Abbreviated Title: mRNA-Based, Cancer Vaccine
CC Protocol Number: 18-C-0074 E
IBC Number: RD-18-I-07
OSP Number: 1803-1739
NCT Number: NCT03480152
Version Date: January 23, 2019

PROTOCOL TITLE

A Phase I/II Trial to Evaluate the Safety and Immunogenicity of a Messenger RNA (mRNA)-Based, Personalized Cancer Vaccine Against Neoantigens Expressed by the Autologous Cancer

NIH Principal Investigator: Steven A. Rosenberg, M.D., Ph.D.
Chief of Surgery, Surgery Branch, CCR, NCI
Building 10, CRC, Room 3-3940
9000 Rockville Pike, Bethesda, MD 20892
Phone: 240-858-3080; E-mail: Steven.Rosenberg@nih.gov

Investigational Agent:

Drug Name:	NCI-4650 (mRNA-based, Personalized Cancer Vaccine)
IND Number:	17858
Sponsor:	Center for Cancer Research
Manufacturer:	ModernaTX, Inc.

PRÉCIS

Background:

- Therapeutic vaccination against cancer has proven very challenging with little clinical benefit.
- Vaccines against non-viral tumors have mainly targeted differentiation antigens, cancer testis antigens, and overexpressed antigens. However, negative selection in the thymus against these normal non-mutated antigens severely limits the ability to generate high avidity anti-cancer T-cells. Such depletion can impair their antitumor activity and limit tumor elimination.
- The National Cancer Institute Surgery Branch (NCI-SB) has developed a pipeline for the identification of immunogenic T-cell epitopes derived from neoantigens.
- In recent studies, we identified the neoantigens recognized by tumor-infiltrating lymphocytes (TIL) that mediated regression in patients with metastatic melanoma. Using whole exome sequencing of a resected metastatic nodule followed by high throughput immunologic screening, we were able to demonstrate that tumor regressions were associated with the recognition by the administered TIL of unique somatic mutations that occurred in the cancer.
- We also found that TIL from 29 of 32 patients with a wide variety of metastatic gastrointestinal cancers contained lymphocytes that recognized unique mutations presented in that patient's cancer.
- We, therefore, aim to use this pipeline to identify immunogenic neoantigens and to predict for neoantigens binding the patient human leukocyte antigen (HLA) molecules from melanoma or epithelial cancer patients and to use these epitopes for a personalized therapeutic messenger RNA (mRNA) vaccine.

Objectives:

- Primary objectives:
  - Determine the clinical response rate in patients with metastatic melanoma, gastrointestinal, or genitourinary cancers who receive NCI-4650.
  - Determine the safety of NCI-4650 in patients with metastatic melanoma, gastrointestinal, or genitourinary cancers.

Eligibility:

- Age ≥ 18 years and ≤ 70 years
- Evaluable metastatic melanoma, gastrointestinal, or genitourinary cancers refractory to standard of care treatment.
- Metastatic cancer lesions suitable for surgical resection to perform whole exome sequencing and preparation of TIL.

Design:

- Patients with metastatic cancer will undergo surgical resection of tumor followed by exome and RNA sequencing to identify expressed mutations. This will be conducted under the NCI-SB cell harvest protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).
- Immunogenic neoantigens will be identified from TIL by high throughput immunologic screening using long peptides and tandem minigenes covering all mutated epitopes.
- Up to 15 predicted neoantigens will be selected based on exome and RNA sequencing and their binding affinity to the patient HLA molecules.
- The mRNA vaccine will be manufactured and supplied as cGMP product by ModernaTX, Inc.
- The patient will be vaccinated with mRNA containing epitopes from immunogenic neoantigens, predicted neoantigens, and mutations in tumor suppressor or driver genes.
- The mRNA vaccine will be administered intramuscularly (IM) for four cycles every two weeks. A patient may receive a second course for a total of eight cycles.
- Blood samples will be taken every two weeks (during the vaccination period) and at each follow-up visit, and patients will be monitored for the quantity and quality of circulating neoantigen-specific T-cells.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

- Determine the clinical response rate in patients with metastatic melanoma, gastrointestinal, or genitourinary cancers who receive NCI-4650.
- Determine the safety of NCI-4650 in patients with metastatic melanoma, gastrointestinal, or genitourinary cancer.

1.1.2 Secondary Objective

- Determine whether administration of NCI-4650, a mRNA-based, personalized cancer vaccine (PCV) presenting immunogenic neoantigens, can increase the quantity and quality of circulating antigen-specific T-cells in patients with solid tumors.

1.2 BACKGROUND AND RATIONALE

1.2.1 Cancer Vaccines

Protective vaccination against infectious diseases has proven to be one of the most effective health measures. Therapeutic vaccination against established diseases such as persistent infections and cancer has proven much more challenging. Cancer vaccines are designed to target antigens that can elicit an immune response that selectively attacks cancer cells. Ideally, these antigens should be exclusively presented on cancer cells. Until recently, vaccines against non-viral tumors mainly targeted differentiation antigens, cancer testis antigens, and overexpressed antigens. However, central immunological tolerance has limited the generation of high-avidity reactivity against these normal antigens[1, 2]. Thus, T-cells prompted using these vaccines underwent depletion of high-avidity clones directed against such antigens. This depletion causes the loss of T-cells bearing high-affinity T-cell receptors (TCR) for their cognate antigens, which have the superior cytotoxic capacity, longer persistence in the tumor microenvironment, and decreased susceptibility to immune suppression[3]. Taken together, such depletion can lead to impaired clinical efficiency following vaccine administration.

In recent years, our group and others have extensively studied the importance of neoantigens as targets for immunotherapy. It is now clear that neoantigen-specific T-cells are present in most cancers. Neoantigens derived from somatic mutations offer a specific and highly immunogenic target for vaccination. Today, with the rapid development of technologies for DNA sequencing, the identification of those mutations becomes widely feasible.

Evidence for the power of neoantigen vaccines in pre-clinical and clinical settings was recently published. The first proof of concept of neoantigen vaccines for cancer prevention was shown in the B16 mouse melanoma model[4]. By prophylactic vaccination with two mutated epitopes, the researchers achieved complete tumor protection in 40% of the mice. In therapeutic settings, they observed significant tumor growth inhibition induced by mutation-specific peptide vaccination. Recently, the same group published evidence for the potential of neoantigen vaccines as tested in three independent murine models[5]. In this paper, the researchers showed that a considerable fraction of the non-synonymous mutations in the cancer were immunogenic, and the majority of them were recognized by $CD4^+$ T-cells. Vaccination with these $CD4^+$ epitopes induced potent tumor control and rejection of established tumors. Similar results were published in another independent mutation-based cancer model in mice. In this work, the authors developed a pipeline

to identify immunogenic mutant peptides using whole-exome and transcriptome sequencing together with mass spectrometry. Again, the researchers were able to show that vaccination with mutation-specific peptides induced therapeutically active T-cell responses. Results of the current pre-clinical data support the evaluation of neoantigen vaccines as an effective cancer treatment.

The first human trial involving a personalized cancer vaccine was conducted by a group from Washington University in St. Louis which reported on a first-in-man clinical trial evaluating neoantigen vaccines in patients with metastatic melanoma[6]. These researchers vaccinated three patients with mature dendritic cells presenting seven predicted mutation-specific HLA-A*02:01 restricted epitopes. They showed that vaccination with high-affinity, patient-specific mutated epitopes augmented T-cell immunity directed against naturally occurring dominant neoantigens and broadened the response by revealing subdominant neoantigens; but no clinical responses were observed. However, this did show that vaccination against mutated epitopes was safe as none of the three patients experienced any autoimmune adverse events (AEs).

Recently, two trials involving personalized cancer vaccines in patients with advanced melanoma were published[7, 8]. The first[7] used synthesized, clinical-grade long peptides targeting up to 20 neoantigens per patient, admixed with the Toll-like receptor 3 (TLR3) and melanoma differentiation-associated protein 5 (MDA-5). This trial demonstrated the feasibility, safety, and immunogenicity of a vaccine that targets up to 20 predicted personal tumor neoantigens where the vaccine-induced polyfunctional $CD4^+$ and $CD8^+$ T-cells targeted 58 (60%) and 15 (16%) of the 97 unique neoantigens used across patients, respectively. These T-cells discriminated mutated from wild-type antigens, and in some cases directly recognized autologous tumor. Of six vaccinated patients, four had no recurrence at 25 months after vaccination, while two with recurrent disease were subsequently treated with anti-programmed cell death-1 (anti-PD-1) therapy and experienced complete tumor regression, with expansion of the repertoire of neoantigen-specific T-cells. The authors concluded that these data provide a strong rationale for further development of this approach, alone and in combination with checkpoint blockade or other immunotherapies.

The second trial[8] implemented an RNA-based poly-neoepitope approach to mobilize immunity against a spectrum of cancer mutations also in patients with melanoma. All patients in this trial developed T-cell responses against multiple vaccine neoepitopes at up to high single-digit percentages. Vaccine-induced T-cell infiltration and neoepitope-specific killing of autologous tumor cells were shown in post-vaccination resected metastases from two patients. The cumulative rate of metastatic events was highly significantly reduced after the start of vaccination, resulting in a sustained progression-free survival. Two of the five patients with metastatic disease experienced vaccine-related objective responses. One of these patients had a late relapse owing to outgrowth of β2-microglobulin-deficient melanoma cells as an acquired resistance mechanism. A third patient developed a complete response to vaccination in combination with PD-1 blockade therapy. These authors concluded that individual mutations can be exploited by way of personalized immunotherapy for patients with cancer.

1.2.2 Identification of Cancer-Specific Immunogenic Epitopes Derived from Somatic Mutations

The NCI-SB has developed a process to identify immunogenic T-cell epitopes derived from neoantigens (**Figure 1**). The identification of these antigens is done in four main stages: (1) Whole exome sequencing and RNA-seq analysis of the tumor and a matched normal apheresis

sample to identify highly expressed somatic mutations; (2) Construction of tandem minigenes and synthesis of long peptides covering these mutations; (3) Expression of the constructs in autologous antigen-presenting cells; and, (4) *In vitro* co-culture assay to identify T-cells recognizing mutated epitopes from TIL[9]. In earlier studies done by the NCI-SB (published and unpublished data), 25 patients with melanoma were screened in the above manner, and 64 antigenic somatic mutations were identified with no overlapping neoantigens between patient tumors[10, 11].

Recently, a study to identify antigenic mutations from patients with epithelial cancers, including those of the gastrointestinal tract, genitourinary tract, and breast, identified 57 non-overlapping somatic mutations. In this study, two patients were identified who developed a T-cell response against the mutated KRAS oncogene[12]. Thus far, more than 121 immunogenic epitopes have been identified from multiple cancer types including melanoma, ovarian, colorectal, lung, and breast cancers[9, 10, 12-15]. This process, which can be applied to any cancer type and can be completed in several weeks, ensures that the selected antigens are not just expressed in the tumor but can also prompt a significant T-cell-mediated immune response.

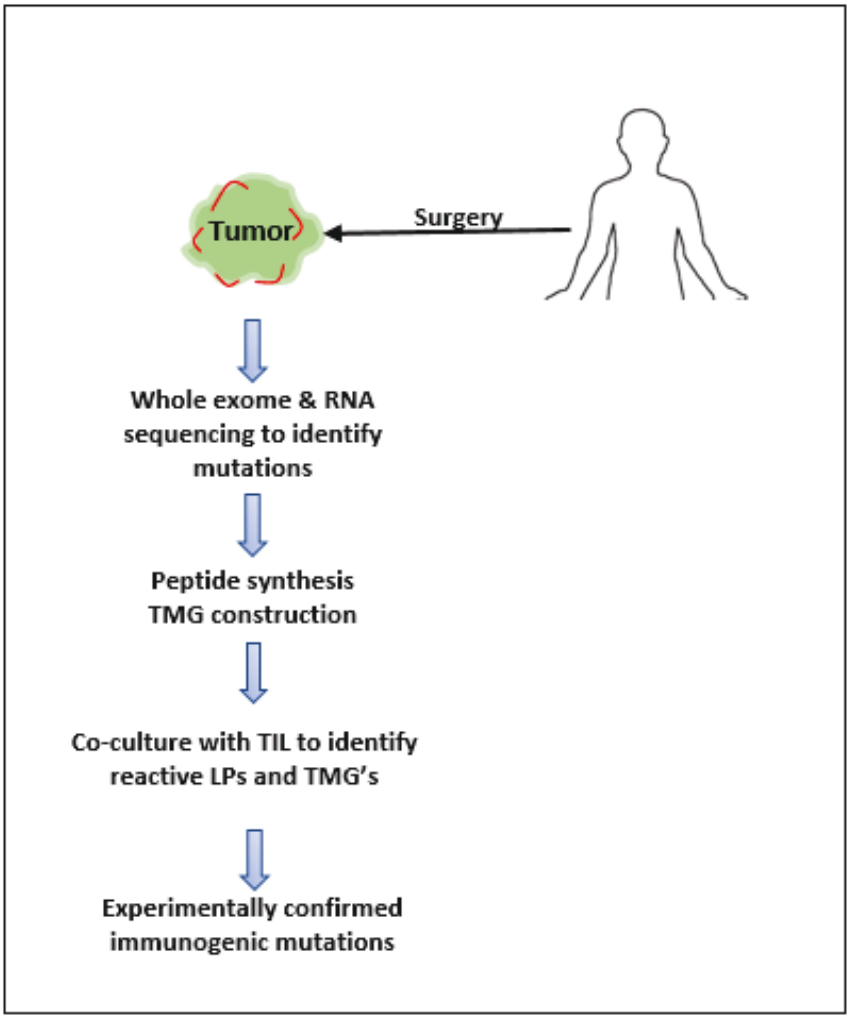


Figure 1. *A pipleline for the identification of immunogenic somatic mutations.*

1.2.3 Overview of mRNA-4650, a mRNA-Based, Personalized Cancer Vaccine

ModernaTX, Inc. (Moderna) is developing a new mRNA technology that allows for induced production of a broad array of secreted, membrane-bound and intracellular proteins both in cell culture and in many animal models and humans. The mRNA vaccines are produced by a well-controlled, characterized, scalable process that is agnostic to the antigen being produced. Additionally, host cell production and presentation of the antigen more closely resemble viral antigen expression and presentation compared to an exogenously produced, purified, and formulated protein antigen. This offers advantages in speed, precision, and adaptability of antigen design and production that cannot be replicated by conventional platforms, which may be especially valuable for personalized cancer vaccines (PCV). This mRNA vaccine production process allows for rapid mRNA production and formulation (within approximately eight weeks) at sufficient quantities to support individualized manufacture and dosing in a clinical study. Moreover, this mRNA-based vaccine technology overcomes the challenges other nucleotide approaches pose such as preexisting immunity or antivector immunity for viral vectors, the risk of genome integration, or the high doses and devices needed (e.g., electroporation) for DNA-based vaccines.

