
Personalized neoantigen-targeting cancer vaccines in ovarian cancer: a phase 1 trial

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Supplementary Information

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Supplementary Information 1: Protocol Amendment Summaries

Protocol v2.0 (8/2/2019) updates:

- New peptide provider (Almac Sciences, Ltd.).
- Added a back-up sequencing vendor (Personalis, Inc.).

Protocol v3.0 (2/19/2020) updates:

- Clarification of timepoints for tumor biopsy, blood collection, and leukapheresis.
- Clarification on processing of tumor and germline cells genetic sequencing
- Clarification of vaccination locations (Table 5.4)
- Updated expected toxicities for Hiltonol (Section 7.1.1.1) and NeoVax (section 7.1.1.2), based on Investigational Drug Brochures (IDB)s.
- Updates on section 8.1.3 (storage and stability) according to Hiltonol IB v16
- Removed duplicative information on Hiltonol availability (section 8.1.6)

Protocol v4.0 (1/17/2021) updates:

- Removed skin biopsies and Delayed Type Hypersensitivity (DTH) testing.
- Clarified tumor biopsy timepoints and samples to be collected (changes in protocol section 10 and the screening ICF): Amendment allowed tumor tissue to be collected through Standard of Care laparoscopies for vaccine generation, due to prior screening failures from inadequate tissue.

Protocol v5.0 (05/20/2021) updates:

- Updated procedures to indicate that eligibility checklist will only be assessed after signing the Main Study Consent (Treatment consent), and not at the Pre-screening Consent (Sections 3 and 4).
- Updated Eligibility criteria (section 3.1) to allow biopsies performed prior to pre-screening consent to be used for vaccine manufacture in both Cohorts A and B. Additionally, improve recruitment in Cohort B, an exclusion for patients with tumors recurring > 18 months after first line treatment was removed.
- Updated Section 5.5 to allow patients to receive the SARS-CoV-2 vaccine in light of the ongoing pandemic.
- Clarified the final NeoVax syringe volume of 0.5mL (+/- 0.1 mL) (Sections 5.4, 8.2.7)
- Clarification of tumor biopsy timepoints and samples to be collected (section 10 and screening ICF)
- Updated to allow for the addition of an HLA-DR helper peptide to vaccines; this was not pursued.

Protocol v6.0 (7/21/21) updates:

- Amendment to allow for the use of PARP inhibitor maintenance after the initial 5 vaccine priming doses, to align with FDA approvals of PARP inhibitors Olaparib and Niraparib as standard of care 1L and 2L maintenance therapies.
- Clarification regarding the eligibility checklist assessment.

- Updates based on the Nivolumab IB v19

Protocol v7.0 (9/14/22) updates:

- Updates to blood collection timepoints for correlative studies
- Cohort B eligibility criteria updated to require recurrence >24 months after completion of 1L platinum-based chemotherapy.
- Clarify that the protocol uses NCI CTCAE 5.0.
- Updates to the Oncovir contact information (Hiltonol supplier).

Protocol v8.0 (3/9/2023) updates:

- Amendment to allow the use of standard of care Bevacizumab maintenance therapy at investigator's discretion for patients in cohort B.
- Updated the indications for PARPi maintenance therapy to align with new FDA guidance (i.e. allowing use in 1L maintenance only in BRCA mutant or HRD tumors; withdrawal of 2L maintenance therapy indications for *BRCA* wildtype tumors).
- Updates to the registration procedures language to better describe the registration process.

Protocol v9.0 (11/7/2023) updates:

- Update the risks related to Nivolumab based on Nivolumab IB v22.
- Notified the IRB that the study team plans to assess samples collected under banking protocol studies to perform analysis related to the 18-318 exploratory objectives.

Protocol v10.0 (12/7/23) updates:

- To align with FDA approvals for Nivolumab dosing, amendment allowed at the investigator's discretion, Nivolumab administration to be switched to every 4 weeks starting at week 24; timing of CA125 and Thyroid function tests were updated to align with this schedule.

Protocol v11.0 (4/1/2024) updates:

- Removed External Safety Monitoring Board (ESMB) language from the protocol (this study is not subject to an ESMB).

Protocol v12.0 (3/20/25) updates:

- Clarification of the schedule of active-follow up after patients have completed all NeoVax with Nivolumab related therapy (every 3 months +/- 2 weeks) and until subsequent therapy. Clarification of long-term follow-up to continue from next subsequent therapy to death, rather than for 5 years or until death.

Supplementary Information 2: Accounting of Primary, Secondary and Exploratory Endpoints.

Primary Endpoints: The primary objectives of the study are to evaluate the feasibility and safety of administering NeoVax with Nivolumab in each of two cohorts of EOC patients: i) patients with newly diagnosed ovarian cancer who are opted for neoadjuvant chemotherapy (Cohort A) and ii) patients with recurrent platinum sensitive ovarian cancer (Cohort B).

- 1) **Accrual Goals:** “The target accrual is 30 patients, i.e. 15 patients per cohort that sign treatment consent to receive NeoVax/Nivolumab treatment.”
[Accrual was stopped early due to funding.](#)
- 2) **Feasibility** will be determined by assessing the percentage of patients prescreened in each cohort who have enough neoantigens for generation of the vaccine, are not platinum refractory, and who initiate vaccination; this will be evaluated using a negative binomial test (one-sided $\alpha = 0.1$). Defining a 45% success rate of prescreening as unacceptable, we will reject this null hypothesis if a maximum of 25 patients are prescreened in order for 15 patients to initiate vaccination (i.e. observed success rate $\geq 60\%$). There will be greater than 90% power to reject the null hypothesis if the true success rate is 70%.
 - a. [Feasibility objectives are addressed in the abstract and Figure 1b.](#) Briefly, Nine of 12 evaluable Cohort B patients were vaccinated (75%, 90% CI 47-93%) and 1 of 9 Cohort A patients were vaccinated (11%, 90% CI 0.6 – 43%).
 - b. [Of note, in Cohort B, 13 patients signed pre-screening consent. One patient subsequently withdrew consent within days, and prior to B1 biopsy. This patient was deemed non-evaluable as they did not undergo pre-screening/biopsy.](#)
- 3) **Safety:** Of patients who sign treatment consent, we will assess the percentage (with 90% confidence intervals [CIs]) of patients from each cohort who successfully complete vaccination and receive Nivolumab therapy without discontinuation due to toxicity. . . We will determine the safety and tolerability of NeoVax/Nivolumab separately for each cohort of EOC patients, using the Common Terminology Criteria for Adverse Events (CTCAE, v.5.0).
 - a. [See Table 2 and associated main-text discussion](#)
 - b. [The most common AEs associated with NeoVax were injection site reactions \(70%\), Myalgias \(50%\), Chills \(50%\) and neutropenia \(50%\).](#)
 - c. [Grade 3 AEs associated with NeoVax: allergic reaction, neutropenia, both occurring in 1 patient, each.](#)
 - d. [Grade 3 AEs associated with nivolumab: abdominal pain and diarrhea occurred in P111.](#)
 - e. [Cohort A: 3 patients signed treatment consent, but two of these patients withdrew consent prior to the first vaccine dose and thus have been deemed unevaluable for safety. Thus, the only vaccinated Cohort A patient received all vaccine doses and Nivolumab, and did not](#)

discontinue due to toxicity. This results in a 90% CI of 5-100% successful treatment without treatment limiting toxicity.

- f. Cohort B: Eight of 9 vaccinated patients received all planned vaccine doses and nivolumab. P111 did not received the final vaccine booster dose due primarily to progression. P111 progressed prior to the first booster dose, and continued post-progression to receive Booster 1 and Nivolumab. In the context of continued CA125 rise and ongoing growth of peritoneal disease, the patient developed ICB-induced diarrhea and abdominal pain (both Grade 3). Therefore, it was deemed that the patient's curtailed NeoVax treatment was attributable primarily to progression, with ICB-related toxicity occurring concomitantly. Thus, all evaluable, treatment-consented cohort B patients completed NeoVax and Nivolumab without discontinuation due to toxicity (100% (8 of 8); 90% CI: 69-100%).

Secondary Endpoints

- 1) To determine anti-tumor activity as assessed by objective response rate (ORR) based on Response Criteria in Solid Tumors (RECIST) and based on immune-related RECIST (irRECIST) for patients with residual measurable disease after completion of chemotherapy.
 - a. There were no RECIST responses, as noted in the text.
- 2) For patients in cohort B who do not have residual measurable disease after completion of chemotherapy, we will determine whether the second progression free survival (PFS) PFS2 (measured as the interval from the start of secondary therapy to the date of the second relapse) is longer than the first PFS (PFS1, measured as the interval from the start of primary therapy to date of first relapse)
 - a. Due to the limited cohort size and therefore statistical power, we have not assessed this endpoint.
- 3) To evaluate progression-free survival (PFS) and overall survival (OS)
 - a. We have reported PFS from vaccine Day 1. Due to limited cohort size and therefore statistical power, we have not assessed OS.
- 4) To evaluate duration of response (DOR)
 - a. As there were no RECIST responders, we have not reported DOR.
- 5) To evaluate ORR among patients who were retreated with NeoVax with Nivolumab at the time of disease progression or recurrence
 - a. No patients were re-treated with NeoVax, and thus we have not reported on this secondary endpoint.

Exploratory Endpoints

- 1) Define the evolution of T cell reactivity, state and repertoire following NeoVax/Nivolumab
 - a. This endpoint is explored in the text, especially in Fig. 2-3 and associated extended data figures.
- 2) Define evolution of the tumor and the tumor microenvironment following standard of care chemotherapy and NeoVax/Nivolumab, and correlate this with T cell and clinical responses
 - a. This endpoint is explored in the text, especially in Fig. 3 and associated extended data figures.
- 3) Identify mechanisms of immune escape to NeoVax/Nivolumab
 - a. This endpoint is explored in the text, especially in Fig. 3-4 and associated extended data figures.

Supplementary Information 3: Major Protocol Deviations

We list here IRB approved major protocol Deviations by date.

- 12/22/2020: IRB was notified that eligibility criteria checklists were not submitted at the time of pre-screening consent. The protocol was subsequently updated to clarify that the eligibility criteria checklist was only meant to be submitted once, at the time of treatment consent.
- 04/07/2021: IRB was notified that P103 accidentally signed the wrong section of the Portuguese short form consent for pre-screening, and did not include a full date with her signature.
- 05/12/2021: IRB was notified that P103's pre-screen reconsent was obtained without appropriate interpreter and witness. An MD signature was also missing.
- 06/14/2021: IRB was notified that cross contamination was identified in the B1 tumor gDNA sample from P104, precluding use for vaccine development.
- 02/25/2022: IRB was notified that P104, P105, P106, P107, P108, and P109 were registered after signing the prescreening consent, without completed eligibility checklists. This deviation was due to an incomplete prior attempt to update the protocol to make clear the eligibility checklists were only to be completed at the time of treatment consent (see 12/22/2020 Deviation). Protocol v 5.0 was submitted to fully correct the language around eligibility checklist submission.
- 04/12/2022: IRB was notified that patient P108 would continue to be treated post-progression.
- 06/07/2022: IRB was notified that patient P108 would continue to be treated post-progression.
- 08/11/2022: IRB was notified that P113 B2 biopsy would fall outside of the pre-specified day 78-92 window by four days, and occur 4 days after the first Nivolumab infusion, due to interventional radiology availability.
- 10/28/2022: IRB was notified that P113 would continue to be treated post-progression.
- 09/07/2023: IRB was notified of missed reconsents for P111 (ICF v 9/17/2021 [Protocol v6.0]), as well as P114, P116, and P117 (ICF v12/2/2022 [Protocol v7.0]). All of the ICFs had been superseded and thus reconsent was no longer possible. At the time, P111, P114, and P117 were off study due to PD.
- 02/02/2024: IRB was notified of a post-progression treatment deviation. Per protocol, post-progression treatment is allowed if patients meet treatment criteria (See *main text, protocol*) and the decision is discussed with both the overall PI and

the IND holder. Patients are then expected to be re-consented and have scans performed within 6 weeks of the initial investigator assessed progression. Following PD for P118 on 12/23/24 the patient was found to meet treatment criteria as assessed by the study PI, however treatment was continued without discussion with the IND holder and without re-consent. Subsequent scans also occurred at 8 weeks, rather than the required 6 weeks. Supplementary Methods: Bulk TCR sequencing

Supplementary Methods: Bulk TCR sequencing Analysis

PBMC from pre-vax leukapheresis 1 (W0), post-boost leukapheresis 2 (W14) and vaccine treatment completion leukapheresis (W22) were thawed and CD3⁺ cells were selected via magnetic cell sorting (Miltenyi CD3 beads) per manufacturer instructions. RNA was isolated via RNA-Easy kit (Qiagen) per manufacturer instructions, with the aid of passage over a QiaShredder (Qiagen) and on-column DNase digestion per manufacturer's instructions (Qiagen). Bulk TCRseq libraries were prepared using TUT-TCRseq protocol. 20ng RNA was used for template switch reverse transcriptase reactions with specific TCR constant region primers. Four replicates were processed for each sample. A UMI primer was added during the 2nd strand synthesis reaction. After exonuclease digestion of the UMI and other primers, PCR1 was performed with nested TCR constant region primers and reaction-specific index primers using repliQa HiFi ToughMix (Quantabio 95200-100). PCR1 products were pooled, cleaned up and further amplified with PCR2 using Illumina adaptor P5 and P7 primers. Final libraries were cleaned up and eluted in Promega Elution buffer. Library quality and quantity were assessed using Bioanalyzer High Sensitivity DNA Kit (Agilent 5067-4626). Oxford nanopore sequencing adapter was ligated using the SQK-LSK114 kit (Oxford Nanopore Technologies) and the NEBNext Companion Module v2 (New England Biolabs E7672) according to manufacturer instructions with 100 fmol input per library. Nanopore sequencing was performed using R10.4 flow cells on a Nanopore GridION instrument. Base-calling was performed using guppy version 6.1.2 with the module dna_r10.4_e8.1_sup. Long read bulk TCR data were processed using MiXCR¹ v4.7.0 preset, '*generic-ont -with-umi*' and aligned using the built-in reference annotation (repseqio.v3.0.1). Additional parameters were included to handle ambiguous base calls and exclude UMIs with single read support (`--rna --tag-pattern '^(UMI:N{20}d)(R1:*)'` --

