H3.3K27M Specific Peptide Vaccine Combined with poly-ICLC with and without PD-1 inhibition using Nivolumab for the Treatment of newly diagnosed HLA-A2 (02:01)+ H3.3K27M Positive Diffuse Intrinsic Pontine Glioma (DIPG) and newly diagnosed HLA-A2 (02:01)+ H3.3K27M Positive Gliomas

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Protocol Signature Page

Protocol No.: PNOC 007 **Version Date:** 3-1-2021

1. I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Institutional Review Board (IRB), and Data Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with Good Clinical Practices (ICH-GCP) and the applicable IRB, ethical, federal, state, and local regulatory requirements.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

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Protocol Signature Page

Protocol No.: PNOC 007 **Version Date:** 3-1-2021

Participating Site(s)

I have read this protocol and agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP) and the applicable IRB, ethical, federal, state, and local regulatory requirements.

Principal Investigator	**Site**
Printed Name	Institution Name
Signature	Date

Abstract

Title	H3.3K27M Specific Peptide Vaccine Combined with poly-ICLC and Nivolumab for the Treatment of newly diagnosed HLA-A2 (02:01)$^+$ H3.3K27M Positive Diffuse Intrinsic Pontine Glioma (DIPG) and newly diagnosed HLA-A2 (02:01)$^+$ H3.3K27M Positive Midline Gliomas
Subject Population	Children and young adults (ages 3-21) with newly diagnosed DIPG or newly diagnosed other midline gliomas, positive for HLA-A2 (02:01)$^+$ and H3.3K27M.
Rationale for Study	Currently, there are no effective therapies for pediatric malignant gliomas. Gliomas located within midline structures – such as DIPG – have a particularly poor prognosis. Immunotherapy, such as active vaccinations, has the potential to develop as an effective and safe modality for these patients. Vaccines using specific peptides, in comparison to whole glioma-derived antigens, are expected to be more feasible because these vaccines may induce glioma-specific immune responses without theoretical concerns of auto-immune encephalitis. In addition, these vaccines can be generated “off the shelf” owing to the use of synthetic antigen peptides and Montanide ISA-51. Use of modified peptides (peptides in which amino acid residues are replaced from the wild-type sequence) may allow us to induce more efficient T cell responses than natural antigens in whole glioma cells. Furthermore, based on recently published laboratory data, administration of poly-ICLC along with the synthetic peptides remarkably enhances the induction of anti-peptide cytotoxic T lymphocyte (CTL) responses and trafficking of antigen-specific T cells to the brain tumor sites. Programmed cell death protein 1 (PD-1) plays an important role in suppressing the immune system by inhibiting T cell inflammatory activity and has been shown to play a role in tumor evasion of the immune system. PD-1 allows tumor cells to “turn off” T cell mediated immune response and prevent immune-mediated attack on the tumor. We hypothesize that the combination of PD-1 inhibition with the H3.3K27M specific peptide vaccine will improve activity of the peptide vaccine, by amplifying the response of peptide-primed T cells against the tumor. Utilizing the H3.3K27M peptide as monotherapy, we have shown that the peptide vaccine approach is reasonably well tolerated in patients with newly diagnosed DIPG (Stratum A) or newly diagnosed other midline gliomas (Stratum B), positive for HLA-A2 and H3.3K27M. Based on these initial safety results, we will now assess the combination of the peptide vaccine with PD-1 inhibition, using nivolumab (Stratum C). We will conduct a multicenter study evaluating the safety and immune activity of a synthetic peptide vaccine specific for the H3.3.K27M epitope given in combination with poly-ICLC and the H3.3.K27M epitope given in combination with poly-ICLC and the PD-1 inhibitor, nivolumab, in HLA-A2 (02:01)$^+$ children with newly

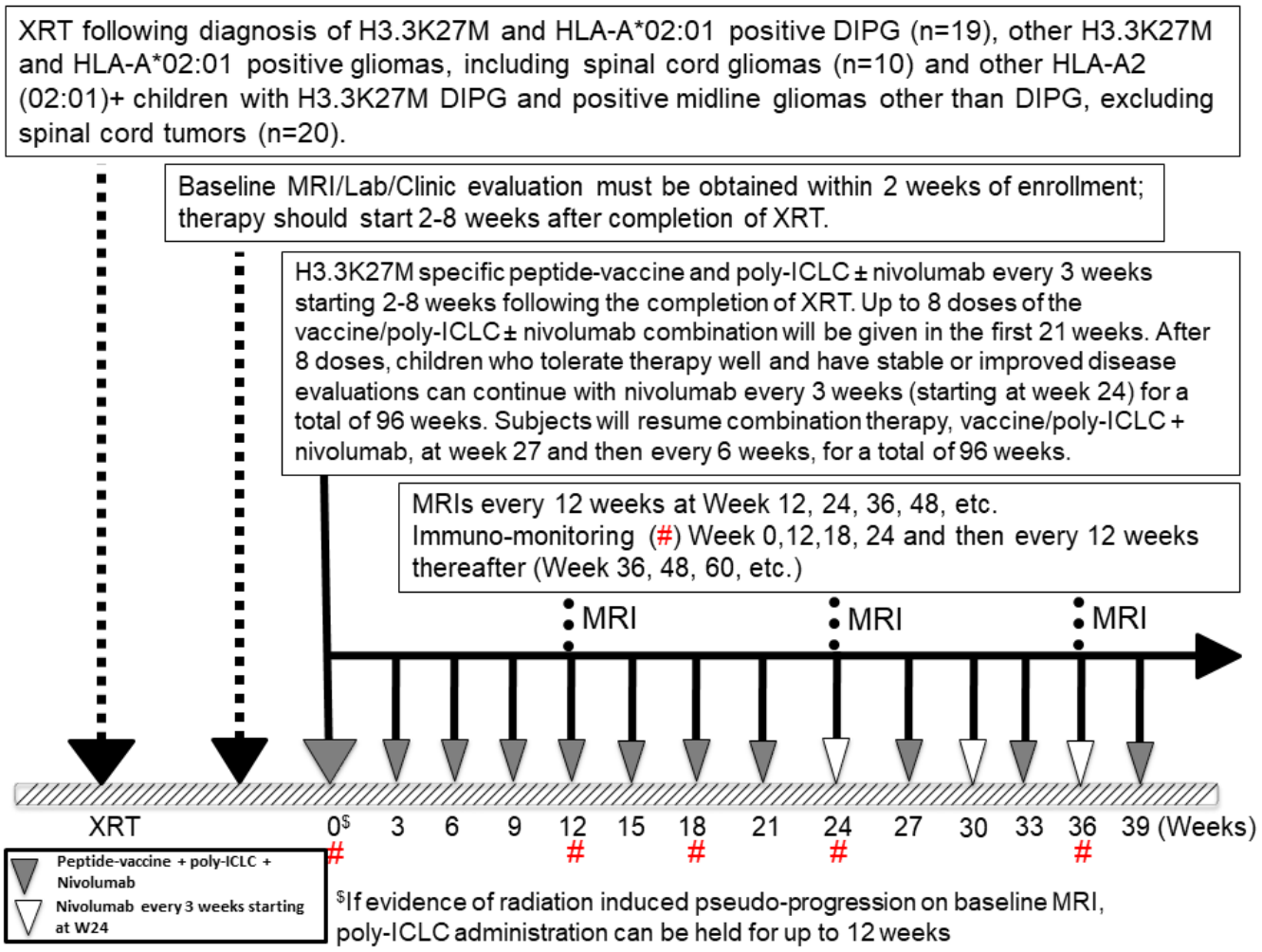
	diagnosed DIPG or other midline gliomas that are positive for H3.3K27M.
Primary Objectives	• **Stratum A** o To determine the overall survival at 12 months (OS12) in HLA-A2 (02:01)$^{+}$ children with DIPG that are treated with repeated administration of the H3.3K27M peptide. o To assess the safety of repeated administration of the H3.3K27M epitope specific vaccine in HLA-A2 (02:01)$^{+}$ children with H3.3K27M positive DIPGs. • **Stratum B** o To assess the safety of repeated administration of the H3.3K27M epitope specific vaccine in HLA-A2 (02:01)$^{+}$ children with H3.3K27M positive midline gliomas other than DIPG. • **Stratum C** o To assess the safety of repeated administration of the H3.3K27M epitope specific vaccine in combination with nivolumab in HLA-A2 (02:01)+ children with H3.3K27M DIPG and positive midline gliomas other than DIPG (excluding primary spinal cord tumors).
Exploratory Objectives	**Stratum A:** • Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)$^{+}$ children with H3.3K27M positive gliomas other than DIPG • Assessment of H3.3K27M infiltrates in subjects with evidence of progression that undergo tissue collection as part of their standard of care. Tumor tissue will be analyzed for H3.3K27M expression status and infiltration of H3.3K27M specific T cells • To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance. **Stratum B:** • Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)+ children with H3.3K27M positive gliomas other than DIPG • Assessment of H3.3K27M infiltrates in subjects with evidence of progression that undergo tissue collection as part of their standard of care. Tumor tissue will

	be analyzed for H3.3K27M expression status and infiltration of H3.3K27M specific T cells • To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance. **Stratum C:** • To determine the OS12 in HLA-A2 (02:01)$^{+}$ children with H3.3K27 positive DIPGs and midline gliomas (excluding primary spinal cord tumors) that are treated with repeated administration of the H3.3K27M peptide and nivolumab. • Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)$^{+}$ children with H3.3K27M DIPG and other positive gliomas other than DIPG (excluding primary spinal cord tumors). • To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance. • To assess Quality of Life (QoL) and cognitive measures in HLA-A2 (02:01)$^{+}$ children with H3.3K27M positive DIPG or other midline gliomas.
Study Design	This is 3-arm, multicenter study that will be conducted through the Pacific Pediatric Neuro-oncology Consortium (PNOC). **Stratum A** consists of HLA-A2 (02:01)$^{+}$ positive subjects with newly diagnosed H3.3K27M positive DIPG. **Stratum B** consists of HLA-A2 (02:01)$^{+}$ positive subjects with newly midline diagnosed H3.3K27M positive gliomas that are not classified as DIPG, including spinal cord tumors. **Stratum C** consists of newly diagnosed DIPG or newly diagnosed other midline gliomas (excluding primary spinal cord tumors), positive for HLA-A2 (02:01) and H3.3K27M. Once we have completed accrual onto strata A and B (subjects who have received vaccine peptide + poly-ICLC alone), we will enroll subjects onto stratum C (subjects will receive the combination of nivolumab and vaccine peptide + poly-ICLC).
Number of subjects	This trial will enroll a total of: **Stratum A** - 19 evaluable HLA-A2 (02:01)$^{+}$ positive subjects with newly diagnosed H3.3K27M positive DIPG. **Stratum B** - 10 evaluable HLA-A2 (02:01)$^{+}$ positive subjects with newly diagnosed midline H3.3K27M positive gliomas, including spinal cord tumors,

	Stratum C - 20 newly diagnosed H3.3K27M and HLA-A2 (02:01)$^{+}$ positive subjects with newly diagnosed DIPG or other midline H3.3K27M positive gliomas (excluding primary spinal cord tumors).
Duration of Therapy	In the absence of tumor progression, subjects may continue treatment for 96 weeks or about ~24 months from the time of study entry.
Duration of Follow up	Subjects will be followed for 24 months after completion of treatment, or until removal from study or death, whichever occurs first.
Duration of study	Enrollment into strata A, B and C will be completed in approximately 4 years and the primary efficacy endpoint is OS12. We therefore anticipate that we will complete the study in approximately 6 years.
Study Drugs	The synthetic H3.3K27M peptide and helper peptide (Tetanus Toxoid) will be included in the vaccine formulation. The combined H3.3K27M and Tetanus Toxoid will be referred to as K27M/TT peptide. These will be emulsified in Montanide prior to administration. Poly-ICLC is a synthetic complex of polyinosinic and polycytidylic acid, stabilized with polylysine and carboxymethyl cellulose. This will be co-administered with K27M/TT peptide to improve therapeutic efficacy. Nivolumab is an anti-PD-1 monoclonal antibody, which has received FDA approval for treatment of metastatic melanoma, non-small cell lung cancer, and other adult malignancies, but has not received approval for pediatric brain tumors.
Safety Assessments	Analyses will be performed for all subjects having received at least one course of study therapy, which constitutes K27M/TT peptide and poly ICLC treatment (Strata A and B) as well as K27M/TT peptide, poly ICLC plus nivolumab (Stratum C). The safety parameters include all laboratory tests and imaging evaluations, physical findings, and spontaneous reports of adverse events reported to the investigator by subjects. Each subject will be assessed for the development of any toxicity or adverse event as outlined in the Study Calendar. The study will use the CTCAE v 5.0 for assessing all adverse events. The Study Chair is responsible for the overall conduct of the study and for monitoring its safety and progress at all participating sites.
Efficacy Assessments	Overall Survival at 12 months for stratum A and stratum C.
Unique Aspects of this Study	This is the first study to evaluate the safety and efficacy of specific synthetic peptide for the H3.3.K27M epitope alone and in combination with PD-1 inhibition with nivolumab in HLA-A2 (02:01)$^{+}$ children with newly diagnosed H3.3K27M

	positive DIPG or other H3.3K27M positive midline gliomas.

Therapy Schema



List of Abbreviations

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CBC	complete blood cell (count)
CR	complete response
CRC	Clinical Research Coordinator
CRF	case report form
CSF	cerebral spinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Lymphocyte
CTMS	Clinical Trial Management System
DIPG	Diffuse intrinsic pontine glioma
DLT	dose limiting toxicity
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
FDA	Food and Drug Administration
GAA	Glioma associated antigens
GBM	Glioblastoma multiforme
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HDFCCC	Helen Diller Family Comprehensive Cancer Center
HGB	Hemoglobin
HGG	High grade glioma
HLA	Human leukocyte antigen
IFA	Incomplete Freund’s adjuvant
IHC	immunohistochemistry
IM	intramuscular
IND	investigational new drug application
IRB	Institutional Review Board
IV	Intravenous
K27M/TT	H3.3K27M/Tetanus Toxoid vaccine

List of Abbreviations

MRI	magnetic resonance imaging
NABTC	North American Brain Tumor Consortium
NABTT	New Approaches for Brain Tumor Therapy
NCI	National Cancer Institute
ORR	overall response rate
OSR	overall survival rate
OS12	Overall survival at 12 months
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PD-1	Programmed cell death protein 1
Poly-ICLC	Polyinosinic-Polycytidylic acid with polylysine and carboxymethylcellulose
PR	partial response
PRC	Protocol Review Committee (UCSF)
RLT	Regimen limiting toxicity
RT-PCR	Reverse transcriptase polymerase chain reaction
SAE	Serious adverse event
SD	stable disease
SD	standard deviation
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SOP	standard operating procedure
Th	T helper cell
TIL	Tumor infiltrating lymphocytes
TLR3	Toll-like receptor 3
TT	Tetanus Toxoid
ULN	upper limit of normal
WBC	white blood cell (count)

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1 Introduction

1.1 Background on Indication

Overview

Malignant gliomas, including glioblastoma (GBM) and diffuse intrinsic pontine gliomas (DIPG) are lethal brain tumors in both adults and children. Indeed, brain tumors are the leading cause of cancer-related mortality and morbidity in children. Children with DIPG have one-year progression-free survival (PFS) rates below 25%, and median overall survival (OS) of 9 to 10 months with current treatment.[1] Histological grading plays no part in this disease.[2] Recent genetic studies have revealed that malignant gliomas in children often show recurrent missense mutations in *H3F3A*, which encodes the replication-independent histone 3 variant H3.3.[2-4] Approximately 30% of pediatric glioblastoma[3] and 70% of DIPG [5] cases harbor the amino-acid substitution from lysine (K) to methionine (M) at the position 27 of H3.3 (K27M mutation, hereafter), which is universally associated with shorter survival in DIPG patients compared with patients with non-mutated H3.3[5].

Recent studies have shown that the phenotypic and molecular features of DIPG are highly similar to other pediatric HGG arising in anatomical midline structures (e.g. thalamus, spinal cord – midline HGG) and that currently these midline HGG are considered to present a spectrum along a single clinico-pathological entity [6-8]. The defining H3K27M mutation of these tumors has been shown to co-segregate non-randomly with specific partner mutations dependent on H3 subtype and anatomical location. H3.3K27M mutations are frequently found in conjunction with alterations in the p53 pathway (TP53, PPM1D, ATM), along with receptor tyrosine kinase amplification/mutation in PDGFRA (DIPG) or FGFR1 (other midline gliomas). By contrast, H3.1K27M mutations are almost exclusively found in DIPGs, are p53 wild-type, and instead harbor alterations in the TGFβ/BMP receptor ACVR1 and downstream PI3-kinase pathway genes (PIK3CA, PIK3R1) [2,9-11]. The H3K27M mutations appear to be early clonal events in midline gliomagenesis, co-segregating mutations are frequently found at subclonal frequencies, and may demonstrate evidence of convergent or parallel evolution across tempero-spatial disease progression [12,13]. The outcome for these tumors is universally poor with OS rates similar to DIPGs – overall survival rates of 30-40% at 12 months and less than 10% survival at 2 years.

