone in newly diagnosed treatment-naive multiple myeloma.

Post marketing reports identified Vogt-Koyanagi-Harada syndrome and hemophagocytic lymphohistiocytosis.

15.18 Drug procurement

Pembrolizumab will be provided free of charge to study participants by Merck.

15.19 Nursing guidelines

15.191 Pembrolizumab side effects vary greatly from those of traditional chemotherapy and can vary in severity from mild to life threatening. Instruct patients to report any side effects to the study team immediately. Side effects may be immediate or delayed up to months after discontinuation of therapy. Most side effects are reversible with prompt intervention of corticosteroids.

15.192 Diarrhea can be seen however is less common than that seen with anti-CTLA-4 agents. However it can be severe, leading to colonic perforation. Instruct patients to report ANY increase in the number of stools and/or change in baseline, blood in the stool, abdominal pain to the study team immediately.

15.193 Rash/pruirits/dermatitis is seen. Patients should report any rash to the study team. Treat per section 9.0 and monitor for effectiveness.

15.194 Monitor LFT’s closely as elevations in these levels could indicate early onset autoimmune hepatitis. Patients should also be instructed to report any jaundice, or right upper quadrant pain to the study team immediately.

15.195 Pneumonitis can be seen and may be mild (only seen on imaging) to severe. Patients should be instructed to report any SOB, dyspnea, cough, chest pain, etc. to the study team immediately. Patients reporting these symptoms should have a pulse ox checked and consider immediate imaging per the treating MD.

15.196 Endocrinopathies (including hypopituitarism, hypothyroidism, hypophysistis, and adrenal insufficiency) are seen with this agent. Patients may present only with the vague sense of fatigue and "not feeling well". Additional symptoms may be that of nausea, sweating and decreased activity tolerance. Instruct patients to report these signs or symptoms immediately and obtain appropriate labs as ordered by MD.

15.197 Patients who are started on steroid therapy for any side effects of pembrolizimab toxicity should be instructed to take the steroids as ordered, and not to discontinue abruptly as symptoms may return and be severe. Patients may be on steroid therapy for weeks. Instruct patients to report any increase or change in side effects with any dosage decrease as patients may need a slower taper.

15.198 Fatigue is common and may or may not be associated with immune related side effects. Assess patient's fatigue level prior to each cycle of therapy and report any changes to the study team.

15.199 Patients should avoid receiving live DC within 30 days of study drug administration or per other study guidelines.

15.200 Patients who have undergone an allogenic bone marrow transplant, have an increased risk of severe complications including early GVHD, and venoocclusive disease, if they have previously been treated with pembrolizumab

15.201 Myocarditis has been reported and associated with pembrolizumab. Instruct patients to report chest pain, SOB, or dyspnea to study team immediately and/or seek emergency medical attention.

15.202 Autoimmune hematologic disorders including ITP and hemolytic anemia have been reported. Monitor blood counts closely and report any abnormalities to the study team.

15.203 Rare neurologic disorders including Guillian-Barre syndrome and myasthenia gravis have been reported. Instruct patients to report any neurologic symptoms including weakness, parasthesias or numbness, tingling to the study team immediately.

15.2 Dendritic Cells

15.21 Background: The DC used in this study is patient's autologous dendritic cells (DC). Dendritic cells are cells manufactured in lab from monocytes removed from patients' blood by leukapheresis. The dendritic cells need to acquire tumor antigens in order to stimulate anti-tumor immunity. Patients will be treated with DC placed into a tumor killed using cryoablation where tumor

antigen exposure will occur *in vivo*.

15.22. Formulation: DC is supplied as recently thawed cells prepared from patient material. Cells are manufactured and released by the Human Cellular Therapy Lab, Mayo Clinic Rochester. Before released for use, cells will undergo testing for sterility and potency.

15.23 Preparation and storage: DC will be prepared and stored at the Human Cellular Therapy Lab, Mayo Clinic Rochester according to approved SOPs included in the IND. The drug will be supplied to the appropriate administration area by personnel from the Human Cell Therapy Lab.

15.24 Administration: Each patient will undergo percutaneous cryoablation of the lymphoma tumor lesion to a cryoablated size of less than or equal to 2 cm in diameter equal to 50 – 75% of the index lymphoma lesion. Incomplete treatment of the index lymphoma tumor lesion will significantly minimize risk of thermal injury to adjacent structures, yet result in desired cytotoxic cell injury. Our general cryoablation technique has been previously described (Atwell, Farrell et al. 2008). Patients will be treated at on the 3W CT scanner. Due to the extended length of the procedure, requiring the patient to lie still (expected duration 45 minutes), sedation will be managed by the Department of Anesthesia. Using ultrasound guidance, a cryoprobe will be placed in the index lymphoma node. The node will be treated using a conventional freeze-thaw-freeze cycle with each freeze stage lasting 10 minutes or until the iceball reaches within 3mm of the outer node margin (as determined by CT monitoring at 2 minute intervals).

