nature portfolio

https://doi.org/10.1038/s41586-024-08507-5

Supplementary information

A neoantigen vaccine generates antitumour immunity in renal cell carcinoma

In the format provided by the authors and unedited

DF/HCC Protocol #: 16-097

TITLE: A Phase I Study combining NeoVax, a Personalized NeoAntigen Cancer Vaccine, with Ipilimumab to Treat High-Risk Renal Cell Carcinoma

Coordinating Center: **Dana Farber Cancer Institute**

Principal Investigator (PI): Toni Choueiri, MD
Dana Farber Cancer Institute
Toni_Choueiri@DFCI.HARVARD.EDU

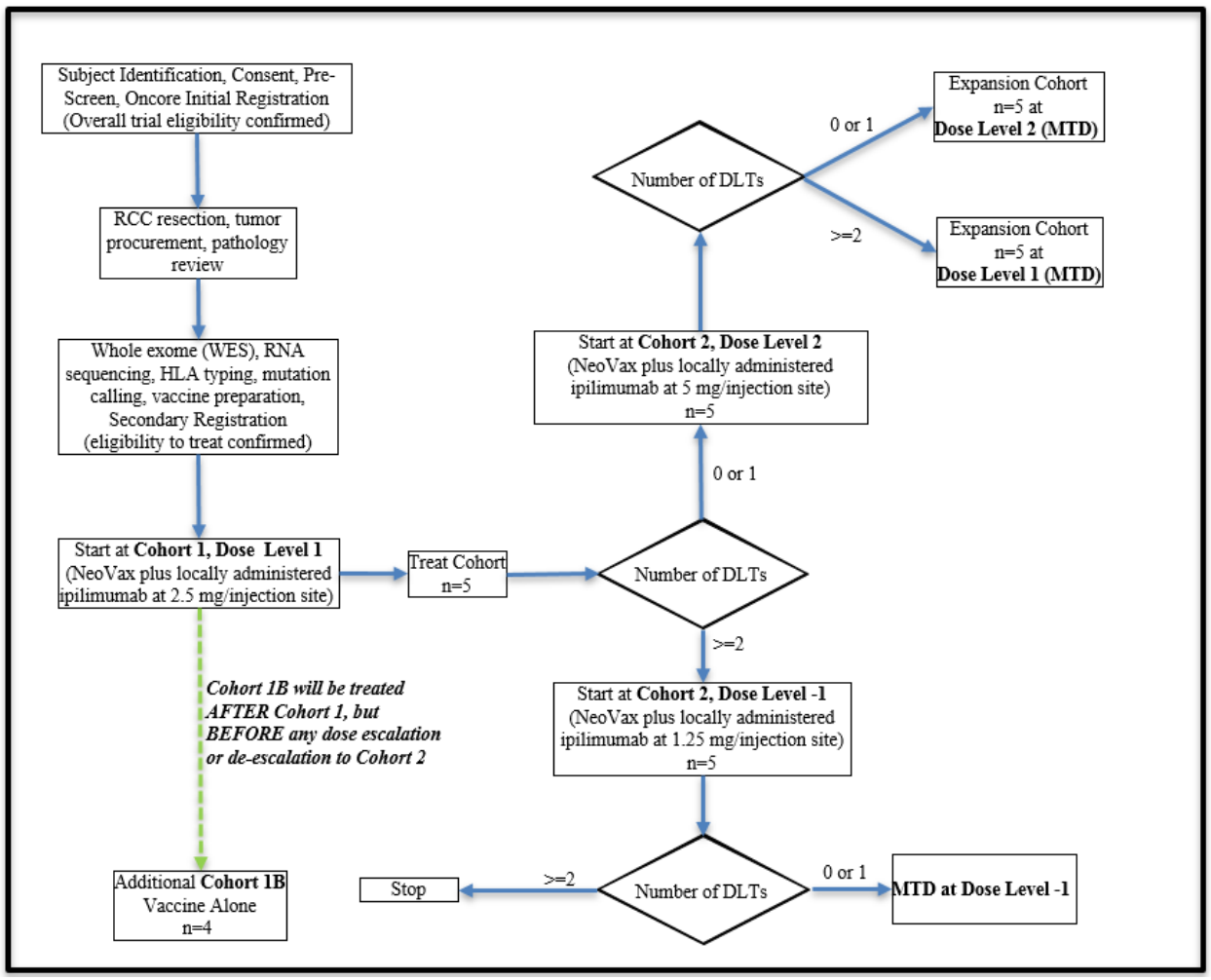
Co-Principal Investigator (PI): Patrick Ott, MD PhD
Dana Farber Cancer Institute
PATRICK_OTT@DFCI.HARVARD.EDU

David Braun, MD PhD
Dana Farber Cancer Institute
David_Braun@dfci.harvard.edu

Biostatician: Yiwen Liu
Dana-Farber Cancer Institute
yiwenliu@ds.dfci.harvard.edu

IND #: 15703
IND Sponsor: Patrick Ott, MD PhD
Protocol Version # / Version Date: Version 7.0 September 30, 2022

SCHEMA A



SCHEMA B

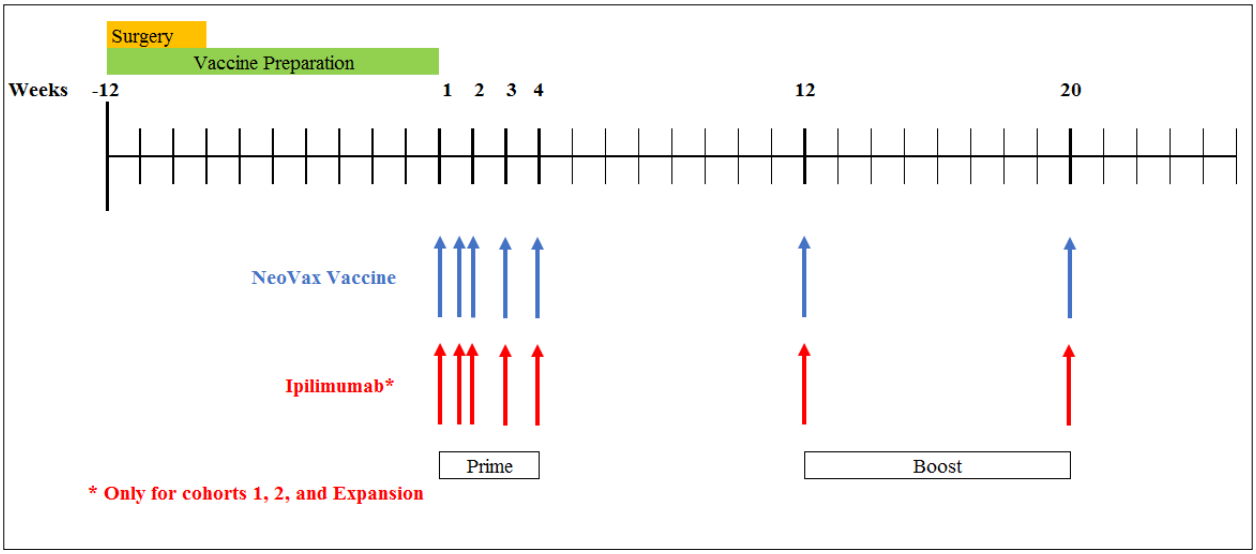


TABLE OF CONTENTS

OBJECTIVES

1.1 Study Design

The study is an open label, phase I trial in which "high-risk" patients with fully resected Renal Cell Carcinoma (RCC) will be immunized with up to 20 peptides that are both specific to the participant's tumor cells (i.e., not found in their normal cells) and unique to the participant (i.e., "personal") and concurrently receive Ipilimumab in close proximity to the vaccine site. 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 participant's tumor cells and are identified through DNA and RNA sequencing. Up to 20 peptides of about 20-30 amino acids in length will be prepared for each participant and will be administered together with the immune adjuvant polyinosinic-polycytidylic (poly-IC) stabilized with poly-L-lysine and carboxymethylcellulose (poly-ICLC). Thus, the personalized NeoAntigen Cancer Vaccine consists of peptides + poly-ICLC and will be termed "NeoVax". Ipilimumab is an antibody against CTLA-4, a molecule on the surface of T cells that limits T cell expansion, and has been approved for the treatment of metastatic melanoma (Yervoy®). Ipilimumab will be delivered via subcutaneous injection in proximity to each vaccination site in order to 1) direct anti-CTLA-4 activity to the vaccine-draining lymph nodes and 2) limit systemic toxic effects. This approach is effective in animal tumor models and is expected to result in significantly reduced levels of systemic Ipilimumab compared to the approved dose/schedule in advanced melanoma (3 mg/kg q3wks for four doses).

Eligible patients will be entered into the trial to undergo surgery with the intent to resect the primary kidney tumor or a metastatic site, leading to "no evidence of disease" (NED). Patients who have undergone surgery, fulfill eligibility criteria, and for whom vaccine is successfully prepared will continue onto the treatment phase of the trial.

The study will be in multiple parts. One part (Cohorts 1 and 2; 10 patients) will identify the maximum tolerated dose (MTD) of locally delivered Ipilimumab from among three possible dose levels. One part (Cohort 1B; 4 patients) will assess the induction of neoantigen-specific cellular immune responses following administration of NeoVax alone (without Ipilimumab). The final part will add an additional 5 patients (Expansion Cohort) at the MTD of locally delivered Ipilimumab to extend the safety and activity analysis of the NeoVax cancer vaccine administered with Ipilimumab.

Five (5) patients will be entered for the initial safety evaluation (Cohort 1, Dose Level 1). Prior to any dose escalation or de-escalation, 4 patients (Cohort 1B) will be treated with NeoVax alone, without Ipilimumab, to assess for the induction of neoantigen-specific cellular immune responses (not for DLT assessment, because no Ipilimumab will be administered to this cohort).

If none or only 1 patient from Cohort 1 experiences a DLT during the first 7 weeks of treatment of Cohort 1, five (5) patients will be entered on Cohort 2 Dose Level 2. If two (2) or more patients in Cohort 1 experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then five (5) patients will be entered on Cohort-1 Dose Level -1.

If none or only 1 patient experiences a DLT on Cohort 2 Dose Level 2, then Dose Level 2 will be the maximum tolerated dose (MTD) and an additional five (5) patients will be entered at that 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 two or more patients in Cohort 2 Dose Level 2 experience dose limiting toxicity (DLT), then Dose Level 1 will be the MTD and an additional five (5) patients will be treated at this dose.

If none or only 1 patient experiences a DLT on Cohort -1, then Dose Level-1 will be the maximum tolerated dose (MTD) and an additional five (5) patients will be entered at that dose level. If two or more patients in Cohort -1 experience dose limiting toxicity (DLT), then the study will be stopped.

The primary objectives of the study are to test the safety and feasibility of administering NeoVax in combination with locally-delivered Ipilimumab, and to determine the maximum tolerated dose (MTD) of locally administered Ipilimumab; 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 and locally delivered Ipilimumab in patients with fully resected, high-risk clear cell renal cell carcinoma (ccRCC).

1.2.2 To determine the maximum tolerated dose (MTD) of locally administered Ipilimumab when delivered with NeoVax.

1.3 Secondary Objectives

1.3.1 To assess the induction of neoantigen-specific cellular immune responses following administration of NeoVax with Ipilimumab.

1.3.2 To determine the proportion of patients alive without recurrence at two years after surgery following administration of NeoVax with Ipilimumab.

BACKGROUND

2.1 Study Disease

Renal cell carcinoma is one of the ten most common cancers and the incidence is increasing. Between 20 to 30% of patients initially present with metastatic disease, which is generally incurable, whereas 30% of patients who undergo curative-intent nephrectomy experience recurrence with distant metastases. Targeted systemic agents such as inhibitors of vascular endothelial growth factor (VEGF) or mammalian target of rapamycin (mTOR) have provided significant clinical benefit for patients with metastatic disease, but tumor responses are not durable and most patients relapse and eventually succumb to the disease[1]. Combinations of existing therapeutic modalities are being investigated in these settings but are associated with significant toxicities and modest added activity. Effective adjuvant treatment of resected, high-risk patients - with presumably micrometastatic, if any, disease burden - is lacking. Sunitinib, which was recently

approved as adjuvant therapy for high risk renal cell carcinoma, is associated with substantial toxicity and has not been shown to confer an overall survival benefit[2,3]. For these reasons, it is not considered standard of care therapy at this time, and innovative approaches are therefore necessary to achieve substantial improvement.

Perhaps more than any other therapeutic approach, immunotherapy has the potential for curative outcome by capturing the diversity and long-lasting memory of the immune system and its ability to destroy malignant tumor cells and prevent relapse. High dose interleukin-2 (HD-IL2) is used in patients with metastatic RCC based on a durable complete remission rate of 7%, but this therapy is only offered in select centers due to its significant toxicity and there is no established biomarker to predict for response[4]. Nevertheless, the results with HD-IL2 provide proof that "first generation" immunotherapy attempts can work in RCC.

Two immunotherapeutic approaches are being actively evaluated in RCC and other diseases – antigen-specific immunization (vaccination) and, more recently, non-specific immune stimulation via treatment with checkpoint blockade antibodies (CPB)[5-7]. Vaccines in RCC to date have provided hints of efficacy but tumor response rates and durability of tumor responses remain low,[8,9] most likely due to the lack of effective tumor-specific immune responses induced with these approaches. Historically, most cancer vaccines have utilized tumor-associated antigens (TAA), a class of native proteins, which are preferentially or selectively expressed on tumor cells, but can also be found on some normal cells. Native proteins generate relatively weak immune responses due to central tolerance (the naturally occurring phenomenon that prevents the immune system from recognizing self-antigens and thereby generating auto-immunity). Despite the relatively weak results achieved with TAA vaccination to date, several pivotal clinical studies are underway in RCC utilizing such native antigens, pointing to the demand for more effective therapies.

2.2 IND Agents

2.2.1 Personalized NeoAntigen Peptides

Sequencing technology has revealed that each tumor contains multiple, patient-specific mutations that alter the protein coding content of a gene[10]. 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[11].

There have been multiple reports indicating that missense mutations or neoORFs in animal tumors induce strong CD8+ cytotoxic T lymphocyte (CTL) reactions, in some cases leading to prevention or eradication of disease[12-15]. More recently Matsushita and colleagues demonstrated that a single amino acid change in a normal murine structural protein (spectrin-β2) was the dominant target for immune attack against a methylcholanthrene-induced transplantable tumor, defining whether the tumor would survive or not following transplantation[16]. Furthermore, spectrin-β2 expression was

also dramatically reduced in escape variants, providing a clear mechanistic example of the immunoediting hypothesis. At the same time, DuPage and colleagues made a similar observation using a small immunogenic neoORF in the mouse (ovalbumin peptide)[17]. Both of these studies indicate that the immuno-edited neoantigen is therefore sufficient as the target of an effective anti-tumor response.

Correspondingly, many 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[18]. These studies began with the first identification of human immunogenic neoantigens[19,20], and the seminal study by Lennerz demonstrating the strength and durability of neoantigen response compared to native protein responses[21], and now have included the observations of an increase in neoantigen-specific CD8+ T cells in a patient responding to anti-CTLA-4 therapy[22] and tumor regression following infusion of a tumor infiltrating lymphocyte (TIL) population highly enriched in a neoantigen directed CD4+ T cell[23]. These observations were made in multiple cancer types for multiple HLA alleles and have been widely observed in tumor infiltrating T cell populations[24-26]. Most of the $CD8^+$ T cell responses show a high degree of 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.

Thus, in animals and in humans, immune responses to both discrete, mutated antigens (such as missense mutations) and expansive novel antigens (neoORFs) are observationally correlated with regression and long-term remission. Extending that correlation among a large set (n=468) of patients found in the Cancer Genome Atlas (TCGA) database, a recent meta-analysis of six tumor types revealed a significant survival advantage (hazard ratio = 0.53; p=0.002) for patients with at least one predicted immunogenic neoepitope compared to patients with no predicted immunogenic epitopes[27].

Three studies in humans have directly assessed the immunotherapeutic potential of mutated antigens. First, 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[28]. The induced $CD8^+$ T cells showed reactivity to the rearranged, mutated portion of the immunoglobulin molecule (the idiotype) and not the germline framework[29]. Second, 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[30-32]. Finally, immunization with a synthetic version of an in-frame junctional deletion variant of the epidermal growth factor receptor (*EGFRviii*) in two studies in glioblastoma patients, a population known to frequently contain this mutation, provided encouraging phase 2 results[33,34]. Importantly, in both studies, evaluation of tumors in patients with tumor recurrence showed that the recurrent tumors almost uniformly (20 of 23) lost expression of *EGFRviii*. This was interpreted as clear evidence of immunoediting due to immune pressure against an immunogenic neoantigen in humans.

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[30,31]. 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[35] as well as multiple long peptides, including neoORFs. In particular, four studies have been conducted with a mixture of 10 overlapping long peptides derived from p53[36-40] and five separate studies with a mixture of 13 long peptides derived from the oncogenic proteins of HPV[30-32,41,42]. 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, [43,44] tumor cell lysates[45] and shed tumor cell line antigens[46]. 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; such complexes also contain many mutated peptides[47]. 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 5.4.1.2) and combined with the immune adjuvant poly-ICLC, a stabilized double-stranded RNA (see Section 2.3 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 participant will receive the full complement of peptides at each immunization.