The adaptive immune system, such as T-lymphocytes (T cells hereafter), is often tolerant to normal self-proteins, but can recognize mutated amino-acids as non-self. Hence cancer-specific mutations can be suitable targets for cancer immunotherapy, such as cancer vaccines and adoptive T cell transfer therapy. Dr. Okada and his colleagues at the University of Pittsburgh recently conducted one of the first vaccine studies in pediatric patients including DIPG and demonstrated robust and specific T cell responses against non-mutated, but immunogenic antigens that are overexpressed in glioma cells.[14] Although these data show safety of vaccines targeting these non-mutated antigens,[14] it has also been reported that immunotherapy targeting non-mutated, non-cancer-specific antigens can cause fatal adverse events due to the off-target effects. Therefore, there is a strong demand for finding cancer-

specific targets given the availability of powerful technologies for inducing immune responses in patients.

Dr. Okada has recently found that a specific peptide that includes the K27M mutation (H3.3K27M epitope, hereafter) can induce specific cytotoxic T lymphocyte (CTL) responses in human leukocyte antigen HLA-A2 $(02:01)^{+}$ donors (please see figure 1 below). Most importantly for the therapeutic relevance, induced CTLs recognize the H3.3K27M epitope that is endogenously expressed by HLA-A2 $(02:01)^{+}$ glioma cell lines that also harbor the K27M mutation. Therefore, H3.3K27M represents a novel, shared neoantigen epitope for T-cell-based immunotherapy.

Pediatric Glioma Vaccines

Cancer vaccines are designed to induce systemic immunity against antigens expressed by tumor cells. Results were recently published on using a series of glioma associated antigens (GAA) for the treatment of children with HGG including DIPG.[14] The GAAs were EphA2, interleukin-13 receptor alpha and survivin. This study was performed on the basis of encouraging results in adult patients with glioma using peptide based vaccines.[15-19] In the pediatric study, children (n=26: 20 children with DIPG and 6 children with other HGG) were treated with a combination of these GAA-derived HLA2 restricted antigens and poly-ICLC. All patients underwent focal radiation therapy as primary treatment modality and vaccinations were started after completion of radiation therapy. Children received subcutaneous injections of GAA restricted peptides, Tetanus toxoid emulsified in Montanide ISA-51 with concurrent intramuscular injections of Toll-like receptor ligand poly-ICLC (for details about the rationale for these compounds please see below). Within this trial a total of 84 children were screened for the HLA-A2 status, of which 42 tested positive. Principal toxicities included grade 1 and 2 injection site reactions in 100% of patients and flu like symptoms such as fatigue, fever, myalgias, chills and headache in 92% of participants. Usually these symptoms resolved with acetaminophen and/or ibuprofen within 1-2 days. Grade 1 gastrointestinal symptoms occurred in 31% of subjects. No grade 3 or higher systemic toxicities or auto-immune reactions occurred in that trial. The median survival was 12.7 months among 20 children with DIPG and 25.1 months among those with HGG. Median survival among patients with pseudoprogression was 19.5 months versus 10.9 months in those without pseudoprogression. Immune responses against at least one of the GAA were present in 13 out of 21 evaluable patients.[14] This study highlights the favorable toxicity profile of peptide-based vaccination strategies but also highlights the complexity of assessing therapy response given the relatively high incidence of pseudoprogression that seem to correlate with improved outcome. It will also be critical to investigate tumor tissue at time of progression to enhance our understanding of therapy resistance.

Checkpoint inhibitors for cancer therapy

Immunotherapy has had significant positive effects on outcomes for some of the most malignant tumors such as metastatic melanoma and non-small-cell lung cancer. The anti-PD-1 antibody, nivolumab, is a second-generation checkpoint inhibitor that has shown antitumor effects in melanoma, renal cell carcinoma and lung cancer among other cancers. Nivolumab is a PD-1 inhibitor that has been tested in the children with hematologic, solid, and CNS tumors as single agent as well as in combination with CTCL4 antibody ipilimumab (NCT02304458; NCT03130959; NCT02927769). The RP2D of nivolumab in the pediatric setting has been reported to be 3mg/kg IV every 2 weeks. Good tolerability was demonstrated in the most recent Children's Oncology Group trial (ADVL1412; NCT02304458), with immune-mediated AEs comparable to adult experience. Nivolumab alone as well as in combination therapy with ipilimumab is currently being tested in pediatric high-grade CNS malignancies in an ongoing BMS sponsored study (NCT03130959). Details about nivolumab and associated toxicity can be found in the Investigator Brochure.

To enhance the activity of the specific K27M/TT vaccine, we will conduct a multicenter study evaluating the safety and immunological activity of a vaccine using a specific synthetic peptide for the H3.3K27M epitope in combination with nivolumab in HLA-A2 $(02:01)^+$ children with newly diagnosed DIPG or other midline gliomas that are positive for the H3.3K27M mutation.

1.2 Background on the Compounds

K27M peptide: It was recently found that a mutated peptide that includes the K27M mutation (the H3.3K27M epitope) can induce specific CTL responses in HLA-$A2^+$ healthy donors (**Figure 1**). Most importantly, induced CTLs lyse the H3.3K27M epitope that is endogenously expressed by the HLA-$A2.1^+$ H3.3K27M^+ glioma cell line in an HLA-class I-dependent manner (**Figure 1C**). To our knowledge, there is not a similar neoantigen-derived epitope for T-cell-based immunotherapy for gliomas.

A recent study extended binding analyses to HLA-A*02:01, *02:02, A*02:03, A*02:06, A*02:07, and A*02:17, and found that the H3.3K27M peptide most effectively binds to the HLA-A*02:01.[20]

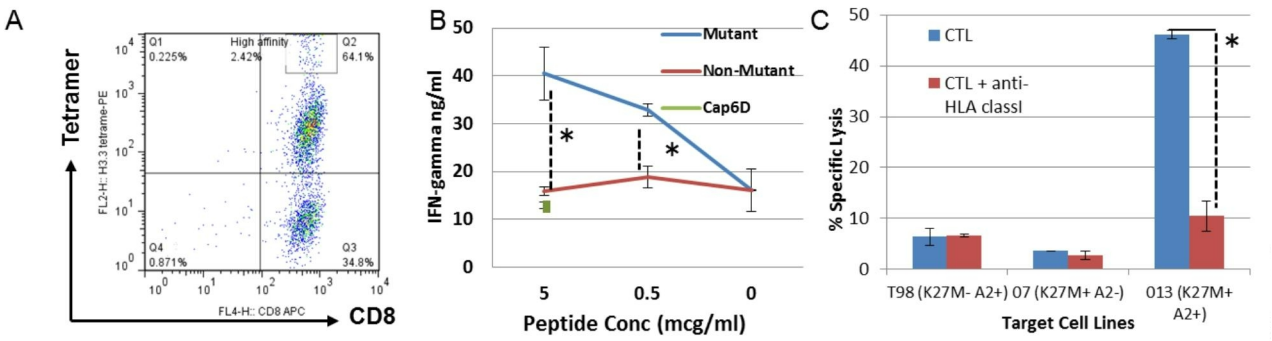


Figure 1. **HLA-A2⁺ donor-derived CTLs specifically recognize HLA-A2⁺ K27M⁺ glioma cells in an HLA-class I-dependent manner**. Peripheral blood mononuclear cells from an HLA-A2⁺ donor were stimulated *in vitro* with the H3.3K27M peptide and evaluated for their reactivity against: (**A**) HLA-A2/H3.3K27M-specific tetramer and anti-CD8 mAb, and (**B**) T2 cells pulsed with the mutant or non-mutated H3.3 peptide by IFN-γ ELISA. In (**A**), among the CD8⁺tetramer⁺ population (64.1% of total lymphocyte-gated cells), there is a tetramer^{high} subpopulation (2.4% of total lymphocyte-gated cells), some of which were used as CTL clones (Aim 2). In (**B**), the Cap1-6D peptide (tested at 5 µg/ml only) is a high avidity HLA-A2.1-binding epitope derived from CEA used as an irrelevant negative control.

(**C**) The CTL line was evaluated for cytotoxicity against glioma cell lines T98 (HLA-A2⁺but K27M-negative), HSJD-DIPG-07 (HLA-A2-negative but K27M⁺), and HSJD-DIPG-13 (HLA-A2⁺ and K27M⁺) lines. CFSE-labeled target cells (10e4/well) were incubated with CTLs at the E/T ratio of 25 for 4 hours. To block the CTL cytotoxicity, anti-HLA-ABC 10µg/ml was added to one group. At the end of incubation, 7-ADD was added into each well and incubated for 10 minutes on ice. The samples were analyzed by flow cytometry, and the killed target cells were identified as CFSE⁺ and 7-ADD⁺ cells. The cytotoxicity was calculated as the percentage of CFSE⁺ and 7-ADD⁺ cells in total HLA-A2⁺ CFSE⁺ cells. (*p<0.05 by Wilcoxon rank-sum tests).

Tetanus Toxoid (TT)-derived helper T cell epitope: A number of studies support the important role of CD4⁺ T-helper (Th) cells in the induction of anti-tumor CD8⁺ CTLs and memory responses.[21-23] Since Th cell epitopes have not been well-characterized, we have selected a well characterized Th epitope from a Tetanus Toxoid (TT) protein in the first pediatric study, to which the vast majority of the population has been sensitized and which is commonly used to induce a Th response to the immunizing peptides in peptide-based cancer vaccines.[21-23] We will apply a similar strategy to the current trial using the specific H3.3K27M specific peptide.

Montanide ISA-51: H3.3K27M and TT peptides will be emulsified in the adjuvant Montanide ISA-51 similar to the GAA peptide-based trial described above. Montanide ISA-51 consists of a mineral oil base similar to incomplete Freund's adjuvant (IFA). However, the Arlacel A emulsifying agent of IFA, which has caused reactions in the past, has been replaced with a purified Mannide monooleate called "montanide", which appears to be safer and has been shown to be well tolerated in the pediatric GAA peptide based trial.[14] Peptide-based vaccines in Montanide ISA-51 have been safely administered in a large number of research patients, including pediatric patients with DIPG and HGG, and have induced T-cell responses against the immunizing peptides in a majority of patients without major toxicities.[14,24,25] Toxicities most commonly observed include local discomfort, induration, and erythema at the injection site as outlined above in detail for the pediatric trial using GAA based peptides.

Poly-ICLC: Polyinosinic-Polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) is a synthetic nucleic acid, and functions as a Toll-like receptor-3 (TLR3) ligand. We propose to combine intramuscular (IM) poly-ICLC administration and H3.3K27M peptide based vaccine because: 1) data show that IM administration of poly-ICLC remarkably and safely improves the therapeutic effects of GAA-based vaccines in mouse models[26] and in humans[14]; and 2) IM poly-ICLC as a single agent has been extensively evaluated in patients with malignant glioma in pilot and multi-center studies through the two NCI-funded brain tumor consortia, the North American Brain Tumor Consortium (NABTC) and the New Approaches for Brain Tumor Therapy (NABTT) consortium.[27] The proposed dose in this trial (30 μg/kg) is based on the safety and biological activity data obtained in these preceding trials. Data demonstrates that concurrent IM injection of poly-ICLC at the time of vaccination provides the maximal adjuvant effect for the induction of antigen-specific CTL response.[26] Based on studies with frequent injections (2-3/week), the main toxicity of poly-ICLC is a flu-like reaction that can include fever, myalgias, arthralgias, malaise, and possibly nausea and vomiting. With much less frequent injections in our treatment plan, we do not anticipate major toxicities with poly-ICLC in the proposed trial, which is well supported by results from the pediatric trial using GAA based epitopes.[14]

In 1996, a multi-institutional trial of single agent poly-ICLC in pediatric patients with brain tumors was initiated. The initial dose studied was 20 micrograms/kg given twice a week IM, which was subsequently escalated to 30 micrograms/kg. The protocol enrolled a total of 46 pediatric patients with either: 1) newly diagnosed and recurrent high-grade gliomas; 2) recurrent low-grade gliomas; 3) newly diagnosed and recurrent brain stem gliomas; 4) any other recurrent brain tumors, or 5) neurofibromatosis-related tumors. There were 16 incidents of Grade 3 or 4 toxicity, with the majority of them not likely related to the therapy. There were 4 cases of Grade 3 hepatic toxicity and one case of Grade 3 fever, which were considered to be possibly or definitely related to the therapy, respectively. A case with Grade 3 and another case with Grade 4 neurological toxicity were noted but both cases manifested clinical and radiological signs of tumor progression. One of 14 patients with HGG had a partial response (PR). One of 5 patients with recurrent low-grade gliomas had a PR and two patients had prolonged stable disease. One patient had an improved and prolonged response when the dose of poly-ICLC was increased from 20 micrograms/kg to 30 micrograms/kg given twice a week.

In a recent pilot study of peptide-based vaccination in combination with poly-ICLC in DIPG patients,[14] concurrent (i.e., the same day) administrations of subcutaneous peptide vaccine and intramuscular (IM) 30 micrograms/kg poly-ICLC safely induced robust vaccine-specific T-cell response. Therefore, we will use the same mode of poly-ICLC administration (i.e., 30 micrograms/kg, IM).

Nivolumab [Bristol-Myers Squibb (BMS)]: Nivolumab is a monoclonal antibody consisting of 4 polypeptide chains, which include 2 identical heavy chains consisting of 440 amino acids and 2 identical light chains. Molecular weight is 146,221 Daltons. The recommended phase 2 dose for nivolumab in pediatric patients based on ADVL1412 is 3 mg/kg every 2 weeks.[28] However, more recent communication with BMS supports a standard dosing of Nivolumab every 3 weeks at 4.5 mg/kg IV. This is based on PK analysis that supports such a dosing schedule as well as scientific rationale to

give nivolumab on the same schedule as the K27M/TT vaccine (communication with BMS). We will co-administer nivolumab with the K27M/TT vaccine.

Justification of Nivolumab 4.5 mg/kg every 3 weeks Dosing in Pediatric Patients Nivolumab [Bristol-Myers Squibb (BMS)]:

The selected nivolumab dose of 4.5 mg/kg every 3 weeks represents the same time-averaged dose as 3 mg/kg every 2 weeks which has been shown to be safe and efficacious in several adult indications, and which is currently being investigated for pediatric indications. The PK of nivolumab is linear, and consequently the time-averaged concentration is expected to be the same for the 4.5 mg/kg every 3 weeks and 3 mg/kg every 2 weeks doses; whereas the peak concentrations are expected to be higher, and the trough concentration lower with 4.5 mg/kg every 3 weeks.

The magnitude of the predicted differences in key measures of exposure were assessed by a nivolumab population pharmacokinetic model was used to predict the nivolumab concentration time profiles after 3 mg/kg every 2 weeks and 4.5 mg/kg every 3 weeks dosing regimens in pediatric subjects with solid tumors. The predicted summary measures of exposure were used to compare the nivolumab exposures achieved with nivolumab 4.5 mg/kg every 3 weeks relative to 3 mg/kg every 2 weeks (Table 1). The difference within exposure measures (Cmin and Cavg) after 4.5 mg/kg every 3 weeks were within ~13% when compared to the exposure measures after 3 mg/kg every 2 weeks , suggesting that efficacy is less likely to be affected from exposure. The comparison of Cmax in pediatric patients dosed with 4.5 mg/kg every 3 weeks with that in pediatric patients dosed with 3 mg/kg every 2 weeks shows higher Cmax up to ~50%. Although the Cmax of nivolumab was expected to be higher with 4.5 mg/kg every 3 weeks compared to 3 mg/kg every 2 weeks , the predicted Cmax with 4.5 mg/kg every 3 weeks (82.3 micrograms/mL) was well below the Cmaxss (387 micrograms/mL) achieved with 10 mg/kg every 2 weeks , which was considered safe and tolerable.

Table 1: Geometric Mean Exposure for Nivolumab after 3 mg/kg Q2W and 4.5 mg/kg Q3W in Pediatric Subjects with Solid Tumors

Exposure Metric	3 mg/kg Q2W (N = 46)		4.5 mg/kg Q3W (N = 46)		% Difference[a]
	Geo. Mean	CV(%)	Geo. Mean	CV(%)	
Cmax1 [µg/mL]	54.8	40.6	82.3	41.1	50.2
Cmind42[µg/mL]	34.6	29.9	30.3	33	-12.4
Cavgd42 [µg/mL]	36.5	25.4	39.5	26	8.22
Cmaxss [µg/mL]	102	32.2	125	32.8	22.5
Cminss [µg/mL]	44.7	42.8	38.9	45.4	-13
Cavgss [µg/mL]	61.6	35	62.4	33.9	1.3

Abbreviations: CavgdX = time-averaged concentration over the first X days; Cmax = peak serum concentration after the first dose, CminX = minimum serum concentration on day X; Q2W = every 2 weeks; Q3W = every 3 weeks; SS = steady state.

[a] Percent difference in geometric mean of 4.5 mg/kg Q3W relative to 3 mg/kg Q2W.

1.3 Rationale for the Proposed Study

We will conduct a phase 1/2 multicenter study evaluating safety and immunological activity of a vaccine using a specific synthetic peptide for the H3.3K27M epitope alone and in combination with PD-1 blockade using nivolumab in HLA-A2 (02:01)+ children with newly diagnosed DIPG or other midline gliomas that are positive for the H3.3K27M mutation through the Pacific Pediatric Neuro-Oncology Consortium (PNOC). Currently, there are no effective therapeutic modalities for pediatric malignant gliomas. Gliomas located within midline structures – such as DIPG tumors – have a particularly poor prognosis. Immunotherapy, particularly active vaccinations, has the potential to develop as an effective and safe modality for these patients. Vaccines using specific peptides, in comparison to whole glioma-derived antigens, are expected to be more feasible because these vaccines may induce glioma-specific immune responses without theoretical concerns of auto-immune encephalitis. In addition, these vaccines can be generated "off the shelf" owing to the use of synthetic antigen peptides and Montanide ISA-51. Use of modified peptides (peptides in which amino acid residues are replaced from the wild-type sequence) may allow us to induce more efficient T cell responses than natural antigens in whole glioma cells. Furthermore, based on recent laboratory data, administration of poly-ICLC along with the synthetic peptides remarkably enhance the induction of anti-peptide CTL responses and trafficking of antigen-specific T cells to the brain tumor sites [18,26,27].

