Title: A Phase I Study With a Personalized NeoAntigen Cancer Vaccine in Melanoma

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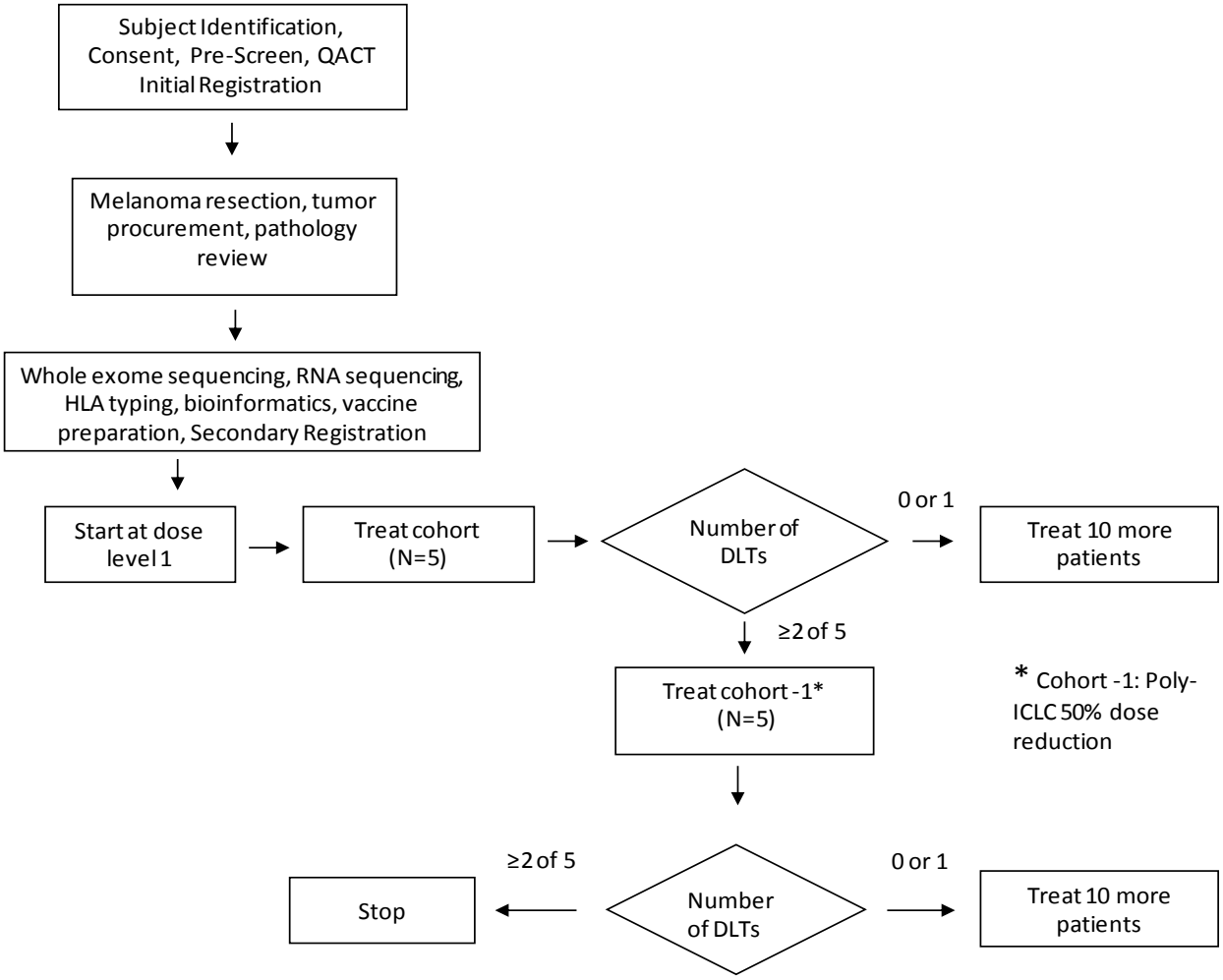
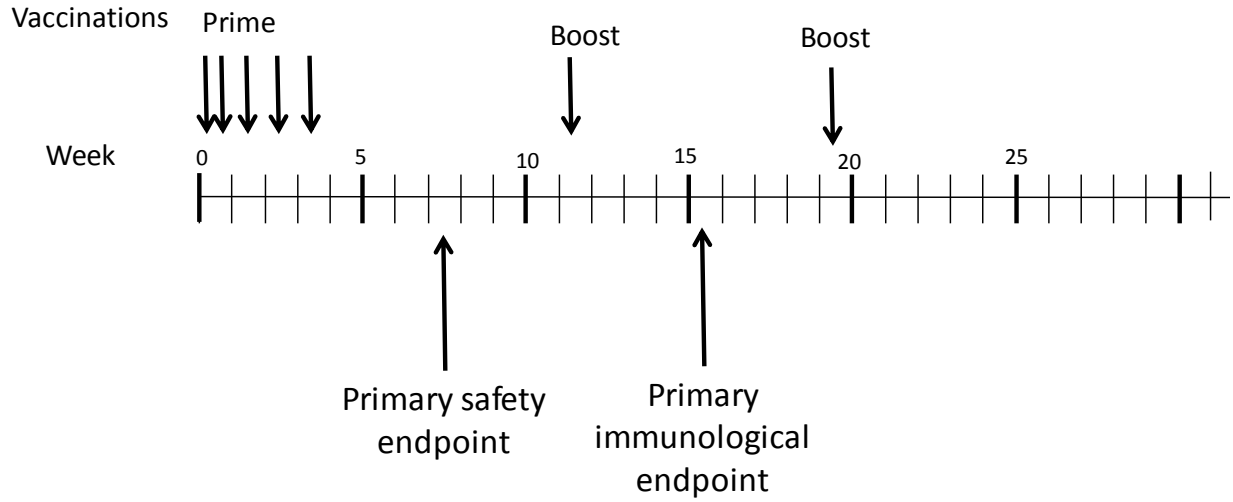
2) Poly-ICLC (Hiltonol ®) DMF BB-MF 14,266 and BB-MF 14,322 Oncovir Inc.

Schema A:



Schema B



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A Personalized NeoAntigen Cancer Vaccine in Melanoma

1. OBJECTIVES

1.1 Study Design

The study is an open label, phase I trial in which patients with high-risk melanoma will be immunized with up to 20 peptides that are both specific to the patient's tumor cells (i.e. – not found in their normal cells) and unique to the patient (i.e. – "personal"). These peptides are encoded by missense mutations, in-frame gene fusions and novel open reading frame mutations (collectively known as "neoantigens") that have occurred within that patient's tumor cells and are identified through DNA and RNA sequencing. Up to 20 peptides about 20-30 amino acids in length will be prepared for each patient and will be administered together with the immune adjuvant poly-ICLC. Thus, the personalized NeoAntigen Cancer Vaccine consists of peptides + poly-ICLC and will be termed "NeoVax".

Eligible patients will be initially entered into the trial to undergo surgery with the intent to resect all melanoma detectable clinically and on radiographic scans. Patients will be reassessed for eligibility prior to the first vaccination and will continue onto the treatment phase of the trial if secondary eligibility criteria are met.

Five patients will be entered for the initial safety evaluation (Cohort 1). If none or only 1 patient experiences a DLT on Cohort 1, an additional 10 patients will be treated at this dose to increase the likelihood of detecting serious toxicities, to complete biologic correlative endpoints and to gain preliminary experience with clinical tumor activity. If two or more patients in Cohort 1 experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then the dose of poly-ICLC will be reduced by 50% and 5 patients will be enrolled on Cohort -1. If none or only 1 patient experiences a DLT on Cohort -1, then an additional 10 patients will be enrolled as described above. If two or more patients in Cohort -1 experience a DLT, then the study will be stopped.

In parallel and under the same IND (#15703), a second phase I trial is being conducted with NeoVax (DF/HCC # 14-362 - A Phase I Study of a Personalized NeoAntigen Cancer Vaccine with Radiotherapy Among MGMT Unmethylated, Newly Diagnosed Glioblastoma Patients). Both trials utilize early stage patients who are otherwise healthy with competent immune systems. For both trials, the NeoVax personalized peptides are being produced by the same process and released to the same specifications, identical amounts are being used and the identical amount of Hiltonol® is added. In both trials the vaccination schedule is identical and the definition of Dose Limiting Toxicities is identical. In both trials, aninitial cohort of five patients is being evaluated for safety.

For the purposes of evaluation of safety in the five patient cohort for either trial, the initial patients from both trials will be combined to achieve the required 5 patient cohort. Once 5 patients from the combined trials have successfully reached the end of the DLT evaluation window (4 weeks after the last priming dose) and if fewer than two DLTs have been observed, treatment can initiate to complete the initial 5 patient cohort and for the additional 10 patients at the selected dose.

The primary objectives of the study are to test the safety and feasibility of administering NeoVax; the secondary objectives include evaluating the immune response to the personalized neoantigens and monitoring disease recurrence.

1.2 Primary Objectives

1.2.1 To evaluate safety and tolerability of administering NeoVax in patients with high-risk melanoma

1.2.2 To determine the feasibility of generating and administering NeoVax in patients with high-risk melanoma

Two feasibility endpoints will be evaluated:

(1) The proportion of patients for whom sequencing and analysis leads to identification of at least 10 actionable peptides to initiate vaccine production

(2) The proportion of patients for whom the time between surgery and vaccine availability is 12 weeks or less (excluding those patients who did not generate at least 10 actionable peptides)

1.3 Secondary Objectives

1.3.1 To assess the induction of specific cellular immune responses following administration of NeoVax

1.3.2 To determine the proportion of patients alive without progression at two years after surgery following administration of NeoVax

2 BACKGROUND

2.1 Study Agents

2.1.1 Personalized NeoAntigen Peptides

Sequencing technology has revealed that each tumor contains multiple, patient-specific mutations that alter the protein coding content of a gene[1]. Such mutations create altered proteins, ranging from single amino acid changes (caused by missense mutations) to addition of long regions of novel amino acid sequence due to frame shifts, read-through of termination codons or translation of intron regions (novel open reading frame mutations; neoORFs). These mutated proteins are valuable targets for the host's

immune response to the tumor as, unlike native proteins, they are not subject to the immune-dampening effects of self-tolerance. Therefore, mutated proteins are more likely to be immunogenic and are also more specific for the tumor cells compared to normal cells of the patient[2].

There have been several reports indicating that tumors expressing missense mutations or neoORFs can be prevented, and in some cases eradicated, by immunization with peptides corresponding to the mutated protein[3-7]. For example, $CD8^+$ cytotoxic T lymphocytes (CTLs) directed at missense mutations in the RNA helicase protein[6] or the ß2-spectrin protein[8] found in particular murine tumors have been identified. Expression of these mutated proteins by the tumor was found to correlate with tumor control or progression.

Correspondingly, several human studies of spontaneous regression and long-term survival have shown that powerful $CD8^+$ T cell responses against mutated epitopes correlate with good clinical responses[9-12]. These observations were made in both melanoma and lung cancer. Most of these $CD8^+$ T cell responses show specificity toward the mutated missense epitope compared to the native epitope, represent a high proportion of circulating T cells, and result in cells that are more abundant and active than $CD8^+$ T cell responses in the same patients directed toward over-expressed native antigens.

Two studies in humans have directly assessed the immunotherapeutic potential of mutated antigens. Follicular lymphoma is characterized by uncontrolled growth of a B cell expressing rearranged immunoglobulin. Purification of that rearranged immunoglobulin and use as a vaccine may improve disease-free survival[13]. The induced $CD8^+$ T cells showed reactivity to the rearranged, mutated portion of the immunoglobulin molecule (the idiotype) and not the germline framework[14]. Moreover, a mix of peptides corresponding to the oncogenic proteins of HPV (a neoORF for humans) has been shown to result in significant remission of premalignant lesions induced by HPV[15-17].

Thus, in animals and in humans, immune responses to both discrete mutated antigens (such as missense mutations) and expansive novel antigen (neoORF) are observationally correlated with regression and long-term remission and, in two cases, were shown to control disease following immunization. Importantly, escape from host immunosurveillance ("immunoediting") can be tracked to alterations in expression of dominant mutated antigens in mice[8,18] and man[19].

Most cancer vaccines employing peptides as immunogens have utilized "short" peptides. These peptides are typically 9 – 10 amino acids in length and capable of direct binding to the HLA molecule on the surface of HLA-expressing cells. "Long" peptides, ~20-30 amino acids in length, have recently been shown to produce a more robust and more durable immune response[15,16]. Long peptides require internalization, processing and cross-presentation in order to bind to HLA molecules; these functions

only occur in professional antigen-presenting cells, such as dendritic cells, which can induce strong T cell responses.