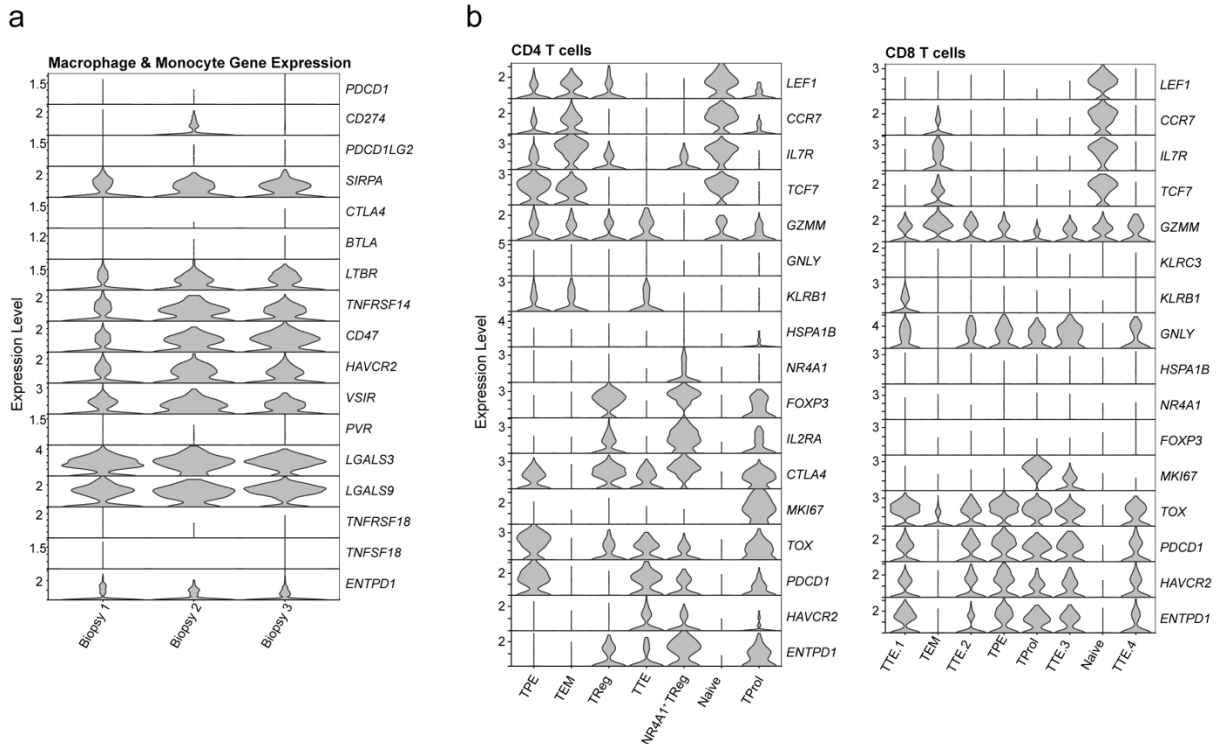
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MassembleContigs.parameters.discardAmbiguousNucleotideCalls=TRUE      -
Massemble.cloneAssemblerParameters.addReadsCountOnClustering=TRUE      -
M.assemble.consensusAssemblerParameters.assembler.minRecordsPerConsensus=2
-MrefineTagsAndSort.parameters.postFilter=null). We clustered all detected clonotypes
with identical CDR3 amino acid sequence, V genes, and J genes for each technical
replicate via the exportClonesOverlap function with the following parameters (-tagCounts
-vGene -jGene --only-productive --criteria "CDR3|AA|V|J" -aaFeatureImputed
VDJTranscriptWithout5UTR) and generated a list of consensus clonotypes for each
biological sample.

```

To identify post-vaccine and post-nivolumab specific expansion, the lower limit of detection was first calculated by patient by rounding down the smallest observed frequency to the nearest order of magnitude². The number of individual T cell clones that expanded after vaccination or nivolumab was calculated using one-sided Fisher's exact tests (fisher.test from the package "stats") on clones with more than 5 reads or 5x above the lower limit of detection at pre- or post-vaccination or post-nivolumab timepoints. Contingency tables were constructed with the number of UMIs of each clone from pre- and post-treatment, and the number of UMIs from all other clones pre- and post-treatment. P-values were then adjusted for multiple comparisons using the Benjamini-Hochberg procedure and all clones with $p_{adj} < 0.05$ were considered significantly expanded. To subset expanded clones to those *de novo* following treatment, clones that were absent or below the LOD at pre-treatment timepoints and detectable post-treatment were selected.

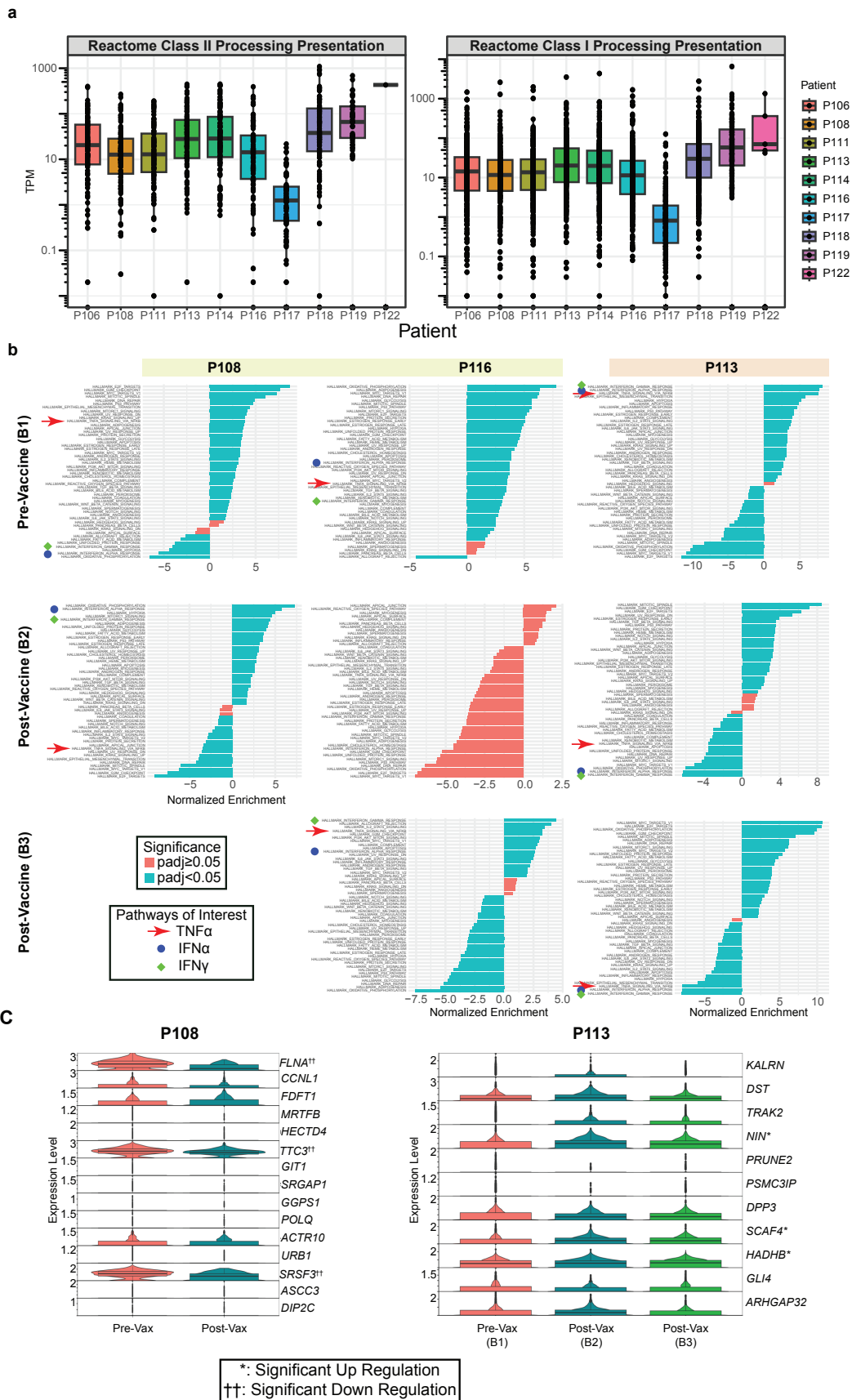
Supplementary Information Figure 1



Supplementary Fig 1: RNA expression in tumor Macrophage/monocytes and TIL.

a. scRNAseq expression of select inhibitory receptor genes from pseudobulk analysis of Macrophage/Monocyte populations at the indicated Biopsy timepoints (cells from $n = 11$ samples from 6 patients; See **Fig. 3a-b**, **Extended Data Fig. 5a**). **b.** scRNAseq pseudobulk expression of T cell markers relevant to T cell clustering³ in CD4+ and CD8+ T cell clusters (cells from $n = 11$ samples from 6 patients; See **Fig. 3a**, **Extended Data Fig. 5b-c**)

Supplementary Information Figure 2



Supplementary Fig. 2. Gene expression programs associated with resistance.

a. RNAseq TPM of genes associated with HLA-II and HLA-I processing and presentation, from pre-vaccine biopsy (B1). **b.** For tumors with serial biopsies, GSEA analysis was used to identify differentially expressed gene sets in tumor cells pre- and post-vaccine. In P113, but not P108 or P116, pathways encompassing IFN γ , IFN α and TNF α were the most significantly differentially expressed between pre- and post-Vaccine (B1 vs. B2 and vs. B3). P-Values calculated with FGSEA v1.24.0⁴ (an adaptive multi-level split Monte Carlo method, with Benjamini-Hochberg correction). **c.** Vaccine target gene expression in tumor cells from Pre and post-vaccine biopsies as measured by scRNAseq. Significant up or down regulation compares post-vax (B2 or B3) to pre-vax (B1) based on Seurat's FindMarkers (Wilcoxon rank sum test, two-sided) to identify differentially expressed genes in tumor cells post-vax versus pre-vax.

Supplementary Table: Primers for *JAK1* SV characterization.

Primer Name	Genomic location (hg38)	Primer Sequence
Int-B-F	chr1:63,277,127-63,277,145	TCACTGGACAGAGGGGAC
Int-B-R	chr1:63,277,257-63,277,276	AGCCGACCTCTGAACTCAAT
JAK1-B-F	chr1:64,882,784-64,882,803	CACTTCAACTCAGCACCCACG
JAK1-B-R	chr1:64,882,980-64,882,999	GTGGATCCAGGCTCAGTTCT
JAK1-C-F	chr1:64,882,603-64,882,623	CTGCCAATGTCCTCAAGTTCA
JAK1-C-R	chr1:64,882,759-64,882,778	CTGAGTGTTGCTTTGTGGCT
Int-I-R	chr1:63,277,400-63,277,419	TTGCACACCACATCACATGC
JAK1-I-R	chr1:64,883,009-64,883,028	AAGATACCAGGGGATGACGC

Expected Amplicon Size:

Target	Primers	Amplicon Size
Intergenic break point	Int-B-F/Int-B-R	150
<i>JAK1</i> break point	JAK1-B-F/JAK1-B-R	216
<i>JAK1</i> common target	JAK1-C-F/JAK1-C-R	175
<i>JAK1</i> intronic SV fusion	Int-I-R/JAK1-I-R	327

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1. Bolotin, D.A., *et al.* MiXCR: software for comprehensive adaptive immunity profiling. *Nat Methods* **12**, 380-381 (2015).
2. Blass, E., *et al.* A multi-adjuvant personal neoantigen vaccine generates potent immunity in melanoma. *Cell* (2025).
3. Oliveira, G., *et al.* Landscape of helper and regulatory antitumour CD4(+) T cells in melanoma. *Nature* **605**, 532-538 (2022).
4. Korotkevich, G., *et al.* Fast gene set enrichment analysis. *bioRxiv*, 060012 (2021).

DF/HCC Protocol #: 18-318
BMS protocol #: CA209-9LW

TITLE: Open-label, Phase I study of NeoVax with Nivolumab in Patients with Ovarian Cancer

Coordinating Center: Dana-Farber/Harvard Cancer Center

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Agents:

- 1) Personalized neoantigen peptides IND # 15703 Dana Farber Cancer Institute
- 2) Poly-ICLC (Hiltonol®) DMF # BB-MF 14,266 and BB-MF 14,302 Oncovir Inc.
- 3) Nivolumab, Bristol Myers Squibb

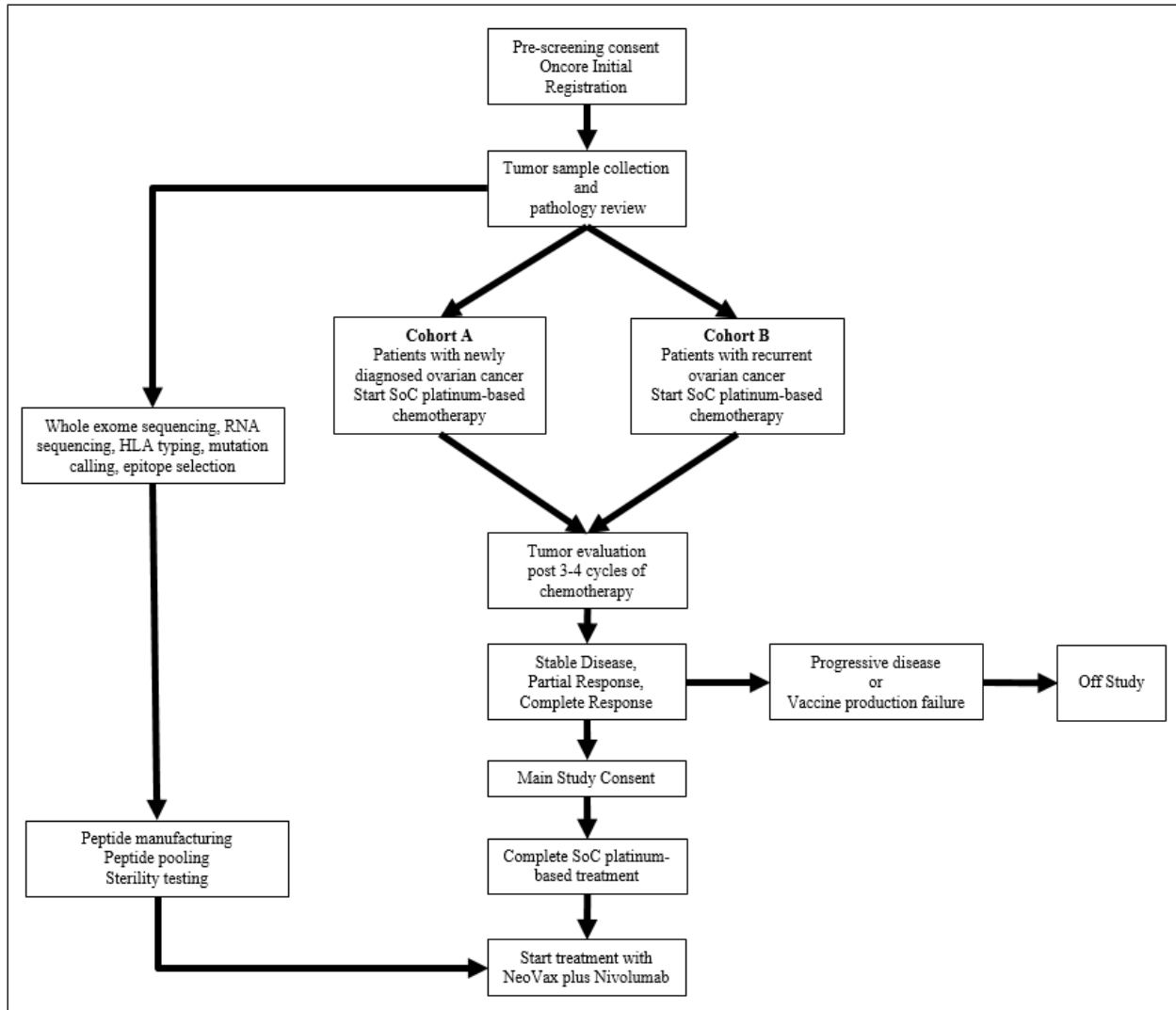
IND #: 15703

IND Sponsor: Patrick Ott, MD, PhD

Original / Version 12.0 / March 20, 2025



SCHEMA A



SCHEMA B

This is an open label Phase I trial of NeoVax with Nivolumab in two cohorts of patients with ovarian cancer:

- i) Cohort A: Patients with newly diagnosed ovarian cancer who are opted for neoadjuvant chemotherapy
- ii) Cohort B: Patients with recurrent platinum sensitive ovarian cancer with progression free interval (PFI) 24 months after completion of first line chemotherapy

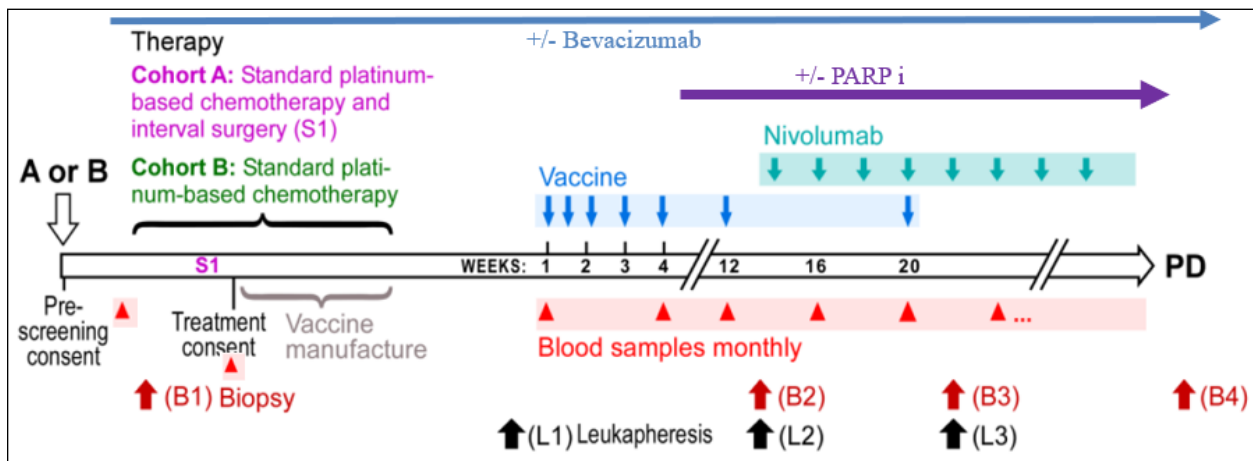


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1. OBJECTIVES

1.1 Study Design

This is an open label Phase I trial of NeoVax with Nivolumab in two cohorts of patients with epithelial ovarian cancer (EOC).

NeoVax refers to the combination of neoantigen vaccine and adjuvant poly-ICLC.

The primary purpose of this study is to demonstrate that the regimen of NeoVax with Nivolumab is safe for the treatment of patients with ovarian cancer. The study will also evaluate the clinical and immune response to treatment of EOC patients. Our main hypothesis is that the combination of the immune stimulating effects of NeoVax coupled with the release of the immune suppression by the PD-1 inhibitor Nivolumab will be safe and may produce clinical responses in EOC.

Two cohorts of patients with EOC will participate in the study:

- i) Cohort A: Patients with newly diagnosed ovarian cancer who are opted for neoadjuvant chemotherapy
- ii) Cohort B: Patients with recurrent platinum sensitive ovarian cancer with progression free interval (PFI) 24 months after completion of first line chemotherapy

The study design for the 2 cohorts is:

i) Cohort A: Patients with newly diagnosed ovarian cancer opted for neoadjuvant chemotherapy:

The study is designed to have a 2-stage consent process. Following the signing of initial pre-screening informed consent, patients will undergo required surgical or core needle biopsy (biopsy B1) (with peripheral blood DNA as matched germline source of DNA) and HLA typing of peripheral blood. Their tumor will undergo DNA and RNA sequencing and neoantigen prediction to determine whether a neoantigen vaccine can be generated. In the meantime, patients will receive the first 3-4 cycles of platinum/taxane chemotherapy followed by assessment of response to chemotherapy and consideration of interval cytoreductive surgery as per standard-of-care (SOC) ^{1, 2}. If generation of NeoVax is not feasible or if patients, in the opinion of the investigator, progress through platinum chemotherapy, then they will not be considered for vaccination and no vaccine will be manufactured. Conversely, if NeoVax can be generated and patients have response or stable disease after 3-4 cycles of platinum/taxane chemotherapy, then they will be offered to sign a treatment consent followed by initiation of manufacture of the vaccine. If patients undergo interval debulking surgery, treatment consent will be offered after surgery is performed.

While the vaccine is manufactured, these patients will complete their standard-of-care therapy, i.e. 3-4 more cycles of chemotherapy. Development of NeoVax takes approximately 12-14 weeks and hence will be available by the time patients complete their standard of care therapy.

3-8 weeks after completion of chemotherapy, patients will undergo vaccination with NeoVax; for the priming phase of vaccination, a total of 5 immunizations will be administered over a 4-week period on days 1, 4, 8, 15, and 22. A booster vaccination will be given at Day 78 (Week 12) and Day 134 (Week 20). On week 14 (i.e. 2 weeks after the first booster dose of NeoVax), patients will begin treatment with Nivolumab at a dose of 240 mg administered by intravenous (IV) infusion over 30 minutes every 2 weeks. Patients will continue Nivolumab every 2 weeks for up to 24 months or until disease progression, initiation of alternate antineoplastic therapy, or death. At the investigator's discretion, on treatment week 24 Nivolumab administration schedule may be switched to every 4 weeks. Subjects will be allowed to start PARPi maintenance as per the FDA approved indication and as per the investigator's discretion starting at or anytime after Day 29 of protocol treatment, i.e. after the 5 immunizations of the priming phase of the vaccination have been administered (refer to Section 5.2). Treatment with PARPi olaparib and niraparib will occur as per standard of care (please refer to olaparib and niraparib package insert).

ii) Cohort B: Patients with recurrent platinum sensitive ovarian cancer: Similar to cohort A, following the signing of initial pre-screening informed consent, patients with recurrent platinum sensitive ovarian cancer, at first recurrence, with progression free interval (PFI) ≥ 6 months and ≤ 18 months, and with measurable and biopsiable disease, will undergo required surgical or core needle biopsy (biopsy B1) (with peripheral blood DNA as matched germline source of DNA) and HLA typing of peripheral blood, followed by 3-4 cycles of SOC platinum based chemotherapy. Their tumor will undergo DNA and RNA sequencing and neoantigen prediction to determine whether a neoantigen vaccine can be generated. If generation of NeoVax is feasible and patients, in the opinion of the investigator, do not progress on 3-4 cycles of SOC platinum-based chemotherapy, then they will be offered to sign a treatment consent followed by initiation of manufacture of the vaccine and administration of 3-4 more cycles of chemotherapy as per standard-of care.

As with cohort A, 3-8 weeks after completion of chemotherapy, patients will undergo vaccination with NeoVax using the same schedule. On week 14 (i.e. 2 weeks after the first booster dose of NeoVax), patients will begin Nivolumab at a dose of 240 mg IV over 30 minutes every 2 weeks. Patients will continue Nivolumab every 2 weeks for up to 24 months or until disease progression, initiation of alternate antineoplastic therapy, or death. At the investigator's discretion, on treatment week 24 Nivolumab administration schedule may be switched to every 4 weeks. Subjects will be allowed to start PARPi maintenance as per the FDA approved indication and as per the investigator's discretion as discussed with cohort A.

Use of Bevacizumab will be allowed at investigator's discretion in Cohort B; bevacizumab will be allowed to be administered during chemotherapy, or after chemotherapy (as maintenance) or throughout (i.e. both during chemotherapy and after chemotherapy) as per the investigator's discretion. Treatment with bevacizumab will occur as per standard of care (please refer to bevacizumab package insert).

At the time of disease progression or recurrence, patients in both cohorts who have experienced a clinical benefit i.e. those with complete response (CR), partial response (PR) or stable disease (SD) for ≥ 6 months by Response Criteria in Solid Tumors (RECIST) or by immune-related

RECIST (irRECIST) or no recurrence for ≥ 12 months may be given the option of undergoing another biopsy for generation of another NeoVax vaccine followed by revaccination with NeoVax followed by Nivolumab using the same schedule as previously administered.

The target accrual is 30 patients, i.e. 15 patients per cohort that sign treatment consent to receive NeoVax/Nivolumab treatment. The 2-consent design (prescreening and therapeutic) ensures that NeoVax manufacture will only proceed for patients without platinum-refractory disease (assessed in the first 3-4 cycles of SOC platinum therapy) and those for which generation of NeoVax is feasible.

1.2 Primary Objectives

The primary objectives of the study are to evaluate the feasibility and safety of administering NeoVax with Nivolumab in each of two cohorts of EOC patients: i) patients with newly diagnosed ovarian cancer who are opted for neoadjuvant chemotherapy (Cohort A) and ii) patients with recurrent platinum sensitive ovarian cancer (Cohort B).

1.3 Secondary Objectives

- To determine anti-tumor activity as assessed by objective response rate (ORR) based on Response Criteria in Solid Tumors (RECIST) and based on immune-related RECIST (irRECIST) for patients with residual measurable disease after completion of chemotherapy
- For patients in cohort B who do not have residual measurable disease after completion of chemotherapy, we will determine whether the second progression free survival (PFS) PFS2 (measured as the interval from the start of secondary therapy to the date of the second relapse) is longer than the first PFS (PFS1, measured as the interval from the start of primary therapy to date of first relapse)
- To evaluate progression-free survival (PFS) and overall survival (OS)
- To evaluate duration of response (DOR)
- To evaluate ORR among patients who were retreated with NeoVax with Nivolumab at the time of disease progression or recurrence

1.4 Exploratory Objectives

- Define the evolution of T cell reactivity, state and repertoire following NeoVax/Nivolumab
- Define evolution of the tumor and the tumor microenvironment following standard of care chemotherapy and NeoVax/Nivolumab, and correlate this with T cell and clinical responses
- Identify mechanisms of immune escape to NeoVax/Nivolumab

2. BACKGROUND

2.1 Study Disease

2.1.1 Ovarian Cancer

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy and the fifth most common cause of female cancer death in the United States³. Delay in diagnosis and emergence of resistance to conventional chemotherapy are primarily responsible for the dire outcome of EOC patients, which has not changed appreciably over the last 3 decades despite advances in surgical and chemotherapeutic approaches⁴⁻⁷, suggesting that alternative approaches are urgently needed.

Approximately 75% of EOC patients are diagnosed with advanced disease which is curable only in a minority of the cases resulting in a modest 5-year overall survival rate of 20-30%^{5, 8}. The standard of care management of EOC consists of primary surgical cytoreduction followed by platinum-based chemotherapy^{5, 9}. Platinum (Carboplatin or Cisplatin) in combination with paclitaxel is the current standard of care chemotherapy for EOC and is administered for a total of 6-8 cycles^{5, 9}. Neoadjuvant chemotherapy is also increasing in usage and is currently selected for 25% of patients with Stage II disease, 40% of patients with Stage III disease, and 50% of patients with Stage IV disease. In that scenario, chemotherapy is administered for 3-4 cycles and if there is significant response and the patient is a good operative candidate, a surgical procedure is performed (interval cytoreduction) followed by completion of 3-4 cycles of chemotherapy. Two large randomized Phase III clinical studies, i.e., the EORTC 55971, and the CHORUS studies, have shown the non-inferiority of neoadjuvant chemotherapy versus primary debulking surgery followed by chemotherapy in EOC^{1, 2}.

2.1.2 Platinum sensitive relapsed ovarian cancer

Based on their response to previous platinum therapy, patients are categorized into platinum sensitive or resistant groups. Patients who have responded to platinum therapy with duration of progression free interval equal or greater than 6 months after completing platinum therapy are termed platinum sensitive, whereas patients with disease recurrence within less than 6 months are termed platinum resistant¹⁰. Platinum resistant patients are not considered candidates for further platinum therapy and are routinely offered other chemotherapeutics, including topotecan, liposomal doxorubicin or paclitaxel alone¹¹⁻¹³. The standard of care chemotherapy for patients that are platinum sensitive consists of a platinum doublet; options include carboplatin/paclitaxel, carboplatin/gemcitabine and carboplatin/liposomal doxorubicin^{14, 15}. Bevacizumab may also be added to carboplatin/paclitaxel or carboplatin/gemcitabine chemotherapy¹⁶.

However, as in the first line setting, despite significant response rates to chemotherapy, patients commonly progress and in the second line setting this routinely occurs sooner than the duration of their first remission (i.e. PFS2 is routinely shorter than PFS1). Therefore, there is a great need for novel treatment approaches to prolong the duration of the response and outcome after platinum therapy for these patients.

2.2 Investigational Agents

2.2.1 NeoVax

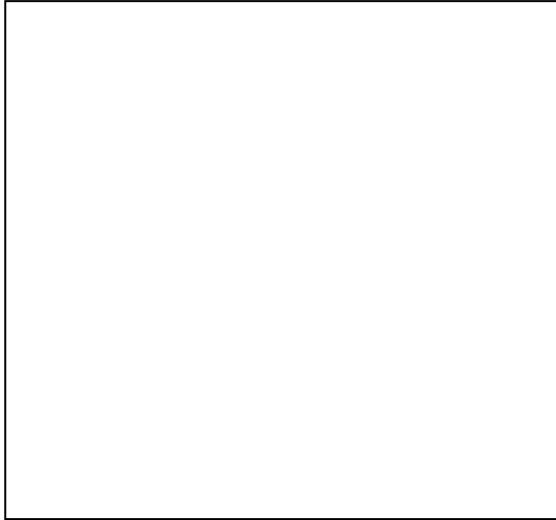
NeoVax is a personalized neoantigen vaccine. It consists of up to 20 synthesized peptides approximately 20 to 35 amino acids in length that are encoded by each patient's mutated tumor deoxyribonucleic acid (DNA) and therefore are not present in normal cells (neoantigens). The NeoVax drug product is administered following mixing with the adjuvant polyinosinic-polycytidylic acid-polylysine carboxymethylcellulose (Poly-ICLC).

Poly-ICLC is an investigational product in the United States. It is a double-stranded RNA mimic that functions as an immune adjuvant. Poly-ICLC activates TLR3 and the RNA helicase domains of melanoma differentiation-associated protein (MDA5) (both members of the PAMP signaling pathway) leading to DC and NK cell activation and production of a natural mix of type I IFNs, cytokines, and chemokines¹⁷. Furthermore, Poly-ICLC exerts a direct, broad, host-targeted anti-infectious and possibly anti-tumor effect¹⁸.

Unlike the majority of previously-used cancer vaccines, this neoantigen peptide vaccine is based on the production of a novel and unique product for each individual patient. The extent of possible tumor mutations and the wide range of patient human leukocyte antigen (HLA) haplotypes makes it highly unlikely that two patients will receive the same vaccine.

Generation of NeoVax begins with whole exome DNA and RNA sequencing of tumor and normal tissue samples from the patient. These data are used to identify coding-sequence mutations that have occurred in the patient's tumor. The peptides are designed specifically from the molecular profile of each individual's tumor. These mutations include single-amino acid missense mutations and novel open reading frames (neoORFs) varying in length from one up to hundreds of amino acids as well as fusion proteins. The vaccine will be composed of a mixture of peptides that are predicted to induce a response in either CD4+ or CD8+ T-cells, as well as peptides predicted to illicit a humoral response. Using a number of filters, up to 30 of the identified mutations are selected and prioritized for peptide synthesis. A primary criterion for peptide selection is the HLA binding affinity of the mutant epitope compared to its native protein. A benchmarked algorithm (netMHCpan) is used to identify mutation-containing epitopes that are predicted to bind to the major histocompatibility complex (MHC) class I molecules of each patient¹⁹. Other key criteria include RNA expression, type of mutation (e.g. missense versus neoORF), the likelihood that the mutation is an oncogenic driver, cellular localization of the expressed mutation and the physical location of the mutant residue(s) on the peptide.

Peptides are synthesized under GMP conditions and are mixed together in up to four pools of up to five peptides each for injection. Each of the up to four pools is injected intradermally and subcutaneously in one of the four extremities or in the flank. The steps for development of NeoVax are summarized in the Figure below.