Using a lipid nanoparticle (LNP), Moderna has generated very high levels of transient expression of several different types of proteins (including those for prophylactic vaccines for infectious diseases), in several animal species as wells as humans. The approach also avoids the use of conventional immunostimulatory compounds for enhancement of immunogenicity. Specifically, Moderna has demonstrated that LNP-based, modified-mRNA vaccine technology can generate robust and protective immune responses in mice, ferrets, and cynomolgus monkey, and initial data from early trials in humans have shown such mRNA-based vaccines to be safe and well-tolerated. As an example, Moderna has shown that a range of doses of formulated mRNA encoding the hemagglutinin (HA) protein of either H7N9 or H10N8, can stimulate rapid, robust, long-lasting, and dose-related immune responses as measured by a hemagglutination inhibition (HAI) test, microneutralization assay, and protection from viral challenge[16]. Importantly, mRNA is a potent and flexible component for vaccination, inducing rapid, durable, and high levels of antigen-specific B- and T-cell responses to protein antigens encoded by mRNA. Moreover, mRNA vaccines can also induce potent T-cell responses to murine and human neoantigens, while not inducing strong responses to epitopes derived from the wild-type sequences[5].

For this clinical trial, Moderna will manufacture NCI-4650 encoding immunogenic and predicted neoantigens expressed by the patient autologous tumor identified by the NCI-SB. The goal of the approach is to prime, activate, and expand a diverse population of neoantigen-specific T-cells against these immunogenic and predicted neoantigens for the treatment of solid tumors. As part of this approach, next-generation sequencing (NGS), HLA epitope prediction, and high throughput epitope screens will be employed to enable robust and consistent vaccine design within this clinical study.

1.2.4 Preclinical Experiments Evaluating the Safety of mRNA-Based, Personalized Cancer Vaccines

To support the proposed Phase I clinical study, the safety and tolerability of a similar, mRNA-based PCV, mRNA-4157 (IND 17390), was evaluated in a Good Laboratory Practices (GLP)-compliant one-month toxicity study, in outbred Sprague Dawley® rats at 0.012, 0.035, and 0.117 mg per dose via IM injection administered weekly for a total of 5 doses, and followed by a

14-day post-study recovery period. These doses approximate to human equivalent doses (HED) of 0.29, 0.97, and 3.51 mg, respectively (based on group mean body weights of 0.46, 0.40, and 0.37 kg in rats, respectively, and using an average human body weight of 70 kg), and thus approximate margins of 7-, 24- and 88-fold over the human starting dose of 0.04 mg. The study involved a more dose-dense regimen than the proposed human dosing frequency of every 2 weeks which is being used herein. Although more frequent administration of antigens can impact immunogenic responses, it is unlikely that more frequent dosing would interfere with immunogenicity of this drug product as the antigens expressed are not intended for the rodent and are selected to cross a broad spectrum of major histocompatibility complex (MHC) specificities. A more aggressive regimen than the intended clinical dosing frequency was selected to thoroughly characterize drug product toxicity. Although the vaccine design of NCI-4650 is 100% personalized (i.e., sequences coding for tumor-associated neoantigens unique to the subject and predicted to be most immunogenic will be administered for each subject), the sequence tested in this toxicology study represented a human-based sequence of comparable size and composition to the NCI-4650 that will be generated for each subject in the clinical study, and was manufactured in a similar manner to how NCI-4650 will be manufactured in this clinical study, with the exception that the immunogenic neoantigens coded by NCI-4650 will be determined by laboratory techniques at the NCI-SB and bioinformatics prediction algorithms described in Section 3.2.R in IND 17390. The primary driver of safety that can be adequately assessed non-clinically is that of the LNP and mRNA chemistry, and any immunological effects of antigen expression is likely to be subject-specific and more adequately assessed clinically.

The results of the repeat-dose toxicity study in rats indicated that mRNA-4157 was tolerated at all dose levels (no mRNA-4157 related mortality was observed). In addition, no changes in body temperature (measured indirectly by clinical observation), serum chemistry, urinalysis, or ocular findings were noted. Weekly administration of mRNA-4157 to Sprague Dawley rats (both male and female) at dose levels of 0.012, 0.035, and 0.117 mg was associated with a dose-dependent slight to mild decrease in body weight gain at doses $\geq$ 0.012 mg and this correlated with reduced food consumption. Clinical observations of injection-site reaction (ISR) were observed at all doses and included erythema (slight to mild) and edema (slight to moderate) that increased in incidence and severity with increasing dose. These clinical observations were associated with gross and microscopic changes at the injection site and associated enlargement of lymph nodes. Hematology changes consistent with inflammation were also observed and included mild to moderate increased white blood cells (WBCs) (driven primarily by neutrophils), and a decrease in lymphocytes. Systemic effects were primarily considered responses to local inflammation at the injection site. At the end of the recovery period, all changes were fully or partially resolved (absolute body weights remained lower than controls).

1.2.5 Preclinical Experiments Evaluating the Immunogenicity of a mRNA-Based, Personalized Cancer Vaccine in Mice

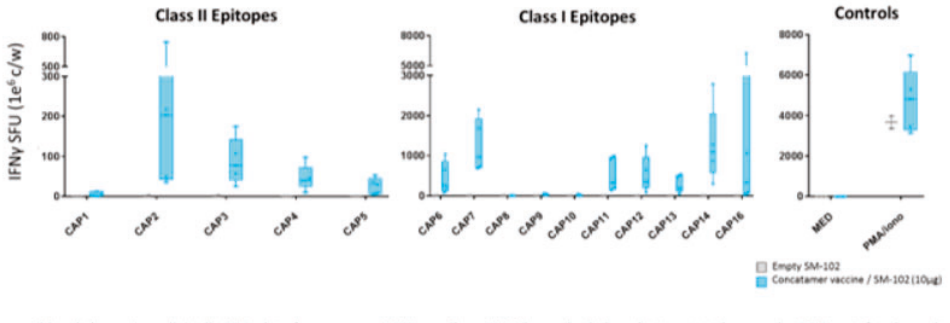
To determine the ability of a poly-epitope concatemer mRNA vaccine to generate T-cell responses to multiple epitopes in C57Bl/6 mice, we produced a concatemer mRNA vaccine containing 15 known mouse epitopes (sourced from Immune Epitope Database [IEDB]) that bind to alleles H-2-Kb, H-2-Db, and H-2-IAb in C57Bl/6 mice (**Table 1**). Mice were vaccinated on Days 0 and 14, and spleens were harvested on Day 21. Splenocytes were restimulated with peptides corresponding to the vaccine epitopes and positive responses were detected via IFN-γ ELISpot assay. Positive T-cell responses were measured for 4 of 5 Class II epitopes targeting

IAb, 3 of 5 Class I epitopes targeting Kb, and 5 of 5 Class I epitopes targeting Db (**Figure 2**). Restimulation with vehicle produced no T-cell responses and robust responses were detected to phorbol myristate acetate/ionomycin in both the empty SM-102 and concatemer vaccine groups. As expected, the negative control SM-102 vaccine containing no mRNA did not induce T-cell responses upon peptide restimulation. Positive T-cell responses were measured for 4 of 5 (CAP2-CAP5) Class II epitopes targeting IAb, 3 of 5 (CAP6, CAP7, and CAP9) Class I epitopes targeting Kb, and 5 of 5 (CAP11-CAP16) Class I epitopes targeting Db (**Figure 2**) when splenocytes from mice vaccinated were restimulated with peptides corresponding with epitopes in the vaccine. These data demonstrate the ability of a poly-epitope concatemer vaccine to elicit robust T-cell responses to multiple epitopes targeting distinct Class I and Class II alleles in a single mRNA construct. The vaccine design used in this study is similar to the proposed design for the clinical trial; these data provide evidence that concatemerizing multiple epitopes together in a single mRNA sequence will lead to effective protein translation, processing, and presentation of the epitopes on MHC molecules. Although capable of eliciting T-cell responses against known T-cell epitopes, we decided to further determine if neoantigen-encoding mRNA vaccines can elicit antigen-specific T-cell responses in C57Bl/6 mice.

Epitope ID #	IEDB Epitope ID #	Peptide Sequence	Binding MHC Allele
CAP 1	243723	WFPAEPEDV	H-2-IAb
CAP2	150992	GRWFMRAAQAVTAVV	H-2-IAb
CAP3	55953	RSRYLTAAAVTAVLQ	H-2-IAb
CAP4	186923	QLIFNSISARALKAY	H-2-IAb
CAP5	86785	NISGYNFSLGAAVKA	H-2-IAb
CAP6	61086	SSIEFARL	H-2-Kb
CAP7	66504	TSYKFESV	H-2-Kb
CAP8	76205	YTVKYPNL	H-2-Kb
CAP9	66266	TSINFVKI	H-2-Kb
CAP10	56055	RTFSFQLI	H-2-Kb
CAP11	58282	SGVENPGGYCL	H-2-Db
CAP12	17516	FQPQNGQFI	H-2-Db
CAP13	53112	RAHYNIVTF	H-2-Db
CAP14	23911	HGIRNASFI	H-2-Db
CAP16	6972	CSANNSHHYI	H-2-Db

Abbreviations: ID, identification; IEDB, Immune Epitope Database; MHC: major histocompatibility complex.

Table 1. *Concatemer mRNA vaccine epitopes.*



Abbreviations: c/w, cells/well; IFNγ, interferon gamma; MED, medium; PMA/iono, phorbol myristate acetate/ionomycin; SFU, spot forming units.

Figure 2. *Concatemer mRNA vaccine epitopes.*

To test the immunogenicity of neoantigen specific mRNA vaccine, mice were vaccinated either subcutaneously (SQ) or intravenously (IV) twice (prime day 0, boost day 14) with three mRNA vaccines (given in a single injection), with each vaccine containing an mRNA sequence encoding 1 of 3 mutant epitopes identified by Yadav and colleagues[17] (**Figure 3**) in the MC38 tumor cell line. Three days after, the boost spleens were harvested and cells were restimulated with peptides corresponding to the mutant or wild-type peptide sequences for the 3 neoantigens. To compare the mRNA vaccine to a common peptide vaccine, mice were also vaccinated intraperitoneally with long peptides corresponding to the neoantigens sequence together with PolyI:C and anti-CD40 antibody. To evaluate the phenotype of the neoantigen-specific T-cells elicited by the vaccine, T-cells were also tested for CD62L and CD44 expression.

A

MC38 Neoantigens

Neo-antigen	Peptide 25mer Sequence
Adpgk	IPVHLELASMTNMELMSSIVHQQVF
Dpagt1	LVISASIIVFNLLELEGDYRDDHIF
Resp1	GRVLELFRAAQLANDVVLQIMELCG

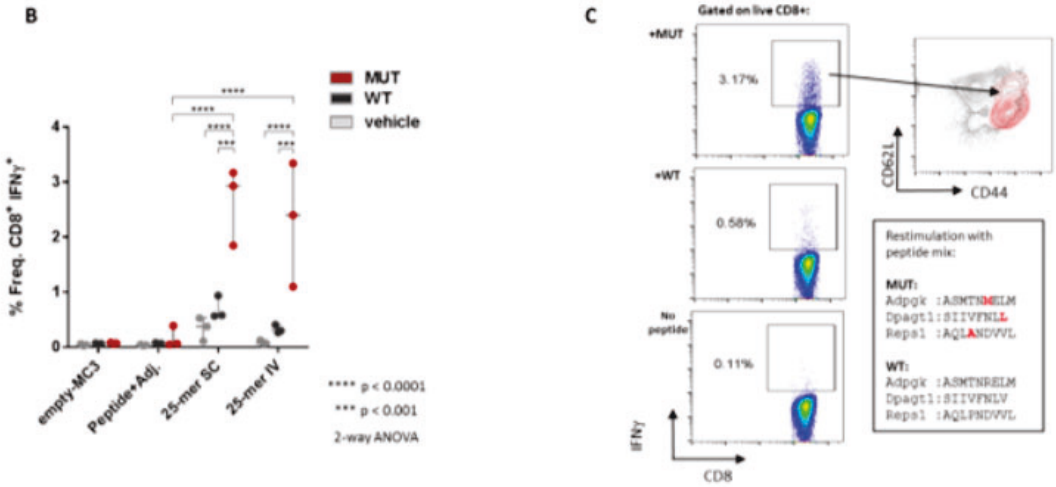


Figure 3. *MC38 neoantigen-specific T-cell responses in C57Bl/6 mice.*

Strong $CD8^+IFN\text{-}\gamma^+$ T-cell responses were detected from splenocytes of mice vaccinated SQ or IV with the combination mRNA vaccine containing the three mutant neoantigens when restimulated with the mutant peptides (**Figure 3**); the mutant-specific responses were significantly higher than the responses measure to wild-type peptides or when there was no peptide restimulation (vehicle). Responses were comparable for IV and SQ administration of the mRNA vaccine, and vaccination with peptide+adjuvant+anti CD40 against the neoantigens resulted in minimal/low specific T-cell responses upon restimulation with mutant peptides (**Figure 3** and **Table 2**) that were significantly lower than the responses measured with mRNA vaccines. The neoantigen-specific T-cells responding to restimulation with mutant peptide were demonstrated to be primarily of the $CD62L^{lo}$ and $CD44^{hi}$ phenotype, resembling an effector memory cell type (representative flow cytometry plots are included in (**Figure 3**).

Vaccination	Mouse1 (% IFNγ)	Mouse 2 (% IFNγ)	Mouse 3 (% IFNγ)	Mean (SD) (% IFNγ)
MUT peptide restimulation				
25-mer SC	2.93	3.17	1.85	2.65 (0.70)
25-mer IV	1.10	3.34	2.40	2.28 (1.12)
Peptide+Adj.	0.067	0.052	0.39	0.17 (0.19)
Empty-MC3	0.085	0.073	0.063	0.07 (0.01)
WT peptide restimulation				
25-mer SC	0.94	0.58	0.57	0.70 (0.21)
25-mer IV	0.30	0.27	0.41	0.33 (0.07)
Peptide+Adj.	0.058	0.069	0.084	0.07 (0.01)
Empty-MC3	0.067	0.047	0.076	0.06 (0.01)
No peptide restimulation				
25-mer SC	0.53	0.11	0.38	0.36 (0.18)
25-mer IV	0.072	0.069	0.13	0.09 (0.03)
Peptide+Adj.	0.033	0.043	0.052	0.04 (0.01)
Empty-MC3	0.044	0.064	0.03	0.05 (0.02)

Abbreviations: 25-mer, 25 amino acids in length; Adj, adjuvant; IFNγ, interferon gamma; IV, intravenous; MC3, DLin-MC3-DMA; MUT, mutant; SC, subcutaneous; WT, wildtype.