Many studies in humans have demonstrated the safety of peptide vaccines. These include studies with multiple short peptides[33] as well as multiple long peptides, including neoORFs. In particular, two studies have been conducted with a mixture of 10 overlapping long peptides derived from p53[28,29] and three separate studies with a mixture of 13 long peptides derived from the oncogenic proteins of HPV[17-19]. In these studies, no toxicity higher than grade 2 was observed and most adverse events were of limited duration and severity. Additionally, many heterologous antigen preparations have been tested in humans. Such preparations include irradiated cell vaccines[20,21] and tumor cell lysates[22]. These heterogeneous vaccines contain mutated antigens, in the form of intact proteins, partially degraded intracellular protein, and peptides found on the surface bound to MHC I. Moreover, they contain over-expressed and selectively-expressed molecules as well as many additional native proteins. In addition, purified heat shock protein (HSP) 96 peptide complexes have been used as antigen[23]; such complexes also contain many mutated peptides. None of these studies have reported significant safety issues directly attributable to the immunogens of the vaccines.

GMP peptides will be prepared by synthetic chemistry, purified by Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), mixed in small groups (see Sect 5.2.1.2 and Appendix B; Section 7.3.4) and combined with the immune adjuvant poly-ICLC, a stabilized double-stranded RNA (see Section 2.1.2 below). The mixtures of peptides and poly-ICLC will be used for vaccination with the intention to induce cellular immune responses directed at these patient/tumor specific mutations. Each patient will receive the full complement of peptides at each immunization.

[redacted]

2.1.2 Poly-ICLC

2.1.2.1 TLR agonists as adjuvants for cancer vaccines

Toll like receptors (TLRs) are important members of the family of pattern recognition receptors (PRRs) which recognize conserved motifs shared by many micro-organisms, termed “pathogen-associated molecular patterns” (PAMPS). Recognition of these “danger signals” activates multiple elements of the innate and adaptive immune system. TLRs are expressed by cells of the innate and adaptive immune systems such as dendritic cells (DCs), macrophages, T and B cells, mast cells, and granulocytes and are localized in different cellular compartments, such as the plasma membrane, lysosomes, endosomes, and endolysosomes[24]. Different TLRs recognize distinct PAMPS. For example, TLR4 is activated by LPS contained in bacterial cell walls, TLR9 is activated

by unmethylated bacterial or viral CpG DNA, and TLR3 is activated by double stranded RNA[25]. TLR ligand binding leads to the activation of one or more intracellular signaling pathways, ultimately resulting in the production of many key molecules associated with inflammation and immunity (particularly the transcription factor NF-κB and the Type-I interferons). TLR mediated DC activation leads to enhanced DC activation, phagocytosis, upregulation of activation and co-stimulation markers such as CD80, CD83, and CD86, expression of CCR7 allowing migration of DC to draining lymph nodes and facilitating antigen presentation to T cells, as well as increased secretion of cytokines such as type I interferons, IL-12, and IL-6. All of these downstream events are critical for the induction of an adaptive immune response.

Among the most promising cancer vaccine adjuvants currently in clinical development are the TLR9 agonist CpG and the synthetic double-stranded RNA (dsRNA) TLR3 ligand poly-ICLC. In preclinical studies poly-ICLC appears to be the most potent TLR adjuvant when compared to LPS and CpG due to its induction of pro-inflammatory cytokines and lack of stimulation of IL-10, as well as maintenance of high levels of co-stimulatory molecules in DCs[26]. Furthermore, poly-ICLC was recently directly compared to CpG in non-human primates (rhesus macaques) as adjuvant for a protein vaccine consisting of human papillomavirus (HPV)16 capsomers. Poly-ICLC was found to be much more effective in inducing HPV specific Th1 immune responses[27].

2.1.2.2 Poly-ICLC – a synthetic TLR3 agonist with strong vaccine adjuvant properties

Poly-ICLC is a synthetically prepared double-stranded RNA consisting of polyI and polyC strands of average length of about 5000 nucleotides, which has been stabilized to thermal denaturation and hydrolysis by serum nucleases by the addition of polylysine and carboxymethylcellulose. The compound activates TLR3 and the RNA helicase-domains of MDA5 and RIG3, both members of the PAMP family, leading to DC and natural killer (NK) cell activation and production of a "natural mix" of type I interferons, cytokines, and chemokines. Furthermore, poly-ICLC exerts a more direct, broad host-targeted anti-infectious and possibly anti-tumor effect mediated by the two IFN-inducible nuclear enzyme systems, the 2'5'-OAS and the P1/eIF2a kinase, also known as the PKR (4-6), as well as RIG-I helicase and MDA5.

In rodents and non-human primates, poly-ICLC was shown to enhance T cell responses to viral antigens[28-31], cross-priming, and the induction of tumor-, virus-, and autoantigen-specific $CD8^+$ T-cells[32-34]. In a recent study in non-human primates, poly-ICLC was found to be essential for the generation of antibody responses and T-cell immunity to DC targeted or non-targeted HIV Gag p24 protein, emphasizing its effectiveness as a vaccine adjuvant.

In human subjects, transcriptional analysis of serial whole blood samples revealed similar gene expression profiles among 8 healthy human volunteers receiving one single s.c. administration of poly-ICLC and differential expression of up to 212 genes between these 8 subjects versus 4 subjects receiving placebo[35]. Remarkably,

comparison of the poly-ICLC gene expression data to previous data from volunteers immunized with the highly effective yellow fever vaccine YF17D[36] showed that a large number of transcriptional and signal transduction canonical pathways, including those of the innate immune system, were similarly upregulated at peak time points.

More recently, an immunologic analysis was reported on patients with ovarian, fallopian tube, and primary peritoneal cancer in second or third complete clinical remission who were treated on a phase 1 study of subcutaneous vaccination with synthetic overlapping long peptides (OLP) from the cancer testis antigen NY-ESO-1 alone or with Montanide-ISA-51, or with 1.4 mg poly-ICLC and Montanide. The generation of NY-ESO-1-specific CD4+ and $CD8^+$ T-cell and antibody responses were markedly enhanced with the addition of poly-ICLC and Montanide compared to OLP alone or OLP and Montanide[37].

2.1.2.3 Pre-clinical toxicology of poly-ICLC

Complete information on the pre-clinical toxicology studies can be found in the poly-ICLC (Hiltonol®) Investigator Brochure (IB). The results of these toxicology studies are summarized in brief below:

Single dose toxicity:

All administrations of poly-ICLC were intravenous (IV).
Rodents. The median lethal dose (LD50) values for single dose poly-ICLC were approximately 15-18.3 mg/kg in rats and 25-30 mg/kg in mice. No necropsies were conducted.
Dogs. In beagle dogs, the lethal dose of single dose poly-ICLC administration was 4.0 mg/kg. Necropsy indicated toxic effects in the gastrointestinal, hepatic, cardiovascular, renal, endocrine, and lymphatic systems. Sublethal doses caused reversible nephrotoxicity and hepatotoxicity. The non-toxic dose for dogs was 0.25 mg/kg.
Primates. In rhesus monkeys, single-dose administration of poly-ICLC was non-toxic at a dose of .5 mg/kg, but lethal at 20 mg/kg. Toxic effects were seen in the gastrointestinal, hepatic, cardiovascular, renal, endocrine, and lymphatic systems.

Repeated dose toxicity:

Rodents. Mice treated with four daily IV doses of up to 3.66 mg/kg poly-ICLC exhibited no toxicity.

Cats. One of two cats treated with the same regimen at 3.33 mg/kg poly-ICLC demonstrated vomiting, an episode of diarrhea, and moderate weight loss.

Non-human Primates. Three rhesus monkeys and 6 macaque monkeys were treated with 5 mg/kg IV of poly-ICLC over 2 days and 3 mg/kg 3 times weekly for 16 weeks, respectively; no significant toxicity was observed other than a single instance of emesis and shivering. Necropsy of the macaques showed no drug-related changes by gross or microscopic pathology of all organs. In two additional studies, macaque monkeys

were treated daily with up to 3 mg/kg IV for 12 days and chimpanzees were treated daily with 3 mg/kg IV for two 6-day periods, then every other day for a total of 7 weeks. At the 3 mg/kg doses, a decrease in hematocrit was seen in both studies. No change in hematocrit was seen at 0.3 mg/kg. In the chimpanzee study, in addition to a decrease in hematocrit, leukocytosis and elevations in alanine aminotrasferase (ALT) and aspartate aminotrasferase (AST) were observed and normalized after termination of dosing. No other significant toxicity was observed in these studies.

2.1.2.4 Clinical toxicology of poly-ICLC

Poly-ICLC is the TLR3 agonist formulation most extensively tested in patients with infectious diseases and in subjects with a variety of different tumor types.

Prior to the availability of recombinant interferon, poly-ICLC was used clinically at high doses $\geq$ 6mg/m^2 (about 170 μg/kg) in patients with a variety of solid tumors and leukemia[38]. Fever, often above 40^{o}C, was a common adverse event and the primary dose-limiting factor. Other common adverse events were flu-like symptoms (nausea, vomiting, arthralgia. myalgia and fatigue) and hypotension, thrombocytopenia and leukopenia. Once recombinant interferon became clinically available, the need to pursue high dose poly-ICLC was eliminated, and it became recognized that lower doses (10 – 50 μg/kg) were highly effective at stimulating host defense and as an immune adjuvant.

By now, more than 400 patients with malignant gliomas have been entered on 7 clinical trials using low dose (1-2 mg total dose) poly-ICLC either as monotherapy or in conjunction with chemotherapy, radiation, or vaccine (Table 1). Furthermore, patients with various other solid tumors (prostate, colorectal, pancreatic, hepatocellular, breast, and ovarian cancers), in addition to patients with HIV/AIDS and multiple sclerosis have been treated on more than 10 additional clinical phase I and phase II studies. Overall, the drug has been well-tolerated across all studies and spectrum of diseases. The most common adverse events attributed as at least possibly related to poly-ICLC have included:

- bone marrow toxicity (leukopenia, neutropenia, thrombocytopenia, and anemia)
- reversible liver toxicity (AST/ALT elevations, LDH and alkaline phosphatase elevation)
- transient discomfort at the injection site
- transient fatigue/malaise
- transient flu-like symptoms

In a recently published phase I clinical trial, 11 patients with ovarian, fallopian tube, or primary peritoneal cancer were treated with poly-ICLC at a total dose of 1.4 mg in combination with Montanide-ISA-51 and synthetic over-lapping long peptides from NY-ESO-1. The vaccine was generally well-tolerated with grade 1-2 injection site

reactions and fatigue as the only adverse events that were considered possibly or definitely drug-related[37]. No grade 3 or 4 adverse events were reported.

In another clinical study which recently completed accrual (ClinicalTrials.gov Identifier NCT01079741) 27 patients with high-risk melanoma received poly-ICLC at a total dose of 1.4 mg in addition to NY-ESO-1 protein ± Montanide-ISA-51 given s.q. every 3 weeks for 4 doses (in addition to 6 patients who received 0.35 mg and 0.70 mg, respectively of poly-ICLC on cohorts 1 and 2 of the dose escalation part of the trial). The vaccine was generally well-tolerated with grade 1 and 2 injection site reactions and transient, grade 1 or 2, flu-like symptoms that resolved after 12-48 hours [redacted].