Following the freezing procedure, the cryoprobe will be actively warmed and then withdrawn. After the node has thawed, a 22 G needle will be placed under ultrasound-guidance into the ablated portion of the node. The DC will be administered through this needle in 10 – 15 divided injections over different areas of the cryoablated region and then flushed with 1 ml of sterile saline.

15.25 Pharmacokinetic information: NOTE: this drug is a biologic made up of cells from the patient. Classical drug pharmokinetics do not apply.

15.26 Potential Drug Interactions: Unknown.

15.27 Known potential toxicities: Unknown.

15.28 Drug procurement: Drug is manufactured on site in the [redacted]

15.3 Pneumococcal 13-valent Conjugate Vaccine (Prevnar13)

15.31 Background: Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein), is a sterile solution of saccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F individually linked to non-toxic diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth.

Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens, and by the saccharide to protein ratios in the individual glycoconjugates.

15.32 Formulation: Supplied as ready-to-use prefilled syringes (10 x 0.5-mL prefilled syringes per package). Pneumococcal 13-valent Conjugate Vaccine is manufactured as a liquid preparation for intramuscular injection. Each 0.5 mL

dose of the vaccine is formulated to contain approximately 2.2 µg of each of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 23F saccharides, 4.4 µg of 6B saccharides, 34 µg CRM_{197} carrier protein, 100 µg polysorbate 80, 295 µg succinate buffer and 125 µg aluminum as aluminum phosphate adjuvant.

15.33 Preparation and storage: Pneumococcal 13-valent Conjugate Vaccine is stored at refrigerated temperatures of 2ºC to 8ºC (36ºF to 46ºF) away from freezer compartment. DO NOT FREEZE. Discard if frozen.

15.34 Administration: Shake vigorously immediately prior to use to obtain a uniform suspension in the vaccine container. The vaccine should not be used if it cannot be resuspended. After shaking, the vaccine is a homogeneous, white suspension. Do not mix the vaccine with other products in the same syringe.

The dose is 0.5 mL to be given intramuscularly. *Do not inject intravenously*, intradermally or subcutaneously. The vaccine should not be injected in the gluteal area or areas where there may be a major nerve trunk and/or blood vessel. Before injection, the skin at the injection site should be cleansed and prepared with a suitable germicide. After insertion of the needle, aspirate and wait to see if any blood appears in the syringe, which will help avoid inadvertent injection into a blood vessel. If blood appears, withdraw the needle and prepare for a new injection at another site.

15.35 Potential Drug Interactions: Patients receiving therapy with immunosuppressive agents (large amounts of corticosteroids, antimetabolites, alkylating agents, cytotoxic agents) may not respond optimally to active immunization.

As with other intramuscular injections, Pneumococcal 13-valent Conjugate Vaccine should be given with caution to patients on anticoagulant therapy.

See complete prescribing information for information regarding co-administration of Pneumococcal 13-valent Conjugate Vaccine with other vaccines.

15.36 Known potential toxicities: There is no safety data available in the adult patient population. Please see Prevnar13® prescribing information for comprehensive toxicity data.

The following systemic events were noted within 2-3 days of the Pneumococcal 13-valent Conjugate Vaccine injection in pediatric patients: fever, irritability, drowsiness, restless sleep, decreased appetite, vomiting, diarrhea, and urticaria-like rash. The following local reactions occurred within 3 days of immunization pediatric patients: erythema, induration, tenderness and interference with limb movement.

15.37 Drug procurement: Prevnar13 will be purchased with study funds and provided free of charge to patients.

15.38 Nursing Guidelines:

15.381 Monitor for injection site reaction. Local reaction is usually seen within 3 days of vaccination, including erythema, induration and tenderness. Treat symptomatically and monitor for signs on infection.

15.382 Patients may experience mild fever. Acetaminophen can be used symptomatically. Monitor for effectiveness.

15.383 Patients may experience “flu-like” symptoms (fever, irritability, decreased appetite, vomiting and diarrhea). Treat symptomatically.

