Abbreviated Title: QuICC Trial

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Title: Phase II Trial of Combination Immunotherapy in Subjects with Advanced Small Bowel and Colorectal Cancers

NCI Principal Investigator: Hoyoung Maeng, MD
Center for Immuno-Oncology (CIO)
Center for Cancer Research (CCR)
National Cancer Institute (NCI)
10 Center Drive
Room 3B37
Bethesda, MD 20892
Phone: 240-781-3253
Email: hoyoung.maeng@nih.gov

Drug Name:	MSB0011359C (M7824)	N-803	NHS-IL12 (M9241)	CV301
IND Number:	19660			
Sponsor:	Center for Cancer Research, NCI			
Manufacturer:	EMD Serono, Inc.	ImmunityBio, Inc.	EMD Serono, Inc.	Bavarian Nordic, Inc.
Supplier:	EMD Serono, Inc.	ImmunityBio, Inc.	EMD Serono, Inc.	Bavarian Nordic, Inc.

Commercial Agents: None

PRÉCIS

Background:

- Metastatic or refractory/recurrent small bowel and colorectal cancers are incurable and poorly palliated by standard therapies. There is an unmet need for active treatments for these tumors.
- To date immunotherapies including anti PD-1 or anti PD-L1 inhibitors have proven largely ineffective for the vast majority of these cancers.
    - In microsatellite stable (MSS) colorectal cancer (>95% of these cancers) the response rate to checkpoint inhibitors has been <5%.
    - Preclinical studies suggest that the use of different combinations of multiple immunotherapy agents may improve anti-tumor efficacy. These studies have employed (1) a vaccine targeting a tumor associated antigen, (2) an IL-15 superagonist (N-803, also known as ALT-803), (3) an anti-PD-L1 MAb or a bifunctional fusion protein targeting PD-L1 and TGFβ (M7824), and (4) a tumor targeted immunocytokine (NHS-IL12).

Objectives:

- To evaluate the objective response rate (ORR) according to Response Evaluation Criteria (RECIST 1.1) of the combination of (1) CV301, a poxviral based vaccine targeting CEA and MUC1, (2) N-803 and (3) M7824; and of the combination of (1) CV301, (2) N-803, (3) M7824 and (4) NHS-IL12 (M9241) in subjects with advanced checkpoint naïve MSS small bowel and colorectal cancers.

Eligibility:

- Age ≥ 18 years old
- Subjects with cytologically or histologically confirmed locally advanced or metastatic small bowel or colorectal adenocarcinomas.
- Prior first line systemic therapy is required unless the participant declines standard treatment after appropriate counseling has been provided.
- Subjects must have measurable disease.

Design:

- This is a phase II trial of combination immunotherapy, with a brief dose escalation portion for Arm 2.
- The trial will be conducted using a Simon optimal two-stage design in each Phase II Arm.
- Participants will be enrolled on the following arms in sequential order: (1) Arm 1: CV301 + M7824 + N-803, (2) Arm 2A and Arm 2B: CV301 + M7824 + N-803 + NHS-IL12; NHS-IL12 dose level will be evaluated in Arm 2A prior to further enrollment in Arm 2B.
- The first six participants on arm 1 will be evaluable for dose limiting toxicities (DLTs) and accrual will only continue to 9 participants on that arm if less than 2 out of the first 6 participants experience a DLT.
- In Arm 2B, participants will receive 4 drug treatments (CV301 + M7824 + N-803 + NHS-IL12), but the dose level of NHS-IL12 will first be determined during a dose escalation portion, Arm 2A. Following determination of the MTD or highest safe dose

evaluated, the 6 participants at that dose level will be included among the initial 9 participants for the first stage of that arm.

- If two or more out of nine participants have objective responses on a given arm that arm will be expanded to enroll 20 evaluable participants.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To evaluate the objective response rate (ORR) according to Response Evaluation Criteria (RECIST 1.1) of the combination of (1) CV301, a poxviral based vaccine targeting CEA and MUC1, (2) N-803 and (3) M7824; and of the combination of (1) CV301, (2) N-803, (3) M7824 and (4) NHS-IL12 in subjects with advanced checkpoint naïve MSS small bowel and colorectal cancers.

1.1.2 Secondary Objectives

- To evaluate the safety of the combination of (1) CV301, (2) N-803 and (3) M7824 in subjects with advanced small bowel and colorectal cancers.
- To evaluate the safety of the combination of (1) CV301, (2) N-803, (3) M7824 and (4) NHS-IL12 in subjects with advanced small bowel and colorectal cancers.
- To assess progression-free survival time (PFS) according to RECIST 1.1 per treatment assignment (three or four drug combination).
- To assess overall survival (OS) per treatment assignment (three or four drug combination).
- To assess duration of response per treatment assignment (three or four drug combination).
- To assess ratio of participants that are hospitalized because of adverse events attributed to disease progression

1.1.3 Exploratory Objectives

- To conduct exploratory immunologic studies to understand and improve the administered treatment, including:

- peripheral immune subset analysis before and on treatment;
- soluble factors circulation (e.g., sCD27 and sCD40 ligand) before and on treatment;
- tumor tissue PD-L1 expression and immune infiltration before and after treatment.

- To assess responses to therapy using iRECIST per treatment assignment (three or four drug combination).
- To assess response to therapy using RECIST and iRECIST in checkpoint refractory participants (including participants with MSI disease)
- To assess responses to therapy by RAS mutation status

1.2 BACKGROUND AND RATIONALE

1.2.1 Colorectal Cancers

Metastatic colorectal cancer is characterized by poor outcomes. The statistics are discussed below and highlight the unmet need for effective treatments in this populations. In the face of the failures of the many tested treatment strategies for these patients, it is clear that innovative and bold approaches will be needed in order to develop treatment regimens that positively affect this population's prognoses.

1.2.1.1 Metastatic Colon Cancer

Each year, approximately 300,000 new cases of colon cancer are diagnosed in the United States and Europe. Following first-line treatment with 5-fluorouracil-based combination chemotherapy plus targeted therapy, median overall survival is approximately 2 years. Once a patient progresses on first-line treatment, overall survival is further diminished to 10-12 months. Oral salvage chemotherapy agents have received regulatory approval in recent years. However, the overall survival benefit seen in phase III studies with these agents is an additional 1-2 months [1-3]. With the exception of microsatellite unstable cases of mCRC, activity of ICI monotherapy is rare [4-6].

1.2.2 Rationale for Combination of Immunotherapy Agents

The vast majority of human carcinomas are characterized as "cold" tumors in that they do not respond to checkpoint MAb therapy. Preclinical studies are now revealing that an effective immuno-oncology strategy for so-called "cold" tumors such as colon carcinoma (MSS) is the use of multiple immune-mediating agents, each targeting different components of the immune system. These include: (a) induction of an immune response via vaccination directed against tumor-associated antigens, (b) potentiation of the systemic immune response by the use of immunocytokines (such as the N-803, an IL-15 superagonist fusion protein) to enhance both NK and CD8+ T-cell responses, (c) potentiation of the immune response in the tumor microenvironment (TME) by the use of the tumor targeting NHS-IL12 immunocytokine, and (d) reduction of immunosuppressive entities in the TME by the use of anti-PD1/PDL1 MAb checkpoint inhibitors, and/or reduction of immunosuppressive cytokines such as TGF-β with the use of a bifunctional anti-PDL1/TGF-β2 "Trap" designated M7824.

The LTIB, in collaboration with the GMB, has been able to develop and/or co-develop these agents via a series of CRADAs. Each has been interrogated in a series of preclinical studies and in Phase I/II clinical studies in the CCR as well as by other investigators. Preclinical studies and early clinical studies are demonstrating the relative lack of additional toxicities with enhanced clinical benefit employing combinations of 2 of these agents. Preclinical studies are now demonstrating

that the combined use of agents from each of the immune-mediating categories described above elicits the most immune-mediated anti-tumor activities.

Avelumab (anti-PD-L1) and M7824 (anti-PD-L1/TGF- βR2)

Studies in the LITB were the first to describe [7, 8] avelumab, a fully human anti-PD-L1 IgG1 MAb, and its ability to mediate ADCC for a range of human carcinoma cells employing NK cells as effectors. Avelumab (Bavencio) has since been approved by the FDA for the therapy of Merkel cell carcinoma (the first drug approved for this indication) and second line urothelial carcinomas. We have also shown that endogenous IL-12 will enhance avelumab-mediated NK lysis, thus forming the basis for the combined use of avelumab or M7824 with NHS-IL12. The first-in-human clinical study of avelumab with over 1,700 patients was led by Gulley with the dose escalation and initial PK and PD done entirely within the CCR [8]. Because of the activity this study was amended to include multiple expansion cohorts internationally. Because PD-L1 is also expressed on some immune cell subsets, and because avelumab can mediate ADCC there was initial concern that Avelumab may lead to depletion of PD-L1 expressing immune cells. Our finding [9] that there were no adverse events in the patients treated with avelumab above that seen with other anti-PD-L/PD-L1 MAbs (which did not mediate ADCC) was important for planning further studies with avelumab or M7824.

TGF-β is a well-known inhibitor of immune cell function, especially in the TME [10-15]. Prior studies have indicated the advantage in the use of a TGF-β inhibitor in combination with checkpoint therapy [16, 17] In collaboration with our CRADA partner EMD Serono, we have co-developed a bifunctional fusion protein aimed at bringing a TGF-β TRAP to tumor cells via binding to PD-L1. M7824 is a novel fusion protein consisting of avelumab linked to the extracellular domain of two TGF-βR2 (serving as a TGF-β TRAP) **(Figure 1)**. We were the first to report [18, 19] that M7824 (a) mediates ADCC, (b) increases tumor cell gene expression of molecules involved in T-cell trafficking to the tumor, (c) enhances TRAIL- and antigen-specific CD8+ T-cell lysis of tumor cells, and (d) reduces TGF-β–induced immunosuppressive activity.

We have also recently demonstrated [20] in in vivo preclinical murine carcinoma models **(Figure 2A and B)** that M7824 has the ability to decrease TGF-β– induced signaling in the TME.

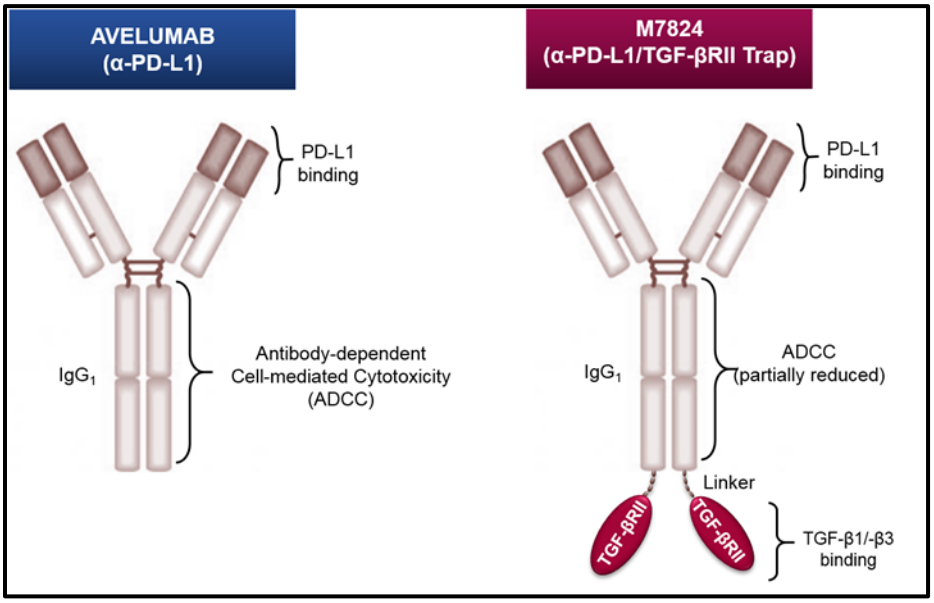


Figure 1 M7824 (anti-PD-L1/TGFβR2): "TRAP".

Specifically, phosphorylation of SMAD2 was significantly decreased in tumors following M7824 treatment, and not with an M7824(mut) molecule, devoid of the PD-L1 binding site **(Figure 2C)**. In both breast and colon carcinoma murine models, M7824 decreased tumor burden and increased survival, which required both CD8+ and NK cell activity **(Figure 2D)**. Two recent studies [21, 22] demonstrated the advantage in anti-tumor activity in the use of the bifunctional agent vs. the use of a PD-1/PD-L1 checkpoint in combination with a systemic TGF-β inhibitor. TGFβ is also known to alter human carcinoma cells from a more epithelial to a more mesenchymal phenotype and such tumor cells are more resistant to a range of therapies **(Figure 3)**. In LTIB studies, TGF-β–induced mesenchymalization of carcinoma cells was shown [23] to be reversed by M7824, rendering tumor cells more susceptible to chemotherapy and immune-mediated killing [18, 20, 23].

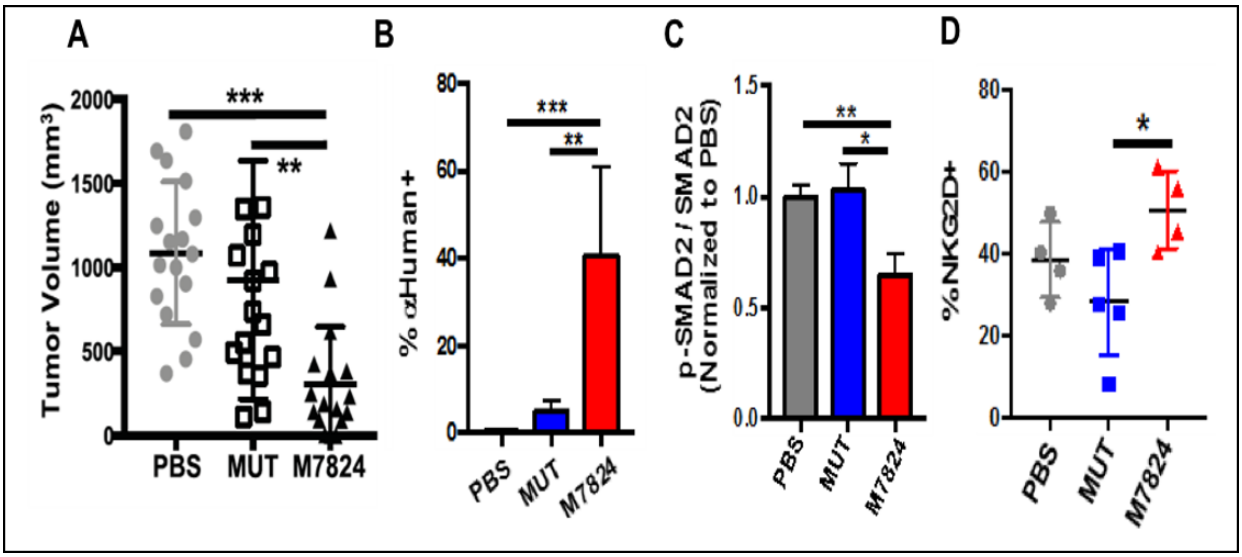


Figure 2 (A) Comparison of anti-tumor activity of M7824, and M7824(mut) devoid of PD-L1 binding site in the EMT6 breast cancer model. **(B)** Accumulation of M7824 in the TME resulting in reduction of SMAD2 signaling **(C)** and increased T- and NK-cell activation **(D)** [20].

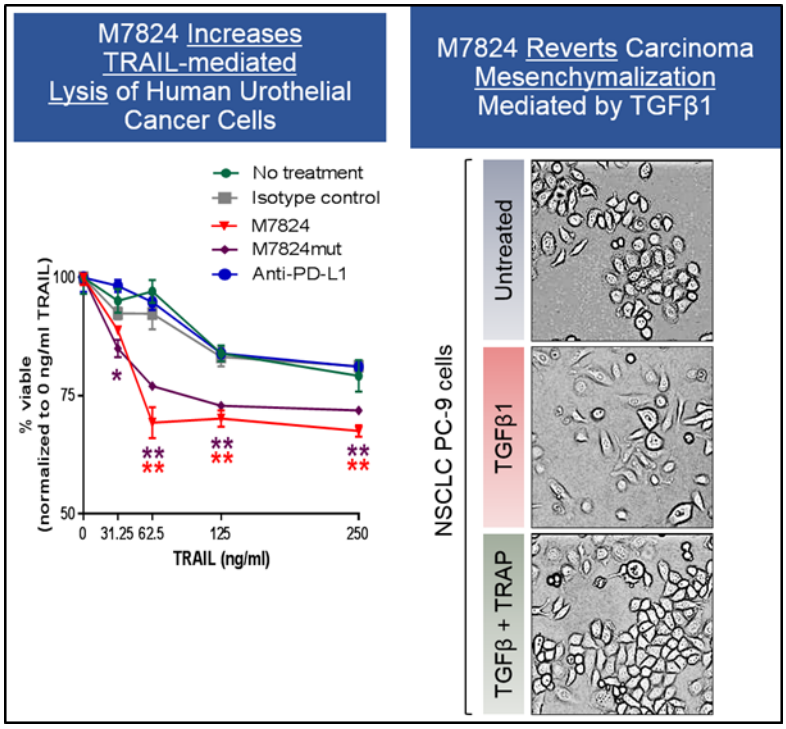


Figure 3 Functions of M7824 [18, 23]

The first-in-human trial of M7824 (anti-PD-L1/TGF-βR2) was conducted at the CCR. Nineteen heavily pretreated patients with solid tumors (non-melanoma) were treated; the MTD was not reached. M7824 was shown to saturate peripheral PD-L1 and sequester all released (acid released) plasma TGFβ1, -β2, and -β3 throughout the dosing period at >1 mg/kg (recommended phase 2

dose is 1200 mg or about 10 mg/kg). There were signs of clinical efficacy across all dose levels **(Figure 4, Figure 5)**, including one ongoing CR (cervical cancer), two durable PRs (pancreatic and anal cancers), one near PR (cervical) and two cases of prolonged stable disease [24]. In the Phase I study and a subsequent Phase II study, greater than 30–40% of patients with HPV+ malignancies showed evidence of clinical response to M7824 therapy. Prior studies by others have shown approximately 15-20% patient responses in this population employing checkpoint inhibition therapy.

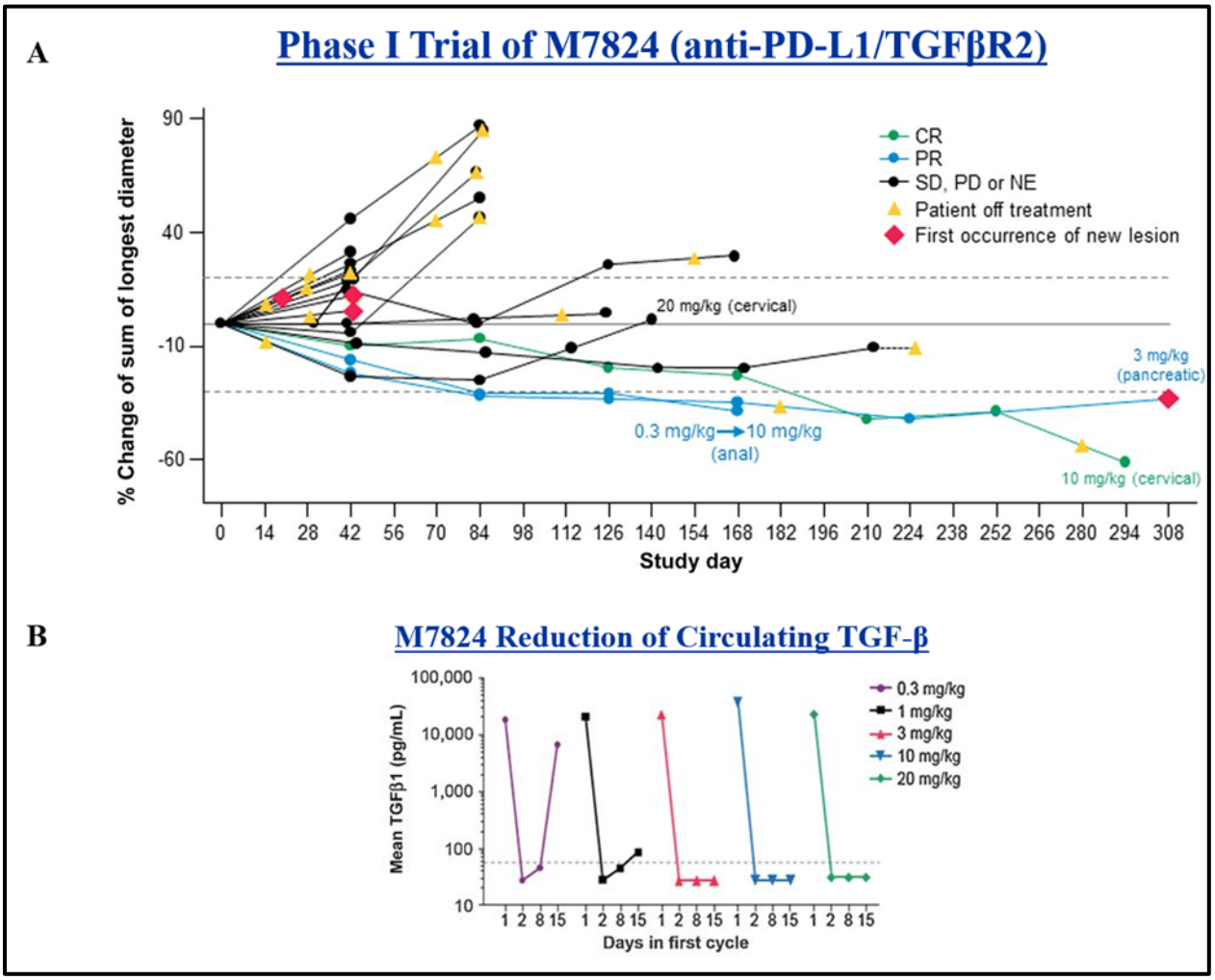


Figure 4 (A), (B) Phase I Trial of M7824 [24]