We have now initiated two Phase I trials of NeoVax for patients with stage III/resectable stage IV melanoma (DFHCC Protocol #13-240; NCT01970358⁴⁸) and newly diagnosed glioblastoma (DFHCC Protocol #14-362; NCT02287428). Between the 2 trials, 15 patients have received vaccination doses safely. On the melanoma trial, we have enrolled 15 patients, 8 of whom have completed dosing (median follow up 21 months). Five of 8 patients remain free of disease while 3 have progressed.

2.2.2 Nivolumab

Nivolumab (OPDIVO®) is a commercially available immune checkpoint modulator manufactured and marketed in the US by Bristol-Myers Squibb Company for the treatment of patients with unresectable or metastatic melanoma and metastatic squamous non-small cell lung cancer (NSCLC) whose disease progressed after prior treatment.

Nivolumab is a human immunoglobulin G4 (IgG4) monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, reversing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor expressed on T cells inhibits T-cell proliferation and cytokine production. Up-regulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors.

2.3 **Rationale**

2.3.1 Immune checkpoint blockade (ICB) has revolutionized treatment of multiple cancers

A number of immune checkpoint inhibitors targeting PD-1, PD-L1 or CTLA4 have been FDA-approved for a variety of solid tumors and hematologic malignancies and have caused a treatment paradigm shift by outperforming standard of care chemotherapy in several clinical settings^{20, 21}. The excitement for this approach stems from evidence of durable responses or remissions even in patients with advanced, widely metastatic tumors that are refractory to chemotherapy or targeted therapies.

2.3.2 Challenge: ICB as monotherapy exhibits only modest activity against ovarian cancer

Ovarian cancer was among the first tumor types where native host anti-tumor cell mediated immune mechanisms were demonstrated to play a role in controlling malignant progression. Specifically, presence of CD3+ and CD8+ tumor infiltrating lymphocytes (TILs) was shown to be associated with improved overall survival (OS) and increased tumor expression of interferon

gamma (IFN- γ) and other lymphocyte-attracting cytokines^{22, 23}. Similarly, the paradigm of regulatory T cells (Treg)-mediated immunosuppression in human malignancies was originally defined in ovarian cancer whereby Treg cells were found to suppress tumor-specific T-cell immunity, contribute to tumor growth *in vivo* and to be associated with poor OS²⁴. Furthermore, expression of both PD-L1 and PD-L2 is associated with poor OS in this disease and PD-L1 expressed on ovarian cancer cells has been shown to inhibit anti-cancer immunity²⁵. Despite these findings which have been repeatedly confirmed in subsequent studies, clinical trials of immune checkpoint inhibitors suggest only modest response in ovarian cancer, with objective response rate (ORR) of 10-15% and infrequent durable responses^{26, 27}. Strategies to improve ICB efficacy while minimizing immune-related toxicities are urgently needed.

2.3.3 Neoantigens are promising targets for anti-tumor immune response because of their tumor-specific expression and high immunogenicity

Somatic mutations that generate completely novel stretches of amino acids (novel open reading frames or “neoORFs”) create new, personal, tumor-specific antigens, also called neoantigens, which can bind personal HLA molecules and be presented for immune recognition²⁸. Neoantigens are optimal targets for anti-tumor immune response because they have bypassed deletion of cognate T cells via thymic tolerance and are absent from normal tissues, thereby both maximizing the affinity of T cell receptors that can be induced and reducing the potential for autoreactivity²⁹. Experiments in animal models first demonstrated that neoantigens are bona fide tumor antigens producing T cell responses that recognize and kill tumor cells. In humans, there is also mounting evidence for the effectiveness of targeting tumor neoantigens and presence of neoantigen responsive T cells has been correlated with positive clinical outcomes in various tumor types^{28, 30, 31}.

2.3.4 Genomics-based identification of neoantigens has reinvigorated the field of cancer vaccination

Studies in the pre next-generation sequencing (NGS) era had already identified tumor neoantigens as a target antigen class of interest³²⁻³⁵ but the technical difficulties of T cell cloning and antigen-specific screening precluded their prompt identification. Two analytical advances have made it possible to systematically identify neoepitopes. First is the increased availability of exome-wide massive parallel tumor sequencing which can be used for comprehensive detection of all coding somatic mutations. The second is the development and maturation of algorithms predicting peptide binding to class I MHC molecules using binding data from a large number of peptides to different MHC molecules. Using these algorithms, protein sequences containing tumor-encoded mutations can be evaluated *in silico* for binding to a specific class I MHC molecule. Such epitopes can then be generated synthetically and used in a personalized vaccine to initiate specific T cell responses targeting tumor cells expressing the mutated proteins. Application of these approaches has led to the discovery:

- i) of an association between tumor neoantigen load and clinical outcome³⁶⁻³⁹;
- ii) that neoantigens are expanded in settings of clinical response to immune-based therapy⁴⁰⁻⁴²;
- iii) that neoantigens can be *in vitro* and *in vivo* targets of cytotoxic T lymphocytes (CTLs)^{18, 43, 44}.

Given the observed potency of T cells recognizing this class of immune targets, we hypothesize that vaccination with neoantigens can both expand pre-existing neoantigen-specific T cell populations and induce a broader repertoire of new T cell specificities in cancer patients, tipping the intra-tumoral balance in favor of enhanced tumor control.

2.3.5 Addressing the challenge: Combining a personalized neoantigen vaccine with ICB in ovarian cancer

We propose a novel immunotherapy strategy against ovarian cancer that involves a personalized neoantigen vaccine consisting of up to 20 synthesized peptides that are encoded by each patient's mutated tumor DNA admixed with immune adjuvant poly-ICLC in combination with an antibody against PD-1. Our main hypothesis is that the combination of the immune stimulating effects of the personalized neoantigen vaccine with the release of the immune suppression by the PD-1 inhibitor may produce clinical responses in EOC. This may occur by expanding existing tumor-reactive T-cells and by broadening the T-cell repertoire to include new tumor-specific T-cells thereby steering the immune response specifically towards the tumor with fewer autoimmune side effects. Furthermore, this strategy may help overcome tumor heterogeneity by generating a diversity of antigen specific T cells that can recognize different tumor clones.

2.3.6 Combination of NeoVax with PD-1 blockade

As stated above, in the phase I trial of NeoVax for patients with stage III/resectable stage IV melanoma (DFHCC Protocol #13-240; NCT01970358), 3 of 8 patients with melanoma who completed dosing of NeoVax developed progressive disease⁴⁸. Subsequently, these 2 patients were treated with the anti-PD-1 antibody pembrolizumab and each achieved a complete radiographic response (CR) after 4 doses of pembrolizumab (CR rate of pembrolizumab as first-line treatment for metastatic melanoma has been reported to be 6%⁴⁵⁻⁴⁷). We investigated in these 2 patients whether the vaccine-induced neoantigen-reactive cells persisted following checkpoint blockade therapy and whether the specificity of responses broadened through epitope spread. We tested samples collected after 9-12 months of treatment with pembrolizumab for reactivity against all 15-16mer assay and class I epitope-length peptides by IFN- γ ELISPOT. In the first patient, these studies revealed CD4+ T cell responses against 25 of 49 assay peptides (with 4 novel responses) and CD8+ T cell responses against 2 of 18 predicted class I epitope peptides. Similarly, for the second patient, CD4+ T cell responses were detected against 15 of 61 assay peptides (with 2 novel responses) and CD8+ T cell responses against 4 of 32 class I epitope peptides (with 2 novel responses). These results not only demonstrate the persistence of the vaccine-induced neoantigen responses over time but also broadening of the repertoire of neoantigen-specific T cells following checkpoint blockade therapy.

2.3.7 Nivolumab Treatment in Combination with a Peptide Vaccine

In a phase I trial (NCT01176474), 49 patients received Nivolumab at doses of 1 mg/kg, 3 mg/kg, and 10 mg/kg in combination with a 4-peptide vaccine consisting of gp100₂₀₉₋₂₁₇ (210M), gp100₂₈₀₋₂₈₈ (288V), MART-1₂₆₋₃₅ (27L), and NY-ESO-1₁₅₇₋₁₆₅ (165V) emulsified in Montanide ISA 51 VG (Weber, 2013). One dose-limiting toxicity (grade-3 bilateral optic neuritis) was observed (Nivolumab at 3 mg/kg), which resolved with a prednisone taper from 60 mg over 4 weeks and topical corticosteroids. Two other patients discontinued treatment secondary to toxicity beyond the DLT period of 12 weeks: One patient receiving Nivolumab at 1 mg/kg had

grade-3 fevers in cycle 2 that required 4 weeks of a prednisone taper from 60 mg for resolution, and one patient receiving Nivolumab at 10 mg/kg had grade-3 pneumonitis after completion of two cycles of therapy requiring a prednisone taper from 120 mg over 2 months for resolution. No dose-limiting immune-related colitis was seen in the study. The rate or severity of Aes in this trial were not different from those seen in trials with Nivolumab alone (such as MDX-1106-03, [NCT00730639]).

2.3.8 Rationale for allowing PARP inhibitor maintenance therapy

One of the primary objectives of our study has been to evaluate the feasibility of incorporating Neovax with Nivolumab in the treatment of epithelial ovarian cancer both in the setting of newly diagnosed disease (cohort A) and in the setting of recurrent platinum sensitive disease (cohort B). However, since initiation of this study, important developments have modified the standard of care treatment of ovarian cancer with the regulatory approval of PARP inhibitors as maintenance therapy after platinum-based chemotherapy.

Specifically:

- a. PARP inhibitor (PARPi) olaparib has now been approved by the Food and Drug Administration (FDA) for the maintenance treatment of adult patients with deleterious or suspected deleterious germline or somatic BRCA-mutated advanced epithelial ovarian, fallopian tube or primary peritoneal cancer who are in complete or partial response to first-line platinum-based chemotherapy. This approval was based on SOLO-1 (NCT01844986), a randomized, double-blind, placebo-controlled, multi-center trial that compared the efficacy of olaparib with placebo in patients with BRCA-mutated advanced ovarian, fallopian tube, or primary peritoneal cancer following first-line platinum-based chemotherapy⁴⁸. Patients were randomized (2:1) to receive olaparib tablets 300 mg orally twice daily (n=260) or placebo (n=131). The primary efficacy outcome was investigator-assessed progression-free survival (PFS) evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. The trial demonstrated a statistically significant improvement in investigator-assessed PFS for olaparib compared to placebo. Estimated median PFS was not reached in the olaparib arm and was 13.8 months in the placebo arm (HR 0.30; 95% CI: 0.23-0.41; p<0.0001).
- b. PARP inhibitor niraparib has now been approved by the FDA for the maintenance treatment of adult patients with advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to first-line platinum-based chemotherapy. Efficacy was investigated in PRIMA (NCT02655016), a double-blind, placebo-controlled trial that randomized 733 patients to niraparib or matched placebo⁴⁹. Patients were in a complete or partial response to first-line platinum-based chemotherapy. The main efficacy outcome measure, progression-free survival (PFS), was first tested in the homologous recombination deficient population, then in the overall population and was determined by blinded independent central review per RECIST 1.1. Tumor samples were tested for homologous recombination deficiency status; homologous recombination deficient was defined by either presence of tumor breast cancer susceptibility gene (tBRCA) mutation or genomic instability score (GIS) ≥ 42 . The trial demonstrated a

statistically significant improvement in PFS for patients randomized to niraparib compared with placebo in the homologous recombination deficient and overall population. Median PFS in the homologous recombination deficient population was 21.9 months (19.3, NE) for patients receiving niraparib compared with 10.4 months (8.1, 12.1) for those receiving placebo (HR 0.43; 95% CI: 0.31, 0.59; $p < 0.0001$). Median PFS in the overall population was 13.8 months (11.5, 14.9) for patients receiving niraparib compared with 8.2 months (7.3, 8.5) for those receiving placebo (HR 0.62; 95% CI: 0.50, 0.76; $p < 0.0001$).

It is important to underscore that the FDA approval of niraparib was regardless of BRCA mutation status and regardless of homologous recombination deficient status. In other words, patients with BRCA mutated and BRCA wildtype as well as patients with homologous recombination deficient and homologous recombination proficient tumors are eligible for niraparib maintenance therapy as per the FDA approved indication. Patients with tumors with BRCA mutations exhibited the most prominent benefit from PARP inhibitor maintenance, with a HR of 0.4 (niraparib maintenance v placebo) in PRIMA and HR of 0.30 (olaparib maintenance vs placebo) in SOLO-1^{48,49}. In the absence of a BRCA1/2 mutations, a larger benefit of niraparib was observed in women with homologous recombination deficient tumors although women with homologous recombination proficient tumors also derived benefit⁴⁹.

Taken together, the approval of olaparib and niraparib as maintenance therapy for patients with newly diagnosed ovarian cancer who are in complete or partial response to first-line platinum-based chemotherapy has changed the treatment paradigm of ovarian cancer. Based on this, in order to evaluate the feasibility of incorporating Neovax with Nivolumab in the treatment of epithelial ovarian cancer (the primary endpoint of this study), we will allow use of PARPi maintenance therapy with olaparib or niraparib as per their FDA approved indications.

Of note, there have been recent concerns about overall survival decrement with PARP inhibitor maintenance therapy in the second line or later in germline (g)BRCA unmutated patients; this does not pertain to the first line, or to gBRCA mutated patients in later lines. The FDA has come to an agreement with GSK (Niraparib; <https://www.gsk.com/en-gb/media/press-releases/gsk-provides-an-update-on-zejula-niraparib-us-prescribing-information/>), and asked Clovis (Rucaparib; <https://d18rn0p25nwr6d.cloudfront.net/CIK-0001466301/139d3568-6b47-428a-8e99-726f06e47a70.pdf>), to voluntarily withdraw the indication for PARPi maintenance therapy in the second line, in gBRCA unmutated patients. Therefore, to align with this FDA guidance, PARPi maintenance therapy will not be allowed in gBRCA unmutated patients in cohort B.

Use of PARPis will be permitted after the priming phase of vaccination is completed. Specifically, subjects will be allowed to start PARPi maintenance as per the FDA approved indication and as per the investigator's discretion starting at or anytime after Day 29 of protocol treatment, i.e. after the 5 immunizations of the priming phase of the vaccination have been administered (refer to Section 5.2). Treatment with PARPi olaparib and niraparib will occur as per standard of care (please refer to olaparib and niraparib package insert).

2.3.9 Safety of addition of PARP inhibitors

As stated above, both PARP inhibitors olaparib and niraparib, are now FDA approved for the maintenance treatment of adult patients with advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to first-line platinum-based chemotherapy. The safety and side effect profile of these agents is well established (please refer to olaparib and niraparib package insert).

As per protocol, on week 14 (Day 92, +/- 3 days), patients are supposed to begin treatment with anti-PD-1 antibody Nivolumab. The safety of combinations of PARP inhibitors with immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway is also well established. Specifically, combinations of PARP inhibitors with PD-1/PD-L1 inhibitors have been evaluated in 4 phase II clinical trials in ovarian cancer, i.e. TOPACIO, MEDIOLA, JAVELIN PARP Medley and by an NCI sponsored study (Lampert et al.), Table below.

Table. Phase II studies of combinations of PARP inhibitors with immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway in ovarian cancer

STUDY	AGENTS	OVARIAN CANCER SETTING	DESIGN	NUMBER OF PATIENTS
TOPACIO ⁵⁰	Pembrolizumab/ Niraparib	Recurrent platinum resistant or platinum ineligible	Phase I/II	N=62
MEDIOLA ^{51,52} NCT02734004	Durvalumab/ Olaparib	Recurrent platinum sensitive	Phase II	N=66
JAVELIN PARP Medley trial NCT03330405 ⁵³	Avelumab/ Talazoparib	Recurrent Platinum Sensitive	Phase I/II	N=40
Lampert et al. ⁵⁴ (NCI study) NCT02484404	Durvalumab/ Olaparib	Recurrent Platinum Sensitive and Resistant	Phase II	N=35

In all these Phase II studies, there were no unexpected toxicities and no new safety signals with the combination of PARP inhibitors with PD-1/PD-L1 inhibitors compared with the safety profiles of either drugs (PARP inhibitors and PD-1/PD-L1 inhibitors) as single agents. Furthermore, in these studies, the incidence of immune related adverse events with the combinations of PARP inhibitors and PD-1/PD-L1 inhibitors was comparable with that of PD-1/PD-L1 inhibitors alone⁵⁰⁻⁵⁴; in other words, no new/additional safety concerns due to immune related adverse events have been observed with the combinations of PARP inhibitors with PD-1/PD-L1 inhibitors.

It is important to underscore that besides ovarian cancer, combinations of PARP inhibitors with PD-1/PD-L1 inhibitors have also been evaluated in other tumor settings. For example, the pembrolizumab/niraparib combination (TOPACIO trial) was also evaluated in 55 patients with breast cancer⁵⁵ and the avelumab/talazoparib (JAVELIN PARP Medley trial) has been evaluated in 22 patients with breast cancer⁵³. Again, there were no unexpected toxicities, no new safety signals and no new/additional safety concerns due to immune related adverse events observed

with the combinations of PARP inhibitors with PD-1/PD-L1 inhibitors compared with either drugs (PARP inhibitors and PD-1/PD-L1 inhibitors) as single agents.

Importantly, a further testament to the safety of the combinations of PARP inhibitors with PD-1/PD-L1 inhibitors is that these combinations are currently being explored in the upfront maintenance setting in patients with newly diagnosed ovarian cancer in three large randomized phase III studies involving collectively more than 3000 patients (see Table below). ATHENA is a large randomized phase III trial with a target enrollment of 1012 patients, of the combination of rucaparib with nivolumab in patients with newly diagnosed ovarian cancer after completion of frontline platinum/taxane combination (NCT03522246). The FIRST trial evaluates the combination of the anti-PD1 agent dostarlimab and niraparib in frontline maintenance (NCT03602859) with a target enrollment of 912 patients, and KEYLYNK-001/ENGOT-OV43 evaluates pembrolizumab and olaparib for the first-line treatment of women with BRCA wild type advanced ovarian cancer (NCT03740165) with a target enrollment of 1284 patients.

Table. Randomized Phase III studies of combinations of PARP inhibitors with PD-1 inhibitors in ovarian cancer

STUDY	AGENTS	OVARIAN CANCER SETTING	DESIGN	NUMBER OF PATIENTS
FIRST NCT03602859	Dostarlimab/ Niraparib	Newly Diagnosed Stage III/IV	Randomized Phase III	N=912
ATHENA NCT03522246	Nivolumab/ Rucaparib	Newly Diagnosed Stage III/IV	Randomized Phase III	N=1012
KEYLYNK- 001/ENGOT-OV43 NCT03740165	Pembrolizumab/ Olaparib	Newly Diagnosed Stage III/IV (BRCAwt)	Randomized Phase III	N=1284