PD-1 (CD279) is a cell surface receptor present on T cells and a subset of B cells. It is a member of the CD28 family of T-cell co-stimulatory receptors that also includes CD28 and CTLA 4. PD-1 signaling has an inhibitory effect in cytokine activation (such as IL-2, IL-10, IL-13, interferon-, and Bcl-xL) that is CD28 mediated and has been shown to inhibit T cell activation as well as decrease expansion of previously activated cells. Inhibition of PD-1 activity appears to stimulate the immune response and enhance T-cell-mediated attack on tumor cells by effector T cell activation and repression of regulator T

cell activity.[29],[30] These data support our hypothesis that PD-1 blockade has the potential to amplify T-cell responses triggered by the K27M/TT vaccine.

Safety update from PNOC 007 (phase 1) cutoff date September 8, 2020: We have shown that treatment with the vaccine and poly-ICLC is safe in children with DIPG (n=19) as well as other midline gliomas including spinal cord tumors (n=10), as of September 8, 2020. With this current amendment, we will test the safety and efficacy of combined therapy of K27M/TT and poly-ICLC with nivolumab. Our hypothesis is that the combination therapy will be more effective compared to vaccine administration alone. We will combine DIPG and other midline glioma in one combined strata given our current experience that supports a favorable safety profile for both Strata A and B and that recent studies have shown similar outcomes for these entities. However H3K27 mutant spinal cord tumors seem to have significantly worse outcome and to date we have very limited safety data in this patient population with single agent vaccine (n=2) and therefore we will exclude these from Stratum C [31,32].

2 Objectives of the Study

2.1 Primary Objectives

Stratum A:

- To assess the safety of repeated administration of the H3.3K27M epitope specific vaccine in HLA-A2 (02:01)$^+$ children with H3.3K27M positive DIPGs.
- To determine the overall survival at 12 months (OS12) in HLA-A2 (02:01)$^+$ children with DIPG that are treated with repeated administration of the H3.3K27M peptide.

Stratum B:

- To assess the safety of repeated administration of the H3.3K27M epitope specific vaccine in HLA-A2 (02:01)$^+$ children with H3.3K27M positive midline gliomas other than DIPG, including spinal cord gliomas.

Stratum C:

- To assess the safety of repeated administration of the H3.3K27M epitope specific vaccine in combination with the PD-1 inhibitor nivolumab in HLA-A2 (02:01)$^+$ children with H3.3K27M positive midline gliomas including DIPG and midline gliomas (excluding primary spinal cord tumors).

2.2 Exploratory Objectives, Other Assessments

Stratum A:

- Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)+ children with H3.3K27M positive gliomas other than DIPG
- Assessment of H3.3K27M infiltrates in subjects with evidence of progression that undergo tissue collection as part of their standard of care. Tumor tissue will be analyzed for H3.3K27M expression status and infiltration of H3.3K27M specific T cells

- To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance.

Stratum B:

- Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)+ children with H3.3K27M positive gliomas other than DIPG
- Assessment of H3.3K27M infiltrates in subjects with evidence of progression that undergo tissue collection as part of their standard of care. Tumor tissue will be analyzed for H3.3K27M expression status and infiltration of H3.3K27M specific T cells
- To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance.

Stratum C:

- To determine the OS12 in HLA-A2 (02:01)$^+$ children with H3.3K27 positive DIPGs and midline gliomas (excluding primary spinal cord tumors) that are treated with repeated administration of the H3.3K27M peptide and nivolumab.
- Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)+ children with H3.3K27M positive gliomas including DIPG or other H3.3K27M positive midline gliomas, excluding primary spinal cord tumors.
- To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance.
- To assess Quality of Life (QoL) and cognitive measures in HLA-A2 (02:01)$^+$ children with H3.3K27M positive DIPG or other midline gliomas.

3 Study Design

This is a 3 strata multicenter study that will be conducted through PNOC. **Stratum A** consists of HLA-A2 (02:01)$^+$, positive newly diagnosed subjects with H3.3K27M positive DIPG, **Stratum B** consists of other HLA-A2 (02:01)$^+$, positive newly diagnosed subjects with H3.3K27M positive midline gliomas, including spinal cord tumors, that are not classified as DIPG, and **Stratum C** consists of other HLA-A2 (02:01)$^+$, positive newly diagnosed subjects with H3.3K27M positive DIPG and other midline gliomas, excluding primary spinal cord tumors. We will enroll subjects onto stratum C once we have completed accrual onto strata A and B.

3.1 Number of Subjects

A total of 19 evaluable H3.3K27M positive HLA-A2 (02:01)$^+$ DIPG patients will be enrolled into stratum A. Stratum B will include 10 evaluable HLA-A2 (02:01)$^+$ patients with midline gliomas other than DIPG that are positive for the H3.3K27M mutation (including spinal cord gliomas). Stratum C will include 20 evaluable HLA-A2 (02:01)$^+$ patients with DIPG and other midline gliomas that are positive for the H3.3K27M mutation (excluding primary spinal cord gliomas).

3.2 Eligibility Criteria

Patients must have eligibility evaluations performed prior to enrollment and must meet all inclusion and exclusion criteria. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

3.2.1 Inclusion Criteria

Stratum A:

- Newly diagnosed children (3-21 years old) with DIPG who are positive for the H3.3K27M mutation (positive testing from a CLIA or equivalent laboratory required) that underwent standard radiation therapy.

Stratum B:

- Newly diagnosed children (3-21 years old) with diagnosis of midline glioma other than DIPG who are positive for the H3.3K27M mutation (positive testing from a CLIA or equivalent laboratory required), including spinal cord gliomas, that underwent standard radiation therapy.

Stratum C:

- Newly diagnosed children 3-21 years of age with diagnosis of DIPG or midline glioma other than DIPG (excluding primary spinal cord gliomas) who are positive for the H3.3K27M mutation (positive testing from a CLIA or equivalent laboratory required), that underwent standard radiation therapy.

The following eligibility criteria apply to strata A, B and C:

- The patient must test positive for HLA-A*02:01 (positive testing from a CLIA or equivalent laboratory required; only the HLA A*02:01 subtype is eligible; other subtypes are excluded)
- The patient must be either off systemic steroids or be on stable dose of dexamethasone or equivalent (max 0.1 mg/kg/day; maximum 4mg/day) at time of enrollment.
- Patients must not have received any prior chemotherapy, immunotherapy or bone marrow transplant for the treatment of their tumor. Prior use of temozolomide during radiation at maximum of the standard pediatric dosing (defined as 90 mg/m^2 /dose continuously during radiation therapy for 42 days) or dexamethasone is allowed.
- Patients must have undergone radiation therapy and surgery as part of their standard of care.

- Stratum A: Radiation therapy must have started within 4 weeks of diagnosis by imaging or surgery, whichever is later.
- Stratum B: For subjects undergoing surgery for more extensive resection, radiation therapy should be started within 4-6 weeks from surgery.
- Stratum C: Radiation therapy must have started within 4 weeks of diagnosis by imaging or surgery, whichever is later. For subjects undergoing surgery for more extensive resection, radiation therapy should be started within 4-6 weeks from surgery.

- Karnofsky ≥50 for patients ≥16 years of age, and Lansky ≥50 for patients < 16 years of age (See Appendix A). Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

- The patient must have adequate organ function defined as:

Adequate Bone Marrow Function Defined as:

- Peripheral absolute neutrophil count (ANC) ≥ 1000/mm^3 **and**
- Platelet count ≥ 100,000/mm^3 (transfusion independent, defined as not receiving platelet transfusions for at least 7 days prior to enrollment).

Adequate Renal Function Defined as:

- Creatinine clearance or radioisotope GFR ≥ 70mL/min/1.73 m^2 **or**
- A serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine	
	Male	**Female**
3 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR utilizing child length and stature data published by the CDC.

Adequate Liver Function Defined as:

- Bilirubin (sum of conjugated + unconjugated) ≤ 1.5 x upper limit of normal (ULN) for age **and**
- SGPT (ALT) ≤ 110 U/L **and**
- Serum albumin ≥ 2 g/dL.

Adequate Pancreatic Function Defined as:

- Serum lipase ≤ ULN at baseline.

Adequate Pulmonary Function Defined as:

- No evidence of dyspnea at rest, no exercise intolerance due to pulmonary insufficiency, and a pulse oximetry of > 92% while breathing room air.

Adequate Neurologic Function Defined as:

- Patients with seizure disorder may be enrolled if seizure disorder is well controlled.

- The effects of the H3.3K27M vaccine and nivolumab on the developing human fetus are unknown. For this reason, females of child-bearing potential and males must agree to use adequate contraception. Adequate methods include: hormonal or barrier method of birth control; or abstinence prior to study entry and for the duration of study participation. 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. Males treated or enrolled on this protocol must also agree to use adequate contraception prior to the study and for the duration of study participation.
- Ability to understand a written informed consent document, and the willingness to sign it. Assent will be obtained when appropriate based on the subjects age.

3.2.2 Exclusion Criteria

- Investigational Drugs
  - Patients who are currently receiving another investigational drug are not eligible.
  - Prior treatment with another investigational drug.
- Anti-cancer Agents
  - Patients who are currently receiving other anti-cancer agents are not eligible.
  - Prior treatment with other anti-cancer agents.
- Patients who have received a live / attenuated vaccine within 30 days of first treatment.
- Patients with evidence of disseminated or leptomeningeal disease
- Patients with a known disorder that affects their immune system, such as HIV or Hepatitis B or C, or an auto-immune disorder requiring systemic cytotoxic or immunosuppressive therapy are not eligible. Note: Patients that are currently using inhaled, intranasal, ocular, topical or other non-oral or non-IV steroids are not necessarily excluded from the study but need to be discussed with the study chair.
- Patients with a ≥ Grade 2 hypothyroidism due to history of autoimmunity are not eligible. (Note: Hypothyroidism due to previous irradiation or thyroidectomy will not impact eligibility).
- Patients who have received prior solid organ or bone marrow transplantation are not eligible.
- Patients with uncontrolled infection.
- Female patients of childbearing potential must not be pregnant or breast-feeding. Female patients of childbearing potential must have a negative serum or urine pregnancy test prior to the start of therapy (as clinically indicated).

3.3 Duration of Therapy

Treatment may continue for 24 months or until:

- Disease progression
- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- Significant patient non-compliance with protocol
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

3.4 Duration of Follow Up

Patients will be followed for 24 months after completion of treatment, or until removal from study or death, whichever occurs first. Patients removed from treatment for unacceptable treatment related adverse event(s) will be followed until resolution or stabilization of all treatment related adverse events to grade 2 or lower, or a minimum of 24 months after removal from treatment.

3.5 Study Timeline

This study aims to enroll 19 HLA-A2 (02:01)$^+$ children with H3.3K27M positive DIPG (Stratum A), 10 HLA-A2 (02:01)$^+$ children with H3.3K27M positive midline gliomas other than DIPG, including spinal cord tumors (Stratum B) and 20 HLA-A2 (02:01)$^+$ children with H3.3K27M positive DIPG or midline gliomas other than DIPG, excluding primary spinal cord tumors (Stratum C). H3.3K27M status needs to be confirmed in a CLIA or equivalent approved laboratory. Eligible patients with positive H3.3K27M need to also test positive for HLA A*02:01in a CLIA or equivalent approved laboratory.[14] We will enroll into the 3 strata separately, and the first 3 patients in each stratum will be observed for RLTs for at least 6 weeks before the next set of patients are enrolled in that stratum. **We will enroll subjects onto stratum C once we have met total accrual onto strata A and B. See** Section 9.3 **for further information.**

Based on our accrual into this trial we anticipate to enroll an average of 1.8 subjects per month. We anticipate that enrollment for strata A, B, and C will be similar and therefore we anticipate that enrollment into the K27M glioma strata will be completed in about two years for each stratum.

3.6 Study Completion

Enrollment into strata A, B and C will be completed in approximately 4 years and the primary efficacy endpoint is OS12. We therefore anticipate that we will complete the study in 6 years.

4 REGISTRATION PROCEDURES

4.1 General Guidelines

Participant must meet all inclusion criteria and no exclusion criteria should apply. The participant or their legal parent/guardian must have signed and dated an approved, current version of the applicable consent and/or assent forms. To allow non-English speaking participants to participate in this study, bilingual health services will be provided in the appropriate language when feasible.

The treating physician must complete and sign the eligibility checklist. A clinical team member (nurse or clinical research coordinator) must also sign. The completed eligibility checklist will be submitted to the PNOC Operations Office for review. The PNOC Operations Office will review the eligibility checklist to ensure that all items on the eligibility checklist are filled out.

Eligible participants will be registered using the UCSF OnCore® database. Treatment on protocol therapy cannot be initiated prior to receiving the registration confirmation email from the PNOC Operations Office.

4.2 Reservation and Registration Process

The wait-list for study slots will be maintained by the PNOC Operations Office. Investigators can view updated information about slot availability and registration process updates on the PNOC Member's SharePoint homepage using their secure login and password, or by emailing a request to [redacted]

To place a participant on the waitlist, please complete the Qualtrics survey (link available on SharePoint). An automatic screening ID will be generated, and emailed to both the Operations Office and the person submitting the form. This screening ID will be used for registration and participant tracking purposes.

To register a participant for the study, limited participant information (confirmation of screening ID, gender, ethnicity, race, month & year of birth, ZIP or country code, disease site, histology, diagnosis date, name of treating physician and study specific information) along with a signed consent form and HIPAA authorization (if applicable to your institutional regulatory guidelines) should be emailed to the PNOC Operations Office [redacted]. All participant PHI must be redacted, and the screening ID included on each source document or consent form page. The participant will be given the status of consented in OnCore®.

When the eligibility checklist has been completed, the member institution PI and/or Coordinator will upload the completed eligibility checklist into the participant's OnCore® record.

Once the eligibility checklist has been confirmed as received, the PNOC Operations Office will send a confirmation e-mail to the institutional PI(s) and Research Coordinator(s) with the participant's study ID and dose information.

Detailed participant registration instructions can also be found on the PNOC Member's SharePoint Wiki.

5 Study Drugs

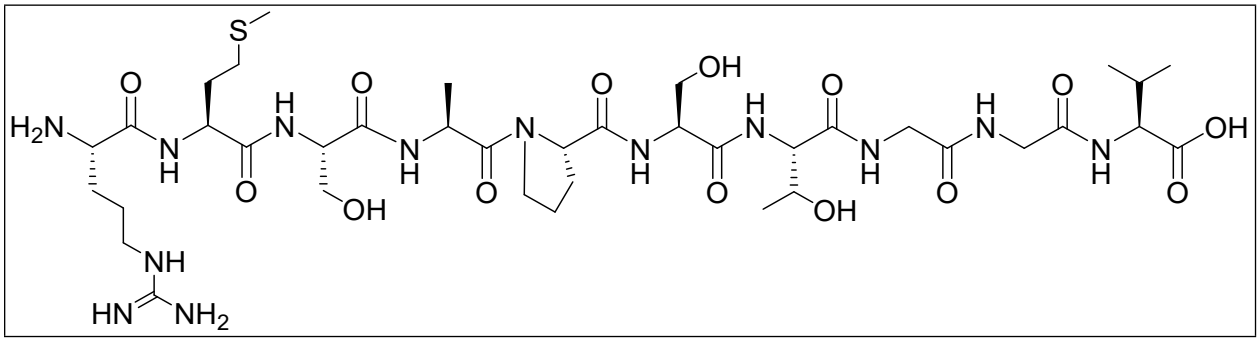
5.1 Description, Supply and Storage of Investigational Drugs

5.1.1 H3.3K27M peptide

The following synthetic H3.3K27M peptide and helper peptides will be included in the vaccine formulation. The combined H3.3K27M and Tetanus Toxoid will be referred to as K27M/TT peptide.

H3.3K27M is a linear, decapeptide containing eight (8) natural amino acids. The product is isolated as its acetate salt form with some residual water as a natural constituent of the drug substance. Figure 2 shows the structure

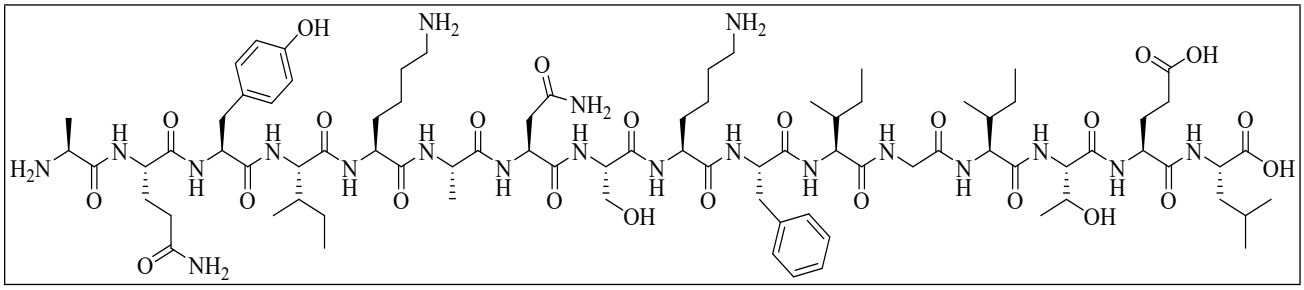
Figure 2: Structure of H3.3K27M



5.1.2 Tetanus Toxoid

Tetanus Toxoid is a linear, hexadecapeptide containing twelve (12) natural amino acids; both the N- and the C-terminus of the sequence are unmodified. The product is isolated as its acetate salt form with some residual water as a natural constituent of the drug substance. Figure 3 shows the structure of Tetanus Toxoid.