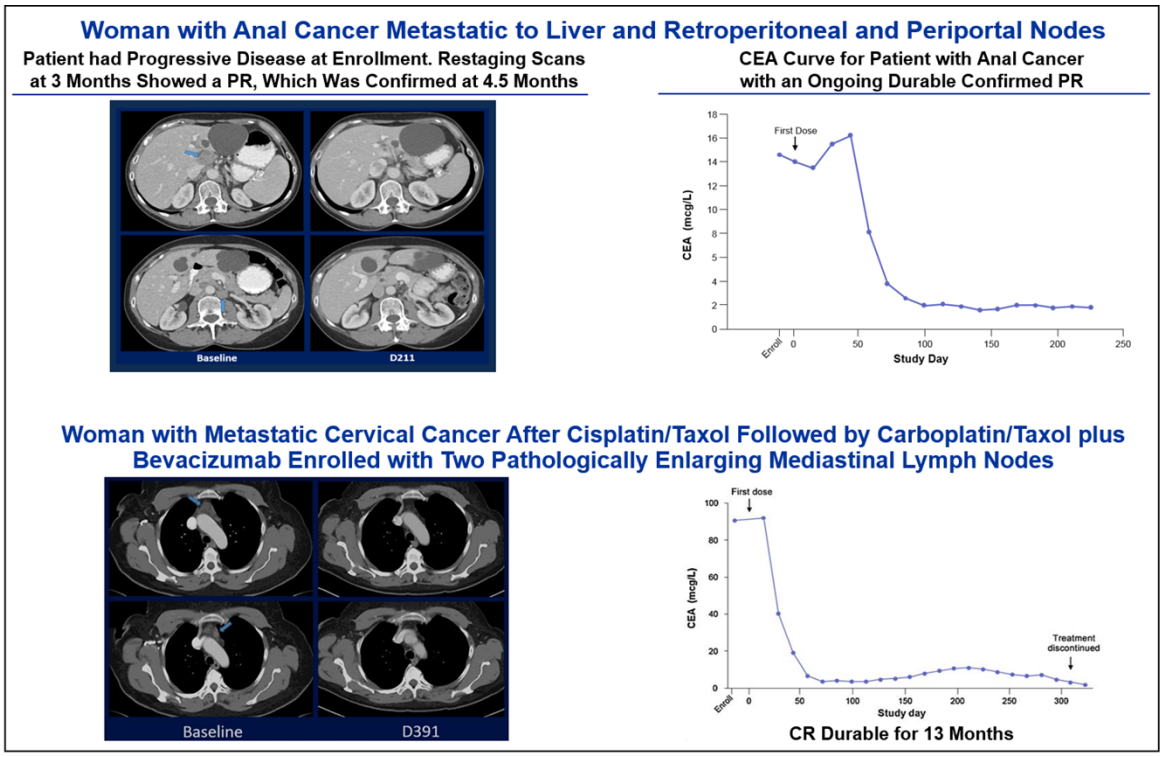


Figure 5: Phase I Trial of M7824 **[**24**]**

N-803 (IL-15 Superagonist)

IL-15 is a well-studied Th1 cytokine. Prior studies by others [25-28] have shown, in both preclinical and clinical studies, the ability of prokaryotic rec. IL-15 protein to enhance the level of both NK and CD8+ T cells. Limitations in the use of rec. IL-15 center around issues of pharmacokinetics and toxicity, and the lack of the IL-15/IL-15R complex for optimal activity. Our interest in IL-15 was also piqued by the ability of avelumab and M7824 to mediate ADCC. N-803 is an IL-15/IL-15Rα-Fc superagonist fusion protein developed by our CRADA partner ImmunityBio, Inc. (formerly Altor Bioscience and NantCell, Inc.). In a series of studies, we have shown [19, 29, 30] that N-803 can (a) significantly increase cytotoxic NK cells, showing enhanced NK lysis of tumor cells on a per-cell basis, (b) increase memory CD8+ T cells, (c) elicit anti-tumor effects in metastatic models of breast and colon carcinoma, and (d) inhibit TGF-β–mediated reduced NK function, leading to increased NK lysis of a range of human carcinoma cell types.

It has now been reported [31] that N-803 can re-induce objective responses to anti-PD-1 immunotherapy after treatment relapse or failure. Historically, about 8% of patients with NSCLC treated past progression had partial responses with continued PD-1 monoclonal antibodies treatment. The objective responses to N-803-nivolumab combination treatment after PD-1 treatment failure coincided directly with initiation of N-803 after progression in patients with relapsed disease treated with uninterrupted nivolumab [31]. Moreover, partial responses were seen in 3 out of 10 (30%) patients whose tumor lacked PD-L1 expression and in 3 out of 11 (27%) patients who had relapsed or refractory disease after previous anti-PD-1 immunotherapy. The level of toxicity observed in the combination was similar to (and perhaps less than) that observed with PD-1/PD-L1 monotherapy. These data suggest that N-803 represents a novel immunotherapeutic

agent that could augment the anti-tumor activity of checkpoint inhibitors in several treatment settings.

Vaccines

Both CEA and MUC-1 have been reported as relevant immunologic targets in colon cancer. Earlier efforts to generate efficient cancer immunotherapy were directed towards the development of PANVAC, a prime and boost approach based on the administration of a replicating-competent Vaccinia-based delivery vector followed by a Fowlpox vector. Both vectors encoded the transgenes for B7.1, ICAM-1 and LFA-3, together with the CEA and MUC-1. In order to establish its therapeutic potential, several preclinical studies were performed, showing that PANVAC activates antigen-specific human T cells *in vitro* [32], elicits antibody and cellular immune responses *in vivo,* and shows significant antitumor efficacy against tumors expressing either human CEA or MUC-1 in mice, resulting in programmed death ligand 1 (PD-L1) up-regulation within the tumor microenvironment. Subsequently, the clinical development program continued with the design of several clinical trials to further confirm the efficacy and safety of this intervention. Overall, the results of Phase I and II clinical trials in different cancer populations employing PANVAC monotherapy or in combination with GM-CSF or docetaxel demonstrate a good safety profile accompanied by some hints of clinical benefits [33, 34].

A second generation cancer immunotherapy strategy was developed, giving rise to CV301 involving a modified vaccinia. CV301 is a recombinant non-replicating poxvirus based immunotherapy encoding the same 5 transgenes used in PANVAC (B7.1, ICAM-1, LFA-3, CEA and MUC-1) and thus designed to activate an antigen-specific response. Therefore, tumors expressing these antigens, including colon cancer, qualify as a potential indication for CV301.

Given the potential risks associated with the replicating capacity of the vaccinia-based PANVAC-V construct, the next generation cancer immunotherapeutic candidate MVA-BN-CV301 was generated by Bavarian Nordic as part of a CRADA with the NCI (LTIB). It constitutes a recombinant non-replicating poxvirus- based immunotherapy derived from the MVA-BN construct. This novel viral vector was developed from the highly attenuated vaccinia virus known as MVA, which after more than 500 serial passages in chicken embryo fibroblasts lost approximately 15% of the original vaccinia genome. Furthermore, MVA-BN differs from other MVA strains in its extensive plaque purification and propagation in serum-free conditions, and has already undergone extensive investigation as a new smallpox vaccine. Within this comprehensive clinical development program, MVA-BN has been administered to more than 7,700 subjects without major safety concerns, hence exhibiting a robust safety profile. Accordingly, besides decreasing the risk of cardiac adverse events associated with earlier generation vaccinia-based strategies, the improved MVA-BN enables previously excluded segments of the general population, such as immunocompromised individuals and persons diagnosed with atopic dermatitis, to receive the potential benefits offered by this novel approach. Therefore, it embodies a highly novel approach and offers a reliable technological platform that enables the safe delivery of different transgenes to produce a tailored immune response against specific antigens.

The new CV301 strategy encodes the same 5 transgenes expressed by the previous PANVAC vector: CEA, MUC-1, B7.1, ICAM-1 and LFA-3. However, these inserts encode each of the transgenes with amino acid sequences that are slightly different from the original vectors. In fact, MUC-1 has been modified with additional agonistic epitopes. Consequently, the CV301 construct is expected to promote an antigen-specific targeted immune response for tumors expressing these

antigens. In addition, a change in biosafety also characterizes the new CV301 construct. Thus, whereas vaccinia possesses a BSL-2, MVA has been categorized as BSL1 in Europe. As well, similarly to the earlier PANVAC concept, CV301 also encompasses a heterologous prime-boost regimen employing an MVA-BN-CV301 priming dose followed by a Fowlpox-based FPV-CV301 boosting dose, thus replacing the former PANVAC-V and PANVAC-F components. Given the existing correspondences between the CV301 and PANVAC vectors, the clinical development program designed for CV301 will rely on the extensive nonclinical and clinical data currently available from PANVAC.

Clinical Safety Data

Analysis of safety data from a Phase 1/1b clinical trial of CV301 in 24 subjects showed no dose limiting toxicities at a dosage up to 1.6 × 109 Inf.U of MVA-BN-CV301.

In both Phase 1b cohorts (CV301 plus nivolumab and CV301 plus pembrolizumab) the safety of the combination regimen was established. There was only one case of DLT reported due to immune mediated events in the nivolumab combination: one case of pneumonitis that further developed into a vasculitis, DIC and ultimately multi organ failure (fatal outcome). Overall, DLT and IMAE were below the pre-specified threshold to continue the trial based on the now established safety profile. In conclusion, there was no increased toxicity of the combination regimens CV301 plus pembrolizumab or nivolumab as compared to ICIs alone (per ICI prescribing information).

The clinical safety data for the MVA-BN backbone vector and its recombinant products tested clinically to date are provided in Section 5.2 of CV301 IB v 5. In summary, no safety concerns, in particular no cardiac concerns have arisen from the development program of the MVA-BN platform, involving more than 51,000 subjects who have received MVA-BN and MVA-BN based recombinant products in completed and ongoing clinical trials.

Six of the seven clinical trials with CV301's predecessor product PANVAC with data available have demonstrated an acceptable safety profile for the vaccines. Across these clinical trials, the most common treatment-related AEs were injection site reaction (including injection site erythema, injection site pain, and injection site swelling), myalgia, fatigue, vomiting, nausea, and abdominal pain. Most treatment-related AEs were Grade 1 or 2 in severity. SAEs related to study treatment, discontinuations due to AE, and deaths related to study treatment were rare (only 1 death due to Grade 5 pulmonary infiltrate was assessed as possibly related to study drug).

Cardiac toxicity:

A detailed analysis of the TEAEs reported under the cardiac disorders SOC in clinical trial TBC-PAN-003 and CTEP No 6977 were performed due to an imbalance of cardiovascular events reported in the PANVAC treatment arms. This detailed analysis concluded that for all reported cardiac SAEs and cases of pericardial effusion, alternative risk factors were present and causality with regard to PANVAC is unlikely. Importantly, there have been no reported observations of myocarditis or pericarditis in any of the PANVAC clinical trials.

Based upon these data and the total clinical experience with poxvirus vectors for anticancer vaccines, including CV301's predecessor product PANVAC, a causal relationship of cardio-toxicity following treatment with CV301 and/or PANVAC is unlikely. Nonetheless, a proactive approach in pharmacovigilance will be applied prospectively, requesting from the investigators a

deeper characterization of every case with a cardiac AE with the working hypothesis that myocarditis/pericarditis may be the underlying cause.

Further toxicities monitored during clinical trials:

Immune-Mediated Adverse Events: Autoimmune diseases and immune-mediated clinical syndromes, emerging since the initiation of trial product, will be reported as potential IMAEs. So far, no signal has emerged from the diverse poxvirus constructs studied in the clinic, but the theoretical possibility exists that breaking the immune tolerance to self-antigens may induce autoimmune phenomena. This possibility deserves close monitoring.

CV-301 Phase I Experience

A phase I study was just completed at the NCI [35]. As expected, there were no grade 3 or greater toxicities reported in the study. There were no dose-limiting toxicities. Twelve patients enrolled on trial [dose level (DL) 1 = 3, DL2 = 3, DL3 = 6). Most side effects were seen with the prime doses and lessened with subsequent boosters. All treatment-related adverse events were temporary, self-limiting, grade 1/2, and included injection-site reactions and flu-like symptoms. Antigen-specific T cells to MUC1 and CEA, as well as to a cascade antigen, brachyury, were generated in most patients. Single-agent BN-CV301 produced a confirmed partial response (PR) in 1 patient and prolonged stable disease (SD) in multiple patients, most notably in *KRAS*-mutant gastrointestinal tumors. Furthermore, 2 patients with *KRAS*-mutant colorectal cancer had

prolonged SD when treated with an anti-PD-L1 antibody following BN-CV301.

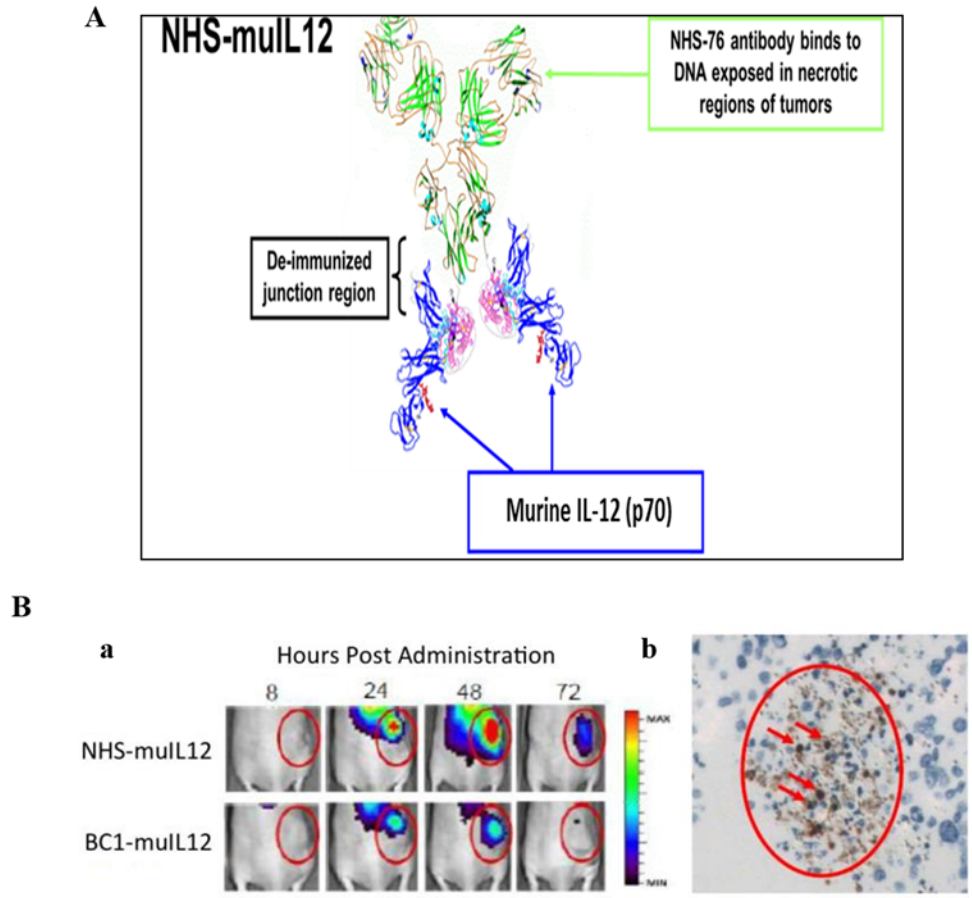


Figure 6 NHS-IL12 Immunocytokine. **(A)** NHS76 is a fully human 2nd generation TNT antibody bound to 2 murine IL-12 (p70) molecules. **(B)** ***a:*** Specific tumor targeting of transplanted lung carcinoma by the MAb NHS-IL12(mu). Control MAb BC1-IL12(mu). ***b:*** NHS-IL12 tumor targeting of nuclear DNA histones.

NHS-IL12 Immunocytokine (M9241)

NHS-IL12 is a tumor targeting immunocytokine that binds to DNA-histone in necrotic areas of tumor. Studies by others have shown the ability of radiolabeled NHS-IL12 to target murine and human tumors **(Figure 6)**. Prior studies in the LTIB have shown the ability of this agent to elicit anti-tumor activity in multiple murine models, and in combination with anti-PD-L1 and with radiation and chemotherapy [36]. J. Strauss (LTIB), in collaboration with J. Gulley (GMB, CCR), conducted the Phase I dose escalation trial of the NHS-IL12 immunocytokine [36]. Due to known prior toxicities using a rec. IL-12 protein, multiple ascending dose levels were employed and the agent was administered every 4 weeks. Time-dependent increase in serum IFN-γ and subsequent rise in IL-10 increased with dose and varied greatly among patients **(Figure 7A)**. Increases in TCR diversity and TIL density in the TME following NHS-IL12 dosing were observed in those patients with elevated IFN-γ serum levels **(Figure 7B)**. Toxicity was acceptable with an MTD of 16.8 mcg/kg. Although no objective tumor responses were seen, of

30 patients with measurable disease, 15 had stable disease, with some durable (from 6–30+ months).

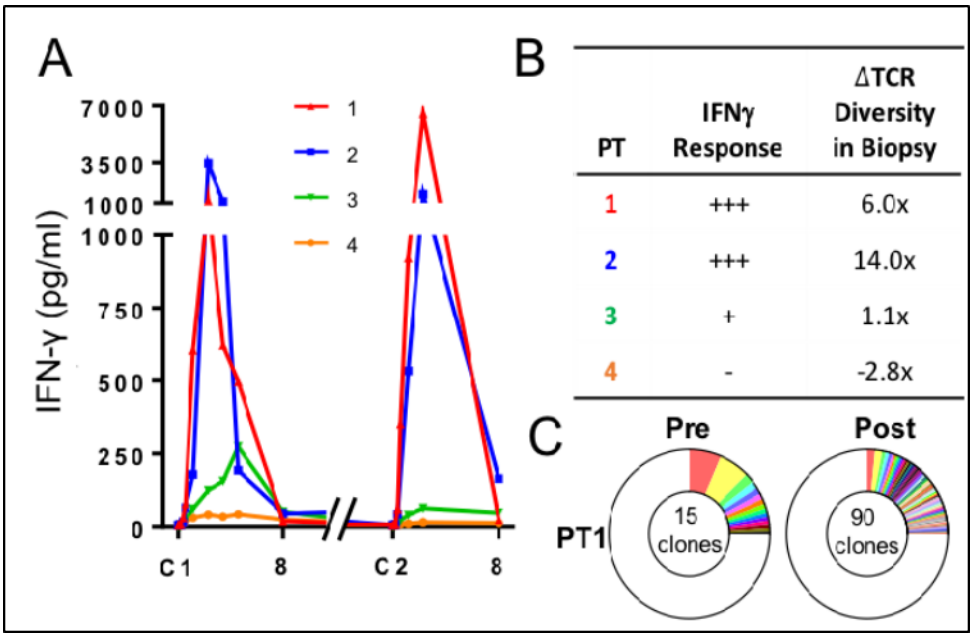


Figure 7 (A) Diversity of IFN-γ spikes in sera among patients 3 days post-NHS-IL12 treatment for cycles 1 (C1) and 2 (C2): as examples, 2 patients with high (pts 1 and 2, red and blue) and low (pts 3 and 4, green and orange) levels are shown. **(B)** Changes in TCR clonal diversity in the tumor biopsy correlate with serum IFN-g responses post-NHS-IL12 treatment. **(C)** # of clones in tumor comprising the top 25% of the repertoire of patient 1 pre- and post-NHS-IL12, demonstrating an increase in TCR diversity in a high IFN-g responder after therapy.

Our recent preclinical studies (**Figure 8**) have also shown that there is enhanced anti-tumor effect of avelumab when used in combination with NHS-IL12 (**Figure 8**) and that this effect is mediated via an IFN-γ–dependent mechanism. Studies in NSG-β2m$^{-/-}$ mice bearing a human carcinoma and reconstituted with human PBMC also showed the enhanced anti-tumor effect of the combination that coincided with increased NK and CD8^{+} in the TME. This further provided the rationale for an ongoing CCR led clinical study of avelumab in combination with NHS-IL12 (NCT02994953), currently evaluating safety and efficacy of the combination. In addition to the combination being found to be safe and well-tolerated, evidence of clinical responses has been observed. As an example, a patient with checkpoint refractory metastatic urothelial cancer with a large sacral mass and retroperitoneal adenopathy at enrollment has had an ongoing complete response for 21+ months and a patient with rapidly progressive small cell bladder cancer with metastases to liver who progressed on standard therapy has an ongoing partial response for 15+ months.

Combination Immunotherapies

As a consequence of the potential multi-functionality of M7824, we have conducted several combination studies employing this agent. We have previously shown [20] enhanced anti-tumor activity when M7824 is combined with vaccine in a metastatic carcinoma murine model **(Figure 9)**. While M7824 can mediate ADCC for a range of human carcinoma cells, it is not as potent as avelumab in this mechanism; the addition of N-803, however, enhanced the ADCC activity of both

avelumab and M7824, but more importantly, increased the tumor lytic ability of M7824 to that equivalent of avelumab with N-803 [29] **(Figure 10)**.

A series of preclinical studies have been carried out employing combinations of vaccine, M7824, N-803, and NHS-IL12. Combining M7824 with the NHS-IL12 molecule greatly enhanced anti-tumor activity **(Figure 11)**. Tumor challenge studies showed the presence of immune memory. This was shown in 3 different murine carcinoma models.