16.0 Statistical Considerations and Methodology

16.1 Overview:

This is a phase I/II study of dendritic cell therapy and Pembrolizumab for patients with non-Hodgkin Lymphoma. The Phase I portion utilizes a 3+3 design with dose de-escalation only to identify the MTD of this treatment regimen. In this study, MTD means the maximum tolerated dose level/schedule. The Phase II portion is designed as an open-label study of patients having non-Hodgkin lymphoma.

16.11 Primary Endpoint:

The primary endpoint of the phase I portion of this trial is to assess DLT to determine the maximum tolerated dose level/schedule (MTD). For the phase II portion of this trial the pembrolizumab primary endpoint is the clinical response of combination therapy with pembrolizumab, cryoablation and intra-tumor injection of autologous DC at MTD dose schedule.

A success will be defined as a confirmed response: PR or CR. Throughout Section 16.0, PR or CR will be considered synonymous with “success” in the phase II portion, unless specified otherwise.

16.12 Sample Size

The phase I portion of this study is expected to require a minimum of 3 and a maximum of 12 evaluable patients. The 3 or 6 patients treated at the MTD in the phase I portion will also be included in the phase II portion.

A maximum of 28 additional evaluable patients will be accrued at the MTD dose level for a maximum of 34 evaluable patients in the phase II portion of this study. We anticipate accruing up to 4 additional patients (1 phase I, 3 phase II) to account for ineligibility, cancellation, major treatment violation, or other reasons. Therefore, a maximum of 13 patients will be accrued in the phase I portion and a maximum of 31 patients will be accrued to the phase II portion for an overall maximum of 44 patients for the entire study.

16.13 Accrual Rate and Study Duration

The anticipated accrual rate is approximately 1-3 patients per month. Therefore, the accrual period for Phase I of this trial is expected to be approximately 12 months and for phase II to be approximately 22 months. If for any reason accrual fails to meet our expectations, we will pursue efforts to open this study at Mayo Clinic Scottsdale, AZ site. The maximum total study duration is expected to be approximately 4 years, or until the last patient accrued has been observed for at least 12 months.

16.2 Phase I Portion

16.21 Study Design

The phase I study is designed to determine MTD and toxicity profile of dendritic cell therapy and pembrolizumab for patients with non-Hodgkin lymphoma using a 3+3 design with dose de-escalation only. Three patients will be treated at dose level 1 and observed for a minimum of six weeks (i.e. two full cycles and completion of Cycle 2 with pembrolizumab, cryoablation, and intra-tumor DC injection) before new patients are treated. Doses will not be escalated in any individual patient.

16.22 MTD Definition

MTD is defined as the dose level that does not induce DLT in at least one-third of patients (1 of 3 or 2 of 6 patients). A total of 6 patients treated at the MTD will be sufficient to identify common toxicities at the MTD. For instance, those toxicities with an incidence of at least 25% will be observed with a probability of at least 82% $(1-(1-0.25)_6)$. Refer to Section 7.61 for definition of DLT.

16.23 MTD Determination

The phase I portion of this study will follow dose levels and schedules as assigned in Section 7. The first cohort of three patients will be treated at dose level 1 and observed for 1 cycle of combination of Pembrolizumab, cryoablation, and intra-tumor DC injection (completion of Cycle 2) to assess toxicity. Decisions on when and how to dose de-escalate are described below.

16.231 If DLT (see Section 7.6) is not observed in any of the 3 patients, dose level 1 will be considered the MTD.

16.232 If DLT is observed in 1 of 3 patients treated at dose level 1, 3 additional patients will be enrolled and treated at the same dose level and evaluated after 1 cycle of combination therapy to assess toxicity.

If no additional DLT is observed, then dose level 1 will be considered the MTD.

If at least one additional DLT is observed in the new cohort of patients treated at dose level 1, then the next 3 patients will be treated at dose level -1.

i) If DLT is not observed in any of the 3 patients treated at dose level -1, then dose level -1 will be considered the MTD.

ii) If DLT is observed in 1 of 3 patients treated at dose level -1, then 3 additional patients will be treated at this dose level. If no additional DLT is observed in the new cohort of patients treated at dose level -1, then dose level -1 will be considered the MTD.

iii) If DLTs are observed in 2 or more of the 6 patients treated at dose level -1, then MTD will have been exceeded.

16.233 If DLT is observed in more than one of the 3 patients treated at dose level 1, then the next 3 patients will be treated at dose level -1. Decision rules for the 3 patients treated at dose schedule 2 follow 16.232 i) – iii) as described above.

16.234 If a patient fails to complete the first cycle of treatment for reasons other than toxicity, the patient will be regarded as inevaluable and will be replaced.