Figure 3: Structure of Tetanus Toxoid



For more information about the K27M/TT peptide vaccine, please see the Investigator's Brochure.

5.1.3 K27M/TT Peptide

5.1.3.2 Availability

The H3.3K27M and Tetanus Toxoid peptide will be synthesized under good manufacturing practice (GMP) conditions by Polypeptide Inc. (San Diego, CA). Recombinant vectors in bacteria or viruses will not be used. The synthetic peptide will be purified by HPLC. The identity of the synthetic peptide will be confirmed by verifying their mass and amino acid sequences by mass spectrometry. Each lot of peptide will be evaluated as required by the FDA for identity, purity, sterility and pyrogenicity. An investigator-sponsored IND application has been filed [redacted] incorporating the clinical protocol and chemistry, manufacturing and control (CMC)

information. Details of the synthesis, certificates of analysis, and technical summaries are included in the Chemistry and Manufacturing section of the IND applications. The bulk peptide has been sent to the University of Pittsburgh Immunologic Monitoring and Cellular Products Laboratory (IMCPL), for vialing and combination with TT helper peptide. Enrolling sites will request the K27M/TT peptide through the PNOC Operations office [redacted] Please refer to the PNOC secure SharePoint Member Website for details on how to request study drug.

5.1.3.3 Agent Ordering and Distribution (K27M/TT Peptide Vaccine)

Final reconstitution with Montanide ISA-51 will occur at each PNOC site's pharmacy after each site has completed training on how to reconstitute K27M/TT with Montanide. The standard operating procedure (SOP) for the final preparation (i.e., reconstitution) has been established to meet the defined specifications of strength, quality and purity as outlined in the CMC section of the IND application. Each site will request Montanide through the PNOC Operations office around the time of enrollment together with the peptide and Poly ICLC (Hiltonol ®). Please refer to the PNOC secure SharePoint Member Website for details on how to request Montanide as well as the SOP for reconstitution.

5.1.3.4 Storage & Stability (Peptides)

The peptides will be vialed under GMP conditions and saved at ≤ -70°C. Stability of lyophilized peptides will be tested annually by mass spectroscopy. Based on data from peptides used in other trials at the University of Pittsburgh, lyophilized peptides are typically stable at least for three years. Lyophilized peptides in unit dosage vials will be reconstituted with saline/10% DMSO, and aliquoted as 1 ml vials as the mixture of the two peptides (750 micrograms/ml K27M peptide and 500 micrograms/ml TT peptide) at the University of Pittsburgh and sent to each PNOC site after a patient has been accrued and enrolled in the trial. The vials will be stored at a temperature ≤ -70°C and protected from light. Vials with reconstituted peptides in saline/10%DMSO expire 6 months from time of reconstitution. Data from Pittsburgh for past peptide-vaccine trials indicate reconstituted peptides are stable for at least 6 months (less than 8% oxidized). For each vaccination, one vial will be thawed and mixed with the same volume of Montanide ISA-51 at the enrolling PNOC site's investigational pharmacy. Once mixed, the vaccine must be administered within 6 hours. The standard operating procedure (SOP) for the final preparation (i.e., reconstitution) has been established to meet the defined specifications of strength, quality and purity as outlined in the CMC section of the IND applications. Please refer to the PNOC secure SharePoint Member Website for details on how to request Montanide as well as the SOP for reconstitution.

5.1.3.5 Other Commercially Available Agents in the Vaccines

Montanide ISA-51 will be purchased from SEPPIC Inc. (www.seppic.com) and stored at UPMC IMCPL. Each enrolling site will receive Montanide from UPMC IMCPL once a patient is enrolled. Please refer to the PNOC secure SharePoint Member Website for details on how to request Montanide as well as for the SOP on how to prepare the final peptide emulsion that will be administered to the patient.

5.1.3.6 Toxicities (K27M/TT Vaccine)

Chronic inflammatory reactions are expected to occur in all patients at their vaccine sites. Induration may persist for months, but is not expected to require additional therapy. Sterile abscesses may occur

in some patients. These are not a basis for discontinuation of the vaccines. However, if the inflammatory reactions are severe, subsequent vaccines may be administered in adjacent skin (< 2 cm from prior injection site) rather than in the same location(s), to minimize additional morbidity. In the event the vaccine cannot be administered within 2 cm of the designated site(s), vaccination site(s) will be assigned on the corresponding location of the contralateral side. Poly-ICLC will be administered intramuscularly on the same side as the vaccine on the same day the vaccine is administered. Complete and updated adverse event information is available in the Investigator's Brochure.

5.1.4 Poly-ICLC

Poly-ICLC is classified as an investigational drug. It is a synthetic complex of polyinosinic and polycytidylic acid, stabilized with polylysine and carboxymethyl cellulose. The thermal denaturation point is 89.5°C, about 40°C above that of plain Poly-ICLC; the resistance to hydrolysis is several times that of the parent compound, and it induces peak levels of about 1000-2000 IU of interferon-□□per mL of serum in monkeys given 1 mg/kg intravenously.

5.1.4.2 Availability (Poly-ICLC)

Poly-ICLC is prepared and packaged in the GMP facility of Bioserv, Corp. It is then tested for activity and pyrogenicity by Oncovir, Inc. Each site will request poly-ICLC from UPMC-IMCPL through the PNOC Operations office after a patient has been enrolled into the trial. To order poly-ICLC please refer to the secure PNOC SharePoint member website.

5.1.4.3 Storage & Stability (Poly-ICLC)

Poly-ICLC is supplied in vials containing 1mL of translucent solution with a concentration of 1.8 mg/mL. It is stable at room temperature for several days, but is better stored refrigerated at about 40°F (not frozen).

5.1.4.4 Toxicities of Poly-ICLC

Flu-like symptoms:

The main toxicity of poly-ICLC is a flu-like reaction including fever, myalgias, arthralgias, malaise, and possibly nausea and vomiting. The severity is dependent on dose, route of injection and overall health of the participant. Early Phase I studies were done to determine the maximum tolerated dose (MTD) under the assumption that this was also the most effective dose. In these studies of cancer patients, it was found that the MTD was about 12 mg/m² IV in patients who were not terminally ill. Patients typically showed fevers of 40°C, myalgias, arthralgias, malaise, and nausea and vomiting. Fever was the primary dose-limiting factor. At this dose, the mean serum IFN level was 2000 IU/mL. While giving exogenous IFN rarely attains this level, levels of 100 IU/mL in response to exogenous IFN are associated with the same types and degree of adverse effects as seen with high dose poly-ICLC. In most of the early cancer trials, about 6 mg/m² poly-ICLC IV was generally used.

It was subsequently shown that low doses (i.e., low dose poly-ICLC) were superior to higher ones for enhancing immune effects, and that the higher dose actually inhibited a number of cell-associated immune functions. It was also found that intramuscular injection produced milder side effects than IV

administration [33]. The most common symptom has been mild, transient discomfort at the injection site. On occasion, 8 to 12 hours after doses of 10 to 50 micrograms/kg IM, patients may also develop a mild flu-like syndrome with fever of less than 38ºC, which may last for another 12 hours, but responds readily to acetaminophen or aspirin. Mild myalgias, arthralgias, sometimes nausea, and malaise are present during this period of time. This flu-like syndrome typically diminishes markedly after the first few poly-ICLC treatments.

Hematologic:

Poly-ICLC has been associated with a coagulopathy in dogs, but not in other species (including primates); and there has been no change in the expected incidence of deep venous thrombosis, pulmonary embolus, or coagulopathy in multiple sclerosis, AIDS or malignant glioma patients on low dose IM poly-ICLC. One paralyzed multiple sclerosis participant treated with 100 micrograms/kg suffered a fatal pulmonary embolus which was not confirmed to be related to the drug-administration. Transient leucopenia, lymphopenia, or granulocytopenia has been reported in malignant glioma patients on poly-ICLC. In the completed North American Brain Tumor Consortium trials (n=76 patients), in which patients received IM poly-ICLC three times/week (20 micrograms/kg), of 786 events reported, there were 23 grade 1 or 2 granulocytopenias, 32 grade 1 or 2 leukopenias, and 17 grade 1 or 2 lymphopenias. Three patients had grade 3 leukopenia. Animal studies demonstrated that a major portion of IFN-induced leucopenia is related to transient sequestering of WBC in lymphoid organs.[30]

Hepatic enzyme elevation:

Mild (grade 1), transient (<7 days) hepatic enzyme elevations were described in a trial of 100 micrograms/kg poly-ICLC given intravenously in multiple sclerosis patients. In three patients this was prolonged >7 days, but in all patients the enzymes returned to normal after temporary discontinuation of the poly-ICLC.

Seizures:

Three glioma patients with epilepsy had seizures during the febrile episode after poly-ICLC, but recovered uneventfully. Whether this reaction represented inadvertent IV injection on those occasions is uncertain.

Pseudo-Tumor Progression:

In the malignant glioma pilot trial in adults[27] a few patients showed increased contrast enhancement with or without increased edema 2-4 months after the initiation of poly-ICLC administration, followed by an apparent tumor response at 6-12 months. Dexamethasone was used if clinically indicated during the time of pseudo-tumor progression. In a more recent NABTC study, in adult patients with advanced recurrent gliomas, several patients have shown a similar phenomenon after several weeks of poly-ICLC therapy, which resolved with or without concomitant steroids (patients continued their poly-ICLC administration during this time). Biopsy of a tumor demonstrating increased contrast enhancement in

one patient receiving dendritic cell-based vaccines showed inflammation rather than recurrent tumor[27]. These findings raise the possibility that vaccines and poly-ICLC may at times be facilitating a relatively early immunologic anti-tumor response, manifested by increased edema and/or contrast enhancement on MRI. Once treatment is started, patients will be closely monitored with serial MRI scans and neurologic evaluations to assess for treatment response as well as pseudo-tumor progression.

To minimize the likelihood that the timing of radiotherapy-induced swelling or increased enhancement may coincide with the pseudo-tumor progression caused by poly-ICLC, we will defer the initiation of poly-ICLC/vaccine for up to 12 weeks post-RT in strata A, B and C in those patients that experience clinically symptomatic radiographic worsening (i.e. increased enhancement/mass effect on MRI scan) immediately following irradiation, to allow this response to resolve.

Experience in pediatric patients with glioma:

As reviewed in Section 1.2, a multi-institutional trial of poly-ICLC in pediatric patients with brain tumors enrolled a total 46 patients. An improved and prolonged response was seen in one patient in whom the poly-ICLC dose was increased from 20 micrograms/kg to 30 micrograms/kg given twice a week IM, so that a dose of 30 micrograms/kg was used in subsequent patients and well tolerated. There were 16 incidents of Grade 3 or 4 reported adverse events but most of them were unlikely to be related to the therapy. There were 4 cases of Grade 3 hepatic toxicity and one case of Grade 3 fever, which were considered to be possibly or definitely related to the therapy, respectively. A case with Grade 3 and another case with Grade 4 neurological toxicity were noted but both cases manifested clinical and radiological signs of tumor progression (personal communication Oncovir).

In the pediatric trial using GAA specific peptides, poly-ICLC (Hiltonol, Oncovir, Washington DC) was also used at a dose of 30micrograms/kg and this dose was well tolerated. Based on these observations, we will also use poly ICLC at 30micrograms/kg in the current protocol[14].

5.1.5 Summary of Common AEs

The most common AEs from the combination of K27M/TT, Montanide and poly-ICLC (≥ 25% of patients) include injection site reaction, white blood cell decreased, vomiting, fatigue, alanine aminotransferase increased, aspartate aminotransferase increased, lymphocyte decreased, headache and skin induration.

Update: As of 12/31/18, treatment with the K27M/TT vaccine and poly-ICLC has been proven to be well tolerated with one RLT in the total 29patients treated on strata A and B, 19 and 10 patients in each stratum, respectively.

5.1.6 Nivolumab

Structure and molecular weight

Nivolumab is a monoclonal antibody consisting of 4 polypeptide chains, which include 2 identical heavy chains consisting of 440 amino acids and 2 identical light chains. Molecular weight is 146,221 Daltons.

Supplier

Nivolumab will be supplied by Bristol-Myers Squibb (BMS). Do NOT use commercially available supply.

Formulation

The agent is a clear to opalescent, colorless to pale yellow liquid, with light (few) particulates. It is available in a 100 mg/10 mL vial containing a sterile, non- pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/mL in sodium citrate, sodium chloride, mannitol, diethylenetriamine pentaacetic acid (pentetic acid) and polysorbate 80 (Tween® 80) to a pH of 6.0. A small amount of overfill (0.7 mL) is included with each vial to account for VNS (vial, needle, syringe) loss. The 10 mL type I flint glass vials are stoppered with butyl rubber stoppers and sealed with aluminum seals.

Storage

Nivolumab vials for injection must be stored at 2°– 8°C (36°– 46°F) and protected from light, freezing and shaking.

Solution Preparation

Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to a final concentration of 1 – 10 mg/mL. Vial contents from different lots should not be mixed in the same infusion.

Stability

The administration of undiluted and diluted nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution of nivolumab injection prepared for dosing may be stored for up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 8 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and under room light. The maximum 8-hour period under room temperature and room light conditions for nivolumab injection in IV bag includes the product administration period (30 minutes). Vials of nivolumab for injection do not contain preservatives or bacteriostatic agents and should be prepared as soon as possible prior to administration using aseptic technique.

5.2 Drug Accountability

Each PNOC site Investigational Pharmacist will manage drug accountability records per site standards.

5.3 Drug Ordering

The K27M/TT peptide vaccine will be ordered from UPMC -IMCPL through the PNOC Operations office. Please refer to the PNOC SharePoint Member Website for detailed order instructions and an order request form.

The poly-ICLC will be ordered by each PNOC site directly through PNOC Operation's Office and shipped from UPMC -IMCPL- please refer to the PNOC SharePoint Member Website for detailed order instructions and an order request form.

Each investigational pharmacy will order Montanide (Seppic, Inc.) from UPMC -IMCPL through the PNOC Operation's Office to generate the K27M/TT peptide-based vaccine per SOP just prior to administration of the vaccine. Please refer to the PNOC SharePoint Member Website for detailed order instructions and an order request form.

Nivolumab: Nivolumab will be ordered via manual drug order form. Drug supply will be shipped by BMS vendor Fisher Scientific. More detailed instructions are available via the PNOC SharePoint Member Website.

6 Treatment Plan

Eligible patients will undergo focal radiation therapy after initial diagnosis as part of their standard of care per institutional guidelines. **Stratum A** patients **must** begin radiation therapy within 4 weeks of diagnosis by imaging or pathology, whichever is later. **Stratum B** patients **must** begin radiation therapy within 4-6 weeks from surgery (for those subjects undergoing surgery for more extensive resection). **Stratum C:** Patients with DIPG **must** begin radiation therapy within 4 weeks of diagnosis by imaging or pathology, whichever is later. Patients with other midline gliomas, excluding primary spinal cord tumors, **must** begin radiation therapy within 4-6 weeks from surgery (for those subjects undergoing surgery for more extensive resection).

Treatment should start 2-8 weeks after completion of radiation therapy for all strata. Children will receive subcutaneous injections of synthetic peptides of the H3.3.K27M epitope and tetanus toxoid-derived helper epitope (K27M/TT) emulsified in Montanide ISA-51 along with concurrent intramuscular injections of immunoadjuvant poly-ICLC every **3 weeks for a total of 8 doses** (please see **Therapy Schema** for trial design). Stratum C patients will also receive nivolumab.

Strata A & B: If the subject demonstrates stable to improved disease to the vaccine therapy and tolerates the therapy well (defined as not having any regimen related toxicities RLT; see below), patients may receive additional vaccinations every 6 weeks after the 8th dose starting at Week 30 for a total of 96 weeks (approximately 2 years).

Stratum C: On the same day (+/- 3 days) as the vaccination with K27M/TT and poly-ICLC, stratum C patients will receive nivolumab 4.5 mg/kg IV, every 3 weeks, through week 21 of the study procedures. If the subject demonstrates stable to improved disease to the vaccine therapy and nivolumab combination therapy and tolerates the therapy well (defined as not having any regimen related toxicities RLT; see below), stratum C patients may continue to receive nivolumab every 3 weeks after the 8th dose for a total of 96 weeks (approximately 2 years). Patients may receive additional vaccination with K27M/TT and poly-ICLC combined with nivolumab every 6 weeks after the 8th dose and will resume combination therapy, starting at Week 27, for a total of 96 weeks (approximately 2 years).

Because the peptide vaccine is sequestered locally and the immune response occurs primarily locally, the dose of the vaccine does not need to be scaled up proportionately to weight or body surface area; however, nivolumab will be based on the weight measured within 7 days prior to each infusion. With regard to the dose of poly-ICLC we will use a fixed amount of 30 micrograms/kg, which was used previously and demonstrated good tolerability with only grade 1-2 adverse events (injection site reactions, flu like and GI symptoms)[14]. The K27M/TT specific peptide will be emulsified in the adjuvant Montanide ISA-51, which consists of a mineral oil base, which has demonstrated to be well tolerated in previous studies[14].