Table 2. *MC38 neoantigen-specific T-cell responses in C57B1/6 mice.*

To summarize, robust neoantigen-specific $CD8^+IFN\text{-}\gamma^+$ T-cell responses were induced in mice vaccinated, either SQ or IV, with the combination vaccine containing three mutant sequences; the T-cell responses were comparable between the SQ and IV vaccinated groups. The neoepitope-specific T-cells generated through mRNA vaccination had an effector memory phenotype which is thought to be necessary for a successful anti-tumor response[18]. The antigen-specific responses to the mutant peptides were significantly higher than responses to restimulation with either the wild-type antigen sequences or vehicle, demonstrating the ability of the neoepitope mRNA vaccine to produce highly-specific responses. In addition to being specific, the response to mRNA vaccination were also significantly higher than responses to the adjuvanted peptide vaccine, validating the use of the mRNA platform in delivering neoepitopes for the generation of antigen-specific T-cell responses in the context of a PCV. These data demonstrate that mRNA vaccines can induce potent and specific T-cell responses to multiple murine neoantigens.

1.2.6 Preclinical Experiments Evaluating the Presentation of CD4 and CD8 Epitopes by Human Dendritic Cells

To test if human CD4 and CD8 epitopes can be processed and presented from mRNA concatemer, a screening system to evaluate the presentation of two CD8 and two CD4 epitopes in one assay was developed. A concatemeric mRNA encoding 25mer long antigens either on the 3' (CA-48) or the 5' (CA-49) of the mRNA backbone was produced. As a control, we used a single 25mer mRNA corresponding to each epitope and a peptide pool containing all four epitopes. To test antigen presentation, dendritic cells (DC) were transfected with the concatemer constructs or loaded with a peptide pool containing all four epitopes. DCs were co-cultured with peripheral blood mononuclear cells (PBMC) transduced with TCRs recognizing the corresponding antigens, and antigen recognition was evaluated by flow cytometry for 41BB expression and ELISA for IFN-γ (**Figure 4**). The results clearly show that both CD8 and CD4 epitopes can be processed and presented efficiently after transfection with concatemers harboring several CD8 and CD4 epitopes. While known that human DC are the major population of antigen-presenting cells to uptake and process mRNA molecules *in vivo*[19], these results support the assumption a concatemer mRNA vaccine can give rise to widespread T-cell activation upon mRNA delivery.

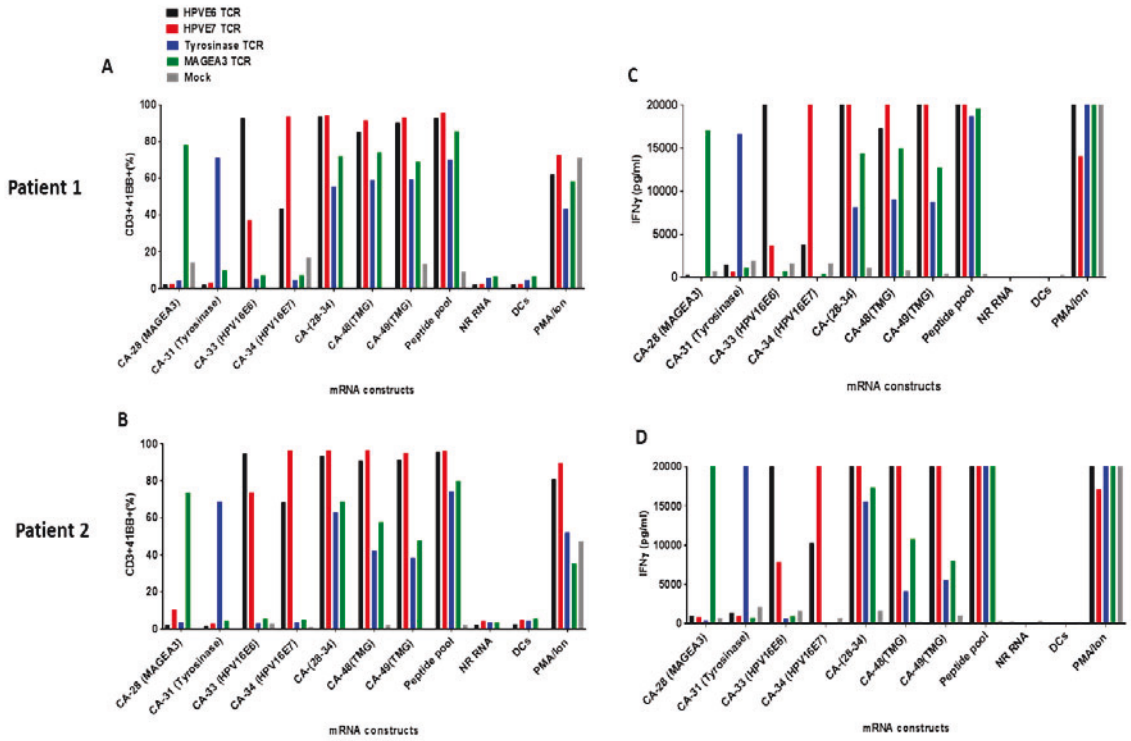


Figure 4. *Presentation of mRNA-derived CD4 and CD8 epitopes by human dendritic cells.*

1.2.7 Rationale for Selecting Defined Epitopes, Predicted Epitopes, and Tumor Suppressor and Driver Neoantigens to Use in this Trial

Historically, preclinical and clinical neoantigen vaccines used epitopes predicted *in silico*. Although *in silico* prediction can select for proper antigens, in many cases there is no evidence that those antigens are processed and presented by the tumor or antigen presenting cells in the microenvironment. As described above, in addition to up to 15 predicted neoantigens, patient TIL will be screened to identify defined mutated antigens (**Table 3**) recognized by T-cells guaranteeing that those antigens are processed and presented for T-cells in the tumor or its draining lymph nodes.

Cancer type	No. of mutations recognized by TIL
Colorectal	38
Pancreas	4
Bile Duct	11
Ovary	7
Endometrium	2
Lung	26
Bladder	3
Esophagus	12
Breast	14
Melanoma	76

Table 3. *Defined mutated antigens recognized by TIL.*

There will be one exception for using predicted or laboratory-derived, immunogenic neoantigens in this trial. There is growing evidence to suggest that targeting tumor driver and suppressor genes can elicit superior clinical response. Mutations in these genes are highly common and have been found in many tumors. When mutations are identified in KRAS, NRAS, p53, EGFR, IDH1, PIK3CA or any other potential driver mutations, nucleotides coding for peptides covering those epitopes will be synthesized and used in the vaccine composition.

Augmenting T-cell responses against defined and driver neoantigens should increase the likelihood of a significant clinical response.

Appendix A describes the three different classes of immunogenic antigens that will be included in the vaccine backbone, and provides the methods for identifying the defined epitopes and selecting the predicted epitopes.

1.2.8 Structure of NCI-4650

A schematic of NCI-4650 is provided in (**Figure 5**). NCI-4650 is chemically identical to naturally occurring mammalian mRNA with the exception that the uridine nucleoside normally

present in mammalian mRNA is fully replaced with N1-methyl-pseudouridine, a naturally occurring pyrimidine base present in mammalian tRNAs[20, 21]. The cap structure used in NCI-4650 is identical to the natural mammalian Cap 1 structure[22, 23]. Each mRNA construct will be between 500 and 3000 nucleotides in length coding for neoantigens between 15 and 41 amino acids in length.

Abbreviations: UTR = untranslated region; ORF = open reading frame.

Figure 5. *Schematic of mRNA-4650 design.*

1.2.9 Risk for NCI-4650 Monotherapy

The risk to subjects receiving NCI-4650 is expected to be low and primarily involving mild to moderate toxicities which have been observed in animal studies and generally observed and expected for other IM administered vaccines. These local reactions may consist of transient and dose-dependent pain, swelling, and erythema. Possible mild to moderate systemic reactions, which are also transient, may include fever, fatigue, chills, headache, myalgias, and arthralgias. Such AEs may, in part, be due to the poor biodegradability of LNP formulations used in other vaccine studies; the LNP that comprises NCI-4650 is designed to have superior biodegradability and thus may have a further improved safety profile. Also, other AEs that have generally been associated with approved IM administered vaccines have included mild hematological and clinical chemistry abnormalities, which are usually reversible. Subjects receiving NCI-4650 could experience signs and symptoms compatible with a C activation-related pseudoallergy (CARPA) response, which has been observed in the administration of approved liposomal products, contrast agents, pegylated proteins, and antibodies[24], as well as for small interfering RNA products formulated in LNPs[25, 26]. The signs and symptoms of CARPA resemble those of an acute hypersensitivity reaction. However, complement activation is far less likely to be associated with clinical signs for LNP products such as NCI-4650, which are administered IM at a much lower dose on a mg/kg basis than other clinical entities that cause CARPA in humans and nonclinical species.

The design of this study allows an escalation of dose with intensive safety monitoring to ensure the safety of subjects, the study will use a traditional “3+3” design. Any potential safety risks, such as ISRs, will be monitored closely throughout the study. Dosing will be staggered by three weeks between the first dose administered to the first patient and the first dose administered to the second patient in Dose Level 2 (starting dose level) of the Phase I Cohort for safety monitoring purposes. Subsequent patients may begin treatment at an interval of one week.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Measurable (per RECIST v1.1 criteria), metastatic melanoma, gastrointestinal, or genitourinary cancer with at least one lesion that is resectable. Only patients with metastatic gastrointestinal cancer will be eligible for enrollment on the Phase I portion of the study. Patients with metastatic melanoma, gastrointestinal, or genitourinary cancer will be eligible for enrollment on the Phase II portion of the study.

2.1.1.2 Confirmation of diagnosis of metastatic cancer by the NCI Laboratory of Pathology.

2.1.1.3 Patients with 3 or fewer brain metastases that are < 1 cm in diameter and asymptomatic are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for one month after treatment for the patient to be eligible. Patients with surgically resected brain metastases are eligible.

2.1.1.4 Prior therapy with at least one first-line standard of care treatment or second-line treatment of proven effectiveness. Patients must have progressive disease after prior treatment. Prior first- or second-line treatments would include the following:

- Patients with metastatic melanoma: Receipt of a checkpoint inhibitor as first-line therapy.
- Patients with metastatic melanoma with an activating mutation of *KIT*: Receipt of imatinib.
- Patients with a BRAF V600 activating mutation: Receipt of appropriate targeted therapy.
- Patients with metastatic gastrointestinal cancer: Receipt of up to two forms of approved first- and/or second-line chemotherapy regimens.
- Patients with metastatic genitourinary cancers: Receipt of a first- or second-line therapy appropriate for their histologic subtype.

2.1.1.5 Age ≥ 18 years and ≤ 70 years.

2.1.1.6 Clinical performance status of ECOG 0 or 1 (see **Appendix B**).

2.1.1.7 Women of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.

2.1.1.8 Serology

- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive may have decreased immune-competence and thus may be less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then the patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

2.1.1.9 Hematology

- ANC > 1000/mm^3 without the support of growth factors.
- WBC ≥ 3000/mm^3
- Platelet count ≥ 100,000/mm^3
- Hemoglobin > 8.0 g/dL. Subjects may be transfused to reach this cut-off.

2.1.1.10 Chemistry

- Serum ALT/AST ≤ 5.0 x ULN
- Serum creatinine < 1.5 x ULN or measured creatinine clearance (calculated using Cockcroft-Gault formula) > 40 mL/min
- Total bilirubin ≤ 2.0 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL.

2.1.1.11 More than four weeks must have elapsed since any prior systemic therapy at the time the patient receives the immunization regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo).

2.1.1.12 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.1.13 Subjects must be co-enrolled on protocol 03-C-0277.

2.1.2 Exclusion Criteria

2.1.2.1 Pregnant or breastfeeding women who do not consent to stop breastfeeding while on study treatment and for 30 days after the use of the investigational vaccine, where pregnancy is confirmed by a positive, rising hCG laboratory test.

2.1.2.2 Women of child-bearing potential, defined as all women capable of becoming pregnant, unless they agree to use an appropriate method of contraception during dosing and for 120 days after the last dose (i.e., final vaccine). Effective contraception methods include a combination of any two of the following (unless method is abstinence or sterilization, in which only one method is required):

- Use of oral, injected, or implanted hormonal methods of contraception, or other forms of hormonal contraception that have comparable efficacy (failure rate < 1%). In case of use of oral contraception, women should have been stable on the same pill for a minimum of 6 months before taking study treatment.
- Placement of an intrauterine device or intrauterine system.
- Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository.
- Total abstinence
- Female sterilization at least eight weeks before taking study treatment.
- Male sterilization (at least six months prior to screening).

2.1.2.3 Sexually active males must use a condom during intercourse during dosing and for 120 days after the last dose (i.e., final vaccine), and should not father a child in this period.

2.1.2.4 Any systemic steroid therapy or other form of immunosuppressive therapy within 7 days of the first dose of the vaccine. A physiologic dose of systemic corticosteroids may be approved. Inhaled or topical steroids, and adrenal replacement steroid doses ≤ 10 mg daily prednisone equivalent are permitted in the absence of active autoimmune disease.

2.1.2.5 Active systemic infections requiring anti-infective treatment, coagulation disorders, or any other active or uncompensated major medical illnesses of the cardiovascular, respiratory, or immune system.

2.1.2.6 Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

2.1.2.7 Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune-competence may be less responsive to the experimental treatment and more susceptible to its toxicities).

2.1.2.8 Any vaccinations four weeks prior to the first vaccination cycle or live vaccines at any time during the study.

2.2 SCREENING EVALUATIONS

Note: Testing for screening evaluation is conducted under the NCI-SB companion protocol, 99-C-0128 (Evaluation for NCI Surgery Branch Clinical Research Protocols).

2.2.1 Within 3 Months Prior to Part Two Registration

- HIV antibody titer, HBsAg determination, and anti-HCV
- Confirmation of diagnosis of metastatic melanoma, gastrointestinal, or genitourinary cancer by the NCI Laboratory of Pathology. (Note: Testing is permitted to be conducted at any time prior to Part Two registration.)