Taken together, we do not anticipate any new safety signals or any new/additional safety concerns due to immune related adverse events with allowing PARPi (olaparib or niraparib) maintenance therapy as per the FDA approved indication.

2.3.10 Rationale for allowing Bevacizumab maintenance therapy

Bevacizumab is supported as a maintenance therapy by the NCCN guidelines in platinum sensitive, recurrent ovarian cancer in the second line. This is based on several randomized phase III trials in the platinum sensitive, recurrent ovarian cancer setting, which showed 1) improved PFS with bevacizumab vs. placebo added to carboplatin + gemcitabine, followed by bevacizumab vs. placebo maintenance (OCEANS trial; PFS HR 0.484; 95% CI, 0.388 to 0.605; $p < 0.0001$); 2) improved OS with bevacizumab vs. placebo added to carboplatin + paclitaxel, followed by bevacizumab or placebo maintenance (GOG-0213; audited treatment free interval >6 months, OS HR 0.823, 95% CI 0.680–0.996; $p=0.0447$); and 3) improved PFS with Bevacizumab combined with Carboplatin + liposomal doxorubicin vs. carboplatin + gemcitabine, both followed by bevacizumab (AGO-OVAR2.21/ENGOT-ov18; PFS HR 0.81, 95% CI 0.68-0.96; $p=0.012$). To align with current SOC maintenance indications for recurrent platinum sensitive ovarian cancer, Bevacizumab will be allowed at investigators discretion in Cohort B; bevacizumab will be allowed to be administered during chemotherapy, or after chemotherapy (as maintenance) or throughout (i.e. both during chemotherapy and after

chemotherapy as maintenance) as per investigator's discretion

2.3.11 Safety of Bevacizumab in combination with immune checkpoint blockade.

The safety and side effect profile of bevacizumab with anti-PD1 checkpoint inhibitor has been established in multiple studies. These include the large randomized phase III IMAGYN050 (1311 patients; first line; bevacizumab and chemotherapy with or without atezolizumab) and ATALANTE/Ov29 (614 patients; recurrent disease; bevacizumab and chemotherapy with or without atezolizumab). Taken together, we do not anticipate any new safety signals or any new/additional safety concerns due to immune related adverse events with allowing bevacizumab maintenance therapy as per the FDA approved indication.

2.4 Correlative Studies Background

2.4.1 Testing the immunogenicity of NeoVax

On the melanoma trial, testing has focused on the induction of neoantigen-specific responses on the 6 patients enrolled in that study. Per protocol, PBMCs for the melanoma study were collected by leukapheresis (pre-vaccination and at week 16 [4 weeks after 1st boost]), and by monthly venipuncture for the duration of the study. Across all 6 melanoma subjects, we have uniformly identified strong, polyfunctional CD4+ and CD8+ T cell responses against multiple neopeptides.

As shown in 2 representative examples (Panel A, Figure below), we have observed high ex vivo responses in PBMCs across multiple peptide pools of overlapping 15-16mers corresponding to the pools of immunizing SLPs. By deconvolution of the peptide pools, we have identified CD4+ and CD8+ T cell responses against 18% (19 of 103) of the immunizing peptides and 19% (16 of 83) predicted epitopes, respectively. The vast majority of T cell lines were specific for the mutated but not corresponding wildtype peptide (Panel B, Figure below).



Detection of immune responses to neopeptides following NeoVax for melanoma patients.

A. *Ex vivo* analysis of PBMCs against pools of peptides (A, B, C, D) encompassing the SLPs formulating the personal vaccines across the duration of vaccine delivery. **B.** Deconvolution against peptide pool responses reveals reactivity against specific mutated but not corresponding wildtype epitopes. **C.** For some neoantigen-specific T cells, reactivity is directed against autologous tumor but not irrelevant tumor.

2.4.2 Physical identification of melanoma neoepitopes confirms endogenous processing and presentation of at least some predicted neoepitopes by tumors

To evaluate if the peptide-reactive T cells above could detect predicted neopeptides as processed and presented antigens, we tested:

- i) if T cells were reactive against expressed minigenes encompassing the mutation. This was confirmed for 15 of 19 CD4 peptides; and for 12 of 12 CD8 peptides;
- ii) if responses could be detected against autologous tumor, i.e. melanoma cell lines generated from these 6 patients. Indeed, we detected recognition of autologous tumor by multiple neoepitope-reactive CD4+ and CD8+ T cells against 2 of 6 tumors, both of which had detectable MHC class I and II molecules; MHC expression was depressed in the other 4 tumors) (Panel C, Figure above).

2.4.3 Sample collection for correlative studies

Tumor and peripheral blood collection includes procurement of:

(i) Peripheral blood mononuclear cells (PBMCs): We will obtain leukapheresis samples from study subjects prior to initiation of vaccination (L1), prior to initiation of Nivolumab (L2; 1-2 weeks after 1st booster vaccine) and 8 weeks after start of Nivolumab (L3; 2 weeks after second vaccine boost). PBMCs will also be obtained by venipuncture at the time the patient signs the screening consent, at the time the patient signs the main consent, and on weeks 1, 3, 12, 20, 28, and every 4 weeks thereafter until disease progression.

(ii) Tumor samples. An initial baseline surgical or core needle biopsy (specimen B1) will be obtained from all study participants from both cohorts. Furthermore, tumor will be obtained from Cohort A patients who undergo interval debulking surgery (specimen S1) after 3-4 cycles of chemotherapy as per standard-of-care (~75% of patients in Cohort A expected to undergo surgery). All patients in both cohorts will also undergo biopsies B2 and B3 at times coincident to leukapheresis L1 and L2 (before and after starting Nivolumab/on vaccine), provided that biopsiable disease is present.

2.4.4 Optional biopsy at the time of progression for evaluation of mechanisms of resistance and generation of a new vaccine

At the time of disease progression or recurrence, patients in both cohorts who have experienced a clinical benefit i.e. those with complete response (CR) or partial response (PR) or stable disease (SD) for ≥ 6 months by Response Criteria in Solid Tumors (RECIST) or by immune-related RECIST (irRECIST) or no recurrence for ≥ 12 months will be given the option to undergo a biopsy (biopsy B4) of their recurrent disease. Specimen B4 will undergo WES and RNA sequencing and histopathologic and IHC evaluation to explore mechanisms of resistance and may also be used to generate another personalized neoantigen vaccine as previously described.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

To be eligible to participate in the study participants must meet the eligibility criteria during screening examination after having consented to the main study consent:

3.1.1 Diagnosis of epithelial ovarian cancer, primary peritoneal or fallopian tube cancer. High grade serous, high grade endometrioid, clear cell and carcinosarcoma (carcinosarcomas only with high grade serous epithelial component) histologies are allowed. Low grade histologies and mucinous histology are not allowed.

3.1.2 Participants must be classified into one of two cohorts:

Cohort A: Patients with newly diagnosed stage IIIC or stage IV epithelial ovarian, fallopian tube, or primary peritoneal cancer who are planned to undergo neoadjuvant chemotherapy. Patients must be candidates for platinum-based chemotherapy and previously untreated. At the time of pre-screening consent, patients must have disease that is amenable to biopsy and are agreeable and can safely undergo biopsy OR have already undergone biopsy with tumor specimen that can be used for vaccine preparation.

Cohort B: Patients with recurrent (first recurrence only) epithelial ovarian, primary peritoneal or fallopian tube cancer with platinum sensitive disease defined as disease progression greater or equal than 24 months after completion of their last dose of first line platinum chemotherapy. Prior hormonal therapy is allowed but no other therapy for recurrence is allowed, including no chemotherapy, no targeted therapy, and no antiangiogenic therapy. Maintenance therapy after first line chemotherapy is allowed. At the time of pre-screening consent, patients must have measurable disease by RECIST 1.1 and disease that is amenable to biopsy and are agreeable and can safely undergo biopsy OR have already undergone biopsy with tumor specimen that can be used for vaccine preparation.

3.1.3 Eastern Cooperative Group (ECOG) performance status ≤ 2 (please see Appendix A)

3.1.4 Age ≥ 18 years

3.1.5 Patients must have adequate organ and bone marrow function:

- Haemoglobin ≥ 8.0 g/dL
- Absolute neutrophil count (ANC) $\geq 1.5 \times 10^9/L$
- White blood cells (WBC) $> 2 \times 10^9/L$
- Platelet count $\geq 100 \times 10^9/L$
- Total bilirubin $\leq 1.5 \times$ institutional upper limit of normal
- AST (SGOT)/ALT (SGPT) $\leq 2.5 \times$ institutional upper limit of normal unless liver metastases are present in which case it must be $\leq 5 \times$ ULN
- Serum creatinine $\leq 1.5 \times$ institutional upper limit of normal (ULN)

- 3.1.6 Negative serum β -HCG or urine pregnancy test
- 3.1.7 Ability to understand and the willingness to sign a written informed consent document.
- 3.1.8 Willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures
- 3.1.9 Women of childbearing potential (WOCBP) must agree to follow instructions for method(s) of contraception for the duration of study treatment with Nivolumab and 5 months after the last dose of study treatment {i.e., 30 days (duration of ovulatory cycle) plus the time required for the investigational drug to undergo approximately five half-lives}.
Patients of non-childbearing potential are defined as those fulfilling at least one of the following criteria:
 - Have undergone a documented hysterectomy and/or bilateral oophorectomy;
 - Have medically confirmed ovarian failure; or
 - Achieved postmenopausal status, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; status may be confirmed by having a serum follicle-stimulating hormone (FSH) level confirming the post-menopausal state.

3.2 Exclusion Criteria

Patients with **any** of the following characteristics/conditions will not be included in the study:

- 3.2.1 Non-epithelial tumors or ovarian tumors with low malignant potential (i.e. borderline tumors)
- 3.2.2 Mucinous or low grade histologies (i.e. low grade serous or low grade endometrioid)
- 3.2.3 Cohort A and B patients who have signed prescreening consent form and then, in the opinion of the investigator, have progressed after 3 or 4 cycles of platinum-based chemotherapy, are ineligible for the treatment part of the study and no vaccine will be manufactured
- 3.2.4 Prior immunotherapy with IL-2, IFN- α , or anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-cytotoxic T lymphocyte associated antigen 4 (anti-CTLA-4) antibody (including Ipilimumab), or any other antibody or drug specifically targeting T cell co-stimulation or immune checkpoint pathways
- 3.2.5 Major surgery (other than debulking surgery for ovarian, primary peritoneal or fallopian tube cancer) for any reason within 4 weeks prior to initiation of vaccination and/or incomplete recovery from surgery

- 3.2.6 Known brain metastases or leptomeningeal metastases because of their poor prognosis and because patients often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. A scan to confirm the absence of brain metastases is not required.
- 3.2.7 Active or history of autoimmune disease (known or suspected). Exceptions are permitted for vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition requiring only hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger.
- 3.2.8 Have a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days prior to the first dose of study drug (Nivolumab). Inhaled or topical steroids and adrenal replacement doses (≤ 10 mg daily prednisone equivalents) are permitted in the absence of active autoimmune disease.
- 3.2.9 Known human immunodeficiency virus (HIV) infection, active chronic hepatitis B or C, life-threatening illnesses unrelated to cancer, or any serious medical or psychiatric illness that could, in the investigator's opinion, interfere with participation in this study.
- 3.2.10 Known allergy to tetanus toxoid
- 3.2.11 Have an uncontrolled intercurrent illness including, but not limited to, ongoing or active infection requiring treatment, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia.
- 3.2.12 Have any underlying medical condition, psychiatric condition, or social situation that, in the opinion of the investigator, would compromise study administration as per protocol or compromise the assessment of aEs.
- 3.2.13 Pregnant women are excluded from this study because Nivolumab, personalized neoantigen peptides, and Poly-ICLC are agents with unknown risks to the developing fetus.
- 3.2.14 Nursing women are excluded from this study because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with Nivolumab, personalized neoantigen peptides, and Poly-ICLC.
- 3.2.15 Have a history of an invasive malignancy, except for the following circumstance: individuals with a history of invasive malignancy are eligible if they have been disease-free for at least 2 years or are deemed by the investigator to be at low risk for recurrence of that malignancy; individuals with the following cancers are eligible if diagnosed and treated carcinoma in situ of the breast, oral cavity or cervix, localized prostate cancer, basal cell or squamous cell carcinoma of the skin.

3.2.16 Participants who are receiving any other investigational agents.

3.3 Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this trial. These diseases do not affect men.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

Registration in Oncore (REG arm) will occur once the patient signs the consent to participate in screening procedures. No eligibility assessment is required at this timepoint.

Once the eligibility criteria is confirmed by the investigator and a member of the study team completes the appropriate protocol-specific eligibility checklist, patients will be registered to either COH A or COH B arms. Eligibility criteria will be assessed within 14 days prior to start treatment with NeoVax.

Following COH A/COH B registration, participants may begin protocol therapy with the NeoVax vaccine after they have completed their standard of care treatment (chemotherapy ± surgery).

Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5. TREATMENT PLAN

Patients in both cohort A and cohort B will initially undergo and complete SOC therapy before initiation of NeoVax and Nivolumab. If clinically indicated patients may start treatment with PARP inhibitors (either Olaparib or Niraparib), starting after they have received the 5 priming doses of NeoVax.

The study is designed to have a 2-stage consent process. Following the signing of initial pre-screening informed consent, patients from both cohorts will undergo required surgical or core needle biopsy (biopsy B1) (with peripheral blood DNA as matched germline source of DNA) and HLA typing of peripheral blood. Their tumor will undergo DNA and RNA sequencing and neoantigen prediction to determine whether a neoantigen vaccine can be generated. Alternatively, archival tissue may be used if the sample is confirmed adequate for vaccine production purposes. In the meantime, patients will receive the first 3-4 cycles of platinum-based chemotherapy followed by assessment of response to chemotherapy and consideration of interval cytoreductive surgery (for cohort A patients) [1, 2]. If generation of NeoVax is not feasible or if patients progress through platinum chemotherapy, then they will not be considered for vaccination and no vaccine will be manufactured. Conversely, if NeoVax can be generated and patients have response or stable disease after 3-4 cycles of platinum-based chemotherapy, then they will be offered to sign the treatment consent followed by initiation of manufacture of the personalized neoantigen peptides. If cohort A patients undergo interval debulking surgery, treatment consent will be offered after surgery is performed.

While the vaccine is manufactured, cohort A and B patients will complete their standard-of-care therapy, i.e. 3-4 more cycles of chemotherapy. Development of NeoVax takes approximately 8-10 weeks and hence will be available by the time patients complete their standard of care therapy.

5.1 Preparation of NeoVax

5.1.1 Tumor and normal tissue procurement and sequencing

Once tumor tissue has been harvested it will be transferred to the BWH Specialized Histopathology Lab (SHL), where it will be fixed in formalin and portions of the fixed tissue will be prepared for whole-exome and transcriptome sequencing in order to identify vaccine targets. Peripheral blood mononuclear cells from a blood draw will be utilized for a source for normal host germline tissue DNA.

Nucleic acid will be extracted from the tissue samples and sequencing will be conducted at either the CLIA-certified laboratory at Broad Institute/Clinical Research Sequencing Platform (CRSP) or Personalis, Inc.

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

Sequencing 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 nucleotide 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, MSIIntinsic, 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 participant²⁰. Additionally, depending on tumor RNA availability, RNA expression (non-CLIA) may be used in neoantigen prediction pipeline as well. In that case, an algorithm incorporating RNA expression data (such as MSIIntrinsicEC) 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 UPLC. Each individual peptide will be analyzed by a variety of qualified assays to assess appearance (visual), purity (UPLC), identity (by mass spectrometry), quantity (elemental nitrogen), residual solvents (by gas chromatography) and trifluoro-acetate counterion and released. This work will be performed by ALMAC Sciences Scotland Ltd. 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.

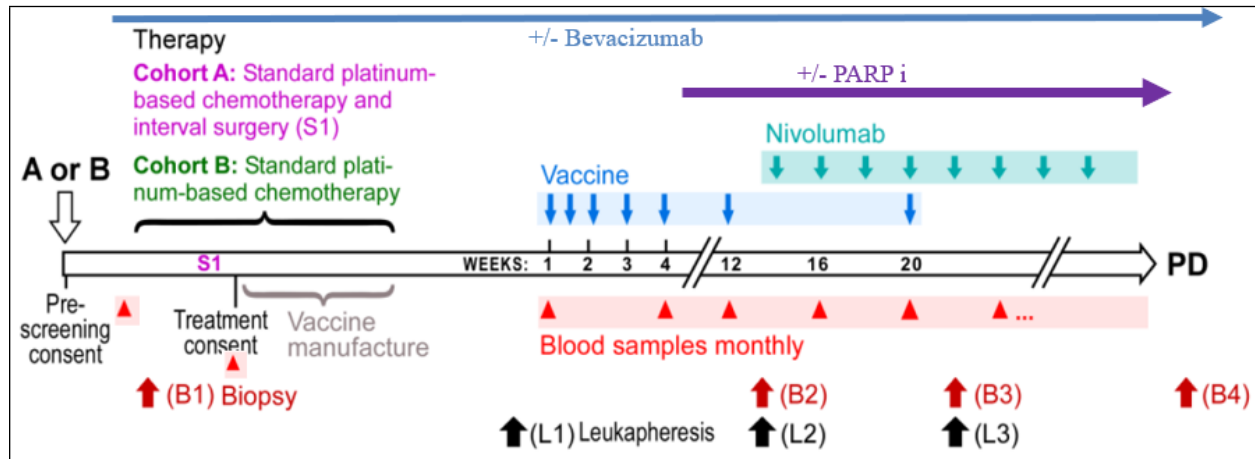
The peptide pools will be prepared by ALMAC Sciences Scotland Ltd. The amounts of each peptide are predicated on a final dose of 300 µg of each peptide per injection. The peptide pools will be prepared by dissolving appropriate quantities of each peptide individually at high concentration (approximately 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. An HLA-DR restricted helper peptide may be added to each peptide pool as a nonspecific helper epitope, depending on several factors, including solubility and quality of synthesizable neoantigen peptides. This peptide has been shown to enhance activation of CD4+ T cells⁷⁸. The un-modified peptide QYIKANSKFIGITEL representing amino acid residues 830–844, has been shown to bind class II MHC molecules expressed on a large proportion of Caucasians⁷⁹⁻⁸¹, although the exact tetanus peptide structure may vary slightly for solubility and stability purposes. For example, the NH2-terminal glutamine (Q) residue is highly susceptible to conversion to pyroglutamate. To stabilize the structure, the peptide is modified by addition of an NH2-terminal alanine residue.

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 DFCI/Research Pharmacy facility where it will be filter sterilized in a laminar flow biosafety cabinet and aliquoted into 2 ml Nunc cryo individual dosing vials for storage. Each pool will utilize a different color-coded cap. Each pool will be tested by UPLC for identity, sterility and endotoxin.

Individual dosing vials will be transported to the DFCI Pharmacy and stored frozen between -70 to -80°C.

5.2 Treatment Regimen

The sequence and timing of administration of standard therapy, NeoVax and Nivolumab are illustrated in the Figure below.



Specifically, 3-8 weeks after completion of chemotherapy for all patients who signed treatment consent in both cohort A and cohort B, NeoVax will be administered using the following schedule:

For the priming phase of vaccination, a total of 5 immunizations will be administered over a 3-week period: Days 1 and 4 (+/- 1 day) of Week 1, Week 2 (Day 8 +/- 1 day), Week 3 (Day 15 +/- 3 days) and Week 4 (Day 22 +/- 3 days).

NeoVax may be administered within +/- 1 day of the scheduled administration date for day 4 and 8, within +/- 3 days of the scheduled administration date for days 15 and 22 (at least 5 days must elapse between day 15 and day 22).