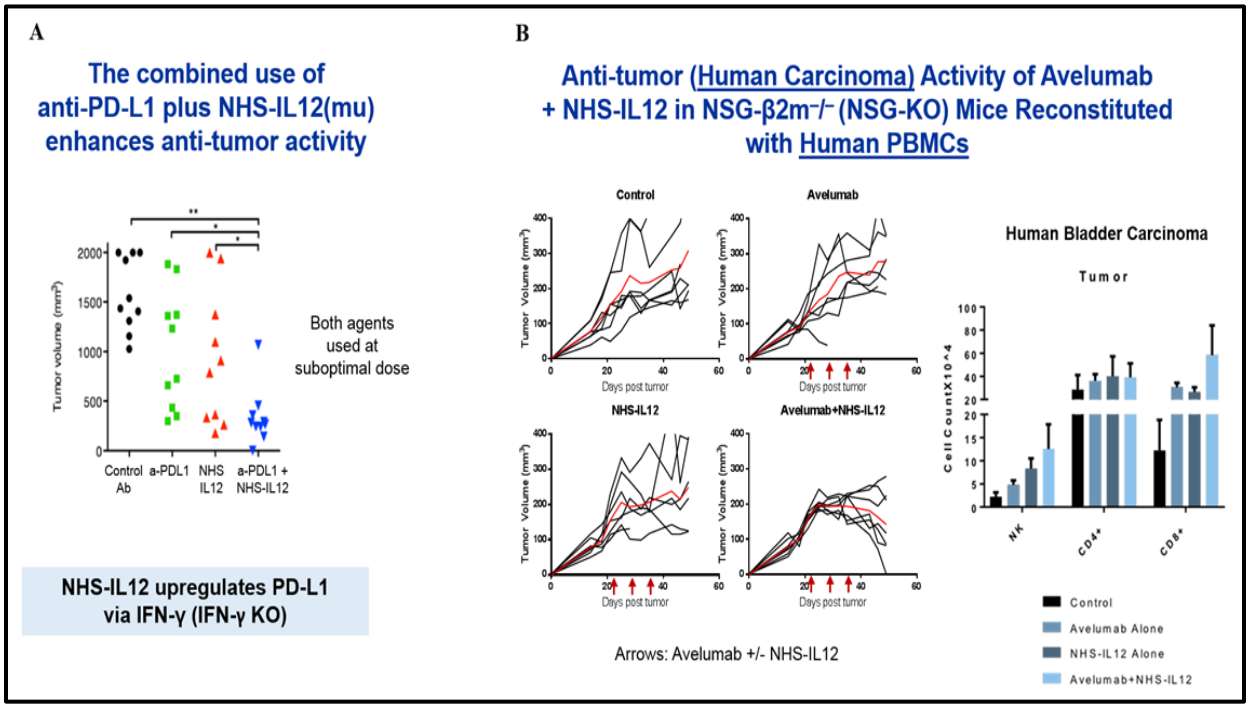


Figure 8 Anti-tumor Activity of Immunocytokine NHS-IL12 + anti-PD-L1. **(A)** [37, 38] **(B)** Ongoing Trial: Avelumab + NHS-IL12 (Strauss et al, unpublished).

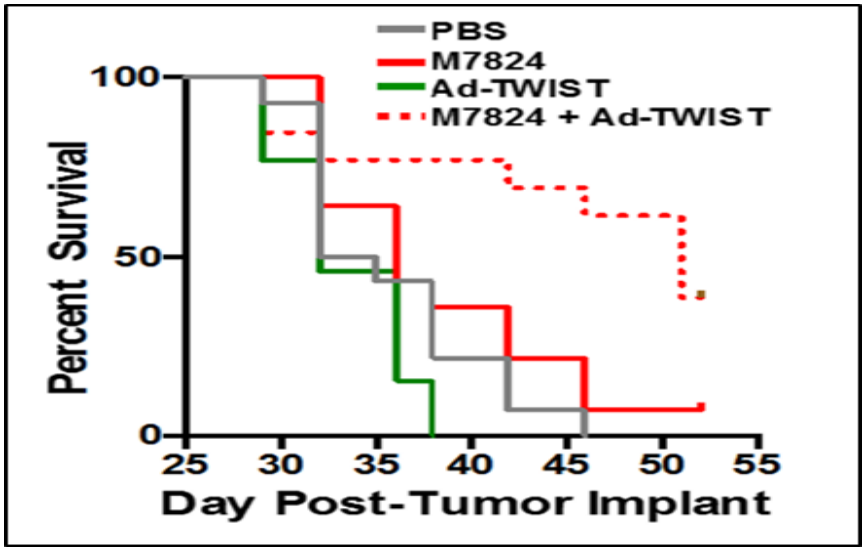


Figure 9 Combined effect of Ad-Twist vaccine and M7824 in EMT6 carcinoma model [20].

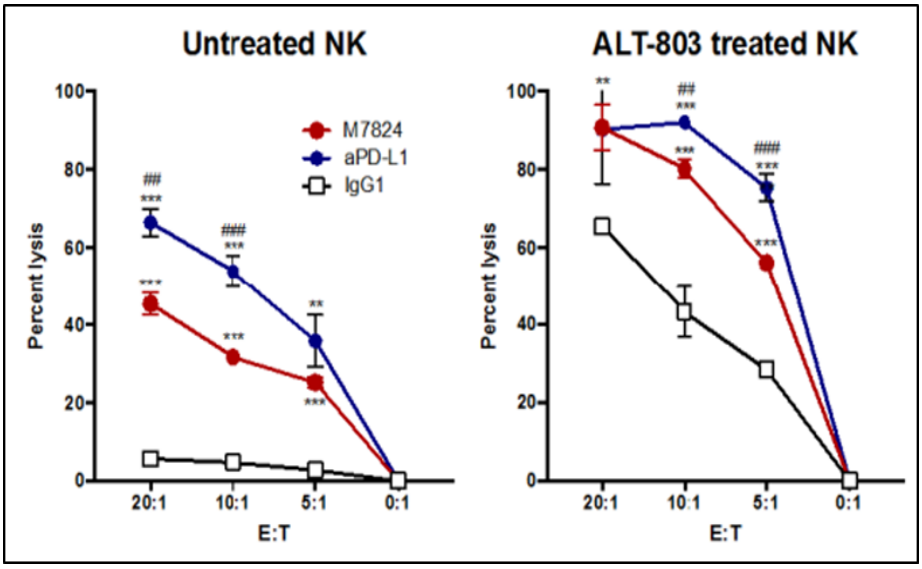


Figure 10 N-803 enhances the NK-mediated lysis of avelumab and M7824 [29].

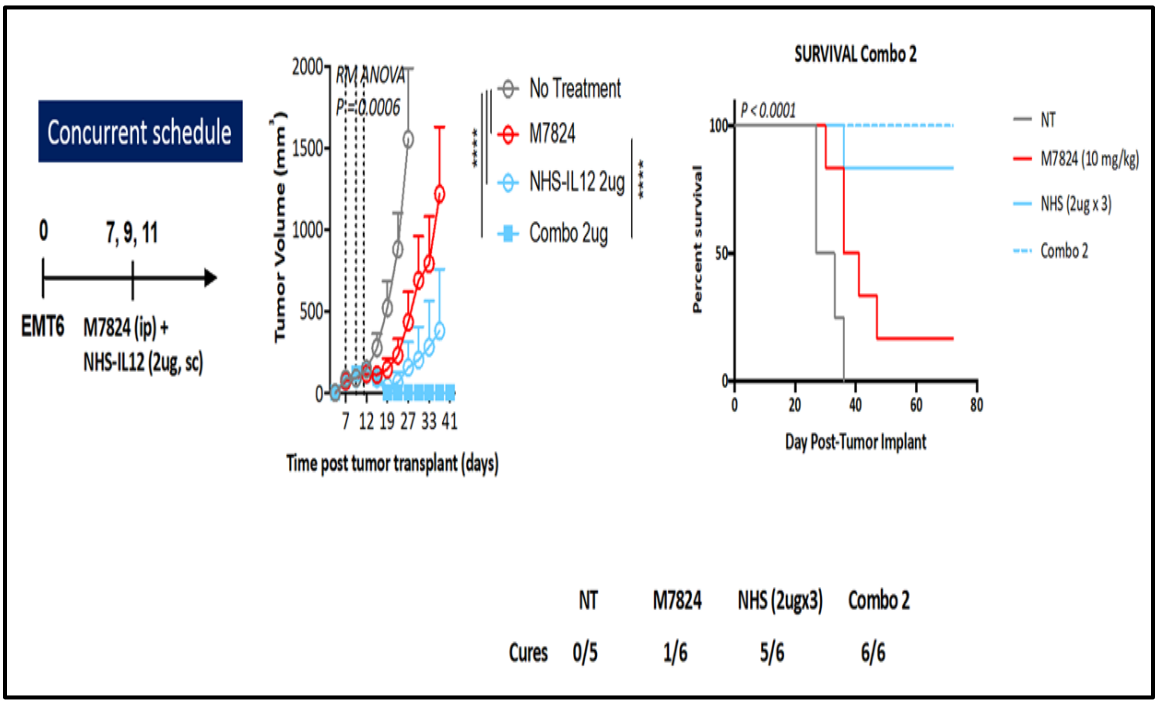


Figure 11 SG51: Anti-tumor effect of M7824 + NHS-IL12 combination treatment in EMT6 murine carcinoma tumors.

Multimodal therapies were also carried out. Employing NSG-β2m–/– mice bearing human carcinomas and reconstituted with human PBMC, the combination of vaccine, N-803, and M7824 resulted in the greatest level of anti-tumor activity compared to the use of individual agents **(Figure 12)**. Employing the MC38 syngeneic murine colon carcinoma model the combination of vaccine, N-803, anti-PD-L1, and NHS-IL12 resulted in precipitous reduction of large tumors not seen with combinations of 3 of these agents **(Figure 13)**. The use of all 4 agents also resulted in an intense influx of CD8+ T cells in tumors not seen with lesser combinations **(Figure 14)**. Tumor challenge studies showed the presence of immune memory.

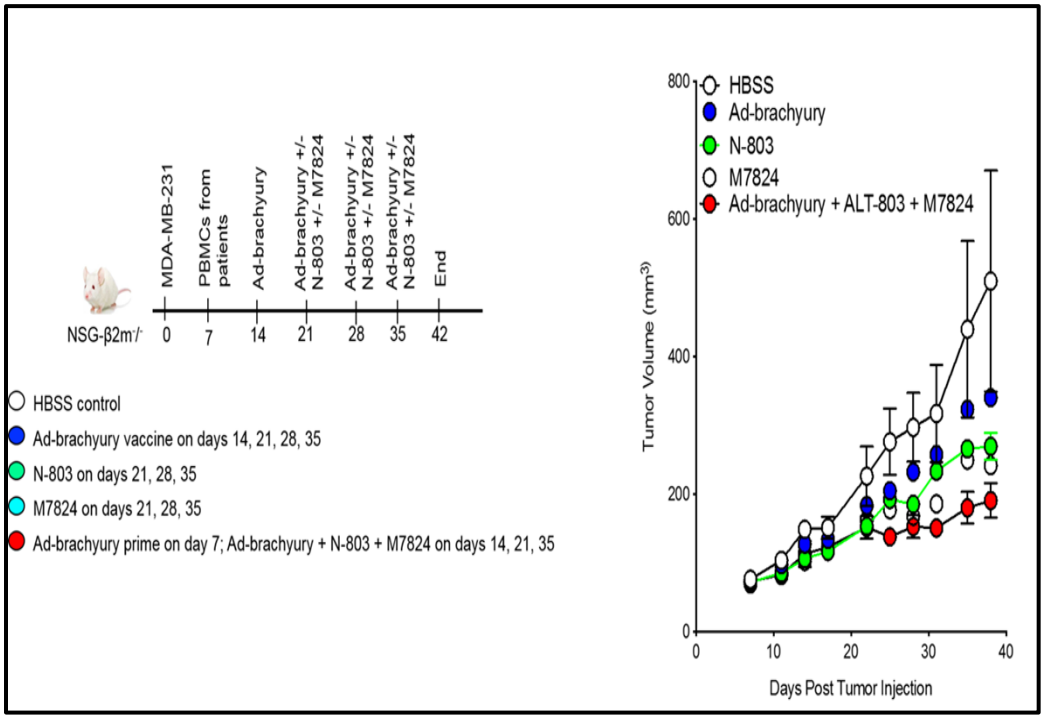


Figure 12 Multimodal therapy in mice reconstituted with PBMCs from patients.

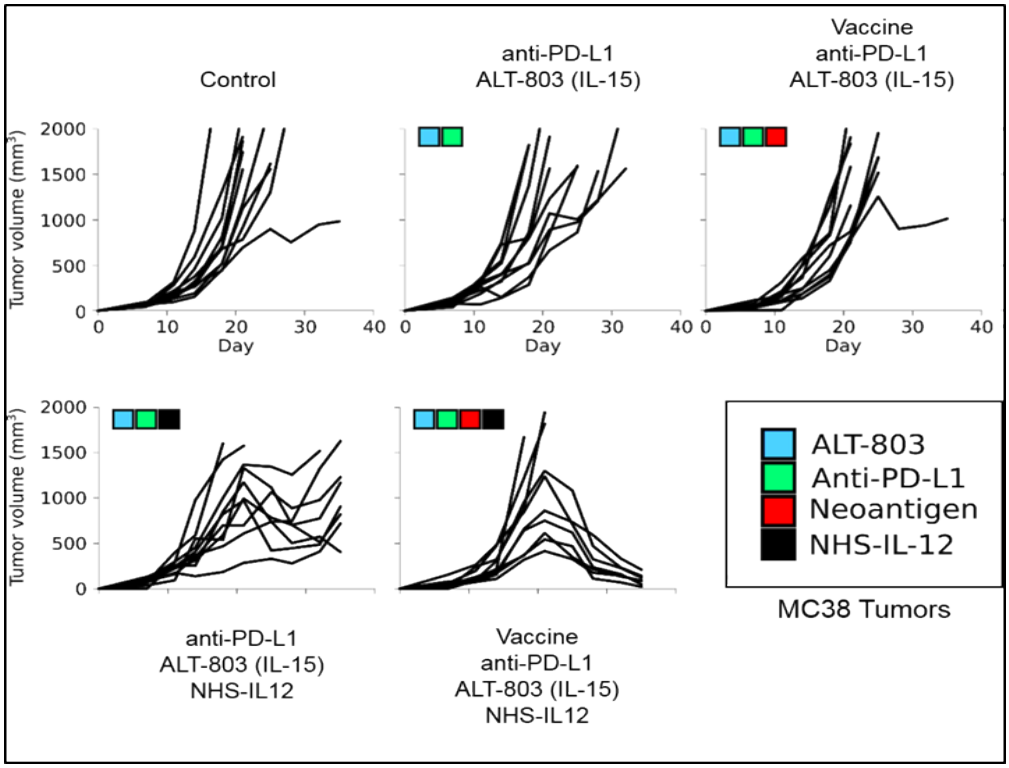


Figure 13 The ability of a neoantigen vaccine to enhance combinatorial anti-tumor activity (Unpublished data).

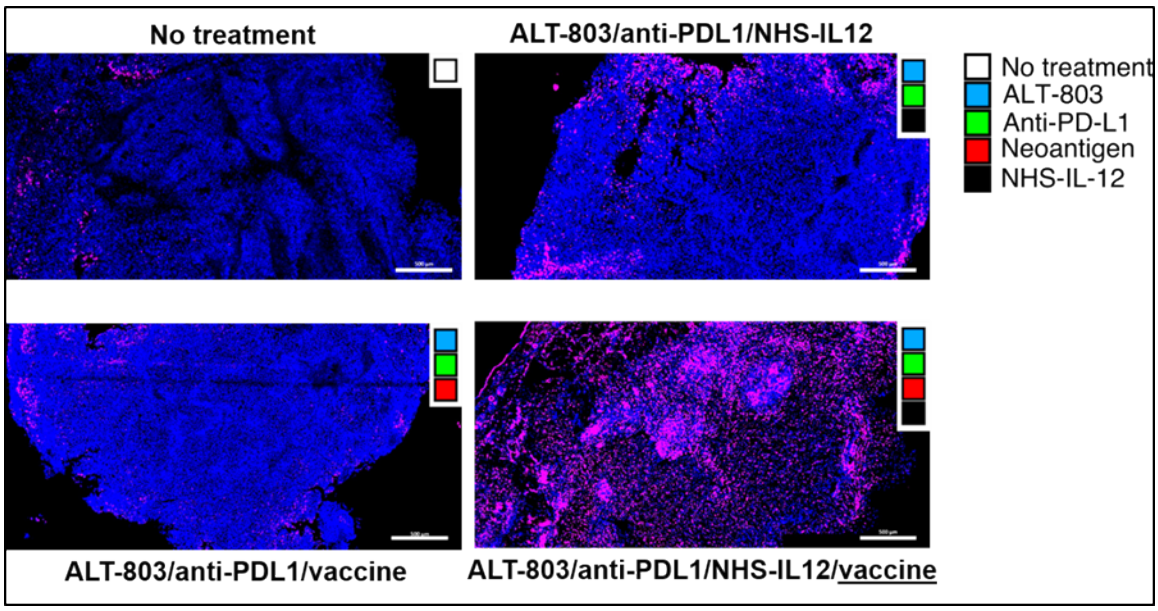


Figure 14 Neoantigen Vaccination in Combination Therapies: Tumor-infiltrating CD8 T Cells. (Unpublished data)

1.2.3 Safety and Efficacy of Immunotherapy Combinations in Humans

As detailed below **(Figure 15)**, evidence of clinical activity has been observed in MSS CRC patients who received anti-tumor vaccine, followed by ICI. These observations are particularly intriguing in the MSS colon cancer population, a group in which ICI activity has been rare [39]. Evidence of clinical activity has also been seen in a MSS, CRC patient who received the combination of M7824 and N-803 following a CEA/ MUC1 based vaccine regimen (**Figure 16**).

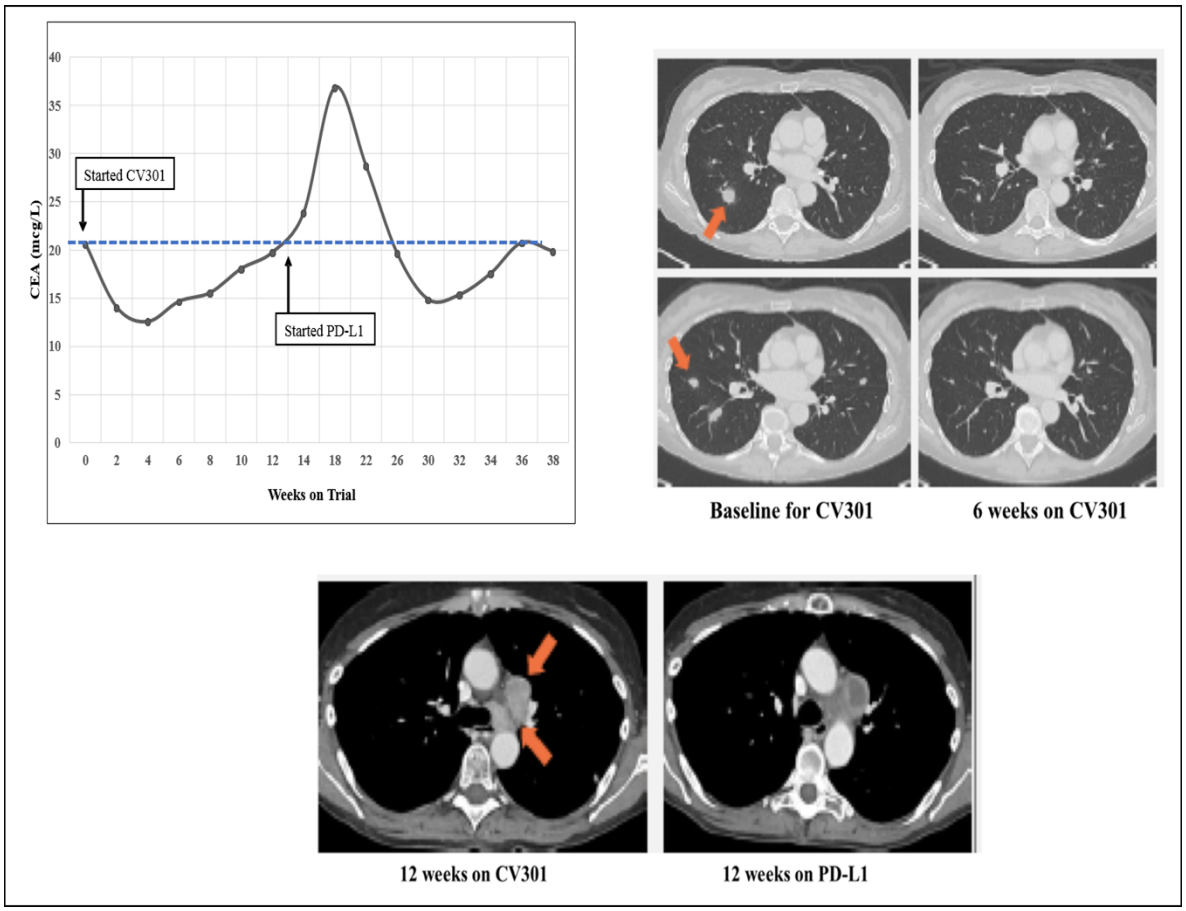


Figure 15 (A) CEA (mcg/L) of a patient with KRAS mutant, MSS colorectal cancer. This patient enrolled on the phase 1 CV301 trial, had an unconfirmed partial response at the first retagging (6 weeks), then developed new mediastinal adenopathy at the 12-week restaging. Patient was then enrolled on a PD-L1 trial and experienced a subsequent decrease in her tumor markers as well as a radiographic response to treatment. Blue dotted line represents baseline CEA. **(B)** Unconfirmed PR on CV301 at 6 weeks on study. **(C)** Developed new mediastinal adenopathy (red arrows) at week 12 on CV301 trial, resulting in patient coming off trial for progressive disease. Patient was then started on another trial with a PD-L1 agent. After 3 months on the PD-L1 trial, mediastinal adenopathy had necrosed and tumor markers declined.