Neon Therapeutics, Inc. has collaborated with DFCI in the development of the NeoVax. DFCI will exclusively license the NeoVax technology to Neon Therapeutics, Inc. for future research that could lead to new clinical trials.

2.2.2 **Poly-ICLC**

2.2.2.1 **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[48]. 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[49]. 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[50]. 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[51].

2.2.2.2 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 poly-lysine 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[52-55], cross-priming, and the induction of tumor-, virus-, and autoantigen-specific $CD8^+$ T-cells[56-58]. 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[59]. Remarkably, comparison of the poly-ICLC gene expression data to previous data from volunteers immunized with the highly effective yellow fever vaccine YF17D[60] 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.

Two studies of poly-ICLC in conjunction with long peptides have been published. 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.[61] In a second human study, poly-ICLC was combined with a MUC1 synthetic long peptide in patients with pre-malignant adenomas. Robust antibody responses were detected in nearly half the patients which inversely correlated with the pre-existing circulating myeloid derived suppressor cell level[62].

Poly-ICLC has also been utilized as an adjuvant for immunization with minimal epitope-loaded patient-derived dendritic cells. Both CD4+ and CD8+ T cell responses to multiple peptides were observed in the majority of patients[63].

2.2.2.3 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 (LD_{50}) 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 0.5 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 aminotransferase (ALT) and aspartate aminotransferase (AST) were observed and normalized after termination of dosing. No other

significant toxicity was observed in these studies.

2.2.2.4 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 ≥ 6mg/m^2 (about 170 μg/kg) in patients with a variety of solid tumors and leukemia[63]. 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[61]. No grade 3 or 4 adverse events were reported.

In another clinical study which recently completed accrual (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.c. 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 (Dr. Nina Bhardwaj, personal communication).

Selected Clinical Trials with Low-Dose Poly-ICLC						
Protocol Title	Phase	Protocol Location	Indication	Status	N	Dosing Schedule
Long-term IM poly-ICLC in Malignant Glioma – a Pilot Study	I-II	Walter Reed	Malignant Glioma	Closed	67	IM 10-50 µg/kg 1-3 X week
Poly-ICLC in Recurrent Malignant Brain Tumors	I-II	MCV	Recurrent Glioma	Closed	99	IM 20 µg/kg 3 X week
Poly-ICLC in Malignant Pediatric Brain Tumors	I-II	L.A. Childrens Hosp	Pediatric Glioma	Closed	46	IM 20 µg/kg 2 X week
Poly-ICLC plus Radiation in Glioblastoma	II	NABTC 2001-05	Glioblastoma	Closed	31	IM 20 µg/kg 3 X week
Poly-ICLC in Recurrent Anaplastic Glioma	II	NABTC 2001-06	Recurrent Anaplastic Glioma	Closed	55	IM 20 µg/kg 3 X week
Poly-ICLC plus Temodar in Newly Diagnosed Glioblastoma	II	NABTT 2005-01	Glioblastoma	Closed	97	IM 20 µg/kg 3 X week
Pilot Study of MUC1 Vaccine plus poly-ICLC in Advanced Prostate Cancer	I-II	UPMC 05-086	Advanced Prostate Cancer	Closed	25	IM 25 µg/kg 2 X week
Poly-ICLC plus Dendritic Cell vaccine in Recurrent Gliomas	I-II	UPMC	Recurrent gliomas	Closed	25	IM 20 mcg/kg
Poly-ICLC plus HSP-HPVE7 Vaccine in Cervical Dysplasia	I	Nventa	Cervical Dysplasia	Closed	24	.05 – 2 mg
IntraTumoral (IT) poly-ICLC plus XRT and TACE in Liver Cancer	I-II	UMDNJ	Hepatoma, Metastatic liver cancers	Closed	24	1 mg IT + 1 mg IM 2 X week
A Randomized Controlled Phase I trial of Nasal Hiltonol	I	NIAID, NIH:	Normal volunteers	Closed	50	0.25-4mg IN
CDX 1307 vaccine with poly-ICLC in metastatic cancers	I	Various	Metastatic Cancers	Closed	20	2 mg IM
Poly-ICLC with glioma associated peptide vaccine	I-II	UPMC	Grade II Gliomas	Closed	20	20 mcg/kg IM 2X Wk
MUC1 100-mer and poly-ICLC Vaccine for Colonic Polyposis	I/II	UPMC	Colonic Adenoma	Closed	45	.5 mg SC
Hiltonol +NYESO1 Protein Vaccine in Ovarian Cancer	I/II	MSKCC, LICR	Ovarian Cancer	Closed	10	1.4 mg SC
Intratumoral PICLC and DC in Pancreatic CA	I/II	MUSC	Pancreatic Cancer	Closed	12	1 mg IT + IM
Intratumoral PICLC and DC in Solid Cancers	I/II	U Navarra Spain	Solid Cancers	Closed	15	1 mg IT
Pilot study of intratumoral and IM Poly-ICLC	I/II	Mt. Sinai MC, NY	Solid Cancers	Closed	8	1 mg IT+ IM

Selected Clinical Trials with Low-Dose Poly-ICLC						
Poly-ICLC + CDX1401 + FLT3	I/II	CITN, Celldex	Melanoma	Closed	60	1 mg IT+ IM
IMA950 vaccine /Poly-ICLC, Keytruda	I/II	U. Geneva Suisse	Glioblastoma	Open	20	1 mg IM
MAGE A3 + Hiltonol in Myeloma	I/II	Univ. of Maryland	Myeloma	Closed	27	1 mg SC
DEC205 GAG Protein / Hiltonol	II	Rockefeller Univ.	HIV vaccine	Closed	40	1.5 mg SC
WT1 vaccine / Hiltonol / Basiliximab	I/II	U. Chicago	AM Leukemia	Closed	7	1 mg SC
In-Situ, IT Autovaccination Hiltonol, FLT3, aPD1	I/II	Mt. Sinai MC, NY	Lymphoma	Open	40	1 mg
In Situ Autovaccination with IT Hiltonol,	II	Multicenter	Solid Tumors	Open	60	1 mg IT + IM
GAPVAC Trial, Europe	I	Multicenter	Glioblastoma	Closed	16	1 mg SC
A Personalized Cancer Neoantigen vaccine + Poly-ICLC, Nivolumab	I/II	Multicenter	Solid Cancers	Open	90	2 mg SC
In situ vaccination with durvalumab±tremelimumab + IT Hiltonol	I/II	UVA, ISMMS	Solid Cancers	Open	36	1 mg IT
Pembrolizumab and poly-ICLC	I/II	Augusta U.	Colon Cancer	Open	45	1 mg IT
IM Poly-ICLC in Low grade Glioma	I/II	UCSD, Emory U	Pediatric Low Grade Glioma	Closed	23	20 mcg/kg IM
IM Hiltonol + aPD1/L1	II	Multicenter	Solid Cancers	Open	60	1 mg 2X wk

2.2.3 **Ipilimumab**

Ipilimumab (BMS-734016, MDX010, MDX-CTLA4) is a fully humanized monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T lymphocyte antigen 4 (CTLA-4, CD152), which is expressed on a subset of activated T cells. CTLA-4 is a negative regulator of T cell activity. Ipilimumab is a monoclonal antibody that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T cell activation and proliferation, including the activation and proliferation of tumor infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell function, which may contribute to a general increase in T cell responsiveness, including the anti-tumor response.

Yervoy™ (ipilimumab) has been approved for use in over 47 countries including the United States

(US, Mar-2011), the European Union (EU, Jul-2011) and Australia (Jul-2011).

2.2.3.1 Preclinical Toxicology of Ipilimumab

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

Ipilimumab binds to human and cynomologous monkey CTLA-4 with high affinity and blocks binding of CD80 (B7.1) and CD86 (B7.2) to CTLA-4. The proposed mechanism of action for ipilimumab is interference of the interaction of CTLA-4 with its natural ligands, CD80/CD86, expressed on antigen presenting cells, which results in blockade of the inhibitory modulation for T cell activation. CTLA-4 blockade by ipilimumab resulted in augmentation of antibody responses to various T cell dependent antigens and suppression of tumor growth in mice.

The cynomologous monkey was selected as the primary toxicology species because ipilimumab binds specifically to macaque CTLA-4, but not to homologous CTLA-4 in other traditional toxicology species[64]. Administration of ipilimumab to cynomologous monkeys resulted in marked enhancement of the immune response to a viral antigen (hepatitis B surface antigen), a cell-based vaccine (SK-mel) and to keyhole limpet hemocyanin.

In IV repeat-dose toxicology studies in monkeys, ipilimumab was tolerated without adverse effects at doses up to 30 mg/kg/day administered every 3 days for 3 doses (peak serum concentrations $\leq$ 682 µg/mL)[65-67] at 10 mg/kg (equivalent to human dose on a body-weight basis) administered weekly for 1 month[68] (mean area under the concentration-time curve 0-168h) and AUC (0-63days) of 31.6 mg·h/mL and 90.6 mg·h/mL respectively), at 1 mg/kg administered weekly for 10 weeks[69] and at doses up to 10 mg/kg/day administered approximately monthly for up to 6 months[66,68,70,71]. In the pivotal 6-month toxicity study (10 mg/kg administered on days 0,28,56,84 and 140), treatment-related findings were limited to decreases in absolute and relative thyroid (44% to 50%) and testicular (27% to 50%) weights.

In an exploratory pharmacology study, severe colitis occurred after the second dose in 1 of 5 monkeys receiving ipilimumab at 10 mg/kg approximately monthly in combination with 3 vaccines. The binding of ipilimumab to CTLA-4 expressed on gut-associated lymphoid tissue was confirmed in human and monkey tissue-binding studies[64,72,73] and suggests that these lymphocytes exist in an activated state, making them susceptible to CTLA-4 blockade. Two gastrointestinal toxicities (diarrhea and colitis) have emerged as significant immune related adverse events in the clinical studies with Ipilimumab (See 2.2.3.2 below).

The effects of ipilimumab on reproduction and development were studied in an enhanced pre and postnatal development study in cynomologous monkeys[74]. Pregnant monkeys received ipilimumab every 21 days from the onset of organogenesis through parturition at doses of 2.6 or 7.2 times the clinical exposure at a dose of 3 mg/kg or 0.9 to 2.1 times higher than the clinical exposure at a dose of 10 mg.kg every 21 days of ipilimumab. Beginning in the third trimester, the ipilimumab groups experienced increased maternal weight decrements; higher incidences of abortion, stillbirth, premature delivery and higher incidences of infant mortality in a dose-related manner.

Infants exposed to 30 mg/kg/q3w had a lower mean body weight at birth that persisted for multiple months. Based on the results for the monkey reproductive system, ipilimumab is not recommended for use during pregnancy unless the potential benefit justifies the potential risk to the fetus.

In summary, other than the events described above, ipilimumab did not result in any adverse toxicities in any other monkeys in general toxicity studies when administered IV at doses up to 30 mg/kg for 1 week, 10 mg/kg weekly for 1 month, 1 mg/kg weekly for 10 weeks, or 10 mg/kg monthly for 6 months.

2.2.3.2 Clinical Toxicology of Ipilimumab

Except for pediatric patients, all the clinical studies reported in the Investigator Brochure utilized Ipilimumab delivered intravenously at doses of 3 mg/kg to 10 mg/kg, usually q3weeks X 4. As described in Section 6.1.3, the starting dose level utilized in this protocol results in one fourth (1/4) the total Ipilimumab exposure during the first three weeks compared to the 3 mg/kg i.v. dosing and one thirtieth (1/30) the exposure over the subsequent 16 weeks. Thus, the safety events observed with Ipilimumab delivered intravenously may be significantly greater than following limited local delivery, a hypothesis of this study.

Complete information on the clinical safety of Ipilimumab delivered intravenously at doses of 3 mg/kg and 10 mg/kg can be found in the Ipilimumab Investigator Brochure (IB) and are summarized in brief below.

Blockade of CTLA-4 by Ipilimumab leads to T cell activation with the potential for clinical inflammatory AEs primarily involving the skin (dermatitis, pruritis), GI tract (diarrhea/colitis), liver (hepatitis), endocrine glands (e.g., hypophysitis, adrenal and thyroid abnormalities) and other less frequent organs (e.g., uveitis/episcleritis). The majority of the inflammatory AEs are reversible with the guidance issued below. In rare cases, these inflammatory AEs may be fatal.

Patients should be assessed for signs and symptoms of enterocolitis, dermatitis, neuropathy and endocrinopathy, and clinical chemistries (including liver function and thyroid function tests) should be evaluated at baseline and before each dose of ipilimumab. GI (diarrhea and colitis) and skin (rash and pruritis)-related toxicities are the most common inflammatory events reported in studies with ipilimumab. Suggested evaluation procedures for suspected GI, liver, skin, endocrine, neurological and ocular toxicities are described below. Early diagnosis and treatment intervention for inflammatory events can help prevent the occurrence of complications, such as GI perforation.

During evaluation of a suspected inflammatory AE, all efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes. Serological, immunological, imaging, and biopsy with histology (e.g. biopsy-proven lymphocytic) data should be used to support the diagnosis of an immune-mediated toxicity or support an alternative cause of the AE.

In general, for severe inflammatory AEs, ipilimumab should be permanently discontinued and systemic high-dose corticosteroid therapy should be initiated. For moderate immune-mediated AEs, ipilimumab should be held or delayed and moderate dose corticosteroids should be considered. Upon improvement, corticosteroids should be tapered gradually over at least 1 month.

Based on limited current clinical experience, corticosteroids do not appear to adversely affect the antitumor response. For example, disease control was maintained in subjects with objective responses who received corticosteroid administration for concomitant serious inflammatory AEs.

The management guidelines for general inflammatory GI, liver, skin, endocrine, and neurological toxicities are provided in detail in Appendix 3 of the Investigators Brochure.

2.2.3.3 Clinical Toxicology of Ipilimumab in combination with a peptide vaccine

A number of clinical trials have evaluated the combination of systemic Ipilimumab with a variety of different vaccines, including peptide vaccines as outlined in the table below. In all cases, no qualitatively or quantitatively different toxicities were observed other than those typically observed with Ipilimumab alone.

Vaccine	Dose and schedule	Reference
gp100 peptide vaccine	3 mg/kg Ipilimumab; iv; q3weeks 1 mg peptide; sc; q3weeks (2 peptides)	2010; Hodi et al[5]
GM-CSF transduced allogeneic prostate cancer cells (GVAX- prostate)	0.3 – 5 mg/kg Ipilimumab; iv; q4weeks GVAX-prostate id; q2weeks for 24 weeks	2012; Van den Eertwegh et al[75]
Prostate-specific antigen and three co-stimulatory molecules (CD58, CD80 and ICAM1) in a poxviral vector (Prostvac)	1 – 10 mg/kg Ipilimumab; iv; q4weeks Prostvac; sc; q4weeks	2012; Madan et al[76]
GM-CSF transduced allogeneic pancreatic ductal adenocarcinoma cells (GVAX-pancreatic)	10 mg.kg Ipilimumab; iv; q3weeks GVAX-pancreatic; id; q3weeks	2013; Le et al[77]
gp100, MART-1 and Tyrosinase peptide vaccine	3 or 10 mg/kg Ipilimumab; iv' q6-8weeks 1 mg each peptide; sc; q2weeks x6 then less frequently	2011; Sarnaik et al[78]

2.2.3.4 Clinical Efficacy of Ipilimumab as Monotherapy in Advanced Melanoma

In a large, double-blind, double-dummy randomized phase 3 study involving 676 patients, Ipilimumab was delivered at 3 mg/kg in patients who had received 1 or more prior therapies. Patients were randomized (3:1:1) to receive Ipilimumab in combination with a peptide vaccine, Ipilimumab alone or the peptide vaccine alone. All patients were HLA A*02:01. Overall survival was compared in the Ipilimumab + vaccine arm vs the vaccine-alone arm. Treatment with Ipilimumab +vaccine resulted in a 32% reduction of risk of death (HR 0.68; 95% CI 0.55-0.85) versus vaccine alone (p = 0.0004). There was no apparent difference between the Ipilimumab + vaccine arm compared to Ipilimumab alone (HR 1.04; 95% CI 0.83-1.30). The median survival of patients on the Ipilimumab + vaccine arm was improved by 4 months (10 months vs 6 months). One of the hallmarks of Ipilimumab efficacy is long-term survival. A large meta-analysis of 1861 advanced melanoma subjects treated with Ipilimumab as part of clinical trials demonstrated a plateau in OS Kaplan-Meier curve at Year 3, which remained flat through Year 10[79].