Table 1 Clinical Trials with Low-Dose poly-ICLC

					Patients			
Protocol Title	Phase	Protocol Location	Indication	Status	N	Active	Year of initation	Dosing Schedule
Long-term IM poly-ICLC in Malignant Glioma – an Open Pilot Study	III	Walter Reed	Malignant Glioma	Closed	67	0	1996	IM 10-50 µg/kg 1-3 X week
Poly-ICLC in Recurrent Malignant Brain Tumors, an Open Label Study	III	MCV	Recurrent Glioma	Closed	99	0	2000	IM 20 µg/kg 3 X week
Poly-ICLC in Malignant Pediatric Brain Tumors	III	L.A. Childrens Hosp	Pediatric Glioma	Closed	46	0	2002	IM 20 µg/kg 2 X week
Poly-ICLC plus Radiation in Glioblastoma	II	NABTC 2001-05	New Glioblastoma	Closed	31	0	2006	IM 20 µg/kg 3 X week
Poly-ICLC in Recurrent Anaplastic Glioma	II	NABTC 2001-06	Recurrent Anaplastic Glioma	Closed	55	6	2006	IM 20 µg/kg 3 X week
Poly-ICLC plus Temodar in Newly Diagnosed Glioblastoma	II	NABTT 2005-01	New Glioblastoma	Closed	97	24	2006	IM 20 µg/kg 3 X week
Pilot Study of MUC1 Vaccine plus poly-ICLC in Advanced Prostate Cancer	III	UPMC05-086	Advanced Prostate Cancer	Open	25	15	2006	IM 25 µg/kg 2 X week
Poly-ICLC plus Dendritic Cell vaccine in Recurrent Gliomas	I	UPMC	Recurrent malignant gliomas	Open	25	20	2007	IM 20 mcg/kg
Poly-ICLC plus HSP-HPVE7 Vaccine in Cervical Dysplasia	I	Nventa	Cervical Dysplasia	Closed	24	5	2007	.05 – 2 mg
IntraTumoral poly-ICLC plus Radiation and TACE in Liver Cancer	I	UMDNJ	Hepatoma, Metastatic pancreatic cancer	Open	31	1	2007	.25-2 mg

Table 1 Clinical Trials with Low-Dose poly-ICLC								
A Randomized Controlled Phase I dose escalation trial of Nasal Hiltonol in normal volunteers.	I	NIAID, NIH:	Normal volunteers	Closed	56	0	2009	.25-4mg IN
PSMA and TARP peptide with poly ICLC adjuvant in … Prostate Cancer.	I/II	Moffit Cancer Center	Prostate Cancer	Open	30		2009	1 mg
CDX 1307 vaccine with poly-ICLC in metastatic cancers	I	Various	Metastatic Cancers	Closed	20		2009	2 mg IM
Poly-ICLC with glioma associated peptide vaccine	I/II	UPMC	Grade II Gliomas	Open	20		2009	20 mcg/kg IM 2X Wk
MUC1 Hundred-mer and poly-ICLC Vaccine for Triple-Negative Breast Cancer.	I/II	Case Western	Triple negative breast cancer	Open	37		2009	50 mg IM
MUC1 100-mer and poly-ICLC Vaccine for Colonic Polyposis	I/II	UPMC	Colonic Adenoma	Open	45		2009	.5 mg SC
Hiltonol +NYESO1 Protein Vaccine in Ovarian Cancer	I/II	MSKCC, LICR	Ovarian Cancer	Closed	28	0	2009	1.4 mg SC

2.2 Study Disease

Melanoma incidence rates have been increasing for at least 30 years. It is estimated that 76,690 individuals will be diagnosed in 2013[39]. The disease is usually curable when detected in its early stages (thin primary tumor, no lymph node involvement). However, the prognosis is not as favorable for thicker (and/or ulcerated) primary melanomas and for melanomas with regional lymph node involvement, in transit disease, or satellite metastatic lesions. Patients with resectable stage IIIB, IIIC face a risk for disease recurrence of 60-80% at two years, with 5-year overall survival rates ranging between 25 to 50%[40,41] Patients with resectable distant metastatic disease have a risk for disease recurrence of approximately 80% at two years, with a 4-year overall survival rate of approximately 30%[42]

For patients with unresectable or metastatic melanoma, recently emerged novel systemic treatment modalities such as CTLA-4, PD-1/PD-L1 blockade, and BRAF and MEK inhibition have markedly widened the spectrum of therapeutic options, although the intention of treatment remains palliation rather than cure for the majority of these patients[43]. In contrast, for patients with surgically resected high risk melanoma, who are potentially curable, IFN-α is the only agent that has proven clinical efficacy in randomized phase 3 studies yet has an unfavorable toxicity profile and only a small, if any, overall survival benefit[44-46]. For patients with resected melanomas in high risk categories, effective systemic

adjuvant treatment strategies are critical in order to improve recurrence rates. Importantly, preventing metastatic disease by effectively reducing recurrence rates in the adjuvant setting (patients with no evidence of disease) may be seen as surrogate for curing patients with disease. Immunotherapy has been recognized decades ago as a potentially effective treatment in melanoma. In the adjuvant setting, the purpose of vaccination is to initiate or re-stimulate endogenous, melanoma-specific T and B cells to react against and eradicate minimal residual disease.

2.3 Rationale

This phase 1 study seeks to demonstrate that the combination of personalized neoantigen peptides and poly-ICLC is safe, feasible, and induces strong tumor-specific T cell immunity.

DNA sequencing, particularly next-generation sequencing technology, has revealed a genetic landscape of cancer that contains many protein coding mutations found uniquely in the tumor of an individual patient. These mutations include both single-amino acid missense mutations (the predominant type) and novel open reading frames created by frame-shift or read-through mutations (neoORFs) varying in length from 1 to up to 100's of amino acids. There is evidence in both animals and humans demonstrating that such mutated epitopes are effective at inducing an immune response[47]. Importantly, strong $CD8^+$ T cell responses against mutated epitopes have been found in patients with melanoma and non small cell lung cancer who either had a spontaneous regression or a dramatic response after adoptive T cell transfer, suggesting that there may be an association with $CD8^+$ T cell activity and good clinical responses[9-12,48,49]. Most of these $CD8^+$ T cell responses showed very good specificity toward the mutated epitope compared to the native epitope, represented a high proportion of circulating T cells, and induced cells that were more abundant and active than $CD8^+$ T cells in the same patients directed toward over-expressed native antigens.

Two studies in humans have directly assessed the immunotherapeutic potential of mutated antigens. Purified rearranged immunoglobulin expressed by malignant B cells in follicular lymphoma has been used as vaccine and has led to good clinical outcomes in some phase 2 and phase 3 studies (possibly dependent on amounts of residual disease)[50]. More recently, a mix of peptides corresponding to the oncogenic proteins of HPV (directly analogous to the neoORF type of mutation) has been shown to result in significant remission of premalignant lesions induced by HPV[15-17]. Thus, in animals and in humans, immune responses to both discrete mutated antigens (such as missense mutations) and expansive novel antigen (neoORF) are observationally correlated with regression and long-term remission and, in two clinical studies, have been shown to control disease following therapeutic vaccination. The direct and comprehensive identification of the many mutated epitopes found in cancer genomes creates the opportunity to use this class of immunogen to improve the immune response and efficacy of cancer vaccines.

Translating sequencing information into a therapeutic vaccine further requires:

(1) *Prediction of mutated peptides that can bind to personal HLA molecules.* Efficiently choosing which particular mutations to utilize as immunogen requires identification of the patient HLA

type and the ability to predict which mutated peptides would efficiently bind to the patient's HLA alleles. Recently, neural network based learning approaches with validated binding and non-binding peptides have advanced the accuracy of prediction algorithms for the major HLA-A and -B alleles[51]. In a pilot study, we prepared seventy-four 9-mer and sixty-three 10-mer peptides predicted by the algorithm NetMHCpan to have affinities to particular HLA alleles below 1000 nM, with most being below 500 nM. Binding of these peptides to their cognate HLA alleles was experimentally determined using a well-established competitive binding assay[52]. Seventy-five percent of 9-mer peptides predicted to have an affinity below 150 nM were experimentally verified to be below 150 nM and 90% of these peptides were shown to have an affinity below 500 nM, a generally accepted threshold . Fifty percent of 9-mer peptides predicted to have an affinity between 150 and 500 nM were shown to have an affinity below 500 nM. Ten-mer peptide predictions were slightly less precise. For predicted high affinity 10-mer peptides (below 150 nM), 70% were shown to have an affinity below 500 nM. However, only 35% of 10-mer peptides predicted to have an affinity between 150 and 500 nM were shown to have an affinity below 500 nM. Thus, 10-mer peptides with predicted affinities above 150 nM will not be utilized. These predictions provide a reliable tool, when correctly applied, to assist in the selection of potentially immunogenic peptides.

(2) *Formulating the drug as a multi-epitope vaccine of long peptides.* For each patient, multiple neoantigens will be targeted. Targeting as many mutated epitopes as practically possible takes advantage of the enormous capacity of the immune system, prevents the opportunity for immunological escape[53] by down-modulation of a particular immune targeted gene product, and compensates for the known inaccuracy of epitope prediction approaches. Synthetic peptides provide a particularly useful means to prepare multiple immunogens efficiently and to rapidly translate identification of mutant epitopes to an effective vaccine. Peptides can be readily synthesized chemically and easily purified utilizing reagents free of contaminating bacteria or animal substances. The small size allows a clear focus on the mutated region of the protein and also reduces irrelevant antigenic competition from other components (unmutated protein or viral vector antigens). "Long" peptides, ~20-30 amino acids in length (as opposed to "short" peptides, ca 8-10 amino acids) have recently been shown to produce a more robust and more durable immune responses[15,16].

(3) *Combination with a strong vaccine adjuvant*. Effective vaccines require a strong adjuvant to initiate an immune response. As described in Section 2.1.2.2, poly-ICLC, an agonist of TLR3 and the RNA helicase domains of MDA5 and RIG3, has shown several desirable properties for a vaccine adjuvant. These properties include the induction of local and systemic activation of immune cells *in vivo*, production of stimulatory chemokines and cytokines, and stimulation of antigen-presentation by DCs. Furthermore, poly-ICLC can induce durable $CD4^+$ and $CD8^+$ responses in humans. Importantly, striking similarities in the upregulation of transcriptional and signal transduction pathways were seen in subjects vaccinated with poly-ICLC and in volunteers who had received the highly effective, replication-competent yellow fever vaccine[35]. Furthermore, >90% of ovarian carcinoma patients immunized with poly-ICLC in combination with a NY-ESO-1 peptide vaccine (in addition to Montanide) showed induction of $CD4^+$ and $CD8^+$ T cell, as well as antibody responses to the peptide in a recent

phase 1 study[37]. Moreover, poly-ICLC has been extensively tested in more than 25 clinical trials to date and exhibited a relatively benign toxicity profile.

2.4 Correlative Studies Background

The primary immunological endpoint of this study will be the assessment of T cell response measured by *ex vivo* IFN-γ ELISPOT. IFN-γ secretion occurs as a result of the recognition of cognate peptides or mitogenic stimuli by $CD4^+$ and/or $CD8^+$ T –cells. A multitude of different $CD4^+$ and $CD8^+$ determinants will likely be presented to T cells *in vivo* since the 20-30-mer peptides used for vaccination should undergo processing into smaller peptides by antigen presenting cells. We hypothesize that the combination of personalized neoantigen peptides, which are novel to the immune system and thus not subject to the immune-dampening effects of self-tolerance, and the powerful immune adjuvant poly-ICLC will induce strong $CD4^+$ and/or $CD8^+$ responses. The expectation is therefore that T cell responses are detectable *ex vivo,* i.e. without the need for *in vitro* expansion of epitope specific T cells through short-term culture. Patients will initially be evaluated using the total pool of peptide immunogens as stimulant in the ELISPOT assay. For patients demonstrating a robust positive response, the precise immunogenic peptide(s) will be determined in follow-up analyses.

The IFN-γ ELISPOT is generally accepted as a robust and reproducible assay to detect *ex vivo* T cell activity and determine specificity. In addition to the analysis of the magnitude and determinant mapping of the T cell response in peripheral blood monocytes, other aspects of the immune response induced by the vaccine are critical and will be assessed. These evaluations will be performed in patients who exhibit an *ex vivo* IFN-γ ELISPOT response in the screening assay. They include the evaluation of T cell subsets (Th1 versus Th2, T effector versus memory cells), analysis of the presence and abundance of regulatory cells such as T regulatory cells or myeloid derived suppressor cells, and cytotoxicity assays if patient-specific melanoma cells lines are successfully established.

3 PATIENT SELECTION

3.1 Eligibility Criteria

Eligibility to participate will be assessed at two timepoints: prior to surgery (Initial Registration) and prior to the first vaccination (Secondary Registration).

3.1.1 Eligibility Criteria for Initial Registration

Patients must meet the following criteria prior to surgery to be eligible for initial registration onto the study:

3.1.1.1 Patient is willing and able to give written informed consent.

3.1.1.2 Patient is agreeable to allow tumor and normal tissue samples to be submitted for complete exome and transcriptome sequencing.

3.1.1.3 Pathologically confirmed, clinically evident (by physical examination or radiographic imaging) stage IIIB, IIIC, and IVM1a and b cutaneous melanoma (anatomic stages T1-4b N1a and T1-4b N2a not included). The current diagnosis may be the patient's first diagnosis of melanoma or recurrent melanoma after previous diagnosis of an earlier stage melanoma.