2.2.2 Within 4 Weeks Prior to Part Two Registration

- Complete history and physical examination. (Note: Patient history may be obtained within 8 weeks prior to Part Two registration.)
- Imaging evaluation to determine baseline disease status. This may include CT, MRI, PET, and/or photography.

2.2.3 Within 7 Days Prior to Part Two Registration

- Screening blood tests:
  - CBC w/differential
  - Chemistries: Creatinine, ALT/GPT, AST/GOT, Total bilirubin
  - Lymphocyte phenotyping (TBNK)
- Urinalysis, with culture if indicated

- Physical examination, including ECOG of 0 or 1 (see **Appendix B**)
- Weight and vital signs
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential

2.3 REGISTRATION AND TREATMENT ASSIGNMENT PROCEDURES

2.3.1 Prior to Registration for this Protocol

Patients will initially sign the consent for and enroll on protocol 03-C-0277 prior to tumor harvest and leukapheresis for generation of the cell product. Patients will then sign the consent for and register for Part One of this treatment protocol. If the mRNA vaccine meets the requirements specified in the Certificate of Analysis (CoA) (**Appendix C**) to include USP <71> sterility testing, the vaccine will be released by Moderna and shipped to the NIH Clinical Center Pharmacy, where it will be dispensed and administered to the patient after receipt by the pharmacy of a physician's order. The patient must meet the eligibility criteria and be registered to Part Two of this treatment protocol before receiving the vaccine. If the mRNA vaccine does not meet the requirements specified in the CoA, the vaccine will not be released by Moderna and the patient will be taken off-study (Section **3.8.2**).

2.3.2 Registration Procedure

Registration will be a two-part process. Authorized staff must register an eligible candidate with the NCI Central Registration Office (NCI-CRO) within 24 hours of signing consent. To initially register a subject after the participant has signed the consent, complete the top portion of the registration Eligibility Checklist from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) indicating that the patient is being registered for Part One and send via encrypted email to the NCI-CRO at ncicentralregistration-l@mail.nih.gov.

Once the mRNA vaccine meets the requirements specified in the CoA and the vaccine is released by Moderna, and eligibility is confirmed after completion of screening studies, authorized staff must complete the remainder of the eligibility checklist, indicating that the patient is being registered for Part Two (treatment on study). Email the completed registration Eligibility Checklist to the NCI-CRO at ncicentralregistration-l@mail.nih.gov.

After confirmation of eligibility at the NCI-CRO, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of registration will be forwarded electronically via email to the research team. A recorder is available during non-working hours.

Subjects that do not meet screening criteria should be removed from the study following the procedure in Section **3.8.3**.

Note: Patients are not considered to be fully enrolled on this treatment protocol unless they meet the eligibility criteria and are registered to Part Two.

2.3.3 Treatment Assignment Procedures

2.3.3.1 Cohorts

Number	Name	Description
1	Phase I Gastrointestinal Cancer	First 12-18 patients enrolled with a diagnosis of gastrointestinal cancer
2a	Phase II Melanoma	Patients with a diagnosis of melanoma
2b	Phase II Gastrointestinal or Genitourinary Cancer	Patients with a diagnosis of gastrointestinal or genitourinary cancer

2.3.3.2 Arms

Number	Name	Description
1	Phase I	Escalating doses of mRNA vaccine
2	Phase II	MTD of mRNA vaccine established in Phase I

2.3.3.3 Randomization and Arm Assignment

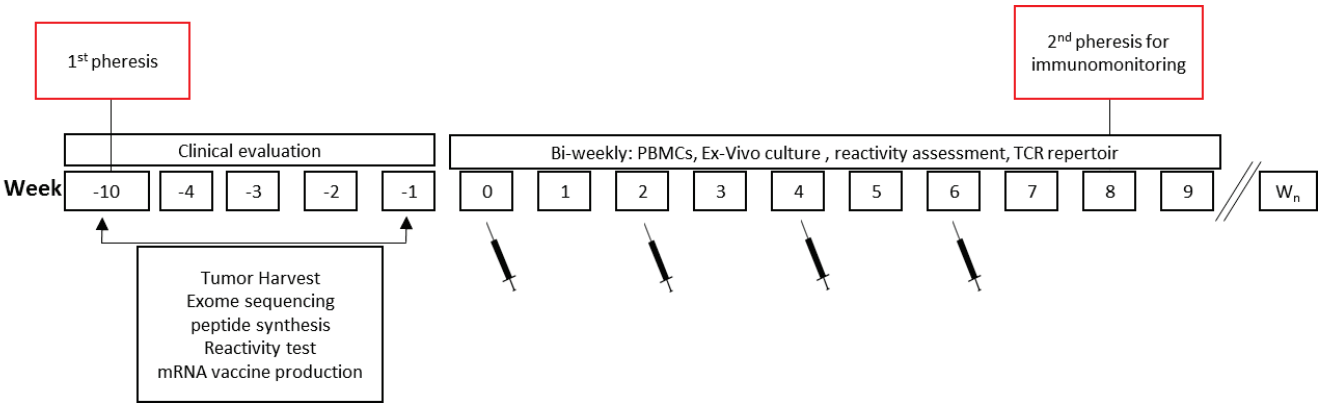
This is a non-randomized study. All patients will be directly assigned based on cohort as follows:

- Subjects in Cohort 1 will be directly assigned to Arm 1.
- Subjects in Cohort 2a will be directly assigned to Arm 2.
- Subjects in Cohort 2b will be directly assigned to Arm 2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Schema



3.1.2 Pre-Treatment Phase: Performed on 03-C-0277

Patients with metastatic melanoma, gastrointestinal, or genitourinary cancers will undergo resection of the tumor and apheresis under protocol 03-C-0277. Whole exome sequencing will be performed under protocol 03-C-0277 to identify all cancer mutations using our standard procedures. TIL will be grown and expanded for this trial according to Standard Operating

Procedures (SOPs) which can be found in BB-IND 17858. After a sufficient yield of TIL ($5x10^7$ cells) is expanded, immunogenic neoantigens will be identified by high throughput immunologic screening using long peptides and tandem minigenes covering all mutated epitopes to identify the exact mutations recognized by autologous T-cells. Up to 15 predicted neoantigens will be selected based on exome and RNA sequencing and their binding affinity to the patient HLA molecules (see **Appendix A**). The final nucleotide sequences of the mutated 15-41mer epitopes and/or driver genes will then be transmitted electronically via Globus, a secure research data management system, to Moderna for production of the NCI-4650 drug product.

3.1.3 Treatment Phase

Patients will receive a mRNA-based vaccine IM at two-week intervals for four cycles. All patients may receive one course of treatment (four vaccination cycles). Immunologic tests will be performed as per Section **5.4** to quantitate circulating T-cells reactive with the immunizing antigens. If a 3-fold increase in the precursor level of pre-existing neoantigen-specific T-cells or new reactivity appears in the blood at detectable levels, patients may be vaccinated with a second and final course of treatment using the same vaccine dose. The second course may start approximately four weeks (± 2 weeks) from the last vaccine dose of the first course.

If the patient receives only one course of treatment (four vaccination cycles), the start date of the course will be the date of the first vaccination; the end date will be the date of the first post-treatment evaluation. If the patient receives a second course (four additional vaccination cycles), the start date of the first course will be the date of the first vaccination. The date of the fifth vaccination will be both the end date of the first course and the start date of the second course. The end date of the second course will be the date of the first post-treatment evaluation.

3.1.4 Dose-Limiting Toxicity

The dose-limiting toxicity (DLT) evaluation period for determination of the maximum tolerated vaccine dose (MTD) will occur during the 21 days after the first vaccination for each patient. A DLT is defined as follows: All grade 3 or greater toxicities related to the mRNA vaccine, **with the exception of**:

- Grade 3 fever
- Grade 3 pruritus/itching
- Grade 3 fatigue
- Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 or less within 7 days.
- Grade 3 autoimmune toxicity that resolves to grade 2 or less within 7 days.
- Events that are clearly related to the patient's disease.

3.1.5 Phase I – Dose Escalation

Subjects will receive their assigned dose level of NCI-4650 as IM injections on Days 0, 14, 28, and 42. The maximum dose of mRNA vaccine for each dose level will be:

Dose Level	NCI-4650, mRNA Vaccine Dose
Level 1	0.04 mg (0.0006 mg/kg based on a 70-kg subject)
Level 2	0.13 mg (0.0019 mg/kg based on a 70-kg subject)
Level 3	0.39 mg (0.0056 mg/kg based on a 70-kg subject)

Moderna is the sponsor of a dose escalation trial utilizing a nearly identical vaccine (mRNA-4157) as the mRNA vaccine used in this protocol (NCI-4650). The dose escalation in both protocols is the same (0.04 mg, 0.13 mg, and 0.39 mg). As indicated in the Moderna Safety Review Committee Dose Level Approval Form dated 21 December 2017, the Moderna Medical Monitor noted that for the three patients enrolled on Dose Level 1 (Part A) in the Moderna trial (0.04 mg mRNA-4157 monotherapy), no DLTs were experienced and the only AEs experienced were grade 1 injection site soreness, which is expected for the vaccine. As a result, Dose Level 1 was declared safe and tolerated. For this trial, because Dose Level 1 was shown to be safe and tolerated, the first two patients were enrolled on Dose Level 2. No DLTs were experienced and the only AEs experienced were grade 1 and 2 related events.

As indicated in the Moderna Safety Review Committee Dose Level Approval Form dated 13 July 2018, the Moderna Medical Monitor noted that for the six evaluable patients enrolled on Dose Level 2 (Part A) in the Moderna trial (0.13 mg mRNA-4157 monotherapy), no DLTs were experienced and the only AEs experienced were mild, non-serious grade 1 events considered to be related to the experimental treatment. Additionally, as indicated in the Moderna Safety Review Committee Dose Level Approval Form dated 07 September 2018, four evaluable patients were enrolled on Dose Level 2 (Part B) in the Moderna trial (0.13 mg mRNA-4157 in combination with pembrolizumab). These four patients experienced only mild, non-serious grade 1 events considered to be related to the experimental treatment, and no DLTs were seen. The following table shows all grade 1 events experienced by all patients enrolled on Dose Level 2 in the Moderna trial, for both parts A and B:

Adverse Events	Incidence	# of Patients
Injection site (edema, erythema, tenderness)	8	3
Diarrhea	2	1
Photosensitivity	1	1
Arthritis	4	3
Finger edema	1	1
Myalgias	4	3
Flu-like symptoms	1	1

For this trial, because Dose Level 2 has been shown to be safe and tolerated, we propose to enroll our third patient (previously anticipated to be the third patient in Dose Level 2) as the first patient in Dose Level 3. The protocol will enroll three patients at Dose Level 3 unless a patient experiences a DLT.

In each dose level, if a patient experiences a DLT, a total of six patients will be treated at that dose to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher dose level. If a level with 2 or more DLTs in 3-6 patients has been identified, patients will be accrued at the next-lowest dose level, for a total of 6 patients. If two DLTs occur in the second dose level (0.13 mg), the dose will be de-escalated to Dose Level 1 (0.04 mg). If two DLTs occur at Dose Level 1, the study will be temporarily halted, as described in Section **3.2**.

The MTD is the highest dose at which ≤ 1 of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels.

Although the MTD may be reached during this clinical study, determining the MTD is not the aim of the study as benefit from this therapeutic vaccine approach may be achieved at lower doses.

3.1.6 Rationale for Dose Level Regimen and Route of Administration

As described in the repeat-dose GLP toxicology studies in outbred Sprague Dawley rats described in Section **1.2.4** and IND 17390, the no-observed-adverse-effect-level (NOAEL) for mRNA-4157 was determined to be 0.035 mg based on the clinical and microscopic findings observed in this study. Based on HED, this represents an approximate 24-fold safety margin over the proposed human starting dose of 0.04 mg. The highest dose in the rat toxicology study was 0.117 mg (0.32 mg/kg), which is an approximate HED of 3.51 mg (0.05 mg/kg based on a 70-kg subject) and is approximately 88-fold higher than the proposed starting clinical dose of 0.04 mg. Thus, it is considered that a starting dose of 0.04 mg has an adequate safety margin.

The highest dose in the GLP rat toxicology study (0.117 mg; which is a HED of 3.51 mg based on a 70-kg subject) is approximately 9-fold higher than the highest planned clinical dose of 0.39 mg. The NCI-SB considers that this represents a sufficient safety margin to the dose levels in this clinical study. The 3+3-guided dose escalation scheme of approximately half-log (3-fold) increments (i.e., 0.04, 0.13, and 0.39 mg) is consistent with FDA guidance for early-phase clinical trials involving cellular and gene therapy products[27].

Subjects will receive their assigned dose level on Day 1 of each 14-day cycle (days 0, 14, 28, and 42). Four cycles of NCI-4650 are planned to boost the intended tumor-killing T-cell population over an extended period by promoting optimal development of T-effector memory cells. The 14-day cycle allows for maximal contraction of the antigen-specific $CD8^+$ T-cells response before boosting, which found to produce best maintenance of $CD8^+$ T-cells with high recall capacity. From a safety perspective, the Manufacturer assessed the toxicity of mRNA-4157 in Sprague Dawley rats at the dose levels of 0.012, 0.035, and 0.117 mg via IM injection every week for 4 weeks (5 doses in total; refer to Section **1.2.4** and IND 17390). This preclinical weekly injection schedule is 2-fold more dose-dense over time than the clinical dosing schedule in this study, and thus provides an additional margin to potential cumulative toxicity.

The IM route of administration was chosen for ease of administration.

3.1.7 Phase II Portion

Patients in Phase II will receive the MTD as determined in Phase I. The Phase II portion of the protocol will consist of two cohorts as defined in Section **2.3.3.1**: Cohort 2a, patients with melanoma and Cohort 2b, patients with gastrointestinal or genitourinary cancer.

The Phase II portion of the study will utilize an optimal design where initially 12 evaluable patients will be enrolled in each cohort. For each cohort, if 0 of the first 12 evaluable patients experience a clinical response, then no further patients will be enrolled; if 1 or more of the first 12 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 21 evaluable patients have been enrolled in each cohort.

Note: As stated in Section **8.2**, up to 6 patients enrolled at the MTD will count towards the accrual in the Phase II portion of the trial if they are evaluable for response and if they would be fully eligible for enrollment in the Phase II portion of the trial.

3.2 PROTOCOL STOPPING RULES

New subject enrollment to the protocol will be temporarily halted if any of the following conditions are met, and consideration will be given to the need for protocol revisions regarding safety, if applicable. Consultation with the FDA may be arranged by the Sponsor before or after the protocol amendment is submitted to the NIH Intramural IRB, as needed.