2.3 Rationale

2.3.1 RCC is responsive to immunotherapy

Several immune-modulating therapies have already been approved for RCC, such as interleukin-2 and interferon-alpha although their utilization is limited due to minimal efficacy or limited access. Ipilimumab, an anti-CTLA4 specific IgG1 monoclonal antibody, blocks a key regulatory pathway that dampens both *de novo* and memory T-cell responses following antigen activation and has been demonstrated to improve overall survival as monotherapy in patients with advanced melanoma[5]. Furthermore, antibodies targeting the PD-1 pathway (another immune checkpoint) have demonstrated impressive anti-tumor activity in multiple cancer types[6,7,80-82] and the combination of CTLA-4 and PD-1 pathway blockade showed apparent synergy in advanced melanoma[80,81,83-85] and in RCC (Hammers et al, ASCO 2014 Abstract #4504; CheckMate-214 trial). Despite this success, the lack of coincident immune stimulation with an effective vaccine may limit the maximal impact of such therapy.

2.3.2 NeoVax is a novel personalized cancer vaccine utilizing the exquisitely tumor-specific neoantigens created by the personal mutations found in each patient's tumor

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 (as discussed in Section 2.1.1). Importantly, strong $CD8^+$ T cell responses against mutated epitopes have been found in patients with multiple tumor types 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[18]. Most of these $CD8^+$ T cell responses showed a high level of specificity towards 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 towards over-expressed native antigens.

Three 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)[86]. 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.[30-32] Finally, a mutated form of the epidermal growth factor receptor (EGFR) commonly found in glioblastoma patients has been used as an immunogen in multiple early clinical trials[87]. Interestingly, evidence for down-regulation of this mutated gene has been found, suggesting immune editing due to immune pressure induced by the vaccine[34]. 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 three 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.

2.3.3 The combination of NeoVax and Locally delivered Ipilimumab may synergistically increase the efficacy of the immune response

Many studies in animals have shown that combining anti-CTLA-4 treatment with a vaccine can substantially augment efficacy[88-90] and anecdotal information suggests a similar effect in humans[91,92]. This potential synergy may depend critically on the antigen as the most dramatic effects of the combination in animals and the effects in humans have typically been observed with complex vaccines such as autologous cellular vaccines and not with an individual tumor-associated antigen (such as gp100 for melanoma). Autologous tumor cell vaccines[91] contain both neoantigens and tumor-associated antigens. NeoVax, by focusing the immune system on neoantigens, presumably the most immunogenic subset of possible tumor antigens, is expected to strengthen and focus the immune response to the tumor, thereby generating more robust clinical activity. Reciprocally, checkpoint blockade during vaccination may increase the breadth and the magnitude of the response to NeoVax and increase memory cell formation. Recently, neoantigen-specific T-cells were observed in an Ipilimumab-treated patient prior to treatment initiation and increased following therapy[93].

Another innovative aspect of the proposed study deals with the route of delivery of Ipilimumab. Although effective, the toxicity profile of intravenously delivered Ipilimumab, the approved delivery route for metastatic disease, can limit its application. For this reason, Ipilimumab will be delivered "locally" in this study by sub-cutaneous injection in proximity to the vaccination site during each vaccine dosing. Large proteins like Ipilimumab enter the circulation following sub-cutaneous injection by transit through the lymphatics and draining lymph nodes[94]. Thus, we expect that this approach will target Ipilimumab to the same draining lymph node as the vaccine and reduce systemic exposure, thereby both maximizing effectiveness and limiting systemic toxicity of Ipilimumab. Multiple animal studies have shown that localized dosing of anti-CTLA-4 is effective. The modes of localized dosing have included: (i) local production of anti-CTLA-4 from the irradiated tumor cell being used as vaccine[95], (ii) "in transit" delivery of anti-CTLA-4 between the tumor (antigen source) and the local draining lymph node[96], and (iii) direct intra-tumoral injection of anti-CTLA-4[97]. Anti-tumor activity with localized administration from irradiated anti-CTLA-4 expressing tumor cells was reduced compared to systemic administration, but it was equal or better in the other two studies. Of note, systemic anti-tumor activity was observed, wherein injection of anti-CTLA-4 antibody in close proximity or into a tumor on one flank of the animal resulted in elimination of the tumor on the opposite flank[96,97] indicating a systemic treatment effect. In each study, overall toxicity attributed to anti-CTLA antibody was decreased, and was associated with dramatically reduced measured levels of circulating anti-CTLA-4 antibody. Although toxicity of anti-CTLA-4 is not typically apparent in the mouse, surrogate markers of toxicity, such as levels of the liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST)[96] or anti-nuclear antibodies (ANA)[95] were higher in animals treated systemically. For the dose of Ipilimumab proposed in this study (5mg per injection site), the total systemic exposure will be approximately 1/6 that of standard intravenous Ipilimumab therapy (please refer to Table 1 in section 7.1.1.3).

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

2.4 Correlative Studies Background

The induction of a neoantigen-specific T cell response following vaccination with NeoVax and simultaneous treatment with locally delivered Ipilimumab will be assessed by IFN-γ ELISPOT and/or tetramer analysis. Comparison will be made between samples taken prior to vaccine administration and after vaccination, beginning 4 weeks after the last priming dose.

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, the powerful immune adjuvant poly-ICLC and the relief of immune checkpoint control by Ipilimumab will induce strong $CD8^+$ and/or $CD4^+$ 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 be evaluated using the predicted epitope short peptides as well as longer peptides which require proteasomal processing as stimulant in the IFN-γ ELISPOT assay. If warranted, the precise immunogenic peptide(s) will be determined in follow-up analyses.

When feasible, HLA-tetramers will be prepared for one or more epitopes and will be used for cell staining and flow cytometry to independently evaluate the level of responding T cells.

In addition to the analysis of the magnitude and determinant mapping of the T cell response in peripheral blood, 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 or tetramer 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 patient-specific tumor cell recognition.

Additional correlative analysis may include autologous tumor cell line generation for T cell recognition and cytolysis assays, single cell proteogenomic profiling of the tumor-immune microenvironment and relevant skin sites (e.g. vaccine site), bulk and single cell T cell receptor (TCR) sequencing with reconstruction and specificity testing, among others.

In patients who require a follow-up biopsy for diagnosis of recurring disease, if sufficient excised tumor tissue is available, T cells from the biopsied tumor will be evaluated for epitope-specificity and activation markers.

PARTICIPANT SELECTION

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

3.1 Eligibility Criteria for Initial Registration

3.1.1 Ability to understand and the willingness to sign a written informed consent document before planned surgery.

3.1.2 Patient should have suspected stage III or stage IV clear cell Renal Cell Carcinoma (ccRCC), with anticipation that all disease can be surgically resected. Confirmation of clear cell histology, final stage (III or IV), and removal of all disease will be done after the surgery, and will be required for further participation in the trial.

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

3.1.4 Patients undergoing a potentially curative metastatectomy are eligible if the tumor tissue from the surgery is enough to make a vaccine.

3.1.5 ECOG performance status ≤1

3.1.6 Age ≥ 18 years.

3.1.7 Participants must have normal organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional upper limit of normal
– creatinine clearance	≥25 mL/min (calculated using the Cockroft-Gault equation)

3.1.8 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 and 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.

3.1.9 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 for the duration of treatment with ipilimumab plus 5 half-lives of ipilimumab (75 days) plus 30 days (duration of ovulatory cycle) for a total of 105 days post-treatment completion. 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.

3.1.10 Male patients must agree to use an adequate method of contraception for the duration of treatment with study drugs plus 5 half-lives of the study drug (75 days) plus 90 days (duration of sperm turnover) for a total of 165 days post-treatment

3.2 **Eligibility Criteria for Secondary Registration**

3.2.1 ECOG performance status ≤1

3.2.2 Participants must have normal organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional upper limit of normal
– creatinine clearance	≥25 mL/min (calculated using the Cockroft-Gault equation)

3.2.3 Patients must have histologically confirmed clear cell renal cell carcinoma (ccRCC), stage III or fully resected stage IV (no evidence of disease), before starting study drugs per AJCC 8th edition.

3.2.4 No evidence of disease (NED) at secondary registration

3.2.5 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.

3.2.6 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, cervical cap with spermicide, male cond

oms, or female condom with spermicide. Spermicides alone are not an acceptable method of contraception.

3.2.7 Male patients must agree to use an adequate method of contraception for the duration of treatment with study drugs plus 5 half-lives of the study drug (75 days) plus 90 days (duration of sperm turnover) for a total of 165 days post-treatment

3.3 **Exclusion Criteria**

Patients who exhibit any of the following conditions at any point prior to first vaccination (i.e. at either initial or secondary registration) will not be eligible for admission to or continuation on the

study:

3.3.1 Prior treatment with immune-modulatory agents including, but not limited to: IL-2, CTLA-4 blockade, PD-1/PD-L1 blockade, CD40 stimulation, or CD137 stimulation.

3.3.2 Prior investigational ccRCC-directed cancer vaccine therapy.

3.3.3 Patients with active brain metastases or leptomeningeal disease.

3.3.4 Prior systemic therapy, including targeted therapy such as VEGF or mTOR inhibitors unless it is >6 months between last dose of drug and first vaccination with NeoVax. Systemic therapy is allowed only if prior therapy was not immune therapy (i.e. VEGF TKI), and it was >6 months ago

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

3.3.6 Previous bone marrow or stem cell transplant.

3.3.7 Concomitant therapy with any anti-cancer agents for ACTIVE cancer treatment, other investigational anti-cancer therapies, or immunosuppressive agents; chronic use of systemic corticosteroids with prednisone >10 mg/day.

3.3.8 Use of a non-oncology vaccine therapy for prevention of infectious diseases (with the exception of vaccination against the SARS-CoV-2 virus for the prevention of COVID-19 disease) is not allowed for 4 weeks prior to day 1, until 8 weeks after last study dose. Given the severity of the COVID-19 pandemic, vaccination specifically against the SARS-CoV-2 virus for the prevention of COVID-19 is ALLOWED in this study.

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

3.3.10 History of or current active autoimmune diseases, [e.g. including but not limited to inflammatory bowel diseases [IBD], rheumatoid arthritis, autoimmune thyroiditis, autoimmune hepatitis, systemic sclerosis (scleroderma and variants), systemic lupus erythematosus, autoimmune vasculitis, autoimmune neuropathies (such as Guillain-Barre syndrome). Vitiligo and adequately controlled endocrine deficiencies such as hypothyroidism are not exclusionary.].

3.3.11 Patients who have had a history of acute diverticulitis, intra-abdominal abscess, GI obstruction and abdominal carcinomatosis which are known risk factors for bowel perforation.

3.3.12 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.

3.3.13 Known chronic infections with HIV, hepatitis B or C. Serology results are not required for initial registration but they must be available before the start of vaccine production.

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

3.3.15 History of current immunodeficiency disease [e.g., splenectomy or splenic irradiation].

3.3.16 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.

3.3.17 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 enfants secondary to treatment of the mother with personalized neoantigen peptides and poly-ICLC, nursing women are excluded from this study.

3.3.18 Individuals with a history of another invasive malignancy are ineligible except for the following circumstances: a) individuals with a history of invasive malignancy are eligible if they have been disease–free for at least 2 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.

3.4 Inclusion of Women and Minorities

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.

REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

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 the 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 10-14 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 3.2 and 3.3), participant will proceed to receive vaccines on study.

4.2 Registration Process for DF/HCC Institutions

The Initial Registration procedures are as follows:

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.

1. Confirm written informed consent from the participant has been obtained prior to the performance of any protocol-specific screening procedures or assessments.
2. Complete the *Initial Eligibility Checklist for Overall Study Participation (for Initial Registration)*
3. Perform subject registration per DF/HCC SOP REGIST-101B

The Secondary Registration procedures are as follows:

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.

1. Complete the *Secondary Eligibility Checklist to Initiate Treatment (for Secondary Registration)*
2. Verify the success of the subject registration in all DF/HCC systems.
3. Perform subject registration per DF/HCC SOP REGIST-101B

TREATMENT PLAN

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for NeoVax and Ipilimumab (or NeoVax alone for Cohort 1B) 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 disease or resectable distant metastatic disease (to obtain tumor for vaccine manufacturing) as defined in 3.2.3 will be identified and enrolled on the study. Tissue procurement will be performed as described in 5.1. After confirmation of complete tumor resection by pathologic review, confirmation of histology and stage, 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 end up providing inadequate DNA and/or RNA for sequencing or for whom vaccine cannot be prepared will be removed from the trial. These slots will be filled with additional patients.

5.1 Preparation of NeoVax

5.1.1 Tumor and normal tissue procurement and sequencing

Principal investigator Dr. Toni Choueiri (DFCI pager 46566) or a clinical research coordinator on his team 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 and pathology staff of the Brigham and Women's Hospital (BWH).

Once adequate tumor for pathological assessment has been harvested as deemed by the surgeon, the remaining tumor tissue will be fixed in formalin and transferred to the DFHCC Hematopathology Core, where portions of the fixed tissue will be prepared for whole-exome and transcriptome sequencing in order to identify vaccine targets. If remaining tumor tissue is adequate, single cells may also be prepared in research laboratories, from which cell lines may be initiated and tumor infiltrating lymphocytes may be isolated. Any remaining tumor will be cryopreserved or fixed in formalin for future exploratory research studies at the discretion of the Principal Investigator. In the event that sequence analysis yields sub-optimal results, cells from the tumor cell lines 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 source for normal host germline tissue DNA.

Samples will be de-identified before being shipped to the the Clinical Research Sequencing Platform (CRSP) and Broad Genomics Platform research lab at the Broad Institute.

Whole-exome sequencing will be conducted in the Clinical Research Sequencing Platform, LLC (CRSP) at the Broad Institute. CRSP is CLIA licensed (License #22D2055652) and CAP accredited (#8707596) and one of tests offered by CRSP is Somatic Whole Exome sequencing, which will be utilized for these trials. CRSP will extract total DNA from blood and tumor tissue using standardized procedures. The DNA samples will be processed through the CLIA Somatic WES test For tumor and normal DNA samples, whole exome capture will be conducted prior to sequencing on Illumina HiSeq or NovaSeq. RNA sequencing is completed at the non-CLIA Broad Genomics Platform research lab. For tumor RNA, a cDNA library will be prepared and may be enriched by transcriptome capture prior to sequencing on Illumina HiSeq or NovaSeq. 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).

5.1.2 Preparation of personalized neoantigen peptides

5.1.2.1 Identification of target epitopes for peptide design

Personalized neoantigen peptides are identified for each patient by first conducting whole exome DNA sequencing of tumor and normal tissue samples from the patient to identify the specific coding-sequence mutations that have occurred in the tumor of that patient, using the cancer genome analysis pipeline. The cancer genome analysis pipeline is a research-grade pipeline (neither validated nor maintained under a CLIA license) that is used to identify candidate mutations. The pipeline characterizes the somatic changes in a tumor sample through analysis of tumor and matched normal sequencing data and generates a MAF (mutation annotated format) file listing the candidate mutations it identified, both somatic single necleotide variations (SSNVs) and somatic small insertions and deletions (indels). In addition, the pipeline's quality control tasks generate several reports flagging potential QC issues such as sample mix-ups, cross-sample contamination and low-quality base reads and alignments. A review of the QC metrics contained in these reports is a step in the bioinformatics SOP which encompasses the running of the pipeline. Orthogonal RNA-seq and confirmatory targeted sequencing will be employed to confirm that the candidate mutations identified by the cancer genome analysis pipeline are present in the tumor and not present in the germline DNA. Candidate missense mutations and indels will be analyzed for the presence of mutation-containing epitopes by the protocol's neoantigen prediction algorithms.

Only confirmed candidate missense mutations and indels will be included in the final vaccine product. Well-established algorithms (such as netMHCpan, MSIntinsic, and/or similar predictive algorithms) will be used to identify mutation-containing epitopes that are predicted to bind to the MHC class I molecules of each participant[98]. Additionally, depending on tumor RNA availability, RNA expression (non-CLIA) may be used in neoantigen prediction pipeline as well. In that case, an algorithm incorparting RNA expression data (such as MSIntrinsicEC) would be used. The bioinformatics pipeline for identification of mutations and prediction of neoantigens consists of tools in a research use (non-CLIA) pipeline.
From this list of candidate mutations, 20 – 40 mutations will be selected by the Epitope Selection Board (comprised of experts in clinical immuno-oncology, immunology, and genomics) at DFCI and prioritized for peptide preparation based on a pre-defined set of criteria including:

- Type of mutation (missense vs neoORF)
- Predicted binding potential of peptides encoded by the mutated region to the MHC class I alleles of the particular individual
- Predicted binding potential of the corresponding native peptide
- The likelihood that the mutation is directly or indirectly related to the tumorigenic phenotype (i.e., an "oncogenic driver" mutation or a mutation in a related biochemical pathway)

- RNA expression
- Biochemical properties of the full peptide (e.g. predicted poor solubility secondary to hydrophobic amino acid number or distribution and/or cysteine content).

Up to forty mutations for each participant will be used to design peptides, each approximately 20 - 30 amino acids in length.

As noted, somatic mutations identified by analysis of whole exome data as described above (including single nucleotide variations, and small insertions and deletions) will be assessed by examining the corresponding RNA-Seq tumor BAM file of the patient, and by performing orthogonal confirmatory targeted sequencing in a research laboratory setting at the Dana Farber Cancer Institute using nucleic acids extracted at the Broad Institute (if there is sufficient additional DNA/RNA available), or from an independent tissue from the same patient. This confirmatory sequencing, using DNA/RNA from both malignant and normal cells, will confirm that the mutation is present in the tumor, and is not present in germline DNA.