3.1.1.4 Complete surgical resection of metastatic disease (lymph node, in transit, satellite lesion(s), distant metastases) with negative margins on resected specimens as confirmed by pathologic review has not been performed, but is deemed feasible by the treating surgical oncologist. Surgical resection of the primary melanoma may or may not have been performed.

3.1.1.5 The patient must be free of unresectable metastatic disease within 4 weeks prior to the surgery being performed with the intention to remove all melanoma. This pre-surgery baseline assessment must be documented by complete physical examination and imaging studies. Imaging studies must include a total body PET-CT in conjunction with a brain MRI (or head CT if brain MRI is contraindicated). If a PET/CT scan cannot be done, a CT of the neck, chest, abdomen, and pelvis should be performed.

3.1.1.6 Patients may have received prior interferon alpha (IFN-α), but must not have received IFN-α in the 4-week period prior to initiation of first vaccination with NeoVax. Patients who have not received prior adjuvant therapy should be informed of the potential therapeutic benefit of IFN-α. Previous radiation therapy, including after the surgical resection, is allowed as long as 14 days have elapsed between the radiation and initiation of first vaccination with NeoVax.

3.1.1.7 Age $\geq$ 18 years.

3.1.1.8 ECOG performance status $\leq$1.

3.1.1.9 Normal organ and bone marrow function as defined below:

- Leukocytes $\geq$ 3,500/mcL
- Absolute lymphocyte count $\geq$ 800/mcL
- Absolute neutrophil count $\geq$ 1,500/mcL
- Platelets $\geq$ 100,000/mcL
- Hemoglobin $\geq$ 9.0 g/dL
- Total serum bilirubin $\leq$ 1.0 x institutional upper limit of normal
- AST (SGOT)/ALT (SGPT) $\leq$ 2.0 x institutional upper limit of normal
- Serum creatinine $\leq$ 1.5 x institutional upper limit of normal

3.1.1.10 Women of childbearing potential (WOCBP) must have a negative pregnancy test (minimum sensitivity 25 IU/L or equivalent of HCG) before entry onto the trial, because the effects NeoVax on the developing human fetus are unknown.

3.1.1.11 Female patients enrolled in the study, who are not free from menses for >2 years, post hysterectomy / oophorectomy, or surgically sterilized, must be willing to use either 2 adequate barrier methods *or* a barrier method plus a hormonal method of contraception to prevent pregnancy or to abstain from sexual activity throughout the study, starting with visit 1 through 4 weeks after the last dose of study therapy. Approved contraceptive methods include for example; intra uterine *dev*ice, diaphragm with spermicide, cervical cap with spermicide, male condoms, or female condom with spermicide. Spermicides alone are not an acceptable method of contraception.

3.1.1.12 Male patients must agree to use an adequate method of contraception starting with the first dose of NeoVax through 4 weeks after the last dose of study therapy.

3.1.2 Eligibility Criteria for Secondary Registration

3.1.2.1 ECOG performance status $\leq$1.

3.1.2.2 Normal organ and bone marrow function as defined below:

- Leukocytes $\geq$ 3,500/mcL
- Absolute lymphocyte count $\geq$ 800/mcL
- Absolute neutrophil count $\geq$ 1,500/mcL
- Platelets $\geq$ 100,000/mcL
- Hemoglobin $\geq$ 9.0 g/dL
- Total serum bilirubin $\leq$ 1.0 x institutional upper limit of normal
- AST (SGOT)/ALT (SGPT) $\leq$ 2.0 x institutional upper limit of normal
- Serum creatinine $\leq$ 1.5 x institutional upper limit of normal

3.1.2.3 Women of childbearing potential (WOCBP) must have a negative pregnancy test (minimum sensitivity 25 IU/L or equivalent of HCG) within 7 days prior to start of study medication, because the effects NeoVax on the developing human fetus are unknown. It is the investigators' responsibility to repeat the pregnancy test should start of treatment be delayed.

3.1.2.4 Female patients enrolled in the study, who are not free from menses for >2 years, post hysterectomy / oophorectomy, or surgically sterilized, must be willing to use either 2 adequate barrier methods *or* a barrier method plus a hormonal method of contraception

to prevent pregnancy or to abstain from sexual activity throughout the study, starting with visit 1 through 4 weeks after the last dose of study therapy. Approved contraceptive methods include for example; intra uterine device, diaphragm with spermicide, cervical cap with spermicide, male condoms, or female condom with spermicide. Spermicides alone are not an acceptable method of contraception.

3.1.2.5 Male patients must agree to use an adequate method of contraception starting with the first dose of NeoVax through 4 weeks after the last dose of study therapy.

3.2 Exclusion Criteria

Patients who exhibit any of the following conditions at any point prior to first vaccination will not be eligible for admission to or continuation on the study:

3.2.1 Prior treatment with immune-modulatory agents including, but not limited to: IL-2, CTLA-4 blockade, PD-1/PD-L1 blockade, CD40 stimulation, CD137 stimulation with the exception of INF-α given as adjuvant treatment for high-risk, surgically resected melanoma

3.2.2 Prior investigational melanoma-directed cancer vaccine therapy

3.2.3 Prior chemotherapy, including targeted therapy such as BRAF or MEK inhibition

3.2.4 Treatment with other investigational products within the last 2 months prior to entry into this study

3.2.5 Previous bone marrow or stem cell transplant

3.2.6 Concomitant therapy with any anti-cancer agents, other investigational anti-cancer therapies, or immunosuppressive agents; chronic use of systemic corticosteroids

3.2.7 Use of a non-oncology vaccine therapy for prevention of infectious diseases during the 4 week period prior to first dose of NeoVax administration. Patients may not receive any non-oncology vaccine therapy during the period of NeoVax administration and until at least 8 weeks after the last dose of study therapy.

3.2.8 History of severe allergic reactions attributed to any vaccine therapy for the prevention of infectious diseases

3.2.9 Mucosal melanoma and uveal melanoma

3.2.10 Active, known, or suspected autoimmune disease or immunosuppressive conditions with the exception of vitiligo, type 1 diabetes, residual autoimmune-related hypothyroidism requiring hormone replacement, or psoriasis not requiring systemic treatment

3.2.11 Concomitant treatment with corticosteroids greater than physiologic doses (used in the management of cancer or non-cancer-related illnesses). Topical (if not including the proposed vaccination sites) or inhalational steroids are allowed.

3.2.12 Known chronic infections with HIV, hepatitis B or C

3.2.13 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia

3.2.14 Any underlying medical condition, psychiatric condition or social situation that in the opinion of the investigator would compromise study administration as per protocol or compromise the assessment of AEs

3.2.15 Pregnant women are excluded from this study because personalized neoantigen peptides and poly-ICLC are agents with unknown risks to the developing fetus. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with personalized neoantigen peptides and poly-ICLC, nursing women are excluded from this study.

3.2.16 Individuals with history of an invasive malignancy except for the following circumstances: a) individuals with a history of invasive malignancy are eligible if they have been disease –free for at least 3 years and are deemed by the investigator to be at low risk for recurrence of that malignancy; b) individuals with any of the following cancers are eligible if diagnosed and treated: carcinoma in situ of the breast, oral cavity or cervix and basal cell or squamous cell carcinoma of the skin.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

This protocol does not exclude women, minorities, or other underrepresented populations. The inclusion and exclusion criteria are not expected to have a negative effect on the recruitment or retention of underrepresented populations.

4 REGISTRATION PROCEDURES

Eligibility will be confirmed and participants registered on 2 separate, serial occasions on this trial:

1. Prior to surgical resection:
   Prior to initial surgical resection, the patient is screened for eligibility using the initial eligibility checklist. Once overall trial initial eligibility is confirmed, patient is registered, undergoes surgical resection and preparation of the participant's vaccine is initiated.

2. Prior to first vaccination:
   As participant's 1st study vaccine dose may not be administered until 10-17 weeks from initial study registration, there will be a secondary "screen" conducted prior to first vaccination to ensure s/he meets criteria to receive study treatment.
   If confirmed eligible for this next phase of the study (eligible to treat as per sections 3.1.2 and 3.2), participant will proceed to receive vaccines on study.

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

The study team will register eligible patients with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Initial Registration must occur prior to surgical resection. Any patient not registered to the protocol before surgical resection begins will be considered ineligible and registration will be denied. Secondary Registration must occur prior to initiation of study vaccine administrations. Any patient not registered to the protocol before receiving protocol vaccine treatment will be considered ineligible and registration will be denied.

For both registration timepoints, an investigator will confirm eligibility criteria and a member of the study team will complete the appropriate protocol-specific eligibility checklist:

- Initial Eligibility Checklist for Overall Study Participation (for Initial Registration)
- Secondary Eligibility Checklist to Initiate Treatment (for Secondary Registration)

Following each registration stage, participants may begin the relevant protocol therapy. Issues that would cause treatment delays beyond this timeframe should be discussed with the Principal Investigator (PI). If a participant does not receive study therapy as outlined in protocol following either registration, the participant's registration must be canceled. Notify the QACT Registrar of registration cancellations as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The Initial Registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any protocol-specific screening procedures or assessments.
2. Complete the QACT protocol-specific eligibility checklist:

   Initial Eligibility Checklist for Overall Study Participation (for Initial Registration)

   using the initial eligibility assessment documented in the patient's medical/research record.

   To be eligible for initial registration to the study, the participant must meet each inclusion and exclusion criteria listed on the initial eligibility checklist.

   Reminder**:** Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.
3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at [redacted].

   For Phase I protocols, attach participant dose level assignment confirmation from the sponsor.
4. The QACT Registrar will (a) review the initial eligibility checklist, (b) initially register the participant on the study, and (c) randomize the participant when applicable.

5. An email confirmation of the registration and/or randomization will be sent to the PI, study coordinator(s) from QACT immediately following the registration and/or randomization.

The Secondary Registration procedures are as follows:

1. Complete the QACT protocol-specific eligibility checklist:
   Secondary Eligibility Checklist to Initiate Treatment (for Secondary Registration)
   using the secondary eligibility assessment documented in the patient's medical/research record.
   To be eligible for secondary registration to the study, the participant must meet each inclusion and exclusion criteria listed on the secondary eligibility checklist.
   Reminder**:** Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.
2. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at [redacted].
   For Phase I protocols, attach participant dose level assignment confirmation from the sponsor.
3. The QACT Registrar will (a) review the secondary eligibility checklist, (b) register the participant on the study, and (c) randomize the participant when applicable.
4. An email confirmation of the registration and/or randomization will be sent to the PI, study coordinator(s) from QACT immediately following the registration and/or randomization.

5 TREATMENT PLAN

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for NeoVax are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Patients with clinically evident locoregional metastatic disease or fully resectable distant metastatic disease as defined in 3.1.3 will be identified and enrolled on the study. After signing consent, the patients will undergo surgery with the intention to remove all melanoma (curative intent). Tissue procurement will be performed as described in 5.1. After confirmation of complete tumor resection by pathologic review and determination of adequacy of DNA and RNA yield from tumor tissue for sequencing, the generation of the vaccine as described in 5.2.1 will be initiated. Patients with tumors that are not completely resected and/or that provide inadequate DNA and/or RNA for sequencing will be removed from the trial and replaced.

5.1 Tissue procurement and sequencing

5.1.1 Tumor and normal tissue harvest

Patients will undergo complete resection of their primary melanoma (if not already removed) and all regional metastatic disease with the intent of rendering them free of melanoma. Dr. Patrick Ott, the principal investigator [redacted] should be contacted at the time of the initial consent to ensure that ancillary studies are coordinated. The study investigators and members of the study team will coordinate tissue acquisition with the surgical staff of the participating institution and the staff of the DFCI/Cell Manipulation Core Facility.

After adequate tumor for pathological assessment has been harvested as deemed by the surgeon, remaining tumor tissue will be placed in sterile media in a sterile container and transferred to the DFCI/Cell Manipulation Core Facility for disaggregation. Portions of the tumor tissue will be used for whole-exome and transcriptome sequencing and cell line and tumor infiltrating lymphocyte generation and any remaining tumor will be cryopreserved or given to the sponsor for protocol-related studies. In the event that sequence analysis yields no results or sub-optimal results, tumor cell line cells or paraffin embedded samples retained by the participating institutions pathology department may be used to prepare additional nucleic acid for sequencing. No saved samples will be used to prepare cellular products for future clinical use. Peripheral blood mononuclear cells from a blood draw will be utilized for a normal tissue sample.