The first day of NeoVax administration will be termed Day 1 (Week 1) of protocol treatment.

Two booster vaccinations will be given at Week 12 (Day 78 +/- 7 days) and Week 20 (Day 134 +/- 7 days), respectively.

Use of PARPi will be permitted after the priming phase of vaccination is completed. Specifically, subjects will be allowed to start PARPi maintenance as per the FDA approved indication and as per the investigator's discretion at any time starting at or anytime after Day 29 of protocol treatment, i.e. after the 5 immunizations of the priming phase of the vaccination have been administered. Treatment with PARPi olaparib and niraparib will occur as per standard of care (please refer to olaparib and niraparib package insert).

Use of Bevacizumab will be allowed at investigator's discretion in Cohort B; bevacizumab will be allowed to be administered during chemotherapy, or after chemotherapy (as maintenance) or throughout (i.e. both during chemotherapy and after chemotherapy) as per the investigator's

discretion. Treatment with bevacizumab will occur as per standard of care (please refer to bevacizumab package insert).

On week 14 (Day 92, +/- 3 days), i.e. 2 weeks after the first booster dose of NeoVax, patients will begin treatment with Nivolumab at a dose of 240 mg administered by intravenous (IV) infusion over 30 minutes every 2 weeks. Patients will continue Nivolumab every 2 weeks +/- 3 days for up to 24 months or until disease progression, initiation of alternate antineoplastic therapy, or death. At the investigator's discretion, on treatment week 24 Nivolumab administration schedule may be switched to every 4 weeks. No dose reductions or escalations will be allowed for NeoVax or Nivolumab.

5.3 Pre-Treatment Criteria

As stated above, vaccination occurs on days 1, 4, 8, 15 and 22 (priming doses) and on Days 78 and 134.

If clinically indicated, patients in either Cohort A or Cohort B may start maintenance with PARP inhibitors (Olaparib or Niraparib) at or anytime after Day 29 (after having received the 5 vaccine doses corresponding to the priming phase).

Olaparib /Niraparib may be initiated at or anytime after Week 5 Day 29 if indicated as per package insert.

Use of Bevacizumab will be allowed at investigator's discretion in Cohort B; bevacizumab will be allowed to be administered during chemotherapy, or after chemotherapy (as maintenance) or throughout (i.e. both during chemotherapy and after chemotherapy) as per the investigator's discretion. Treatment with bevacizumab will occur as per standard of care (please refer to bevacizumab package insert).

Nivolumab will be initiated on day 92 and repeated every 2 weeks. The day of every vaccination and every Nivolumab dose, the following criteria should be fulfilled for vaccination and/or Nivolumab to occur:

- Absolute neutrophil count $\geq 1,000/\text{mcL}$
- Platelets $\geq 100,000/\text{mcL}$
- Total bilirubin below or equal to the upper institutional limit of normal
- AST (SGOT)/ALT (SGPT) $< 2\times$ institutional upper limit of normal (if a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, drug-related Grade < 3 toxicity)
- Creatinine $\leq 1.5 \times$ institutional ULN
- Amylase or lipase $< \text{Grade } 2$
- All toxicities of previous therapy must have resolved to $\leq \text{grade } 2$ unless otherwise specified
- ECOG performance status of 2 or less
- No evidence of life-threatening medical problems
- Non-skin, drug-related AE grade < 2 (Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay)

- Skin, drug-related AE < Grade 3
- Resolution of any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

5.4 Agent Administration

Treatment Description					
Agent	Treatment phase	Schedule	Allowable Treatment Admin Window	Dose	Route
NeoVax (peptides + poly-ICLC)	Prime	Day 1	N/A	Poly-ICLC: 8 x 0.125 mL	i.d. / s.c. injections into up to 4 different anatomic sites
		Days 4 and 8	±1 day	Peptides: (up to 4 peptide pools) x (up to 5 peptides per pool) x (300µg per peptide/0.75 ml)	
		Days 15 and 22	±3 days (at least 5 days must elapse between doses)		
	Boost	Days 78 and 134	±7 days	Total NeoVax Volume: up to 8 x 0.5 ml (+/- 0.1 mL)	
Olaparib OR Niraparib	N/A	If clinically indicated, stating at or anytime after Day 29 and continued until disease progression or unacceptable toxicity	±3 days	As per package insert	p.o.
Nivolumab	N/A	Day 92 and then every 2 weeks for up to 24 months or until disease progression; Nivolumab administration schedule may be switched to every 4 weeks starting on Day 162	±3 days	240 mg every 2 weeks OR 480 mg every 4 weeks (starting on Day 162)	IV over 30 minutes (+/- 10 min)
Bevacizumab	N/A	May be administered during chemotherapy, or after chemotherapy (as maintenance) or throughout (i.e. both during chemotherapy and after chemotherapy)	N/A	As per package insert	IV

5.4.1 NeoVax administration

Each of the up to 4 NeoVax pools (2 syringes per pool) will be assigned to one of four extremities. At each immunization timepoint, 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 (+/- 0.1 mL) 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, 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.).

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.

Patients should be observed in the clinic for at least 60 minutes after the last NeoVax injection and vital signs should be checked at 30 minutes (+/- 10 minutes), and 60 minutes (+/- 10 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.

Information regarding the number and location of the injections, the date of vaccination, route of administration, total volume of vaccine administered, and vaccine site injection reactions will be collected for each vaccination and recorded in the patient medical record.

If the dosing of PARP inhibitor is due at the same day as the study drugs, then subjects should take it prior to the study visit, i.e. before administration of the study medications.

5.4.2 Olaparib / Niraparib administration

Starting at or anytime after Day 29 Olaparib or Niraparib will be administered orally to those patients whom, based on investigator's criteria could benefit from it.

If the dosing of PARP inhibitor is due at the same day as the study drugs, then subjects should take it prior to the study visit, i.e. before administration of the study medications.

Olaparib and Niraparib are administered as per package insert.

5.4.3 Nivolumab Administration

Nivolumab is to be administered as a 30-minute (+/-10 min) IV infusion. For details on prepared drug storage, preparation, and administration, please refer to the Nivolumab package insert. If Nivolumab is given the same day as NeoVax, Nivolumab infusion should start within 10 minutes after NeoVax observation period.

If the dosing of PARP inhibitor is due at the same day as the study drugs, then subjects should take it prior to the study visit, i.e. before administration of the study medications.

Nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding polyethersulfone membrane in-line filter at 240 mg or 480mg. It is not to be administered as an IV push or bolus injection. Nivolumab injection will be infused diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 0.35 mg/mL. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

5.4.4 Bevacizumab Administration

For details on prepared drug storage, preparation, and administration, please refer to the Bevacizumab package insert.

If Bevacizumab is given the same day as NeoVax, Bevacizumab infusion should start within 10 minutes after NeoVax observation period.

If Bevacizumab is given the same day as Nivolumab, Bevacizumab infusion should start 30 minutes after conclusion of Nivolumab infusion.

If Bevacizumab is given the same day as Nivolumab and NeoVax, then Nivolumab infusion should start within 10 minutes after NeoVax observation period. Bevacizumab infusion should start 30 minutes after conclusion of Nivolumab infusion.

5.5 **General Concomitant Medication and Supportive Care Guidelines**

5.5.1 Prohibited Concomitant Therapy

The following therapies are not permitted during the study:

- Immunotherapeutic agents including, but not limited to, Ipilimumab, and Pembrolizumab.
- Non-oncology vaccine therapy. Annual flu shots are not allowed for 4 weeks prior to day 1, until 8 weeks after last vaccine 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 SARS-CoV-2 virus for the prevention of COVID-19 is ALLOWED in this study.
- Systemic corticosteroids (≥ 10 mg daily prednisone equivalents) or other immunosuppressive medications with the exception of steroids for the treatment of Nivolumab-related immune adverse events.
- Any concurrent antineoplastic therapy (i.e., chemotherapy, hormonal therapy, immunotherapy, radiation therapy except for palliative radiation therapy, or standard or investigational agents for treatment of cancer) or natural herbal products or other “folk remedies”. Supportive care for disease-related symptoms may be offered to all subjects on the trial.

5.5.2 Allowed Concomitant Therapy

Medications and treatments other than those specified above are permitted during the study. All intercurrent medical conditions and complications of the underlying malignancy will be treated at the discretion of the Investigator according to acceptable local standards of medical care. Patients should receive analgesics, antiemetics, anti-infectives, antipyretics, and blood products as necessary. All concomitant medications, including transfusions of blood products, will be

recorded in the eCRF from Day 1 (first dose of study treatment) through 100 days after the last dose of Nivolumab.

Patients should inform the Investigator of any new medication or significant non-drug therapies (i.e. blood transfusions) including vitamins, supplements, etc. administered after the start of study treatment.

Subjects are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). A brief course of corticosteroids for prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g. delayed-type hypersensitivity reaction caused by contact allergen) is permitted.

5.6 Criteria for Taking a Participant Off Protocol Therapy

As discussed above, cohort A and B patients who have signed prescreening consent form and then, in the opinion of the investigator, have progressed after 3 or 4 cycles of platinum-based chemotherapy, are ineligible for the treatment part of the study and no vaccine will be manufactured.

For patients who sign treatment consent form and initiate protocol therapy, duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment will proceed and may continue for up to 24 months or until one of the following criteria applies:

- Disease progression (please refer to section 5.10 regarding option of revaccination). If Week 12 scans result in PD with confirmatory scan at Week 16 showing PD, subjects must discontinue all protocol treatment (vaccine and nivolumab) despite only receiving the first dose of nivolumab (on Week 14). The timing of nivolumab has been positioned so as to be given after the first booster dose of the vaccine.
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with scheduled vaccinations and/or Nivolumab infusions
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment 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.

When a participant is removed from protocol therapy and/or is off of the study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

5.7 Duration of Follow Up

Participants will be followed for 5 years after removal from protocol therapy or until death, whichever occurs first.

Active Follow-Up: Participants removed from active study treatment will enter active follow-up and will be followed every 3 months (+/- 2 weeks) until initiation of a new therapy for progressive disease, withdrawal of consent, or death (whichever comes first). Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. All participants will be followed until resolution or stabilization of any serious adverse events occurring during treatment or starting within 100 days of last study drug.

Long-Term Follow-Up: Participants removed from active follow-up for progression/initiation of a new therapy will enter long-term follow-up, where they will be followed via medical record review until death for survival. All patients will be followed for survival. Updates to the CRFs are to be made roughly every six months.

5.8 Criteria for Taking a Participant Off Study

As discussed above, cohort A and B patients who have signed prescreening consent form and then, in the opinion of the investigator, have progressed after 3 or 4 cycles of platinum-based chemotherapy, are ineligible for the treatment part of the study and no vaccine will be manufactured.

Participants will also be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

An ODQ Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the DF/HCC website at:

<http://www.dfhcc.harvard.edu/research/clinical-researchsupport/document-library-forms-sops-etc/>

For Decentralized Subject Registrations, the research team updates the relevant Off Treatment/Off Study information in OnCore.

5.9 Treatment after progression

Accumulating evidence indicates that a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of progressive disease (PD). While subjects are treated with NeoVax and/or Nivolumab, they will be permitted to continue treatment beyond

initial PD as assessed by the investigator's clinical judgment and local standards of care as long as they meet the following criteria:

- Investigator-assessed clinical benefit and do not have rapid disease progression
- Tolerance of study drug
- Stable performance status
- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (e.g., CNS metastases)

The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment. All decisions to continue treatment beyond initial progression must be discussed with the overall principal investigator as well as with the IND15703 holder. Subjects will be re-consented with an ICF describing any reasonably foreseeable risks or discomforts. Decisions to continue treatment beyond initial progression will be documented in the study records. A radiographic assessment/scan should be performed within 6 weeks of initial investigator assessed progression to determine whether there has been a decrease in the tumor size or continued PD. The assessment of clinical benefit should be balanced by clinical judgment as to whether the subject is clinically deteriorating and unlikely to receive any benefit from continued treatment with NeoVax and Nivolumab.

For the subjects who continue on study therapy beyond progression, further progression is defined as an additional 10% increase in tumor burden from time of initial PD. This includes an increase in the sum of diameters of all target lesions and/ or the diameters of new measurable lesions compared to the time of initial PD. Upon documentation of further progression, please refer to section 5.9.

New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden if the longest diameter increases to at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). In situations where the relative increase in total tumor burden by 10% is solely due to inclusion of new lesions which become measurable, these new lesions must demonstrate an absolute increase of at least 5 mm.

5.10 Option for revaccination after progression

At the time of disease progression, patients in both cohorts who have experienced a clinical benefit may be given the option of undergoing another biopsy for generation of another vaccine NeoVax followed by revaccination with NeoVax followed by Nivolumab using the same schedule as previously administered.

Clinical benefit is defined as:

- i) For patients with measurable disease at the time of initiation of protocol therapy, clinical benefit is defined as complete response (CR) or partial response (PR) or stable disease (SD) for $\geq$

6 months by Response Criteria in Solid Tumors (RECIST) or by immune-related RECIST (irRECIST)

ii) For patients without measurable disease at the time of initiation of protocol therapy, clinical benefit is defined as no recurrence for ≥ 12 months.

Patients who have experienced clinical benefit (as defined above) and develop progression, either at the time of initial progression or at the time of confirmed progression (see section 5.8 above), may be given the option of another biopsy and generation of another NeoVax.

These patients will continue treatment with Nivolumab until the new vaccine is being developed as long as they meet the following criteria:

- Investigator-assessed clinical benefit and do not have rapid disease progression
- Tolerance of study drug
- Stable performance status
- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (e.g., CNS metastases)

When the vaccine is developed, it will be administered to the patients using the exact same schedule as described in Section 5.1; the first dose of NeoVax will be given so that it coincides with a dose of Nivolumab which will continue to be administered every 2 weeks. Follow up of these patients will continue as previously and as described in section 10. Patients that go on to retreatment with the new NeoVax vaccine must meet the eligibility criteria listed on sections 3.1 and 3.2 of the protocol.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 NeoVax and Nivolumab Dose Modifications

Nivolumab dose reductions or dose escalations are not permitted. If at any point, treatment with Nivolumab is held (see section 6.3 for criteria requiring dose delay), treatment with NeoVax must also be held. When blood counts have recovered, or toxicities have resolved as described in section 6.3 and 6.5, Nivolumab will be resumed at full dose.

6.2 Treatment Modification for NeoVax Related Adverse Events

Treatment with NeoVax should be held for any of the following:

- Grade 3 or higher injection site reaction
- Grade 3 or higher myalgias not adequately managed by analgesia
- Grade 3 or higher fevers not adequately managed by antipyretic treatment

Resume NeoVax in patients when aEs resolve to Grade 1 or less. If grade 2 or greater symptoms last more than 7 days during priming vaccination, contact Overall PI and continue to hold dose. As referenced in section 6.1, if Nivolumab is held, vaccination will be held until Nivolumab is resumed.

6.3 Treatment Modification for Olaparib-Related Adverse Events

For dose modifications please refer to the package insert.

6.4 Treatment Modification for Niraparib-Related Adverse Events

For dose modifications please refer to the package insert.

6.5 Treatment Modification for Bevacizumab-related Adverse Events

Please refer to Bevacizumab package insert.

6.6 Nivolumab

6.6.1.1 Dose Modification and Guidelines for Management of Nivolumab-related Adverse Events

Management algorithms (listed in Appendix B) have been developed to assist investigators in assessing and managing the following groups of aEs:

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

6.6.1.2 Treatment of Nivolumab Related Infusion Reactions

Since Nivolumab 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 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (CTEP Active Version (version 5.0) 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; infusion interruption not indicated; intervention not indicated) Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional Nivolumab administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion 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 the Nivolumab infusion, 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. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further Nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional Nivolumab 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 and/or brief interruption of infusion]; 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).

Immediately discontinue infusion of Nivolumab. 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. Nivolumab 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).

7. 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. In this trial, AE monitoring and reporting will start from Day 1 when NeoVax is administered; AE monitoring and reporting during standard of care therapy (i.e. chemotherapy $\pm$ surgery) will not be performed.

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

7.1 Expected Toxicities

7.1.1 Adverse Event List

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 for poly-ICLC