- During the Phase I portion of the study – If two DLTs, as described in Section **3.1.4**, occur at Dose Level 1.
- During the Phase II portion of the study – Once five or more patients have been enrolled, if 20% or more patients cumulatively enrolled develop a DLT, as described in Section **3.1.4**.
- During the Phase I and II portions of the study – Any death that is at least possibly attributed to the investigational agent and which occurs within 30 days of receiving the investigational agent.

3.3 DRUG ADMINISTRATION

Patients will receive the mRNA vaccine on days 0, 14 (± 5 days), 28 (± 5 days), and 42 (± 5 days). The vaccine will be administered as an IM injection.

The patient's vaccine is delivered to the Patient Care Unit by the research nurse. Prior to the injection, the vaccine identity label is double-checked by two authorized staff (MD or RN), and an identification of the product and documentation of administration are entered in the patient's chart.

- The NCI-4650 mRNA vaccine will be administered as an outpatient procedure in the 3SE Day Hospital in the NIH Clinical Center.
- For all subjects, NCI-4650 will be administered by IM injection into the deltoid muscle (alternating deltoid muscle sites should be used for each subsequent injection, and injection sites may also be rotated to thigh muscles).
- The dose volume for Dose Levels 1 and 2 will be held constant at approximately 0.3 mL per injection. The dose volume for Dose Level 3 (PN 85002) will be at least 0.39 mL.
- The NCI Pharmacy Manual contains specific instructions for the preparation and administration of each dose level of NCI-4650.
- The NCI Pharmacy Manual contains details regarding sterility release of NCI-4650.

3.4 BASELINE EVALUATIONS

3.4.1 Within 7 Days Prior to the First Vaccination (Day 0)

- Physical examination, including ECOG (see **Appendix B**)
- Weight and vital signs
- Baseline blood tests:
  - CBC w/differential
  - Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
  - PT-INR/PTT
  - Anti-CMV antibody titer, HSV and VZV serology, and EBV panel (Note: Patients who are known to be positive do not need to be retested. May be performed within 3 months prior to Part Two registration.)
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- Urinalysis, with culture if indicated
- Chest x-ray
- EKG
- Repeat imaging if not performed within 4 weeks of Day 0. This may include CT, MRI, PET, and/or photography.

3.4.2 Within 2 Days Prior to the First Vaccination (Day 0)

- Lymphocyte phenotyping (TBNK)
- Review of baseline symptoms
- Research samples, as described in Section **5.3**

3.5 ON-STUDY EVALUATIONS

3.5.1 Within 2 Days Prior to Each Subsequent Vaccination (Days 14, 28, & 42 (± 5 days))

- Physical examination, including ECOG (see **Appendix B**)
- Weight and vital signs
- Toxicity assessment, including a review of systems
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein

- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- Research samples, as described in Section **5.3**

3.5.2 After Each Vaccination

- Vital signs will be monitored hourly (± 15 minutes) for two hours. The post-administration monitoring period may be adjusted by the PI.

3.6 POST-TREATMENT (FOLLOW-UP) EVALUATIONS

3.6.1 First Follow-up Evaluation

All patients (unless the patient receives a second course of treatment) will return to the NIH Clinical Center for their first follow-up evaluation on day 56 (± 5 days), which is approximately two weeks after the fourth vaccination. If a patient receives a second course of four vaccines, the first follow-up evaluation will be two weeks (± 5 days) after the eighth vaccination.

At the first follow-up evaluation, patients will undergo:

- Physical examination, including ECOG (see **Appendix B**)
- Weight and vital signs
- Toxicity assessment, including a review of systems
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
- Lymphocyte phenotyping (TBNK)
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- Research samples, as described in Section **5.3**. Approximately 72 mL of blood will be obtained. PBMC will be cryopreserved so that immunologic testing may be performed. This will be performed on protocol 03-C-0277.
- Imaging studies as performed at baseline to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.
- A 5-liter apheresis may be performed. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. This will be performed on protocol 03-C-0277.

3.6.2 Subsequent Follow-up Evaluations

All patients will return to the NIH Clinical Center for their second follow-up evaluation one month (± 5 days) after the first follow-up evaluation. Subsequent follow-up evaluations will take place every 1-2 months for the first year, and then annually for up to five years or until clinically proven progressive disease, whichever comes first. Patients will be co-enrolled and followed on the NCI-SB long-term follow-up protocol 09-C-0161 (Follow-up Protocol for Subjects Previously Enrolled on NCI Surgery Branch Studies). As noted in Section **3.6.1**, additional

apheresis may be performed only at the first follow-up evaluation. It will not be performed at any other post-treatment evaluations.

Note: Patients may be seen more frequently as clinically indicated. Patients who are unable or unwilling to return for follow-up evaluations may be followed via phone or email contact. Patients may be asked to send laboratory, imaging, and physician exam reports performed by their treating physician.

At each scheduled follow-up evaluation, patients will undergo:

- Physical examination, including ECOG (see **Appendix B**)
- Weight and vital signs
- Toxicity assessment, including a review of systems
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
- Lymphocyte phenotyping (TBNK)
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential (complete at all follow-up evaluations within 120 days after the last vaccine)
- Research samples, as described in Section **5.3**. Approximately 72 mL of blood will be obtained at each follow-up visit. PBMC will be cryopreserved so that immunologic testing may be performed. This will be performed on protocol 03-C-0277.
- Imaging studies as performed at baseline to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.

3.7 STUDY ASSESSMENT CALENDAR

Assessments	Screening (Prior to Part Two registration)			Baseline (Prior to first vaccine)		On-Study/Course of Treatment (1 vaccine = 1 cycle)[1]				Post-Treatment Follow-up	
	Within 3 Months	Within 4 Weeks	Within 7 Days	Within 7 Days	Within 2 Days	Day 0	Day 14 (±5 d)	Day 28 (±5 d)	Day 42 (±5 d)	Day 56[2] (±5 d)	Subsequent Follow-up Visits[3]
Confirmation of diagnosis by NCI Lab of Pathology[4]	X										
Medical history[5]		X									
Physical exam		X	X	X			X	X	X	X	X
Performance status (ECOG)[6]			X	X			X	X	X	X	X
Weight			X	X			X	X	X	X	X
Vital signs[7]			X	X		X	X	X	X	X	X
β-HCG pregnancy test[8]			X	X			X	X	X	X	X[9]
Urinalysis[10]			X	X							
EKG				X							
Toxicity assessment[11]					X[12]		X	X	X	X	X
Serology											

1 Assessments must be performed within 2 days prior to each vaccination on Days 14, 28, & 42. Patients may receive a 2nd course of treatment for a total of 8 cycles.
2 If a patient receives a second course of four vaccines, the first follow-up evaluation will be two weeks (± 5 days) after the eighth vaccination.
3 All patients will return to the NIH Clinical Center for their second follow-up evaluation one month (± 5 days) after the first follow-up evaluation. Subsequent follow-up evaluations will take place every 1-2 months for the first year, and then annually for up to five years or until clinically proven progressive disease, whichever comes first. Patients may be seen more frequently as clinically indicated. Patients who are unable or unwilling to return for follow-up evaluations may be followed via phone or email contact. Patients may be asked to send laboratory, imaging, and physician exam reports performed by their treating physician.
4 Confirmation of diagnosis of metastatic melanoma, gastrointestinal, or genitourinary cancer. Testing is permitted to be conducted at any time prior to Part Two registration.
5 Patient history may be obtained within 8 weeks prior to Part Two registration.
6 ECOG of 0 or 1 (see **Appendix B**).
7 After each vaccination, vital signs will be monitored hourly (± 15 minutes) for two hours. The post-administration monitoring period may be adjusted by the PI.
8 Serum or urine; on all women of child-bearing potential.
9 Complete at all follow-up evaluations within 120 days after the last vaccine.
10 With culture if indicated.
11 Including a review of systems.
12 Review of baseline symptoms.

Assessments	Screening (Prior to Part Two registration)			Baseline (Prior to first vaccine)		On-Study/Course of Treatment (1 vaccine = 1 cycle)[1]				Post-Treatment Follow-up	
	Within 3 Months	Within 4 Weeks	Within 7 Days	Within 7 Days	Within 2 Days	Day 0	Day 14 (±5 d)	Day 28 (±5 d)	Day 42 (±5 d)	Day 56[2] (±5 d)	Subsequent Follow-up Visits[3]
HIV antibody titer, HBsAg, anti-HCV	X										
Anti-CMV antibody titer, HSV and VZV, EBV panel[13]				X							
Laboratory Procedures											
CBC w/differential			X	X			X	X	X	X	X
Blood chemistries[14]			X	X			X	X	X	X	X
PT-INR/PTT				X							
Lymphocyte phenotyping (TBNK)			X		X					X	X
Additional apheresis[15]										X	
Correlatives[16]											
CPT tubes (SB-CPF)					X		X	X	X	X	X
SST tubes (Figg Lab)					X		X	X	X	X	X
Imaging											
CT, MRI, PET, and/or photography		X[17]		X[18]						X[19]	X[19]
Chest x-ray				X							
Treatment/Intervention											
NCI-4650 mRNA vaccine[20]						X	X	X	X		

13 Patients who are known to be positive do not need to be retested. May be performed within 3 months prior to Part Two registration.
14 **Screening:** Creatinine, ALT/GPT, AST/GOT, Total bilirubin. **All other times:** Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein.
15 A 5-liter apheresis may be performed. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. This will be performed on protocol 03-C-0277, and will only be performed at the first follow-up evaluation.
16 Research samples, as described in Section **5.3**.
17 Imaging evaluation to determine baseline disease status.
18 Repeat imaging if not performed within 4 weeks of Day 0.
19 As performed at baseline to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g., brain MRI, bone scan.
20 Administered intramuscularly.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete an evaluation safety visit approximately 30 days following the first follow-up evaluation (at the second follow-up evaluation).

3.8.1 Criteria for Removal from Protocol Therapy

Patients will be taken off treatment for the following:

- Completion of second follow-up evaluation
- Grade 3 or 4 toxicity due to the vaccine, excluding those known events such as local injection site reactions, skin rash, pruritus, fatigue, fever and local adenopathy.
- Progression of disease
- Patient requests to be withdrawn from protocol therapy
- Investigator discretion
- Positive pregnancy test

3.8.2 Off-Study Criteria

Patients will be taken off-study for the following:

- Vaccine does not meet CoA requirements
- Completion of study follow-up period
- Progression of disease
- Patients begins a new therapy for their cancer
- Patient requests to be withdrawn from the study
- Significant noncompliance
- Investigator discretion
- Patient lost to follow-up
- Patients who require the use of any medications listed in Section **4** for clinical management.
- Death

All patients will be co-enrolled on protocol 09-C-0161. Patients who are taken off-study for progressive disease or study closure on the treatment protocol will be followed on protocol 09-C-0161.

Once a subject is taken off-study, no further data can be collected on this treatment protocol.

3.8.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify the NCI-CRO when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Update Form from the website

(http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to the NCI-CRO at ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

Use of all medications taken at any time from consent to the last safety follow-up visit must be recorded in the subject's electronic case report form (eCRF). This will include all prescription drugs, herbal products, vitamins, minerals, and over-the-counter (OTC) medications. Any changes in concomitant medications also will be recorded in the subject's eCRF.

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the investigator, including palliative radiation. Blood transfusions are allowed at any time during the study, except to meet eligibility criteria.

- Antineoplastic systemic chemotherapy or biological therapy is prohibited.
- Systemic corticosteroids are prohibited for any purpose other than to treat symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the investigator. If in the interest of safety, the investigator must treat the subject immediately.
- Inhaled or topical steroids, and adrenal replacement steroid doses ≤ 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease.
- Subjects already taking erythropoietin at the time of screening for the study may continue, provided they have been taking it for more than one month at the time study treatment is started. Prophylactic erythropoietin should not be started during screening or the first two weeks of study treatment but may be started thereafter.
- Granulocyte colony stimulating factors should not be used prophylactically during the first two weeks of study treatment. Use of prophylactic colony stimulating factors may be considered thereafter following discussion with the investigator.
- Live vaccines within 30 days prior to the first dose of NCI-4650 and while participating in the study are prohibited. Examples of live vaccines include but are not limited to the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, Bacillus Calmette-Guérin, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines, and are prohibited.
- Radiation therapy to a symptomatic solitary lesion or to the brain may be considered on an exceptional case-by-case basis after documented consultation with the investigator. The subject must have clear measurable disease outside the radiated field. Administration of palliative radiation therapy will be considered clinical progression.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

5 BIOSPECIMEN COLLECTION

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence, as well as to support long-term research efforts within the NCI-SB and with collaborators as specified in protocol 03-C-0277.

The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

Samples will be ordered in CRIS and tracked through the Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without NIH Intramural IRB notification and an executed MTA.

5.1 SAMPLES SENT TO FIGG LAB

- Venous blood samples will be collected in either a 4-mL or an 8-mL SST tube to be processed for serum and stored for future research.
    - Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pick-up.
    - For sample pick-up, page 102-11964.
    - For immediate help, call 240-760-6180 (main Blood Processing Core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
    - For questions regarding sample processing, contact the Blood Processing Core (BPC) at NCIBloodcore@mail.nih.gov or 240-760-6180.
    - The samples will be processed, barcoded, and stored in the Figg lab until requested by the investigator.

5.2 SAMPLES SENT TO SURGERY BRANCH CELL PRODUCTION FACILITY

- Venous blood samples will be collected in 8-mL CPT tubes to be processed and stored for future research.
    - Record the date and exact time of draw on the tube. Blood tubes are kept at room temperature until pick-up.
    - Samples will be picked up by the research nurse or designee and transported to the Surgery Branch Cell Production Facility (SB-CPF) within 24 hours of blood draw.
    - The samples will be processed, barcoded, and stored in SB-CPF.

5.3 SAMPLE COLLECTION SCHEDULE

Collection Point	Test/Assay	Volume Blood (approx.)	Type of Tube	Location of Specimen Analysis
Prior to Each Vaccination	Immune-Monitoring	64 mL 8 mL	CPT SST	SB-CPF Figg Lab

Each Follow-up Evaluation	Immune-Monitoring	64 mL 8 mL	CPT SST	SB-CPF Figg Lab

5.4 IMMUNOLOGICAL TESTING

- Apheresis may be performed prior to and approximately 2 weeks following the administration of the final vaccine product. At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be cryopreserved for immunological monitoring of cell function.
- A variety of tests including evaluation of specific lysis and cytokine release, metabolomic and bioenergetic studies (using Seahorse), intracellular FACS of cytokine production, ELISA-spot assays, and lymphocyte subset analysis may be used to evaluate the immunological correlates of treatment. In general, differences of 2- to 3-fold in these assays over the baseline measurement are indicative of true biologic differences.