16.24 Analysis Plans

All the relevant results pertaining to toxicity, MTD, response, timed endpoints and laboratory correlates will be examined in an exploratory and hypothesis-generating fashion. The small sample size and the heterogeneous patient population associated with phase I studies restricts the generalizability of the results. Any notable statistical result should only be viewed as preliminary evidence for the Phase II component rather than a definitive finding in and of itself.

16.241 Adverse Events / Toxicity Profile (Goal 2.1.11)

The primary endpoint of the phase I portion is the incidence of dose-limiting toxicity. A dose-limiting toxicity is defined in section 7.61. Toxicities will be assessed using the CTEP Active Version of the CTCAE. Non-hematologic toxicities will be evaluated via the ordinal CTC standard toxicity grading. Hematologic toxicity measures of thrombocytopenia, neutropenia, and leukopenia will be assessed using continuous variables as the outcome measures (primarily nadir) as well as categorization via CTC standard toxicity grading.

These data will be assessed in evaluating the tolerability of the regimen for future studies. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be considered evaluable for dose-limiting toxicity. Incidence of DLT will be estimated by the number of patients with DLT divided by the total number of evaluable patients. No more than three patients will receive the first cycle of combination immunotherapy (Pembrolizumab and cryoablation and DC injections, i.e. Cycle 2 of the overall treatment plan) at any time during Phase I of the study.

For a comprehensive picture of toxicity profile, we will also summarize the incidence of other significant toxicities as defined in Section 8 or as a Grade 3 or higher adverse event otherwise not described in Section 8, that is possibly, probably, or definitely related to treatment. The number and severity of all adverse events (overall and by dose level) will be tabulated and summarized in this patient population. The Grade 3+ adverse events will also be described and summarized in a similar fashion. This will provide an indication of the level of tolerance for this treatment combination in this patient group. Frequency distributions, graphical techniques and other descriptive measures will form the basis of these analyses. Further longitudinal analysis techniques will be employed to explore the adverse event profile over time.

16.242 Feasibility (Goal 2.1.21)

The feasibility of the regimen will be estimated by the number of patients who received at least one dose of intratumoral DC injection divided by the total number of patients who received leukapheresis. Patient accrual rate and study completion rates will also be evaluated using summary statistics to determine if further studies of this regimen will be feasible.

16.243 Quality of Life (Goal 2.1.22)

Quality of life as measured using the FACT-Lym will be utilized. The assessment will be scored according the scoring algorithm. Changes from baseline will be calculated at each assessment time points. Mean change scores at each time point will be calculated to determine if quality of life is reduced over the course of treatment. Longitudinal techniques will be employed to describe changes over time.

16.3 Phase II Portion

16.31 Decision Rule

Pembrolizumab monotherapy induces an ORR of 39% in patients with

ipilimumab-naïve melanoma by central independent RECIST review. The potential activity of this agent in non-Hodgkin lymphoma (NHL) has not been tested. Similar check-point inhibitor Nivolumab (BMS) has reported ORR of 28% in B-cell NHL (ASH 2014). We estimate that Pembrolizumab may have similar single agent activities in NHL. Therefore we hypothesize that an ORR of 53% with this combination immunotherapy approach will be worthy of further testing in this disease population. We will explore if there is a difference in histologies enrolled in this study. Any specific NHL type with a clinical response defined as PR or CR in 2 or more patients may be expanded into a larger cohort for formal evaluation of ORR at the discretion of the PI and the Sponsor.

For phase II, the largest ORR where the proposed treatment regimen would be considered ineffective in this population is 30%, and the smallest ORR that would warrant subsequent studies with the proposed regimen in this patient population is 53%. The following one-stage design requires 28 evaluable patients (22 - 25 from phase II and 3 - 6 from the MTD portion of phase I provided that these patients have follicular lymphoma) to test the null hypothesis that the true ORR in this patient population is at most 30.

16.311 Interim Analysis: No interim analysis was planned for this study due to the following reasons: i) patients will be treated and evaluated for response up to two years from enrollment. ii) the total expected accrual time for this phase is only 18 – 24 months. If we were to conduct an interim analysis, all patients in the trial would have been accrued by the time patients in the interim analysis set have reached the primary endpoint for analysis.

16.312 Final Decision Rule: If 12 or fewer of 28 patients achieve overall response, then the proposed treatment is considered ineffective; and if 13 or more patients achieve overall response, then the treatment is conserved promising and warrants further study.

16.313 Over Accrual: If more than the target number of patients are accrued, the additional patients will not be used to evaluate the stopping rule or used in any decision making process. If more than the target number of patients are accrued, the additional patients will not be used to evaluate the stopping rule or used in any decision making processes; however, they will be included in final endpoint estimates and confidence intervals.