5.1.2 Nucleic acid will be extracted from the tissue samples and sequencing will be conducted at the CLIA-certified laboratory at Broad Institute. For tumor and normal DNA samples, whole exome capture will be conducted prior to sequencing on Illumina HiSeq. For tumor RNA, a cDNA library will be prepared on poly-A selected RNA prior to sequencing on Illumina HiSeq. If the quantity or quality of DNA or RNA isolated from the tissue sample is inadequate for exome or cDNA library preparation and sequencing, then DNA or RNA may be extracted from the patient-specific tumor cell line (if generated) or paraffin embedded samples.

5.2 Preparation of NeoVax

5.2.1 Preparation of personalized neoantigen peptides

5.2.1.1 Identification of target epitopes for peptide design

[redacted]

[redacted]

5.2.1.2 Peptide synthesis

[redacted]

5.2.2 Preparation of NeoVax peptide pools

[redacted]

5.2.3 Pretreatment criteria

Preparation of the final NeoVax product will begin upon confirmation that the patient is medically cleared to undergo vaccine administration (confirmed arrival in the clinic,

stable vital signs, no new acute medical issues or laboratory abnormalities potentially interfering with vaccine administration).

5.2.3.1 Day 1

Laboratory parameters must be reviewed and must be within the ranges as listed in 3.1.1.9 prior to administration of the study agents. No specific prophylactic or supportive care is necessary prior to administration of the study agents.

5.2.3.2 Subsequent treatment days

Laboratory parameters must be reviewed and must be within the ranges as listed in 3.1.1.9 prior to administration of the study agents. No specific prophylactic or supportive care is necessary prior to administration of the study agents, however, patients who have developed flu-like symptoms that were controlled with acetaminophen or non-steroidal anti-inflammatory drugs during previous treatments may be pre-treated with acetaminophen (650 mg PO) or ibuprofen (400-600mg PO) as per the investigator's discretion.

5.2.4 Preparation of the dosage form

[redacted]

5.3 Administration

Treatment Description					
Agent	**Treatment phase**	**Schedule**	**Premedications Precautions**	**Dose**	**Route**
NeoVax (peptides + poly-ICLC)	Prime	Days 1, 4, 8, 15, 22*	None	Poly-ICLC: 4 x 0.5 mg (total dose 2 mg) Peptides: 4 x 300µg per peptide Vol = 4 x 1 ml	injections into 4 different anatomic sites (0.5 ml i.d. & 0.5 ml s.c.)
NeoVax (peptides + poly -ICLC)	Boost	Days 78 and 134**	None	Poly-ICLC: 4 x 0.5 mg (total dose 2 mg) Peptides: 4 x 300µg per peptide Vol = 4 x 1 ml	injections into 4 different anatomic sites (0.5 ml i.d. & 0.5 ml s.c.)

*± 3 day treatment window is allowed except for days 4 and 8 (± 1 day)
** ± 7 day treatment window is allowed

5.3.1 Injections

Each of the up to 4 NeoVax syringes will be assigned to one of four extremities. At each immunization, each NeoVax syringe will be administered to the assigned extremity as follows: using one syringe/needle per site, 0.5 mL will be administered intradermally (i.d.) and 0.5 mL will be administered subcutaneously (s.c.) using the same syringe/needle at the same injection site (i.e. NeoVax A will be injected i.d. and s.c. into left arm on day 1, 4, 8 etc., NeoVax B will be injected i.d. and s.c. into right arm on days 1, 4, 8 etc.). Alternative anatomical locations for patients who are status post complete axillary or inguinal lymph node dissection or other contraindications that prevent injections to a particular extremity are the left and right midriff, respectively.

5.3.2 Treatment window

NeoVax may be administered within 1 day of the scheduled administration date for days 4 and 8, within 3 days of the scheduled administration date for days 15 and 22 and within 7 days for days 78 and 134.

5.3.3 Observation post vaccination

Patients should be observed in the clinic for at least 60 minutes after the last of the 4 injections and vital signs should be checked once between 30 and 90 minutes after the

last injection for each clinic visit. Monitoring for immediate adverse events should include attention to possible injections site reaction or a systemic reaction. In the absence of the occurrence of an adverse event, patients will be discharged from the outpatient clinic.

5.4 Dose schema and staggering of patients

5.4.1 DLT and expansion cohorts

Five patients will be entered for the initial safety evaluation (Cohort1). If two or more patients in Cohort 1 experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then that cohort will be determined to have unacceptable toxicity; otherwise, an additional 10 patients will be treated at the initial dose to increase the likelihood of detecting serious toxicities, to complete biologic correlative endpoints, and to gain preliminary experience with clinical tumor activity. If Cohort 1 is found to have unacceptable toxicity, then 5 patients may be enrolled on cohort -1 shown below. For Cohort -1, the dose of poly-ICLC is dropped by 50% relative to Cohort 1 but the dose of peptides remains the same. If two or more patients in Cohort -1 experience a DLT, then the study will be stopped. If none or only one patient experiences a DLT on Cohort -1, then 10 additional patients will be enrolled for additional toxicity evaluation, to complete biologic correlative endpoint, and to gain preliminary experience with clinical tumor activity. If four or more patients in the expansion cohort experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then that cohort will be determined to have unacceptable toxicity and further dosing of all patients will be stopped.

Dose De-escalation Schedule 1 cycle = 22days		
	Dose Level	Dose of poly-ICLC
	- 1	0.25 mg per injection site (4 injections, total dose 1 mg Vol = 4 x 0.875 ml
Starting Dose →	1	0.5 mg per injection site (4 injections, total dose 2 mg

5.4.2 Staggering of patients in DLT cohorts

5.4.3 The first three patients of Cohort 1 and Cohort -1 will be enrolled in a staggered fashion: Patients 2, 3 and 4 will not start treatment until the week after the previous

patient has completed the priming phase (scheduled for day 22). **Prime/boost schedule**

Vaccine will be administered following a prime/boost schedule. Priming doses of vaccine will be administered on days 1, 4, 8, 15, and 22 as shown above (Section 5.3, Administration and Schema B). In the boost phase, vaccine will be administered on days 78 (week 12) and 134 (week 20).

5.4.4 Definition of evaluable patients

All patients receiving at least one dose of vaccine will be evaluable for toxicity. Patients will be evaluable for immunologic activity if they have received all vaccinations during the priming phase and the first boost vaccination.

A minimum of 3 vaccinations and absence of DLTs are required for a patient to complete the 7-week DLT observation period successfully.

5.5 Definition of Dose-Limiting Toxicity

Dose-limiting toxicity (DLT) is based on the CTEP Active Version (version 4.0) of the NCI Common Terminology Criteria for Adverse Events (CTCAE). DLT refers to toxicities experienced within 49 days (7 weeks) of treatment initiation ("DLT window"). If treatment has to be delayed because of a toxicity that does not fulfill the criteria of a DLT, the DLT window should be extended by that time period of delay. A DLT will be defined as follows:

1. Grade 3 or 4 toxicity that is definitely, probably, or possibly related to the administration of vaccine, excluding:
   - Transient ($\leq$ 72 hours) flu-like syndrome;
   - Grade 3 nausea, vomiting, constipation, or diarrhea that returns to grade 2 (or lower) level within 48 hours;
   - Grade 3 rash that resolves to grade 2 or grade 1 within $\leq$ 14 days.

2. Grade 3 or 4 abnormal laboratory value that is definitely, probably, or possibly related to the administration of vaccine if it persists for more than 7 days, requires hospitalization or medical intervention (except for Grade 3 electrolyte abnormality that lasts $\leq$ 72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical intervention).

3. Any grade 3 or grade 4 toxicity that is considered, in the opinion of the investigator, to be dose-limiting.

4. Any death related to study treatment.

Management and dose modifications associated with the above adverse events are outlined in Section 6 (Expected Toxicities and Dosing Delays/Dose Modifications).

5.6 General Concomitant Medication and Supportive Care Guidelines

Acetaminophen or non-steroidal anti-inflammatory drugs (NSAIDS) may be used for prevention or treatment of flu-like illness symptoms. If clinically necessary, corticosteroids, antihistamines and non-steroidal anti-inflammatory drugs, including COX-2 inhibitors may be used at the discretion of the treating physician. Investigators may prescribe all other concomitant medications or treatments deemed necessary to provide adequate patient care.

Management of more serious toxicities will be directed by a study investigator and will be in accordance with standard of care clinical practice.

5.7 Duration of Therapy

Duration of therapy will depend on tolerability of the immunizations and evidence of disease recurrence as judged by the treating investigator. In the absence of treatment delays due to adverse events, treatment will be given until the day 134 vaccination (the 2nd booster vaccination) or until one of the following criteria applies:

- Disease recurrence, if it is deemed by the treating investigator to be in the best interest of the patient to discontinue study treatment;
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s);
- Patient demonstrates an inability or unwillingness to comply with protocol requirements;
- Patient decides to withdraw from the study; or
- General or specific changes in the patient's condition which render the patient unacceptable for further treatment in the opinion of the treating investigator.

5.8 Duration of Follow Up

Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

Every 3 months (“close observation”) follow-up visits should continue until initiation of a new therapy for recurrent disease or withdrawal of consent, whichever occurs first, for a maximum of 2 years after the surgery. Patients will undergo disease restaging scans every 6 months for 2 years after the surgery.

5.9 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.7 applies. The reason for study removal and the date the patient was removed must be documented in the study-specific case report form (CRF).

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Patrick Ott, MD, PhD at DFCI [redacted].

6 EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Active Version (version 4.0) of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used.

All adverse events experienced by patients will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Patients continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appears below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1.1 Adverse Events for poly-ICLC

Poly-ICLC is an investigational agent; however there has been significant experience in previous clinical trials as discussed in 2.1.2.4. The safety profile has been acceptable.

These reactions have been generally mild to moderate and transient for most patients in previous studies.

Localized reactions:

- Transient (<24 h) injection fluid accumulation
- Erythema
- Induration
- Pruritus
- Edema
- Localized rash

Systemic reactions:

Flu-like symptoms including malaise, fatigue, myalgia/arthralgia, headache, fever and chills.

Hepatobiliary Disorders:

- Alanine aminotransferase (ALT) increased
- Aspartate aminotransferase (AST) increased
- Lactate dehydrogenase (LDH) elevated
- Alkaline Phosphatase increased

Bone Marrow:

- Transient Leukocyte count decreased, Neutrophil count decreased, Platelet count decreased, and Anemia

6.1.2 Adverse Events for Personalized NeoAntigen Peptides

Localized reactions or systemic reactions are not expected, but may occur:

Localized reactions:

- Erythema
- Induration
- Pruritus
- Edema
- Localized rash

Systemic reactions:

Flu-like symptoms including malaise, fatigue, myalgia/arthralgia, headache, fever and chills.

Autoimmune diseases:

This is a theoretical possibility for which patients will be monitored. In principle, the mechanisms that allow the immune system to recognize tumor antigens could also lead to breakdown of tolerance to native or self-antigens, generating an autoimmune reaction.

6.2 Toxicity Management

6.2.1 Treatment with corticosteroids and antihistamines are not recommended for initial treatment, but may be instituted at the discretion of the treating physician if clinically necessary.

6.2.2 Local applications of cold compresses and moisturizing creams are preferable over systemic agents for the management of local reactions.

6.2.3 Patients who develop flu-like symptoms that are controlled with acetaminophen or non-steroidal anti-inflammatory drugs given at the discretion of the investigator may be pre-treated with acetaminophen (650 mg PO) or ibuprofen (400-600 mg PO).

6.3 Dose Modifications/Delays

6.3.1 If a patient develops grade 3 toxicity attributable to the vaccine, additional treatment will be withheld until the toxicity has resolved or improved to grade 1. If the toxicity constitutes a DLT as defined in 5.5, the patient will be removed from the study. If the toxicity does not fulfill the criteria of a DLT, the next vaccine administration may be delayed for a maximum duration of 14 days. If vaccine dosing has to be delayed more than 2 times because of a toxicity that is not considered a DLT, the patient will be removed from the study.

6.3.2 If a patient develops grade 4 toxicity attributable to the vaccine (with the exception of certain grade 4 laboratory abnormalities as defined in section 5.5), he or she will be removed from treatment.

6.3.3 If two or more grade 4 or greater toxicities attributable to the vaccine are observed, the trial will be suspended to investigate the causes of these (unexpected) toxicities.