NOTE: Analysis of mutations will be limited to comparison of normal and tumor sequence information for each participant. Although alignments to a reference genome will be used during sequence analysis, no mutational comparison of the reference genome to either normal or tumor DNA will be conducted. Therefore, germline mutations potentially indicative of disease susceptibility will not be identified.

5.1.2.2 **Peptide synthesis**

GMP peptides will be synthesized by standard solid phase synthetic peptide chemistry and purified by RP-HPLC. Each individual peptide will be analyzed by a variety of qualified assays to assess appearance (visual), purity (RP-HPLC), identity (by mass spectrometry), peptide purity (analytical UPLC), residual solvents (by gas chromatography) and trifluoro-acetate counterion (RP-HPLC) and released. This work will be performed by Almac Sciences, Ltd. or an alternative peptide vendor able to synthesize peptides that will be used in clinical research studies under cGMP conditions. Synthesis will be initiated with up to 24 peptides if possible so that additional peptides are immediately available for replacement of insoluble peptides if needed (See Section 5.1.2.3).

5.1.2.3 **Preparation of NeoVax peptide pools**

It is intended to immunize patients with as many peptides as possible, up to a maximum of 20. Peptides will be mixed together in up to 4 pools of up to 5 peptides each. The selection criteria for each pool will be based on the particular MHC allele to which the peptide is predicted to bind. Peptides predicted to bind to the same MHC allele will be placed into separate pools whenever possible in order to limit antigenic competition. Some of the neoORF peptides may not be predicted to bind to any MHC allele of the patient. These peptides will still be utilized however, primarily because they are completely novel and therefore not subject to the immune-dampening effects of central tolerance, thus having a high probability of being immunogenic. NeoORF peptides also carry a dramatically reduced potential for autoimmunity as there is no equivalent molecule in any normal cell. In addition, there can be false negatives arising from the prediction algorithm and it is possible that the peptide will contain a HLA class II epitope (HLA class II

epitopes are not reliably predicted based on current algorithms). All peptides not identified with a particular HLA allele will be randomly assigned to the individual pools.

The peptide pools will be prepared at Almac Sciences, Ltd. (or alternative GMP peptide vendor). The amounts of each peptide are predicated on a final dose of 300 μg of each peptide per injection. The peptide pools will be prepared by dissolving appropriate quantities of each peptide individually at high concentration (approximately 50 mg/ml) in dimethyl sulfoxide (DMSO) and dilution with 5% dextrose in water (D5W)/ 5 mM succinate to a final concentration of 2 mg/ml. Any peptides that do not demonstrate clear solutions upon dilution will be discarded and replaced with another peptide if available; if no additional peptides are available, D5W/succinate will be used. Equal quantities of each of up to 5 peptides will then be admixed, effectively diluting each peptide to a concentration of 400 μg/ml.

The bioburden in the pooled peptides will be reduced by filtration through a 0.2 μm sterilizing filter. The pooled and filtered bulk will be shipped to the DFCI Pharmacy where final strerile filtration in a laminar flow biosafety cabinet and aliquoting into 2 ml Nunc cryo individual dosing vials for storage will be performed.

An aliquot of each pool will be transferred to the Cell Manipulation Core Facility (DFCI/CMCF) and tested for sterility and endotoxin testing (the Certificate of Analysis contains the solvent result). Individual dosing vials will be stored at the DFCI Pharmacy at -70 to -80ºC.

5.2 Administration of NeoVax (all Cohorts) and Ipilimumab (Cohort 1, Cohort 2, Expansion Cohort)

5.2.1 Pre-Treatment Criteria

Preparation of the final NeoVax product (all Cohorts) and syringes containing Ipilimumab (Cohort 1, Cohort 2, Expansion Cohort) 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.

5.2.1.1 Day 1

On Day 1 laboratory parameters do not need to re-meet eligibility criteria prior to administration of the study agents. No specific prophylactic or supportive care is necessary prior to administration of the study agents. Ipilimumab injections (Cohort 1, Cohort 2, Expansion Cohort) should be administered within 30 minutes of peptide injections.

5.2.1.2 Subsequent treatment days

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.

Ipilimumab injections should be administered within 30 minutes of peptide injections.

5.2.2 Preparation of the NeoVax dosage form for intradermal and for subcutaneous delivery

The final step in the preparation of NeoVax (mixing with poly-ICLC, dividing into an intradermal dose and subcutaneous dose for each peptide pool) is conducted on the day of the scheduled vaccine administration. For each patient, up to four distinct pools (labeled "A", "B", "C" and "D") of up to 5 synthetic peptides each will have been prepared and filter sterilized at the GMP peptide manufacturer and stored at -80^{o}C. Poly-ICLC has been made available for this protocol by Oncovir, Inc and is stored at the DFCI Pharmacy (See 8.1.3).

On the day of immunization, the complete vaccine consisting of the peptide component(s) and poly-ICLC will be prepared in a laminar flow biosafety cabinet. One vial each (A, B, C and D) will be thawed at room temperature in a biosafety cabinet. 0.75 ml of each peptide pool will be withdrawn from the vial into separate syringes. Separately, four 0.25 ml aliquots of poly-ICLC will be withdrawn into separate syringes. The contents of each peptide-pool containing syringe will then be gently mixed with a 0.25 ml aliquot of poly-ICLC by syringe-to-syringe transfer.

Next, 0.5mL (+/- 0.1mL) from each of the four syringes ("NeoVax A" containing peptide pool A and poly-ICLC, "NeoVax B" containing peptide pool B and poly-ICLC, "NeoVax C" containing peptide pool C and poly-ICLC, and "NeoVax D" containing peptide pool D and poly-ICLC) will be transferred to four corresponding 1mL syringes, to be used for intradermal delivery (i.e. 0.5mL from syringe "NeoVax A" into a new syringe "NeoVax A", 0.5mL from syringe "NeoVax B" into a new syringe "NeoVax B", etc.). Overall, there will be two syringes per pool (NeoVax_A, NeoVax_B, NeoVax_C, NeoVax_D), each containing 0.5 mL (+/- 0.1mL) NeoVax (peptide pool + poly-ICLC), with one syringe per pool for subcutaneous administration, and the other syringe per pool for intradermal administration.

5.2.3 Preparation of Ipilimumab for subcutaneous delivery

For all cohorts except Cohort 1B. On each dosing day, a 200mg/40mL vial or 50mg/10 mL vial containing 5 mg/ml Ipilimumab will be used to prepare 4 syringes, each containing:

Cohort 1 Dose Level 1: 2.5 mg (0.5 ml)
Cohort 2 Dose Level 2: 5 mg (1mL)
Cohort 2 Dose Level -1: 1.25 mg (0.25mL)

If there are less than 4 peptide pools available, the number of Ipilimumab syringes prepared will be equal to the number of NeoVax syringes prepared.

5.2.4 Dose Preparation Procedures for intradermal and subcutaneous delivery

Cohort 1, Cohort 2, Expansion Cohort: Each vaccination treatment consists of up to 12 separate injections– up to 8 injections containing peptides from one of the up to four peptide pools combined with the adjuvant with two injections performed for each peptide pool (one subcutaneous injection, and one intradermal injection), and up to 4 subcutaneous injections of Ipilimumab within 1cm of each vaccine site.

Cohort 1B: Each vaccination treatment consists of up to 8 separate injections– up to 8 injections containing peptides from one of the up to four peptide pools combined with the adjuvant with two injections performed for each peptide pool (one subcutaneous injection, and one intradermal injection).

5.2.5 Preparation of peptide mixture for delayed-type hypersensitivity (DTH) assessment

On the day of the DTH assessment, a mixture containing all peptides will be prepared in a laminar flow biosafety cabinet. One vial of each of the four peptide pools (A, B, C and D) will be thawed at room temperature in a biosafety cabinet. 0.25 ml of each peptide pool will be withdrawn from the vial into a single syringe, and gently mixed together. 100µL of this mixture (containing all peptides) will then be transferred into a new 1mL syringe (labelled "NeoVax_DTH"), to use for the DTH assessment.

5.3 Agent Administration

5.3.1 Injections

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 on Schema B. In the boost phase, vaccine will be administered on days 78 (week 12) and 134 (week 20).

<table>
<tr><th colspan="6">Treatment Description for Cohorts 1, 2, and Expansion Cohort</th></tr>
<tr><th>Agent</th><th>Treatment phase</th><th>Schedule</th><th>Allowable Treatment Admin Window</th><th>Dose</th><th>Route</th></tr>
<tr><td rowspan="4">Cohorts 1, and 2 Levels 1, 2, and -1 NeoVax (peptides + poly-ICLC) and Ipilimumab</td><td rowspan="3">Prime</td><td>Day 1</td><td>N/A</td><td rowspan="4">Poly-ICLC:
8 x 0.125 mL

Peptides:
(4 peptide pools) x (up to 5 peptides per pool) x (300µg per peptide peptide/0.75 ml)

<u>Total NeoVax Volume</u> =
up to 8 x 0.5 ml (+/- 0.1 ml)

Ipilimumab:
Dose Level -1
4 x 1.25 mg (0.25 mL)
Dose Level 1
4 x 2.5 mg (0.5 mL)
Dose Level 2
4 x 5 mg (1 mL)</td><td rowspan="4">NeoVax
i.d. / s.c. injections into up to 4 different anatomic sites.

Ipilimumab
injected s.c.

The two NeoVax and the Ipilimumab injections are administered within 1 cm of each other.</td></tr>
<tr><td>Days 4 and 8</td><td>± 1 day</td></tr>
<tr><td>Days 15 and 22</td><td>± 3 days
(at least 5 days must elapse between doses)</td></tr>
<tr><td>Boost</td><td>Days 78 and 134</td><td>± 3 days</td></tr>
<tr><td>NeoVax peptides</td><td>DTH</td><td>7-14 days after day 78</td><td>N/A</td><td>100 µL from a mixture of all peptides</td><td>Intradermallly on the right forearm</td></tr>
</table>

<table>
<tr><th colspan="6">Treatment Description for Cohort 1B</th></tr>
<tr><th>Agent</th><th>Treatment phase</th><th>Schedule</th><th>Allowable Treatment Admin Window</th><th>Dose</th><th>Route</th></tr>
<tr><td rowspan="4">Cohort 1B, NeoVax (peptides + poly-ICLC)</td><td rowspan="3">Prime</td><td>Day 1</td><td>N/A</td><td rowspan="3">Poly-ICLC:
8 x 0.125 mL

Peptides:
(4 peptide pools) x (up to 5 peptides per pool) x (300µg per peptide peptide/0.75 ml)

<u>Total NeoVax Volume</u> =
up to 8 x 0.5 ml (+/- 0.1 ml)</td><td rowspan="3">NeoVax
i.d. / s.c. injections into up to 4 different anatomic sites.

The two NeoVax injections are administered within 1 cm of each other.</td></tr>
<tr><td>Days 4 and 8</td><td>± 1 day</td></tr>
<tr><td>Days 15 and 22</td><td>± 3 days
(at least 5 days must elapse between doses)</td></tr>
<tr><td>Boost</td><td>Days 78 and 134</td><td>± 3 days</td></tr>
<tr><td>NeoVax peptides</td><td>DTH</td><td>7-14 days after day 78</td><td>N/A</td><td>100 µL from a mixture of all peptides</td><td>Intradermallly on the right forearm</td></tr>
</table>

Each of the up to 4 NeoVax pools (2 syringes per pool) will be assigned to one of four extremities. At each immunization, each NeoVax syringe will be administered to the assigned extremity as follows: using two syringes/needles per site, the first syringe, containing 0.5 mL (+/- 0.1mL) NeoVax (peptide pool + poly-ICLC), will be administered intradermally (i.d.), and the second syringe, containing the remaining 0.5 mL (+/- 0.1mL) of NeoVax, will be administered subcutaneously (s.c.) at the same injection site (i.e. 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.). Following each NeoVax injection, Ipilimumab (volume determined be dose level) will be administered subcutaneously, within 1cm of each NeoVax injection. 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. If a prior skin biopsy was obtained, then the area should be examined for palpable scar tissue, and the vaccine administration in that extremity should be at least 2cm from the scar tissue associated with the prior biopsy site, to avoid injection into scar tissue.

To evaluate for delayed-type hypersensitivity (DTH) responses, patients will receive an injection intradermally on the right forearm (unless contraindicated) 7-14 days after the first boost. DTH administration will consist of 100 µL of a mixture of all personalized neoantigen peptides ("NeoVax_DTH" syringe). Injection sites will be examined for any inflammatory reaction 48-72 hours later, and skin punch biopsies at the injection site will be taken for histologic and laboratory examination; thus, all DTH administrations should occur on a Monday or Tuesday to facilitate follow up evaluation. DTH neoantigen injection site should be labeled and circled for punch biopsy (two 5mm punch biopsies).
DTH Assessment will be performed by a trained physician or midlevel dermatology provider. Skin biopsies will be performed by a trained physician or midlevel dermatology provider.

5.3.2 Treatment window

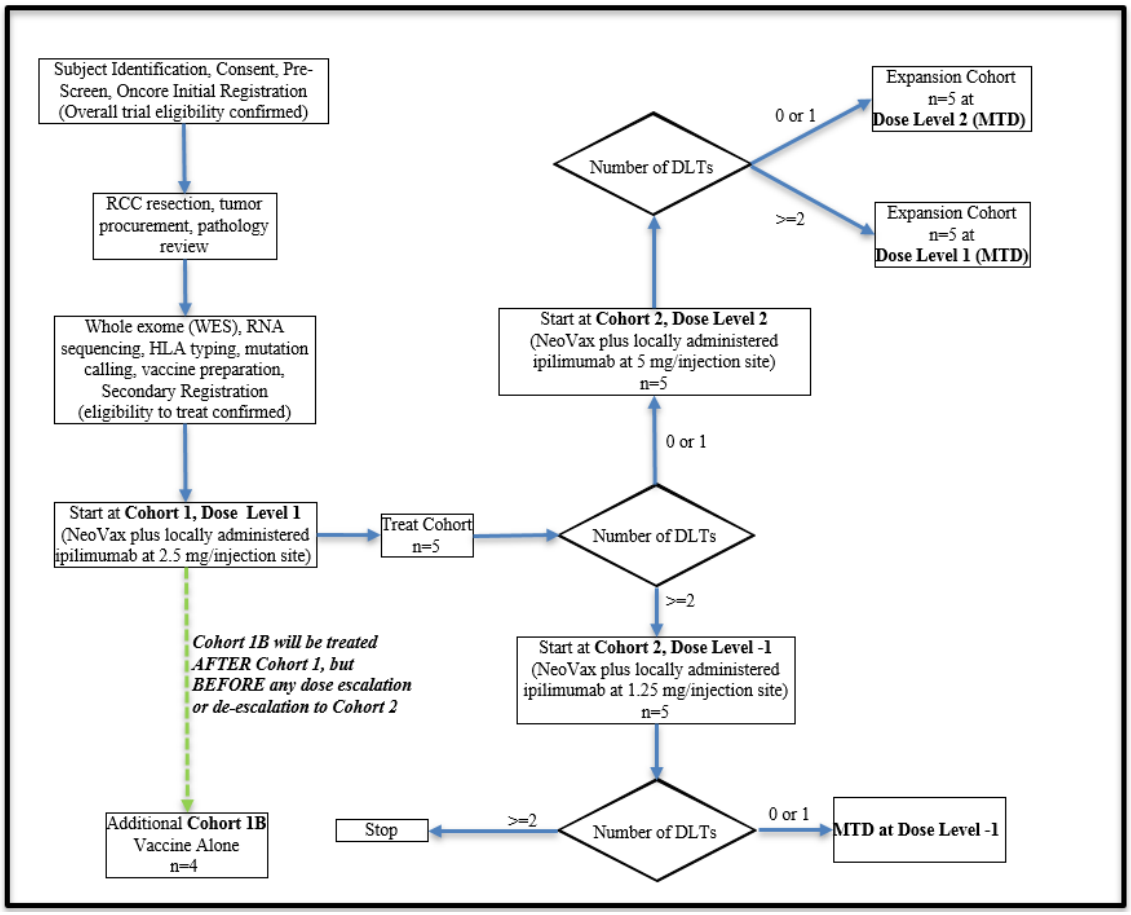
NeoVax (and Ipilimumab for Cohort 1, Cohort 2, and Expansion Cohort) may be administered within 1 day of the scheduled administration date for days 4 and 8, within 3 days of the scheduled administration date for days 15, 22 (dose on days 15 and 22 but must be at least 5 days apart), 78, and 134.

5.3.3 Observation post vaccination

Patients should be observed in the clinic for at least 60 minutes after the last of the 8 injections and vital signs should be checked at 30 (+/-15) and 60 (+/-15) 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 at DFCI.

5.4 Dose Schema and staggering of patients

5.4.1 DLT and expansion cohorts



Five (5) patients will be entered for the initial safety evaluation (Cohort 1). If two or more patients in Cohort 1 Dose Level 1 experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then that cohort will be determined to have unacceptable toxicity.