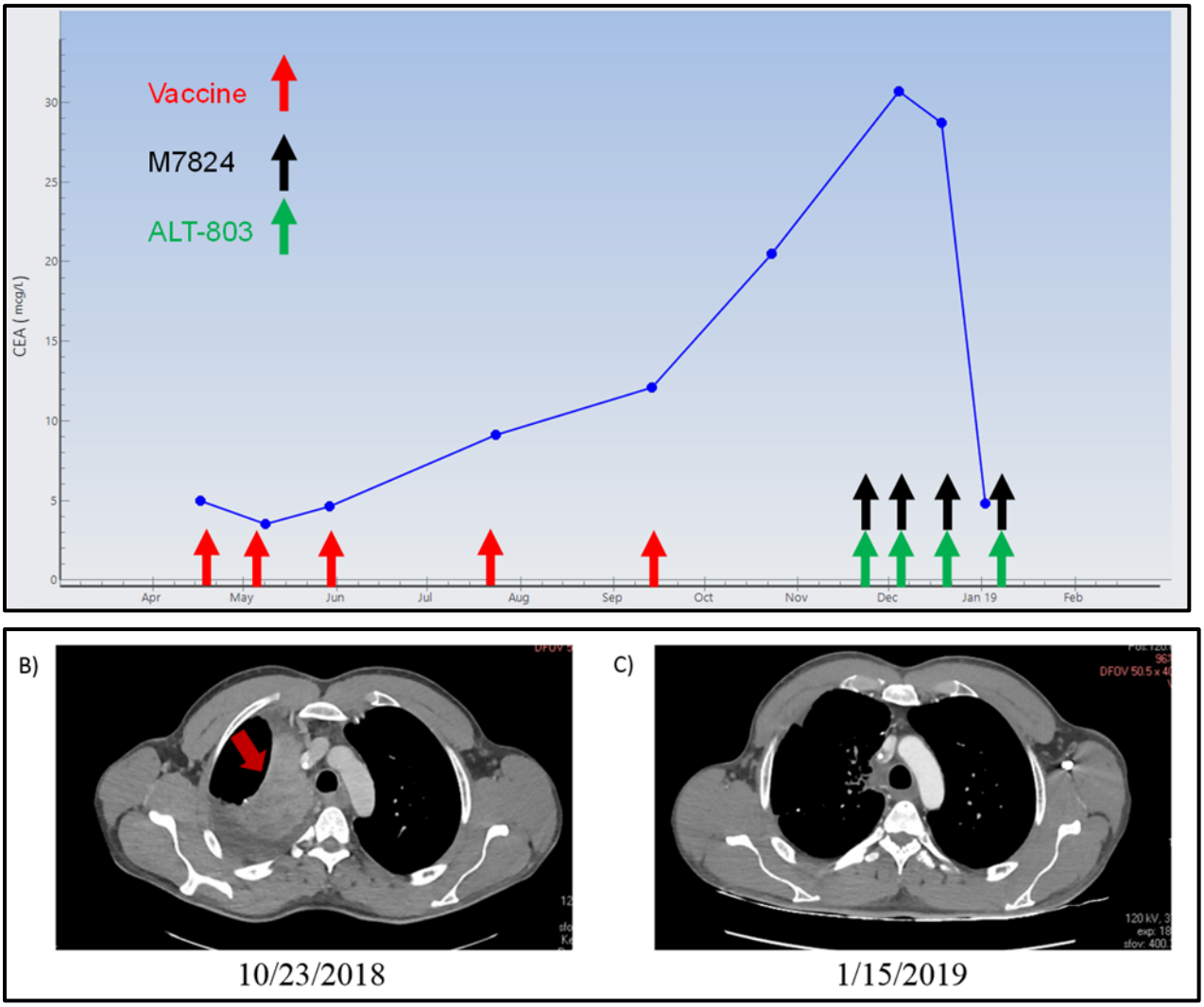


Figure 16 (A) CEA (mcg/L) of a patient with NRAS mutant, MSS colorectal cancer **(B)** Presence of large right sided hilar mass prior to starting M7824 + N-803 **(C)** Substantial tumor reduction at first restaging following starting M7824 + N-803.

This patient enrolled on the phase 1 trial of a CEA/MUC1based vaccine regimen, had evidence of disease progression, and switched over to another trial of M7824 plus N-803. Shortly after starting M7824 plus N-803 his tumor marker was found to drop precipitously. In addition, his first restaging scan showed a substantial partial response which was confirmed on his next scan.

Many of these novel immunotherapy agents have been tested clinically in combination with other immunotherapy agents and demonstrated an acceptable safety profile (**Table 1**).

Table 1

Nivolumab + N-803 [40]	NCT02523469
Avelumab (anti-PD-L1) + NHS-IL12	NCT02994953
CV301 (Poxviral vaccine) + M7824	NCT03315871
N-803 + M7824	NCT03493945
Poxviral vaccine + N-803 + M7824	NCT03493945

Safety data with the combination of Nivolumab and N-803 has been reported [31]. Twenty-three patients were enrolled and 21 patients treated at four dose levels of N-803 (6,10,15, and 20 mcg/kg) in combination with nivolumab at standard FDA approved dosing. No dose limiting toxicities were observed. And the maximum tolerated dose was not reached. The most common adverse events were injection site reactions (in 19 [90%] of 21 patients) and flu-like symptoms (in 15 [71%] of 21 patients). The most common grade 3 adverse events, occurring in two patients each were lymphopenia and fatigue. A grade 3 myocardial infarction occurred in one patient (only case of treatment discontinuation due to AE). No grade 4 or 5 adverse events were recorded. One case of grade 2 pneumonitis occurred which resolved without steroids. Otherwise no other notable autoimmune toxicity was observed.

Thirty-three patients have been treated with the combination of avelumab (anti PD-L1) and NHS-IL12. 26 patients in four dose levels of NHS-IL12 (4, 8, 12, or 16.8 mcg/kg) in combination with standard dosing of avelumab 10 mg/kg IV every 2 weeks and 7 additional patients in a fifth dose level which received NHS-IL12 at 16.8 mcg/kg in combination with avelumab 800 mg flat dosing weekly. One dose limiting toxicity occurred at DL3 (grade 3 autoimmune hepatitis). No dose limiting toxicities were observed at any other dose level. No grade 4 or 5 adverse events were recorded. The one case of grade 3 autoimmune hepatitis was the only notable autoimmune toxicity observed.

Six patients have been treated with the combination of CV301, a poxviral vaccine, and M7824. No dose limiting toxicities have been observed. One patient did have a transient asymptomatic grade 3 lipase elevation which did not require treatment hold and self-resolved. No grade 4 or 5 adverse events were recorded. Aside from the one case of transient asymptomatic lipase elevation no other notable autoimmune toxicities were observed.

Thirteen patients have been treated with the combination of two dose levels of N-803 (10 and 15 mcg/kg) and M7824 at 1200 mg flat dose. No dose limiting toxicities have been observed. One patient had grade 3 enterocolitis which resolved with steroid taper. No grade 4 or 5 adverse events were recorded. Aside from the one case of enterocolitis no other notable autoimmune toxicities were observed.

Nine patients have been treated with the combination of the same poxviral vaccine vector employed in CV301 composed of an MVA component as four injections ($4x10^8$ IU/0.5 mL) each on day 1 and 15 followed by an FPV component ($1x10^9$ IU/0.5 mL), in addition to N-803 at 15 mcg and M7824 at 1200 mg flat dose. No dose limiting toxicities have been observed.

The level of toxicity observed with all of these combinations were similar to (and perhaps less than) that observed with PD-1/PD-L1 monotherapy.

1.2.4 Clinical Rationale for using M7824 over other anti PD-L1 agents in Immunotherapy Combinations

M7824 is being chosen over other anti PD-1/L1 agents as M7824 has been well tested in the CCR having had the first in human phase I trial done here and also having shown early promising efficacy in a number of tumor types often beyond the efficacy observed with avelumab or other anti PD-L1 agents. As an example in a subset analysis of patients treated within the dose-escalation cohort with human papillomavirus (HPV)-associated disease (n = 17), including patients with anal, cervical, and squamous cell carcinoma of the head and neck, there was an ORR of 35.3%, and an ORR of 41.7% in patients with known HPV-positive disease (n = 12) which exceeded the expected ORR of 15-20% expected with single agent PD-L1 inhibition [41]. In another cohort of patients with advanced NSCLC and PD-L1 expressing tumors the confirmed ORR was 40.7% in patients treated at the recommended phase II dose (1200 mg every 2 weeks) which also compared favorably to the 15-20% expected response rate with single agent PD-L1 inhibition. In patients with high-PD-L1 expressing tumors, the ORR was 71.4% (n = 7) [42]. Based upon its potentially enhanced efficacy, M7824 is being used as the anti PD-1/L1 agent of choice in a number of combination trials either ongoing or planned in the CCR. In addition, once the safety profile of M6903 is well defined in combination with M7824 we plan to further expand on this combination by evaluating it in the context of additional IO agents such as vaccine therapies.

1.2.5 Dose Rationale

In the phase I dose escalation trial of combination N-803 and M7824, escalating doses of N-803 were evaluated at 10 and 15 mcg/kg given with a standard flat RP2D of M7824 at 1200 mg. No DLTs were observed. Therefore, a dose of 15 mcg/kg of N-803 is being chosen for this trial for Arm 1 in combination with standard RP2D of M7824 at 1200 mg. In the phase I dose escalation trial of combination Avelumab (anti-PD-L1) + NHS-IL12, escalating doses of NHS-IL12 were evaluated at 8,12, and 16.8 mcg/kg given every 4 weeks in combination with standard dosing of Avelumab. The combination including NHS-IL12 at the highest dose evaluated (16.8 mcg/kg given every 4 weeks) was found to be well tolerated. The only DLT observed on the trial was a transient grade 3 AST/ALT elevation at NHS-IL12 at 12 mcg/kg but otherwise no other DLT was observed and the MTD was not reached. On another trial (20C0045) evaluating the combination of a therapeutic cancer vaccine plus NHS-IL12 at 16.8 mcg/kg and M7824 no DLTs were observed in the six participant lead in safety cohort, although the majority of original participants (11/19; 57.9%) treated on that trial needed dose reductions of NHS-IL12 when given in combination with the therapeutic cancer vaccine and M7824 due to grade 2 fever or flu like symptoms. After further evaluation a dose of NHS-IL12 at 8 mcg/kg was found to be very tolerable when combined with a therapeutic cancer vaccine and M7824 on that other trial. Therefore, based on that data we evaluated NHS-IL12 at a dose of 8 mcg/kg on both this study and the 20C0045 study. However, after evaluating a total of 39 patients on the 20C0045 study including 21 patients with starting dose of 16.8 mcg/kg and 18 patients with starting dose of 8 mcg/kg we observed what looks to be reduced efficacy in patients receiving 8 mcg/kg as a starting dose with only 3/18 patients responding with the 8 mcg/kg starting dose as compared to 11/21 with the 16.8 mcg/kg starting dose. Therefore, based on this observation that the possibility that a lowering starting dose of NHS-

IL12 may reduce efficacy in combination with vaccine and M7824, we are amending to allow treatment (effective with Protocol Amendment Version 10.20.2021) with a limited number of doses of NHS-IL12 at 16.8 mcg/kg (four doses total) before reducing NHS-IL12 to 8 mcg/kg on both this study and the 20C0045 study. This is based upon responding patients in the first 21 patients on the 20C0045 study which received anywhere between 1-4 doses of NHS-IL12 at 16.8 mcg/kg before dose reductions were made.

Although N-803 and NHS-IL12 have potential overlapping toxicities (e.g., grade 1-2 flu like symptoms), it should be noted that no DLTs were observed in the six patient safety lead in combination of NHS-IL12 at 8 mcg/kg and N-803 at 10 mcg/kg. The NHS-IL12 and N-803 doses are being given on alternating schedules to minimize overlapping toxicities. Additionally, it was noted that 3/6 patients on this dose level (NHS-IL12 at 8 mcg/kg + N-803 10 mcg/kg in combination with vaccine and M7824) had bleeding events which did not meet DLT criteria but nonetheless limited patients' ability to receive further treatment with M7824 as part of this combination. Therefore, we are planning to dose reduce M7824 to 300 mg when evaluating the next and highest dose level on Arm 2A which also includes NHS-IL12 at 16.8 mcg/kg (for up to a total of 4 doses before reducing to 8 mcg/kg) + N-803 at 10 mcg/kg. A dose of M7824 at 300 mg has been found to be clinically active and anecdotally result in less bleeding.

1.2.6 Immune Assays

Multiple immune assays have been developed in the LTIB to better define the mechanism(s) involved in the use of specific novel agents, as monotherapy or in combination therapies, both for preclinical and clinical studies (Section **5.1**). In addition to analyses of biopsies, analyses of the peripheral immunome can provide valuable information in that multiple samples can be analyzed over the course of a given therapy vs. pre-therapy. The LTIB has now developed and employed [8, 9, 24, 43] a flow cytometry–based assay that can analyze 123 immune cell subsets in human PBMC from one vial of processed PBMC (approximately 10^7 cells). This assay will be used in this clinical trial to detect multiple subsets of CD4+ T cells (n=32), CD8+ T cells (n=29), Tregs (n=7), B cells (n=5), NK (n=20), NKT (n=4), DC (n=10), and MDSC (n=16) to better understand the role of each agent. We also plan to evaluate changes in TCR clonal diversity both in biopsies and the periphery. One example involves an ongoing first-in-human trial of the NHS-IL12 tumor-targeting immunocytokine. TCR diversity increased 6-14–fold in biopsies of patients with a high or intermediate IFN-γ response but were unchanged or decreased with a low IFN-γ response. These findings also correlated with TIL in biopsies. We are also currently employing NanoString analyses to identify a gene signature in biopsies and PBMC, pre- and post-treatment. The GMB will conduct multiplexed, multispectral imaging of FFPE tissue to evaluate multiple immune parameters within the TME before and after treatment in patients with available tissue.

1.2.7 Summary

- We are proposing a novel immunotherapy combination to help overcome multiple barriers in an effective anti-tumor immune response.
    - (1) vaccines targeting tumor associated antigens to prime an anti-tumor immune response
    - (2) N-803 to expand the response and activate T effector cells and NKs
    - (3) NHS-IL12 and M7824 to enable activated immune cells to work better in the TME

- Preclinical studies have shown that the addition of M7824 to vaccine therapy improves anti-tumor activity
- Preclinical studies have shown that the addition of N-803 to vaccine therapy and M7824 further improves anti-tumor activity
- Preclinical studies have shown that the addition of NHS-IL12 to vaccine therapy, N-803 and checkpoint therapy still further improves anti-tumor activity
- Clinical data suggests that M7824 is at least as effective if not more effective at producing anti-tumor responses than other standard anti PD-L1 agents in a number of tumor types
- Clinical data evaluating the safety of combination immunotherapy has shown multiple combinations of these agents to be safe and overall well tolerated including:
    - Vaccine + M7824
    - NHS-IL12 + Checkpoint therapy
    - N-803+ M7824
    - Vaccine + N-803 + M7824
- There has been anecdotal evidence of clinical activity with combination immunotherapy
    - Vaccine + anti PD-L1
    - Vaccine + M7824 + N-803

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Subjects with cytologically or histologically confirmed locally advanced or metastatic small bowel or colorectal adenocarcinoma

2.1.1.2 Subjects must have received two prior lines of systemic therapy unless the subject is not eligible to receive standard therapy or declines standard treatment

2.1.1.3 Subjects must have measurable disease

2.1.1.4 ECOG performance status ≤ 2 (**Appendix A**: Performance Status Criteria)

2.1.1.5 Adequate hematologic function at screening, as follows:

- Absolute neutrophil count (ANC) ≥1 x 10^9/L
- Hemoglobin ≥ 9 g/dL
- Platelets ≥ 75,000/microliter

2.1.1.6 Adequate renal and hepatic function at screening, as follows:

- Serum creatinine ≤ 1.5 x upper limit of normal (ULN) OR
  Measured or calculated creatinine clearance ≥ 40 mL/min for participant with creatinine levels > 1.5 X institutional ULN (GFR can also be used in place of creatinine or CrCl);
- Bilirubin ≤ 1.5 x ULN OR in subjects with Gilbert's syndrome, a total bilirubin ≤ 3.0 x ULN
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 x ULN, unless liver metastases are present, then values must be ≤ 3 x ULN)

2.1.1.7 The effects of the immunotherapies on the developing human fetus are unknown; thus, women of childbearing potential and men must agree to use highly effective contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study treatment and up to two months after the last dose of study drug. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.8 Participants serologically positive for HIV, Hep B, Hep C are eligible as long as the viral loads are undetectable by quantitative PCR. HIV positive participants must have CD4 count ≥ 200 cells per cubic millimeter at enrollment, be on stable antiretroviral therapy for at least 4 weeks and have no reported opportunistic infections or Castleman's disease within 12 months prior to enrollment.

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

2.1.2 Exclusion Criteria

2.1.2.1 Participants with prior investigational drug, chemotherapy, immunotherapy or any prior radiotherapy (except for palliative bone directed therapy) within the past 28 days prior to the first drug administration except if the investigator has assessed that all residual treatment-related toxicities have resolved or are minimal and feel the participant is otherwise suitable for enrollment. Additionally, current therapies (e.g., maintenance capecitabine) may be continued where in the opinion of the investigator stopping such therapies may increase the risk of disease progression. Also, participants may continue adjuvant hormonal therapy in the setting of a definitively treated cancer (e.g., breast).

2.1.2.2 Participants with microsatellite unstable or mismatch repair deficient disease.

2.1.2.3 Major surgery within 28 days prior to the first drug administration (minimally invasive procedures such as diagnostic biopsies are permitted).

2.1.2.4 Known life-threatening side effects resulting from prior checkpoint inhibitor therapy (e.g., colitis, pneumonitis, fulminant hepatitis which led to permanent discontinuation of prior checkpoint therapy). Autoimmune toxicity which was not life threatening (e.g., arthritis) or did not lead to discontinuation of prior checkpoint therapy is allowed.

2.1.2.5 Known active brain or central nervous system metastasis (less than a month out from definitive radiotherapy or surgery), seizures requiring anticonvulsant treatment (<3 months) or clinically significant cerebrovascular accident (<3 months). In order to be eligible participants must have repeat CNS imaging at least a month after definitive treatment showing stable CNS disease. Participants with evidence of intratumoral or peritumoral hemorrhage on baseline imaging are also excluded unless the hemorrhage is grade ≤ 1 and has been shown to be stable on two consecutive imaging scans.

2.1.2.6 Pregnant women are excluded from this study because these drugs have not been tested in pregnant women and there is potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to

treatment of the mother with these immunotherapies, breastfeeding should be discontinued if the mother is treated on this protocol.

2.1.2.7 Active autoimmune disease that might deteriorate when receiving an immunostimulatory agent with exception of:

- Diabetes type I, eczema, vitiligo, alopecia, psoriasis, hypo- or hyperthyroid disease or other mild autoimmune disorders not requiring immunosuppressive treatment;
- Subjects requiring hormone replacement with corticosteroids are eligible if the steroids are administered only for the purpose of hormonal replacement and at doses ≤ 10 mg of prednisone or equivalent per day;
- Administration of steroids for other conditions through a route known to result in a minimal systemic exposure (topical, intranasal, intro-ocular, or inhalation) is acceptable;
- Subjects on systemic intravenous or oral corticosteroid therapy with the exception of physiologic doses of corticosteroids (≤ the equivalent of prednisone 10 mg/day) or other immunosuppressive agents such as azathioprine or cyclosporin A are excluded on the basis of potential immune suppression. For these subjects these excluded treatments must be discontinued at least 1 weeks prior to enrollment for recent short course use (≤ 14 days) or discontinued at least 4 weeks prior to enrollment for long term use (> 14 days). In addition, the use of corticosteroids as premedication for contrast-enhanced studies is allowed prior to enrollment and on study.

2.1.2.8 Subjects with a history of serious intercurrent chronic or acute illness, such as cardiac or pulmonary disease, hepatic disease, bleeding diathesis or recent (within 3 months) clinically significant bleeding events or other illness considered by the Investigator as high risk for investigational drug treatment.

2.1.2.9 Subjects unwilling to accept blood products as medically indicated.

2.1.2.10 History of second malignancy within 3 years of enrollment except for the following: adequately treated localized skin cancer, cervical carcinoma in situ, superficial bladder cancer, or other localized malignancy which has been adequately treated or malignancy which does not require active systemic treatment (e.g., low risk CLL).

2.1.2.11 Subjects with a known severe hypersensitivity reaction to a monoclonal antibody (grade ≥ 3 NCI-CTCAE v5) will be evaluated by the allergy/immunology team prior to enrollment.

2.1.2.12 Receipt of any organ transplantation requiring ongoing immunosuppression.

2.1.2.13 Receipt of prior lymphodepleting chemotherapy (e.g., cyclophosphamide or fludarabine at standard lymphodepleting doses).

2.1.3 Recruitment Strategies

This study will be listed on www.clinicaltrials.gov. This study will be posted on NIH websites and on NIH social media forums. Participants will be recruited from the current participant population at NIH. Any recruitment materials will be submitted to the IRB for approval prior to distribution.

2.2 SCREENING EVALUATION

2.2.1 Screening activities prior to the obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent or the consent for study # 01C0129 (provided the procedures are permitted on that study) on which screening activities will be performed within 28 days prior to enrollment. Assessments performed at outside facilities or on another NIH protocol within the timeframes listed may also be used to determine eligibility once a participant has signed the consent.

- Complete medical history and physical examination (including height, weight, vital signs, and ECOG performance status).
- CT of chest, abdomen and pelvis or MRI, if clinically indicated.
- A brain CT scan in participants with known CNS disease as described in Section **2.1.2.5** / MRI scan if clinically indicated.
- EKG.
- Clinical laboratory tests (within 16 days prior to enrollment).
  - Chemistry: sodium, potassium, chloride, bicarbonate, calcium, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, total protein, albumin, and total and direct bilirubin.
  - Hematology: complete blood count (CBC) with differential.
  - CD4 (if HIV positive).
- Coagulation panel: PT, INR, and PTT.
- Urine or serum pregnancy test (β-HCG) for females of childbearing-potential and women < 12 months since the onset of menopause (within 16 days prior to enrollment).
- Urinalysis.
- HBV, HCV, HIV testing including viral load in positive participants (within 3 months prior to enrollment).
- Histologic confirmation (at any time point prior to enrollment). If there is no available tumor sample or pathology report, a biopsy will be performed to confirm the diagnosis.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a transient lab abnormality may be rescreened.

2.3.2 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Cohort 1	Subjects with small bowel or colon adenocarcinoma receiving fixed doses of treatment.
2	Cohort 2	Subjects with small bowel or colon adenocarcinoma receiving quadruple therapy during dose escalation of NHS-IL12.

Arms

Number	Name	Description
1	Arm 1	CEA/ MUC1 Vaccines + M7824 + N-803 (Triple Therapy).
2	Arm 2A	CEA/ MUC1 Vaccines + M7824 + N-803 + NHS-IL12 (Quadruple Therapy); dose escalation of NHS-IL12
3	Arm 2 B	CEA/ MUC1 Vaccines + M7824 + N-803 + NHS-IL12 (Quadruple Therapy); fixed dose of NHS-IL12

Arm Assignment

Assignment of participants will be sequentially to arm 1, then 2A, then 2B as follows:

Participants in Cohort 1 will be assigned to Arm 1 first.

Then participants in Cohort 2 will be assigned to Arm 2A until the MTD or maximum safe dose of NHSIL12 has been determined in the quadruple combination.

Then, following completion of Arm 2A, participants in Cohort 1 will be assigned to Arm 2B.

If there is a pause in accrual to Arm 1 after the first Simon II stage step (due to pending clinical outcomes needed for the determination to expand Arm 1) accrual may continue to Arm 2A and 2B in the interim and be switched back to Arm 1 if and when the decision is made to expand Arm1.