6.1 Dosage and Administration

6.1.1 Dosage

6.1.1.2 Poly-ICLC

Justification for the proposed dose of poly-ICLC (30 micrograms/kg IM) is provided in Section 1.2 and Section 5.1.4.4 (Experience in pediatric patients with glioma). The first course of poly-ICLC administration (30 micrograms/kg IM) will be administered on the day of the first K27M/TT vaccine, unless the patient has evidence of radiation induced pseudoprogression as outlined in Section 5.1.4.4 (Pseudo-tumor progression). If that is the case, administration of poly-ICLC can be deferred for up to 12 weeks. For each of the following repeated vaccinations, poly-ICLC (30 micrograms/kg IM) will be administered on the day of the vaccine. This design is based on a recent pilot study of peptide-based vaccines in DIPG patients, in which the combination of peptide-vaccine and IM administration of poly-ICLC (30 micrograms/kg) administered on the same day induced robust antigen-specific T-cell responses.[14]

Dose should be verified with each administration as per standard chemotherapy procedures. Dose adjustments should be done if there is a ≥ 10% weight change from the prior dose.

Pretreatment with acetaminophen or with any NSAID should be given before each poly-ICLC dose.

Reported adverse events and potential risks are detailed in Section 5.1.4.4. Appropriate dose modifications for poly-ICLC are described in Section 6.2.1.

6.1.1.3 K27M/TT Peptide Vaccine

Because the peptide vaccine is sequestered locally, and the immune response occurs primarily locally and in the draining lymph nodes, the dose of the vaccine does not need to be scaled up proportionately to the size (by weight or body surface area) of the recipient, as might be done for a drug whose effect is related to its distribution in body fluid. Because direct toxicity of the peptide is not expected, dose escalation is not as meaningful as it would be with a drug with a narrow therapeutic index. The proposed dose is based on past trials, in which up to 1 mg for each of tumor-antigen peptide emulsified in Montanide ISA-51 were safely administered. The 1 ml K27M/TT solution ordered by each site contains 750 micrograms/ml K27M peptide and 500 micrograms/ml TT peptide. Eight hundred (800) microliters (µL) of that aqueous solution, which corresponds to 600 micrograms K27M peptide and 400 micrograms TT will be mixed 1:1 with Montanide ISA-51 to form one water-in-oil emulsion. The injection volume is 800 microliters (µL), which contains a final peptide amount of 300 micrograms and 200 micrograms of the TT. Ideally, the vaccine should be administered within 2 hours after mixing, however it is stable up to 6 hours at room temperature. If the vaccine is not administered within 6 hours after mixing, it should be discarded.

6.1.1.4 Nivolumab

Nivolumab will be given intravenously every 3 weeks at 4.5 mg/kg per dose over 30 minutes. Premedication is not required as infusion reactions are rare, but anaphylactic precautions should be observed during each infusion with nivolumab. If grade ≥ 2 infusion reaction occurs, the infusion should be stopped and supportive care given based on institutional guidelines. Please refer to Section 6.2.3 for dose modifications for nivolumab. The drug dose should be adjusted based on the patient's actual weight in kilograms measured within 7 days prior to the beginning of each infusion.

6.1.2 Treatment Administration

Treatment will be administered on an outpatient basis. Patients will be vaccinated subcutaneously in the upper arm or thigh. Since we mix the K27M peptide and the TT-helper peptide in one emulsion (0.8 mL), there will be one injection for the peptide vaccination and one intramuscular (IM) injection for poly-ICLC. Poly-ICLC is expected to enhance the antigen-presentation process in the draining lymph nodes, and therefore poly-ICLC should be administered intramuscularly (IM) within close vicinity to the peptide-injection site (less than 3 cm from the center of the peptide injection sites).

In each vaccination, the location of vaccination will remain at the same site. Numbing measures such as creams, ice or mechanical devices are not permitted. Per Section 5.1.3.6, if the skin reaction from the previous vaccine does not allow the next administration at the same site, the vaccine may be administered in adjacent skin (< 2 cm from the prior injection site). If the vaccine cannot be administered within 2 cm of the designated site, the vaccine site will be administered on the contralateral side. Poly ICLC will be administered intramuscularly on the same side and on the same day as the K27M/TT vaccine was administered.

The K27M/TT vaccine will be administered every three weeks starting 2-8 weeks following the completion of radiation therapy. Vaccine will be administered every 3 weeks up to and including Week

21 (8 doses). For strata A and B, vaccination will resume on Week 30 and every 6 weeks thereafter. For stratum C, vaccination will resume on Week 27 and every 6 weeks thereafter.

Poly-ICLC is administered IM using sterile technique, as supplied from the vial and in the amount prescribed for the participant's weight (typically, one vial is sufficient for one dose). The poly-ICLC treatments will be administered on the same day as the vaccine, sequentially after K27M/TT administration. Vital signs will be monitored before and for at least 20 minutes after each treatment.

Nivolumab should be given intravenously on the same day as the vaccine or within 3 days (+/- 3 days) of vaccination. It can be given before or after the vaccination but should ideally be given on the same day as vaccination with K27M/TT and poly-ICLC.

Nivolumab Injection, 100 mg/10 mL (10 mg/mL) and 40 mg/4 mL (10 mg/mL) nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding in-line filter at the protocol-specified doses. It is not to be administered as an IV push or bolus injection; any programmable infusion pump may be used. Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 0.35 mg/mL. Dilution and infusion of nivolumab should be carried out per institutional standard and as per section 5.1.5. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent. Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets, and glass bottles.

6.2 Dose Modifications and Dosing Delays

The following dose modification rules will be used with respect to potential toxicity. Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0).

6.2.1 Dose Modifications for Poly-ICLC

For grade 2 or greater constitutional symptoms (see Section 5.1.4.4) that are considered related to treatment that persist for greater than 48 hours after the injection, the next poly-ICLC dose should be given at two-thirds of the original dose (20 micrograms/kg). If further dosing is well tolerated, the original dose may be subsequently re-instituted at the discretion of the investigator. All dosage changes will be recorded in Advarra. If grade 2 or greater symptoms again occur despite one dose reduction and last > 48 hours, the patient will not receive any more poly-ICLC but can continue to receive the K27M/TT peptide if patient is stable.

In the case of treatment-related hepatic enzyme elevation > 5x baseline (grade 3 or greater), or any related, intolerable grade 2 or greater non-hematologic toxicity that lasts for $\geq$ 7 days, poly-ICLC will be held until that toxicity has reduced to Grade 1 or less. Poly-ICLC will then be re-administered at two-thirds of the original dose (20 micrograms/kg), and the participant will be closely observed. If the same dose-limiting toxicity again recurs despite the dose reduction, the participant will continue to receive the K27M/TT peptide without poly-ICLC if patient is stable.

For treatment-related grade 3 or greater hematologic toxicity, the next dose should be reduced to two thirds of the original dose (20 micrograms/kg) as long as the toxicity has resolved to grade 1 or less by the time the next dose is due. If the toxicity has not resolved by the time the next dose is due, the patient is off treatment with poly-ICLC but can continue with the K27M/TT peptide vaccination per schedule.

See Section 6.2.4 below for pseudo-tumor progression guidelines (presumed or proven) that require a temporary discontinuation of treatment.

6.2.2 Dosing Delay for the Peptide Vaccines (K27M/TT emulsified in Montanide ISA-51)

Possible adverse events by Montanide ISA-51-based peptide vaccines are summarized in Section 6.1.1.3. The toxicity profile may partially overlap with that of poly-ICLC, though the attribution to either or both of these agents may be apparent if the reactions/events are localized to the administration site or follow specific timings after the administration (e.g. fever after poly-ICLC administration). In circumstances where poly-ICLC administration is suspended, if the event is not attributable to the peptides/Montanide ISA-51 vaccine, vaccine administration should continue on schedule. In circumstances where assessment of an adverse event is limited, such as by intercurrent illness, or when laboratory studies are required to assess for other causes of toxicity, the vaccine schedule may be interrupted for up to 6 weeks. If vaccine administration is delayed by longer than 6 weeks due to an adverse event other than for pseudo-tumor progression, regardless of attribution, treatment must be discontinued.

6.2.3 Dose modification for Nivolumab

See Appendix I for management algorithms.

6.2.4 Pseudo-progression (please refer to the flow diagram in Table 7.1)

If pseudo-tumor progression is suspected, i.e., increased contrast enhancement with or without increased edema of the primary tumor approximately ≤ 6 months following the initiation of protocol treatment, the patient may be placed on dexamethasone or equivalent, and/or the dose increased up to 0.3 mg/kg/day, maximum of 12 mg/day, or started on Avastin or equivalent (10mg/kg every 2 weeks for a total of 3 doses) if clinically symptomatic. The recommendation is not to start steroids/Avastin or equivalent if the patient is clinically stable and to continue peptide vaccination. If there is evidence of clinical deterioration, the peptide vaccination will be discontinued and only restarted if the child is on Decadron less than max 0.1 mg/kg/day; maximum 4mg/day and if patient stabilizes within 8 weeks. If the repeat MRI scan is unchanged or worse, and/or the patient's clinical status has not improved despite the maximum allowed steroid dose, a biopsy (or resection, if clinically indicated) should be performed to differentiate between pseudo- and true tumor progression. If for some reason (e.g., patient refusal or medical/surgical contraindication), a biopsy (or resection) cannot be performed, the patient will be taken off study due to presumed tumor progression. When a biopsy or resection is performed, the histopathological specimen will be carefully examined for evidence of: inflammatory/lymphocytic infiltration (pseudo-tumor progression). If inflammatory/ lymphocytic

infiltration and/or necrosis comprise the majority of the specimen, patients may remain on study and restart treatment following resolution of toxicity to grade 1 or less at the discretion of the study chair. Such patients should restart treatment at two-thirds of the poly-ICLC dose (20 micrograms/kg). If the majority of the resected specimen consists of persistent/recurrent tumor, the patient will be considered to have true tumor progression and will be taken off study.

Patients with suspected pseudoprogression should be discussed with the study chair.

Please refer to Section 9.3.1.4 for details regarding assessment of progressive disease.

Prior to any therapy decision, any cases of suspected tumor progression or pseudo-tumor progression should be reviewed by the study chair/co-chair to determine whether the subject should remain in the trial.

6.3 Regimen Limiting Toxicity (RLT)

Each patient receiving K27M/TT peptide vaccination and poly-ICLC or K27M/TT peptide vaccination and poly-ICLC in combination with nivolumab will be evaluable for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings, and spontaneous reports of adverse events reported to the investigator by patients.

RLT includes any of the following events that occur during the first 8 vaccine doses (the entirety of the time from the 1st vaccine administration through the 8th vaccine administration) and up to 30 days after the 8th vaccine administration and are considered either possibly, probably, or definitely to the vaccine:

- Any grade 2 or greater autoimmune reaction.
- Any grade 3 or greater hematologic or non-hematologic toxicity with the exception of lymphopenia.
- Any life-threatening event.
- Any other unexpected Grade 2 or greater neurological deficit, which is possibly, probably, or definitely related to the vaccine therapy that does not respond within 21 days trial of 0.3mg/kg day dexamethasone or equivalent (max 12mg/day) or Avastin equivalent (10mg/kg every 2 weeks for a maximum of 3 doses)

6.4 Suspected Myocarditis

Given the potential risk of immune-mediated myocarditis (inflammation of the heart) from check point inhibitor, patients will be evaluated by CPK and cardiac troponins from baseline and throughout treatment. Should myocarditis be suspected or as otherwise clinically indicated, treatment should be held and patient evaluated with a cardiology consult and TDI-derived strain echocardiography or cardiovascular magnetic resonance imaging should be considered.

7 Study Procedures and Observations

7.1 Schedule of Procedures and Observations

The study-specific assessments are detailed in this section and outlined in Table 6.1 and Table 6.2. Screening assessments must be performed within 14 days prior to enrollment unless otherwise noted or in the case of H3.3K27M and HLA confirmation. Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

A written, signed, informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A copy of the signed ICF will be given to the subject, and a copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the UCSF Helen Diller Family Comprehensive Cancer Center Clinical Trial Management System (CTMS) that is being used for PNOC related trials. The system is password protected and meets HIPAA requirements.

7.1.1 Eligibility Assessments

The Eligibility procedures and assessments must be completed within 14 days prior to enrollment unless otherwise noted.

Strata A & B:

- Complete medical history, including history of prior treatments and initial and/or other pre-radiotherapy MRIs
- Performance status
- Brain MRI; spine MRI only if clinically indicated
- Concomitant medications
- Complete blood count (CBC) with differential and platelet count
- Blood chemistry assessment, including:
    - Aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, creatinine and albumin
- Females of child-bearing potential will have a serum or urine beta-HCG pregnancy test

Stratum C:

- Complete medical history, including history of prior treatments
- Brain MRI; spine MRI only if clinically indicated
- Performance status
- Pulse oximetry
- Concomitant medications
- Complete blood count (CBC) with differential and platelet count
- Blood chemistry assessment, including:
    - Aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, creatinine, lipase, amylase, albumin, and C-reactive protein (CRP)

- TSH (if TSH is abnormal also measure free T4)
- Urine analysis (UA)
- Females of child-bearing potential will have a serum or urine beta-HCG pregnancy test.

7.1.2 Treatment Period

7.1.2.2 Study Procedures: After enrollment and prior to vaccination

- PNOC Health Related Quality of Life & Neurocognitive Measures (+7 days after registration; see Appendix H) – for Stratum C only.
- Brain MRI; spine MRI only if clinically indicated – within 14 days of treatment start.

7.1.2.3 Study Procedures Day 1 of Week 0 (all strata, unless otherwise indicated)

Laboratory assessments do not need to be repeated if completed within 7 days prior.

Prior to Vaccination:

- Baseline toxicity assessment including assessment of any residual toxicity relating to prior radiation therapy.
- Physical examination including neurological examination
- Vital signs
- Pulse oximetry (Stratum C only)
- Performance status
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including:
    - ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, albumin, glucose, potassium, sodium, chloride, bicarbonate
    - Lipase, amylase, and CRP (Stratum C only)
    - CPK and cardiac troponins (Stratum C only)
- TSH (if TSH is abnormal also measure free T4) (Stratum C only)
- Urine analysis (UA) (Stratum C only)
- Correlative immune monitoring assessments
- Circulating tumor DNA
- Serum or urine pregnancy test for females of childbearing age (**within 24 hours prior** to the initial administration of study drug)

Vaccination:

- Vaccination with K27M/TT

- IM injection of poly-ICLC (if evidence of pseudo-progression after radiation therapy on Eligibility MRI, poly-ICLC can be held for up to 12 weeks)

Post Vaccination:

- All patients will be closely observed for adverse events for at least 20 minutes following each vaccination. Any time thereafter, patients should report any adverse events to the research coordinator or research clinician.

Nivolumab (**+/- 3 days;** Stratum C only)**:**

- Infusion of nivolumab on the same day as K27M/TT vaccination and poly-ICLC injection or within 3 days (+/- 3 days) of vaccination. Infusion can occur before or after vaccination.

7.1.2.4 Study Procedures Day 1 of Weeks 3, 6, 9, 12, 15, 18, 21 (- 3 days) **(all strata, unless otherwise indicated)**

Laboratory assessments do not need to be repeated if completed within 7 days prior.

Prior to vaccination:

- Physical examination including neurological examination
- Vital signs
- Pulse oximetry (Stratum C only)
- Performance status
- Evaluation of adverse events
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including:
    - Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, glucose, potassium, sodium, and chloride, bicarbonate
    - Lipase, amylase, and CRP (Stratum C only)
    - CPK and cardiac troponins (Stratum C only)
- TSH (if TSH is abnormal also measure free T4) (Stratum C only)
- UA (Stratum C only)
- Serum or urine pregnancy test for females of childbearing age
- ECHO and ECG, as clinically indicated (Stratum C only)

Vaccination:

- Vaccination with K27M/TT
- IM injection of poly-ICLC

Post Vaccination:

- All patients will be closely observed for adverse events for at least 20 minutes following each vaccination. Any time thereafter, patients should report any adverse events to the research coordinator or research clinician.

Nivolumab (**+/- 3 days**; Stratum C only):

Infusion of nivolumab on the same day as K27M/TT vaccination and poly-ICLC injection or within 3 days (+/- 3 days) of vaccination. Infusion can occur before or after vaccination.

7.1.2.5 Study Procedures Day 1 of Week 24 (- 3 days)

Laboratory assessments do not need to be repeated if completed within 7 days prior.

- Physical examination including neurological examination
- Vital signs
- Pulse oximetry (Stratum C only)
- Performance status
- Evaluation of adverse events
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including:
    - Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, glucose, potassium, sodium, chloride, and bicarbonate
    - Lipase, amylase, and CRP (Stratum C only)
    - CPK and cardiac troponins (Stratum C only)
- TSH (if TSH is abnormal also measure free T4) (Stratum C only)
- UA (Stratum C only)
- Serum or urine pregnancy test for females of childbearing age
- Echo/ECG, as clinically indicated (Stratum C only)

Nivolumab (**+/- 3 days**; Stratum C only)

7.1.2.6 Study Procedures Day 1 of Week 27, 30, 33, 36, 39 etc. through Week 96 (- 3 days)

Laboratory assessments do not need to be repeated if completed within 7 days prior.