Before any dose escalation or de-escalation of Ipilimumab, an additional cohort of four (4) patients will be treated with NeoVax alone (no ipilimumab) – Cohort 1B. Of note, Cohort 1B will be used for assessment of the induction of cellular immune responses, but will not be used for DLT assessment (because no Ipilimumab is being administered)

After completion of Cohort 1B, the escalation or de-escalation of Ipilimumab in subsequent cohorts will be based on the number of DLTs in the original Cohort 1 (NeoVax administered with Ipilimumab). If Cohort 1 is found to have unacceptable toxicity, then five (5) patients may be enrolled on Cohort 2 Dose Level -1 (shown above). For Cohort 2 Dose Level -1 the dose of Ipilimumab is dropped to 50% relative to Cohort 1 Dose Level 1 but both the dose of Poly-ICLC and peptides remains the same.

If two or more patients in Cohort 2 Dose Level -1 experience a DLT, then the study will be stopped. If none or only one patient experiences a DLT on Cohort 2 Dose Level -1, then five (5) 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.

If none or only one patient experiences a DLT on Cohort 1 Dose Level 1, then five (5) more patients may be enrolled on Cohort 2 Dose Level 2. If two or more patients in Cohort 2 Dose Level 2 experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then that cohort will be determined to have unacceptable toxicity.

If Cohort 2 Dose Level 2 is found to have unacceptable toxicity, then five (5) more patients may be enrolled on Expansion Cohort at Dose Level 1. If none or only one patient experiences a DLT on Cohort 2, then 5 additional patients will be enrolled on Expansion Cohort at Dose Level 2 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 Escalation schedule (Cohort 1, Cohort 2, Expansion Cohort)		
	Dose Level	Dose of Ipilimumab
	-1	1.25 mg (0.25 mL)
Starting Dose -->	1	2.5 mg (0.5 mL)
	2	5 mg (1.0 mL)

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 5.4.1, and Schema B). In the boost phase, vaccine will be administered on days 78 (week 12) and 134 (week 20).

5.4.2 Definition of Dose Limiting Toxicity (DLT)

Dose limiting toxicity (DLT) are defined for the **combination of Ipilimumab and NeoVax**. 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 (≤ 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 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

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.5 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.

Use of a non-oncology vaccine therapy for prevention of infectious diseases is not allowed for 4 weeks prior to day 1, until 8 weeks after last study dose (with the exception of vaccination against

the SARS-CoV-2 virus for the prevention of COVID-19 disease). Given the severity of the COVID-19 pandemic, vaccination specifically against the SARS-CoV-2 virus for the prevention of COVID-19 is ALLOWED in this study.

5.6 Criteria for Taking a Participant Off Protocol 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. Patients may be treated beyond disease recurrence if the patient is clinically stable, and would not otherwise receive systemic therapy, per the clinician's judgement
- 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.

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

For Decentralized Subject Registrations, the research team updates the relevant Off Treatment/Off study information in OnCore as per DF/HCC REGIST-102.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Toni Choueiri at 617-632-5456.

5.7 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 follow-up and CT 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. After 2 years, observation/follow up/imaging will be at the discretion of the treating physician.

DOSING DELAYS/DOSE MODIFICATIONS

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for

Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to Ipilimumab). All study drugs must be delayed until treatment can resume.

Because of the potential for clinically meaningful Ipilimumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories (listed in Appendix B).

Dose delay criteria apply for all drug-related AEs. Ipilimumab must be delayed until treatment can resume.

6.1 Treatment of Ipilimumab Related Infusion Reactions (Cohort 1, Cohort 2, Expansion Cohort)

Since Ipilimumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 injection reactions should be reported as an SAE if criteria are met. Injection reactions should be graded according to NCI CTCAE (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 guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For Grade 1 symptoms: (Mild reaction; intervention not indicated)
Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future injections: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional Ipilimumab administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or injection interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours).

Stop Ipilimumab injections, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. The following prophylactic premedications are recommended for future injections: diphenhydramine 50 mg (or equivalent)

and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional Ipilimumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.
For Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated).

Stop Ipilimumab injections. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Ipilimumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

6.2 NeoVax with or without Ipilimumab should be delayed for the following:

Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:

- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related AE

Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:

- Grade 3 lymphopenia or leukopenia does not require dose delay.
- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity.
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity.
- Asymptomatic amylase or lipase do not require a treatment delay

Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

6.3 Criteria to Resume Treatment

NeoVax and Ipilimumab are treated as a combination for Cohort 1, Cohort 2, Expansion Cohort. The listed criteria for holding/restarting treatment is for BOTH NeoVax and Ipilimumab for Cohort 1, Cohort 2, Expansion Cohort (a patient cannot continue on one drug alone), or for JUST NeoVax for Cohort 1B (where Ipilimumab is not administered).

Subjects may resume treatment when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol. However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes.

If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

6.4 Discontinuation criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and injection reactions
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or injection reaction of any duration requires discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:

  - AST or ALT > 8 x ULN
  - Total bilirubin > 5 x ULN
  - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
- Any dosing interruption lasting > 6 weeks with the following exceptions:
  - Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
  - Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
  - Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Principal Investigator, presents a substantial clinical risk to the subject with continued Ipilimumab dosing

ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

7.1.1 Adverse Events Lists

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.

7.1.1.1 Adverse Event List(s) for poly-ICLC

Poly-ICLC is an investigational agent; however there has been significant experience in previous clinical trials as discussed in 2.2.2.4. 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
- Bruising
- Induration
- Pruritus
- Edema
- Localized rash
- Perioral numbness

Systemic reactions:
Flu-like symptoms, including:

- Malaise
- Fatigue
- Myalgia/arthralgia
- Headache
- Fever
- Chills
- Dizziness
- Elevated blood pressure
- Elevated pulse rate
- Shortness of breath

7.1.1.2 Adverse Event List for Personalized NeoAntigen Peptides

Localized reactions or systemic reactions are expected but they are anticipated to be mild to moderate in the majority of cases:

Localized reactions:

- Erythema
- Bruising
- Induration
- Pruritus
- Edema
- Localized rash
- Perioral numbness

Systemic reactions:
Flu-like symptoms, including:

- Malaise
- Fatigue
- Myalgia/arthralgia
- Headache
- Fever
- Chills
- Arthralgia
- Dizziness
- Flushing
- Elevated blood pressure
- Elevated pulse rate
- Shortness of breath

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.

7.1.1.3 **Adverse Event List for Ipilimumab (Cohort 1, Cohort 2, Expansion Cohort)**

Localized reactions:

- Erythema
- Induration
- Pruritus
- Edema
- Localized rash

The systemic exposure of patients to Ipilimumab will be significantly lower than the exposure according to approved dosing regimen for melanoma (3 mg/kg q3weeks x4) as shown in the Table below.

Dose Description and Comparison to Approved Ipilimumab Dosing Regimen (Cohort 1, Cohort 2, Expansion Cohort) *

	Priming Phase			Boosting Phase		
Cohort Dose Level	**Localized Ipilimumab****	**Approved Ipilimumab*****	**Relative Exposure******	**Localized Ipilimumab****	**Approved Ipilimumab*****	**Relative Exposure******
Cohort 1 Dose Level 1	0.5 ml per injection site 50 mg over first 22 days	210 mg over first 21 days	1/4	0.5 ml per injection site 20 mg over next 16 weeks	630 mg over next 6 – 12 weeks	1/30
Cohort 2 Dose Level -1	0.25 ml per injection site 25 mg over first 22 days	210 mg over first 21 days	1/8	0.25 ml per injection site 10 mg over next 16 weeks	630 mg over next 6 – 12 weeks	1/60
Cohort 2 Dose Level 2	1.0 ml per injection site 100 mg over first 22 days	210 mg over first 21 days	1/2	1.0 ml per injection site 40 mg over next 16 weeks	630 mg over next 6 – 12 weeks	1/15

* The NeoVax dose is identical for all injections:
- 300 µg of each of 5 peptides per injection site
- 0.5 mg Poly-ICLC (Hiltonol®) per injection site

** Localized Ipilimumab dosing (not Cohort 1B): 5 mg/ml dosage form; 4 injection sites/day; 5 injection days

*** Approved Ipilimumab dosing: 3 mg/kg IV, q3weeks X4

**** Relative exposure is calculated as the mass of Ipilimumab delivered subcutaneously divided by that delivered systemically

At the maximum dose of Ipilimumab planned (5 mg Ipilimumab at each vaccination site with the vaccine), the maximal exposure would be 140 mg over 20 weeks, about 6-fold lower than the approved regimen [840 mg for a 70 kg patient given over 10 – 16 weeks].
Thus, systemic reactions are not expected, but may occur:

- Gastrointestinal
- Renal
- Pulmonary
- Hepatic
- Endocrinopathies
- Skin
- Neurological

7.2 Adverse Event Characteristics

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

from the CTEP web site
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - 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.

7.3 Procedures for AE and SAE Recording and Reporting

7.3.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.

7.3.2 Investigators **must** report to the Overall PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.3 DF/HCC Adverse Event Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.3.4 Protocol-Specific Adverse Event Reporting

The following information regarding injection site reaction(s) will be collected during the visits belonging to the vaccine treatment period for both peptide pools and ipilimumab injections:

- Erythema/redness: yes/no. If **yes** length /width will be provided
- Induration/swelling: yes/no. If **yes** length /width will be provided
- Warmth: yes/no
- Pruritus: yes/no
- Other (if applicable)

7.4 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.

7.5 Reporting to Study Sponsor

7.5.1 Non-Serious Adverse Events

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

7.5.2 Serious Adverse Events

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 – that are *Unexpected* and there is a *Reasonable Possibility* that the Adverse Event is related to the study intervention

- All Grade 4 (life-threatening or disabling) Events – Report all events that are Unexpected. Events that are expected and listed within the protocol and /or current consent form do not need to be reported to the DFCI IRB. Please not, an event that present at a higher severity than what is currently listed within protocol and/or current consent asexpected would be considered unexpected and reportable.

- 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 and Study Sponsor (Dr. Patrick Ott, DFCI) 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:

DF/HCC Overall PI	**Study Sponsor**
Toni Choueiri, MD	Patrick Ott, MD PhD
Office: 617-632-5456	Office: 617-632-5990

Fax: 617-632-2165
Email: Toni_Choueiri@dfci.harvard.edu

Fax: 617-632-6727
Email: Patrick_Ott@dfci.harvard.edu

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.

All SAEs, whether related or unrelated to Ipilimumab and all pregnancies must be reported to **BMS** (by the investigator or designee) within 24 hours. All SAEs should be reported via confirmed facsimile (fax) transmission, or scanned and reported via electronic mail to:

SAE Email Address: Worldwide.Safety@BMS.com
SAE Fax Number: 609-818-3804

BMS will be provided with a simultaneous copy of all adverse events filed with the FDA. All SAEs should simultaneously be faxed or e-mailed to BMS (see email or Fax Number above).

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

7.6 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).

7.7 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.8 Reporting to the Food and Drug Administration (FDA)

Patrick A. Ott, MD, PhD, as IND sponsor, is responsible for submitting an IND safety report when any of the following criteria are met:

- Suspected adverse reaction that is both serious and unexpected
- Findings from clinical studies or findings from animal or in-vitro testing that suggest a significant risk in humans exposed to the drug
- An increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure
- Unexpected fatal or life-threatening suspected adverse reactions represent especially important safety information and must be reported no later than seven (7) calendar days after the sponsor's initial receipt of the information
- A follow up report may be submitted as relevant information becomes available

- It is the responsibility of the sponsor to ensure that all investigators participating in a multi-center trial are promptly informed of significant new adverse effects or risks with respect to the drug used in a study protocol

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

PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in Section 7.1.

8.1 Poly-ICLC (Hiltonol®)

8.1.1 Description

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

8.1.2 Form

Poly-ICLC is supplied by Oncovir in single-dose vials containing 1 mL of 1.8 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.

8.1.3 Storage and Stability

Poly-ICLC is normally shipped or stored refrigerated at about 40ºF (2-8°C) but should not be frozen. Based on extended formal stability data at +5°C ± 3°C and at +25°C ± 2°C, it is justified to accept temperature excursions during transport, shipment and storage of Hiltonol at clinical sites up to +27°C for up to 5 days.
Likewise, temperature deviations between 0°C and +2°C are acceptable, and have no effect on product quality.

8.1.4 Compatibility

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

8.1.5 **Availability**

Poly-ICLC is an investigational agent and has been purchased 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. Vials of poly-ICLC sufficient for this clinical trial will be shipped from the Hiltonol® storage facility (Bellwyck Packaging, Mississauga, Ontario, Canada) to the DFCI Pharmacy and BIDMC Pharmacy. Poly-ICLC can be ordered directly from Oncovir, Inc, by submitting a purchase order to:

Oncovir, Inc.
c/o Andres M. Salazar, M.D.
3203 Cleveland Ave, NW
Washington, DC 20008
202-342-1726

8.1.6 **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).

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 sttled 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.

8.1.7 **Administration**

Following mixing with personalized neoantigen peptides, the vaccine (peptide + poly-ICLC) will be administered as follows: using two syringes/needles per site, the first syringe, containing 0.5 mL (+/- 0.1 mL) of NeoVax (peptide pool + poly-ICLC), will be administered intradermally (i.d.), and the second syringe, containing the remaining 0.5 mL (+/- 0.1 mL) of NeoVax (volume determined by dose level), will be administered subcutaneously (s.c.) at the same injection site, within 1cm.

8.1.8 **Accountability**

The Pharmacy department will maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.9 Destruction and Return

At the end of the study, unused supplies of poly-ICLC will be held by the IND holder for future research use.

8.2 Personalized neoantigen peptides

8.2.1 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.

8.2.2 Form

The four peptide pools, each containing up to 5 peptides, will each be provided in one bulk aliquot for use in Drug Product preparation; they will be packaged with a tamper evident seal, labelled and shipped under appropriate temperature controlled conditions to DFCI Pharmacy dept.

Bulk aliquots for up to 4 different peptide pools will identified with the patient's Oncore ID. Final sterile filtration will be performed by the Pharmacy department prior to aliquoting into vials. The vials containing peptide pools are to be stored at -80°C (+/-5°C) until use.

8.2.3 Storage and Stability

Personalized neoantigen peptides are stored frozen at -80°C (+/-5°C). 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.

8.2.4 Compatibility

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

8.2.5 Availability

The personalized neoantigen peptides is prepared by Almac Sciences, Ltd.or an alternative GMP peptide vendor under contract with DFCI and will be supplied directly to the DFCI or an alternative GMP fill facility. No other institutions will be involved in delivering personalized neoantigen peptides under this protocol.

8.2.6 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 **5.3.4** and **5.3.5**. 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.

Dose Preparation Procedures

Each vaccination treatment consists of up to 8 separate injections – each injection containing peptides from one of the up to four peptide pools combined with the adjuvant, and two injections performed for each peptide pool (one subcutaneous injection, and one intradermal injection).

Personalized Neoantigen peptides + Poly-ICLC

- On the day of vaccination, remove one Personalized Neoantigen peptide vial for each of the up to four peptide pools from the box and allow to thaw and warm to ambient conditions (15°C to 30°C) by placing them at room temperature for 1 to 4 hours. Accelerating aids such as a water bath should not be used. Once thawed, Personalized Neoantigen peptides cannot be re-frozen or refrigerated.
- On the day of vaccination, remove one vial of the Poly-ICLC (adjuvant) from the refrigerated storage, remove from box so that air can circulate around and allow it to warm to room temperature for at least 1 hour and a maximum of 2 hours prior to dose preparation.
- The vaccine dose should be prepared in a biosafety cabinet and using aseptic handling procedures in order to prevent contamination. Place the vials of Personalized Neoantigen peptides, Poly-ICLC vial, mixing syringes, needles and dosing syringe into the biosafety cabinet. The Personalized Neoantigen peptides and Poly-ICLC will be mixed by using two connected syringes and passing the suspension back and forth between the syringes. The suspension will have an opaque white appearance.

  i. Gently swirl the thawed Neoantigen peptides vial. Using a syringe with a luer-lock dispensing needle withdraw 0.75 mL from the Neoantigen peptides vial.

  ii. Gently swirl the Poly-ICLC vial. DO NOT INVERT the Poly-ICLC vial. The vial must remain upright and tipped to an approximately 30-degree angle to remove enough adjuvant for each of the four pools. One vial of Poly-ICLC should provide enough adjuvant for each of the four peptide pools. Insert a new syringe with a luer-lock 21gauge 1 ½ inch needle into the vial placing the tip of the needle near the bottom of the vial. Withdraw the Poly-ICLC to the 0.25 mL mark on the syringe. An air bubble may be in place between the stopper of the plunger and the 0.25mL mark on the syringe. Please DO NOT attempt to remove the air bubble. This air bubble will be removed during the next mixing step. Removing the air bubble at this step will limit the amount of Poly-ICLC which can be removed from the vial.

  iii. The contents of the two syringes are connected to a Braun Medical (Bethlehem, PA) Fluid Dispensing Connector, Product code FDC 1000, REF 415080. The peptides and Poly-ICLC are mixed by gently transferring the contents back and forth between the syringes, thru the Fluid Dispensing Connector.

  iv. Draw 0.5 mL (+/- 0.1 mL) into one of the syringes (for subcutaneous administration), and 0.5 mL (+/- 0.1mL) into the other syringe (for intradermal administration). Remove the syringes from the device. Attach a 27 gauge 1/2-inch or 5/8-inch stainless steel needle to each syringe.

v. Repeat the syringe preparation and labeling for each peptide pool. Prepared dosing syringes may be stored at room temperature for up to 6 hours prior to administration.