7 DRUG FORMULATION AND ADMINISTRATION

7.1 Poly-ICLC (Hiltonol ®)

7.1.1 Description

Poly-ICLC is a synthetic, nuclease resistant, hydrophilic complex of polyinosinic and polycytidylic acid, stabilized with poly-L-lysine and carboxymethylcellulose.

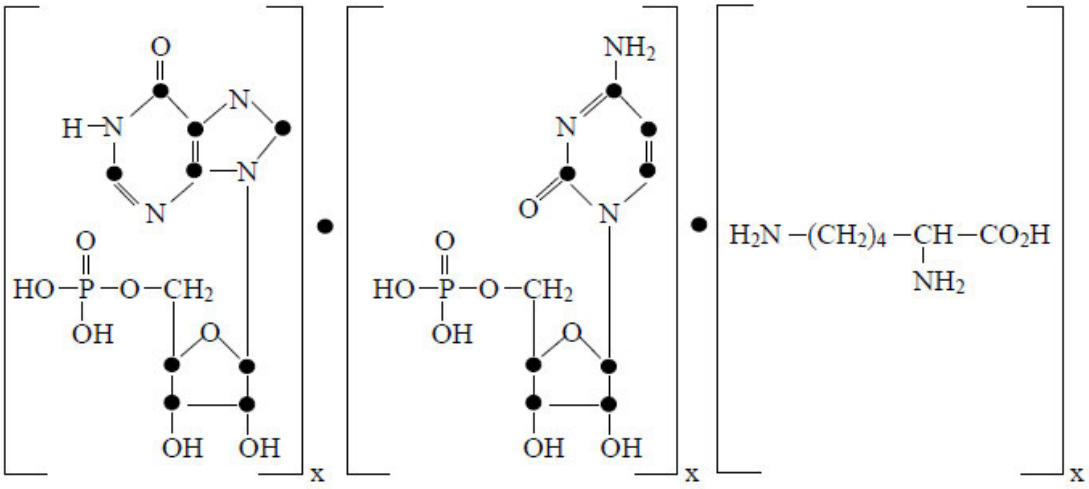
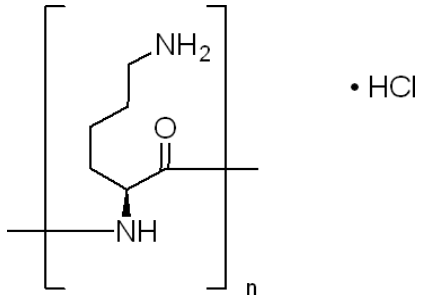
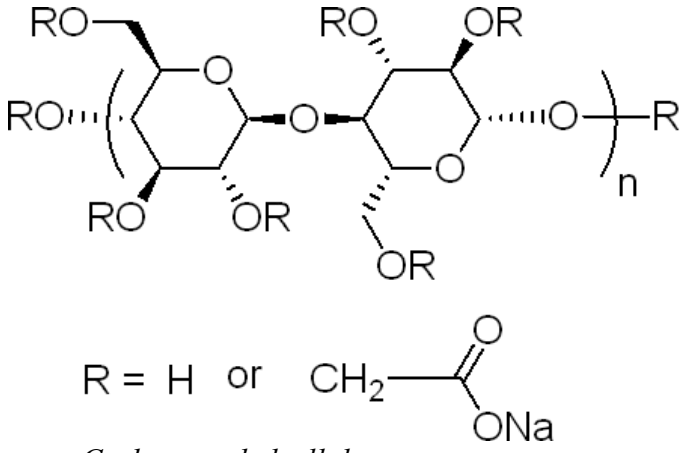


Figure 1: Structure of poly-ICLC



-Lysine



Carboxymethylcellulose

7.1.2 Form

Poly-ICLC is supplied by Oncovir in single-dose vials containing 1 mL of 2 mg/mL opalescent white suspension. Each mL of poly-ICLC for injection contains 2 mg of poly-IC, 1.5 mg poly-L-lysine, and 5 mg sodium carboxymethylcellulose in 0.9% sodium chloride solution, adjusted to pH 7.6-7.8 with sodium hydroxide.

7.1.3 Storage and Stability

Poly-ICLC is stable at room temperature for brief periods (days). For long term storage, it should be refrigerated at ca. 40ºF (2-8ºC). Poly-ICLC should NOT be frozen.

7.1.4 Compatibility

Poly-ICLC will be mixed with personalized neoantigen peptides just prior to use to prepare the final product (NeoVax).

7.1.5 Handling

There are no specific handling instructions for poly-ICLC.

7.1.6 Availability

Poly-ICLC is an investigational agent and has been purchased from Oncovir, Inc. Vials of poly-ICLC sufficient for this clinical trial have been shipped from the Hiltonol® storage facility (Bellwyck Packaging, Mississauga, Ontario, Canada) to the DFCI Pharmacy and are currently stored under conditions appropriate for this investigational agent.

7.1.7 Preparation

Poly-ICLC is supplied as an opalescent white suspension. It will be directly withdrawn from the vial under sterile conditions and mixed with the personalized neoantigen peptides (see section 5.2.1.3).

7.1.8 Administration

Following mixing with personalized neoantigen peptides, the vaccine (peptide + poly-ICLC) will be administered as follows: using one syringe/needle per site 0.5 mL will be administered intradermally and 0.5 mL will be administered subcutaneously.

7.1.9 Ordering

Poly-ICLC is an investigational agent that has been obtained from Oncovir, Inc under a Clinical Trials Agreement between DFCI and Oncovir, Inc, dated May 2012. Under that agreement Oncovir, Inc will grant access to the Drug Master File for poly-ICLC for regulatory purposes and provides DFCI information necessary to pursue the clinical use of poly-ICLC. Poly-ICLC can be ordered directly from Oncovir, Inc, by submitting a purchase order to:

[redacted]

7.1.10 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.)

7.1.11 Destruction and Return

At the end of the study, unused supplies of poly-ICLC will be held by the IND holder for future use until they are out of date at which point they will be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

7.2 Personalized neoantigen peptides

7.2.1 Description

Personalized neoantigen peptides are comprised of up to 20 distinct peptides unique to each patient. Each peptide is a linear polymer of ~20 - ~30 L-amino acids joined by standard peptide bonds. The amino terminus is a primary amine (NH_2-) and the carboxy terminus is a carbonyl group (-COOH). Only the standard 20 amino acids commonly found in mammalian cells are utilized (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid , glycine, histidine, isoleucine, leucine lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine). The molecular weight of each peptide varies based on its length and sequence and is calculated for each peptide.

7.2.2 Form

[redacted]

7.2.3 Storage and Stability

Personalized neoantigen peptides are stored frozen at -70 to -80ºC. The thawed vials and the final mixture of personalized neoantigen peptides and poly-ICLC can be kept at room temperature but should be used within 6 hours after preparing the peptide + poly-ICLC mixture.

7.2.4 Compatibility

Personalized neoantigen peptides will be mixed with poly-ICLC just prior to use to prepare the final product (NeoVax).

7.2.5 Handling

There are no specific instructions for handling personalized neoantigen peptides.

7.2.6 Availability

The personalized neoantigen peptides are prepared by CSBio, Inc under contract with DFCI and will be supplied directly to the DFCI. It will be supplied from DFCI to other participating institutions.

7.2.7 Preparation

The final step in the preparation of NeoVax (mixing with poly-ICLC) is conducted on the day of the scheduled vaccine administration as described in 5.2.1.1. Briefly, one vial each of the frozen personalized neoantigen peptides pools will be thawed the personalized neoantigen peptides will be combined and mixed with poly-ICLC within a properly certified biosafety cabinet.

7.2.8 Administration

Following mixing with personalized neoantigen peptides, the vaccine (peptide + poly-ICLC) will be administered as follows: using one syringe/needle per site 0.5 mL will be administered intradermally and 0.5 mL will be administered subcutaneously.

7.2.9 Ordering

The order for personalized neoantigen peptides to CSBio, Inc will be placed by the DFCI / Cell Manipulation Core Facility separately for each individual patient. Delivery time is anticipated to be 4 – 8 weeks.

7.2.10 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.)

7.2.11 Destruction and Return

Unused supplies of personalized neoantigen peptides will be released to the sponsor for further research or destroyed according to institutional policies. If unused personalized neoantigen peptides are destroyed this will be documented in the Drug Accountability Record Form.

8 CORRELATIVE/SPECIAL STUDIES

8.1 Pharmacodynamic Studies

The immunization strategy is a "prime-boost" approach, involving an initial series of closely spaced immunizations to induce an immune response followed by a period of

rest to allow memory T-cells to be established. This will be followed by a booster immunization, and the T-cell response 4 weeks after this boost (16 weeks after the first vaccination) is expected to generate the strongest response and will be the primary immunological endpoint. Immune monitoring will be performed in a step-wise fashion as outlined below to characterize the intensity and quality of the elicited immune responses. Peripheral blood will be collected and PBMC will be frozen at two separate time points prior to the first vaccination (baseline) and at different time points thereafter as illustrated in Schema B and specified in the study calendar. Immune monitoring in a given patient will be performed after the entire set of samples from the induction phase and the maintenance phase, respectively, have been collected.

If sufficient tumor tissue is available, a portion of the tumor will be used to develop autologous melanoma cell lines for use in cytotoxic T-cell assays.

8.1.1 Screening *ex vivo* IFN-γ ELISPOT

For each patient, a set of screening peptides will be synthesized. The screening peptides will be 15-17 amino acids in length, overlapping by 11 amino acids and covering the entire length of each peptide or the entire length of the neoORF for neoORF-derived peptides. The entire set of patient-specific screening peptides will be pooled together at approximately equal concentration and a portion of each peptide will also be stored individually. Purity of the peptide pool will be ascertained by testing PBMC from 5 healthy donors with established low background in ex vivo IFN-γ ELISPOTs. Initially, PBMC obtained at baseline and at week 16 (the primary immunological endpoint) will be stimulated for 18 hours with the complete pool of overlapping 15-mer peptides (11 amino acids overlap) to examine the global response to the peptide vaccine. Subsequent assays may utilize PBMC collected at other time points as indicated.

If no response is identified at the primary immunological endpoint using the ex vivo IFN-γ ELISPOT assay, PBMC will be stimulated *in vitro* with the peptide pool for a longer time period (up to 10 days) and re-analyzed.

We are broadly interested in the immunogenic potential of the predicted neoantigens that have been used to formulate the study subject's personal vaccines. Given that patients will have completed vaccine dosing, subsequent profiling of the subject's immune responses necessarily will include analysis of antigen-specific immunity to the precise vaccine antigens that had been administered. The unused peptides are thus valuable reagents to enable these informative immune studies. For this reason, and under the request of the Overall PI, personalized neoantigen peptides may be transferred from the pharmacy department to the research lab.

8.1.2 Deconvolution of epitopes in follow-up *ex vivo* IFN-γ ELISPOT assays

Once an ex vivo IFN-γ ELISPOT response elicited by a overlapping peptide pool is observed (defined as at least 55 spot forming units / 10^6 PBMC or increased at least 3

times over baseline), the particular immunogenic peptide eliciting this response will be identified by de-convoluting the peptide pool based into sub-pools based on the immunizing peptides and repeating the *ex vivo* IFN-γ ELISPOT assays.
For some responses, an attempt will be made to precisely characterize the stimulating epitope by utilizing overlapping 8-10 mer peptides derived from confirmed, stimulating peptides in IFN-γ ELISPOT assays

8.1.3 Additional assays conducted on a case-by case basis for appropriate samples

- The entire 15mer pool or sub-pools will be used as stimulating peptides for intracellular cytokine staining assays to identify and quantify antigen-specific CD4+, CD8+, central memory and effector memory populations
- Similarly, these pools will be used to evaluate the pattern of cytokines secreted by these cells to determine the T_H1 vs T_H2 phenotype
- Extracellular cytokine staining and flow cytometry of unstimulated cells will be used to quantify Treg and myeloid-derived suppressor cells (MDSC).
- If a melanoma cell line is successfully established from a responding patient and the activating epitope can be identified, T-cell cytotoxicity assays will be conducted using the mutant and corresponding wild type peptide
- PBMC from the primary immunological endpoint will be evaluated for "epitope spreading" by using known melanoma tumor associated antigens as stimulants and by using several additional identified mutated epitopes that were not selected to be among the immunogens
- Peptide ELISAs will be used to monitor humoral responses to the immunizing peptides

8.1.4 Immunohistochemistry

Immuno-histochemistry of tumor samples will be conducted to quantify CD4+, CD8+, MDSC, and Treg infiltrating populations.