16.314 Power and Significance Level: the power calculation is based solely on Phase II. We plan to accrue a total of 28 evaluable patients (including follicular lymphoma patients from the MTD cohort in phase I) for Phase II of the trial. This sample size provides 81% power at a one-sided type I error of 0.05 to conclude that the ORR is higher than 30% if the true ORR is 53%. Secondary analysis will be performed in which Bayesian hierarchical models (Thall et al. 2003) will be used to estimate subtype-specific ORRs for the purposes of examining differences in response and guiding future plans for subtype-specific expansion studies.

16.315 Other considerations: Adverse events, quality/duration of response, and patterns of treatment failure observed in this study, as well as scientific

discoveries or changes in standard care will be taken into account in any decision to terminate the study.

16.32 Analysis Plan

16.321 Primary Outcome Analyses:

16.3211 Definition: The primary endpoint of this trial is the proportion of complete responses of combination therapy with pembrolizumab, cryoablation and intra-tumor injection of autologous DC at MTD dose schedule. A success is defined as a PR or CR as the objective status during therapy. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response.

16.3212 Estimation: The proportion of successes will be estimated by the number of successes divided by the total number of evaluable patients. Confidence intervals for the true success proportion will be calculated.

16.322 Secondary Outcome Analyses:

These analyses will include all patients meeting the eligibility criteria who have signed a consent form and have begun treatment. Analyses will include all patients enrolled in the Phase II portion of the study and analysis of each cohort separately.

16.323 Complete response (Goal 2.2.21): The response rate will be calculated, as above, in each individual cohort as supplementary to the primary analysis.

16. 324 Progression free survival, treatment free survival, and duration of response (Goal 2.2.22):

Survival time is defined as the time from registration to death due to any cause. The distribution of survival time will be estimated using the method of Kaplan-Meier (Kaplan E 1958). In addition, the overall survival rate at 2 years after registration will be reported.

Treatment free survival time is defined for all evaluable patients who have achieved a response as the time from registration to next treatment or death due to any cause. The distribution of treatment-free survival will be estimated using the method of Kaplan-Meier. In addition, the disease-free survival rate at 2 years after registration will be reported.

Duration of response is defined for all evaluable patients who have achieved a PR or CR as the date at which the patient's objective status is first noted to be a response to the earliest date progression is documented. The distribution of duration of complete response will be estimated using the method of Kaplan-Meier.

16.325 Adverse Events (Goal 2.2.23): All eligible patients that have initiated treatment will be considered evaluable for assessing adverse event rate(s). The maximum grade for each type of adverse event at each evaluation will be recorded for each patient, and frequency tables will be reviewed to determine patterns. Additionally, the relationship of the adverse event(s) to the study treatment will be taken into consideration.

Longitudinal analysis techniques will be utilized to determine the effect

of time on treatment adverse events.

16.326 Quality of Life (Goal 2.2.24):
Quality of life as measured using the FACT-Lym will be utilized. The assessment will be scored according to the scoring algorithm. Changes from baseline will be calculated at each assessment time points. Mean change scores at each time point will be calculated to determine if quality of life is reduced over the course of treatment. Longitudinal techniques will be employed to describe changes over time.

16.4 Analysis of other endpoint

16.41 Definitions and Analyses of Radiologic Endpoints

Measurements of index lesion(s) (non-cryoablated lesion) will be evaluated over time for each patient as a marker of systemic immune and treatment response to Pembrolizumab and localized treatment with cryoablation and DC. The index lesion(s) will be selected by the treating physician/investigator and are non-cryoablated node. The percent change from baseline in index lesion measurements will be assessed over time. Differences in values over time will be summarized descriptively and graphically. In addition, patients will be classified into one of the following categories based on the best response seen in the index lesion(s):

- Complete response: disappearance of the node
- Partial response: ≥50% of decrease in measured dimension
- Stable: between 50% decrease to ≤25% increase in dimension
- Progression: >25% increase in measured dimension

While the evaluation of index lesion(s) may be part of the assessment of overall response, it is not the sole criteria for time to progression. The whole body CT or PET/CT will be used to assess overall clinical response and time to progression using the standard Cheson criteria, as detailed in Section 11.

16.42 Correlative Endpoints

Change in immunologic correlates before and after vaccination treatment will be evaluated and summarized both quantitatively and graphically. Each of the correlative endpoints will be summarized individually, but will also be evaluated in terms of their relationships to one another; i.e., we will use Spearman rank correlation coefficient to assess the correlations between baseline levels as well as between changes before and after treatment in these immunologic markers. In addition, these immunologic markers will be correlated with cancer and treatment- related outcomes (e.g. response, toxicities). Relationships will also be explored graphically using scatter plots. Given the limited sample size, these analyses will be considered exploratory.