8.2.7 Administration

Following mixing with personalized neoantigen peptides, the vaccine (peptide + poly-ICLC) will be administered as follows: using two syringes/needles per site, the first syringe for each site, containing 0.5 mL (+/- 0.1 mL) of NeoVax (peptide pool + poly-ICLC), will be administered intradermally (i.d.), and the second syringe for each site, containing the remaining 0.5 mL (+/- 0.1 mL) of NeoVax, will be administered subcutaneously (s.c.) at the same injection site, within 1cm.

Each pool of Neoantigen peptides (two syringes per pool) will be administered to one of the four limbs (A - Right Arm, B - Left Arm, C - Right Leg, D - Left Leg) by subcutaneous injection (one syringe per pool) and intradermal injection (one syringe per pool). In the setting where a limb is unavailable for injection, the injection should be administered to the nearest flank. The same pool should be administered to the same limb across doses.

8.2.8 Ordering

The order for personalized neoantigen peptides to Almac Sciences, Ltd. or an alternative GMP peptide vendor will be placed by the DFCI PI or designee separately for each individual patient. Delivery time is anticipated to be 4 – 6 weeks.

8.2.9 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage).

8.2.10 Destruction and Return

At the end of the study, unused supplies of personalized neoantigen peptides will be held by the IND holder for future research use. Vials containing peptide pools will be de-identified before being transferred to the IND holder.

8.3 Ipilimumab (Cohort 1, Cohort 2, Expansion Cohort)

8.3.1 Description

Ipilimumab is a fully human monoclonal immunoglobulin G1κ antibody that targets the human cytotoxic T lymphocyte antigen 4 (CTLA-4) cell surface membrane receptor.

8.3.2 Form

Ipilimumab Injection Drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 5 mg/ml (0.9% sodium chloride injection; USP, or 5% dextrose injection).

The solution is a clear to slightly opalescent colorless to pale yellow liquid and may contain a small amount of translucent-to-white particles. BMS will provide 50mg/10ml or 200mg/40mL vials for this study.

8.3.3 Storage and Stability

Ipilimumab must be stored at 2º-8ºC (36º-46ºF) and protected from light and freezing. Partially used vials should be discarded after single use.

8.3.4 Compatibility

Ipilimumab will not be mixed with any other study agents.

8.3.5 Handling

Vials containing Ipilimumab should not be shaken and should be discarded if cloudy.

8.3.6 Availability

In this protocol, Ipilimumab is an investigational product and will be supplied by Bristol Myers Squibb to the participating institution's pharmacy.

8.3.7 Preparation

Ipilimumab will be used directly from the supplied vial; no further dilution will be required. The specified volume of Ipilimumab (0.25mL – 1mL, depending on dose level) will be drawn directly into a syringe from the supplied vial. The needle for withdrawal from the vial will be removed. Attach a 27 gauge 1/2-inch or 5/8-inch stainless steel needle to each syringe.

8.3.8 Administration

The specified volume of Ipilimumab will be drawn directly into a syringe from the supplied vial as described in section 8.3.7. Filled syringes should be used for injection as soon as possible after preparation. The 2 NeoVax and the Ipilimumab injections are administered within 1 cm of each other.

8.3.9 Ordering

Ipilimumab will be obtained from Bristol Myers Squibb (BMS) as an Investigator Initiated Study. Ipilimumab can be ordered from BMS by providing a completed Drug Shipment Form.

8.3.10 Accountability

The Pharmacy department will maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage).

8.3.11 Destruction and return

At the end of the study, unused supplies of Ipilimumab will be will be held by the IND holder for future research use.

BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 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

The induction of a neoantigen-specific T cell response following vaccination with NeoVax and simultaneous treatment with locally delivered Ipilimumab will be assessed by ELISPOT and/or tetramer analysis. Comparison will be made between samples taken prior to vaccine administration and after vaccination, beginning 4 weeks after the last priming dose. Assays will be conducted for all 10 patients in the expansion cohort only.Peripheral blood mononuclear cells will be collected at multiple time points for each patient with the purpose of:

1) *Characterizing CD8+ and CD4+ T cell responses against personal immunizing neoepitopes and defining the extent to which these cellular immune responses are associated with anti-tumor responses*.

   a. A series of assay peptides will be prepared based on the sequence of the immunizing peptides in NeoVax. These include both "short" epitope-length peptides (typically 9 – 10 amino acids) as well as longer peptides (15-17 amino acids) spanning the entire length of each immunizing peptide in an overlapping fashion.

   b. Both types of peptides will be used in IFN-γ ELISPOT assays to quantitate CD8+ and CD4+ responses and to identify which neoepitopes are immunostimulatory. Initially, assays will be performed *ex vivo* (i.e. – without *in vitro* antigen-primed expansion of T cells prior to cytokine analysis); *in vitro* stimulation will only be utilized if reactivity is not detected *ex vivo*. Positive responses will be defined as ≥ 55 SFU/10^6 PBMC *or* 3 times baseline level. When necessary, pools of assay peptides generating a positive response will be de-convoluted so the particular stimulating neoantigen mutations can be identified. The proportion of patients with a positive response will be presented with a 90% exact binomial

confidence interval. The breadth of the personal neoepitope-specific T cell responses (number of neoepitopes generating a positive response per patient) will be quantified.

c. To directly track and measure the size of T cell populations responding to neoantigens, we will prepare fluorescent-labeled tetramers for direct flow-cytometric characterization of responding CD8+ T cells. Tetramer staining will be conducted for patients for whom neoantigen epitope loaded MHC complexes can be prepared, based on knowledge of the predicted epitope and the availability of soluble MHC molecules to prepare tetramers (~ 14 soluble HLA A and 10 soluble HLA B). A positive response will be defined as a 3-fold increase in the percentage of positive T cells compared to samples prior to vaccination or >0.015% of total T cells if levels of T cells specific to the respective antigen are undetectable in pre-vaccination samples (< 0.005% of total).

d. Combinations of IFN-γ ELISPOT, tetramer staining assays, tumor cell recognition and assays of cytolysis will be used to define the cytolytic potential of neoepitope-specific T cells for autologous tumor.

e. Selected responses (as measured by IFN-γ ELISPOT, tetramer staining or cytolysis) will be tracked longitudinally to assess the durability of antigen-specific T cell responses.

2) Assessing *the phenotype of immune cells induced by vaccination for selected samples*

a. Flow cytometric analysis and intracellular cytokine staining of pre- and post-treatment antigen-stimulated peripheral blood T cells will be performed to characterize and quantify various T cell sub-populations ($CD4^+$, CD8+, T_H1 and T_H2, central and effector memory [CD45RA, CCR7]). Tumor-infiltrating lymphocytes (TILs) will also be assessed pre-treatment, and when possible, post-treatment in the event of tumor recurrence.

b. Extracellular staining and flow cytometry of unstimulated cells will be used to quantify Treg and myeloid-derived suppressor cells (MDSC).

3) Defining the TCR repertoire and specificity of vaccine-induced T cells.

The fundamental basis of cytolytic T cell recognition of target tumor cells is the highly specific interaction of the T cell receptor (TCR) with the peptide/MHC complex found on the tumor cell. Through either targeted deep sequencing of the Vβ subfamily of the T cell receptor (TCR) and/or paired single cell CDR3αβ TCR sequencing in peripheral blood samples collected before and after vaccination or in TIL populations, the global changes in TCR repertoire as well as changes in the abundance of individual T cell clones will be determined. This information may provide a complementary approach to monitoring the induction of neoantigen-reactive T cells. Specific TCRs may then be reconstructed for specificity testing, including autologous tumor assays using autologous tumor cell lines

*4) Detecting tumor antigens and characterizing the tumor-immune microenvironment***.**

Immunoprecipitation of HLA class I molecules, followed by peptide elution and mass-spectrometry based detection may allow for the physical detection of presented tumor epitopes.

Further, through single cell proteogenomic techniques, the tumor-immune microenvironment can be comprehensively characterized.

5) Antigen presentation of administered vaccine

A comprehensive single cell proteogenomic profiling of the immune cells at relevant skin sites (i.e. vaccine site, DTH site) will be performed to characterize antigen presenting cells and T cells that may ultimately be important for vaccine responses

STUDY CALENDAR

Study Stages	Initial Screening	Vaccine preparation	Secondary Screening	Vaccine Treatment Period													Long Term Evaluation[1] Every 3 months
				Priming Phase							Boosting Phase						
Week	N/A			1		2	3	4	5	8	12	13	16	20	21	24	Every 12 weeks[1]
Day[2]	N/A			1	4[3]	8[3]	15[4]	22[4]	29[4]	50[4]	78[4]	85[4]	106[4]	134[4]	141[4]	162[4]	
Informed consent	X[5]																
Medical History	X																
Concomitant Medications	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam / vitals	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Staging scans	X[6]		X							X[7]			X[7]			X[7]	X
CBC w/ diff and plts	X[6]		X	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X
Serum Chemistry[8]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TSH			X							X			X			X	X
ACTH[9]		X															
β-HCG[10]	X		X														
ECG[11]			X														
Autoimmune panel[12]		X															
Infectious panel[13]		X															
HLA class I and II typing[14]	X																
Surgical tumor biopsy[15]	X																
Pathology review	X																
Blood draw for germlinetissues sequencing[16]	X																
Vaccine manufacturing[17]		X															
Leukapheresis			X[18]										X[18]				
Blood draw for immune response monitoring[19]			X[20]	X[22]			X[21]			X	X		X[23]	X		X	
NeoVax Vaccine administration[2,3,4]				X	X	X	X	X			X			X			
Ipilimumab administration[2,3,4,26]				X	X	X	X	X			X			X			
Adverse Events assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X
DTH assessment[24]												X					
DTH skin biopsy[24]												X					
Skin biopsies at injection sites[25]			X					X									

1 Active Follow-Up: Participants removed from active study treatment will be followed at least every 3 months (+/- 2 weeks) for the first 2 years after surgery until initiation of a new therapy for recurrent disease, withdrawal of consent, or death (whichever comes first).

2 All study procedures should be performed within +/- 3 day window unless specified otherwise.

3 allowable treatment/visit window: +/- 1 day,

4 allowable treatment/visit window: +/- 3 days, unless otherwise specified; at least 5 days must elapse between Day 15 & Day 22 priming vaccines. Days 78, 134, and 141 have a +/- 7 day window

5 within 4 weeks prior to initial study registration. The screening period should be performed within 30 days since the date of consent

6 Initial screening scans (PET/CT or CT chest-abdomen-pelvis) for baseline assessment must be performed no earlier than 6 weeks prior to study start (day of initial consent). Additional imaging, including MRI brain and bone scan, should only be performed if clinically indicated, as part of standard-of-care diagnostic evaluation. Re-staging scans (CT chest-abdomen-pelvis) for Secondary Registration may be performed within -14 to -1 days prior starting treatment.
MRI abdomen-pelvis can substitute for CT abdomen-pelvis for any scan

7 Assessment may be performed up to 7 days prior.

8 Glucose, urea nitrogen, creatinine, sodium, potassium, calcium, total and direct bilirubin, AST, ALT, alkaline phosphatase.

9 ACTH: Before the start of vaccine manufacturing and as clinically indicated.

10 Women of child-bearing potential (WOCBP) must have a negative serum β-HCG pregnancy test; follow up pregnancy tests required for WOCBP only.

11 ECG: Baseline and as clinically indicated.

12 ANA, anti-thyroglobulin antibodies, rheumatoid factor. These assessments are not required before initial registration but they must be available before the start of vaccine manufacturing

13 HBsAg, HBcAb, HCV, HIV I/II Ab. These assessments are not required before initial registration but they must be available before the start of vaccine manufacturing

14 Molecular HLA typing to be performed by Tissue Typing Laboratory (HLA-A, HLA-B, HLA-C, and HLA-DR).

15 Complete or partial resection of primary/metastatic RCC should occur within 6 weeks of study initial enrollment. After confirmation of clear cell histology and tumor cellularity by BWH Histopathology, sections of tumor tissue will be shipped to CRSP, LLC at the Broad Instiute for WES, and to Broad Genomics Platform research labs for RNA-sequening.

16 2x 10 ml K2 EDTA tubes will be drawn and transmitted to the Broad Institute and CAMD for whole exome sequencing / DNA extraction

17 Vaccine production should begin immediately after patient is registered to trial. Vaccine production should be completed about 8-12 weeks after initial registration.

18 Patients will undergo leukapheresis procedure prior to vaccination with NeoVax to collect sufficient amounts of PBMCs to allow comprehensive immune monitoring. The pre-NeoVax leukapheresis should be performed up to 10 days prior to initiation of NeoVax; 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 50ml blood draw should be performed no later than 14 days after the day of discontinuation.

19 Unless otherwise specified, 200ml blood will be drawn: 2 x 10mL Streck tubes + 18 x 10 mL EDTA tubes (prior to NeoVax administration on days of vaccination).

20 If pre-NeoVax leukapheresis was performed, only 2 x 10 mL Streck tubes will drawn. If NO leukapheresis performed, then 135mL of blood will be collected in K2 EDTA tubes on day -14.

21 If pre-NeoVax leukapheresis was performed, 200mL of blood will be collected (2 x 10mL Streck tubes + 18 x 10 mL EDTA tubes). If NO leukapheresis was performed, then 135mL of blood will be collected into EDTA tubes.

22 If pre-NeoVax leukapheresis was not performed, 135 ml blood will be collected in K2 EDTA tubes.

23 If week 16 leukapheresis is performed 2 streck tubes will be collected. If week 16 leukapheresis cannot be performed, 200 ml of blood will be collected in Streck tubes and K2 EDTA tubes.

24 Delayed Type Hypersensitivity (DTH) will be assessed after 1st Boost or at time of recurrence (whatever occurs first). 100µL containing personalized neoantigen peptides will be administered i.d. at the right forearm (unless contraindicated) within 7 to 14 days after the 1st Boost. Patient will come back 48-72 hours after neoantigen peptides administration for DTH evaluation. Skin punch biopsies (two 5mm biopsies) at the injection site will be taken, and injection site will be evaluated for any inflammatory reaction.

25 Skin biopsies near vaccine injection sites (two 5 mm punch biopsies) will be performed during secondary screening, between 48-72 hours after 5th vaccination and between 48-72 hours after injection of all pooled peptides for DTH assessment. The biopsy at secondary screening and after 5th vaccination should be at the same vaccine site (right proximal thigh unless contraindicated). Additionally, if a different vaccine site appears to clinically have a higher degree of inflammation, additional punch biopsy (two 5mm) of that site may be taken.

26 Ipilimumab is not be administered to patients in Cohort 1B

MEASUREMENT OF EFFECT

For the purposes of this study, participants should be re-evaluated for disease recurrence every 3 months.

11.1 Antitumor Effect – Solid Tumors

Recurrence will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of recurrence (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly recurrence occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

MIBG (meta-iodobenzylguanidine). The following is recommended, to assure high quality images are obtained. Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Participants and/or parents are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained. I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150 μCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain). The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.2 Response Criteria

The appearance of one or more new lesions is considered disease recurrence.

11.1.2.1 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply 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 PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, recurrence should be declared using the date of the initial scan on which the lesion was discovered.

DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The ODQ will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. 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 participant safety will be addressed with the Overall PI and study team. The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy 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 Data Sharing Agreement

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 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.

12.5 National Institutes of Health (NIH) Genomic Data Sharing:

Rapid progress in understanding and treating cancer will occur when some of the genetic information derived from tissues and blood can be shared with other researchers. In particular, the National Institutes of Health (NIH) and other organizations have developed special data (information) repositories that analyze data and collect the results of certain types of genetic studies. These central banks will store genetic information, and provide them to qualified researchers to do more research. Therefore, we may share results with these public databases. Some of this information may be made available over the internet and will be freely available to anyone who is interested (an open access database). Other, more detailed information may only be accessed by scientists at other research centers who have received special permission to review study participants' de-identified data (a controlled access database).

Study participant's information will be sent only with a code number attached. Name or other directly identifiable information will not be shared with these repositories or with other investigators. There are many safeguards in place to protect participant's information while they are stored in these repositories and used for research. There is a slight risk of loss of privacy when sharing this information with these banks but we have established procedures to encode study participant information and to protect participant data. The repositories also have robust procedures in place to protect the confidentiality of the stored data. We will do everything we can

to protect study participant's data but we cannot absolutely guarantee its privacy or predict how genetic information will be used in the future.

STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The primary clinical goal of this Phase I trial is to assess safety of the vaccine (neoantigen peptides and poly-ICLC) and locally-delivered Ipilimumab.

Primary Endpoint: Rate of DLT and MTD Determination

Adverse events classified as dose limiting toxicity (DLT) and the assessment time for DLT are summarized in Section 5.5.