9 STUDY CALENDAR

	Initial Screening	Study Drug Preparation	Second Screening	Priming Phase							Boosting Phase						Long Term Evaluation	
Day[1]				1	4	8	15	22	29	50	78	85	106	134	141	162	190	Every 3 months[17]
Week				1	1	2	3	4	5	8	12	13	16	20	21	24	28	40-96
Informed Consent	X																	
Medical History/Physical exam[2]	X		X[19]	X	X	X	X	X	X	X	X	X		X	X	X	X	X
Staging scans[3]	X									X						X		X[18]
Autoimmune panel[4]	X																	
Preganancy testing[5]	X		X[19]															
CBC w/diff, plts	X		X[19]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Chemistry[6]	X		X[19]	X	X	X	X	X	X	X	X	X		X	X	X	X	X
Infectious panel[7]	X																	
Concomitant medications	X		X[19]	X	X	X	X	X	X	X	X	X		X	X	X	X	X
HLA class I and II testing[8]	X																	
Blood draw for normal tissue sequencing[9]	X																	
Melanoma resection[10]		X																
Pathology review		X																
Vaccine preparation[11]		X																
Leukapheresis[12]			X										X					
Blood draw for immune response monitoring			X[13]	X[13]			X[14]			X[15]	X[15]		X[16]	X[15]		X[15]		
Vaccine administration				X	X	X	X	X			X			X				
Adverse event evaluation				X	X	X	X	X	X	X	X	X		X	X	X	X	X

[1]All study procedures should be performed within a +/- 3 day window unless specified otherwise.

[2]History and Physical examination include ECOG Performance Status and Vital Signs

[3]Initial screening scans (PET/CT or CT neck, chest, abdomen, and pelvis, and brain MRI for baseline assessment must be performed ≤4 weeks prior to surgery. Day 50 re-staging scans can be done between day 43 and 50; day 190 scans can be done between day 183 and day 190.

[4]ANA, anti-thyroglobulin antibodies, rheumatoid factor.

[5]Women of child-bearing potential must have a negative serum β-HCG pregnancy test during screening and within 7 days of starting treatment.

[6]Glucose, urea nitrogen, creatinine, sodium, potassium, calcium, total and direct bilirubin, AST, ALT, alkaline phosphatase

[7] HBSAg, HBcAb, HCV, HTLV I/II, RPR, HIV 1 and 2 Ab test

[8]Molecular HLA typing to be performed by Tissue Typing laboratory

[9]10 ml (one purple capped tube) of blood will be drawn and transmitted to the Broad Institute for whole exome sequencing.
[10]Resection of locoregional metastatic disease (regional lymph nodes, in transit metastases, satellite lesions, limited distant metastases) and primary tumor (if not yet removed) should occur within 3 weeks of study enrollment.
[11]Vaccine preparation should begin immediately after the surgical specimen is deemed adequate for vaccine preparation and it has been determined that the primary melanoma and metastatic disease have been completely removed. Vaccine preparation should be completed within 8-10 weeks after this assessment.
[12]Patients will undergo leukapheresis procedure to collect sufficient amounts of PBMCs to allow comprehensive immune monitoring. The pre-treatment leukapheresis should be performed no sooner than 2 weeks after the surgery, prior to the first vaccine administration. A +/- 7 day window is allowed for the day 106 leukapheresis. If a patient is discontinued from active treatment (during Priming or Boosting Phase) and has not yet undergone the day 106 leukapheresis, the leukapheresis or a 50 ml blood draw should be performed no later than 14 days after the day of discontinuation.
[13]135 ml of blood should be drawn if pre-treatment leukapheresis cannot be performed. If pre treatment Leukapheresis was performed 20 ml should be drawn.
[14]If pre-treatment leukapheresis cannot be performed, there is no blood draw. If leukapheresis was performed, 60 ml of blood will be collected.
[15]200 ml
[16]200 ml of blood should be drawn if week-16 leukapheresis cannot be performed. If week -16 leukapheresis can be performed, there is no blood draw.
[17] Follow-up: Every 3 months re-staging follow-up visits (+/- 1 week) should continue until initiation of a new therapy for recurrent disease, withdrawal of consent, or death whichever occurs first for a total of 2 years after treatment initiation.
[18] Every 6 months
[19] Assessments such as: Medical History / Physical Examination, lab tests and Con Meds can be done on Day 1 visit (previous starting treatment) if needed

10 MEASUREMENT OF EFFECT

10.1 Antitumor Effect– Solid Tumors

For the purposes of this study, patients should be re-evaluated for disease recurrence every 12 weeks.

10.1.1 Definitions

Evaluable for toxicity. All patients who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for disease recurrence. All patients who receive at least one dose of study treatment will be evaluable for disease recurrence from the time of their first treatment.

Disease recurrence: The finding of a new lesion on clinical examination or on imaging should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate disease recurrence. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if disease recurrence is confirmed, recurrence should be declared using the date of the initial scan on which the lesion was discovered.

11 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the patient and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.3.1 Expected adverse event

For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

11.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all patient evaluation time points during the study.

All AEs and SAEs whether reported by the patient, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the patient's medical record and on the appropriate study-specific case report forms.

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 website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 Reporting Requirements

The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

11.4 Reporting to the Study Sponsor

11.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.
- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.
- All Grade 5 (fatal) Events – When the patient is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the patient is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., patient sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Patrick Ott, MD
[redacted]

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe

whether the event has resolved or continues, if and how the event was treated, and whether the patient will continue or discontinue study participation.

Oncovir, Inc. will be provided with a simultaneous copy of all adverse events filed with the FDA.

11.4.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

11.6 Reporting to the Food and Drug Administration (FDA)

Patrick Ott, MD, PhD as IND sponsor, will be responsible for all communication with the FDA. She will report to the FDA any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the investigational agent will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by paper mail using Form FDA 3500A (Mandatory Reporting Form for investigational agents). If a faxed copy of Form FDA 3500A is requested by the agency, a paper copy will also be submitted as a follow-up. Forms are available at http://www.fda.gov/medwatch/getforms.htm.

11.7 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.8 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last

study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the patient is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the patient's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Patients should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a patient has discontinued or terminated study participation that may reasonably be related to the study.

12 DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation

Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about patient safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date patient accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 External Safety Monitoring Board

DFCI will be subject to independent monitoring conducted by ESMB.

DFCI shall make reasonable efforts to ensure that the integrity of the research is not directly influenced by its interest in the technology being investigated by ensuring that the trial will be subject to independent data monitoring and auditing by an independent board, the cost of which shall be borne by DFCI. This board shall consist of three members. None of the independent board members have any relationship to either DFCI or Neon. The ESMB shall be review subject enrollment practices to ensure compliance with any ICOI management plan; review and evaluate the accumulated DFCI study data for accuracy, participant safety, study conduct and compliance with any ICOI management plan; monitor individual and institutional compliance with ICOI management plan. The ESMB shall report to the ICOIC bi-annually for each trial.

The ESMB may interact with DSMC and/or CTO if deemed necessary to accomplish their functions.

12.4 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or

DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

Monitoring plan for 13-240 protocol has been developed in conjunction with the DFCI CTO. The monitoring for 13-240 will be completed by a CTO monitor. Monitoring plan for this study will follow a risk-based monitoring approach with a focus on consent/eligibility documentation, safety, endpoints and full source data verification on approximately 50% of participants.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of patient registration and will continue during protocol performance and completion.

12.5 Data sharing

DFCI may share patient specific data with Neon Therapeutics, Inc. This data may contain PHI, limited identifiable patient's information as well as de-identified information. DFCI will release only the minimum necessary identifiable health care information to Neon Therapeutics, Inc. Informed consent will be requested and obtained from former participants, active participants and future participants about the sharing of their information with Neon Therapeutics, Inc.

Data submission will be performed via secure cloud-based service for document transfer and storage. Implemented security of the cloud-based system includes encrypted data transfer, two factor authentication and integration with Active Directory security groups. Neon ensures limited system access to authorized individuals through the use of security groups to agreed-upon members. Data will be backed up as part of the cloud based document storage service and the same security setting will apply to the cloud backup.

13 REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of patient information related to the study (e.g., advertisements used to recruit patients) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be

reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All patients must be provided a consent form describing this study and providing sufficient information for patients to make an informed decision about their participation in this study. The formal consent of a patient, using the IRB approved consent form, must be obtained before the patient is involved in any study-related procedure. The consent form must be signed and dated by the patient or the patient's legally authorized representative, and by the person obtaining the consent. The patient must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 50 – Protection of Human Subjects www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
    - Title 21 Part 56 – Institutional Review Boards www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
    - Title 21 Part 312 – Investigational New Drug Application www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html
- State laws
- DF/HCC research policies and procedures http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research patient. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research patient. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

14 STATISTICAL CONSIDERATIONS

14.1 Study Design/Endpoints

The primary goals of this Phase I trial are: a) to assess safety of the vaccine and determine the recommended dose of poly-ICLC and b) to assess the feasibility of making vaccine containing an adequate number of actionable peptides and in a timely manner.

A. To evaluate safety and tolerability of administering NeoVax in patients with high-risk melanoma.

Dose-limiting toxicity (DLT) will be defined as any of the following toxicities observed:

1. Grade-3 or -4 toxicity that is definitely, probably, or possibly related to the administration of vaccine, excluding:
   - Transient (≤ 72 hours) flu-like syndrome
   - Grade-3 injection site reaction (localized rash, erythema, edema, induration) that resolves to grade 2 (or lower) within 72 hours
   - Grade-3 nausea, vomiting, constipation, or diarrhea that returns to grade 2 (or lower) within 48 hours
   - Grade-3 rash that resolves to grade 2 or grade 1 within 14 days

2. Grade-3 or -4 abnormal laboratory value that is definitely, probably, or possibly related to the administration of vaccine if it persists for more than 7 days, requires hospitalization or medical intervention (except for Grade-3 electrolyte abnormality that lasts ≤ 72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical intervention)

3. Any grade-3 or grade- 4 toxicity that is considered, in the opinion of the investigator, to be dose-limiting

4. Any death related to study treatment

Five patients will be entered for the initial safety evaluation (Cohort 1). We have chosen dose escalation cohorts of 5 patients, rather than a more traditional 3+3 design, because the determination of whether to treat at a different dose in this trial is made over a longer period of time due to the staggered enrollment of patients, the time required for vaccine preparation, and the extended period for safety evaluation. These larger cohort sizes help us avoid having to pause and then restart enrollment to a dosing cohort several months later. If two or more patients in Cohort 1 experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then that cohort will be determined to have unacceptable toxicity; otherwise, an additional 10 patients will be treated at this dose level to increase the likelihood of detecting serious toxicities, to complete biologic correlative endpoints, and to gain preliminary experience with clinical tumor activity. If Cohort 1 is found to have unacceptable toxicity, then 5 patients will be enrolled on Cohort -1. If two or more patients in Cohort -1 experience a DLT, then the study will be stopped. If zero or one patient experiences a DLT in Cohort -1, then 10 additional patients will be enrolled in an expansion cohort.

The table below shows the probability of moving to the dose expansion phase (0 or 1 patients with DLT out of 5 patients) for various possible values of the true, but unknown, toxicity rate.

True rate of DLT (%)	**Probability of Dose Expansion (%)**
10	92
20	74
30	53
40	34
50	19
60	9

If the true probability of DLT is 10% or less, then the probability of proceeding to dose expansion is at least 92%. The probability of dose expansion is less than 50% if the true rate of dose-limiting toxicities is 32% or greater.

Minimum vaccination requirement to pass DLT observation period: A minimum of 3 vaccinations and absence of DLTs are required for a patient to complete the 7-week DLT observation period successfully.

Expansion Cohort: When the recommended dose has been determined, an expansion cohort of 10 patients will be enrolled to assess the performance of the vaccine and to gain additional information about toxicities.

Stopping rule for Expansion Cohort: If four or more patients in the expansion cohort experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then the dose expansion cohort will be determined to have unacceptable toxicity and the study will be stopped prior to completion of enrollment of the expansion cohort. The table below summarizes the probability of observing 4 or more DLTs within the 10 patients of the expansion cohort for varying underlying rates of toxicity.

	True, but unknown, toxicity rate					
	5%	10%	20%	30%	36%	40%
Probability of observing 4 or more patients with DLT	0.001	0.013	0.121	0.350	0.513	0.618

If the true probability of DLT in the expansion cohort is higher than 35%, then the probability is at least 50% of observing 4 or more patients with DLT within the 10 patients of the expansion cohort.