2.4 BASELINE EVALUATION

All subjects are required to complete baseline evaluations within one week prior to the first planned dosing of the study drug (any screening evaluation done within this time period can also serve for the baseline evaluation):

- Physical exam including weight, ECOG performance status and vital signs.
- Concomitant Medications and Baseline Signs and Symptoms evaluation.
- Urine or serum pregnancy test (β-HCG) for females of childbearing-potential and women < 12 months since the onset of menopause.
- Chemistry: sodium, potassium, chloride, bicarbonate, calcium, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, total protein, albumin, and total and direct bilirubin.
- ACTH, TSH, free T4, lipase, amylase, CRP.
- Hematology: CBC with differential.

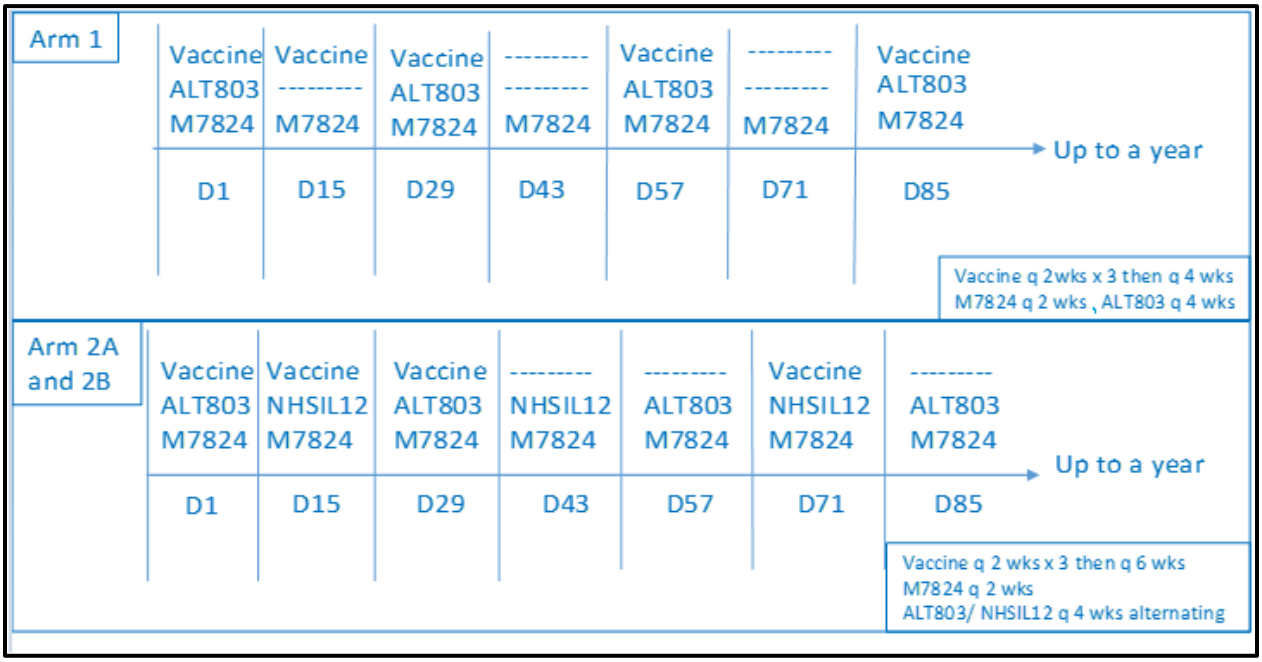
3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

- This is a phase II trial of combination immunotherapy, with a brief dose escalation portion for Arm 2.
- The trial will be conducted using a Simon optimal two-stage design in each Phase II Arm.
- Participants will be enrolled on the following two arms in sequential order: (1) CV301+ M7824 + N-803, (2) CV301 + M7824 + N-803 + NHS-IL12 (**Figure 17**).
- Given the known overlapping toxicities of NHS-IL12 and N-803 (e.g., flu like symptoms), as of the amendment dated 10.20.2021 the second arm will evaluate the safety of the combination of standard dosing of vaccines and M7824 with N-803 at 10 mcg/kg and escalating doses of NHS-IL12 at 8 and 16.8 mcg/kg using a modified 3+3 design (six patients per dose level; if < 2/6 patients with DLTs may move up to higher dose level; if > 1/6 patients with DLTs may move to lower dose level). Dose level 1 on Arm 2A (including N-803 at 10 mcg/kg and NHS-IL12 at 8 mcg/kg) was found to be safe without any DLTs. Therefore, we plan to escalate to dose level 2 on Arm 2A evaluating the combination of N-803 at 10 mcg/kg and NHS-IL12 at 16.8 mcg/kg (for up to 4 consecutive doses followed by 8 mcg/kg thereafter) with standard dosed vaccine + M7824 at 300 mg). The maximum tolerated dose level deemed safe on Arm 2Awill be expanded to nine evaluable patients and used for the Simon optimal two-stage design. If dose level 2 on Arm 2A is determined to be the maximum tolerated dose and expanded to nine evaluable patients in Arm 2B as

part of the Simon two stage design then dose level 1 on Arm 2A may also be expanded up to nine evaluable patients for further immune correlative assessments.

- The first six participants on arm 1 will be evaluable for dose limiting toxicities (DLTs) and accrual will only continue to 9 participants on that arm if less than 2 out of the first 6 pts experience a DLT.
- In Arm 2A, participants will receive the combination of 4 treatments, but the dose level of NHS-IL12will be determined during a dose escalation using a modified 3+3 design (six patients per dose level; if < 2/6 patients with DLTs may move up to higher dose level; if > 1/6 patients with DLTs may move to lower dose level). Following determination of the MTD or highest safe dose evaluated, the 6 participants at that MTD or highest safe dose level will be included among the initial 9 participants for the first stage of Arm 2B.
- There will be a minimum of 28 days between the sixth participant (last participant for determining safety) enrolled to Cohort 1 and assigned to Arm1 and the first participant enrolled on cohort 2 and assigned to Arm 2A
- There will be a minimum of one week between the first three subjects enrolled in Arm 1 and for each dose level of Arm 2A. The fourth through sixth participant of Arm 1 or each dose level of Arm 2A may enroll no sooner than 48 hours after the most recent enrollee. Once a dosage combination has been determined to be safe in six participants further participants may enroll at any time to that dosage combination.
- In the second stage of the Simon optimal two-stage design, if two or more out of nine participants have objective responses on a given arm that arm will be expanded to enroll 20 evaluable participants
- It is possible that the response rates observed with combination immunotherapy will be less in participants who have previously received checkpoint therapy and not be truly reflective of a population of participants who have not had prior checkpoint therapy. While checkpoint experienced participants will not be excluded from enrollment, the primary efficacy analyses will be limited to checkpoint naïve participants with MSS colon cancer and efficacy analyses in the checkpoint refractory participants will be only exploratory.
- Participants with small bowel cancers are being evaluated in this trial as well as participants with colorectal cancer but if efficacy is not observed in the first stage of the Simon II step design for either Arm 1 or Arm 2B than participants with small bowel cancer may be replaced with participants with colorectal cancer at the discretion of the investigator.



Of note each Day # in the figure above has a corresponding Week # in the **Study Calendar** (D1 = W1, D15 = W3, D29 = W5, etc.).

*Note: ALT803 is also known as N-803

Dose Escalation Schedule				
Dose Level	MVA as 4 injections 4 x 10^8 IU/0.5 mL each) on D1 and D15, followed by FPV (1 x 10^9 IU/ 0.5mL)	N-803	M7824	NHS-IL12
Arm 1		15 mcg/kg	1200 mg	n/a
Arm 2A, Dose Level 1		10 mcg/kg	1200 mg	8 mcg
Arm 2A, Dose Level 2		10 mcg/kg	300 mg	16.8 mcg
Arm 2B		10 mcg/kg	300 mg	MTD

Figure 17

3.1.1 Dose Limiting Toxicity

Dose limiting toxicities (DLT) (For first 6 participants in Arm 1, and all participants during dose escalation in Arm 2A):

Dose-limiting toxicity (DLT) will be defined as any one of the following adverse events, possibly attributable to study drugs, that occur within 28 days of the start of therapy:

- Any Grade 3 or greater adverse drug reactions (ADRs) as defined by CTCAE v5.0 and assessed as possibly attributed to an agent except for any of the following:
    - Grade 3 flu-like symptoms or fever, as well as associated symptoms of fatigue, headaches, nausea, emesis which can be controlled with conservative medical management.
    - Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor.
    - Grade 3 Hgb decrease (< 8.0 g/dL) not attributed to grade 3 or greater bleeding events that is clinically manageable with blood transfusions or erythroid growth factor use does not require treatment discontinuation.
    - Grade 3 laboratory values laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline grade within 14 days.
    - Grade 4 laboratory values that are asymptomatic and have no clinical correlate (e.g., lymphopenia).
    - Keratoacanthoma and squamous cell carcinoma of the skin.
    - Any endocrinopathy that can be medically managed with hormone replacement
    - Any grade 3 adverse drug reaction which can be medically managed with minimal risk to the participant (e.g., placement of a pleural catheter for recurrent inflammatory pleural effusions) and resolves to at least grade one or baseline grade within 14 days.

Criteria are based on the NCI Common Terminology Criteria for Adverse Events, Version 5.

Where clinically appropriate subjects on all arms will receive one year of treatment during which subjects will be followed with surveillance scans. On a case by case basis treatment beyond a year is allowed per investigator discretion. Subjects with evidence of disease progression after completing a year of treatment will be allowed retreatment on the same arm.

Patents might be taken off treatment for disease progression prior to completing one year of treatment, but where clinically appropriate, treatment beyond radiographic progression is allowed if in the opinion of the investigator the subject is benefiting from treatment.

Subjects will be taken off treatment if unacceptable toxicity.

Both arms of this trial will be conducted using a Simon two-stage phase II trial design in which 9 evaluable participants will enroll and if 2 or more of the 9 have an objective response, then accrual would continue until a total of 20 evaluable participants have been treated on a given arm. If there are 5 or more of 20 (25.0%) who experience a response in a given arm, this would be sufficiently interesting to warrant further study.

In the event that a participant develops a response (>30% decrease in target lesions as compared with baseline scan) following initial progression or “pseudo-progression”, this response will be considered as an objective response in the determination to expand accrual.

3.2 DRUG ADMINISTRATION

3.2.1 General Rule

A window of +/- 14 days for a scheduled treatment is allowed in the event of scheduling issues (i.e., holiday, bad weather or other scheduling issues). The minimum time between administrations of a similar agent is 11 days.

3.2.2 CV301

The CV301 vaccine regimen consists of MVA-BN-CV301 (priming vaccine) and FPV-CV301 (booster vaccine).

MVA-BN-CV301 is administered as four subcutaneous injections (4 x 10^8 infectious units/0.5 mL each) on D1 and D15. Starting on D29, FPV-CV301 (1 x 10^9 infectious units/ 0.5mL) is administered every 4 weeks on Arm 1 or every 6 weeks on Arm 2A and 2B for up to one year.

MVA-BN-CV301 is administered as one injection in each of the following locations: upper left arm, upper right arm, upper left thigh and upper right thigh (i.e., four injection sites per dose). Alternate subcutaneous sites may be used with approval of the principle investigator.

FPV-CV301 is administered as a subcutaneous injection preferably in the upper arm (i.e., one injection site per dose). An alternate subcutaneous site may be used with approval of the principle investigator.

3.2.3 M7824

Subjects will receive M7824 via IV infusion over 1 hour (-10 minutes / +20 minutes, that is, over 50 to 80 minutes) once every 2 weeks. M7824 will be administered as a "flat" dose of either 300 mg or 1,200 mg independent of body weight depending on dose level. M7824 is administered as an intravenous infusion with a mandatory 0.2 micron in-line filter.

In order to mitigate potential infusion-related reactions, premedication with an antihistamine and with acetaminophen (for example, 25-50 mg diphenhydramine and 500-650 mg acetaminophen within approximately 30 to 60 minutes prior to dosing of M7824 is optional and at the discretion of the Investigator.

3.2.4 N-803

N-803 will be given via subcutaneous injection at a dose of 15 mcg/kg every four weeks on Arm 1 and 10 mcg/kg every four weeks on Arm 2A and Arm 2B.

N-803 dosing will be calculated using a weight obtained within one week prior to the first dose. The dose will be re-calculated at the beginning of each subsequent cycle in the event of a 10% or greater weight change. If the weight at the beginning of each subsequent cycles is within 10%, the previous dose may be used or the dose may be re-calculated based on local site procedures.

Injection should occur in the abdomen and injection sites should be rotated per institutional guidelines and each injection site separated by at least 1 inch. Depending on the injection volume required, the dose of N-803 may be administered as a single injection or multiple injections at the discretion of the investigator.

3.2.5 NHS-IL12

NHS-IL12 will be administered at a dose of 8 or 16.8 mcg/kg by SC injection every 4 weeks on Arm 2A and Arm 2B depending on dose level assignment. The SC injection may be given in the upper arm, anterolateral thigh or abdomen and should preferably be at least 5 cm away from any other subcutaneous injection. The dose of NHS-IL12 will be calculated based on the weight of the subject determined within 72 hours prior to the day of drug administration. The dose of NHS-IL12 used for the previous administration can be repeated if the change in the subject's weight is 10% or less than the weight used for the last dose calculation.

Note: Vital signs will be obtained prior to treatment and 30 minutes after administration of the last study drug. Participants should be observed for 30 minutes after the administration of the last study drug.

3.3 STUDY INTERVENTION COMPLIANCE

All study drug administration will be given at NIH and documented in the electronic medical record.

3.4 DOSE MODIFICATIONS

3.4.1 Discontinuation

Treatment with individual agents will be discontinued in the case of:

- Any Grade 4 or higher adverse drug reactions (ADRs) as defined by CTCAE v5.0 and assessed as possibly related to that agent by the Investigator, except for laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline grade within 7 days without medical intervention".
- Any Grade 3 ADRs possibly attributed to an agent except for any of the following:
    - Grade 3 flu-like symptoms or fever, as well as associated symptoms of fatigue, headaches, nausea, emesis which can be controlled with conservative medical management.
    - Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor.
    - Grade 3 Hgb decrease (< 8.0 g/dL) that is clinically manageable with blood transfusions or erythroid growth factor use does not require treatment discontinuation.
    - Grade 3 laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline grade within 14 days.
    - Keratoacanthoma and squamous cell carcinoma of the skin.
    - Any endocrinopathy that can be medically managed with hormone replacement
    - Any grade 3 adverse drug reaction which can be medically managed with minimal risk to the participant (e.g., placement of a pleural catheter for recurrent inflammatory pleural effusions) and resolves to at least grade one or baseline grade within 14 days.

3.4.2 Dose Delay

Individual agents should be withheld for any Grade 2 or 3 ADR possibly attributed to that agent until resolution to Grade ≤ 1 unless the ADR in the opinion of the investigator is not clinically relevant or can be medically managed with minimal risk to the participant. Should a clinically

relevant grade 2 or 3 ADR persist for more than 4 weeks, consideration should be given to discontinuing treatment with that individual agent at the discretion of the investigator.

For non-medical logistical reasons, unrelated acute illnesses, or palliative radiation, scheduled assessments and dosing can be delayed up to 2 months. Where at all possible, dosing should be restarted to keep in line with the original treatment schedule.

N-803 may be held or delayed on the day of a planned dose for either of the following situations:

- the participant has a fever of > 101°F (38.3 °C);
- if in the opinion of the treating physician, holding would be of benefit to the participant;
- hypotension (systolic blood pressure < 90 mm Hg).

N-803 dosing should be held for hypotension (defined as systolic blood pressure less than 90 mm Hg) if in the presence of any clinically significant symptoms (in the opinion of the treating physician), until the systolic blood pressure reading is stable. If mild dehydration is suspected, an IV fluid bolus may be used per standard of care.

3.4.3 Dose Modification

Participants with dose modifications or skipped doses within DLT period (for first 6 participants in Arm 1 and all participants during dose escalation in Arm 2A) for reasons other than a DLT (e.g., logistical reasons) who do not experience a DLT within the DLT window will not be evaluable for DLTs and will be replaced for the DLT assessment. Participants who have dose modifications or skipped doses within the DLT period for logistical reasons and nevertheless experience a DLT within the DLT period will be considered DLT evaluable.

CEA/ MUC1 vaccines: Vaccine doses will not be modified but doses may be skipped per investigator discretion.

N-803: Doses may be reduced to 4, 6 or 8 mcg/kg dose or doses may be skipped. On Arm1, if participants do not tolerate N-803 at 15 mcg/kg every 4 weeks, N-803 may be given on an alternative treatment schedule at lower doses every 2 weeks per investigator discretion.

M7824: Doses may be reduced to a 200 or 300 mg flat dose or doses may be skipped.

NHS-IL12: Doses may be reduced to a 4,6, 8 or 12 mcg/kg dose or doses may be skipped.

Stopping Rule: Any death at least possibly related to study regimen will prompt a suspension of accrual and reassessment of available safety information prior to reopening study to accrual.

As a general rule consideration should be given to holding doses of drugs when a toxicity greater than grade 1 at least possibly attributed to a given drug occurs until resolution of that toxicity to at least grade 1 or better. If this occurs with N-803 or NHS-IL12 than consideration should be given to restarting at a reduced dose when restarting treatment with those agents. On Arm1, N-803 may be restarted at any scheduled visit as long as there is at least 14 days between doses of this agent. Dose reductions for vaccine and M7824 are not allowed. If repeated grade 2 adverse events occur than consideration should be given to permanently discontinuing a given agent.

3.4.4 Guidelines for Management of Infusion Reactions

(In addition to the below guidelines, investigators may also use NCCN, ASCO, SITC or FDA guidelines for Immune Related Adverse Events)

NCI-CTCAE Grade	**Treatment Modification for Infused Agent (M7824)**
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Consider decreasing the infusion rate of the particular agent by 50% and monitoring closely for any worsening.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, i.v. fluids); prophylactic medications indicated for ≤ 24 hours.	Consider temporarily discontinuing infusion of the particular agent. Consider resuming infusion of the particular agent at 50% of previous rate once infusion related reaction has resolved or decreased to at least Grade 1 in severity, and monitor closely for any worsening.
Grade 3 or Grade 4 – severe or life-threatening *Grade 3:* Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); hospitalization indicated for clinical sequelae. *Grade 4*: Life-threatening consequences; urgent intervention indicated.	Stop the infusion immediately and disconnect infusion tubing from the subject. For grade 3 events: Consider withdrawing immediately from treatment with that particular agent and not offering any further treatment with that agent based upon if the clinical condition can be safely managed. For grade 4 events: Withdraw immediately from treatment and do not offer further treatment with that agent.

If the infusion rate of M7824 has been decreased by 50% or interrupted due to an infusion reaction, keep it decreased for the next scheduled infusion. If no infusion reaction is observed in the next scheduled infusion, the infusion rate may be returned to baseline at the subsequent infusions based on investigator's medical judgment.

If hypersensitivity reaction occurs, the subject should be treated according to the best available medical practice.

3.4.5 Guidelines for Management of Immune-Mediated Adverse Reactions

Gastrointestinal irAEs		
Severity of Diarrhea/Colitis (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 Diarrhea: < 4 stools/day over Baseline Colitis: asymptomatic	Consider continuing M7824 Symptomatic treatment (e.g. loperamide)	Consider close monitoring for worsening symptoms Consider educating subject to report worsening immediately If worsens: Treat as Grade 2, 3 or 4.
Grade 2 Diarrhea: 4 to 6 stools per day over Baseline; IV fluids indicated < 24 hours; not interfering with ADL Colitis: abdominal pain; blood in stool	Consider withholding M7824 Symptomatic treatment	If improves to Grade ≤ 1: Consider resuming therapy If persists > 7 days or recurs: Consider treating as Grade 3 or 4.
Grade 3 to 4 Diarrhea (Grade 3): ≥ 7 stools per day over Baseline; incontinence; IV fluids ≥ 24 h; interfering with ADL Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: life-threatening, perforation	Consider withholding or permanently discontinuing M7824 for Grade 3 events based upon if the clinical condition can be safely managed. For grade 4 events permanently discontinue treatment. Consider 1.0 to 2.0 mg/kg/day prednisone IV or equivalent Consider adding prophylactic antibiotics for opportunistic infections Consider lower endoscopy	If improves: Consider continuing steroids until Grade ≤ 1, then tapering over at least 1 month; consider resuming therapy following steroids taper (for initial Grade 3). If worsens, persists > 7 days, or recurs after improvement: Consider adding infliximab 5mg/kg (if no contraindication). Note: infliximab should not be used in cases of perforation or sepsis.