A total of 15 patients will be enrolled in this study, as summarized in the study schema above. The table below shows the probability of observing 0 or 1 patients with dose-limiting toxicity (DLT); and therefore either altering the ose or proceeding to the expansion cohort

True but unknown probability of DLT	0.1	0.2	0.3	0.4	0.5
Pr(0 or 1 DLT in 5 patients)	0.92	0.74	0.53	0.34	0.19

With this design, observing more than a single DLT at a given dose level is unacceptable. If the true but unknown rate of DLT is low, i.e., 10%, the probability of observing either no DLTs or a single DLT in 5 patients is 0.92. This would lead to escalation of the ipilimumab dose from 2.5 mg/injection site to 5.0 mg/injection site if these results are obtained at dose level 1; at dose level 2, the same results would lead to an expansion cohort of 5 patients at the 5.0 mg/injection site dose. This would imply 5 additional patients in whom to assess the toxicity of this dose, and a total of 10 patients treated at the same dose level in whom to assess immune correlates. Should escalation occur, but the 5.0 mg/injection site be too toxic to move forward (2 or more DLT), the 2.5 mg/injection site dose would move to an expansion cohort of at least 5 patients, depending on the timing of DLT in the patients treated at dose level 2. Similarly, if dose level 1 proves too toxic to move forward, but dose level -1 is safe, we would still have an expansion cohort of 5 patients for additional toxicity characterization, and 10 patients treated at the same dose level in whom to assess immune correlates. If the true but unknown rate of DLT is 30%, the probability of observing 2 or more DLT will be 0.60. This design is slightly more permissive than the typical 3+3 design, but accommodates our sample size constraints.

The table below shows the probability of observing at least 1 patients with toxicity of interests out of 5 patients at the MTD in the expansion cohort for various possible values of the true, but unknown, toxicity rate.

True but unknown probability of toxicity	0.1	0.2	0.3	0.4	0.5
Pr(at least 1 in 5 patients)	0.41	0.67	0.83	0.92	0.97

13.2 Analysis of Secondary Endpoints

Secondary endpoints in this clinical trial will: (a) assess the induction of IFN-γ T-cell response or tetramer staining to the assay peptides, and (b) estimate the proportion of patients alive without disease recurrence at two years after resection. Analyses of these secondary endpoints will be based on 10 patients treated at the MTD.

The induction of IFN-γ T-cell response will be based on ELISPOT assessments taken prior to vaccine administration and at week 16. Our primary assessment will focus on CD8+ T-cell response, which is measured by the proportion of immunizing peptides that elicit a T-cell response either directly *ex vivo* (primary endpoint for our comparison of the vaccine strategy with Ipilumumab and the vaccine strategy alone) or after *in vitro* stimulation. In our prior study in melanoma, there were no CD8+ *ex vivo* responses to any of the 91 immunizing peptides tested across the vaccinated patients; after *in vitro* stimulation, 15 of these peptides (16%) elicited a CD8+ response. We conservatively estimate that the vaccine for each of the patients in this study will be based on at least 10 peptides. We therefore assume that we will be contrasting a pool of 100 peptides among patients treated with vaccine at the MTD of ipilumumab, and a pool of 40 peptides among patients treated with vaccine alone. We will use the Fisher exact test, at a one-sided 0.05 significance level, to compare proportions of immunizing peptides eliciting *ex vivo* reponse among CD8+ T-cells. Assuming a rate of 5% rate with vaccine alone, we will have 90% power to detect an increase to 25%. Using the same testing structure, we will also compare rates of CD8+ T-cell response after *in vitro* stimulation. We assume a null hypothesis of 25% responses with vaccine alone, because the upper 95% exact binomial confidence bound for our observed melanoma response of 15 of 91 peptides is 24%. We will have 83% power to detect an increase from 25% to 50%. This enables us to assess the utility of adding Ipilumumab to vaccine in order to enhance CD8+ T-cell response.

A patient level analysis will be presented descriptively. The proportion of patients who achieve more than 55 SFU/106 PBMC or 3 times their baseline level will be presented with a 90% exact binomial confidence interval. That interval will be no wider than 56 percentage points among the 10 patients treated with vaccine and Ipilumumab at the MTD, and no wider than 80 percentage points among the 4 patients treated with vaccine alone.

The proportion of patients alive without disease recurrence at two years after resection will be estimated, and confidence limits provided, for each treatment strategy. This assessment will occur at the latest two years after the time the final patient is resected, or earlier if the rate of disease Recurrence or death permits full ascertainment of this endpoint. Because of the small sample size, we will require complete follow up to two years for all patients who do not experience an event in the first two years. The proportion of patients alive and disease-free at two years will be presented with exact binomial 90% confidence intervals. If all 10 patients are alive without recurrence two years after resection, the lower 90% confidence bound would be 79%. Recurrence-free survival will be also be calculated for all patients using all follow-up time available as the time between pathological confirmation of complete resection and the first of disease recurrence or death; patients alive at last contact will be censored. This will be presented graphically using the method of Kaplan and Meier.

13.3 Analysis of Correlative Endpoints

Longitudinal analyses of PBMC immune response kinetics to the peptide pool and to individual epitopes 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, T-regs, etc.), myeloid-derived suppressor cells (MDSC), CTL response, and T cell activation status will be summarized using descriptive statistics.

13.4 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.

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

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 Principal Investigator and co-Principal Investigator and authors can 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. It is presumed that the Principal Investigator and co-Principal Investigator will decide on the authorship for the abstract and/or presentation, and/or publications.

REFERENCES

1. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245-52.
2. Haas NB, Manola J, Uzzo RG, et al. Adjuvant sunitinib or sorafenib for high-risk, non-metastatic renal-cell carcinoma (ECOG-ACRIN E2805): a double-blind, placebo-controlled, randomised, phase 3 trial. Lancet 2016;387:2008-16.
3. Ravaud A, Motzer RJ, Pandha HS, et al. Adjuvant Sunitinib in High-Risk Renal-Cell Carcinoma after Nephrectomy. N Engl J Med 2016;375:2246-54.
4. Rammensee HG, Singh-Jasuja H. HLA ligandome tumor antigen discovery for personalized vaccine approach. Expert Rev Vaccines 2013;12:1211-7.
5. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
6. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The New England journal of medicine 2012;366:2455-65.
7. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine 2012;366:2443-54.
8. Rosenberg SA. Raising the bar: the curative potential of human cancer immunotherapy. Sci Transl Med 2012;4:127ps8.
9. Phan GQ, Attia P, Steinberg SM, White DE, Rosenberg SA. Factors associated with response to high-dose interleukin-2 in patients with metastatic melanoma. J Clin Oncol 2001;19:3477-82.
10. Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. Science 2007;318:1108-13.
11. Hacohen N, Fritsch EF, Carter TA, Lander ES, Wu CJ. Cancer Immunology at the Crossroads: Functional Genomics Getting Personal with Neoantigen-Based Therapeutic Cancer Vaccines. Cancer Immunology Research 2013;1:11-5.
12. Dubey P, Hendrickson RC, Meredith SC, et al. The immunodominant antigen of an ultraviolet-induced regressor tumor is generated by a somatic point mutation in the DEAD box helicase p68. The Journal of experimental medicine 1997;185:695-705.
13. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. Nat Rev Immunol 2007;7:118-30.
14. Mandelboim O, Vadai E, Fridkin M, et al. Regression of established murine carcinoma metastases following vaccination with tumour-associated antigen peptides. Nat Med 1995;1:1179-83.
15. Castle JC, Kreiter S, Diekmann J, et al. Exploiting the mutanome for tumor vaccination. Cancer research 2012;72:1081-91.
16. Matsushita H, Vesely MD, Koboldt DC, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 2012;482:400-4.
17. DuPage M, Mazumdar C, Schmidt LM, Cheung AF, Jacks T. Expression of tumour-specific antigens underlies cancer immunoediting. Nature 2012;482:405-9.
18. Fritsch EF, Rajasagi M, Ott PA, Brusic V, Hacohen N, Wu CJ. HLA-Binding Properties of Tumor Neoepitopes in Himans. Ca Immunol Res 2014.

19. Wölfel T, Hauer M, Schneider J, et al. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 1995;269:1281-4.
20. Coulie PG, Lehmann F, Lethe B, et al. A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. Proc Natl Acad Sci U S A 1995;92:7976-80.
21. Lennerz V, Fatho M, Gentilini C, et al. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. Proceedings of the National Academy of Sciences of the United States of America 2005;102:16013-8.
22. Smedby KE, Baecklund E, Askling J. Malignant lymphomas in autoimmunity and inflammation: a review of risks, risk factors, and lymphoma characteristics. Cancer Epidemiol Biomarkers Prev 2006;15:2069-77.
23. Tran E, Turcotte S, Gros A, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014;344:641-5.
24. Gros A, Robbins PF, Yao X, et al. PD-1 identifies the patient-specific CD8+ tumor-reactive repertoire infiltrating human tumors. The Journal of clinical investigation 2014;124:2246-59.
25. Robbins PF, Lu YC, El-Gamil M, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nature medicine 2013;19:747-52.
26. Rajasagi M, Shukla SA, Fritsch EF, et al. Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia. Blood 2014.
27. Brown SD, Warren RL, Gibb EA, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. Genome research 2014;24:743-50.
28. Schuster SJ, Neelapu SS, Gause BL, et al. Vaccination with patient-specific tumor-derived antigen in first remission improves disease-free survival in follicular lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011;29:2787-94.
29. Baskar S, Kobrin CB, Kwak LW. Autologous lymphoma vaccines induce human T cell responses against multiple, unique epitopes. J Clin Invest 2004;113:1498-510.
30. Welters MJ, Kenter GG, Piersma SJ, et al. Induction of tumor-specific CD4+ and CD8+ T-cell immunity in cervical cancer patients by a human papillomavirus type 16 E6 and E7 long peptides vaccine. Clinical cancer research : an official journal of the American Association for Cancer Research 2008;14:178-87.
31. Kenter GG, Welters MJ, Valentijn AR, et al. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. Clinical cancer research : an official journal of the American Association for Cancer Research 2008;14:169-77.
32. Kenter GG, Welters MJ, Valentijn AR, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Engl J Med 2009;361:1838-47.
33. Sampson JH, Aldape KD, Archer GE, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. Neuro-oncology 2011;13:324-33.

34. Sampson JH, Heimberger AB, Archer GE, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:4722-9.
35. Berd D, Murphy G, Maguire HC, Jr., Mastrangelo MJ. Immunization with haptenized, autologous tumor cells induces inflammation of human melanoma metastases. Cancer Res 1991;51:2731-4.
36. Chung Y, Qin H, Kang CY, Kim S, Kwak LW, Dong C. An NKT-mediated autologous vaccine generates CD4 T-cell dependent potent antilymphoma immunity. Blood 2007;110:2013-9.
37. Chen M, Huang L, Wang J. Deficiency of Bim in dendritic cells contributes to overactivation of lymphocytes and autoimmunity. Blood 2007;109:4360-7.
38. Linard B, Bezieau S, Benlalam H, et al. A ras-mutated peptide targeted by CTL infiltrating a human melanoma lesion. J Immunol 2002;168:4802-8.
39. Detanico T, St Clair JB, Aviszus K, Kirchenbaum G, Guo W, Wysocki LJ. Somatic mutagenesis in autoimmunity. Autoimmunity 2013;46:102-14.
40. Wick DA, Webb JR. A novel, broad spectrum therapeutic HPV vaccine targeting the E7 proteins of HPV16, 18, 31, 45 and 52 that elicits potent E7-specific CD8T cell immunity and regression of large, established, E7-expressing TC-1 tumors. Vaccine 2011;29:7857-66.
41. Antonelli LR, Gigliotti Rothfuchs A, Goncalves R, et al. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. J Clin Invest 2010;120:1674-82.
42. Karges B, Muche R, Riegger I, et al. Injection of acidic or neutral insulin and pain: a single-center, prospective, controlled, noninterventional study in pediatric patients with type 1 diabetes mellitus. Clin Ther 2006;28:2094-101.
43. Soiffer R, Hodi FS, Haluska F, et al. Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2003;21:3343-50.
44. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004;10:909-15.
45. Matsumura M, Fremont DH, Peterson PA, Wilson IA. Emerging principles for the recognition of peptide antigens by MHC class I molecules. Science 1992;257:927-34.
46. Saeterdal I, Gjertsen MK, Straten P, Eriksen JA, Gaudernack G. A TGF betaRII frameshift-mutation-derived CTL epitope recognised by HLA-A2-restricted CD8+ T cells. Cancer Immunol Immunother 2001;50:469-76.
47. Ascierto PA, Napolitano M, Celentano E, et al. Regulatory T cell frequency in patients with melanoma with different disease stage and course, and modulating effects of high-dose interferon-alpha 2b treatment. J Transl Med 2010;8:76.
48. Kawai T, Akira S. TLR signaling. Seminars in immunology 2007;19:24-32.
49. Adams S. Toll-like receptor agonists in cancer therapy. Immunotherapy 2009;1:949-64.

50. Bogunovic D, Manches O, Godefroy E, et al. TLR4 engagement during TLR3-induced proinflammatory signaling in dendritic cells promotes IL-10-mediated suppression of antitumor immunity. Cancer Res 2011;71:5467-76.
51. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. Am J Pathol 1994;145:148-56.
52. Boscardin SB, Hafalla JC, Masilamani RF, et al. Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses. The Journal of experimental medicine 2006;203:599-606.
53. Soares H, Waechter H, Glaichenhaus N, et al. A subset of dendritic cells induces CD4+ T cells to produce IFN-gamma by an IL-12-independent but CD70-dependent mechanism in vivo. The Journal of experimental medicine 2007;204:1095-106.
54. Trumpfheller C, Caskey M, Nchinda G, et al. The microbial mimic poly IC induces durable and protective CD4+ T cell immunity together with a dendritic cell targeted vaccine. Proc Natl Acad Sci U S A 2008;105:2574-9.
55. Trumpfheller C, Finke JS, Lopez CB, et al. Intensified and protective CD4+ T cell immunity in mice with anti-dendritic cell HIV gag fusion antibody vaccine. The Journal of experimental medicine 2006;203:607-17.
56. Salem ML, Kadima AN, Cole DJ, Gillanders WE. Defining the antigen-specific T-cell response to vaccination and poly(I:C)/TLR3 signaling: evidence of enhanced primary and memory CD8 T-cell responses and antitumor immunity. J Immunother 2005;28:220-8.
57. Tough DF, Borrow P, Sprent J. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science 1996;272:1947-50.
58. Petricciani J, Egan W, Vicari G, Furesz J, Schild G. Potency assays for therapeutic live whole cell cancer vaccines. Biologicals 2007;35:107-13.
59. Caskey M, Lefebvre F, Filali-Mouhim A, et al. Synthetic double-stranded RNA induces innate immune responses similar to a live viral vaccine in humans. The Journal of experimental medicine 2011;208:2357-66.
60. Gaucher D, Therrien R, Kettaf N, et al. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. The Journal of experimental medicine 2008;205:3119-31.
61. Johnson LA, Morgan RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 2009;114:535-46.
62. Okada H, Kalinski P, Ueda R, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011;29:330-6.
63. Robinson RA, DeVita VT, Levy HB, Baron S, Hubbard SP, Levine AS. A phase I-II trial of multiple-dose polyriboinosic-polyribocytidylic acid in patieonts with leukemia or solid tumors. Journal of the National Cancer Institute 1976;57:599-602.
64. IB BMSR. BMS-734016 (MDX-010) Comparative Tissue-Binding Study with Mouse, Rat, Rabbit, Monkey and Human Tissues (Study DS05067), Report2005.

65. IB BMSR. Study SBi 0919-128: A 2-week toxicity study of human anti-CTLA4 administered by intravenous injection to cynomologous monkeys.2000.
66. IB BMSR. Study SBi 0992-128: An immunogenic study following combined intravenous/intramuscular administration of MAb10D1/HBsAg, respectively, to cynomologous monkeys.2001.
67. IB BMSDR. Covance Study No. 7114-100: 14 day intravenous toxicity study with mAB 10D1 in cynomologous monkeys.2000.
68. Document BMS. Study No. DS0604: BMS-663513 and BMS-734016 (MDX-010): One-month intravenous combination toxicity in monkeys2007.
69. IB BMSR. Study SBi 1416-128: A repeat dose toxicology and effficacy study MDX-010 or MDX-010-HYB in combination with HBsAg and SKMel vaccines in cynomologous monkeys2003.
70. IB BMSDR. Repeated-dose (q3d x 3, 14-day) toxiciyt study of anti-CTLA4/10D1 administered via intravenous injection to cynomologous monkeys (Study 126-002). Report Redfield Laboratories2000.
71. Document BMS. Study 01-3460: MDX-CTLA4: A 6-Month intravenous toxicology study in cynomologous monkeys2002.
72. IB BMSDR. Cross-reactivity study of fluoresceinated human monoclonal antibody 10D1 with normal human tissues (Study IM578) Report Pathology Associates International 2000.
73. IB BMSDR. Cross-reactivity study of fluoresceinated MDX-010 with limited normal human tissues (Study IM933), Report Pathology Associates International2003.
74. IB BMSDR. Ipilimumab (BMS-734016): Intravenous study of pre- and postnatal development in cynomologous monkeys with a 6-month postnatal evaluation.2011.
75. van den Eertwegh AJ, Versluis J, van den Berg HP, et al. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol 2012;13:509-17.
76. Madan RA, Mohebtash M, Arlen PM, et al. Ipilimumab and a poxviral vaccine targeting prostate-specific antigen in metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol 2012;13:501-8.
77. Le DT, Lutz E, Uram JN, et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother 2013;36:382-9.
78. Sarnaik AA, Yu B, Yu D, et al. Extended dose ipilimumab with a peptide vaccine: immune correlates associated with clinical benefit in patients with resected high-risk stage IIIc/IV melanoma. Clin Cancer Res 2011;17:896-906.
79. Schadendorf D, Hodi FS, Robert C, et al. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2015;33:1889-94.
80. Walter S, Weinschenk T, Stenzl A, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 2012;18:1254-61.
81. Ott PA, Hodi FS, Robert C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients.