16.5 Data & Safety Monitoring:

16.51 Routine monitoring of accrual and adverse events will be completed by the study statistical team on a weekly basis to assess patient safety.

16.52 Formal monitoring of accrual, adverse event, and any endpoint problems that might be developing will be conducted by the Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB). The DSMB is responsible for reviewing accrual and safety data for this trial at least twice a year, based on

reports provided by the MCCC Statistical Office.

16.53 Adverse Event Stopping Rules (These rules apply to each arm independently): The stopping rules specified below are based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (i.e. an adverse event with attribute specified as "possible", "probable", or "definite") that satisfy either of the following:

- Phase I portion:
  - Every 3 patients in the phase I portion of the trial will be monitored according to the criteria in Section 7.0 and Section 16.23.
  - Death (other than death related to progressive disease) that occurs within 30 days of the DC cell infusion.
- Phase II portion:
  - If 4 or more patients in the first 12 treated patients experience a grade 4 or higher non-hematologic adverse event at least possibly related to treatment excluding infections.
  - If after the first 12 patients have been treated, 30% of all patients experience a grade 4 or higher non-hematologic adverse event at least possibly related to treatment excluding infections
  - Death (other than death related to progressive disease) that occurs within 30 days of the DC cell infusion.

We note that we will review grade 4 and 5 adverse events deemed "unrelated" or "unlikely to be related", to verify their attribution and to monitor the emergence of a previously unrecognized treatment-related adverse event.

16.54 Results Reporting on [redacted]

At study activation, this study will have been registered within the [redacted] website. The Primary and Secondary Endpoints (i.e., "Outcome Measures") along with other required information for this study will be reported on [redacted] For purposes of timing of the Results Reporting, the initial estimated completion date for the Primary Endpoint of this study is 4 years after the study opens to accrual. The definition of "Primary Endpoint Completion Date" (PECD) for this study is at the time the last patient registered has been observed for 12 months.

16.6 Inclusion of Minorities

16.61 This study will be available to all eligible patients, regardless of race or ethnic origin.

16.62 Although the planned analysis will, as always, look for differences in treatment effect based on racial groupings the sample size is not increased in order to provide additional power for subset analyses.

16.63 Based on prior studies involving similar disease sites conducted at Mayo Clinic in Rochester, MN, we expect about 3% of patients will be classified as minorities by race/ethnicity and about 40% of patients will be women. Expected sizes of racial subsets are shown in the following table:

	Sex/Gender			
Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Hispanic or Latino	0	0	0	0
Not Hispanic or Latino	18	26	0	44
Ethnic Category: Total of all subjects	18	26	0	44
Racial Category				
American Indian or Alaskan Native	0	0	0	0
Asian	0	0	0	0
Black or African American	0	1	0	1
Native Hawaiian or other Pacific Islander	0	0	0	0
White	18	25	0	43
Racial Category: Total of all subjects*	18	26	0	44

Ethnic Categories:	**Hispanic or Latino –** a person of Cuban, Mexican, Puerto Rico, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino." **Not Hispanic or Latino**
Racial Categories:	**American Indian or Alaskan Native –** a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment. **Asian –** a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.) **Black or African American –** a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American." **Native Hawaiian or other Pacific Islander –** a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands. **White –** a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens

17.1 Summary Table of Research Tissue Specimens to be collected for this Protocol

17.11 Tumor Biopsy Schedule: phase I dose schedule 1 and Phase II

Correlative Study (Section for more information)	Mandatory or Optional	Type of Tissue to Collect	Block, Slides, Core, etc. (# of each to submit)	Prior to Day 1 Cycle 1	Day 1 Cycle 6 and Cycle 19	PD	Process at site? (Yes or No)	Temperature Conditions for Storage/ Shipping[1]
Immunohistochemistry, immunofluorescence, gene sequencing for tumor antigen and T cell receptors (17.2)	Mandatory	Lymphoma tumor in normal saline	Excisional biopsy[2] or large bore needle core biopsy	X	X	X	No	Ambient

1. All specimens should be sent to Mary Solseth, IMPACT Lab, Mayo Clinic, 200 First Street SW – Hilton 2, Rochester, MN 55905.
2. Excisional tumor resection will be performed if possible in place of core needle biopsy for Cycle 1 and PD only.