Dermatological irAEs		
Grade of Rash (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 to 2 Covering ≤ 30% body surface area	Consider continuing M7824 Consider symptomatic therapy (for example, antihistamines, topical steroids)	If persists > 1 to 2 weeks or recurs: Consider withholding M7824 and OX40 therapy Consider skin biopsy Consider 0.5-1.0 mg/kg/day prednisone or equivalent. Once improving, consider tapering steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and consider resuming M7824 therapy following steroids taper. If worsens: Consider treating as Grade 3 to 4.
Grade 3 to 4 Grade 3: Covering > 30% body surface area; Grade 4: Life threatening consequences	Consider withholding or permanently discontinuing M7824 for Grade 3 events based upon if the clinical condition can be safely managed. For grade 4 events permanently discontinue treatment. Consider skin biopsy Consider dermatology consult Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent Consider adding prophylactic antibiotics for opportunistic infections	If improves to Grade ≤ 1: Consider tapering steroids over at least 1 month; consider resuming therapy following steroids taper (for initial Grade 3).
Pulmonary irAEs		
Grade of Pneumonitis (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 Radiographic changes only	Consider withholding M7824 Consider Pulmonary and Infectious Disease consults	Consider re-assessing at least every 3 weeks If worsens: Consider treating as Grade 2 or Grade 3 to 4.
Grade 2 Mild to moderate new symptoms	Consider withholding M7824 Consider pulmonary and Infectious Disease consults	If improves:

	Consider monitoring symptoms daily; consider hospitalization Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent Consider adding prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	When symptoms return to Grade ≤ 1, consider tapering steroids over at least 1 month, and then consider resuming therapy following steroids taper If not improving after 2 weeks or worsening: Consider treating as Grade 3 to 4.
Grade 3 to 4 Grade 3: Severe new symptoms; New/worsening hypoxia; Grade 4: Life-threatening	Consider withholding or permanently discontinuing M7824 for Grade 3 events based upon if the clinical condition can be safely managed. For grade 4 events permanently discontinue treatment. Consider hospitalization. Consider pulmonary and Infectious Disease consults. Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent Consider adding prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	If improves to Grade ≤ 1: Consider tapering steroids over at least 1 month If not improving after 48 hours or worsening: Consider adding additional immunosuppression (for example, infliximab, cyclophosphamide, IV immunoglobulin, or mycophenolate mofetil)
Hepatic irAEs		
Grade of Liver Test Elevation (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin > ULN to 1.5 x ULN	Consider continuing M7824	Consider continued liver function monitoring If worsens: Consider treating as Grade 2 or 3 to 4.
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or total bilirubin > 1.5 to ≤ 3 x ULN	Consider withholding M7824	If returns to Grade ≤ 1: Consider resuming therapy. If elevation persists > 7 days or worsens: Consider treating as Grade 3 to 4.
Grade 3 to 4 AST or ALT > 5 x ULN and/or total bilirubin > 3 x ULN	Consider withholding or permanently discontinuing M7824 for Grade 3 events based upon if the clinical condition can be safely managed. For grade 4 events permanently discontinue treatment. Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent	If returns to Grade ≤ 1: Consider tapering steroids over at least 1 month If does not improve in > 7 days, worsens or rebounds: Consider adding mycophenolate mofetil 1 gram (g) twice daily

	Consider adding prophylactic antibiotics for opportunistic infections Consider consulting gastroenterologist/ hepatologist Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	If no response within an additional 7 days, consider other immunosuppressants per local guidelines.
Renal irAEs		
Grade of Creatinine Increased (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 Creatinine increased > ULN to 1.5 x ULN	Consider continuing M7824	Continue renal function monitoring If worsens: Consider treating as Grade 2 to 3 or 4.
Grade 2 Creatinine increased > 1.5 and ≤ 6 x ULN	Consider withholding M7824 Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent. Consider adding prophylactic antibiotics for opportunistic infections Consider renal biopsy	If returns to Grade ≤1: Consider tapering steroids over at least 1 month, and consider resuming therapy following steroids taper. If worsens: Treat as Grade 4.
Grade 3-4 Creatinine increased > 6 x ULN	Consider withholding or permanently discontinuing M7824 for Grade 3 events based upon if the clinical condition can be safely managed. For grade 4 events permanently discontinue treatment. Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent. Consider adding prophylactic antibiotics for opportunistic infections Consider renal biopsy Consider Nephrology consult	If returns to Grade ≤1: Consider tapering steroids over at least 1 month.
Cardiac irAEs		
Myocarditis	**Initial Management**	**Follow-up Management**
New onset of cardiac signs or symptoms and / or new laboratory cardiac biomarker elevations (e.g. troponin, CK-MB, BNP) or cardiac imaging abnormalities suggestive of myocarditis.	Consider withholding or permanently discontinuing M7824 based upon if the clinical condition can be safely managed. Consider hospitalization	If symptoms improve and immune-mediated etiology is ruled out, consider re-starting therapy.

	In the presence of life threatening cardiac decompensation, consider transfer to a facility experienced in advanced heart failure and arrhythmia management. Consider cardiology consult to establish etiology and rule-out immune-mediated myocarditis. Consider myocardial biopsy if recommended per cardiology consult.	If symptoms do not improve/worsen, viral myocarditis is excluded, and immune-mediated etiology is suspected or confirmed following cardiology consult, consider managing as immune-mediated myocarditis.
Immune-mediated myocarditis	Consider withholding or permanently discontinuing M7824 based upon if the clinical condition can be safely managed. Consider guideline based supportive treatment as appropriate as per cardiology consult. Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent Consider adding prophylactic antibiotics for opportunistic infections.	Once improving, consider tapering steroids over at least 1 month. If no improvement or worsening, consider additional immunosuppressants (e.g. azathioprine, cyclosporine A).

*Local guidelines, or eg. ESC or AHA guidelines
ESC guidelines website: https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines
AHA guidelines website: http://professional.heart.org/professional/GuidelinesStatements/searchresults.jsp?q=&y=&t=1001

Endocrine irAEs

Endocrine Disorder	Initial Management	Follow-up Management
Grade 1 or Grade 2 endocrinopathies (hypothyroidism, hyperthyroidism, adrenal insufficiency, type I diabetes mellitus)	Consider continuing M7824 Consider endocrinology consult if needed Consider starting thyroid hormone replacement therapy (for hypothyroidism), anti-thyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for Type I diabetes mellitus) as appropriate. Consider ruling-out secondary endocrinopathies (i.e. hypopituitarism / hypophysitis)	Consider continuing hormone replacement/suppression and monitoring of endocrine function as appropriate.
Grade 3 or Grade 4 endocrinopathies (hypothyroidism, hyperthyroidism, adrenal insufficiency, type I diabetes mellitus)	Consider withholding or permanently discontinuing M7824 for Grade 3-4 events based upon if the clinical condition can be safely managed.	Consider resuming therapy once symptoms and/or laboratory tests improve to Grade ≤ 1 (with or without hormone replacement/suppression).

	Consider hospitalization Consider endocrinology consult Consider starting thyroid hormone replacement therapy (for hypothyroidism), anti-thyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for type I diabetes mellitus) as appropriate. Consider ruling-out secondary endocrinopathies (i.e. hypopituitarism / hypophysitis)	Consider continuing hormone replacement/suppression and monitoring of endocrine function as appropriate.
Hypopituitarism/Hypophysitis (secondary endocrinopathies)	If secondary thyroid and/or adrenal insufficiency is confirmed (i.e. subnormal serum FT4 with inappropriately low TSH and/or low serum cortisol with inappropriately low ACTH): • Consider referring to endocrinologist for dynamic testing as indicated and measurement of other hormones (FSH, LH, GH/IGF-1, PRL, testosterone in men, estrogens in women) • Consider hormone replacement/suppressive therapy as appropriate • Consider performing pituitary MRI and visual field examination as indicated **If hypophysitis confirmed:** • Continue M7824 if mild symptoms • Consider withholding M7824 and if moderate, severe or life-threatening symptoms of hypophysitis. Consider hospitalization. Consider initiating corticosteroids (1 to 2 mg/kg/day prednisone or equivalent) followed by corticosteroids taper during at least 1 month. • Consider adding prophylactic antibiotics for opportunistic infections.	Consider resuming therapy once symptoms and hormone tests improve to Grade ≤ 1 (with or without hormone replacement). In addition, for hypophysitis with abnormal MRI, consider resuming M7824 only once shrinkage of the pituitary gland on MRI/CT scan is documented. Consider continuing hormone replacement/suppression therapy as appropriate.

Other irAEs (not described above)		
Grade of other irAEs (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 2 or Grade 3 clinical signs or symptoms suggestive of a potential irAE	Consider withholding M7824 pending clinical investigation	If irAE is ruled out, consider managing as appropriate according to the diagnosis and consider re-starting therapy If irAE is confirmed, consider treating as Grade 2 or 3 irAE.
Grade 2 irAE or first occurrence of Grade 3 irAE	Consider withholding M7824 based upon if the clinical condition can be safely managed. Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent Consider adding prophylactic antibiotics for opportunistic infections Specialty consult as appropriate	If improves to Grade ≤ 1: Consider tapering steroids over at least 1 month and resuming therapy following steroids taper.
Recurrence of same Grade 3 irAEs	Consider permanently discontinuing M7824 based upon if the clinical condition can be safely managed; Consider 1.0 to 2.0 mg/kg/day prednisone or equivalent Consider adding prophylactic antibiotics for opportunistic infections Specialty consult as appropriate	If improves to Grade ≤ 1: Consider tapering steroids over at least 1 month.
Grade 4	Permanently discontinue M7824 Consider to 2.0 mg/kg/day prednisone or equivalent and/or other immunosuppressant as needed Consider adding prophylactic antibiotics for opportunistic infections Consider specialty consult as appropriate	If improves to Grade ≤ 1: Consider tapering steroids over at least 1 month
Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks for reasons other than hormonal replacement for adrenal insufficiency **Persistent Grade 2 or 3 irAE lasting 12 weeks or longer**	Consider permanently discontinuing M7824 Consider specialty consult as appropriate	

Abbreviations: ACTH=adrenocorticotropic hormone; ADL=activities of daily living; ALT=alanine aminotransferase; AST=aspartate aminotransferase; BNP=B-type natriuretic peptide; CK-MB=creatine kinase MB; CT= computed tomography; FSH=follicle-stimulating hormone; GH=growth hormone; IGF-1=insulin-like growth factor 1; irAE=immune related adverse event; IV=intravenous; LH=luteinizing hormone; MRI=magnetic resonance imaging; NCI CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events; PRL=prolactin;T4=thyroxine; TSH=thyroid stimulating hormone; ULN=upper limit of normal

3.5 STUDY CALENDAR

Procedure	Screening[1]	Baseline / Week 1[2]	Week (2N+1)[3]	EOT[4]	Safety follow up[5]	Long Term follow up[6]
Treatment[3]		X	X			
NIH Advance Directives Form[7]		X				
Medical History	X					
Height	X					
History (e.g., bleeding history) and physical exam, weight, vital signs, ECOG	X	X	X	X	X	
HIV, HCV, HepB	X					
EKG	X	X		X	X	
CBC with differential	X	X	X	X	X	
Biochemical profile[8]	X	X	X	X	X	
Tumor Markers[9]		X	X	X		
CD4[10]	X	X	X[10]	X		
ACTH, TSH, Free T4, lipase, amylase, CRP		X	X[11]			
Urinalysis	X					
Urine or serum pregnancy testing in women of childbearing potential (see Section **2.2**)	X	X	X			
PT, INR, aPTT	X	X	X	X	X	
Tumor Evaluation (CT Scan / MRI)[12]	X		X			X
Brain CT / MRI [13]	X					
Concomitant Medications		X	X	X	X	
Adverse Events		X	X	X	X	
Optional biopsy for immune analysis[14]		X	X			
Research Blood[15]		X	X	X		
Telephone Follow Up					X	X

1. Screening evaluations performed within 28 days prior to the first drug administration, unless specified in Section **2.2.**

2. Baseline evaluations performed within 1 week prior to the first drug administration.

3. Schedule of therapeutic agents administered will be as outlined in Section **3.2**, **Figure 17**, depending upon the arm of study participant has been assigned to. Of note each week in the study calendar above has a corresponding day # in **Figure 17** (W1 = D1, W3 = D15, W5 = D29, etc.). CV301 will be given every 2 weeks for the first 3 doses followed by every 4 weeks on Arm 1 and every 6 weeks on Arm 2A and 2B thereafter. M7824 infusion will be every two weeks for both arms. Subjects assigned to Arm 1 will receive N-803 every 4 weeks. Subjects assigned to Arm 2 will receive N-803 or NHS-IL12 on an alternating schedule every 2 weeks (each of these agents will be given individually every 4 weeks). Administration of study agents and indicated evaluations can be done up to 14 days earlier or delayed up to 14 days due to holidays, inclement weather, conflicts, or similar reasons. The timing of subsequent administrations is adjusted to maintain a minimum of 11 days between treatments of similar agents.

Note: Vital signs will be obtained prior to treatment and 30 minutes after administration of the last study drug. Participants should be observed for 30 minutes after the administration of the last study drug.

4. EOT – End of treatment visit: Where feasible, on the day of or within 7 days of the decision to discontinue treatment prematurely before completion of one year of treatment. Does not need to be completed if drug is withheld after one year of treatment.

5. 28 days (+/- 7 days) after last treatment. If subjects are not willing to come to NIH for FU visit, they will be contacted by phone to assess adverse events.

6. Participants who have come off treatment for disease progression will be followed by phone annually for survival. Participants who have not progressed on treatment will continue to be followed and scanned per investigator discretion until progression. Those that completed one year of treatment will be invited for an additional year of treatment at the time of progression. Initial and follow up courses of treatment may extend beyond a year per investigator discretion.

7. As indicated in Section **12.3**, all subjects will be offered the opportunity to complete an NIH advanced directive form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required

8. Biochemical profile: sodium, potassium, chloride, bicarbonate, calcium, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, total protein, albumin, and total and direct bilirubin. Serial Troponins and echocardiogram may also be added as clinically indicated if there is evidence or concern for myopericarditis.

9. Evaluate CEA, CA19-9, CA125 and CA15-3 at baseline and follow elevated values thereafter.

10. In HIV positive participants; Every 8 weeks

11. Every 8 weeks.

12. Every 8 weeks. In the event of a PR or CR tumor imaging assessments may be performed every 3 months (+/- 2 weeks) at the discretion of the investigator. Tumor assessment should be continued beyond end of treatment in participants who have not experienced PD until they experience PD in order to assess PFS. MRI will be used when CT scan is not an option to follow the disease clinically. Gadolinium will be used only for clinically indicated MRIs. Bone scans and other imaging assessments may also be performed as clinically indicated.

13. In participants with known CNS disease as described in Section **2.1.2.5**. MRIs may be performed as clinically indicated in this population.

14. Optional biopsies at baseline and as close as possible to first imaging restaging.

15. Where feasible, research blood for all study assessments (see Section **4.2**) will be collected at weeks 1, 3, 5, 7 and every 6 weeks thereafter in selected participants per PI discretion.

3.6 COST AND COMPENSATION

3.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by an insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.6.2 Compensation

There will be no compensation provided on this study.

3.6.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Regardless of reason for removal from study therapy, participants will be asked to have a 28 day follow up safety visit. Participants who refuse to return for this visit will be asked to review any safety concerns by phone within this time period.

3.7.1 Criteria for Removal from Protocol Therapy

- Clinical or radiographic progression of disease except when the investigator feels the subject is still benefiting from treatment. (It is generally preferable for participants to remain on treatment past initial radiographic progression in case there is pseudo - progression, except when the investigator feels that the clinical picture warrants changing therapy at initial progression).
- Unacceptable toxicity possibly attributed to all active therapies.
- Participant requests to be withdrawn from active therapy.
- Start of another systemic anticancer treatment (with the exception of continuation of maintenance therapy) or participation in another investigational therapeutic trial. Focal palliative radiotherapy, ablation, or surgery to a site of disease will not necessitate removal from protocol therapy. Investigator discretion.
- Positive pregnancy test.

3.7.2 Off-Study Criteria

- Screen failure.
- PI decision to end the study.
- Participant requests to be withdrawn from study.
- Participant lost to follow up.
- Investigator discretion.

- Death.

3.7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 4 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the next two weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

Any medications (other than those excluded by the clinical trial protocol) that are considered necessary to protect subject welfare or alleviate symptoms and will not interfere with the trial medication may be given at the Investigator's discretion.

Palliative radiotherapy delivered in a normal organ-spearing technique may be administered during the trial. The assessment of PD will not be based on the necessity for palliative radiotherapy.

4.1 THE FOLLOWING TREATMENTS SHOULD NOT BE ADMINISTERED DURING THE TRIAL:

- Other immunotherapies or immunosuppressive drugs (for example, chemotherapy or systemic corticosteroids except for prophylaxis or treatment of allergic reactions, endocrine replacement therapy at low dose prednisone [≤ 10 mg daily] or equivalent, for the treatment of irAEs, or for short courses (≤ 14 days) as appropriate medical therapy for unrelated medical conditions (e.g., asthma). Steroids with no or minimal systemic effect (topical, inhalation) are allowed.
- Prophylactic use of corticosteroids for infusion related reactions. Corticosteroid administration prior to CT scans in participants with intravenous contrast allergy is allowed.
- Any live vaccine therapies for the prevention of infectious disease. Administration of inactivated vaccines is allowed (for example, inactivated influenza vaccines or locally approved COVID vaccines).
- Systemic anticancer treatment.

4.2 N-803

Central nervous function may be affected with N-803. Caution should be used with psychotropic medications. Nephrotoxic, myelotoxic, cardiotoxic, or hepatotoxic medications should be avoided if possible as they may further increase toxicities that have been associated with N-803. Interferon-alfa is prohibited while on study. Beta-blockers and other antihypertensives may potentiate the hypotension seen with N-803. Therefore, administration of these agents should be avoided during N-803 treatment period, unless clinically indicated. If a participant who is to receive N-803 is on a beta-blocker or other antihypertensive, that agent may be discontinued starting on the day of the first treatment with N-803, if the investigator determines that stopping the agent is safe. Anti-hypertensive management decisions will be made on a case by case basis. For example, an anti-hypertensive may be resumed or added at any point if the investigator believes it to be clinically indicated e.g., if a participant experiences uncontrolled hypertension while receiving N-803 treatment on study. For participants on antihypertensives at baseline, the agent will be resumed if clinically indicated by blood pressure readings in the days following N-803 treatment cessation. If a localized skin rash at the injection site occurs that is >6 cm and symptomatic, it may be treated with 0.05% clobetasol propionate or 0.1% triamcinolone cream or similar product at the discretion of the treating physician.

5 CORRELATIVE STUDIES FOR RESEARCH / PHARMACOKINETICS STUDIES

5.1 BIOSPECIMEN COLLECTION

Test/assay	Volume (approx.) per Timepoint	Type of tube [a]	Collection point	Location of specimen analysis*
Standard and 123 immune cell subsets by FACS	60-80 mL blood for PBMCs	Sodium heparin (green top) tubes	See study calendar **3.5**	LTIB
Functional Analysis of immune cell subsets by FACS				LTIB
Antigen Specific Immune Response by cytokine staining assay				LTIB
T cell clonality by immunseq platform				LTIB and NCI Frederick Genomic Core Facility
RNA expression level of 770 genes				LTIB and NCI Frederick Genomic Core Facility
Soluble Factors (to include sCD27 and sCD40 ligand) by ELISA	8 mL blood for serum	SST		LTIB
	10 mL blood for plasma	EDTA tube		

Test/assay	Volume (approx.) per Timepoint	Type of tube [a]	Collection point	Location of specimen analysis*
Immune Markers by IHC	Tumor samples	N/A		GMB TIME Lab
RNA expression level of 770 genes	Tumor samples	N/A		LTIB and NCI Frederick Genomic Core Facility
T cell clonality by immunseq platform	Tumor samples	N/A		LTIB and NCI Frederick Genomic Core Facility

*Research blood samples will be sent to the Clinical Services Program – Leidos Biomedical Research, Inc. (CSP) (Section **5.3.1**) for barcoding, initial processing and storage. Tissue will be sent to the Laboratory of Pathology as described in Section **5.3.2**. From these facilities, coded, linked samples will be sent to the designated labs for analysis upon request.

[a] Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1.1 Immune Phenotyping

Exploratory immunologic studies will be conducted to evaluate the study drug's effect on the immune response before and after treatment, to gain insight into potential biomarkers, and help improve the administered therapy. Blood will be collected as per Study Calendar 3.5. The following immune assays may be performed at the Laboratory of Tumor Immunology and Biology (LTIB) at the NCI's Center for Cancer Research (CCR) in select participants where adequate samples are available:

1. PBMCs may be analyzed for changes in standard immune cell types (CD4 and CD8 T cells, natural killer [NK] cells, regulatory T cells [Tregs], myeloid-derived suppressor cells [MDSCs], and dendritic cells) as well as 123 immune cell subsets, using multi-color flow cytometry.
2. PBMCs from selected subjects may be analyzed for function of specific immune cell subsets, including CD4 and CD8 T cells, NK cells, Tregs, and MDSCs using flow-based assays.
3. PBMCs may be analyzed for tumor antigen-specific immune responses using an intracellular cytokine staining assay. PBMCs will be stimulated in vitro with overlapping 15-mer peptide pools encoding the tumor-associated antigens such as CEA, MUC-1 and Brachyury; control peptide pools will involve the use of human leukocyte antigen peptide as a negative control and CEFT peptide mix as a positive control. CEFT is a mixture of peptides of CMV, Epstein-Barr virus, influenza, and tetanus toxin. Post-stimulation analyses of CD4 and CD8 T cells will involve the production of IFN-γ, IL-2, TNF, and the degranulation marker CD107a. If sufficient PBMCs are available, assays may also be performed for the development of T cells to other tumor-associated antigens.

5.1.2 Soluble Factors

Samples for soluble factor analysis will be collected as per Study Calendar **3.5**.

-Sera and/or plasma may be analyzed pre- and post-therapy for the following soluble factors: sCD27, sCD40 ligand using commercial ELISA kits.

-Sera and/or plasma may be analyzed for changes in cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers using ELISA or multiplexed assays (e.g., Mesoscale, Luminex, cytokine bead array).

5.1.3 Analyses of Tumor Tissue for Immune Markers

Study of immune infiltration as well as PD-L1 status within the tumor microenvironment pre vs. post treatment by IHC and/or multiplex immunofluorescence may be performed by the GMB TIME Lab. Where available, archival tumor samples will be requested. For participants with lesions amenable to biopsy, two optional biopsies may be performed at baseline and at first restaging. Tumor samples will be sent to the Laboratory of Pathology for disease evaluation; remaining samples will be used for research. Tissue samples for research may also be stored in CSP as described in Section **5.3.1**.

5.2 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.2.1 Description of the Scope of Genetic/Genomic Analysis

5.2.1.1 RNA and T-cell Receptor Clonality Analysis of Blood and Tumor Tissue

Where possible, RNA expression and T-cell receptor clonality analysis will be done on the peripheral blood as well as archived tumor tissue or optional biopsies to help further evaluate changes in immune response and RNA expression levels with treatment as well as to determine tumor and infiltrating lymphocyte characteristics which may be predictive of response to treatment. In addition, these analyses will also be used to gauge resistance mechanisms and additional targets for future therapy. Coded, linked samples may be analyzed for RNA expression levels using the Nanostring platform and T-cell receptor clonality using the ImmunoSeq platform (LTIB and NCI Frederick Genomic Core Facility).

NCI Fredrick Genomic Core Facility:
Leidos Biomedical Research, Inc:
Dr. Xiaolin Wu
ATRF, Rm C3016
8560 Progress Drive
Frederick, MD 21701
Ph. 301-846-7677

5.2.2 Description of How Privacy and Confidentiality of Medical Information/Biological Specimens will be Maximized

Confidentiality for genetic samples will be maintained as described (Section **5.3.1** and **5.3.2**).

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a 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 the National Institutes for Health (NIH) without appropriate approvals and/or agreements, if required.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**

5.3.1 Sample Management and Storage at Clinical Services Program – Leidos Biomedical Research, Inc. (CSP)

Clinical Services Program - Leidos Biomedical Research, Inc.
Attn: Theresa Burks
1050 Boyles Street
Bldg. 496/Room 121
Frederick, MD 21702

On days samples are drawn, Jen Bangh at CSP (part of NCI Frederick Central Repositories) should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same-day courier delivery of the specimens.