Clinical cancer research : an official journal of the American Association for Cancer Research 2013;19:5300-9.
82. Linnemann C, Heemskerk B, Kvistborg P, et al. High-throughput identification of antigen-specific TCRs by TCR gene capture. Nat Med 2013;19:1534-41.
83. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. The New England journal of medicine 2015.
84. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. The New England journal of medicine 2012;366:925-31.
85. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus Ipilimumab in Advanced Melanoma. The New England journal of medicine 2013.
86. Kannan S, Neelapu SS. Vaccination strategies in follicular lymphoma. Current hematologic malignancy reports 2009;4:189-95.
87. Brenner M. T cell receptors and cancer: gain gives pain. Nat Med 2010;16:520-1.
88. Krown SE, Kerr D, Stewart WE, 2nd, Field AK, Oettgen HF. Phase I trials of poly(I,C) complexes in advanced cancer. J Biol Response Mod 1985;4:640-9.
89. Levine AS, Levy HB. Phase I-II trials of poly IC stabilized with poly-L-lysine. Cancer Treat Rep 1978;62:1907-12.
90. Hearn A, York IA, Bishop C, Rock KL. Characterizing the specificity and cooperation of aminopeptidases in the cytosol and endoplasmic reticulum during MHC class I antigen presentation. J Immunol 2010;184:4725-32.
91. Hodi FS, Butler M, Oble DA, et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci USA 2008;105:3005-10.
92. Franki SN, Steward KK, Betting DJ, Kafi K, Yamada RE, Timmerman JM. Dendritic cells loaded with apoptotic antibody-coated tumor cells provide protective immunity against B-cell lymphoma in vivo. Blood 2008;111:1504-11.
93. van Rooij N, van Buuren MM, Philips D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 2013;31:e439-42.
94. Walter S, Herrgen L, Schoor O, et al. Cutting edge: predetermined avidity of human CD8 T cells expanded on calibrated MHC/anti-CD28-coated microspheres. J Immunol 2003;171:4974-8.
95. Hawkins MJ, Levin M, Borden EC. An Eastern Cooperative Oncology Group phase I-II pilot study of polyriboinosinic-polyribocytidylic acid poly-L-lysine complex in patients with metastatic malignant melanoma. J Biol Response Mod 1985;4:664-8.
96. Byrne KT, Turk MJ. New perspectives on the role of vitiligo in immune responses to melanoma. Oncotarget 2011;2:684-94.
97. Senzer NN, Kaufman HL, Amatruda T, et al. Phase II clinical trial of a granulocyte-macrophage colony-stimulating factor-encoding, second-generation oncolytic herpesvirus in patients with unresectable metastatic melanoma. J Clin Oncol 2009;27:5763-71.
98. Lundegaard C, Lund O, Nielsen M. Prediction of epitopes using neural network based methods. J Immunol Methods 2011;374:26-34.

99. Abelin JG, Keskin DB, SarkizovaS et al. Mass Spectrometry Profiling of HLA-Associated Peptidomes in Mono-allelic Cells Enables More Accurate Epitope Prediction. Immunity. 2017 Feb 21;46(2):315-326

APPENDICES

APPENDIX A: Performance Status Criteria

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

APPENDIX B1: GI Adverse Event Management Algorithm

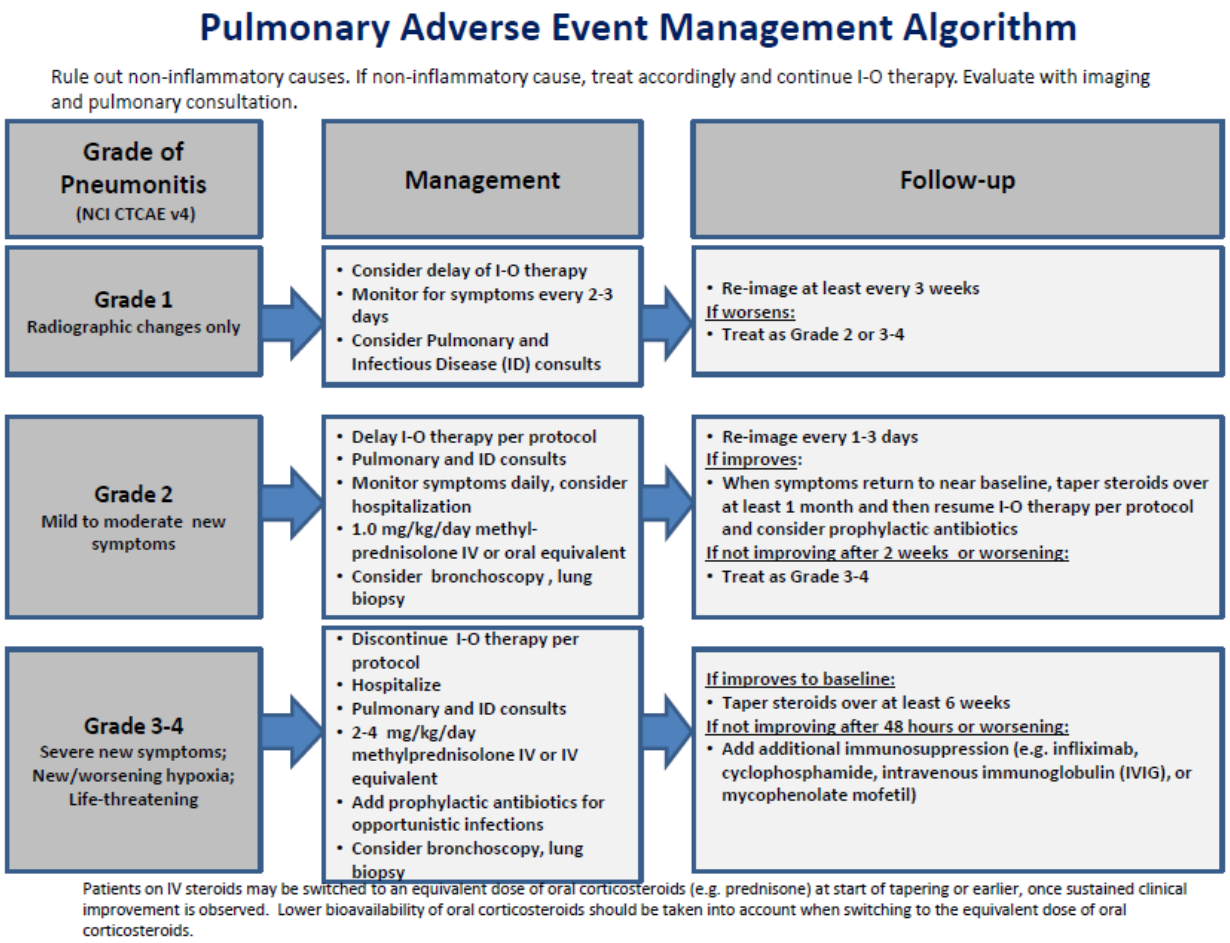
GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

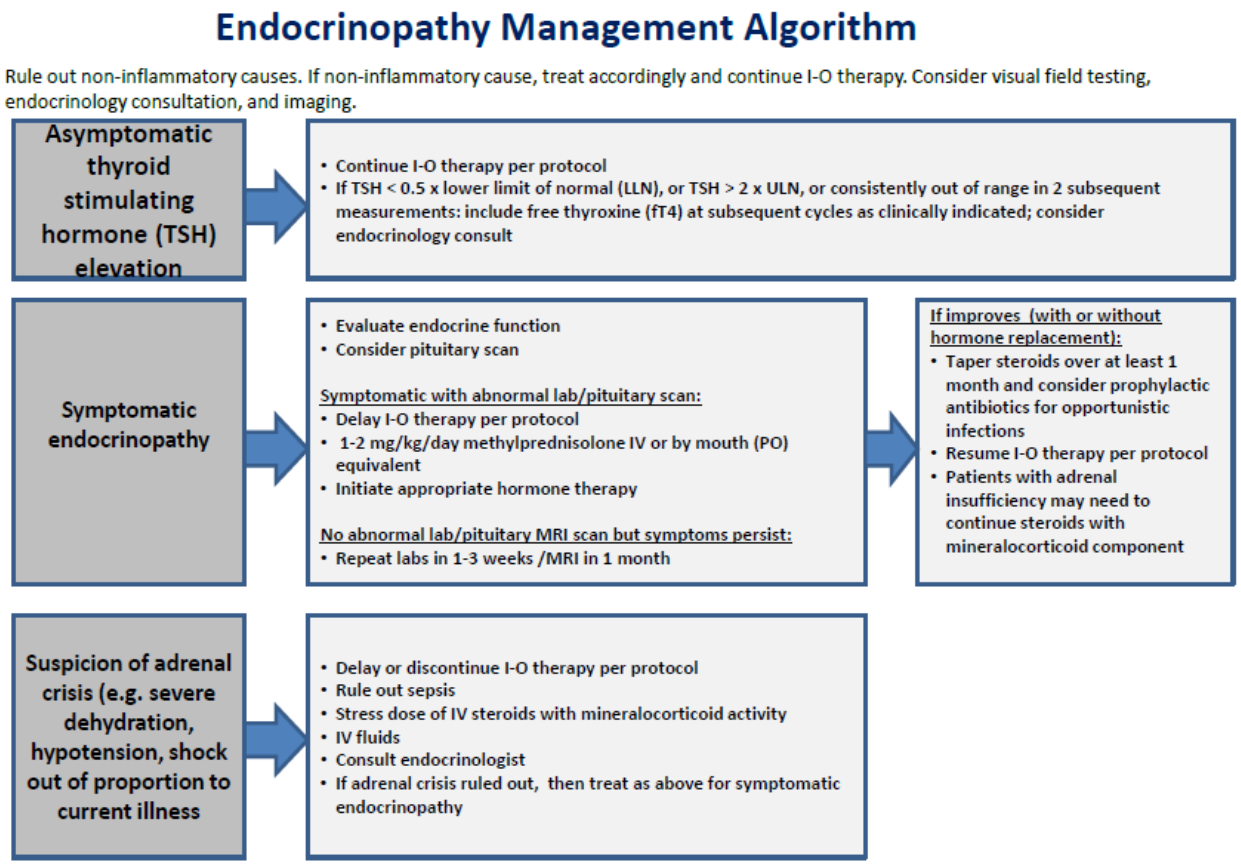
Grade of Diarrhea/ Colitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Diarrhea: < 4 stools/day over baseline; Colitis: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade (G) 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hours (hrs); not interfering with ADL Colitis: abdominal pain; blood in stool	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as grade 3/4
Grade 3-4 Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; interfering with activities of daily living (ADL) Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

APPENDIX B2: Pulmonary Adverse Event Management Algorithm



APPENDIX B3: Endocrinopathy Management Algorithm



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

APPENDIX B4: Hepatic Adverse Event Management Algorithm

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

Grade of Liver Test Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin (T. bili) > ULN - 1.5 x ULN	• Continue I-O therapy per protocol	• Continue liver function tests (LFT) monitoring per protocol If worsens: • Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN	• Delay I-O therapy per protocol • Increase frequency of monitoring to every 3 days	If returns to baseline: •Resume routine monitoring, resume I-O therapy per protocol If elevations persist > 5-7 days or worsen : • 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
Grade 3-4 AST or ALT > 5 x ULN and /or T.bili >3 x ULN	• Discontinue I-O therapy* • Increase frequency of monitoring to every 1-2 days • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent** • Add prophylactic antibiotics for opportunistic infections • Consult gastroenterologist	If returns to grade 2: • Taper steroids over at least 1 month If does not improve in >3-5 days, worsens or rebounds: • Add mycophenolate mofetil 1 gram (g) twice daily (BID) • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.
**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

APPENDIX B5: Neurological Adverse Event Management Algorithm

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

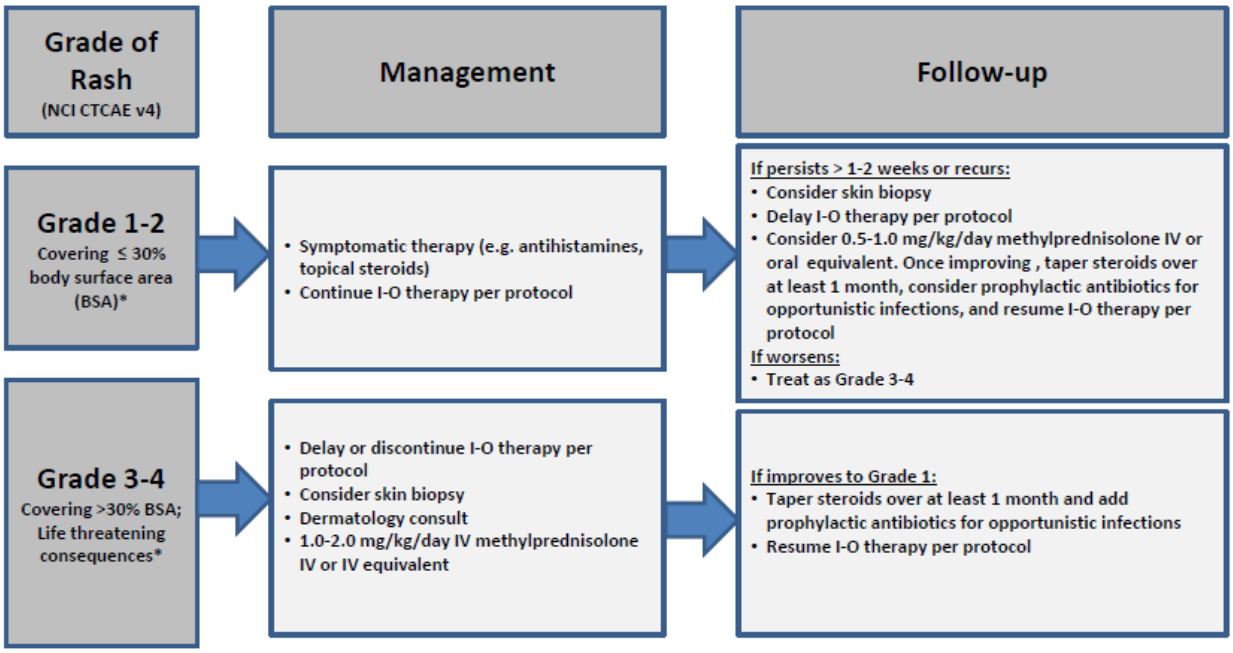
Grade of Neurological Toxicity (NCI CTCAE v4)	Management	Follow-up
Grade 1 Asymptomatic or mild symptoms; Intervention not indicated	• Continue I-O therapy per protocol	Continue to monitor the patient. If worsens: • Treat as Grade 2 or 3-4
Grade 2 Moderate symptoms; Limiting instrumental ADL	• Delay I-O therapy per protocol • Treat symptoms per local guidelines • Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or PO equivalent	If improves to baseline: •Resume I-O therapy per protocol when improved to baseline If worsens: • Treat as Grade 3-4
Grade 3-4 Severe symptoms; Limiting self-care ADL; Life-threatening	• Discontinue I-O therapy per protocol • Obtain neurology consult • Treat symptoms per local guidelines • 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections	If improves to Grade 2: • Taper steroids over at least 1 month If worsens or atypical presentation: • Consider IVIG or other immunosuppressive therapies per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

APPENDIX B6: Skin Adverse Event Management Algorithm

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.

APPENDIX B7: Renal Adverse Event Management Algorithm

Renal Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy

Grade of Creatinine Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 Creatinine > upper limit of normal (ULN) and > than baseline but ≤ 1.5x baseline	• Continue I-O therapy per protocol • Monitor creatinine weekly	<u>If returns to baseline</u>: •Resume routine creatinine monitoring per protocol <u>If worsens:</u> •Treat as Grade 2 or 3/4
Grade 2-3 Creatinine > 1.5x baseline to ≤ 6x ULN	• Delay I-O therapy per protocol • Monitor creatinine every 2-3 days • 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider renal biopsy	<u>If returns to Grade 1:</u> •Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol <u>If elevations persist > 7 days or worsen:</u> •Treat as Grade 4
Grade 4 Creatinine > 6x ULN	• Discontinue I-O therapy per protocol • Monitor creatinine daily • 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent • Consult nephrologist • Consider renal biopsy	<u>If returns to Grade 1</u> : Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.