5.5 SAMPLE STORAGE, TRACKING, AND DISPOSITION FOR SURGERY BRANCH CELL PRODUCTION FACILITY

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the SB-CPF. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW NCI-SB laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the BPC for storage. Samples will be barcoded and stored onsite or offsite at NCI Frederick Central Repository Services in Frederick, MD. All collected samples (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed and reported as such to the NIH Intramural IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the NIH Intramural IRB.

Blood and tissue collected during the course of this study will be stored, tracked, and disposed of as specified in protocol 03-C-0277.

5.6 SAMPLE STORAGE, TRACKING, AND DISPOSITION FOR FIGG LAB

5.6.1 Sample Data Collection

All samples sent to the BPC will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC, and data will be updated to the NCI-SB central computer database weekly. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password-restricted login screen.

All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the Clinical Center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.6.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the NIH Intramural IRB-approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator (PI) to ensure that the samples requested are being used in a manner consistent with NIH Intramural IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following NIH Intramural IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed and reported as such to the NIH-IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the NIH Intramural IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

Blood and tissue collected during the course of this study will be stored, tracked, and disposed of as specified in protocol 03-C-0277.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password-protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The PI, associate investigators (AI), research nurses, and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards.

Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant. Data will be entered into the NCI CCR C3D database.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for AEs from the time the patient receives the first mRNA vaccine dose to the time of the second follow-up evaluation, or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

All AEs must be recorded on the AE case report form unless otherwise noted below in Section **6.1.1**.

End of study procedures: Data will be stored according to HHS and FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the NIH Intramural IRB will be notified.

6.1.1 Exclusions to Routine Adverse Event Recording

There are no exclusions to routine AE reporting/recording for this protocol.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

Coded, linked human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long-term research efforts within the NCI-SB, and coded, linked data may also be shared with collaborators as specified in protocol 03-C-0277.

6.2.2 Genomic Data Sharing Plan

The NIH Genomic Data Sharing Policy does not apply to this study.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response approximately two weeks following the last vaccination, then one month later x1, then every 1-2 months for the first

year, and then annually for up to five years or until clinically proven progressive disease, whichever comes first. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks (but not less than 4 weeks) following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[28] 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 v1.1 criteria.

6.3.1 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:

- By chest x-ray: ≥ 20 mm
- By CT scan:
  - Scan slice thickness 5 mm or under: ≥ 10 mm
  - Scan slice thickness > 5 mm: double the slice thickness
- With calipers on clinical exam: ≥ 10 mm

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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, and abdominal masses (not followed by CT or MRI) are considered as non-measurable.

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.

6.3.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. 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 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.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

6.3.3 Response Criteria Definitions

6.3.3.1 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 of 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 of diameters while on study.

6.3.3.2 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 PI).

6.3.3.3 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 Patients 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 weeks confirmation**
CR	Non-CR/Non-PD	No	PR	≥ 4 weeks 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 weeks 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 v1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration*." Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients 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.

6.3.4 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).

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.

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.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. 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 website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected Adverse Reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed; or, if an Investigator's Brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the Investigator's Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An unanticipated problem or protocol deviation is serious if it meets the definition of a serious adverse event or if it compromises the safety, welfare, or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the Sponsor, it results in any of the following:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization

- Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-Threatening Adverse Drug Experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the NIH Intramural IRB-approved research protocol.

7.1.9 Non-Compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, NIH Intramural IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

1. Is unexpected in terms of nature, severity, or frequency in relation to:
    a. the research risks that are described in the NIH Intramural IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
    b. the characteristics of the subject population being studied; **AND**
2. Is related or possibly related to participation in the research; **AND**
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NIH INTRAMURAL-IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.2.1 NIH Intramural IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The protocol PI will report in the NIH Problem Form to the NIH Intramural IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease

- All protocol deviations
- All unanticipated problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NIH Intramural IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NIH Intramural IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation, and any corrective action.
2. A summary of any instances of non-compliance.
3. A tabular summary of the following adverse events:
    - All grade 2 **unexpected** events that are possibly, probably, or definitely related to the research
    - All grade 3 and 4 events that are possibly, probably, or definitely related to the research
    - All grade 5 events regardless of attribution
    - All serious events regardless of attribution.

Note: Grade 1 events are not required to be reported.

7.2.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

7.3 IND SPONSOR REPORTING CRITERIA

From the time the subject receives the investigational agent/intervention to the time of the second follow-up evaluation, the investigator must immediately report to the sponsor, using the mandatory MedWatch Form FDA 3500A or equivalent, any serious adverse event, whether or not considered drug-related, including those listed in the protocol or Investigator's Brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur after the second follow-up evaluation, only those events that have an attribution of at least possibly related to the agent/intervention will be reported.

Required timing for reporting per the above guidelines:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day.

Events will be submitted to the Center for Cancer Research (CCR) at CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

All serious adverse events will be reported by the Sponsor (Center for Cancer Research) as per FDA 21 CFR 312.32.

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal Exposure

If a patient becomes pregnant during study treatment, the treatment should be discontinued immediately. Pregnancies occurring during study treatment and for the first 120 days after the last vaccination should be reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent should be documented using the MedWatch Form FDA 3500A or equivalent.

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within one day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1-5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 120 days after the last vaccination.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 120 days after the last dose should, if possible, be followed up and documented.

7.4 Safety Reporting Criteria to the Pharmaceutical Collaborator

The Sponsor (Center for Cancer Research) will provide Moderna with: (1) copies of all safety reports concurrently with their submission to the FDA; (2) a cumulative listing of adverse events (including suspected adverse reactions) on a quarterly basis; (3) notice within 48 hours of case completion with respect to a serious adverse event; and, (4) any other information affecting the safety of human subjects. This information will be submitted to Moderna at drugsafety@modernatx.com.

Note: The quarterly cumulative listing of adverse events (including suspected adverse reactions) will be submitted by the PI/research team to the Sponsor for reporting to Moderna.

7.5 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.5.1 Serious Adverse Event Reports to IBC

The PI (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of the gene transfer product as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the MedWatch Form FDA 3500A or equivalent.

7.5.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the PI (or delegate) shall submit the information described below. Alternatively, the NIH Intramural IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.5.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- Title and purpose of the trial
- Clinical site
- Principal Investigator
- Clinical protocol identifiers
- Participant population (such as disease indication and general age group, e.g., adult or pediatric)
- Total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- Status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed
- If the trial has been completed, a brief description of any study results.

7.5.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- Narrative or tabular summary showing the most frequent and most serious adverse experiences by body system

- Summary of all serious adverse events submitted during the past year
- Summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- If any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- Brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.6 DATA AND SAFETY MONITORING PLAN

7.6.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about enrollment and dose escalation, if applicable, will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the PI or AI. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and/or scientific conduct of the trial, or protocol deviations will be reported to the NIH Intramural IRB using iRIS.

The PI will review AE and response data on each patient to ensure safety and data accuracy. The PI will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.6.2 Sponsor Monitoring Plan

As a Sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subject's protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

7.6.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the SMC. Initial review will occur as soon as possible after the annual NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the PI and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL CONSIDERATIONS

8.1 STATISTICAL HYPOTHESIS

8.1.1 Primary Efficacy Endpoints

- Determine the clinical response rate in patients with metastatic melanoma, gastrointestinal, or genitourinary cancers who receive NCI-4650.
- Determine the safety of NCI-4650 in patients with metastatic melanoma, gastrointestinal, or genitourinary cancer.

8.1.2 Secondary Efficacy Endpoint

- Determine whether administration of NCI-4650, a mRNA-based, PCV presenting immunogenic neoantigens, can increase the quantity and quality of circulating antigen-specific T-cells in patients with solid tumors.

8.2 SAMPLE SIZE DETERMINATION

The study will be conducted using a phase I dose escalation followed by a phase II Simon minimax design in two cohorts: Cohort 2a, patients with melanoma and Cohort 2b, patients with gastrointestinal or genitourinary cancer.

Following dose escalation, the objective will be to determine if NCI-4650, given at the MTD, is associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR+CR rate (p1=0.20) in each of the two cohorts in Phase II.

For each cohort in the Phase II portion of this study, using a two-stage Simon minimax design, with alpha=0.10 (10% probability of accepting a poor therapy) and beta=0.20 (20% probability of rejecting a good therapy), initially 12 evaluable patients will be enrolled. If 0 of the first 12 evaluable patients experience a clinical response, then no further patients will be enrolled in that cohort. If 1 or more of the first 12 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 21 evaluable patients have been enrolled in that cohort. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If 1-2 of the 21 evaluable patients have a clinical response, then this will be considered inadequate for further investigation. If 3 or more of the 21 evaluable patients have a clinical response, then this will indicate that this strategy provides a new approach that may be worthy of further consideration in that cohort. Under the null hypothesis (10% response rate), the probability of early termination per cohort is 54.0%.

The dose escalation portion of the study may require 6 patients per dose level. For purposes of sample size estimation, we will assume that as few as 12 and no more than 18 patients will be

required to perform the initial safety evaluation. In order to complete the dose escalation phase and Phase II, a total of up to 18+46 =64 patients may be required (allowing up to 2 inevaluable patients per Phase II cohort). Up to 6 patients enrolled at the MTD will count towards the accrual in the Phase II portion of the trial if they are evaluable for response and if they would be fully eligible for enrollment in the Phase II portion of the trial. Provided that about 2-3 patients per month will be able to be enrolled onto this trial, approximately 2-3 years may be needed to accrue the maximum number of required patients. However, as adequate responses to proceed to the second stage of accrual may not occur, the trial may end up accruing many fewer patients.

8.3 Populations for Analyses

8.3.1 Evaluable for Toxicity

All patients will be evaluable for toxicity from the time of their first mRNA vaccine dose.

8.3.2 Evaluable for Objective Response

Only those patients who have measurable disease present at baseline, have received at least one course of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated in Section **6.3.3**.

8.3.3 Evaluable for Non-Target Disease Response

Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one course 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.

8.4 Statistical Analyses

8.4.1 General Approach

In the Phase I cohort, the toxicities experienced by patients at each dose level will be reported per dose level. In the two Phase II cohorts, clinical responses will be determined and the fraction with a clinical response will be reported, along with confidence intervals.

8.4.2 Analysis of the Primary Efficacy Endpoints

In the Phase I cohort, the toxicities experienced by patients at each dose level will be reported per dose level. The grade of the toxicity as well as the type of toxicity will be tabulated per dose level.

In the Phase II cohorts, the fraction of patients who experience a clinical response in each cohort will be reported along with 80% and 95% two-sided confidence intervals.

8.4.3 Analysis of the Secondary Efficacy Endpoint

The study will aim to determine whether treatment administration can increase the quantity and quality of circulating antigen-specific T-cells, by measuring the reactivity of circulating cells against the immunizing peptides in Elispot and 41BB upregulation assays. The comparisons will be done using descriptive statistics, without formal statistical testing required.

8.4.4 Safety Analyses

Safety will be monitored by identifying the type and severity of any adverse events. The fraction of patients who experience a DLT will be identified at a given dose level, with information reported about the number and grade of each type of DLT identified.

8.4.5 Intention-to-Treat Analysis

An intention-to-treat (ITT) analysis will be conducted of all patients enrolled to the study. A full CONSORT flow diagram will be included in all ITT analyses.

8.4.6 Planned Interim Analyses

As noted in Section **8.2**, results after the first stage of a Simon minimax two-stage design in each Phase II cohort will be examined to determine if the minimum number of responses has been obtained.

8.4.7 Sub-Group Analyses

There are no planned analyses based on demographic characteristics since the power to do subgroup analyses would be limited and make interpretation difficult.

9 COLLABORATIVE AGREEMENTS

9.1 MODERNATX, INC.

CTA #01051-17

Tumor specific antigens (TSAs) containing between 320 and 639 amino acids will be sent to Moderna (formerly Moderna Therapeutics, Inc.) for the manufacture of a single concatemeric mRNA construct to be formulated into a vaccine for the same cancer patient. Sequencing data will be transferred electronically via the secure, encrypted Globus file transfer system (Globus.org) under the current NCI license for this transfer software. The vaccine product and associated data will be returned to the NCI-SB.

CDA #14358-18

The following data will be transferred to Moderna: (1) raw sequencing data, including tumor DNA whole exome, tumor RNA whole transcriptome, and normal DNA whole exome sequencing data in FASTQ and/or BAM format; (2) somatic variant calling results in VCF format; (3) gene expression quantification results in plain text format; (4) patient HLA types (major alleles: HLA-A, -B, -C, -DRB1, and any other HLA class II alleles that are typed, in 4 digits resolution (e.g., HLA-A:02:01)) in plain text format; (5) a list of all neoantigens that had been tested; and, (6) a list of all neoantigens tested to be immunogenic (i.e., true positives). Moderna will compare the neoantigen ranks predicted by Moderna's algorithm with the true positive and negative data from the NCI-SB. Moderna will also perform an investigation on features that are important for neoantigens to be immunogenic.

Data will be transferred electronically via a secure encrypted Amazon Web Service S3 bucket or via the secure, encrypted Globus file transfer system (Globus.org) under the current NCI license for this transfer software. No physical specimens will be shipped under this agreement. Results from Moderna's neoantigen prediction algorithm (a prioritized list of neoantigens for each patient), and a comparison with NCI-SB's results, will be returned to the NCI-SB.

10 HUMAN SUBJECTS PROTECTIONS

10.1 Rationale for Patient Selection

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand, and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

10.2 Participation of Children

Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic cancer, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

10.3 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (see Section **10.5**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have a pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and OHSRP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 Evaluation of Benefits and Risks/Discomforts

The experimental treatment has a chance to provide clinical benefit though this is unknown. The NCI-SB has extensive experience with adoptive cell therapy (ACT) following treatment with high-dose aldesleukin, however this experimental treatment does not include the standard NCI-SB preparative regimen or high-dose aldesleukin. Although we have seen responses to prior NCI-SB ACT treatments, we do not know if this change in our process will improve patient outcome. The risks associated with ACT are substantial, including, a delay in treatment due to the need to harvest and grow the cells, a surgical procedure (possible major) to obtain tumor for

the cell product, the possibility that a cell product cannot be generated, infection due to the surgical procedure, and death. The risks in this treatment are detailed in Section **11**.