All data associated with the participant samples is protected by using a secure database. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.

Samples will be tracked and managed by Central Repository database, where there is no link to personal identifiable information. All samples will be stored in either a -80ºC freezer or vapor phase liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

NCI Frederick Central Repositories (managed under a subcontract) store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited-access facilities with sufficient security, backup, and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

Specimens are stored in accordance with applicable HHS and FDA Protection of Human Subjects Regulations in accordance with the subcontractor's Federal-wide Assurance. The subcontractor's role limited to clinical research databases and repositories containing participant specimens. The subcontractor does not conduct or have any vested interest in research on human subjects but does provide services and support the efforts of its customers, many of which are involved in research on human subjects.

It is the intent and purpose of the subcontractor to accept only coded, linked samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens as well as to maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory,

aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdrawal request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No participant-specific information is encoded in this ID.

Investigators are granted view, input, and withdrawal authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

5.3.2 Procedures for Storage of Tissue Specimens in the Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissues are stored for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the participant was enrolled.

5.3.3 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. If the participant withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the time the study consent is signed through 28 days after removal from study treatment or until off-study, whichever comes first. Adverse events that are serious need to be recorded through 28 days after the last intervention. Beyond 28 days after the last intervention and through long term follow up (survival of subject), only adverse events which are serious and related to the study intervention need to be recorded.

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 participant's outcome.

End of study procedures: Data will be stored according to HHS, 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, this will be reported expeditiously per requirements in Section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through*:*

- An NIH-funded or approved public repository: clinicaltirals.gov
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

6.3.1 Antitumor Response

Tumor assessments may include the following evaluations: physical examination (with photograph and measurement of skin lesions, as applicable); cross-sectional imaging using computed tomography (CT) or magnetic resonance imaging (MRI) scan of the chest, abdomen, and pelvis (pelvis scan is optional unless known pelvic disease is present at baseline); nuclear bone scan for subjects with known/suspected bone lesions; and CT or MRI scan of the brain (only as clinically warranted based on symptoms/findings). The preferred method of disease assessment is CT with contrast. If CT with contrast is contraindicated, CT of the chest without contrast and MRI scan of the abdomen/pelvis is preferred.

At baseline, tumor lesions will be selected and categorized as target or non-target lesions. Target lesions include those lesions that can be accurately measured in at least 1 dimension as ≥ 20 mm with conventional techniques or ≥ 10 mm with CT scan. Malignant lymph nodes with a short axis diameter ≥ 15 mm can be considered target lesions. Up to a maximum of 2 target lesions per organ and 5 target lesions in total will be identified at baseline. These lesions should be representative of all involved organs and selected based on their size (those with the longest diameter) and their suitability for accurate repeated measurements. A sum of the longest lesion diameter (LLD) for all target lesions will be calculated and reported as the baseline sum LLD. For malignant lymph nodes identified as target lesions, the short axis diameter will be used in the sum of LLD calculation. All other lesions (or sites of disease) should be identified as non-target lesions (including bone lesions).

All post-baseline response assessments should follow the same lesions identified at baseline. The same mode of assessment (e.g., CT) used to identify/evaluate lesions at baseline should be used throughout the course of the study unless subject safety necessitates a change (e.g., allergic reaction to contrast media).

For the primary endpoint antitumor activity will be evaluated with target and/or non-target lesions according to RECIST Version 1.1.

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥ 20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥ 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.

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

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

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

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

6.3.3 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.

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT 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).

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

6.3.4 Response Criteria

All the scans performed at Baseline and other imaging performed as clinically required (other supportive imaging) need to be repeated at subsequent visits. In general, lesions detected at Baseline need to be followed using the same imaging methodology and preferably the same imaging equipment at subsequent tumor evaluation visits.

Brain CT / MRI scan should be performed, if clinically indicated by development of new specific symptoms or on the discretion of the Principal Investigator. For each subject, the Investigator will designate 1 or more of the following measures of tumor status to follow for determining response: CT or MRI images of primary and / or metastatic tumor masses, physical examination findings, and the results of other assessments. All available images collected during the trial period will be considered. The most appropriate measures to evaluate the tumor status of a subject should be used. The measure(s) to be chosen for sequential evaluation during the trial have to correspond to the measures used to document the progressive tumor status that qualifies the subject for enrollment. The tumor response assessment will be assessed and listed according to the Study Calendar **3.5**.

The foreseen treatment duration is until disease progression verified by a scan subsequent to the initial documentation of PD, unacceptable toxicity, or any criterion for withdrawal from the trial occurs (see Section **6.3**). Before stopping the treatment, progressive disease should be confirmed by imaging 4 to 6 weeks (preferably 6 weeks, but not later) after progression has been diagnosed according to RECIST 1.1 [44, 45]. If progression is based on the occurrence of a new lesion in an area not scanned at Baseline, a further on-study scan 6 weeks later should be considered before performing the 28-Day Safety Follow-up visit. Treatment may be continued despite progression according to RECIST 1.1 at any time if:

- There are no new or concerning symptoms.
- There is no decrease in ECOG PS.

- The Investigator does not consider it necessary to administer a salvage therapy.

The treatment should be stopped immediately, if the subject does not tolerate M7824 or if therapeutic failure occurs, which requires urgent treatment with an additional drug or results in clinically significant progression / deterioration.

Tumor responses to treatment will be assigned based on the evaluation of the response of target, non-target, and new lesions according to RECIST 1.1 (all measurements should be recorded in metric notation).

- To assess objective response, the tumor burden at baseline will be estimated and used for comparison with subsequent measurements. At baseline, tumor lesions will be categorized in target and non-target lesions according to RECIST 1.1.

Results for these evaluations will be recorded with as much specificity as possible so that pre and post-treatment results will provide the best opportunity for evaluating tumor response.

Any CR or PR should be confirmed according to RECIST 1.1. In the case of a PR or CR, a confirmatory CT or MRI scan should be done no sooner than 4 weeks (preferably at the scheduled 6-week interval).

The Investigator may perform scans in addition to a scheduled trial scan for medical reasons or if the Investigator suspects PD.

As an exploratory endpoint antitumor activity will also be evaluated according to iRECIST

Using iRECIST criteria the following will be incorporated into assessment:

1. An increase in the sum of target lesions of more than 20%, unequivocal increase in non-target lesions or new lesions result in iUPD (unconfirmed progressive disease); iUPD can be assigned multiple times as long as iCPD (confirmed progressive disease) is not confirmed at the next assessment.

2. Progression is confirmed in the target lesion category if the next imaging assessment after iUPD (4–8 weeks later) confirms a further increase in sum of measures of target disease from iUPD, with an increase of at least 5 mm. Progression is confirmed in the non-target lesion category if subsequent imaging, done 4–8 weeks after iUPD, shows a further unequivocal increase in non-target lesions. Progression is confirmed in the new lesions category if at next assessment additional new lesions appear or an increase in size of previously seen new lesions is seen (≥5 mm for sum of new lesion target).

3. However, the criteria for iCPD (after iUPD) are not considered to have been met if complete response, partial response, or stable disease criteria (compared with baseline and as defined by RECIST 1.1) are met at the next assessment after iUPD. The status is reset (unlike RECIST 1.1, in which any progression precludes later complete response, partial response, or stable disease). iCR, iPR, or iSD should then be assigned; and if no change is detected, then the timepoint response is iUPD.

6.3.5 Response Criteria by RECIST 1.1

6.3.5.1 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.

6.3.5.2 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 participant to be considered in complete clinical response.

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

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

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

6.3.5.3 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 participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

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

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD

Non-Target Lesions	New Lesions	Overall Response
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		

6.3.5.4 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.

6.3.5.5 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant 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 web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at:

https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI Clinical Director Reporting

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 Institutional Biosafety Committee (IBC) Reporting Criteria

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of CV301 vaccine 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 CV301 vaccine but are not fatal or life-threatening, much 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 FDA Form 3500a.

7.4.2 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 Principal Investigator (or delegate) shall submit the information described below. Alternatively, the 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.

7.4.2.1 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:

- the title and purpose of the trial;
- clinical site;
- the Principal Investigator;
- clinical protocol identifiers;

- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the 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;
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed; and
- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

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

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system;
- a summary of all serious adverse events submitted during the past year;
- a 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; and
- a 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.

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis (approximately weekly) when participants are being actively treated on the trial to discuss each participant. Decisions about dose level enrollment and dose escalation will be made based on the toxicity data from prior participants.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Data Safety Monitoring Board (DSMB) – Sponsor (OSRO)

The DSMB is an independent group of at least 3 experts that monitors participant safety and advises the Sponsor. DSMB members will be separate and independent of study staff participating in this trial and should not have scientific, financial, or other conflicts of interest related to this

trial. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of data from this trial. A quorum will consist of a simple majority.

The DSMB will review cumulative safety data from this trial at least annually.

The DSMB will meet when trial halting criteria (see Section **3.4.3)** are met, or as requested by the sponsor or PI.

The DSMB will have a final review meeting at the end of the study.

Procedures for DSMB reviews/meetings will be defined in the DSMB charter. The DSMB will review applicable data, including, but not limited to, enrollment, demographics, dosing data, clinical laboratory data, and safety data, at scheduled timepoints during this trial as defined in the DSMB charter. The DSMB will review blinded aggregate data in the open session of the DSMB meetings.

Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by the Sponsor. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study product administration, and to continue, modify, or terminate this trial.

8 SPONSOR/PROTOCOL SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a participant or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Adverse Event of Special Interest (AESI)

Mucosal bleeding events which are at least possibly related to study drug (M7824) will be captured as AESIs. These events will not require expedited reporting to the study sponsor unless they also meet SAE criteria.

8.1.3 Serious Adverse Event (SAE)

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 event (see Section **8.1.4**);
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.

- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for participant convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant 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 participant or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.4 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the participant or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death (21CFR312.32).

8.1.5 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.6 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site

principal investigator or sub-investigator.

- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to Section **6.1**. All serious adverse events recorded must be reported to the sponsor from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in Section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in Section **8.4**.

All SAE reporting must include the elements described in Section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Waiver of Expedited Reporting to CCR

As overall survival, which includes death due to disease progression and hospitalization due to disease progression are part of the study objectives, and captured as an endpoint in this study, death/hospitalization due to disease progression will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to Section **8.3.**

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

Reporting will be per the collaborative agreement.

8.6 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.6.1 Maternal Exposure

If a participant becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (Section **8.1.3**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.6.2 Paternal Exposure

Male participant should refrain from fathering a child or donating sperm during the study and for 2 months after the last dose of M7824.

Pregnancy of the participant's partner is not considered to be an AE. The outcome of all pregnancies occurring from the date of the first dose until 2 months after the last dose should, if possible, be followed up and documented.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance to 21 CFR 31.2.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING PLAN

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and

- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site. Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable) and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

The primary objectives of this trial are to determine the objective response rate of combination immunotherapy in two separate arms evaluating triple or quadruple therapy in participants with checkpoint naïve MSS small bowel or colorectal cancer. A secondary hypothesis (objective) will be to obtain preliminary data on duration of response, PFS, OS and ratio of participants that are hospitalized because of adverse events attributed to disease progression for each of these arms. An exploratory hypothesis (objective) will be to evaluate exploratory immunologic studies to understand and improve the administered treatment.

10.2 SAMPLE SIZE DETERMINATION

Initially, Arm 1 will be conducted using the phase II design described below. Upon its completion, a dose level escalation of NHS-IL12 will be conducted, called Arm 2A. This arm will enroll up to 18 participants in the three dose levels and the participants at the highest safe dose level (or MTD)

will be included in the first stage of the phase II evaluation of the quadruple therapy (Arm 2B) as described below.

In participants with microsatellite stable small bowel or colon cancer, the clinical response rate to current single agent therapies is very low (<5%). It would be desirable if by using a combination of multiple agents, the response rate would be improved. To establish the efficacy of each of the proposed combinations (triple or quadruple therapy), the primary objective would be to determine if the proposed combination would rule out a 10% response rate and result in a response rate consistent with 40%. As such, participants on each arm (Arm 1 or 2B) will be treated in a trial conducted using a Simon optimal two-stage phase II trial design (Simon R, Controlled Clinical Trials 10:1-10, 1989) to rule out an unacceptably low PR+CR rate of 10% (p0=0.10) in favor of an improved response rate of 40% (p1=0.40). With alpha=0.05 (probability of accepting a poor treatment=0.05) and beta = 0.10 (probability of rejecting a good treatment=0.10), the first stage for each arm will enroll 9 evaluable participants, and if 0 to 1 of the 9 have a clinical response, then no further participants will be accrued in that cohort. For evaluation of the quadruple therapy, the first 6 participants may include those at the MTD from Arm 2A, assuming they are fully evaluable for response. For simplicity, once these participants are included in the Phase II evaluation, they will be considered as if they were in Arm 2B in the descriptions below. If 2 or more of the first 9 participants have a response, then accrual would continue until a total of 20 evaluable participants have been treated on that arm. As it may take up to several months to determine if a participant has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 2 to 4 participants with a response out of 20 participants in a given arm, this would be an uninterestingly low response rate. If there were 5 or more of 20 (25.0%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials in this disease type. Under the null hypothesis (10% response rate), the probability of early termination is 77.5%.

It is anticipated that 2-3 participants per month may enroll onto this trial. Thus, it is expected that the trial can enroll up to 46 evaluable participants (up to 12 for safety evaluation during dose escalation in Arm 2A, 40 for primary efficacy analyses in Arm 1 and Arm 2B together, minus 6 included from the dose escalation Arm 2A) in approximately two years. To allow for a small number of screen failures and participants who will be inevaluable for the primary objective analysis (e.g., checkpoint refractory), the accrual ceiling will be set at 80 participants.

10.3 POPULATIONS FOR ANALYSES

All participants who receive any investigational treatment will be evaluable for safety and toxicity evaluations. All participants will be evaluable for toxicity from the time of their first treatment with any agent.

For efficacy, a modified intention to treat population will be used. Only those participants who have measurable disease present at baseline and have had their disease re-evaluated will be considered evaluable for response. (Note: Participants who discontinue treatment before the first scan due to treatment related toxicity or exhibit objective disease progression prior to first restaging will also be considered evaluable.)

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

The fraction of participants with response to the triple or quadruple therapy will be reported. For those enrolled during the first 6 participants on Arm 1 or during the dose escalation for NHS-IL12 (Arm 2A), the participants will be evaluated with respect to the grades and types of toxicities obtained. The results will be presented descriptively and tabled if appropriate.

10.4.2 Analysis of the Primary Endpoints

The percentage of subjects that achieve an objective confirmed complete or partial overall tumor response using RECIST Version 1.1 will be evaluated per arm (Arm 1 and 2B). If anti-tumor responses are observed, the 95% confidence interval of the response rate will be evaluated.

It is possible that the response rates observed with combination immunotherapy will be less in participants whom have previously received checkpoint therapy and not be truly reflective of a population of participants whom have not had prior checkpoint therapy. While checkpoint experienced participants will not be excluded from enrollment, the primary efficacy analyses will be limited to checkpoint naïve participants with MSS colon cancer and efficacy analyses in the checkpoint refractory.

10.4.3 Analysis of the Secondary Endpoints

Data will be obtained on duration of response, PFS, OS and ratio of participants that are hospitalized because of adverse events attributed to disease progression.

10.4.3.1 Duration of Response

The duration of overall response will be evaluated by arm for those with a PR or greater in Arm 1 and Arm 2B. 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 PD is objectively documented and is evaluated using the Kaplan-Meier method.

10.4.3.2 Progression-Free Survival

PFS will be evaluated by arm using Kaplan-Meier methods. PFS will be defined as the time from the date of first treatment to the date of disease progression or death (any cause) whichever occurs first. Subjects who do not have disease progression or have not died at the end of follow up will be censored at the last known date the subject was progression free.

10.4.3.3 Objective Response

Objective response is a complete or partial radiographic response as defined by RECIST 1.1 (Section **6.3.5**).

10.4.3.4 Overall Survival

OS will be evaluated using Kaplan-Meier methods. OS will be defined as the time from the date of first treatment to the date of death (any cause). Subjects who are alive at the end of follow up will be censored at the last known date alive.

10.4.4 Safety Analyses

Safety endpoints will be analyzed as summary statistics during treatment and/or as change scores from baseline assessments. AEs will be coded as defined in the Medical Dictionary for Regulatory Activities (MedDRA). All AEs will be recorded and tabulated following each treatment. AEs will

be recorded by severity, frequency, and relationship to the study intervention and will be presented by System Organ Class (SOC) designations and preferred term groupings. Information on each AE will include start date, stop date, severity, relationship, expectedness, outcome, and duration. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent AEs will be presented either in a Table or a Listing.

In addition, overall safety will be assessed by descriptive analyses using tabulated frequencies of AEs by grade using CTCAE Version 5 within dose cohorts and for the overall study population in terms of treatment-emergent AEs, SAEs, and clinically significant changes in safety laboratory tests, physical examinations, ECGs, and vital signs.

10.4.5 Baseline Descriptive Statistics

Baseline Characteristics will be described.

10.4.6 Planned Interim Analyses

Interim assessment of efficacy will be made according to the Simon two stage Phase II designs of each arm described.

10.4.7 Tabulation of Individual Participant Data

Individual responses in a cohort may be depicted within a larger group using a waterfall plot or spider diagram.

10.4.8 Exploratory Analyses

The following are the planned exploratory objectives and endpoints:

To conduct exploratory immunologic studies to understand and improve the administered treatment, including:

- peripheral immune subset analysis before and on treatment;
- soluble factors circulation (e.g., sCD27 and sCD40 ligand) before and on treatment;
- tumor tissue PD-L1 expression and immune infiltration before and after treatment.

Where feasible, exploratory immune analyses will be conducted to evaluate anti-tumor immune responses. Immune response will be assessed among all subjects treated in each phase II cohort. The magnitude of immune responses will be described. A subject will be considered evaluable for immune response if they receive at least one dose of treatment. The percentage of subjects with a positive immune response will be evaluated separately by arm. For flow cytometry analyses on PBMC samples, Student t-tests or Wilcoxon rank sum tests as appropriate will be performed on percentages of TNF-α and/or IFN-γ expressing cells among the different cohorts to determine any significant differences in cell populations between arms For antigen specific T cell responses, a positive immune response is defined by CMI reactivity in ex vivo stimulation using a flow cytometric readout (cytokine production or CD107 expression). Antigen-specific peptide challenge assays require a readout of >250 reactive T-cells/million cells above the background [46]. In general, evaluations of soluble factors and tumor tissue immune infiltration will be compared before vs. after treatment using appropriate paired tests. Any statistical analyses performed on these exploratory endpoints will be done without formal adjustment for multiple comparisons but in the context of the number of tests performed [46].

To assess responses to therapy using iRECIST per treatment assignment (three or four drug combination).

To assess response to therapy using RECIST and iRECIST in checkpoint refractory participants (including participants with MSI disease).

To assess responses to therapy by RAS mutation status.

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

A CRADA (02666) is in place with EMD Serono for the supply of M7824 and NHS-IL12.

A CRADA (02561) is in place with Bavarian Nordic for the supply of CV301 (MVA-BN-CV301 and FPV-CV-301).

A CRADA (03058) is in place with ImmunityBio, Inc. for the supply of N-803.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. Efforts will be made to extend accrual to a representative population. Due to impaired cellular immunity which may affect the efficacy of treatment, participants with poorly controlled HIV as well as patents with detectable viral loads of hepatitis B and C will be excluded.

As there is a risk of severe bleeding with this study drug, participants must be willing to receive blood transfusions if medically necessary for their own safety. Participants must be able to receive blood transfusions in order to minimize the risks of receiving M7824.

12.2 PARTICIPATION OF CHILDREN

Individuals under the age of 18 will not be eligible to participate in this study because of unknown toxicities in pediatric participants.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to provide consent are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. In the event this occurs, the subjects will remain in the study because there is a prospect of direct benefit from research participation. 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. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see Section **12.5.1** for consent procedure.

12.4 RISK/BENEFIT ASSESSMENT

12.4.1 Known Potential Risks

Some of the procedures performed on this study are not known to be associated with risk. These include urine tests and EKGs. Below are a list of procedures and study interventions that are associated with risk.

12.4.1.1 Study Drug Risks

Risks include the possible occurrence of any of a range of side effects which are listed in Sections **14.1.2**, **14.2.2**, **14.3.2** and **14.4.2**.

Participants may be harmed from being in this study by toxicity due to the drug or combination of drugs given during this study. M7824 is similar to immune check point inhibitors. There are preliminary data to suggest that not all patients benefit from immune check point inhibitors nor M7824. Additionally, there are preliminary data to suggest that an unexpectedly rapid progression of disease occurs in some patients receiving immunotherapy such as immune checkpoint inhibitors.

12.4.1.2 Risk of Optional Biopsies

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent.

12.4.1.3 Risks of Exposure to Ionizing Radiation

This research study involves: 7 possible additional CT CAP scans, 1 brain CT scan, and 2 CT guided biopsies collected for research purposes.

Subjects undergoing these scans and biopsies will be exposed to 9.4 rem. The CT scans and CT guided biopsies in this study will expose the research participant to 31.3 years' worth of background radiation. This level of exposure results in an increased risk of cancer.

12.4.1.4 CT Scan Risk

CT scans create low levels of radiation, which has a small potential to cause cancer and other defects. However, the risk associated with scan is small.

12.4.1.5 Risks Due to Contrast Agents for CT

Contrast agents can cause allergic reactions and kidney damage. Allergic reactions can include mild itching associated with hives but can also result in a serious life-threatening emergency from difficulty breathing. If this occurs, it is treatable.

12.4.1.6 Risk of MRI

People are at risk for injury from the MRI magnet if they have some kinds of metal in their body. People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss.

There are no known long-term risks of MRI scans.

12.4.1.7 Risk of Gadolinium Enhanced MRI

The gadolinium infusion may cause mild symptoms such as coldness in the arm during the injection, a metallic taste, headache, and nausea. There are risks of an IV catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling.

Procedure-related risks from MRI and gadolinium enhanced MRI will be explained fully during informed consent.

12.4.1.8 Risks of Blood Collection

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop.

The amount of blood that may be drawn from adult participants for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. Specific volumes are also listed in table in Section **5.1**.

12.4.1.9 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.4.2 Known Potential Benefits

The potential benefit to a participant that goes onto study is a reduction in the bulk of their tumor which may or may not have favorable impact on symptoms and/or survival.