Poly-ICLC is an investigational agent; however there has been significant experience in previous clinical trials as discussed in 2.2.1. 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 not expected, but may occur:

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

Hypersensitivity reactions represent allergic reactions. Hypersensitivity reactions typically occur in an immediate fashion (i.e. within the same day as the vaccination) although they may be delayed with onset of symptoms from one day to weeks after vaccination. Symptoms typically include mild to moderate pruritus, erythema, flushing, rash, perioral numbness, lightheadedness, shortness of breath, myalgia and increased pulse, blood pressure and temperature. Precautions should be taken to prepare for the possibility of hypersensitivity reactions after NeoVax plus Montanide administration. Patients should be observed for a minimum of one hour following vaccination in order to evaluate and treat any immediate hypersensitivity reactions. The use of all routine supportive medication is permitted for hypersensitivity reactions, although routine use of prophylactic antihistamines, non-steroidal anti-inflammatory agents and concomitant corticosteroids are to be avoided if at all possible. If used, doses of corticosteroids should be the minimum necessary for appropriate clinical management. If any new administration or increased dose of systemic corticosteroids is necessary, every effort should be taken to taper, and preferably discontinue treatment with them as quickly as clinically feasible.

Autoimmune diseases: 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. Thus, symptoms that mimic autoimmune diseases or other autoimmune reactions are a theoretical possibility for which participants will be monitored.

7.1.1.3 Adverse Events for Olaparib

The most common side effects of Lynparza are:

- Nausea or vomiting. Tell your healthcare provider if you get nausea or vomiting. Your healthcare provider may prescribe medicines to treat these symptoms.
- low number of red or white blood cells
- Tiredness or weakness
- Sore throat or runny nose
- Diarrhea
- Joint, muscle, and back pain
- Headache
- Constipation
- Changes in the way food tastes
- Loss of appetite
- Mouth sores
- Respiratory tract infections
- Changes in kidney function blood test
- Low number of platelets
- Indigestion or heartburn
- Myelodysplastic Syndrome
- Acute Myeloid Leukemia
- Pneumonitis

7.1.1.4 Adverse Events for Niraparib

- Blood and lymphatic system disorders
- Thrombocytopenia
- Anemia
- Neutropenia
- Leukopenia
- Cardiac Disorders
- Palpitations
- Gastrointestinal Disorders
- Nausea
- Constipation
- Vomiting
- Abdominal pain/distention
- Mucositis/stomatitis
- Diarrhea

- Dyspepsia
- Dry mouth
- General disorders
- Fatigue/Asthenia
- Metabolism and Nutrition Disorders
- Decreased appetite
- Infections and Infestations
- Urinary tract infection
- Investigations
- AST/ ALT elevation
- Musculoskeletal and Connective Tissue Disorders
- Myalgia
- Back pain
- Arthralgia
- Nervous system Disorders
- Headache
- Dizziness
- Dysgeusia
- Psychiatric Disorders
- Insomnia
- Anxiety
- Respiratory, Thoracic, and Mediastinal Disorders
- Nasopharyngitis
- Dyspnea
- Cough
- Skin and Subcutaneous Tissue Disorders
- Rash
- Vascular Disorders
- Hypertension
- Abnormal Laboratory Findings
- Decrease in hemoglobin
- Decrease in platelet count
- Decrease in WBC count
- Decrease in absolute neutrophil count

7.1.1.5 Adverse Event list for Bevacizumab

Please refer to Bevacizumab package insert.

7.1.1.6 Adverse Event List for Nivolumab

Categorization of aEs Potentially Associated with Nivolumab

In order to characterize aEs of special clinical interest that are potentially associated with the use of Nivolumab, the Sponsor identified select aEs based on the following 4 guiding principles:

- aEs that may differ in type, frequency, or severity from aEs caused by non-immunotherapies
- aEs that may require immunosuppression (e.g., corticosteroids) as part of their management
- aEs whose early recognition and management may mitigate severe toxicity
- aEs for which multiple event terms may be used to describe a single type of AE, thereby necessitating the pooling of terms for full characterization

Based on these guiding principles and taking into account the types of aEs already observed across studies of Nivolumab monotherapy, endocrinopathies, diarrhea/colitis, hepatitis, pneumonitis, nephritis, and rash are currently considered to be select aEs. Multiple event terms that may describe each of these are grouped into endocrine, GI, hepatic, pulmonary, renal, and skin select AE categories, respectively. The list of aEs belonging to select AE categories may evolve as more safety information becomes available in the Nivolumab program.

Hypersensitivity/infusion reactions were also analyzed along with the select AE categories because multiple AE terms may be used to describe these events, and therefore, pooling of terms provides a more complete characterization of the events. Hypersensitivity/infusion reactions do not, otherwise, meet criteria to be considered select aEs.

Serious Adverse Events Reported from Clinical Trials with Nivolumab Monotherapy

The following serious aEs have been reported in clinical studies in which Nivolumab was given as monotherapy. The frequency of ADRs is defined using the following convention: very common ($\geq 1/10$); common ($\geq 1/100$ to $< 1/10$); uncommon ($\geq 1/1,000$ to $< 1/100$); rare ($\geq 1/10,000$ to $< 1/1,000$); and very rare ($< 1/10,000$). There are no very common or very rare SAEs associated with Nivolumab monotherapy. For further information, including SAEs that are rarely associated, please reference the IB.

The following **common SAE** is associated: pneumonitis

The following **uncommon SAEs** by system organ class are associated:

- Endocrine: Adrenal insufficiency, Hypothyroidism
- Gastrointestinal: Abdominal pain, Colitis, Diarrhea, Nausea, Pancreatitis, Vomiting
- General and Administrative Site Conditions: Mucosal inflammation, Pyrexia
- Hepatobiliary: Abnormal hepatic function
- Immune system: Hypersensitivity
- Injury, Poisoning and Procedural Complications: Infusion-related reaction
- Metabolism and Nutrition: Dehydration, Hyperglycemia
- Renal and Urinary: Acute renal failure, Tubulointerstitial nephritis
- Respiratory, Thoracic and Mediastinal Disorders: Dyspnea, Hypoxia, Interstitial lung disease, Lung infiltration, Respiratory failure

Non-serious Adverse Events Reported from Clinical Trials with Nivolumab Monotherapy

The following non-serious aEs have been reported in clinical studies in which Nivolumab was given as monotherapy. The frequency of ADRs is defined using the following convention: very common ($\geq 1/10$); common ($\geq 1/100$ to $< 1/10$); uncommon ($\geq 1/1,000$ to $< 1/100$); rare ($\geq 1/10,000$ to $< 1/1,000$); and very rare ($< 1/10,000$).

The following **very common** non-serious aEs are associated:

- Fatigue
- Nausea
- Pruritus

The following **common** non-serious aEs are associated:

- Arthralgia
- Asthenia
- Cough
- Decreased appetite
- Diarrhea
- Pyrexia
- Rash

A complete list of adverse events observed with Nivolumab are detailed in the Investigator's Brochure (IB).

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 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 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 section 7.3 (Serious Adverse Event).
- **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 Adverse Event Reporting

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

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 100 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 Reporting to BMS

SAEs, whether related or not related to study drug, and pregnancies must also be reported to BMS and Oncovir, Inc. All SAEs should be followed to resolution or stabilization.

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

7.4 Reporting to the Food and Drug Administration (FDA)

Patrick Ott MD PhD, as the IND Sponsor, will be responsible for all communications with the FDA. The study sponsor will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

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

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

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

7.5 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy. The Overall PI and the IND Sponsor (Patrick Ott, MD) will be notified with a simultaneous email of any event filed with Risk Management.

7.6 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **aEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the 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 40°F (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.

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 Handling

There are no specific handling instructions for poly-ICLC.

8.1.6 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. Poly-ICLC can be ordered directly from Oncovir, Inc, by submitting a purchase order to:

Oncovir, Inc.
esalazar@oncovir.com
mvandermast@oncovir.com

8.1.7 Preparation

Poly-ICLC is supplied as an opalescent white suspension. It will be directly withdrawn from the vial under sterile conditions and mixed with the personalized neoantigen peptides (see section 5.5.1).

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

8.1.8 Administration

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 (+/- 0.1 mL) 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, will be administered subcutaneously (s.c.) at the same injection site

8.1.9 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)

8.1.10 Destruction and Return

At the end of the study, unused supplies of poly-ICLC will be held by the IND holder for future use until they are out of date at which point they will be destroyed according to

institutional policies. Destruction will be documented in the Drug Accountability Record Form.

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 (NH₂-) 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 be identified with the patient's study ID number (i.e. 18-318). 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. 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 Handling

There are no specific instructions for handling personalized neoantigen peptides.

8.2.6 Availability

The personalized neoantigen peptides is prepared by a GMP peptide provider 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.

8.2.7 Preparation

The final step in the preparation of NeoVax (mixing with poly-ICLC) is conducted on the day of the scheduled vaccine administration (as described in 5.5). 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 injections - 2 syringes from each peptide pool – containing peptides combined with the adjuvant.

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 21 gauge 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 0.5mL (+/- 0.1 mL) 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.

8.2.8 Administration

Following mixing with personalized neoantigen peptides, the vaccine (peptide + poly-ICLC) will be administered intradermally and subcutaneously.

8.2.9 Ordering

The order for personalized neoantigen peptides will be placed by the DFCI Scientific Project Manager or designee separately for each individual patient. Delivery time is anticipated to be 4 – 8 weeks.

8.2.10 Accountability

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

8.2.11 Destruction and Return

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

8.3 Olaparib

Please refer to the Olaparib Insert package.

8.4 Niraparib

Please refer to the Olaparib Insert package.

8.5 Bevacizumab

Please refer to the Bevacizumab Insert package.

8.6 Nivolumab

8.6.1 Description

Nivolumab is a fully human monoclonal immunoglobulin G4 (IgG4-S228P) antibody that targets the programmed death (PD-1) cell surface membrane receptor.

8.6.2 Study Drug Packaging and Labeling

Nivolumab Injection Drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/ml. The solution is a clear to opalescent, colorless to pale liquid and may contain particles. It is supplied in 100mg, 10mg/ml vials stoppered with butyl stoppers and sealed with aluminum seals.

8.6.3 Storage and Stability

Nivolumab must be stored at 2°-8°C (36°-46°F) and protected from light and freezing. IV bags containing undiluted and diluted solutions of Nivolumab prepared for dosing may be stored up to 20 hours in a refrigerator at 2°-8°C (36°-46°F) and used within 8 hours at room temperature and under room light. The maximum 8-hour period under room temperature and room light conditions for undiluted and diluted solutions of Nivolumab injection in the IV bag should be inclusive of the product administration period.

8.6.4 Compatibility

Nivolumab will not be mixed with any other study agents.

8.6.5 Availability

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

8.6.6 Handling and Preparation

Nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding (eg, polyethersulfone membrane) in-line filter at the protocol-specified doses and infusion times. It is not to be administered as an IV push or bolus injection. When the dose is based on patient weight (ie, mg/kg), nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% sodium chloride injection or 5% dextrose injection to protein concentrations as low as 0.35 mg/mL. When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL. For patients weighing less than 40 kg, the total volume of infusion must not exceed 4 mL per kg of patient weight.

During drug product preparation and handling, vigorous mixing or shaking is to be avoided. Instructions for dilution and infusion of nivolumab injection will be provided to the clinical site.

Care must be taken to ensure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent. Nivolumab infusions are compatible with polyvinyl chloride or polyolefin containers and infusion sets, and glass bottles.

8.6.7 Administration

Nivolumab injection is to be administered as an IV infusion, using a volumetric pump with a 0.2 / 0.22 micron pore size, low-protein binding polyethersulfone membrane in-line filter at the protocol-specified doses.

8.6.8 Ordering

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

8.6.9 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.)

8.6.10 Destruction and Return

At the end of the study, unused supplies of Nivolumab will be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

Tumor and peripheral blood collection include procurement of (see Figure below):

i) Peripheral blood mononuclear cells (PBMCs):

We will obtain 3 leukapheresis samples (L1, L2 and L3):

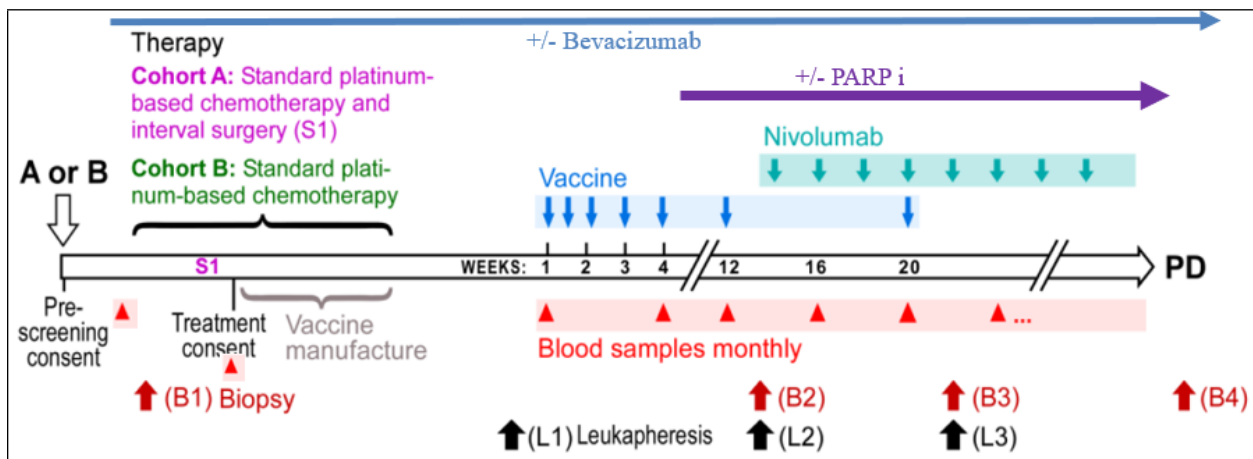
- a. L1: The week prior to Day 1 (i.e. sometime between Day -7 to Day 0)
- b. L2: The week prior to initiation of Nivolumab on Day 92 (i.e. sometime between Day 85 to Day 97)
- c. L3: The week prior to Day 148 (i.e. sometime between Day 140 to Day 147)

ii) PBMCs will also be obtained by venipuncture at the time the screening consent is signed, at the time the main consent is signed, and on weeks 1, 3, 12, 20, 28, and every 4 weeks thereafter until disease progression.

iii) Tumor biopsies. An initial baseline surgical or core needle biopsy (specimen B1) will be obtained from all study participants from both cohorts. Furthermore, tumor will be obtained from Cohort A patients who undergo interval debulking surgery (specimen S1) after 3-4 cycles of chemotherapy as per standard-of-care. All patients in both cohorts will also undergo biopsies B2 and B3 at times coincident to leukaphereses L2 and L3 (see i) above), provided that biopsiable disease is present.

Furthermore, at the time of disease progression or recurrence, patients in both cohorts who have experienced a clinical benefit i.e. those with complete response (CR) or partial response (PR) or stable disease (SD) for ≥ 6 months by Response Criteria in Solid Tumors (RECIST) or by immune-related RECIST (irRECIST) or no recurrence for ≥ 12 months will be given the option to undergo a biopsy (biopsy B4) of their recurrent disease. Specimen B4 will undergo WES and RNA sequencing and histopathologic and IHC evaluation to explore mechanisms of resistance and may also be used to generate another personalized neoantigen vaccine as previously described.

iv) The research team may use samples from 18-318 participants collected under DFCI banking protocols 02-051, 13-064, and/or MGB banking protocol 2019P000017 to perform analysis related to the 18-318 correlative studies.



9.2 Analysis of the evolution of T cell reactivity, state and repertoire following NeoVax/Nivolumab

We will address the following questions:

Q1: Does combination of NeoVax/Nivolumab amplify and broaden neoantigen specificity of T cells compared to NeoVax alone? As per our recently completed proof-of-concept study testing NeoVax alone in advanced melanoma [48], we have established robust experimental workflows to test patient PBMCs for evidence of T cell reactivity against predicted personal tumor neoantigens, through detection of IFN γ (or TNF- α , IL2) secretion via *ex vivo* or *in vitro* pre-stimulation ELISPOT or by intracellular cytokine secretion assays. We will follow an alternative more streamlined strategy which can rapidly characterize the breadth of immune responses while also providing additional information on T cell clonality that we can feasibly gain from small input cellular material (i.e from core biopsies), described below.

A new workflow to characterize and detect T cell responses, based on single cell TCR analysis.

An analysis of the global T cell receptor (TCR) repertoire diversity of a PBMC sample can provide an immunologic “snapshot” of T cell state within that sample. The unique sequence of individual TCRs endows each T cell with a molecular ‘barcode’ by which antigen-reactive T cells can be enumerated and tracked. Thus, we will characterize TCR repertoires of circulating neoantigen-reactive T cells as an approach to delineate the number of distinct neoantigen-reactive T cell clones stimulated by vaccination and/or checkpoint blockade (CPB) per patient. The detection of contracting or expanding numbers of sequencing reads with specific TCR sequences in association with treatment would indicate the dynamics of specific T cell clones. Various commercial approaches for sequencing-based TCR profiling are now available. For example, the Wu lab has worked with Adaptive Biotechnologies to perform deep sequencing and analysis of TCRs to examine repertoire diversity following allogeneic hematopoietic stem cell transplantation for CLL. However, for a more cost-effective approach compatible with limiting input DNA quantities, the Wu lab has recently developed new experimental pipelines for bulk low-input and **single cell (sc)TCR** analysis. A key advantage of scTCR analysis is that **we gain both matched TCR- α and β chain information per cell**, such that TCRs can be reconstructed. A key benefit of constructing lines of single TCR specificity for neoepitopes is that it enhances and standardizes the sensitivity for detecting reactivity against immunizing peptides. We have therefore devised a workflow that leverages information from scTCRs to rapidly capture CD4+ and CD8+ T cell clone information and provides a path **to readily link an antigen-specific immunologic response to specific TCR sequences** (Figure 1A).

Figure 1. A. TCR cloning into a readout cell line. **B.** Patient with enriched TCRs amongst neoantigen peptide pool-reactive T cells, per single cell paired TCR sequencing analysis. **C.** Testing of cloned TCRs against candidate neoepitopes reveals reactivity for 3 of 6 to peptide 17C.

Based on our completed melanoma NeoVax studies, a sensitive approach to detect neoantigen T cell reactivity is to perform T cell cytokine secretion assays after one round of *in vitro* stimulation against the immunizing peptides. The first step still relies on bulk cell testing of post-vaccination PBMC to determine which specific pools generate T cell reactivity. Once stimulating antigen pools are identified, we will then deconvolute the antigen specificities within those pools through: (i) single cell flow cytometric sorting of up to 2 plates of 384 cytokine-secreting CD4 or CD8+ cells into plates (Figure 2A). The isolated cells will be submitted for single cell library generation with the addition of Illumina sequencing-compatible barcodes and adaptors, and then sequenced on a Miseq instrument. (ii) determination of the TCR α and β sequences by alignment of sequenced reads to the International Immunogenetics (IMGT) database. Figure 1B shows an example of this workflow, in which single cell sequencing of neoantigen-peptide pool-reactive T