Stratum C only

- Physical examination including neurological examination
- Vital signs

- Pulse oximetry
- Performance status
- Evaluation of adverse events
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including:
    - Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, glucose
    - Lipase, amylase, and CRP
    - CPK and cardiac troponins
- TSH (if TSH is abnormal also measure free T4)
- UA
- Serum or urine pregnancy test for females of childbearing age
- Echo/ECG, as clinically indicated (Stratum C only)

Nivolumab (**+/- 3 days**; Stratum C only)

Vaccination (every 6 weeks, weeks 27, 33, 39, 45, 51, 57, 63, etc. through Week 96:

- Vaccination with K27M/TT
- IM injection of poly-ICLC

Post Vaccination:

- All patients will be closely observed for adverse events for at least 20 minutes following each vaccination. Any time thereafter, patients should report any adverse events to the research coordinator or research clinician.

7.1.2.7 Study Procedures Day 1 of Weeks 12, 24, 36, 48, 60, 72, 84, 96 (- 7 days) (All strata)

Laboratory assessments do not need to be repeated if completed within 7 days prior.

- Brain MRI (-14 days)
- Spine MRI if clinically indicated (-14 days)
- Circulating tumor DNA
- Immuno-monitoring assessments (also to be collected at Week 18)

7.1.3 Every 3 months (+/- 14 days)

- PNOC Health Related Quality of Life & Neurocognitive Measures (PEDs-QL, PROMIS, and ADHD; see Appendix H) – Stratum C only.

7.1.4 Every 6 months for two years then every 12 months after two years, while on treatment (+/- 14 days)

- PNOC Health Related Quality of Life & Neurocognitive Measures (ABAS-3 and BRIEF; see Appendix H) – Stratum C only.

7.1.5 End-of-Treatment Study Procedures (-14 days) (all strata, unless otherwise indicated)

The following assessments should be completed on the last day of treatment if not assessed within 14 days prior.

- Evaluation of clinical response or deterioration (including MRI, if MRI not done within 30 days prior)
- Physical examination including neurological examination
- Vital signs
- Pulse oximetry (Stratum C)
- Performance Status
- Evaluation of adverse events; all Adverse Events (AEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing, whether related or not related to study drug will be recorded.
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including:
    - Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, glucose, potassium, sodium, chloride, and bicarbonate
    - Lipase, amylase, and CRP (Stratum C only)
    - CPK and cardiac troponins (Stratum C only)
- TSH (if TSH is abnormal also measure free T4) (Stratum C only)
- UA (Stratum C only)
- Serum or urine pregnancy test for females of childbearing age
- ECHO/ECG, as clinically indicated (Stratum C only)
- PNOC Health Related Quality of Life & Neurocognitive Measures (see Appendix H) (Stratum C only)

Vaccination (if last day of treatment):

- Vaccination with K27M/TT
- IM injection of poly-ICLC

Post Vaccination:

- All patients will be closely observed for adverse events for at least 20 minutes following each vaccination. Any time thereafter, patients should report any adverse events to the research coordinator or research clinician.

Nivolumab (**+/- 3 days;** Stratum C only)**:**

- Infusion of nivolumab on the same day as K27M/TT vaccination and poly-ICLC injection or within 3 days (+/- 3 days) of vaccination. Infusion can occur before or after vaccination.

7.1.6 30 Day Toxicity Check

To be completed 30 days (+7 days) after last day of treatment; this may be done by telephone contact.

- Evaluation of adverse events (All study drug related adverse events must continue to be followed until resolution or return to baseline).
- Review of concomitant medications

7.1.7 Every 12 months during Follow-up (+/- 14 days)

- PNOC Health Related Quality of Life & Neurocognitive Measures (PEDs-QL, PROMIS, ADHD, ABAS-3 and BRIEF; see Appendix H*)* – Stratum C only.

7.1.8 Long Term/Survival Follow-up Procedures

Subjects in the follow up phase will be followed by chart review and/or telephone contact until an off-study criterion is met to collect survival information (including disease status and any new treatment started) every three months (+/- 2 weeks) for 24 months. Any study drug related adverse events will be followed until resolution or return to baseline. PNOC Health Related Quality of Life & Neurocognitive Measures will also be collected according to the schedule delineated in Appendix H for patients enrolled in Stratum C only.

The requested follow-up data is to be submitted quarterly from the date the patient went off treatment. This information will be recorded in Advarra® eCRFs due at Follow-up.

7.2 Off Study Criteria

Subjects will be considered Off Study for the following reasons:

- Subject determined to be ineligible.
- Parent, subject, or guardian withdraws consent for continued participation.
- Subject death while on study.

Subjects who expire without confirmation of disease status will be considered progressive disease at the time of death.

The date and reason for the subject coming off study must be documented in the ‘Follow-Up’ section of OnCore® as well as the ‘PNOC End of Treatment eCRF’ in Advarra®. No data will be collected after the “off study” date.

Table 6.1 Schedule of Study Procedures and Assessments (Strata A and B)

Study Day/Visit	Eligibility (-14 days)[1]	Day 1 on Weeks 0, 3, 6, 9, 12, 15, 18, 21, 24 (-3 days)	Day 1 on Weeks 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96 (-3 days)[2]	End of Treatment Visit (-14 days)[3]	30 Day Toxicity Check (+7 days)[4]	Follow-Up[5]
Clinical procedures						
HLA typing	X[6]					
H3.3K27M mutation status	X[6]					
Consent Form	X[1]					
Physical Exam[7]		X	X	X		
Vital Signs		X	X	X		
Medical History[8]	X					
Performance Status	X	X	X	X		
Adverse Event assessment		X[9]	X	X	X	X
Concomitant Medications	X	X	X	X	X	
Vaccination[10] • Vaccine • Poly-ICLC[11]		X				
Evaluation of clinical response				X		
Survival[5]						X
Laboratory procedures						
CBC w/ Diff + plts	X	X[12]	X[12]	X[12]		
Blood Chemistry	X[13]	X[12,13]	X[12,13]	X[12,13]		
Pregnancy test[14]	X	X	X	X		
Imaging procedures						
Brain MRI/Spine MRI	X[15]	X[15]	X[15]	X[4,15]		
Specimen Collection						
Immune monitoring tests[16]		X	X			
Blood for ctDNA [17]		X	X			
Tumor Tissue Collection[18]			X			

1. Eligibility tests and procedures must be performed within 14 days prior to enrollment. Consent to study therapy must be signed.
2. If the subject demonstrates no evidence of disease progression and tolerates the therapy well (defined as not having any regimen related toxicities (RLT); see Section 6.3) after the 8th dose (Week 21), patients may receive additional vaccinations starting at Week 30, every 6 weeks, for a total of 96 weeks (approximately 2 years).
3. End of treatment procedures to occur on last day of treatment. These do not need to be repeated if occurred within 14 days prior. Brain/Spine MRI to be repeated if not done within 30 days prior to the end of treatment visit.
4. 30-day toxicity check may be done via telephone if needed (+7 days). Related AEs must continue to be followed until resolution or return to baseline
5. Subjects in the follow up phase will be followed by chart review and/or telephone contact until an off- study criterion is met to collect survival information every three months for 24 months.
6. Subjects must be positive for HLA-A2 and H3.3K27M. HLA A*02:01 typing (based on sequencing) and H3.3K27M mutation status must be done in a CLIA- or equivalent approved laboratory. If H3.3K27M mutation analysis is to be performed at UCSF, pathology and imaging (MRI), and tissue must be submitted.
7. To include neurological exam.
8. To include history of prior treatments and any residual toxicity relating to prior radiation therapy and baseline conditions assessment.
9. Adverse assessments to be assessed at all visits following Week 0 vaccine administration.
10. All patients will be closely observed for adverse events for at least 20 minutes following each vaccination. Vaccine will not be administered on Week 24 or Week 27. Vaccination will resume on Week 30 and every 6 weeks thereafter, for a total of 96 weeks (approximately 2 years).
11. If there is evidence of pseudo-progression after radiation therapy on baseline MRI, poly-ICLC can be held for up to 12 weeks.
12. Laboratory assessments do not need to be repeated if completed within 7 days prior.
13. Blood chemistry to include: At screening: aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, creatinine and albumin. Week 0 and beyond: alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, total protein, albumin, glucose, potassium, sodium, chloride, bicarbonate.
14. May be serum or urine pregnancy test; for females of child-bearing potential.
15. Spinal MRI only required if clinically indicated. Post radiation therapy MRI must occur within 14 days of enrollment and within 14 days of treatment start date (Day 1 Week 0). MRI(s) to be obtained at baseline and then every 12 weeks thereafter (-14 days) – at Weeks 12, 24, 36, 48, etc.
16. To be obtained at weeks 0 (prior to vaccine), 12, 18, and 24 and then every 12 weeks thereafter each time a MRI is performed (-7 days) (i.e. Week 36, 48, 60, etc.). See Section 9 for further details.
17. To be obtained at baseline (Week 0 Day 1) and then every 12 weeks (-7 days).
18. If a patient has evidence of progressive disease and is undergoing tumor tissue resection as part of standard of care, a sample should be sent to UCSF for further analysis to assess antigen loss and immune cell infiltration. Please see Section 9.2.2 for further details. Please refer to PNOC SharePoint member website for shipping information.

Table 6.2 Schedule of Study Procedures and Assessments (Stratum C)

Study Day/Visit	Eligibility (-14 days)[1]	Day 1 on Weeks 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 33, 39, 45, 51, 57, 63, 69, 75, 81, 87, 93, and 96 **(-3 days)[2]**	End of Treatment Visit (-14 days)[3]	30 Day Toxicity Check (+7 days)[4]	Follow-Up[5]
Clinical procedures					
HLA typing	X[6]				
H3.3K27M mutation status	X[6]				
Consent Form	X				
Physical Exam[7]		X	X		
Vital Signs		X	X		
Pulse oximetry	X	X	X		
Medical History[8]	X				
Performance Status	X	X	X		
Adverse Event assessment[9]	X	X	X	X	X
Concomitant Medications	X	X	X	X	
Drug administration • Vaccine[10] • Poly-ICLC[11] • Nivolumab[20] *(every 3 weeks through 96 week study)*		X	X		
Evaluation of clinical response			X		
Survival[5]					X
Laboratory procedures					
CBC w/ Diff + plts	X	X[12]	X[12]		
Blood Chemistry	X[13]	X[12,13]	X[12,13]		
Pregnancy test[14]	X	X	X		
Urinalysis	X	X	X		
CPK & cardiac troponins		X	X		
Imaging/cardiology procedures					
ECG[15]		X	X		

ECHO[15]		X	X		
Brain MRI/Spine MRI	X[16]	X[16]			
Quality of Life Assessments					
QOL Assessments(see Appendix H for specific timepoints)	X	X	X		X
Specimen Collection					
Immune monitoring tests[17]		X			
Blood for ctDNA [18]		X			
Tumor Tissue Collection[19]	X				

1. Eligibility tests and procedures must be performed within 14 days prior to enrollment. Consent to study therapy must be signed. H3.3K27M sequencing and HLA typing may be done prior to 14 days within enrollment.
2. If the subject demonstrates no evidence of disease progression and tolerates the therapy well (defined as not having any regimen related toxicities (RLT); see Section 6.3) after the 8th dose (Week 21), patients may continue to receive nivolumab every 3 weeks after the 8th dose for a total of 96 weeks. Patients may receive additional vaccination with K27M/TT and poly-ICLC combined with nivolumab every 6 weeks after the 8th dose (starting at Week 27) for a total of 96 weeks.
3. End of treatment procedures to occur on last day of treatment. These do not need to be repeated if occurred within 14 days prior. Brain/Spine MRI to be repeated if not done within 30 days prior to the end of treatment visit.
4. 30-day toxicity check may be done via telephone if needed (+7 days). Related AEs must continue to be followed until resolution or return to baseline
5. Subjects in the follow up phase will be followed by chart review and/or telephone contact until an off-study criterion is met to collect survival information every three months for 24 months.
6. Subjects must be positive for HLA-A*02:01 and H3.3K27M. HLA A*02:01 typing (based on sequencing) and H3.3K27M mutation status must be done in a CLIA- or equivalent approved laboratory. If H3.3K27M mutation analysis is to be performed at UCSF, pathology and imaging (MRI), and tissue must be submitted.
7. To include neurological exam.
8. To include history of prior treatments and any residual toxicity relating to prior radiation therapy and baseline conditions assessment.
9. Adverse assessments to be collected following the subject's written consent to participate in the study through 100 days of discontinuation of dosing, whether related or not related to study drug.
10. All patients will be closely observed for adverse events for at least 20 minutes following each vaccination. Vaccine will not be administered on Week 24. Vaccination will resume on Week 27 and every 6 weeks thereafter, for a total of 96 weeks (approximately 2 years).
11. If there is evidence of pseudo-progression after radiation therapy on baseline MRI, poly-ICLC can be held for up to 12 weeks.
12. Laboratory assessments do not need to be repeated if completed within 7 days prior.
13. Blood chemistry to include: **At screening**: aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, creatinine, albumin, lipase, amylase, C-reactive protein (CRP), and TSH. **Week 0 and beyond**: alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, total protein, albumin, glucose, potassium, sodium, chloride, bicarbonate, lipase, amylase. CRP, TSH (if TSH is abnormal also measure free T4), urine analysis (UA).

14. Required within 24 hours prior to the initial administration of nivolumab, then every 3 weeks. May be serum or urine pregnancy test; for females of child-bearing potential.
15. Day 1, each visit starting at Week 3 (excluding Week 24): as clinically indicated. **End of Treatment**: as clinically indicated.
16. Spinal MRI only required if clinically indicated. Post radiation therapy MRI must occur within 14 days of enrollment and within 14 days of treatment start date (Day 1 Week 0). MRI to be obtained at baseline and then every 12 weeks thereafter (-14 days) – at Weeks 12, 24, 36, 48, etc.
17. To be obtained at weeks 0 (prior to vaccine), 12, 18, and 24 and then every 12 weeks thereafter each time a MRI is performed (-7 days) (i.e. Week 36, 48, 60, etc.). See Section 9 for further details.
18. To be obtained at baseline (Week 0 Day 1) and then every 12 weeks (-7 days).
19. If a patient has evidence of progressive disease and is undergoing tumor tissue resection as part of standard of care, a sample should be sent to UCSF for further analysis to assess antigen loss and immune cell infiltration. Please see Section 9.2.2 for further details. Please refer to PNOC SharePoint member website for shipping information.
20. Nivolumab to be given every 3 weeks. Nivolumab will be given IV before or after vaccine and poly-ICLC injection (+/- 3 days of vaccination and poly-ICLC injection).

7.3 Dietary Restrictions

There are no dietary restrictions on this protocol.

7.4 Prohibited Medications

- Interferon therapy (e.g. Intron-A®)
- Anti–cancer directed therapy including chemotherapy – with the exception of Avastin for treatment of pseudo-progression.
- Allergy desensitization injections
- Corticosteroid medications administered parenterally with the exception of corticosteroids treatment for treatment of pseudo-progression or treatment with inhaled, intranasal, ocular, or topical steroids. These cases should be discussed with the study chair.
- Growth factors (e.g. Procrit®, Aranesp®, Neulasta®)
- Interleukins (e.g. Proleukin®)
- Other investigational medications
- Illicit drugs
- Any live / attenuated vaccine (e.g. varicella, zoster, yellow fever, rotavirus, oral polio and measles, mumps, rubella (MMR)) during treatment and until 100 days post last dose (**Stratum C only**).

Medications associated with prolonged QTc should be avoided. Please refer to Appendix G for a list. Because the lists of these agents change frequently, it is important to regularly consult frequently updated medical references. For the most current list of medications, please refer to the following reference:

Woosley, RL and Romero, KA, **www.Crediblemeds.org**, QTdrugs List, Accession Date December 2nd, 2016, AZCERT, Inc. 1822 Innovation Park Dr., Oro Valley, AZ 85755

8 Quality of Life Surveys

Health-related quality of life (HRQOL) is a construct based on the impact of health and illness on an individual's QOL, as assessed by dimensions of physical, psychological, and social health [1]. Several studies have shown that compared to healthy controls or other cancer survivors, survivors of pediatric brain tumors have the lowest HRQOL [2,3]. For example, children with brain tumors under active therapy are frequently viewed as socially isolated and/or often absent from school by their peers [4]. Cosmetic effects of radiation or chemotherapy treatment (e.g. permanent or temporary alopecia) often occur [5], adding to social burdens and contributing to social isolation. Historically though, HRQOL measures have rarely been included as clinical trial endpoints [6-9]. Fortunately, this trend is slowly changing.

Several criteria are considered when evaluating the utility of an HRQOL assessment tool. These include: reliability and validity of the measure in the population for which it is used, the option for use of proxy report, development and age appropriate versions as well as the inclusion of both a generic core (i.e. questions relevant in assessing the HRQOL of any sick child) and disease-specific modules (i.e. questions specific to brain tumor patients), costs of the study, and availability of forms in parents' native language [1,10]. An important note regarding HRQOL measures is that, though the option for parent or proxy reporting is typically necessary, self-report is preferred as parents may view the impact of the disease differently than the child [11]. Additionally, HRQOL measures should not be too generic. For this reason, HRQOL measures should include disease-specific modules to avoid missing clinically significant changes that are disease dependent [7]. This approach might be particularly important in clinical trials where detecting even small changes related to an individual disease or treatment is necessary [12].

There are several cancer-centric assessment tools that satisfy the criteria above [13-20]. The Pediatric Functional Assessment for patients with Brain Cancer (Peds-FACT-Br) is specific to children with brain tumors and English versions are free-of-charge, making this an attractive assessment tool for HRQOL. Unfortunately, there have been limited studies assessing its validity among different age groups [19].