10.5 Risk/Benefit Analysis

Because all patients in this protocol have metastatic or recurrent/refractory locally advanced cancer and limited life expectancies the potential benefit is thought to outweigh the potential risks. The risk/benefit analysis for adults with the capacity to consent, as well as for adults who may become unable to provide consent, is greater than minimal risk because the study treatment may be associated with severe side effects, with the prospect of direct benefit because the treatment may reduce the cancer burden.

10.6 Consent Process and Documentation

Patients are initially consented on protocols 99-C-0128 and 03-C-0277. If the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with the informed consent document for this protocol, who is requested to review it and to ask questions prior to agreeing to participate. The specific requirements, objectives, and potential advantages and disadvantages will be presented to the patient. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The PI, AI, or clinical fellow is responsible for obtaining written consent from the patient.

10.6.1 Telephone Consent for Part One Registration

Because many patients will have previously been treated on another NCI-SB protocol prior to beginning treatment on this protocol, these patients may not be onsite at the NIH Clinical Center in order to sign the informed consent document for this protocol. In these circumstances, in order to complete Part One of the registration procedure (see Section **2.3.2**), the informed consent process and a detailed description of the protocol treatment will be discussed with the potential participant via phone.

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy of the informed consent will be returned via mail to the subject.

The informed consent process will be documented on a progress note by the consenting investigator.

10.6.2 Informed Consent of Non-English Speaking Subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant NIH Intramural IRB-approved consent document, the PI and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant NIH Intramural IRB-approved consent document. Signed copies of both the English version of the consent and the translated short form

will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long-translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24-48 hours if possible).

We request prospective NIH Intramural IRB approval of the use of the short form process for non-English speaking subjects and will notify the NIH Intramural IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

11.1 CELL PREPARATION

The procedure for growing and expanding the autologous young TIL is similar to what is approved by the Food and Drug Administration and used in other NCI-SB TIL clinical studies. TIL will be grown and expanded for this trial according to SOPs which can be found in BB-IND 17858.

11.2 INTRAMUSCULAR INJECTION PREPARATION

11.2.1 NCI-4650, mRNA-Based Personalized Cancer Vaccine

Description: The CoA for the NCI-4650 mRNA vaccine is in **Appendix C**, and the SOPs for the preparation of the mRNA vaccine are found in BB-IND 17858. This product will be provided for investigational use under BB-IND 17858, which is sponsored by the CCR. The vaccine is composed of cGMP grade mRNA. Neoantigens will be identified from a sequence comparison of a patient's tumor and normal DNA sample. An algorithm will predict mutated sequences with a high probability of binding to a patient's specific HLA repertoire. Neoantigens will be prepared as peptides, either as 25mers, with the mutated amino acid residue situated at position 13, or as minimal epitopes. The patient's TIL, cultured from a biopsy sample, will be screened for their ability to recognize these neoantigens pulsed onto dendritic cells. Neoantigens recognized by the patient's TIL will be used to formulate the vaccine. The vaccine will be formulated for each administration as indicated below. The identified immunogenic neoantigen sequences will be sent electronically to Moderna for vaccine manufacturing. Upon receipt of the final NCI-4650 product, vaccine will be administered via intramuscular injection.

Mode of Action: NCI-4650 is taken by antigen-presenting cells that process and present the neoantigens on HLA molecules to tumor-specific cytotoxic T-lymphocytes, leading to a major histocompatibility complex-restricted cytotoxic response against tumor cells bearing these neoantigens.

Formulation: NCI-4650 is a novel mRNA-based, PCV directed against subject-specific tumor neoepitopes. The individual mRNA sequences are designed for each subject based on the pipeline for the identification of immunogenic T-cell epitopes derived from neoantigens recognized by TIL developed by the NCI-SB. Each NCI-4650 vaccine consists of mRNA encoding neoantigens designed specifically to each individual subject's tumor mutanome, and HLA type. Each subject-specific NCI-4650 vaccine is formulated in LNPs comprised of four

lipid excipients: SM-102; 1,2-dimyristoyl-*rac*-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG 2000); 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); and cholesterol. SM-102 is a novel, ionizable lipid that complexes with the mRNA component of NCI-4650 (mRNA product intermediate [MPI]) to promote the formation of the LNPs. The commercially-available lipids, DMG-PEG 2000, DSPC, and cholesterol, also contribute to the overall pharmaceutical properties of the LNPs.

NCI-4650 is provided as a sterile liquid for injection in a 93 mM Tris, 7% propylene glycol, 1 mM DTPA buffer, at a concentration of 1.0 mg RNA/mL.

Stability: Please refer to the NCI Pharmacy Manual.

Storage: NCI-4650 must be stored in a restricted access area under the storage conditions indicated on the product label and in the NCI Pharmacy Manual. Vials should be stored frozen at -20°C (± 5°C) (-13°F to 5°F).

How Supplied: NCI-4650 is supplied as solution for injection in 2-mL glass vials (with a septum stopper). Each vial contains 0.6 mL.

Preparation: The dose volume for Dose Levels 1 and 2 will be held constant at approximately 0.3 mL per injection. The dose volume for Dose Level 3 (PN 85002) will be at least 0.39 mL. The NCI Pharmacy Manual contains specific instructions for the preparation and administration of each dose level of NCI-4650. The NCI Pharmacy Manual also contains details regarding sterility release of NCI-4650.

Administration: For all subjects, NCI-4650 will be administered by IM injection into the deltoid muscle (alternating deltoid muscle sites should be used for each subsequent injection, and injection sites may also be rotated to thigh muscles).

Toxicities: Risk to subjects receiving NCI-4650 is expected to be low and primarily involving mild to moderate injection site reactions, which have been observed in animal studies and generally observed and expected for other IM administered vaccines. These local reactions may consist of transient and dose-dependent pain, swelling, and erythema. Possible mild to moderate systemic reactions, which are also transient, may include fever, fatigue, chills, headache, myalgias, and arthralgias. Such AEs may, in part, be due to the poor biodegradability of LNP formulations used in other vaccine studies; the LNP that comprises NCI-4650 is designed to incorporate biodegradable properties that may have an improved safety profile. In addition, other AEs that have been generally associated with approved IM administered vaccines have included mild hematological and clinical chemistry abnormalities, which are usually reversible. Subjects receiving NCI-4650 could experience signs and symptoms compatible with a C activation-related pseudoallergy (CARPA) response, which has been observed in the administration of approved liposomal products, contrast agents, pegylated proteins, and antibodies, as well as for small interfering RNA products formulated in LNPs. The signs and symptoms of CARPA resemble those of an acute hypersensitivity reaction. However, complement activation is far less likely to be associated with clinical signs for LNP products such as NCI-4650, that are administered IM at a much lower dose on a mg/kg basis than other clinical entities that cause CARPA in humans and nonclinical species.

Special Handling: Please refer to the NCI Pharmacy Manual.

12 REFERENCES

1. Klein L, Hinterberger M, Wirnsberger G, Kyewski B. Antigen presentation in the thymus for positive selection and central tolerance induction. Nature reviews Immunology. 2009;9(12):833-44.

2. Abramson J, Giraud M, Benoist C, Mathis D. Aire's partners in the molecular control of immunological tolerance. Cell. 2010;140(1):123-35.

3. Bos R, Marquardt KL, Cheung J, Sherman LA. Functional differences between low- and high-affinity CD8(+) T cells in the tumor environment. Oncoimmunology. 2012;1(8):1239-47.

4. Castle JC, Kreiter S, Diekmann J, Lower M, van de Roemer N, de Graaf J, et al. Exploiting the mutanome for tumor vaccination. Cancer Res. 2012;72(5):1081-91.

5. Kreiter S, Vormehr M, van de Roemer N, Diken M, Lower M, Diekmann J, et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature. 2015;520(7549):692-6.

6. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science. 2015;348(6236):803-8.

7. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017;547(7662):217-21.

8. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Lower M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature. 2017;547(7662):222-6.

9. Gros A, Parkhurst MR, Tran E, Pasetto A, Robbins PF, Ilyas S, et al. Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. Nature medicine. 2016.

10. Lu YC, Yao X, Crystal JS, Li YF, El-Gamil M, Gross C, et al. Efficient identification of mutated cancer antigens recognized by T cells associated with durable tumor regressions. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(13):3401-10.

11. Robbins PF, Lu YC, El-Gamil M, Li YF, Gross C, Gartner J, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med. 2013;19(6):747-52.

12. Tran E, Ahmadzadeh M, Lu YC, Gros A, Turcotte S, Robbins PF, et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. Science. 2015;350(6266):1387-90.

13. Prickett TD, Crystal JS, Cohen CJ, Pasetto A, Parkhurst MR, Gartner JJ, et al. Durable Complete Response from Metastatic Melanoma after Transfer of Autologous T Cells Recognizing 10 Mutated Tumor Antigens. Cancer immunology research. 2016;4(8):669-78.

14. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science. 2014;344(6184):641-5.

15. Cohen CJ, Gartner JJ, Horovitz-Fried M, Shamalov K, Trebska-McGowan K, Bliskovsky VV, et al. Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes. The Journal of clinical investigation. 2015;125(10):3981-91.

16. Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, Hassett KJ, et al. Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses. Molecular therapy : the journal of the American Society of Gene Therapy. 2017;25(6):1316-27.

17. Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature. 2014;515(7528):572-6.

18. Klebanoff CA, Gattinoni L, Restifo NP. CD8+ T-cell memory in tumor immunology and immunotherapy. Immunol Rev. 2006;211:214-24.

19. Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC, et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature. 2016;534(7607):396-401.

20. Rozenski J, Crain PF, McCloskey JA. The RNA Modification Database: 1999 update. Nucleic acids research. 1999;27(1):196-7.

21. Kariko K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005;23(2):165-75.

22. Kozak M. An analysis of vertebrate mRNA sequences: intimations of translational control. The Journal of cell biology. 1991;115(4):887-903.

23. Fechter P, Brownlee GG. Recognition of mRNA cap structures by viral and cellular proteins. The Journal of general virology. 2005;86(Pt 5):1239-49.

24. Szebeni J. Complement activation-related pseudoallergy: a stress reaction in blood triggered by nanomedicines and biologicals. Molecular immunology. 2014;61(2):163-73.

25. Fitzgerald K, Frank-Kamenetsky M, Shulga-Morskaya S, Liebow A, Bettencourt BR, Sutherland JE, et al. Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial. Lancet. 2014;383(9911):60-8.

26. Coelho T, Adams D, Silva A, Lozeron P, Hawkins PN, Mant T, et al. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. The New England journal of medicine. 2013;369(9):819-29.

27. Yang S, Wang SJ, Ji Y. An integrated dose-finding tool for phase I trials in oncology. Contemporary clinical trials. 2015;45(Pt B):426-34.

28. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47.

13 APPENDICES

13.1 APPENDIX A: METHODS FOR SELECTING DEFINED EPITOPES, PREDICTED EPITOPES, AND TUMOR SUPPRESSOR AND DRIVER NEOANTIGENS

Three different classes of immunogenic antigens will be included in the vaccine backbone:

1. Defined epitopes
2. Up to 15 predicted epitopes
3. Any driver or suppressor genes identified by WES

Identifying Defined Epitopes[12, 14]

Whole exome sequencing is performed on a patient's tumor DNA as well as their matched normal DNA, extracted from the patient's PBL. RNA sequencing is performed on the patient's tumor sample only. Sequencing is performed on an Illumina Nextseq500 or Nextseq550. The output from the sequencer is de-multiplexed and converted to fastq format using Illumina's bcl2fastq program. Reads are then trimmed for quality and to remove and adapter sequence with Trimmomatic software (USADELLAB.org). Once trimmed, Exome reads are aligned to hg19 genome using novoalign from novocraft (novocraft.com) to create initial starting bams. RNA-Seq reads are aligned to hg19 using STAR (https://github.com/alexdobin/STAR) 2pass alignment process. Both RNA-Seq and Exome bam files are preprocessed according to the GATK best practices protocol. Exome SNVs are called using Strelka, Somatic Sniper, Varscan2, and Mutect, and In/Dels are called using Strelka and Varscan2. For single nucleotide variants (SNVs) cutoff criteria for evaluation of a variant is Tumor, and normal coverage of 10 or greater, Tumor variant read count of 4 or greater, tumor variant frequency of 7% or greater and two or greater callers calling that variant. For neoantigens arise from insertions/deletions (In/Dels), the criteria are the same except there are no caller criteria. RNA variants are called with Varscan with no cutoffs. Somatic variants are annotated using Annovar against three separate reference databases (Refgene, Ensembl, UCSC). All variants that pass cutoff criteria and those found in COSMIC regardless of cutoff criteria have neoepitopes generated using an in-house python script. This script produces 25mers with 12 aa flanking the mutation on either side where possible. In the event, it is not possible due to the mutation be located closer than 12 aa from the beginning or end of a transcript, the maximum number of aa that can flank are used. For In/Del mutations the corresponding change is made to the cDNA sequence, and then 12 aa before the mutation (where possible) are extracted as well as all amino acids beyond the mutation up until the first stop codon is encountered. If no stop codon is encountered, the neoepitope will encompass all sequence up to the end of the cDNA transcript. For each non-synonymous substitution mutation identified by next-generation sequencing, both a "minigene" construct encoding the corresponding amino acid change flanked by 12 amino acids of the wild-type protein sequence or a crude 25 mer peptide are made. Multiple minigenes (10-15) are strung together to generate one TMG construct. These minigene constructs are codon optimized, synthesized, and cloned in-frame into a modified pcDNA3.1 vector. This modified vector contains a signal sequence and a DC-LAMP trafficking sequence to enhance processing and presentation, in addition to a poly-A tail to enhance mRNA stability. The nucleotide sequence of all TMGs is verified by standard Sanger sequencing (Genscript). Approximately one μg of the linearized plasmid is used for the generation of IVT RNA using the mmessage mmachine T7 Ultra kit (Life Technologies) as directed by the manufacturer. RNA purity and concentrations are

assessed using a NanoDrop spectrophotometer. RNA is then aliquoted into microtubes and stored at -80 degrees Celsius until use. Crude peptides are pooled at a final concentration of approximately 3 mg/mL per peptide (10-18 peptides per pool).