Total Sample Size: The final sample size for this trial will depend upon the number of dose-escalation cohorts; however, the number of patients enrolled will be between 4 and 20. The minimum number of patients to be enrolled is based on observing DLTs after the first dose of NeoVax in the first two patients of cohort 1 and similarly in cohort-1. The maximum would occur if Cohort 1 and Cohort -1 were enrolled, with an additional 10 patients at the recommended dose.

B: To determine the feasibility of generating and administering NeoVax in patients with high-risk melanoma

Two feasibility endpoints will be evaluated:

1) The proportion of patients for whom sequencing and analysis leads to identification of at least 10 actionable peptides to initiate vaccine production;

2) The proportion of patients for whom the time between surgery and vaccine availability is 12 weeks or less (excluding those patients who did not generate at least 10 actionable peptides).

We will base the primary assessment of feasibility on all patients enrolled with a confirmation of completely resected tumor and with adequate DNA and RNA for sequencing. The statistical considerations for feasibility that follow are based on 15 patients.

However, the decision rule used to assess feasibility will be adjusted to maintain the operating characteristics if the number of patients is greater than 16.

We would consider NeoVax to be feasible if no more than 50% of patients have fewer than 10 actionable peptides or have NeoVax available more than 12 weeks after confirmation of complete resection. If 4 or fewer of the first 15 patients have insufficient vaccine or first dose delays of more than 12 weeks, then the upper bound of the one-sided 90% exact confidence interval will be less than 50% and we will consider the process feasible. If 5 or more of the first 15 patients have insufficient or delayed vaccine, we will consider the vaccine not feasible.

The planned vaccination regimen will be administered even if the feasibility endpoints are not met. However, the treatment will not be started in a given patient if three or fewer actionable peptides are identified.

14.2 Analysis of Secondary Endpoints

Secondary endpoints in this clinical trial will: (a) assess the induction of IFN-γ T-cell response to the total peptide pool, and (b) estimate the proportion of patients alive without disease progression at two years after resection. Analyses of these secondary endpoints will be based on the 15 patients treated at the recommended vaccine dose.

The induction of IFN-γ T-cell response will be based on ELISPOT assessments taken prior to vaccine administration and at week 16. The proportion of patients who achieve more than 55 SFU/10^6 PBMC *or* 3 times their baseline level will be presented with a 90% exact binomial confidence interval. Based on a cohort of size 15, the confidence interval will be no wider than 0.46.

The proportion of patients alive, without disease progression at two years after resection will be estimated using the method of Kaplan-Meier. The endpoint will be calculated as the time between pathological confirmation of complete resection and the first of disease recurrence or death. Patients who are disease-free at the time of study reporting will have their follow-up censored at the time of the last study visit. Patients who were disease-free at the time of their last assessment and who have either been lost to follow-up or withdrawn consent will have their follow-up censored at the time of the last study assessment. The proportion of patients alive and disease-free at two years will be presented with 90% confidence intervals estimated using log(-log(DFS)) methodology.

14.3 Analysis of Correlative Endpoints

Longitudinal analyses of PBMC immune response kinetics to the peptide pool will be presented graphically and descriptively at each time point. Changes in the magnitude of the response relative to pre-treatment at several different time points after vaccination will be summarized descriptively. Changes in response between pre-treatment and 16 weeks post-vaccination, which is the time of the primary immunologic endpoint, will be assessed using the Wilcoxon signed-rank test.

Immune studies of T cell subpopulations ($CD4^+$, $CD8^+$, central/effector memory cells, Th1/Th2 cells, Tregs, etc.), myeloid-derived suppressor cells (MDSC), CTL response, and T cell activation status will be summarized using descriptive statistics.

14.4 Analysis of Safety and Toxicity Data

Analysis set and grouping for the analyses: All patients who receive one or more doses of NeoVax will be included in the safety analysis. All listings and tables will be presented by dose level.

Adverse events (AEs): All adverse events recorded during the study will be summarized. The incidence of treatment emergent adverse events will be summarized by dose level according to primary system organ class, severity based on the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, type of adverse event, and relationship to the vaccine. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by primary system organ class, type of adverse event, and dose level.

DLTs will be listed and their incidence summarized by primary system organ class, worst grade based on the CTCAE version 4.0, type of adverse event, and by dose level. Any other information collected (e.g. start/end dates and duration of adverse event, severity or relatedness to study medication) will be listed, as appropriate.

Laboratory abnormalities: All laboratory values will be converted into SI units, as appropriate, and the severity grade calculated using CTCAE, version 4.0. Parameters for which a grading does not exist will be classified into low/normal/high group by means of laboratory normal ranges. For each laboratory test (e.g., hematology, biochemistry) a listing of laboratory values will be provided by laboratory parameter, patient and treatment group. The frequency of notable lab abnormalities (i.e., newly occurring CTCAE grade-3 or -4 laboratory toxicities), will be displayed by parameter, cycle, and dose level. Similarly, the frequency of all laboratory abnormalities will be displayed by parameter, worst CTCAE version 4.0 grade experienced and dose level. A separate listing will display notable laboratory abnormalities (i.e., newly occurring CTCAE grade-3 or -4 laboratory toxicities). Laboratory data will be summarized by presenting grade shift tables for those parameters for which CTCAE version 4.0 allows classification. All remaining data will be summarized by presenting shift tables based on normal ranges.

Laboratory data will be also be displayed by presenting summary statistics of raw data and change from baseline values (means, medians, standard deviations, ranges).

15 PUBLICATION PLAN

This study is intended for publication, even if terminated prematurely. Publication may include any or all of the following: posting of a synopsis online, abstract and/or presentation at a scientific conference, or publication of a full manuscript. The Sponsor will work with the Principal Investigator and authors to submit a manuscript describing study results within 24 months after the last data become available; which for vaccine trials, may take up to several months after the last patient visit.

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17 APPENDICES

17.1 Appendix A: Performance Status Criteria

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

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DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**13-240**
Protocol Name:	**Ph I Study with Personalized NeoAntigen Cancer Vaccine in Melanoma**
DFCI Site PI:	Patrick Ott, MD
DFCI Research Nurse:	; Kristina Kelley, RN; Christine Wong, RN; Jennifer Rowan, RN

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy
This is a PI Initiated Study & Therefore There is No Alert Page

SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	This personalized NeoAntigen Cancer Vaccine approach has not been done in humans before. The vaccine is a tumor antigen and will hopefully induce immune responses against tumor cells that are much stronger than what has previously been achieved with traditional tumor vaccine approaches. The anticipated strong Immune responses should allow for clinical benefit. Please refer to Section 1.1 One Cycle = 22 Days (Section 5.4.1)
Dose Calc	***Please refer to Section 5.2.4
Study Drug Administration	***Agent *Administration* Guidelines are found in Sections 5.3, 5.2.3.1, 5.2.3.2, 5.3.1, 5.2.4, & 7.1.8 **Priming Doses** : NeoVax (peptides + poly-ICLC) • Laboratory parameters must be reviewed and must re-meet Eligibility Criteria. (Section 5.2.3.1) • Subsequent treatment days, Laboratory parameters must continue to re-meet Eligibility Criteria.(Section 5.2.3.2) • 4 syringes containing 1 ml each of mixture for Neovax A, B, C, &D will be prepared for sub cu injections. • Administered Days 1, 4, 8, 15, & 22 via sub cu injections into 4 different anatomic sites. • Each of the 4 NeoVax syringes will be assigned to one of four extremities. (Example; NeoVax A will be injected into left arm on day 1, 4, 8 etc., NeoVax B will be injected into right arm on days 1, 4, 8, etc.) • NeoVax may be administered within **1 day** of the scheduled administration date for days 4 & 8, within **3 days** of the scheduled administration date for days 15 & 22 and **7 days** for days 78 & 134 (Section 5.3.2) • For **EACH** clinic visit Participants are to be **observed** in the clinic for at least 60 minutes after the last of the 4 injections. (Section 5.3.3) • At **EACH** clinic visit **Vital Signs** are to be checked once between 30 and 90 minutes after the last injection for each clinic visit. (Section 5.3.3) **Boost Phase:** • **NeoVax** will be administered on Days 78 (week 12) and-134 (week 20) (Section 5.4.3)
Dose Mod & Tox	*Dose Modifications/Dosing Delay for Toxicity* are outlined in Section 6.0, 6.3 • DLT observation period is 7 weeks. (Section 5.4.4) • Toxicities will be graded using the NCI CTCAE version 4.0 (Section 5.5) • Maximum delay of NeoVax vaccine is 14 days. (Section 6.3.1) • Maximum number of times NeoVax may be delayed is 2. (Section 6.3.1)
Concom Meds	****Concomitant Therapy* Guidelines are in Section 5.6 • Participants who have developed flu-like symptoms that were controlled with acetaminophen or non-steroidal anti-inflammatory drugs during previous treatments, Per Investigator's discretion, may be pre-treated with acetaminophen or NSAID. (Sections 5.2.3.2 & 6.2.3)
Req Data	*Study Calendar and Assessment Required data* are outlined in Section 9.0 • All Study procedures should be performed within a +/- 3 day window unless specified otherwise. (Section 9)
Charting Tips	Please be sure to DOCUMENT study medication **actual** UP/DOWN times in medical record • If there is a discrepancy in the infusion time, delay in administration, or the infusion takes longer than is permitted by the guidelines of the protocol, please document the reason for the discrepancy in the medical record. Please be sure to also DOCUMENT any required observation periods, any additional vital signs, routes of administration, or injection sites

ONCOVIR

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Note To Dispensors: Poly-ICLC Suspension Inspection prior to use

To: Pharmacy

From: Andres M. Salazar, MD, Scientific Director

cc: Pharmacy Sites

Date: 28 February 2017

Re: Visual inspection of Poly-ICLC vials lot PJ 215-1-10-01 prior to use

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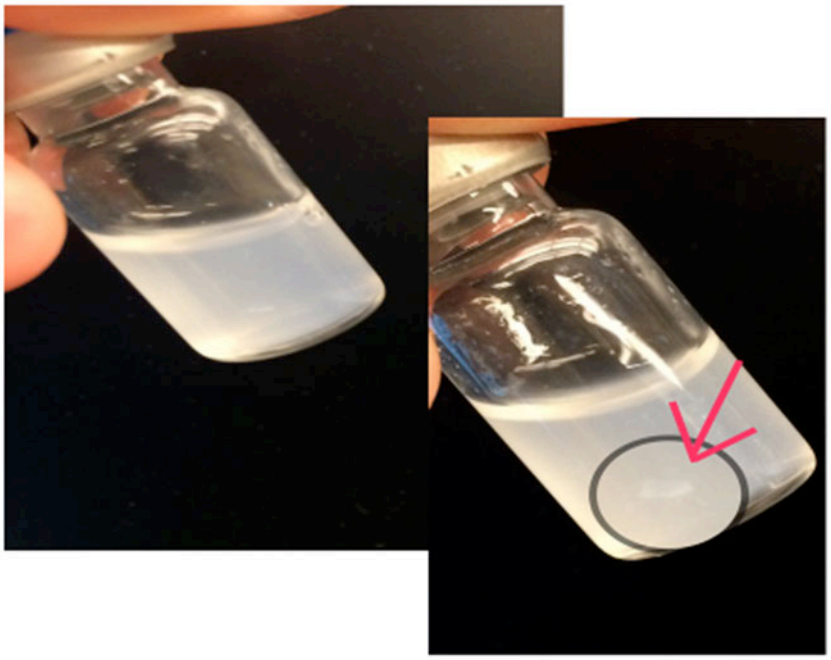
Poly-ICLC presents as an opalescent, or white, viscous uniform coarse particulate suspension in a 2 mL glass vial. Recently it has been observed that some product vials exhibit some settling of the suspension around the lower rim of the product vial as well as some larger clumps as seen in the photo below .

Upon allowing the vial to reach room temperature (approximately 1-2 hours), the vial should be gently inverted about 10 times to insure suspension of the formulation. Gently tap the vial to gather the liquid suspension to the bottom of the vial to aid in extracting the required volume for dose preparation. If visible particulates or aggregates are present when measured against a black background and are not resuspendable or the settled material fails to re-suspend, the vial should be placed in quarantine and not used for clinical administration. Obtain a new vial of Poly-ICLC for dosage preparation.

Please report to the sponsor and/or Oncovir, Inc any instances of Poly-ICLC failures to re-suspend and/or observations of insoluble particulates or agglomerates.



Signature:

Andres M. Salazar, M.D.
CEO, Scientific Director,
Oncovir, Inc.