Pediatric brain tumor survivors live with chronic neurocognitive effects. A core set of cognitive processes appears particularly affected in these children including attention, information processing speed, and working memory. With close monitoring of cognitive development, weaknesses can be readily identified so that appropriate interventions and support can be put in place. Within PNOC, we will focus on the validated measures described in Appendix H. See Appendix H for the references contained within this section.

9 Monitoring of Response

The blood draws performed at weeks 0, 12, 18, 24 and every 12 weeks thereafter at subsequent MRIs as part of immune monitoring will be used in the following two analyses.

9.1 Immuno-monitoring

9.2 Enzyme Linked Immuno-SPOT (ELISPOT) Assays

Frequencies of H3.3K27M-responsive T-lymphocyte precursors in peripheral blood mononuclear cells (PBMC) prior to and after administration of the K27M-peptide based vaccine can be measured by ELISPOT assay. The biological responses measured by ELISPOT will be done at the same time point at least for one individual participant to avoid inter-assay variability. Successful vaccination stimulates clonal populations of T cells that are capable of secreting cytokines in an antigen-specific, MHC-restricted fashion. We will utilize the ELISPOT assay to evaluate H3.3K27M-specific immune

responses of $CD8^+$ T-cell populations as well as $CD4^+$ T cells that react against the helper TT peptide. We will evaluate IFN-γ production to assess Type-1 T-cell response. Hence, even though our plans for peripheral blood drawing should provide us with sufficient PBMC to run both assays, if we still encounter a shortage of PBMC, IFN-γ ELISPOT will be prioritized over tetramer assays.

ELISPOT Assay: ELISPOT provides a measure of the induction of tumor-specific $CD8^+$ and $CD4^+$ T-cells by immunization. Blood specimens from the selected subjects will be obtained pre-, during and post-vaccination. ELISPOT assay will be performed to measure the frequency of a population of T cells capable of responding to the tumor specific antigen by secreting IFN-γ.

A subject will be considered to have responded, if at any of post-vaccine time point against the H3.3K27M antigen, the number of spots is double that at baseline, and there are at least 10 spots/20,000 cells, and if the number of the post-vaccine spots is at least three times the standard-deviation of the pre-vaccine value. This definition provides some protection against false positive response.

9.2.1 Tetramer Analysis of K27M-reactive T cells in Participant's PBMC

Tetramer analyses allow us to evaluate the presence of H3.3K27M-specific $CD8^+$ T-cells in peripheral blood with a great sensitivity without *in vitro* re-stimulation of the cells. It is expected, that significant (a log or more) increase in the frequency of peptide-responsive $CD8^+$ T cells will be observed in some, but not all, patients immunized with tumor-antigen based vaccines. In an exploratory manner, these PBMCs will also be evaluated for surface expression of an integrin receptor very late antigen (VLA)-4, which has been implicated to confer T-cell homing to CNS tumors[28] and chemokine receptors (e.g. CXCR3 and CCR5).

$CD8^+$ cells from PBMC will be positively selected on immunobeads (this procedure is established and available at UCSF). The enriched $CD8^+$ cell fraction will be tested by flow cytometry for the percentage of cells expressing CD8 surface antigen.

In addition to CD8, VLA-4 ($\alpha_4\beta_1$ integrins) and LFA-1 expression on tetramer-positive T cells will be examined as markers dictating preferential homing to the brain tumor sites.

Tetramer assays will be done with PBMC samples obtained at baseline and specified time points after vaccinations. We will define a single time-point positive response for a peptide to be (1.5 + B)% of all $CD8^+$ cells positive by tetramer assay, where B is the percent positive at baseline, which is usually less than 0.1%. In analogy to the definition of ELISPOT response, a patient will be considered to have responded if he/she has two consecutive single time-point responses for the K27M peptide. Response rates will be reported as for ELISPOT.

Flow cytometric analyses of lymphocyte subsets

Number of $CD4^+$, $CD8^+$ T cells as well as $CD4^+$/$Foxp3^+$ T regulatory cells at serial time points pre- and post-vaccines will be evaluated. These data will be evaluated in an exploratory manner.

9.2.2 Evaluation of Primary and Recurrent Tumor Tissues

We will evaluate H3.3K27M expression in the patients' available tumor tissues (either pre-vaccine or after progression post-vaccines; or both) by immunohistochemistry (IHC) and reverse transcriptase-polymerase chain reaction (RT-PCR).

If tumors recur following vaccinations, it will be critical to evaluate how tumors escape the effects of vaccines. To this end, we will evaluate the following specific issues as much as the tissue-availability allows:

- **Antigen-loss**: We will assess, using IHC and RT-PCR, whether the recurrent tumors express H3.3K27M.

- **Immune cell infiltration:** One reason tumors may escape a vaccine-induced immune response is through the failure of reactive T cells to infiltrate the tumor. To examine this, whenever freshly resected tumor tissues (not fixed or frozen) or fresh or flash frozen are available, we will isolate tumor infiltrating lymphocytes (TILs) and characterize their numbers, phenotype, and antigen-specificity using HLA-A2 tetramers for H3.3K27M. Using multi-color flow-cytometry, we will also determine the function and viability of tetramer^{+} TILs by staining for perforin/IFN-γ and Annexin-V, respectively. Control tissues will include pre-vaccine tumors (if available) and recurrent tumors from patients not in the vaccine trial. These studies will allow us to evaluate whether vaccine-induced T-cells efficiently traffic to the brain tumor site and maintain their function and viability.

9.3 Evaluation of Efficacy (or Activity)

Immunotherapeutic approaches for children with gliomas are a relatively new era and therefore we have limited experience on how to monitor response. Recently the Immunotherapy Response Assessment in Neuro-Oncology (iRANO) working group has proposed guidelines on how to monitor and assess efficacy in adult brain tumor patients treated with immunotherapy as a development of the iRANO criteria, that were established to better account for the phenomenon of pseudo-progression in adult patients [34,35]. The pediatric neuro-oncology working group (RAPNO) is currently working on their guidelines for response criteria for pediatric neuro-oncology patients[36]. Our limited experience with immunotherapies have shown that early radiological changes could be misinterpreted as progression due to therapy induced inflammation and long-term benefit can still be seen even after initial progression or even after the appearance of new lesions [14,37]. The response criteria are adapted from iRANO.

9.3.1 Antitumor Effect

9.3.1.2 Definitions

Evaluable for toxicity

All patients will be evaluable for toxicity from the time of their first treatment with the study therapy.

Evaluable for objective response

Only those patients who have measurable disease present at baseline, have received at least one dose of the vaccine and Poly-ICLC in combination with nivolumab, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to week 12 at the first scheduled MRI will also be considered evaluable.)

9.3.1.3 Disease Parameters

In order to completely document the assessment of response, the two-dimensional tumor measurements for all target lesions upon which the assessments of tumor response are based should be explicitly noted in the radiology report for the baseline and all subsequent follow-up exams. Reports for the follow-up exams should reiterate the measurements obtained at baseline for each target lesion. Newly occurring lesions should also be enumerated in these reports, and changes in lesions should be described.

Tumor response criteria for this study are to be determined by changes in size using the maximal 2-dimensional cross-sectional tumor measurements, T x W (product of the longest diameter of the tumor [width (W)] and its longest perpendicular diameter [transverse (T)], using either T1 or T2 weighted images (which ever gives the best estimate of tumor size). This will allow comparison with historical studies as outlined in the statistical design section, such as CCG-9941, ACNS0126, ACNS0927 and ADVL1217 which used cross-sectional measurements in their determination of response status.

The following section describes the methodology.

1. For MRI imaging, the longest diameter can be measured from the axial plane or the plane in which the tumor is best seen or measured, provided the same plane is used in follow ups.
2. The longest measurement of the tumor (or width, W) should be determined.
3. The 2 perpendicular measurements should be determined (transverse (T) measurement-perpendicular to the width (W) in the selected plane

Measurable disease

Measurable disease is defined as the lesions (or lesions) that can be accurately measured in at least 2 dimensions by MRI (no less than double the slice thickness).

All tumor measurements will be recorded in millimeters or decimal fractions of centimeters.

Target lesions

All measurable lesions up to a maximum of 5 lesions total representative of all involved areas should be identified as target lesions and recorded and measured at baseline. Target lesions will be selected on the basis of their size (largest) and their suitability for accurate repeated measurements.

Non-measurable disease

Non-measurable disease is all other lesions (or sites of disease), including leptomeningeal disease.

Methods for Evaluation of Measurable Disease

All measurements will be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations will be performed as closely as possible to the beginning of treatment.

The same method of assessment and the same technique will be used to characterize each identified and reported lesion at baseline and during follow-up.

9.3.1.4 Response Criteria

Complete Response (CR)

Disappearance of all target and non-target lesions, determined by two separate observations conducted not less than 4 weeks apart. There can be no appearance of new lesions.

Partial Response (PR)

At least a 50% decrease in the size of target lesions, taking as reference to the baseline MRI. There can be no appearance of new lesions.

Progressive Disease (PD)

At least a 50% increase in the sum of the size of target lesions, taking as reference the smallest target size since the treatment started that is confirmed on a 3 month follow up scan as long as the patient is NOT experiencing significant neurological decline (defined as CTCAE grade 3 or higher). Please also refer to Table 7.1.The confirmatory scan requirement will assure that patients are not prematurely assigned to have PD. Also, the appearance of new lesions might be part of an immune response and if the patient is clinically stable, these should be confirmed on a 3 month follow scan to assess for true progressive disease versus pseudo-progression. This will apply to subjects that demonstrate worsening of the MRI within 6 months of start of therapy. Subjects who develop worsening radiographic findings > 6 months from start of immunotherapy are expected to have a low likelihood of ultimately deriving benefit from the therapy and should be considered PD based on imaging if they have a 50% increase in size of the target lesion or if new lesions appear.

Patients who experience significant clinical decline or those who have radiographic progression on the 3 month follow up scan should be classified as progressive disease and the date of progression should be entered as the first MRI that showed progressive disease.

If the follow up 3 month scan shows stabilization or reduction of tumor size in the setting of stable clinical examination and absence of increased steroid use treatment, the patient will be classified as having pseudo-progression and will continue on study therapy.

If feasible, we recommend obtaining tissue if imaging is concerning for progression, as tissue evaluation remains the gold standard to differentiate between pseudoprogression versus true progression. If pathology mainly consists of recurrent tumor, the subject should be considered to have true tumor progression and be taken off study. If the tissue mainly consists of gliosis and inflammation (consistent with treatment effect) the subject should be classified has having pseudo-progression and should remain on study. Subjects that have tissue available will be centrally reviewed at UCSF.

In cases for which it remains difficult to differentiate between progression versus pseudo- progression, the PI must discuss with the study chair the possibility of continuation of therapy. Images will also be

centrally reviewed at UCSF. Continuation of therapy might be considered if the patient derives clinical benefit with acceptable toxicity after a review by the study chair.

Please refer to outline below to assess PD. This is applicable for imaging findings within the first 6 months since start of immunotherapy.

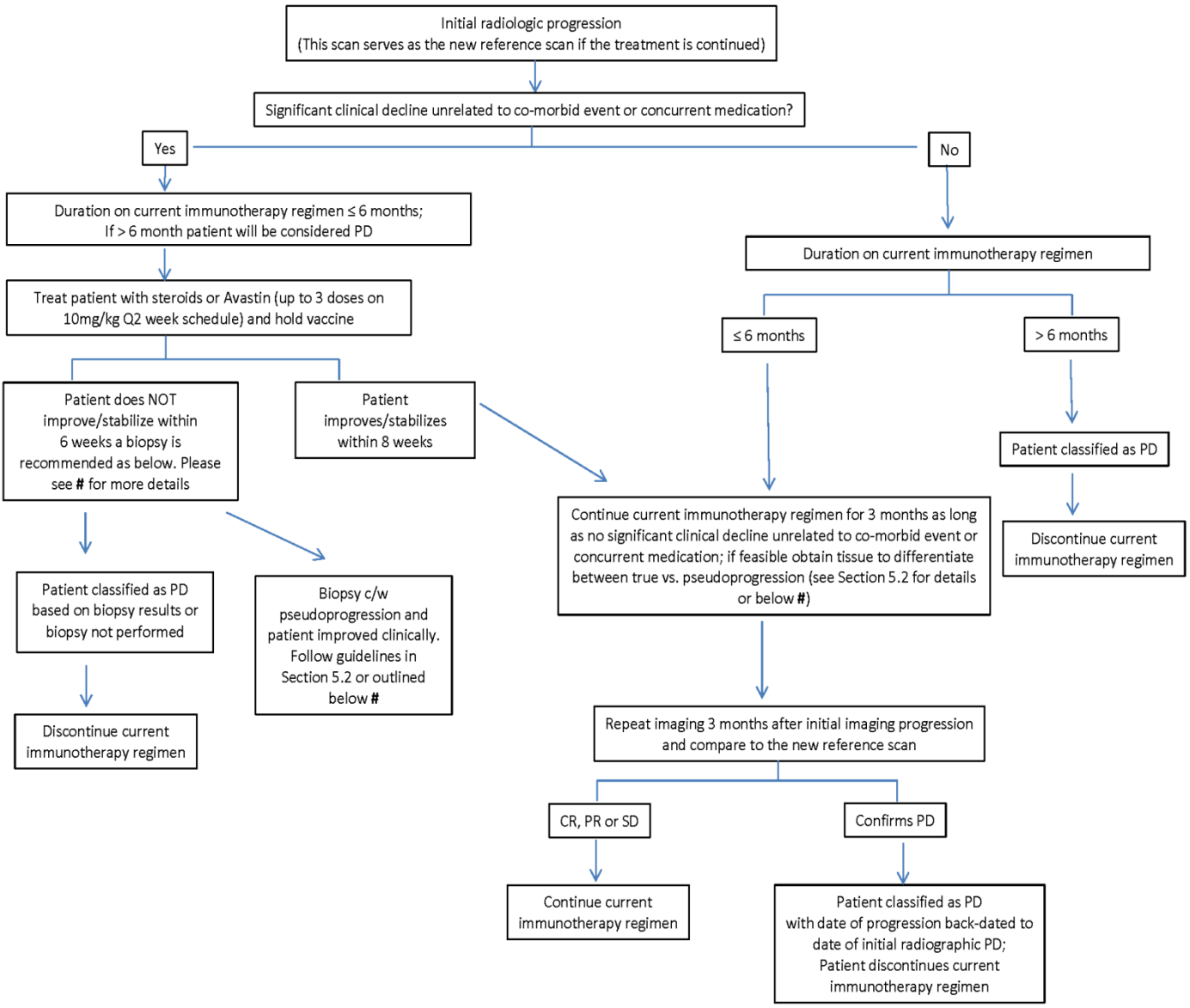
Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest target size since the treatment started.

Evaluation of Best Overall Response

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

Table 7.1 Response Criteria



Biopsy requirements: If the repeat MRI scan is unchanged or worse, and/or the patient's clinical status has not improved despite the maximum allowed steroid dose (refer to Section 5.2.4), a biopsy (or resection, if clinically indicated) should be performed to differentiate between pseudo- and true tumor progression. If for some reason (e.g., patient refusal or medical/surgical contraindication), a biopsy (or resection) cannot be performed, the patient will be taken off study due to presumed tumor progression. When a biopsy or resection is performed, the histopathological specimen will be carefully examined for evidence of: inflammatory/lymphocytic infiltration (pseudo-tumor progression). If inflammatory/ lymphocytic infiltration and/or necrosis comprise the majority of the specimen, patients may remain on study and restart treatment following resolution of toxicity to grade 1 and if steroids were successfully weaned. Such patients MUST be discussed with the study chair and team. Please also refer to Section 5.2.4 under Pseudo-progression.

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 recurrent 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.

Progression-Free Survival

Progression-free survival (PFS) is defined as the duration from diagnosis to time of progression.

Overall Survival

Overall survival (OS) is defined as the duration of time of diagnosis to time of death.

9.3.1.5 Imaging Analyses

At the end of the study, images will be evaluated by central review including assessments of standard MR imaging parameters. Statistical correlations between these imaging parameters and outcome will be performed. Imaging from initial diagnosis, or otherwise prior to radiotherapy, if performed, must be submitted for best comparison and analyses.

9.4 Evaluation of Safety

Analyses will be performed for all patients having received at least one course of study therapy. The study will use the CTCAE v5.0 for reporting of all adverse events.

The Study Chair is responsible for the overall conduct of the study and for monitoring its safety and progress at all participating sites.

9.5 Definitions of Adverse Events

9.5.1 Adverse Event

An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. More specifically, an adverse event (can be any unfavorable and unintended sign e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use

in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

9.5.2 Adverse Reaction

An adverse reaction is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

9.5.2.2 Suspected

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

9.5.2.3 Unexpected

An adverse event or suspected adverse reaction is considered *unexpected* if it is not listed in the investigator brochure or package insert(s), or is not listed at the specificity or severity that has been observed, or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the Investigator Brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered *unexpected* for reporting purposes.

9.5.2.4 Serious

- An adverse event or suspected adverse reaction is considered *serious* if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death

- Life-threatening adverse event
- Inpatient hospitalization ≥ 24 hours or prolongation of existing hospitalization by ≥ 24 hours
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect

Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

9.5.2.5 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 patient 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.

9.6 Recording of an Adverse Event

All clinically significant adverse events will be entered into Advarra®, whether or not the event is believed to be associated with use of the study drug. Data about these events and their severity will be recorded using the NCI CTCAE v5.0. All clinically significant Adverse Events (AEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported, whether related or not related to study drug.