12.4.3 Assessment of Potential Risks and Benefits

Advanced small bowel and colorectal cancers are in need of improved therapy options. Preclinical studies suggest that the use of a combination of multiple immunotherapy agents may have improved anti-tumor efficacy.

A number of clinically appropriate strategies to minimize risks to participants have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. Overall, the potential benefits of the combination of a vaccine targeting tumor associated antigens (CV301), a bifunctional fusion protein targeting PD-L1 and TGF beta (M7824), an IL-15 superagonist (N-803), and a tumor targeted immunocytokine (NHS-IL12) in subjects with advanced small bowel and colorectal malignancies for participants retaining the ability to consent and those who lose capacity to consent during the course of the trial outweigh the risks associated with this combination immunotherapy proposed in this study.

12.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant as described below.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or
- iMedConsent platform (which is 21 CFR Part 11 compliant).

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when at the same location but is not required.

Both the investigator and the subject will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

As there is an optional biopsy for research in this protocol, the participant will be asked to sign a separate consent at the time of the procedure. If the participant refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

12.5.1 Consent Process for Participants Who Decline Other Treatment Options

Participants who are eligible because they decline the treatment options described in the eligibility criteria (see Section **2.1**) will have consent obtained in the presence of an independent consent monitor from the Bioethics department or Human Subjects Protection Unit (HSPU).

12.5.2 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.5**.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility
- Participant death at least possibly attributed to study treatment
- A higher than expected rate (>15-20%) of grade 3 or greater bleeding events due to study treatment or the occurrence of a grade 5 bleeding event due to study treatment.

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB andas applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 CV301 (IND # 19660)

14.1.1 Source

CV301 (MVA-BN-CV301 and FPV-CV-301) will be supplied by the manufacturer, Bavarian Nordic.

14.1.2 Toxicity

A phase I study was just completed at the NCI with no dose limiting toxicities (unpublished). As expected, CV-301 was well tolerated in all 12 participants with no DLT noted. The only attributable toxicities were grade 1 and 2, and primarily injections site reactions and flu-like symptoms, with other rare toxicities such as headache and nausea (also grade 2 or less) also reported. There were no grade 3 or greater toxicities reported in the study.

Initial safety data show that the most frequently occurring treatment-related AEs were temporary and self-limiting, grade 1 or 2 in severity and included injection site reactions (injection site erythema, pruritus, pain, induration and swelling) and general symptoms including fever/chills, flu-like symptoms, headache, fatigue/weakness, nausea/vomiting, myalgia and arthralgia. There was no occurrence of any of the pre-specified adverse events of special interest (immune-related events and cardiac events). There were no related SAEs. There were no dose limiting toxicities.

MVA-BN-CV301 (Prime Vaccine)

The most common side effects associated with MVA-BN and other MVA-BN-derived vaccines include mild to moderate flu-like symptoms such as: fever, chills, muscle or joint ache, and tiredness (fatigue). In addition, some localized reactions at the site where the vaccine is injected under skin (subcutaneously). These injection site reactions may include any or all of the following: swelling, localized pain, hardness of a small area of skin around the injection site induration), and redness.

FPV-CV301 (Boost Vaccine)

The most common side effects from the use of fowlpox vaccines are mild and may include the following: fever, tiredness (fatigue), muscle or joint ache, headache, and flu-like symptoms.

In addition, some localized reactions at the site where the vaccine is injected under skin (subcutaneously). These injection site reactions may include any or all of the following: swelling, localized pain, hardness of a small area of skin around the injection site induration), and redness.

Pooled ADR data of all completed MVA-BN trials

The following two tables from the CV301 Investigator Brochure (IB) v5 summarize the pooled ADR data of all completed MVA-BN trials. The safety profile of each of the trials with recombinant MVA-BN based vaccines is comparable to the safety profile as displayed in the table, as the occurrence of the ADRs is considered to be a reaction to the vector rather than the insert based on previous experience with recombinant

Suspected Adverse Drug Reactions Reported by ≥ 1% of Subjects in the Completed MVA-BN Clinical Trials* (N=7863)**

Preferred Term (PT)	No. of reports by subjects	Frequency %

Injection site pain	6385	81.2
Injection site erythema	5049	64.2
Injection site swelling	3804	48.4
Injection site induration	3315	42.2
Injection site pruritus	2935	37.3
Myalgia	2524	32.1
Fatigue	2425	30.8
Headache	2274	28.9
Nausea	1102	14.0
Rigors/chills	684	8.7
Body temperature increased	269	3.4
Appetite disorder	218	2.8
Arthralgia	206	2.6
Injection site nodule	195	2.5
Injection site discolouration	191	2.4
Pain in extremity	147	1.9
Injection site haematoma	107	1.4
Pyrexia	99	1.3
Axillary pain	91	1.2
Injection site warmth	85	1.1

* POX-MVA-001, -002, -004, -005, -006, -007, -008, -009, -010, -011, -013, -023, -024, -027, -028, -029, -030, -036, -037, -03X, HIV-NEF-004 and HIV-POL-002.

** 8 subjects exposed but not included in analysis. 7 subjects in POX-MVA-009 received Dryvax either on the same day or within 7 days after MVA-BN administration and were therefore not included to avoid a potential bias in the adverse event reporting. 1 subject in POX-MVA-029 was not vaccinated according to the randomization, therefore removed from analysis set. Source: MVA-BN IB Ed. 24.0

ADRs for FPV (Originating from the PROSTVAC program)

General disorders and administration site conditions:	Injection site reactions, injection site erythema, injection site induration*, injection site pain, injection site pruritus, injection site swelling, pyrexia *, chills, peripheral edema, fatigue, influenza-like illness
Gastrointestinal disorders:	Nausea*
Nervous system disorders:	Dizziness
Musculoskeletal and connective tissue disorders:	Myalgia*

Additional safety information on vaccinia and FPV can be found in the PROSTVAC Investigator's Brochures.

14.1.3 Formulation and Preparation

MVA-BN-CV301 and FPV-CV301 encode the human MUC-1 and the human CEA gene in combination with human TRICOM. No marker gene is present in both recombinant viruses.

MVA-BN-CV301 is a liquid-frozen, highly attenuated, live recombinant virus based on the viral vector MVA-BN. It is administered as s.c. injection. Packaging and vials will be labeled according to the respective product specifications.

One MVA-BN-CV301 vaccine vial has a nominal titer of 4 x 10^8 infectious units (Inf.U) in 0.5 mL of the drug product.

FPV-CV301 is a liquid-frozen, highly attenuated, live recombinant virus. It is administered as s.c. injection. The packages and vials will be labeled according to the respective product specifications.

One FPV-CV301 vaccine vial has a nominal virus titer of 1 x 10^9 Inf.U in 0.5 mL of the drug product.

MVA-BN-CV301 and FPV-CV301 are each supplied in 2 mL type I borosilicate glass vials closed with sterile bromobutyl rubber stoppers, crimped with aluminum caps and covered with polypropylene closures.

14.1.4 Stability and Storage

Supplies of both the MVA-BN-CV301 and the FPV-CV301 vaccines will be shipped temperature controlled and monitored to the clinical trial site. Once at the site, the package should be handed over to personnel in charge of vaccine preparation (e.g., the pharmacist or representative). Site personnel are responsible for proper storage of vaccine upon receipt.

Both the MVA-BN-CV301 and the FPV-CV301 vaccines must be shipped to site and stored at a temperature of -20°C +/- 5°C) or -80°C +/- 5°C, avoiding direct light. A vial must not be re-frozen once it has been thawed. A provisional shelf life of 2 years at -80°C ± 10°C has been given. Expiry date may be extended due to real time stability data. Storage at -20°C ± 5°C is limited to 12 months after moving from -80°C ± 10°C to -20°C ± 5°C.

14.1.5 Administration Procedures

Each dose of MVA-BN-CV301 consists of 4 injections administered subcutaneously (one in each arm and one in each leg).

Each dose of FPV-CV301 consists of 1 injection administered subcutaneously (preferably in the non-dominant arm).

14.1.6 Incompatibilities

Not available.

14.2 M7824 (MSB0011359C) (IND # 19660)

14.2.1 Source / Acquisition and Accountability

M7824 is manufactured and supplied for the trial by EMD Serono Research and Development Institute.

M7824 will be provided to the clinical trial site by the manufacturer. The investigator or designee (e.g., pharmacist) will maintain an ongoing inventory of the investigational product supply according to standard site procedures. The investigational product will be dispensed at the direction of an investigator for administration to a study participant enrolled on the clinical trial. Disposal of expired or unused product will be returned to the manufacturer or disposed of according to standard site procedures based on agreement between the manufacturer and the site.

14.2.2 Toxicity

The immunoglobulin portion of M7824 molecule is identical to avelumab (Bavencio). Respective warnings and precautions for grade 2 or higher immune-mediated pneumonitis, immune-mediated

colitis, immune-mediated endocrinopathies, immune-mediated hepatitis) and infusion reactions are included in the prescribing for Bavencio (bavencio.com). Participants will be pre-medicated to prophylax against infusions reactions. The following additionally significant immune-mediated adverse reactions have occurred in less than 1% of 1738 participants treated with BAVENCIO: myocarditis with fatal cases, myositis, psoriasis, arthritis, exfoliative dermatitis, erythema multiforme, pemphigoid, hypopituitarism, uveitis, Guillain-Barré syndrome, and systemic inflammatory response. The above irAEs are all considered an anticipated risk of treatment with M7824 and thus will not be considered DLTs.

In a phase 1, open-label 3+3 dose-escalation study of M7824 in 16 participants, 3 participants experienced grade 3 drug-related adverse events including skin infection secondary to grade 2 bullous pemphigoid, lipase increased, and colitis with associated anemia. There were no grade 4 – 5 treatment related adverse events. Please see table below for details.

Treatment-related adverse events

	3 mg/kg (n = 3)		*10 mg/kg (n = 3)*		*20 mg/kg (n = 7)*		*Total (n = 16)*	
	Any Grade	Grade 3	Any Grade	Grade 3	Any Grade	Grade 3	Any Grade	Grade 3
Participants with any event**	**2 (66.7)**	**1 (33.3)**	**1 (33.3)**	**0 (0.0)**	**4 (57.1)**	**2 (28.6)**	**7 (43.8)**	**3 (18.8)**
Anemia					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Bullous pemphigoid	1 (33.3)						1 (6.3)	
Colitis					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Dermatitis acneiform			1 (33.3)				1 (6.3)	
Dyspnea exertional***					1 (14.3)		1 (6.3)	
Hyperthyroidism					1 (14.3)		1 (6.3)	
Hypophosphatemia					1 (14.3)		1 (6.3)	
Hypothyroidism			1 (33.3)		1 (14.3)		2 (12.5)	
Infusion-related reaction					1 (14.3)		1 (6.3)	
Keratoacanthoma					1 (14.3)		1 (6.3)	
Lipase increase					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Nausea	1 (33.3)						1 (6.3)	

	3 mg/kg (n = 3)		*10 mg/kg (n = 3)*		*20 mg/kg (n = 7)*		*Total (n = 16)*	
Pruritus	1 (33.3)						1 (6.3)	
Rash maculo-papular	1 (33.3)		1 (33.3)				2 (12.5)	
Skin infection	1 (33.3)	1 (33.3)					1 (6.3)	1 (6.3)
Vomiting	1 (33.3)						1 (6.3)	

**There were no treatment-related AEs in the 3 participants treated with 1 mg/kg M7824.

***The differential for this dyspnea was pneumonitis vs. lymphangitic spread of disease (disease progression).

As of August 2017, > 500 participants have been treated with M7824 across multiple solid tumor expansion cohorts. The safety profile is consistent with other monotherapy checkpoint inhibitors, with the exception of keratoacanthomas and cutaneous squamous cell carcinomas which have occurred in approximately 3-5% of participants, and are well managed with surgical excision. These lesions have not been a criterion for treatment discontinuation, but thus far have all spontaneously regressed following treatment discontinuation.

In addition, after discussion among NCI investigators on multiple protocols using M7824, multiple bleeding events ranging from low grade gingival bleeding and epistaxis to more serious hemoptysis, GI bleeding and hematuria have been observed. Some of these events can be attributed to bleeding events related to cancer directly and others bleeding events can be attributed to colitis or cystitis which is a known toxicity of anti-PD-L1 agents including M7824. However, there remains the possibility that M7824 may increase the overall risk of bleeding in ways that may not be directly related to direct tumor bleeding or inflammatory bleeding events described with checkpoint inhibitors like M7824. It is hypothesized that this possible increased bleeding risk may be due to TGF beta inhibition which has an effect on angiogenesis; bleeding has also been observed in participants receiving M7824 and may be drug-related (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool). Accordingly, participants will be notified of the same possible risk in the informed consent document for this study (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool).

At least 2 instances of nodular regenerative hyperplasia have been observed with the use of this agent.

14.2.3 Formulation and Preparation

M7824 is provided as a sterile liquid formulation and packaged at a 10 mg/mL concentration in USP/ Ph Eur type I 50R vials that are filled with drug product solution to allow an extractable volume of 60 mL (600 mg/60 mL). The vials are closed with rubber stoppers in serum format complying with USP and Ph Eur with an aluminum crimp seal closure. Each single-use vial contains 600mg of M7824, formulated as 10mg/mL of active, 6% (w/v) Trehalose, 40 mM NaCl, 5 mM Methionine, 0.05% (w/v) Tween 20, 10 mM LHistidine at pH 5.5.

The liquid formulation is diluted directly with 0.9% sodium chloride solution for injection. The estimated volumes of delivery are anticipated to be no more than 250mL. The verified concentration range in the infusion solution is 0.16 mg/mL to 9.6 mg/mL.

14.2.4 Stability and Storage

M7824 must be stored at 2℃ to 8℃ until use. Product stored at room temperature for extended periods of time might be subject to degradation. M7824 must not be frozen. Rough shaking of the reconstituted solution must be avoided.

The chemical and physical in-use stability for the infusion solution of M7824 in 0.9% sodium chloride for injection has been demonstrated for a total of 72 hours at room temperature; however, from a microbiological point of view, the diluted solution should be used immediately and is not intended to be stored unless dilution has taken place in controlled and validated aseptic conditions. No other drugs should be added to the infusion containers containing M7824. See Manual of Preparation of approved ancillary supplies.

14.2.5 Administration Procedures

See Section **3.2**.

14.2.6 Incompatibilities

Not available.

14.3 N-803 (IND # 19660)

14.3.1 Source / Acquisition and Accountability

N-803 is manufactured and supplied by ImmunityBio, Inc.

N-803 will be provided to the clinical trial site by the manufacturer. The investigator or designee (e.g., pharmacist) will maintain an ongoing inventory of the investigational product supply according to standard site procedures. The investigational product will be dispensed at the direction of an investigator for administration to a study participant enrolled on the clinical trial. Disposal of expired or unused product will be returned to the manufacturer or disposed of according to standard site procedures based on agreement between the manufacturer and the site.

14.3.2 Toxicity

In participants receiving the 10 mcg/kg subcutaneous injection, the following were reported grade 1 and 2 toxicities: worsening anemia, nausea/vomiting, constipation, fatigue, fever, peripheral IV infiltration, injection site reactions, pain/aches, sinusitis, hypoalbuminemia, hypocalcemia, hypophosphatemia, decreased iron, headaches, dysgeusia, and cough. Grade 3 hypertension and decreased lymphocyte count were also reported. Atrial fibrillation, neutropenia and febrile neutropenia have also been reported as potential risks of N-803.

N-803 has been tested in combination with the anti-PD-1 agent, nivolumab at a dose of 240 mg i.v. every 4 weeks, in a phase 1 dose expansion study (n=21) with N-803 given weekly for 5 weeks on, 1 week off, for up to 5-6 months for pre-treated, advanced/metastatic NSCLC (NCT02523469). Fifteen participants received higher N-803 dosing: 15 mcg/kg (n=6) and 20 mcg/kg (n=9). Among all doses, there were no DLTs and no Grade 4 or 5 adverse events. Grade 3 adverse events were fever (n=1), flu-like illness (n=2), lymphopenia (n=2), fatigue (n=2), back pain (n=1), and dizziness (n=1). Grade 1 or 2 large injection site reactions were observed in 14 participants.

14.3.3 Formulation and Preparation

N-803 is a soluble complex consisting of two protein subunits of a human IL-15 variant associated with high affinity to a dimeric human IL-15 receptor α (IL-15Rα) sushi domain/human IgG1 Fc fusion protein. The IL-15 variant is a 114 aa polypeptide comprising the mature human IL-15 cytokine sequence with an Asn to Asp substitution at position 72 of helix C (N72D). The human IL-15Rα sushi domain/human IgG1 Fc fusion protein comprises the sushi domain of the human IL-15 receptor α subunit (IL-15Rα) (aa 1-65 of the mature human IL-15Rα protein) linked with the human IgG1 CH2-CH3 region containing the Fc domain (232 amino acids). Aside from the N72D substitution, all of the protein sequences are human. Based on the amino acid sequence of the subunits, calculated molecular weight of complex comprising two IL-15N72D polypeptides and a disulfide linked homodimeric IL-15RαSu/IgG1 Fc protein is 92.4 kilodaltons (kDa). Each IL-15N72D polypeptide has a calculated molecular weight of approximately 12.8 kDa and the IL-15RαSu/IgG1 Fc fusion protein has a calculated molecular weight of approximately 33.4 kDa. Both the IL-15N72D and IL-15RαSu/IgG1 Fc proteins are glycosylated resulting in an apparent molecular weight of N-803 as approximately 113 kDa by size exclusion chromatography. The isoelectric point (pI) determined for N-803 ranges from approximately 5.5 to 6.5. Thus, the fusion protein is negatively charged at pH 7. The calculated molar extinction coefficient at A280 for N-803 is 116,540 M-1, or 1.26 OD280 for a 1 mg/mL solution of N-803, or one OD280 is equivalent to 0.79 mg/mL solution of N-803.

The biological drug product, N-803, is formulated in a phosphate buffered saline solution. The drug substance is produced by a recombinant mammalian cell line and is manufactured using protein-free media.

N-803 is supplied at a concentration of 2 mg/mL and lot FG-16-0182-2 is administered undiluted. For lots other than FG-16-0182-2, the manufacturer will provide additional preparation instructions.

14.3.4 Stability and Storage

Study medication is provided in a 2mL vial containing 0.6 mL of N-803 at a concentration of 2 mg/mL. Study medication must be maintained at a temperature between 2°C and 8°C. The duration of time during which the product remains stable at room temperature will be obtained from Altor.

14.3.5 Administration Procedures

N-803 is administered subcutaneously.

14.3.6 Incompatibilities

Not available.

14.4 NHS-IL12 (IND # 19660)

14.4.1 Source / Acquisition and Accountability

NHS-IL12 is manufactured and supplied by EMD Serono.

NHS-IL12 will be provided to the clinical trial site by the manufacturer. The investigator or designee (e.g., pharmacist) will maintain an ongoing inventory of the investigational product supply according to standard site procedures. The investigational product will be dispensed at the direction of an investigator for administration to a study participant enrolled on the clinical trial.

Disposal of expired or unused product will be returned to the manufacturer or disposed of according to standard site procedures based on agreement between the manufacturer and the site.

14.4.2 Toxicity

Subjects (n=59) were treated subcutaneously with NHS-IL12 in a single ascending dose cohort followed by a multiple ascending dose cohort (n=37 with every 4-week dosing). The primary objective of this trial was to determine MTD as defined by the number of DLTs. None of the subjects treated with single or multiple doses up to 12.0 μg/kg experienced a DLT. At 16.8 μg/kg, 1/6 subjects had a DLT (grade 3 increase in alanine transaminase [ALT]). At 21.8 μg/kg, 2/6 subjects had a DLT (grade 3 increase in aspartate transaminase [AST] and ALT; grade 3 increase in lipase without clinical signs of pancreatitis). MTD was 16.8 μg/kg. The most frequently observed treatment-related adverse event (TRAE) was decreased lymphocyte count (27/59 subjects; 45.8%). Other TRAEs included decreased white blood cells (WBCs) (24/59; 40.7%), fever and elevated AST (21 each; 35.6%), elevated ALT (20; 3.9%), and anemia and flu-like symptoms (18 each; 30.5%) (Tab. Among the cohort receiving 16.8 μg/kg, the most frequently reported TRAEs were elevated AST (75%), decreased WBCs and elevated ALT (68.8% each), and decreased lymphocyte count and fever (62.5% each). At least one grade ≥ 3 TRAE was seen in 12/59 subjects (20.3%). These included decreased lymphocyte count (5; 8.5%), decreased neutrophil count (4; 6.8%), elevated ALT (3; 5.1%), decreased WBCs (2; 3.4%), and hypokalemia, hyperhidrosis, elevated alkaline phosphatase, AST, and lipase (1 each; 1.7%). All grade ≥ 3 TRAEs were transient; only hyperhidrosis was symptomatic. One grade 4 TRAE was observed (asymptomatic decreased lymphocyte count); no grade 5 TRAE was observed.

14.4.3 Formulation and Preparation

NHS-IL12 is formulated as a 1.5 mg/mL solution and is supplied by the Sponsor in single-use glass vials with a rubber stopper and sealed with an aluminum Flip Off® crimp seal closure.

The contents of the NHS-IL12 vials are sterile and nonpyrogenic, and do not contain bacteriostatic preservatives. Any spills that occur should be cleaned up using the facility's standard cleanup procedures for biologic products.

Rough shaking of the solution must be avoided.

NHS-IL12 drug product is ready-to-use. However, for doses below 300 μg, NHS-IL12 drug product must be diluted with 0.9% saline solution (sodium chloride injection). Detailed information on medical devices to be used for the preparation and administration of NHS-IL12 will be provided in the Manual of Operations.

Any unused portion of the solution should be discarded in biohazard waste disposal with final disposal by accepted local and national standards of incineration.

14.4.4 Stability and Storage

NHS-IL12 drug product must be stored at 2°C to 8°C until use, with a temperature log maintained daily. All medication boxes supplied to the study site must be stored carefully, safely, and separately from other drugs.

The chemical and physical in-use stability of NHS-IL12 diluted and undiluted solution in syringes has been demonstrated for 24hours at 25°C. However, to reduce the risk of microbial contamination, the solution should be used immediately unless medication preparation has been

performed in controlled and validated aseptic conditions. Rough shaking of the solution must be avoided.

14.4.5 Administration Procedures

NHS-IL12 is administered subcutaneously.

14.4.6 Incompatibilities

Not available.

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16 APPENDICES

16.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

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