To isolate TILs, surgically resected tumors are cut into approximately 1-2 mm fragments and placed individually into wells of a 24-well plate containing 2 mL of complete media (CM) containing high dose IL-2 (6000 IU/mL, Chiron). CM consisted of RPMI supplemented with 10 percent in-house human serum, two mM L-glutamine, 25 mM HEPES and ten µg/mL gentamicin. In some cases, after the initial outgrowth of TIL (between 2-4 weeks), selected cultures are rapidly expanded in gas permeable G-Rex100 flasks using irradiated PBMC at a ratio of 1 to 100 in 400 mL of 50/50 medium, supplemented with 5 percent human AB serum, 3000 IU/mL of IL-2, and 30 ng/mL of OKT3 antibody (Miltenyi Biotec). 50/50 media consisted of a 1-to-1 mixture of CM with AIM-V media. All cells are cultured at 37 degrees Celsius with 5 percent CO2. Monocyte-derived, immature dendritic cells are generated using the plastic adherence method. Apheresis samples are thawed, washed, set to 5-10e6 cells/mL with neat AIM-V media (Life Technologies) and then incubated at approximately 1e6 cells/cm2 in an appropriate sized tissue culture flask and incubated at 37 degrees Celsius, 5 percent CO2. After 90 minutes, non-adherent cells are collected, and the flasks are vigorously washed with AIM-V media, and then incubated with AIM-V media for another 60 min. The flasks are then washed again with AIM-V media and then the adherent cells are incubated with DC media. DC media comprised of RPMI containing 5 percent human serum, 100 U/mL penicillin and 100 µg/mL streptomycin, 2 mM L-glutamine, 800 IU/mL GM-CSF (Leukine) and 200 U/mL IL-4 (Peprotech). On days 2-3, fresh DC media is added to the cultures. Fresh or freeze/thawed DCs are used in experiments on day 4-6 after culture initiation. DCs are harvested, washed 1x with PBS, and then resuspended in Opti-MEM (Life Technologies) at 10-30e6 cells/mL. IVT RNA (8 µg) is aliquoted to the bottom of a 2 mm gap electroporation cuvette, and 100 µl of APCs are added directly to the cuvette. The final RNA concentration used in electroporations is thus 80 µg/mL. Electroporations is carried out using a BTX-830 square wave electroporator. DCs are electroporated with 150 V, 10 ms, and 1 pulse. Following electroporation, cells are immediately transferred to polypropylene tubes containing DC media supplemented with the appropriate cytokines. Transfected cells are incubated overnight (12-14 hours) at 37 degrees Celsius, 5 percent CO2. Cells are washed 1x with PBS prior to use in co-culture assays. For peptide pulsing, DCs are harvested and then resuspended at 0.5e6 cells/mL with DC media containing the appropriate cytokines. Long peptides (usually 25mers, Genscript) are dissolved with DMSO and pulsed onto the APCs at approximately 10 µg/mL and incubated overnight at 37 degrees Celsius with 5 percent CO2. The following day, APCs are washed 1x prior co-culture with T cells. For co-culture with APCs, approximately 3.5e4 to 7e4 DCs are used per well of a 96-well plate. Prior to processing the ELISPOT plates, cells are harvested from the plate and processed for flow cytometry analysis described below. All co-cultures are performed in the absence of exogenously added cytokines. For IFN-γ ELISPOT assays, ELIIP plates (Millipore, MAIPSWU) are pre-treated with 50 µl of 70% ethanol per well for 2 min, washed 3x with PBS, and then coated with 50 µl of 10 µg/mL IFN-γ capture antibody (Mabtech, clone: 1-D1K) and incubated overnight in the fridge. Prior to co-culture, the plates are washed 3x with PBS, followed by blocking with 50/50 media for at least 1 h at room temperature (RT). After 20-22 hours of co-culture, cells are harvested from the ELISPOT plates into a standard 96-well round bottom plate for flow cytometry analysis, and then the ELISPOT plates are washed 6x with PBS + 0.05% Tween-20 (PBS-T), and then incubated for 2 hours at RT with 100 µl/well of a 0.22 µm filtered 1

µg/mL biotinylated anti-human IFN-γ detection antibody solution (Mabtech, clone: 7-B6-1, diluent consisted of 1X PBS supplemented with 0.5% FBS). The plate is then washed 3x with PBS-T, followed by a 1-hour incubation with 100 µl/well of streptavidin-ALP (Mabtech, diluted 1:3000 with above diluent). The plate is then washed 5x with PBS followed by development with 100 µl/well of 0.45 µm filtered BCIP/NBT substrate solution (KPL, Inc.). The reaction is stopped by rinsing thoroughly with cold tap water. ELISPOT plates are scanned and counted using an ImmunoSpot plate reader and associated software (Cellular Technologies, Ltd). Expression of the T-cell activation markers OX40 and 4-1BB is assessed by flow cytometry at approximately 18-24h post-stimulation. OX40 is predominantly expressed by activated effector CD4+ T cells, while 4-1BB can be expressed by both activated CD4+ and CD8+ T cells. Cells that were harvested from the ELISPOT plate are pelleted, washed with FACS buffer (1X PBS supplemented with 1 percent FBS and 2 mM EDTA), and then stained with the appropriate antibodies for approximately 30 minutes, at 4 degrees Celsius in the dark. Cells are washed at least once with FACS buffer prior to acquisition on a BD FACSCanto II flow cytometer. The number of live T-cell events collected is usually ranged between 2e3-8e3. Any positive reactivity (3-fold over background of DC only) identified by ELISPOT, 41BB or OX40 is considered as a TIL reactivity against a mutated epitope. To identify the specific antigen, individual peptides comprising each positive TMG or peptide pool are used for co-culture with the reactive TIL. Once identified, an HPLC purified mutated and wild type peptides are synthesized and used to validate immunogenic peptide integrity. A defined neoantigen is considered as a mutated peptide recognized by TIL and showed no reactivity to the wild type epitope. Any defined epitope identified will be included in the vaccine backbone.

The average turnaround time from TIL harvest to identification of immunogenic epitopes is 7-14 weeks (**Table A-1**).

	# pts	Median	Range
Total time from operative resection to clinical decision to treat/not treat with TIL	**20**	**10 weeks**	**7-14 weeks**
Exome Sequencing	20	21 days	8-35 days
Bioinformatic Analysis	20	3 days	1-6 days
Neoepitope Screening	**19**	**7 weeks**	**4-11 weeks**
Reagent Design	20	11 days	2-40 days
Reagent Manufacture:			
Tandem Mini-Genes (external/commercial)	19	22 days	9-41 days
Long peptides (external/commercial)	15	35 days	20-54 days
Long peptides (internal/SB)	13	8 days	7-15 days
Laboratory Assays	19	20 days	4-54 days

Data collected from twenty consecutive operative resections designated for neoantigen-based adoptive cell transfer protocols from 8/2016-3/2017.

Table A-1. Turnaround time for defined neoantigen discovery pipeline.

Selecting Predicted Epitopes

A patients HLAs are predicted from the exome tumor sample, the exome normal sample and the tumor RNA using PHLAT (https://sites.google.com/site/phlatfortype/). If there are differences in allelic predictions the top two most frequently predicted locus for each HLA are used. The 25mers previously generated are then run through netMHCpan-3.0 for each class I HLA and minimal epitopes of 8,9,10,11,12 mer lengths are generated. All predicted binders not containing the mutated amino acid are removed. All 25mers are also ran through netchop-3.1 and scores for proteosomal cleavage for each amino acid are recorded. In order to determine which variants should be screened several steps are taken filtering is performed:

1. Retained variants must possess at least one transcript which has evidence of coding for a protein.
2. Retained variants must be found to be present in at least two of the annotation databases (NCBI, UCSC, Ensembl). The rationale behind this is to remove genes or transcripts with less support
3. Retained variants must not been seen in our sequencing data at a rate greater than 2.5%. This excludes known driver genes as described in Wellcome Sanger's cancer genome census list. For MHC Ranking we created an observed positive rate table from the analysis of > 8000 screened 25mers. A combination of filters was applied to the data and the observed positive rate for all 25mers that remain after that filtering.
    - Filters:
        a. Exome pass 1,0 = yes, no
        b. RNA seen 1,0 = yes, no-filter
        c. Gene expression quartile >= 1,2,3,4
        d. netMHCpan3.0 rank <= 5, 4, 3, 2, 1, .75, .50, .25, 0.1
        e. netCHOP Cterm score>= 0, .1, .2, .3, .4, .5, .6, .7, .8, .9
        f. netCHOP 20S score >= 0, .1, .2, .3, .4, .5, .6, .7, .8, .9
        g. # of minimals that meet last 3 criteria >= 1, 2, 3, 4, 5

Taking all possible combinations there are ~45000 different filtering combinations. Each 25mer is analyzed to see what filtering groups it fits into and the highest observed positive rate from these groups is then assigned to the 25mer. After all retained 25mers have an observed positive rate. The variants with evidence in RNA-seq data are taken 1st sorted by the observed rate and ranked in descending order. After that the same is done to the remaining 25mers. The first 15 ranked 25mers will be included in the vaccine backbone.

13.2 APPENDIX B: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	**Descriptions**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

13.3 APPENDIX C: MRNA-4650 CERTIFICATE OF ANALYSIS

moderna

100 Tech Drive • Norwood, MA 02062
phone 617-714-6500 • fax 617-583-1998

CERTIFICATE OF ANALYSIS

Product Description: mRNA-4650 Labeled Drug Product
Moderna Part #: 85002
Manufacturer Name: Moderna
Container/Closure: 2R Vial, 13mm Stopper, 13mm Flip Cap
Manufacturer Lot #: 85002xxxyy
Moderna Lot #: 85002xxxyy
Product Specification #: SPC-0380
Date of Manufacture: DDMMMYYYY
Expiry Date: DDMMMYYYY

Product Attribute	Method (Test Method #/Revision #)	Specification	Result	Source Data
mRNA-4650 mRNA Product Intermediate (MPI)				
Residual DNA template	qPCR Moderna SOP-0491	< 1% (w/w)		ARN 18-00175-034
Residual total protein	Fluorescence (NanoOrange) Moderna SOP-0521	< 1% (w/w)		ARN 18-00175-035
% 5′ Capped	RP-HPLC Moderna SOP-0467	≥ 85% Cap1		ARN 18-00258-023
% Poly A Tailed RNA (% Tailless RNA)	RP-HPLC Moderna SOP-0291	Report % area main peak (Report % area pre-main peak)		ARN 18-00258-038
mRNA-4650 Drug Product – Final Vial				
Appearance	Visual Moderna SOP-0278	White to off-white dispersion. May contain visible, white or translucent product-related particulates		ARN 18-00258-045
Total RNA content	AEX-HPLC Moderna SOP-0235	0.8 – 1.2 (mg/mL)		ARN 18-00258-026
Identity	Reverse Transcription Sanger Sequencing Moderna SOP-0492	Sequence matches 100% description of the patient-specific coding region		ARN 18-00175-038
Purity	RP-HPLC Moderna SOP-0383	≥ 50% sum of main peak areas		ARN 18-00258-029
Product-related Impurities	RP-HPLC Moderna SOP-0383	Report % Area for each impurity group Impurity Group 1 (pre-main peak 1) Impurity Group 2 (post-main peak 2) Impurity Group 3 (mRNA-adduct species)		ARN 18-00258-029
% RNA Encapsulation	Fluorescence (Ribogreen) Moderna SOP-0298	≥ 80%		ARN 18-00258-042
pH	USP <791> Moderna SOP-0288	7.0 – 8.0		ARN 18-00258-044

VERSION NUMBER 1.0

Page 1 of 3

moderna

100 Tech Drive • Norwood, MA 02062
phone 617-714-6500 • fax 617-583-1998

CERTIFICATE OF ANALYSIS

Product Description: mRNA-4650 Labeled Drug Product
Moderna Part #: 85002
Manufacturer Name: Moderna
Container/Closure: 2R Vial, 13mm Stopper, 13mm Flip Cap
Manufacturer Lot #: 85002xxxyy
Moderna Lot #: 85002xxxyy
Product Specification #: SPC-0380
Date of Manufacture: DDMMMYYYY
Expiry Date: DDMMMYYYY

Product Attribute	Method (Test Method #/Revision #)	Specification	Result	Source Data
Osmolality	USP <785> Freezing point depression Moderna SOP-0279	1110 - 1510 mOsm/kg		ARN 18-00258-047
Particle Size	Dynamic Light Scattering Advantar Moderna SOP-0107	50 – 110 nm		ARN 18-00258-036
Polydispersity	Dynamic Light Scattering Moderna SOP-0107	Report results		ARN 18-00258-036
Product Related Particles 0.8 - 10 µm	Coulter Counter Advantar Moderna SOP-0344	Report Results		ARN 18-00258-027
In Vitro Translation (Potency)	Cell Free Translation Moderna SOP-0489	Molecular weight range (Target ±35%)		ARN 18-00175-040
Lipid Identification SM-102 Cholesterol DSPC PEG2000-DMG	UPLC-CAD Moderna SOP-0502	Matches retention time of standard Matches retention time of standard Matches retention time of standard Matches retention time of standard		ARN 18-00258-041
Lipid Content SM-102 Cholesterol DSPC PEG2000-DMG	UPLC-CAD Moderna SOP-0502	8.3 – 13.9 mg/mL 3.4 – 5.8 mg/mL 1.9 – 3.1 mg/mL 0.9 – 1.5 mg/mL		ARN 18-00258-041
Lipid Impurities	UPLC-CAD Moderna SOP-0502	Report % area and RRT of individual impurities Report % area of total impurities		ARN 18-00258-041

moderna

100 Tech Drive • Norwood, MA 02062
phone 617-714-6500 • fax 617-583-1998

CERTIFICATE OF ANALYSIS

Product Description: mRNA-4650 Labeled Drug Product
Moderna Part #: 85002
Manufacturer Name: Moderna
Container/Closure: 2R Vial, 13mm Stopper, 13mm Flip Cap
Manufacturer Lot #: 85002xxxyy
Moderna Lot #: 85002xxxyy
Product Specification #: SPC-0380
Date of Manufacture: DDMMMYYYY
Expiry Date: DDMMMYYYY

Product Attribute	Method (Test Method #/Revision #)	Specification	Result	Source Data
Particulate Matter ≥ 25 µm ≥ 10 µm	USP <788> Method 2 Moderna SOP-0509	≤ 300 per container ≥ 25 µm ≤ 3000 per container ≥ 10 µm		ARN 18-00258-0020
Bacterial Endotoxins	USP <85>, EP 2.6.14 Moderna SOP-0352	≤ 10 EU/mL		ARN 18-00263-040
Sterility	USP <71>, EP 2.6.1 Moderna SOP-0378	No Growth		ARN 18-00263-018
Rapid Sterility	BacT/Alert Moderna SOP-0508	No Growth		N/A

The material meets specification: Yes ☐ No ☐

Revision History:

1.0 – Original

Department	Signature	Date
QC/QA Review:		
QA Review:		