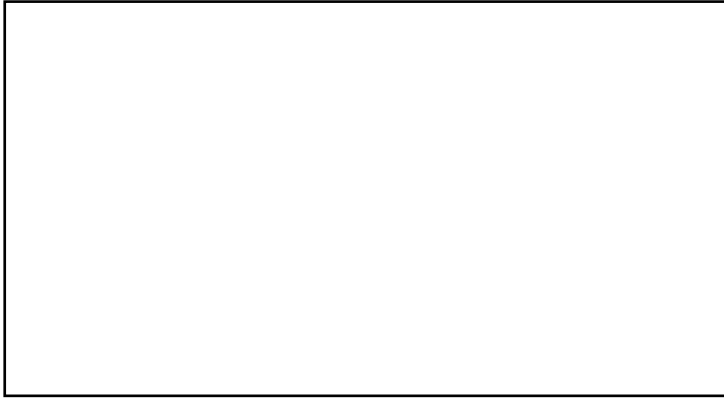


Figure 2. **A.** Workflow for single cell TCR analysis; **B.** Single cell isolation of hematopoietic (CD45+), tumor cells (CD45 neg) and CD3+ T cells from core biopsies, with index sorting by flow cytometry, performed on neovax-candidates with melanoma; **C.** Tetramer isolation of

cells from a patient in our melanoma study revealed many enriched TCRs. (iii) cloning of TCRs on-demand once CDR3 sequence information is available and their subsequent expression in readout cells, from which we can then test the specificity of TCRs. As all TCRs share the same modular structure, our strategy is to express any TCR of interest on a Jurkat $\Delta\alpha\beta$ reporter cell line (lacks TCR expression) through lentiviral transduction following construction by Golden Gate assembly from a

library of plasmids (encoding the various V α and V β regions, the TCR constant region, leader sequence for surface expression) and 2 custom-synthesized 80bp oligonucleotides that encode the desired CDR3 α and β regions. We will select the top 48 enriched TCRs originating from pool-reactive T cells per patient. The assembled TCR-encoding plasmids will be transfected as a pool, and transformants will be selected by PCR-based screening/barcoded sequencing of the transformants to identify correctly assembled TCR plasmids. They will then be expressed in the TCR-less Jurkat cells, for co-culture in 96-well format with patient B cells (expanded under CD40L-stimulation)[49], nucleofected with a minigene encoding the epitopes contained within the pool. Positivity will be detected by IL2 secretion. Positively-reactive TCR-expressing cells will be further expanded and their specificity deconvoluted against APCs pulsed with specific candidate neopeptides. In the example shown in Figure 1C, 3 of 6 unique expressed TCRs all recognize a single predicted neopeptide (17c).

Through this scTCR sequencing-based approach, we will serially analyze peripheral blood-derived T cells to enumerate the number of T cell clones (defined by unique TCRs with >10 reads representing them) present in neoantigen-reactive CD4+ or CD8+ T cells isolated from cryopreserved PBMC collected before and after vaccination (L2 vs L1) and then further changes before and after Nivolumab (L3 vs L2). We will thus identify the numbers of immunizing neoepitopes stimulating responses and of T cell clones matched to individual immunizing peptides and determine if these numbers are expanded following combination with Nivolumab. With TCR sequence as a molecular ‘barcode’, we can also more finely track the kinetics of individual neoantigen-reactive clones over time through the testing of intervening timepoints with clonotype-specific PCR-based detection.

Q2. Can we detect the tumor-specificity and cytotoxicity and avidity of neoantigen-specific T cell lines? Per patient, we expect some TCRs to be more enriched at L2 (after vaccine, before Nivolumab), and others to be enriched at L3 (post-vaccine/on Nivolumab). For up to 5 patients per cohort, we will incubate reactive TCR-defined cell lines (up to the top 10 enriched TCRs from L2 and up to 10 from L3 per patient) with autologous tumor cells that are generated through organoid culture of primary tumor samples from B1. We will assay for IL2 secretion to determine if T cells recognize endogenously processed antigens that are displayed by tumor

cells. To assess *tumor cytotoxicity*, we will express the discovered TCRs in autologous CD8+ T cell lymphoblasts (using standard protocols) and test them in Europium or chromium release cytotoxicity assays. Functional avidity measures the sensitivity of a T cell response to an antigen, and this measure has been shown to correlate well with effector T cell function and protection. Quantifying the functional avidity of the discovered neoantigen-specific TCR allows us to assess the sensitivity of T cell responses elicited by the distinct neopeptides. We will also assess neoantigen-TCR expressing T cells from L2 and L3 co-cultured with autologous PBMCs pulsed with a gradient of peptide concentrations, and IFN- γ secretion will be measured. Published virus-specific TCR with low and high avidity will be used as controls[50]. Overall, since neoantigens have been likened to pathogens in terms of immunogenicity, we expect most discovered TCRs to have relatively high functional avidity, but we also expect variation in the degree of functional avidity across the TCRs. We will assess if there are general avidity differences amongst TCRs enriched following NeoVax alone, compared with NeoVax followed by Nivolumab.

Q3: How do the composition, number and state of immune cells infiltrating tumor biopsies evolve in relationship to NeoVax +/- Nivolumab? We have developed single cell transcriptome approaches that enable us to directly interrogate the gene expression state of small and rare populations. We recently reported the single cell transcriptome analysis results of neoantigen-specific CD4+ T cells, directly isolated *ex vivo* from melanoma NeoVax treated patients by tetramer sorting following vaccination, and we could identify broad changes in the transcriptional profile of post vaccination T cells compared to pre-vaccination (Figure 2C) [48]. We now propose to perform serial single cell transcriptome analysis of tumor and non-tumor (immune cells) fresh core needle biopsies at B1, B2 and B3. As shown in Figure 2B, we have established an approach for directly processing freshly isolated core biopsies from melanoma patients, in which disaggregated samples are stained with CD45 (a marker of hematopoietic cells) and CD3. As single cells, we will isolate CD45+CD3-, CD45+CD3+ and CD45- cells into lysis buffer in wells of 384-well plates; these plates are cryopreserved until the time of processing. The cells can then undergo processing using the CELSeq2 protocol [51], and sequenced by Next-Seq. In close collaboration with our long-time computational analysis colleague Dr. Peter Kharchenko [52, 53], we have developed analytic workflows for scRNA-seq data generated by the CEL-Seq2 platform. Using nonlinear dimensionality reduction by t-distributed stochastic neighbor embedding (t-SNE) analysis of scRNA-seq data, we will determine the composition and state of immune cells.

To confirm that the infiltrating T cells are neoantigen-reactive, we will determine the TCR repertoire of the isolated T cells and compare the extent to which TCR sequences match between peripheral blood and tumor, which would indicate the presence of neoantigen-specific T cells in multiple tissue compartments. If new TCR sequences are detected in the TILs that are not observed in peripheral blood, we will clone them and confirm their reactivity against neoepitopes and against tumor cells.

In conjunction with these transcriptome-based studies, we will confirm the protein-level expression of markers and the architecture of immune cell populations within the tumor through immunohistochemical evaluation of tumor biopsy material. We will evaluate expression of CD3, CD4, CD8, and ask whether tumor-infiltrating T cells before and after platinum exposure, vaccine and nivo change in their states of differentiation (CD45RO), activation, suppression

(FOXP3, PD-1, PD-L1/L2, CTLA4, TIM-3) and cytolytic activity (granzyme B, perforin). At the same time, we will assess if tumor-associated macrophages, NK cells and dendritic cells within the tumor micro-environment change in their number and/or pattern distribution (through the staining of CD68, CD163, CD83, CD56, CD33, CD11b, MHC class I and II).

9.3 Characterization of the genetic evolution of patient tumor cells following SOC chemotherapy and NeoVax/Nivolumab, and with disease progression

A general challenge to cancer therapy is the genomic heterogeneity of tumor cell populations, and the ability for this heterogeneity to fuel clonal evolution and escape from the selective pressure exerted by therapy. The Wu lab has led the field in defining genomic heterogeneity and clonal evolution in chronic lymphocytic leukemia[54-56] and her expertise can now be leveraged in the current temporal analysis of EOC in relationship to SOC and novel immunotherapy. Conceptually, a defining feature of the NeoVax/Nivo combination is its ability to overcome immunologic escape by addressing multiple targets simultaneously.

We will:

i) Define the changes in the somatic mutation landscape across treatment course for EOC patients. Patients on this trial are uniformly exposed to platinum-based chemotherapy and subjected to immune pressure from NeoVax/Nivolumab. We expect ~ 10 of 30 enrolled patients across cohorts to have biopsiable material at B2 and B3. Our analyses of tumor evolution will thus focus on these 10 patients. Using established analytic pipelines, we will analyze WES data generated from genomic DNA extracted from serial tumor biopsies (B1, S and B2/B3; only 250 ng DNA needed) and matched PBMC (as a source of germline DNA), sequenced to a median coverage depth of 150x to define subclonal status of mutations using ABSOLUTE[57]. Through clustering analysis, we will determine if significant changes in the cancer cell fractions (CCFs) of sSNVs and copy number alterations are present at the defined time points[54]. We will enumerate genomic features such as mutation rate, and the numbers of clones and drivers per timepoint. Of high relevance to EOC, we will assess if there are mutations and copy number changes in the homologous recombination (HR) pathway including BRCA1, BRCA2 and Fanconi Anemia gene alterations and in other DNA repair pathways that may be abnormal in EOC such as mismatch repair and nucleotide repair pathways[58, 59], and in other key oncogenic pathways implicated in ovarian cancer (PI3K, Notch and RB pathways)[60].

ii) Determine if changes in expression of genes relevant to immunogenicity are detectable. In addition to examining the single cell transcriptomes of immune cell populations, we will also examine expression heterogeneity and its evolution in single cell tumor transcriptomes from serial biopsies. Given that the CXCL10/CXCR3 axis is important for the chemotaxis of CD4+ and CD8+ T-lymphocytes, and CXCL10 and CCL5 overexpression in cancer cells has been associated with the presence of CD8+ lymphocytes in various cancers[61, 62], we will assess CXCL10 and CCL5 expression in tumor cells from B1, S and B2 specimens. We will also evaluate the expression of genes associated with tumor cytotoxicity including genes of the IFN- γ , the TNFR and the STING/TBK1/IRF3 pathways on the tumor cells [63, 64]. Of note, activation of PD-L1 in a STING-dependent manner has been reported in DNA repair-deficient cancers[63].

iii) Immunogenomic evaluation of progressive disease. These analyses will focus on specimens B4 which will undergo WES and RNA sequencing, and histopathologic evaluation. We will examine the generated sequencing data for: (i) evidence of known mechanisms of resistance to CPB [65, 66], namely acquired deficiencies in antigen presentation (e.g. loss of major histocompatibility expression [67]), or dysregulation of antigen processing machinery via mutation in beta-2-microglobulin or insensitivity to the pro-apoptotic effects of toxic granules (e.g. granzymes), death receptors (e.g., Fas and tumor necrosis factor–related apoptosis-inducing ligand [TRAIL]), or interferons (e.g. acquired JAK1 and JAK2 mutations leading to abrogation of IFN- γ signaling)[68-70]; and (ii) evidence of loss of tumor antigenicity due to immune selection of cancer cells which lack or mutate immunogenic tumor associated antigens or neoantigens [71], a mechanism which would be highly relevant for NeoVax resistance. We will perform IHC studies to examine for: (i) evidence of upregulation of expression of other immune checkpoint molecules expressed on tumor cells and surrounding stromal cells and of expression of negative regulatory markers present on TILs (e.g. PD-1, LAG-3, TIM-3, CD244, VISTA)[72]; and (ii)for evidence of immune elimination due orchestration by the tumor of an immunosuppressive microenvironment[70, 73] e.g. via upregulation of indoleamine 2,3-dioxygenase [74], induction of tumor-promoting M2 macrophages (through IL-4, IL-13, IL-10 and M-CSF), recruitment of Tregs through production of CCL22[73], and induction of myeloid-derived suppressor cells [75, 76].

10. STUDY CALENDAR

The study calendar and biomarker sampling schedule is presented in the Table below. All study procedures should be performed within ± 5 days of the scheduled time unless specified otherwise.

Flowchart of Events

Procedures	Screening Consent	Standard of Care Chemotherapy ± surgery	Treatment Consent ¹	Standard of Care Chemotherapy ± surgery	Pre-Treatment Day -14 to Day 0; eligibility assessment	Study Treatment													
						Vaccination only phase (i.e. only NeoVax)								Nivolumab / Va					
Week	N/A	N/A	N/A	N/A	N/A	1	2	3	4	5	12	13	14	16	18	20			
Day	N/A	N/A	N/A	N/A	N/A	1	4	8	15	22	29	78	85	92	106	120	134		
Informed consent	X		X																
Demographics					X														
Medical History					X														
Concomitant medications					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs ²					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height					X														
Weight					X														
ECOG					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC w/diff, PLTs					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chemistries ³					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hepatitis B & C ⁴					X														
Pregnancy test ⁵					X				X		X				X		X		
CA125 ⁶						X				X	X		X						
Thyroid function tests (TSH, FT4) ⁷													X						
12-lead ECG					X														
AE evaluation ⁸					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CT/MRI ⁹					X							X							
HLA Class I and II typing ¹⁰	X																		
Tumor biopsy ¹¹ B1, B2, B3, and B4		X										X							
Tumor biopsy S1 ¹¹				X															
Blood draw for vaccine manufacturing ¹²	X																		
Leukapheresis ¹³					X								X						
Blood draw for immune response monitoring ¹⁴	X		X			X		X			X						X		
NeoVax Vaccine administration ¹⁵						X	X	X	X	X		X					X		
Olaparib / Niraparib ¹⁶											X								
Nivolumab administration ¹⁷													X	X	X	X			
SoC Chemotherapy		X		X															
Bevacizumab ¹⁸		X ¹⁸		X ¹⁸										X ¹⁸					
Follow up ¹⁹																			

¹Please refer to protocol section 5

²Include temperature, diastolic/systolic blood pressure, pulse, respiratory rate, oxygen saturation.

³Serum chemistry including*: glucose, urea nitrogen, creatinine, sodium, potassium, calcium, total and direct bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase.

*On the day of NeoVax/Nivolumab or Nivolumab administration the serum chemistry should include the assessment of **amylase and lipase**.

Samples will be collected during screening and up to 24 hours prior to dosing. If dosing falls on a Monday, the collection window may be extended to collect samples on the previous Friday.

⁴Includes hepatitis B (HBV) surface antigen and Hepatitis C antibody. If Hepatitis C antibody is positive, the confirmatory HCV RNA test should be done and if it is negative, then participants are eligible.

⁵Women of childbearing potential must have a negative serum β -HCG or urine pregnancy test during screening; this must be repeated every 4 weeks (+/- 1 week) during treatment and at approximately 4 weeks and 10 weeks after the last dose.

⁶CA125 assessment will be performed every 4 weeks after week 28

⁷Thyroid function tests assessment will be performed every 12 weeks after week 28

⁸AE monitoring and reporting will start from Day 1 of NeoVax administration and will continue for 100 days following receipt of the last dose of protocol therapy.

⁹CT or MRI of other areas of disease (e.g., neck) should be obtained as clinically indicated. If intravenous contrast is contraindicated, a non-contrast CT and MRI may be used to evaluate sites of disease where a CT without contrast is not adequate. The imaging modality should remain consistent throughout the study and should be the same modality used at screening. The window for CT/MRIs is ± 7 days. During follow up phase CT/MRI assessment will be performed every 3 months after week 24. The window for CT/MRIs is ± 7 days.

¹⁰HLA typing should be collected any time after the patient consents to participate in screening procedure

¹¹Tumor biopsies:

- Biopsy B1 is mandatory and will be performed during screening period. Tumor cells may be collected either through core biopsy or laparoscopy. If tumor biopsy is collected through core biopsy all tumor tissue will be put in formalin. If tumor biopsy is collected through laparoscopy the best tumor specimen will be put in formalin; the remainder will be put in DMEM
- Biopsy S1 may be performed on patients in Cohort A; if possible, tumor specimen will be collected during surgery and put in DMEM for study correlatives
- Biopsy B2 will be performed anytime between Days 78 (after vaccination) and 92 if it is safe and if there is biopsiable disease.
- Biopsy B3 will be performed anytime between Days 134 and 148 if it is safe and if there is biopsiable disease.
- Biopsy B4 is optional and would be performed at the time of disease progression; tumor may be collected either through core biopsy or laparoscopy. If tumor biopsy is collected through core biopsy all tumor tissue will be put in formalin. If tumor biopsy is collected through laparoscopy the best tumor specimen.

¹²Blood draw for vaccine manufacturing: the 2x10 mL EDTA should be kept frozen at CRL

¹³3 Leukapheresis (L1, L2 and L3) should be obtained at the following timepoints:

- L1: Prior to NeoVax Day 1 (i.e. sometime between Day -7 to Day 0). If Leukapheresis procedure cannot be performed, 200 mL should be collected instead.
- L2: The week prior to initiation of Nivolumab on Day 92 (i.e. sometime between Day 85 to Day 97). If Leukapheresis procedure cannot be performed, 200 mL should be collected instead
- L3: The week prior to Day 148 (i.e. sometime between Day 140 to Day 147). If Leukapheresis procedure cannot be performed, 200 mL should be collected instead.

Along with each leukapheresis (L1, L2, L3), 20 mL of blood will be collected in EDTA tubes and sent to IAL for sample processing.

¹⁴Blood for research purposes will be collected and processed at the IAL during the following timepoints a:

- Screening consent: 60 mL
- Treatment consent 60 mL
- Study treatment Day 1: 60 mL
- Study treatment Days 15, 78, and 134: 200 mL
- Week 28 and every 4 weeks until PD: 60 mL

¹⁵NeoVax vaccination windows for:

Priming phase: Week 1 Day 4 (+/- 1 day), Week 2 Day 8 (+/- 1 day), Week 3 Day 15 (+/- 3 days) and Week 4 Day 22 (+/- 3 days). At least 5 days must elapse between day 15 and day 22

Booster phase: Week 12 Day 78 (+/- 7 days) and Week 20 Day 134 (+/- 7 days)

¹⁶Olaparib/ Niraparib (if clinically indicated): Stating at or any time after Day 29 and continued until disease progression or unacceptable toxicity

¹⁷Nivolumab administration will start at week 14 Day 92 (+/- 3 days) and will be given every 2 weeks (+/- 3 days) for up to 24 months or until disease progression, initiation of alternate antineoplastic therapy, or death. Starting on Week 24 Day 162 Nivolumab administration schedule may be switched to once every 4 weeks (+/- 3 days) at investigator's discretion

¹⁸Use of Bevacizumab will be allowed at investigator's discretion in Cohort B; bevacizumab will be allowed to be administered during chemotherapy, or after chemotherapy (as maintenance) or throughout (i.e. both during chemotherapy and after chemotherapy) as per the investigator's discretion

¹⁹Active Follow Up: Every 3 months every 3 months (+/- 2 weeks) until initiation of a new therapy for progressive disease, withdrawal of consent, or death (whichever comes first).

Long-Term Follow-Up: Participants removed from active follow-up for progression/initiation of a new therapy will enter long-term follow-up, where they will be followed via medical record review until death for survival. All patients will be followed for survival. Updates to the CRFs are to be made roughly every six months (CT scans, labs, vitals, ECOG are not required).