17.12 Tumor Biopsy Schedule: Phase I dose schedule 2

Correlative Study (Section for more information)	Mandatory or Optional	Type of Tissue to Collect	Block, Slides, Core, etc. (# of each to submit)	Prior to Day 1 Cycle 1	Day 1 Cycle 8 and Cycle 19	PD	Process at site? (Yes or No)	Temperature Conditions for Storage /Shipping[1]
Immunohistochemistry, immunofluorescence, gene sequencing for tumor antigen and T cell receptors (17.2)	Mandatory	Lymphoma tumor in normal saline	Excisional biopsy[1] or large bore needle core biopsy	X	X	X	No	Ambient

1. All specimens should be sent to Mary Solseth, IMPACT Lab, Mayo Clinic, 200 First Street SW – Hilton 2, Rochester, MN 55905.

17.13 Tumor biopsy schedule: Phase II

Phase II tumor biopsy will follow Phase I dose schedule 1 (See Section 17.11)

17.2 Background and Methodology

17.21 Analysis of tumor biopsies

We will examine tumors for tumor-infiltrating lymphocyte and dendritic cell phenotype and compare changes prospectively over time. Immuno-histochemistry, flow, and gene sequencing analysis will be performed. Exploratory biomarkers to predict treatment response include but are not limited to tumor-infiltrating CD4 and CD8 T cells, NK cells, and PD-1/PDL1/PDL2 expression on lymphoma and stromal cells. Leftover samples may be used for genomic or proteomic analysis.

17.22 Translational Genomics Research Institute

De-identified tissue samples will be sent for analysis to [redacted] lab at the Translational Genomics Research Institute (TGen).

Translational Genomics Research Institute (TGen)
[redacted]

18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Data submission instructions for this study can be found in the Case Report Form packet.

18.2 Event monitoring

See Section 4.4 and data submission table in the case report form packet for the event monitoring schedule.

18.3 CRF completion

This study will use Medidata Rave for remote data capture (rdc) of all study data.

18.4 Site responsibilities

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation

This study requires supporting documentation of histologic diagnosis and staging which includes the most recent tumor tissue biopsy pathology report, bone marrow biopsy report, and imaging reports. To submit these materials, they can be uploaded into the Supporting Documentation form in Medidata Rave. These reports should be submitted within 14 days of registration.

For response to treatment, supporting documentation includes imaging reports and, if applicable, a bone marrow biopsy report.

For patients who progress after study therapy, supporting documentation including imaging reports is required.

18.6 Labelling of materials

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.7 Incomplete materials

Any incomplete case report forms will be considered "not received" and will not be edited or otherwise processed until the case report forms are completed. A list of the missing case report forms will be available to the appropriate co-sponsor/participant in the Medidata Rave Task Summary

18.8 Overdue lists

A list of overdue materials and forms for study patients will be generated monthly. The listings will be sorted by location and will include the patient study registration number. The appropriate co-sponsor/participant will be responsible to submit the overdue material.

19.0 Budget Considerations

19.1 Costs charged to patient: Routine clinical care
Pembrolizumab will be provided free of charge by Merck

19.2 Tests to be research funded: Leukapheresis
Cryoablation/Anesthesia
DC and Prevnar13 injections
Correlative studies outlined in Section 14.0.

19.3 Other budget concerns: This study is supported by the following:

- The Schulze Family Foundation Career Development Grant in Individualized Medicine and The University of Iowa/Mayo Clinic Lymphoma SPORE through its grant with the National Cancer Institute (CA97274).
- Biocompatibles Inc., a BTG International group company (for a limited number of patients).

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Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

From [redacted]

Appendix II NYHA Classification

Class I: NO Symptoms with ordinary activity

Class II: Symptoms with ordinary activity

Class III: Symptoms with minimal activity

Class IV: Symptoms at rest

Appendix III FACT-Lym

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

PHYSICAL WELL-BEING

		Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy.....	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment.....	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed.....	0	1	2	3	4

SOCIAL/FAMILY WELL-BEING

		Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends.....	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends.....	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness.....	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support).....	0	1	2	3	4

Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box and go to the next section.* ☐					
GS7	I am satisfied with my sex life.....	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	EMOTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness.....	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling.....	0	1	2	3	4
GF3	I am able to enjoy life.....	0	1	2	3	4
GF4	I have accepted my illness.....	0	1	2	3	4
GF5	I am sleeping well.....	0	1	2	3	4

GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now.....	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	ADDITIONAL CONCERNS	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
P2	I have certain parts of my body where I experience pain...	0	1	2	3	4
LEU1	I am bothered by lumps or swelling in certain parts of my body (e.g., neck, armpits, or groin)	0	1	2	3	4
BRM3	I am bothered by fevers (episodes of high body temperature).....	0	1	2	3	4
ES3	I have night sweats	0	1	2	3	4
LYM1	I am bothered by itching.....	0	1	2	3	4
LYM2	I have trouble sleeping at night	0	1	2	3	4
BMT6	I get tired easily	0	1	2	3	4
C2	I am losing weight	0	1	2	3	4
Ga1	I have a loss of appetite	0	1	2	3	4
HI8	I have trouble concentrating	0	1	2	3	4
N3	I worry about getting infections	0	1	2	3	4
LEU6	I worry that I might get new symptoms of my illness.....	0	1	2	3	4
LEU7	I feel isolated from others because of my illness or treatment.....	0	1	2	3	4
BRM9	I have emotional ups and downs	0	1	2	3	4
LEU4	Because of my illness, I have difficulty planning for the future	0	1	2	3	4

Appendix IV Patient Information Sheet

You have been given a booklet to complete for this study. The booklet contains some questions about your 'quality of life' as a patient receiving treatment for cancer. Your answers will help us to better understand how the treatment you are receiving is affecting the way you feel.

1. Please complete this booklet **before** receiving your next treatment.

2. The booklet contains the Functional Assessment of Cancer Therapy – Lymphoma questionnaire.

3. Please follow the directions at the top of each page of the questionnaire.

4. It is very important that you return the booklet to us, whether you finish the study or not.

5. Please return the booklet to your health care provider when finished.

Thank you for taking the time to help us.

Appendix V DEAUVILLE 5-POINT SCALE

DEAUVILLE 5-POINT SCALE
Score 1: No Uptake
Score 2: Uptake < mediastinum
Score 3: Uptake > mediastinum but < liver
Score 4: Moderately increased uptake > liver
Score 5: Markedly increased uptake > liver and/or new lesions related to lymphoma
Score X: New areas of uptake unlikely to be related to lymphoma

Appendix VI Ann Arbor Staging System

Ann Arbor Staging for Lymphoma:

NOTE: The staging does not take into account the grade (growth rate) of the tumor tissue, or prognostic factors, such as bulky disease, LDH, age, symptomatic. See FLIPI

Bone marrow involvement and other so-called extranodal involvement is not unexpected for lymphoma can be reversed with treatment.

- **Stage I -** disease in single lymph node or lymph node region.
- **Stage II** - disease in two or more lymph node regions on same side of diaphragm.

  Note: Stage II *contiguous* means two or more lymph nodes in close proximity (side by side).
- **Stage III -** disease in lymph node regions on both sides of the diaphragm are affected.
- **Stage IV** - disease is wide spread, including multiple involvement at one or more extranodal (beyond the lymph node) sites, such as the bone marrow (which is involved commonly), liver, pleura (thin lining of the lungs).

Appendix VII FLIPI, IPI, and MIPI Tables and MIPI calculator

International Prognostic Index (IPI) (Shipp, 1993)

Risk Factors	**0 points**	**1 point**
Age	≤60 yrs.	>60 yrs.
Tumor Stage	1 or 2	3 or 4
Serum LDH	≤1 x normal	> 1 x normal
Performance Status	0 or 1	2 - 4
Number of extranodal sites	≤1	>1

Total number of risk factors = sum of the number of points for each prognostic factor.

Follicular Lymphoma International Prognostic Index (FLIPI) (Solal-Celigny, 2004):

Risk Factors	**0 points**	**1 point**
Age	<60 yrs.	≥60 yrs.
Tumor Stage	1 or 2	3 or 4
Serum LDH	≤1 x normal	> 1 x normal
Hemoglobin	≥ 12	< 12
Number of nodal groups	≤4	>4

Total number of risk factors = sum of the number of points for each prognostic factor

MIPI Calculator: Please click on link below:

[redacted]

MCL International Prognostic Index (MIPI) (Hoster, 2008):

Points	**Age (years)**	**ECOG PS**	**LDH/ULN**	**WBC, 10^9 /L**
0	< 50	0-1	< 0.67	< 6.700
1	50-59		0.67-0.99	6.700-9.999
2	60-69	2-4	1.00-1.49	10.000-14.999
3	≥70		≥1.50	≥15.000

For each prognostic factor, 0 to 3 points were given to each factor and points were summed up to a maximum of 11. Patients with 0 to 3 points in summary were classified as low risk, patients with 4 to 5 points as intermediate risk, and patients with 6 to 11 points as high risk.