11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by standard criteria. For the purposes of this study, participants will be reevaluated on Day 78 (week 11) and then every 12 weeks (± 4 days). In addition to a baseline

scan, confirmatory scans should also be obtained (not less than 4) weeks following initial documentation of objective response. The next planned scans may be used as the confirmatory scans.

11.1 Antitumor Effect – Solid Tumors

Response and progression 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 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥ 10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area may be considered measurable if progression has been observed in the lesions following completion of the radiation course.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

‘Cystic lesions’ thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 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 each identified and reported lesion 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.

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 date 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.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 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, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks from baseline
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD**	Yes or No	PD	
Any	Any	Yes	PD	
* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion. ** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression. <u>Note:</u> Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “<i>symptomatic deterioration</i>.” Every effort should be made to document the objective progression even after discontinuation of treatment.				

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*

Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6 Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression or censored at date of last disease evaluation for those without progression reported.

11.1.7 Immune related Response Criteria (irRECIST)

Immune related response criteria using unidimensional measurements may be used to describe tumor shrinkage (Nishino *et al.*, 2014). This methodology is the same as described above for RECIST except:

- New lesions do not automatically denote disease progression
- The measurement of longest diameter of new measurable lesions are included in the sum of the measurements of the original target lesions.

The same method of assessment and the same technique should be used to characterize each identified and reported target lesion(s) at baseline and throughout the trial.

irRECIST criteria are defined as follows:

- Overall immune-related complete response (irCR): Complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes also must have a reduction in short axis to <10 mm.
- Overall immune-related partial response (irPR): Sum of the diameters (longest for non-nodal lesions, shortest for nodal lesions) of target and new measurable lesions decreases $\geq 30\%$.
- Overall immune-related stable disease (irSD): Sum of the diameters (longest for nonnodal lesions, shortest for nodal lesions) of target and new measurable lesions neither irCR, irPR, (compared to baseline) or immune-related progressive disease (irPD, compared to nadir).
- Overall immune-related progressive disease (irPD): Sum of the diameters (longest for non-nodal lesions, shortest for nodal lesions) of target and new measurable lesions increases $\geq 20\%$ (compared to nadir), confirmed by a repeat, consecutive observation at least 4 weeks from the date first documented.

New measurable lesions: Incorporated into tumor burden (i.e, added to the target lesion measurements). A lymph node has to be ≥ 15 mm in short axis to be a measurable new lesion and its short axis measurement is included in the sum. Up to 2 new lesions per organ and up to 5 new lesions in total can be added to the measurements.

New non-measurable lesions: Do not define progression but preclude irCR.

Overall responses derived from changes in index, non-index, and new lesions are outlined in the following table.

Overall Response Derived from Changes in Index, Non-index and New Lesions

Measurable response	Non-measurable response		Overall response using irRECIST ^b
Index and New Measurable Lesions (Tumor Burden) ^a	Non-Index Lesions	New, nonmeasurable Lesions	
Decrease 100%	Absent	Absent	irCR
Decrease 100%	Stable	Any	irPR
Decrease 100%	Unequivocal progression	Any	irPR
Decrease $\geq 30\%$	Absent/stable	Any	irPR
Decrease $\geq 30\%$	Unequivocal progression	Any	irPR
Decrease <30% and increase <20%	Absent/stable	Any	irSD
Decrease <30% and increase <20%	Unequivocal progression	Any	irSD
Increase $\geq 20\%$	Any	Any	irPD

a. Decrease assessed relative to baseline.
b. Response (irCR and irPR) and progression (irPD) must be confirmed by a second, consecutive assessment at least 4 weeks apart.

Best immune related responses are classified as immune related CR (irCR – disappearance of all lesions) or immune related PR (irPR ($\geq 30\%$ reduction from baseline)).

Immune-related progression-free survival (irPFS) is defined as time from registration (or randomization) to the earlier of immune-related progression of disease (irPD) or death due to any cause. Participants alive without immune-related disease progression are censored at date of last disease evaluation.

11.1.8 Response Review

All films will be reviewed, and response evaluated by the DF/HCC Tumor Imaging Metrics Core (TIMC).

12. 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 Office of Data Quality 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 Office of Data Quality in accordance with DF/HCC SOPs.

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 level 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 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, and any applicable regulatory requirements. All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is an open label Phase I trial of NeoVax with Nivolumab in two cohorts of patients with epithelial ovarian cancer (EOC).

The primary purpose of this study is to demonstrate that the regimen of NeoVax with Nivolumab is feasible and safe for the treatment of patients with ovarian cancer. The study will also evaluate the clinical and immune response to treatment of EOC patients. Our main hypothesis is that the combination of the immune stimulating effects of NeoVax coupled with the release of the immune suppression by the PD-1 inhibitor Nivolumab will be feasible and safe and may produce clinical responses in EOC.

Two cohorts of patients with EOC will participate in the study:

- i) Patients with newly diagnosed ovarian cancer who are opted for neoadjuvant chemotherapy (Cohort A)
- ii) Patients with recurrent platinum sensitive ovarian cancer with progression free interval (PFI) 24 months after completion of first line chemotherapy (Cohort B).

The study is designed to have a 2-stage consent process (pre-screening and treatment consent) which ensures that NeoVax manufacture will only proceed for patients without platinum-refractory disease (assessed in the first 3-4 cycles of SOC platinum therapy) and those for which generation of NeoVax is feasible.

The primary objectives of the study are to evaluate the feasibility and safety of administering NeoVax with Nivolumab in each of two cohorts of EOC patients: i) patients with newly diagnosed ovarian cancer who are opted for neoadjuvant chemotherapy (Cohort A) and ii) patients with recurrent platinum sensitive ovarian cancer (Cohort B).

The secondary objectives include:

- To determine anti-tumor activity as assessed by objective response rate (ORR) based on Response Criteria in Solid Tumors (RECIST) and based on immune-related RECIST (irRECIST) for patients with residual measurable disease after completion of

chemotherapy.

- For patients in cohort B who do not have residual measurable disease after completion of chemotherapy, we will determine whether the second progression free survival (PFS) PFS2 (measured as the interval from the start of secondary therapy to the date of the second relapse) is longer than the first PFS (PFS1, measured as the interval from the start of primary therapy to date of first relapse).
- To evaluate progression-free survival (PFS) and overall survival (OS).
- To evaluate duration of response (DOR).
- To evaluate ORR among patients who were retreated with NeoVax with Nivolumab at the time of disease progression or recurrence.

The exploratory objectives include:

- Define the evolution of T cell reactivity, state and repertoire following NeoVax/Nivolumab
- Define evolution of the tumor and the tumor microenvironment following standard of care chemotherapy and NeoVax/Nivolumab, and correlate this with T cell and clinical responses
- Identify mechanisms of immune escape to NeoVax/Nivolumab

13.2 Sample Size, Accrual Rate and Study Duration

The target accrual is 30 patients, i.e. 15 patients per cohort that sign treatment consent to receive NeoVax/Nivolumab treatment. The sample size of each Cohort in the phase I study is selected to be 15 patients in order to have a high probability of observing at least one serious toxicity event when the incidence is low. Specifically, with 15 patients who sign treatment consent per cohort, the probability of observing at least one serious toxicity event is 0.79 if the true toxicity rate is 10% and 0.54 if the true toxicity rate is as low as 5%.

Feasibility will be determined by assessing the percentage of patients prescreened in each cohort who have enough neoantigens for generation of the vaccine, are not platinum refractory, and who initiate vaccination; this will be evaluated using a negative binomial test (one-sided $\alpha = 0.1$). Defining a 45% success rate of prescreening as unacceptable, we will reject this null hypothesis if a maximum of 25 patients are prescreened in order for 15 patients to initiate vaccination (i.e. observed success rate $\geq 60\%$).

It is anticipated that approximately 3 subjects will be consented per month to prescreening. We further anticipate an attrition rate of 25% due to platinum-refractoriness (10% in Cohort A; 10-20% in Cohort B) or insufficient number of neoantigens to generate the vaccine. We thus expect to complete accrual of 30 patients (15/cohort) consented to vaccine/Nivolumab treatment within 12-15 months.

13.3 Analysis of Primary Endpoints

The target accrual of patients treated with NeoVax/Nivolumab is 15 patients per cohort. The 2-consent design (prescreening and therapeutic) ensures that vaccine manufacture will only proceed for patients without platinum-refractory disease (assessed in the first 3 cycles of SOC

platinum therapy, ~10% entering Cohort A; 10-20% in Cohort B) and with an adequate number of candidate neoantigens for generation of the vaccine.

Feasibility will be determined by assessing the percentage of patients prescreened in each cohort who have enough neoantigens for generation of the vaccine, are not platinum refractory, and who initiate vaccination; this will be evaluated using a negative binomial test (one-sided $\alpha = 0.1$). Defining a 45% success rate of prescreening as unacceptable, we will reject this null hypothesis if a maximum of 25 patients are prescreened in order for 15 patients to initiate vaccination (i.e. observed success rate $\geq 60\%$). There will be greater than 90% power to reject the null hypothesis if the true success rate is 70%. Of patients who sign treatment consent, we will assess the percentage (with 90% confidence intervals [CIs]) of patients from each cohort who successfully complete vaccination and receive Nivolumab therapy without discontinuation due to toxicity.

We will determine the safety and tolerability of NeoVax/Nivolumab separately for each cohort of EOC patients, using the Common Terminology Criteria for Adverse Events (CTCAE, v.5.0). With 15 patients per cohort, the probability of observing at least one serious toxicity event is 0.79 if the true toxicity rate is 10% and 0.54 if the true toxicity rate is as low as 5%. Safety analysis will be performed on all patients who receive at least one dose of the NeoVax. The overall incidence of treatment-emergent AEs, SAEs, AEs of at least Grade 3 severity, related AEs, and AEs leading to withdrawal of treatment will be described. Treatment emergent AEs will be those that start or worsen on or after the first day of treatment with NeoVax; related AEs will be those with an investigator determination of related to treatment. Adverse event tabulations will be performed using time periods relative to the start of vaccine administration as well as start of Nivolumab.

The rates of autoimmune effects and discontinuation will be estimated in the combined population of Cohorts A and B and reported with two-sided 90% confidence intervals. The following table displays the level of precision when acceptable discontinuation rates are observed

Number of events	Estimated Rate	90% CI
0	0.0%	0% – 9.5%
1	3.3%	0.2% - 14.9%
2	6.7%	1.2% - 19.5%

13.4 Analysis of Secondary Endpoints

Although this study will not formally assess clinical activity, and is not powered to do so, we will nonetheless examine if preliminary signal of clinical activity from NeoVax/Nivolumab in either cohorts is evident, either as radiographic response among patients with residual disease after platinum chemotherapy or as prolongation of PFS2 vs PFS1 among Cohort B patients free of residual disease after platinum chemotherapy.

Secondary objectives are to look for early evidence of clinical activity defined as objective response rate (ORR) based on RECIST v1.1 and irRECIST criteria, and to summarize outcomes

including DOR and PFS and OS using Kaplan-Meier estimates. For Cohort B, descriptive statistics will be used to explore the relationship between second PFS (PFS2, measured as the interval from the start of secondary therapy to the date of the second relapse) and first PFS (PFS1, measured as the interval from the start of primary therapy to date of first relapse). Inferences on the paired endpoints will use Pepe and Fleming (1989) tests for differences between integrated weighted Kaplan-Meier estimates. Although this study will not formally assess clinical activity, and is not powered to do so, with complete information i.e. following all 15 patients from PFS1 and PFS2 and without censoring, there is 80% power to see a 50% improvement in the hazard of progression (HR = 0.5) under strong positive correlation ($\theta = 0.3$) using the frailty model defined by Jung [77].

There will be no intent to treat analysis. Efficacy is a secondary endpoint and will be based on the subjects for which vaccine was manufactured and then vaccinated. Patients which are not vaccinated because the vaccine could not be manufactured, will not be included in the analysis of efficacy.

13.5 Analysis of Exploratory Endpoints

Descriptive statistics will summarize and compare changes in immune parameters before and after the vaccine, after addition of Nivolumab and upon disease progression, including CD8+ and CD4+ T cell response measured by ex vivo IFN- γ ELISPOT assessed through spot counts. Reporting will include medians and inter-quartile ranges, as well as tables depicting shifts from earlier time points. Nonparametric tests (e.g., Wilcoxon signed rank test) will be used to determine differences in the ELISPOT data between time points. With 15 paired observations, there will be 80% power to detect differences that are 0.75 or greater in magnitude using standardized units (two-sided alpha = 0.05). If Gaussian assumptions are met under Box-Cox transformations of any endpoint, linear mixed effects models may be used to explore longitudinal trends over serial measurements.

Genetic alterations, gene expression and IHC data will be analyzed for changes after exposure to chemotherapy (S vs B1), after exposure to NeoVax/Nivo (B2 vs S and B2 vs B1), and upon development of disease progression/recurrence with descriptive statistics and frequency distributions used for reporting as appropriate. PD-L1 expression and other IHC assessments in the paired biopsies (B2 vs B1) will be correlated with T cell and clinical responses using Fisher's Exact tests (for dichotomous variables such as PD-L1 positivity/negativity using cut-offs of 1% and 10%), descriptive statistics and frequency distributions used for reporting as appropriate. With 15 paired biopsies, there will be 80% power to detect differences that are 0.75 or greater in magnitude using standardized units (two-sided alpha = 0.05).

Given that this is a phase I trial with limited sample size, we do not anticipate being able to conduct statistical inferences which can account for false discovery and family-wise errors with multiple comparisons. However, the structure of correlation of all the aforementioned features with response will be explored using standard agglomerative hierarchical models and other unsupervised learning techniques as a hypothesis-generating exercise.

13.6 Reporting and Exclusions

13.6.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment (Day 1 of NeoVax) until 100 days after completion of the last dose of Nivolumab.

13.6.2 Evaluation of the Primary Efficacy Endpoint

All of the participants who met the eligibility criteria and who receive at least one dose of NeoVax will be included in the main intent-to-treat analysis of the response rate.

14. PUBLICATION PLAN

The results should be made public within 12 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. Eur J Immunol 1989;19:2237-42

APPENDIX A PERFORMANCE STATUS CRITERIA

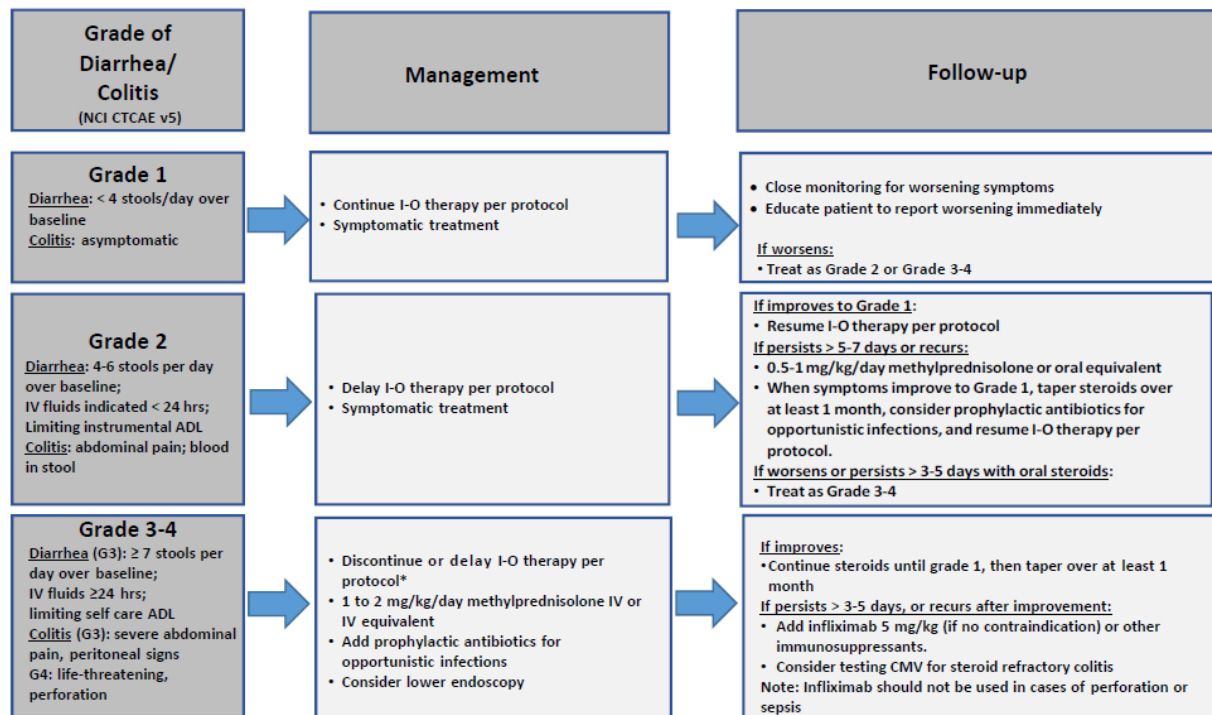
ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or 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 B ADVERSE EVENT MANAGEMENT

Appendix B1:

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.



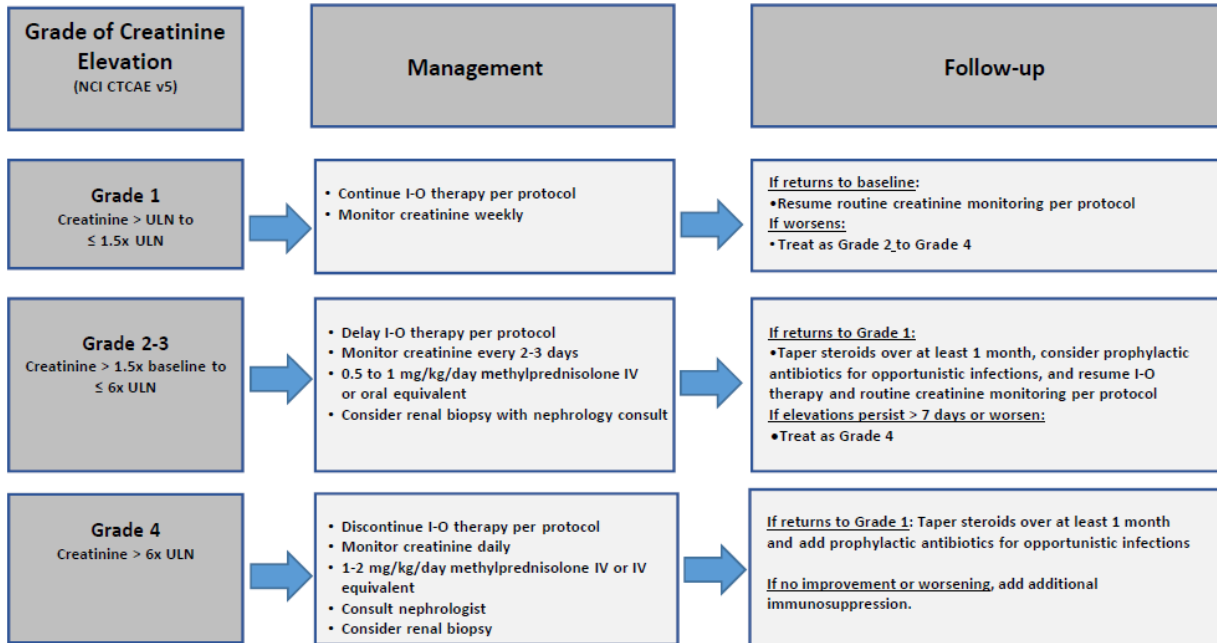
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

* Discontinue for Grade 4 diarrhea or colitis. For Grade 3 diarrhea or colitis, 1) Nivolumab monotherapy: Nivolumab can be delayed. 2) Nivolumab+ Ipilimumab combination: Ipilimumab should be discontinued while nivolumab can be delayed. Nivolumab monotherapy can be resumed when symptoms improve to Grade 1. Please refer to protocol for dose delay and discontinue criteria for other combinations.

Appendix B2:

Renal Adverse Event Management Algorithm

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

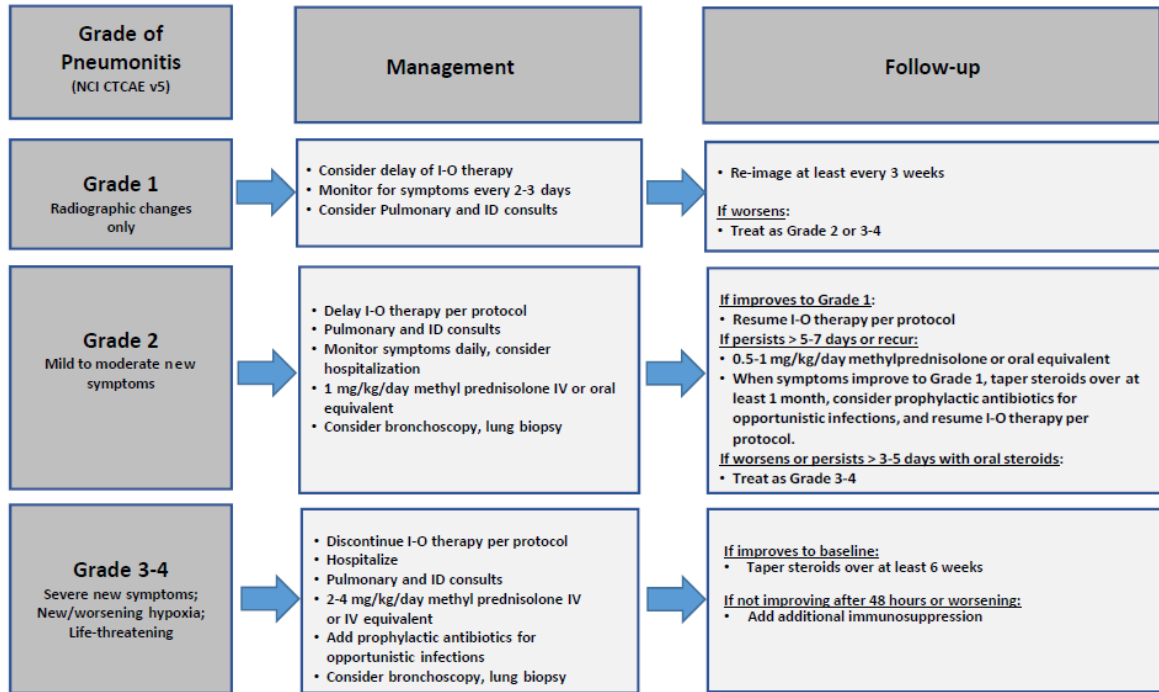


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

Appendix B3:

Pulmonary Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Evaluate with imaging and pulmonary consultation.

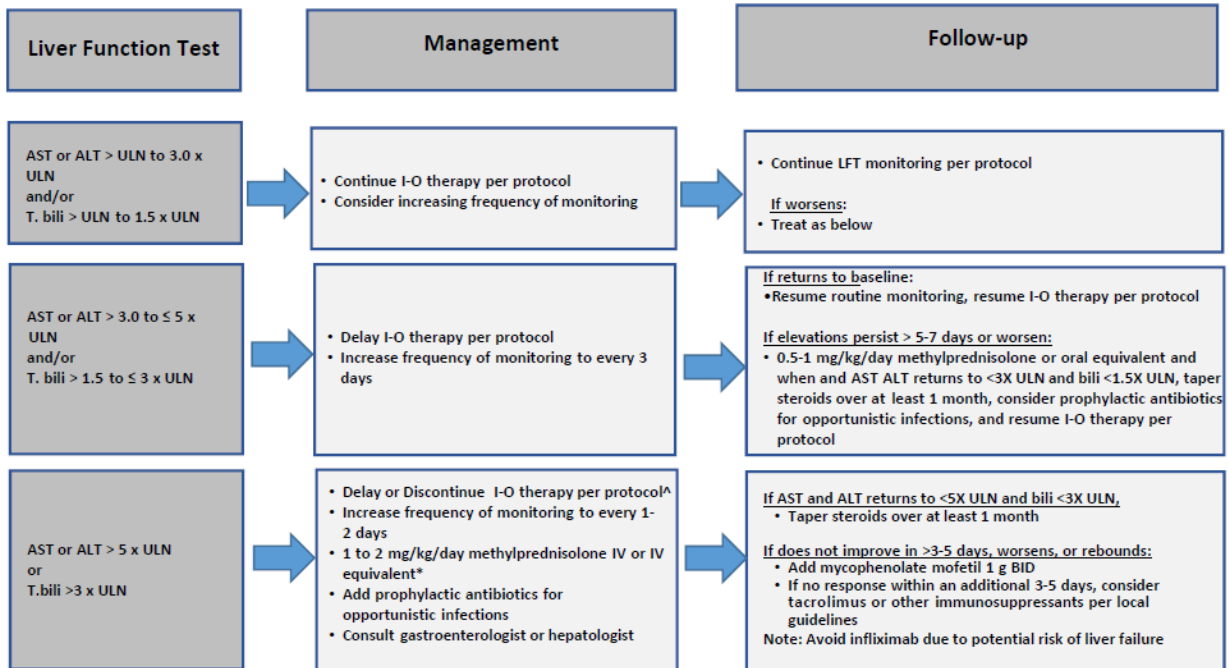


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

Appendix B4:

Hepatic Adverse Event Management Algorithm

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



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

^Λ Please refer to protocol dose delay and discontinue criteria for specific details.

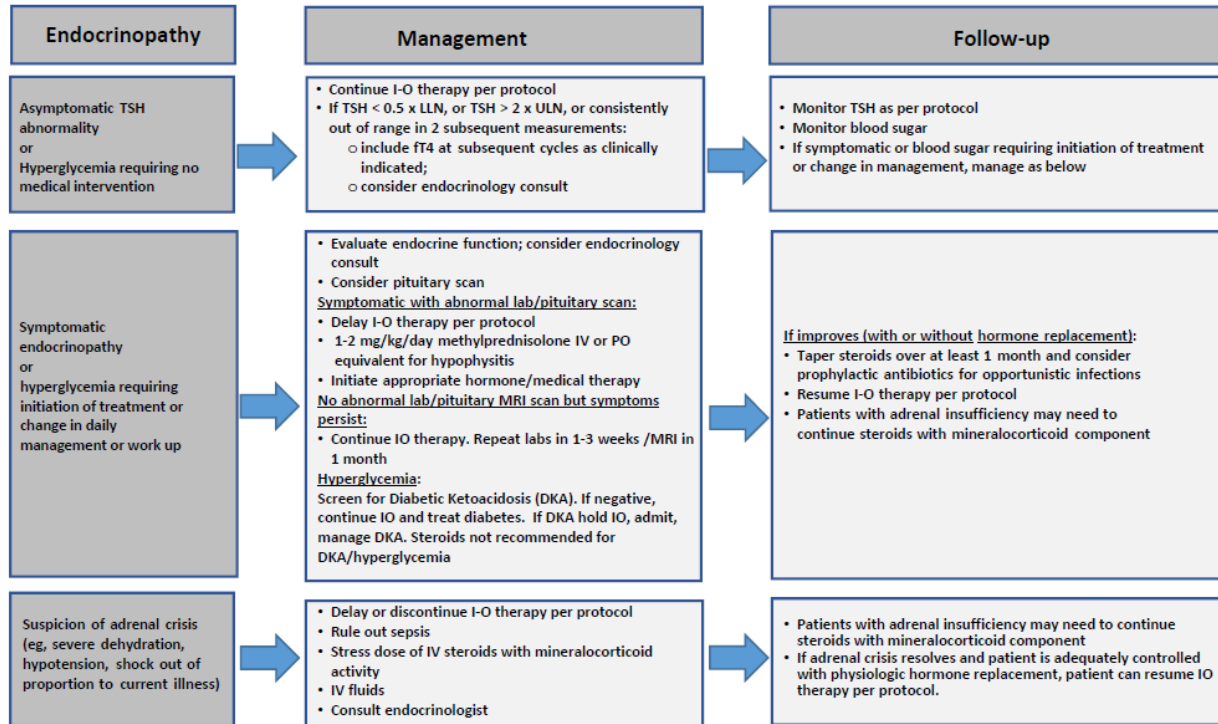
*The recommended starting dose for AST or ALT > 20 x ULN or bilirubin >10 x ULN is 2 mg/kg/day methylprednisolone IV.

For subjects with HCC, please refer to the protocol for specific details.

Appendix B5:

Endocrinopathy Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Consider visual field testing, endocrinology consultation, and imaging.

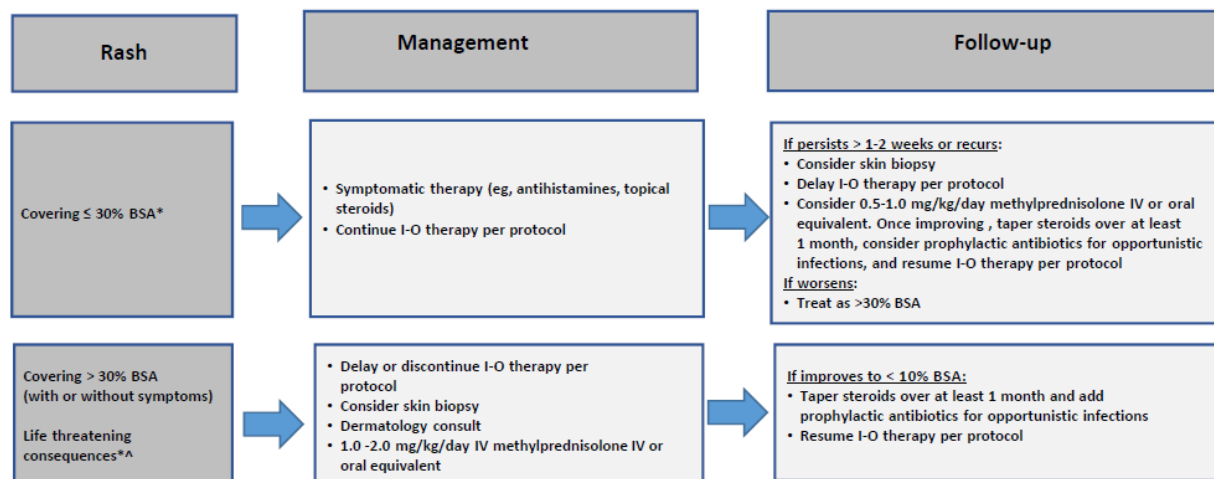


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

Appendix B6:

Skin Adverse Event Management Algorithm

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, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

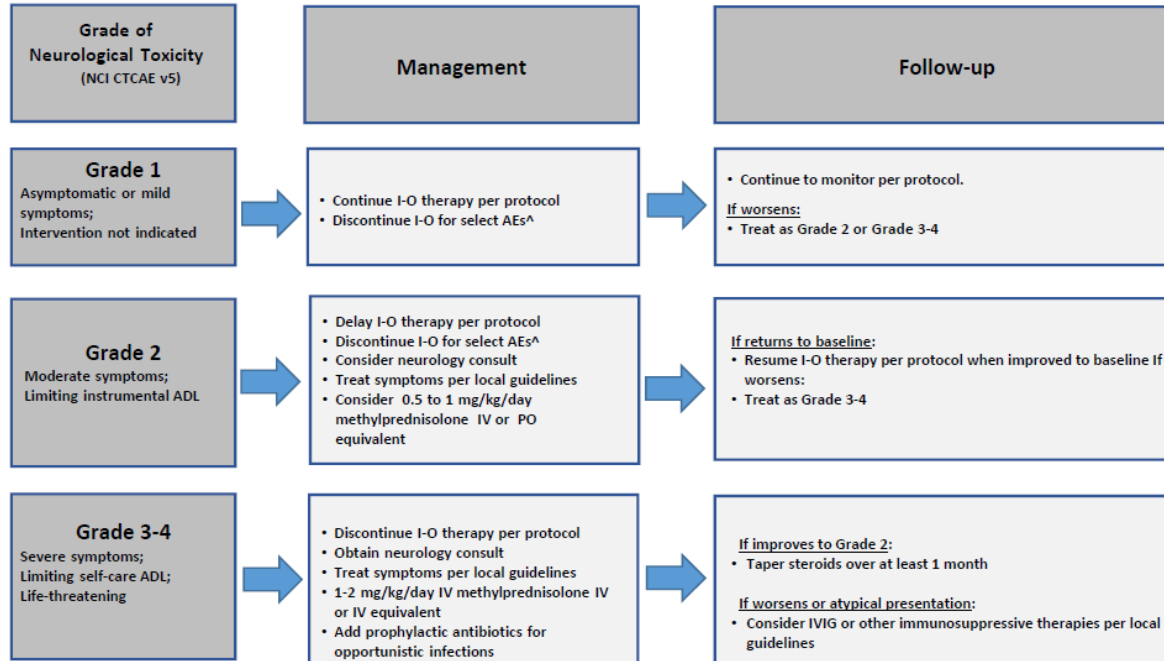
*Refer to NCI CTCAE v5 for term-specific grading criteria.

^If Steven-Johnson Syndrome (SJS), toxic epidermal necrosis (TEN), Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS, TEN, or DRESS is diagnosed, permanently discontinue I-O therapy.

Appendix B7:

Neurological Adverse Event Management Algorithm

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



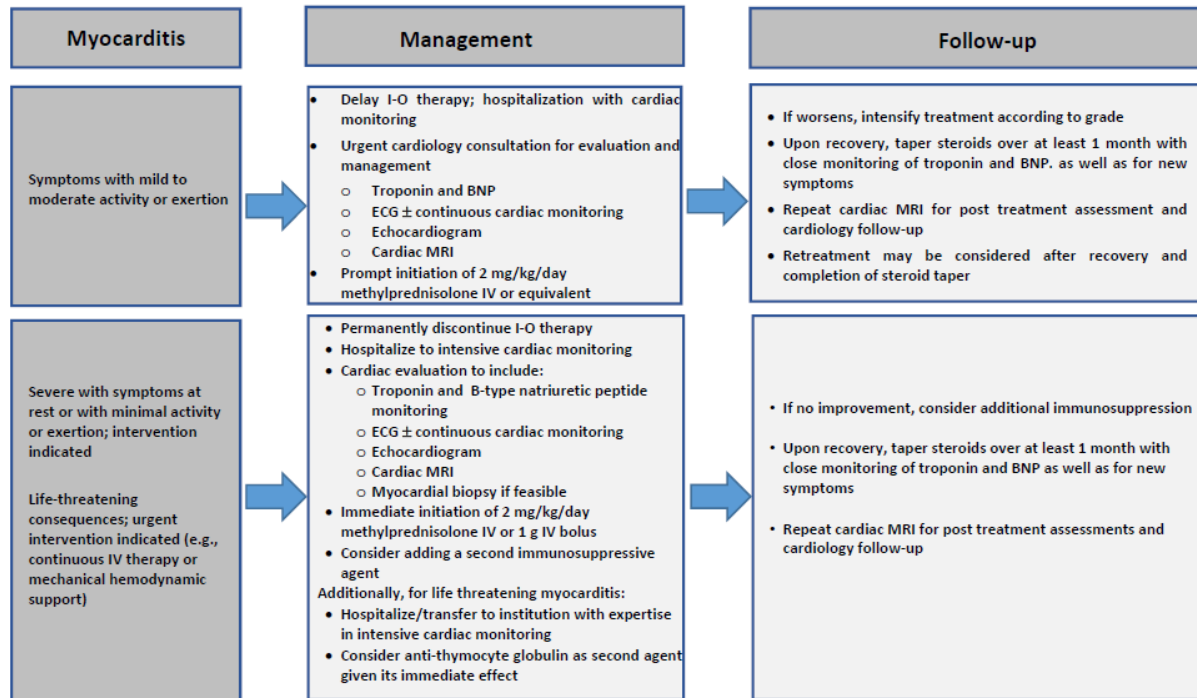
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg. prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

[^]Discontinue for any grade myasthenia gravis, Guillain-Barre syndrome, treatment-related myelitis, or encephalitis.

Appendix B8:

Myocarditis 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 (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. Prophylactic antibiotics should be considered in the setting of ongoing immunosuppression.