The Investigator will assign attribution of the possible association of the event with use of the investigational drug, and this information will be entered into Advarra® using the classification system listed below:

Relationship	**Attribution**	**Description**
Unrelated to investigational drug/intervention	Unrelated	The AE *is clearly NOT related* to the intervention
Related to investigational drug/intervention	Possible	The AE *may be related* to the intervention
	Probable	The AE *is likely related* to the intervention
	Definite	The AE *is clearly related* to the intervention

,Signs or symptoms reported as adverse events will be graded and recorded by the Investigator according to the CTCAE. When specific adverse events are not listed in the CTCAE they will be

graded by the Investigator as *none*, *mild*, *moderate* or *severe* according to the following grades and definitions:

Grade 0 No AE (or within normal limits)

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Grade 2 Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death related to AE

9.7 Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management until resolved or return to baseline. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. For selected adverse events for which administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

9.8 Adverse Event Monitoring

PNOC uses the web-based Advarra® Clinical Trials Management System for monitoring and recording of Adverse Events (AEs) including all adverse reactions considered “serious” (also called Serious Adverse Events, or SAEs).

All Adverse Events entered into Advarra® will be reviewed on a weekly basis by the PNOC Operations Office. The PNOC Operations Office will discuss the toxicity, grade, and relationship to study intervention for all AEs in question.

In addition, all Serious Adverse Events (SAEs) will be reviewed and monitored by the UCSF DSMC on an ongoing basis, and will be discussed at the UCSF DSMC meeting, which take place every six (6) weeks. SAEs must be entered into the OnCore clinical trial management system in addition to the Advarra EDC for the purpose of the UCSF’s DSMC monitoring. Please see Appendix E PNOC Data Safety and Monitoring Plan for more information.

9.9 SAEs and Expedited Reporting

All Adverse Events which meet the definition of ‘Serious’ as well as other medically significant events described below require expedited reporting to PNOC. Please contact the PNOC Operations Office [redacted] with any questions regarding expedited reporting requirements for this study.

Serious Adverse Events (SAEs)

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

Death

Life-threatening adverse event

In-patient hospitalization ≥ 24 hours or prolongation of existing hospitalization by ≥ 24 hours

A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function

Congenital anomaly/birth defect or cancer

Event that changes the risk/benefit ratio of the study

Important medical events that may not result in death, are life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

All SAEs (see above definition) on any PNOC trial, regardless of relationship, must be reported to PNOC via OnCore, the Advarra EDC system, and Email [redacted] within one business day of first PI awareness, even if the SAE is ongoing. The SAE must be followed until resolution:

a) **Advarra:** All SAEs must be entered into the SAE CRF in the Advarra EDC. The Advarra SAE record should be updated immediately as new information becomes available until the SAE is resolved.

b) **OnCore:** All SAEs must be entered into the Subject Console in OnCore (https://oncore.ucsf.edu/ > Subject Console > SAE Tab on left). The OnCore SAE record should be updated immediately as new information becomes available until the SAE is resolved. (Toxicity segment MUST be completed. Don't forget to click "Add" button.) Please refer to the "PNOC OnCore SAE Entry Guide: Field by Field" found in the PNOC SharePoint Member site documents area for more information.

c) **Email:** Please also email [redacted] with the following information:

In the subject line: "SAE: Patient PNOC ID" (e.g. "SAE: PNOC007-1")

In the body of the email: registration number, weight, study drug dose with frequency and route, dates of use, site PI's attribution, outcome (ongoing, resolved etc.). Please also provide a comprehensive event description (including whether event subsided when treatment was halted, and if re-introduction was attempted and if so, if event recurred), pertinent labs or tests with dates, concomitant medications, and any other relevant history. The lot number or other unique information about the study drug should also be provided. Any information that is not available at the time of the initial notification must be provided as soon as possible on an ongoing basis until the SAE and all queries have been resolved.

Site IRB: Each PNOC site is also responsible for following their own IRB guidelines for reporting SAEs.

All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to PNOC, whether related or not related to study drug.

See additional details for SAE reporting in Section 9.9

SAE Data Entry in AE CRF:

All SAEs must also be entered into the AE CRF for that Cycle (Subject Console > Forms by Status/Forms by Visit). This entry must take place within 10 days of the last day of the Cycle in which the SAE occurred, or as soon as possible in the case of an SAE that was discovered late. Please reference the "PNOC SAE Reporting and Entry" in SharePoint for more information.

SAE Deviations:

If the protocol procedures around SAEs are not followed (e.g. reporting timelines or dose modifications), a Deviation may also need to be entered in OnCore (Subject Console > Deviation Tab on left)/the Advarra EDC. Please reference the "PNOC Deviation Reporting Guidelines" in SharePoint for more information.

Email notification to PNOC operations office [redacted] **within one business day** of first PI awareness.

9.10 PNOC Reporting

9.10.1 Reporting to the UCSF Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study, or within 30 days after the last administration of the study drug(s), and is determined to be related either to the investigational drug or to any research related procedure, the Study Chair and the PNOC Operations Office must be notified by the member institution **within 1 business day**. The Study Chair or the PNOC Operations Office must then notify the UCSF DSMC Chair, or qualified alternate, within 1 business day of this notification. The contact may be by phone or e-mail. Each participating site will follow their institutional reporting guidelines to institutional DSMC.

9.10.2 PNOC Reporting to UCSF Institutional Review Board (IRB)

The PNOC operation office must report events meeting the UCSF IRB definition of "Unanticipated Problem" (UP) **within 10 business days** of awareness of the event.

Each participating site will follow their institutional reporting guidelines to the IRB.

9.10.3 Sponsor-Investigator (PNOC) Reporting to the Food and Drug Administration (FDA)

All SAEs on any PNOC trial, regardless of relationship, must be reported to the PNOC Operations Office via OnCore, the Advarra EDC, and Email [redacted] within one (1) business day of first PI awareness, even if the SAE is ongoing. The SAE must be followed until resolution.

The submitting PNOC site must include as much of the following information as possible in the initial notification email and in the entry: registration number, weight, study drug dose with frequency and route, dates of use, site PI's attribution, outcome (ongoing, resolved etc.). Please also provide a comprehensive event description (including whether event subsided when treatment was halted, and if re-introduction was attempted and if so, if event recurred), pertinent labs or tests with dates, concomitant medications, and any other relevant history. The lot number or other unique information about the study drug should also be provided. Any information that is not available at the time of the initial notification must be provided as soon as possible on an ongoing basis until the SAE and all queries have been resolved.

As this study is being conducted under an IND, the PNOC Operations Office is responsible for determining whether or not the suspected adverse reaction meets the criteria for Expedited IND Safety Reporting in accordance with Federal Regulations (21 CFR §312.32).

The PNOC Operations Office will be responsible for IND Safety Reporting to the FDA for any suspected adverse reaction at any PNOC site that is determined to be serious, at least possibly related to the study drug, and unexpected. The PNOC Operations Office needs to ensure that the event meets all three definitions (as defined below by FDA): **Suspected adverse reaction, Unexpected, and Serious.**

When the PNOC Operations Office receives notification of an SAE, they will alert the Study Chair and Co-Chair as well as the PNOC Lead and Co-Lead (the "study team") within one (1) business day. The Study Chair/Co-Chair and PNOC Lead/Co-Lead will be required to respond regarding the relationship and expectedness of the SAE within one (1) business day of receiving all the information needed to make a determination.

If the majority of the study team decides the adverse event **does not** meet all three of the definitions, the SAE will not be submitted as an Expedited IND Safety Report. However, standard PNOC procedures for reviewing an SAE will still be followed, as below.

If the majority of the study team decides the adverse event **does** meet all three definitions, PNOC Operations Office will submit MedWatch Form 3500A to the FDA within ten (10) business days of the determination for general related, unexpected SAEs, or within three (3) business days for any unexpected fatal or life-threatening suspected SAEs.

Any relevant additional information that pertains to a previously submitted IND Safety Report will be submitted to FDA as a Follow-up IND Safety Report as soon as possible after the information becomes available

9.10.4 Sponsor-Investigator (PNOC) Reporting to Bristol-Myers Squibb (BMS)

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to BMS Worldwide Safety, whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (e.g., a follow-up skin biopsy).
- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.
- If the BMS safety address is not included in the protocol document (e.g., multicenter studies where events are reported centrally), the procedure for safety reporting must be reviewed/approved by the BMS Protocol Manager. Procedures for such reporting must be reviewed and approved by BMS prior to study activation.

An appropriate SAE form (e.g. ex-US = CIOMS form or USA = Medwatch form) should be used to report SAEs to BMS. If you prefer to use your own Institutional form, it must be reviewed by BMS prior to study initiation. Note: Please include the BMS Protocol number on the SAE form or on the cover sheet with the SAE form transmission.

  - The CIOMS form is available at: http://www.cioms.ch/index.php/cioms-form-i
  - The MedWatch form is available at: MedWatch 3500 Form

- For studies with long-term follow-up periods in which safety data are being reported, include the timing of SAE collection.
- The Sponsor will reconcile the clinical database SAE cases (case level only) transmitted to BMS Global Pharmacovigilance ([redacted]). Frequency of reconciliation should be every 3 months and prior to the database lock or final data summary. BMS GPV&E will email, upon request from the Investigator, the GPV&E reconciliation report. Requests for reconciliation should be sent to [redacted]. The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS
- In accordance with local regulations, BMS will notify investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (i.e., not previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse

Reaction (SUSAR). Investigator notification of these events will be in the form of a SUSAR Report.

- Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (e.g., animal) study, important safety recommendations from a study data monitoring committee, or sponsor decision to end or temporarily halt a clinical study for safety reasons.
- Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.
- In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours \ 1 Business Day of becoming aware of the event. SAEs must be recorded on either CIOMS, MedWatch, or approved site SAE form.

Pregnancies must be reported and submitted to BMS on any of the following form(s):

1. MedWatch or, CIOMS or
2. BMS Pregnancy Surveillance Form or,
3. Approved site SAE form

*** *Note*: Reporting requirements will vary by product. Please check with your ISR Lead for applicable reporting timeframe.**

SAE Email Address: [redacted]

SAE Facsimile Number: [redacted]

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours \ 1 Business Day to BMS using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

DEFINITIONS

The protocol must include a definition for Serious Adverse Events (SAE)**.**

SERIOUS ADVERSE EVENTS

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, potential drug-induced liver injury (DILI), and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (e.g., death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

NOTE: (PI determines if this information regarding hospitalizations are considered SAEs and should be included in the protocol. This is supplemental information that is included in BMS-sponsored trials)

The following hospitalizations are not considered SAEs in BMS clinical studies:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

The following hospitalizations are not considered SAEs in BMS clinical studies:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

10 Statistical Considerations and Evaluation of Results

10.1 Study Endpoints

10.1.1 Primary Endpoints

- Stratum A:
  - OS12 in HLA-A2+ children with H3.3K27M positive DIPG
  - Safety and toxicity measurements of repeated administration of the H3.3K27M epitope specific vaccine using CTCAE version 5.0 in HLA-A2+ children with H3.3K27M positive DIPG
- Stratum B:
  - Safety and toxicity measurements of repeated administration with the H3.3K27M epitope specific vaccine using CTCAE version 5.0 in HLA-A2 (02:01)+ children with other H3.3K27M positive midline gliomas, including spinal cord tumors
- Stratum C:
  - Safety and toxicity measurements of repeated administration with the H3.3K27M epitope specific vaccine in combination with nivolumab using CTCAE version 5.0 in HLA-A2 (02:01)+ children with H3.3K27M positive DIPG or other H3.3K27M positive midline gliomas, excluding primary spinal cord tumors (Stratum C)

10.1.2 Exploratory Endpoints

Stratum A:

- Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)+ children with H3.3K27M positive gliomas other than DIPG

- Assessment of H3.3K27M infiltrates in subjects with evidence of progression that undergo tissue collection as part of their standard of care. Tumor tissue will be analyzed for H3.3K27M expression status and infiltration of H3.3K27M specific T cells
- To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance.

Stratum B:

- Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)+ children with H3.3K27M positive gliomas other than DIPG
- Assessment of H3.3K27M infiltrates in subjects with evidence of progression that undergo tissue collection as part of their standard of care. Tumor tissue will be analyzed for H3.3K27M expression status and infiltration of H3.3K27M specific T cells
- To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance.

Stratum C:

- To determine the OS12 in HLA-A2 $(02:01)^{+}$ children with H3.3K27 positive DIPGs and midline gliomas (excluding primary spinal cord tumors) that are treated with repeated administration of the H3.3K27M peptide and nivolumab.
- Induction of the H3.3K27M epitope-specific CTL response in post vaccine PBMCs in HLA-A2 (02:01)+ children with H3.3K27M positive gliomas including DIPG or other H3.3K27M positive midline gliomas, excluding primary spinal cord tumors.
- To archive tumor and normal DNA from each subject at time of initial diagnosis along with serial blood draws following therapy to determine whether circulating tumor DNA (ctDNA) sequences in the subject's blood serve as biomarkers of tumor burden, response to therapy, or development of drug resistance.
- To assess Quality of Life (QoL) and cognitive measures in HLA-A2 $(02:01)^{+}$ children with H3.3K27M positive DIPG or other midline gliomas.

10.1.3 Stratification Factors

This trial will enroll into 3 strata based on disease location and treatment intervention – **Stratum A:** DIPG; **Stratum B:** any other H3.3K27M positive midline glioma, including spinal cord tumors; and **Stratum C:** DIPG and any other H3.3K27M positive midline glioma, excluding primary spinal cord tumors.

10.1.4 Design

We will enroll into the 3 strata separately: Stratum A consists of HLA-A02:01$^+$ positive newly diagnosed patients with H3.3K27M positive DIPG, stratum B consists of other HLA-A02:01$^+$ positive newly diagnosed patients with H3.3K27M positive gliomas that are not classified as DIPG, including spinal cord tumors and stratum C consists of subjects with HLA-A02:01$^+$ H3.3K27M positive DIPG or other HLA-A02:01$^+$ H3.3K27M positive midline gliomas, excluding primary spinal cord tumors. The separation of strata A and B is based on anticipation that toxicity of the vaccine and Poly-ICLC could be significantly different based on the primary tumor location. Toxicities resulting from pseudoprogression or local reaction (such as edema or inflammation) may manifest differently when tumors are located within the brainstem (as in DIPG) versus outside the brainstem, such as the spine or other part of the brain. Treatment related reaction in the brainstem may be more toxic due to a more restricted space and closer proximity to high-risk anatomic structures. The first 3 patients in each stratum will be observed at least for 6 weeks before the next set of patients are enrolled.

- If there are no RLTs in the first 3 subjects, enrollment will commence continuously within each stratum.
- If 2 or 3 RLTs occur in the first 3 subjects, accrual will be suspended and the investigator, PNOC leadership and Sponsor will make decisions regarding continuation of study accrual.
- If a RLT occurs in 1 of the first 3 patients, an additional 3 patients will be enrolled in that stratum and observed for at least 12 weeks. In this case:
    - If fewer than 2 of the first 6 patients in each stratum experience RLT with at least 12 weeks of follow-up, the remaining patients will be enrolled.
    - If a RLT occurs in ≥ 2 of the first 6 patients accrual will be suspended and the investigator, PNOC leadership and Sponsor will make decisions regarding continuation of study accrual.

If enrollment commences, all additional patients will be monitored continuously for treatment-related adverse events by the study PIs Drs. Okada and Mueller. With six patients, there is an 88% probability of detecting 1 or more RLTs with an underlying rate of 30%. There is a 74% probability of detecting 1 or more RLTs with an underlying rate of 20%. If all 19 patients are enrolled in Stratum A, there is an 86% probability of detecting 1 or more RLTs with an underlying rate of 10% and a 62% probability with an underlying rate of 5%. If all 10 patients are enrolled in Strata B, there is a 65% probability of detecting 1 or more RLTs with an underlying rate of 10% and a 40% probability with an underlying rate of 5%. If all 20 patients are enrolled in Strata C, there is an 88% probability of detecting 1 or more RLTs with an underlying rate of 10% and a 64% probability with an underlying rate of 5%.

Enrollment into Stratum C will start once Strata A and B have completed accrual.

10.2 Determination of Sample Size and Accrual Rate

10.2.1 Sample Size and Power Estimate

For the primary endpoint for stratum A, we will assess the efficacy endpoint (OS12) in 19 evaluable patients with H3.3K27M positive DIPG. Prior research has shown overall survival for children with DIPG ranges from 30-40% at 12 months[38,39]. The most recent Children's Oncology Group study that treated children newly diagnosed with DIPG with a combination of radiation therapy and temozolomide resulted

in an OS12 rate of 40% (SD ± 6.5%)[1]. Review of outcomes of DIPG patients on PNOC studies of children and young adults with newly diagnosed DIPG who received standard of care radiation therapy revealed an OS12 rate of 50% (PNOC003 - NCT02274987 and PNOC007 - NCT02960230, unpublished data). For PNOC003 patients were enrolled prior to the start of radiation therapy - similar to the COG study- and none of the enrolled patients came off study due to progression during or at completion of radiation therapy making this a reasonable control group for this study.

With a null hypothesis that OS12 is 40%, a sample size of 19 patients achieves 80% power to detect a difference of 30% using a one sided exact binominal test[40]. The target significance level is 5% and the actual significance level is 3.5% with this test. If 12 or more patients are alive at 12 months, the null hypothesis that OS12 is 40% will be rejected. Patients lost to follow-up before 12 months will be considered events. OS12 will be estimated from the Kaplan-Meier method. Two-sided 95% CI will be computed based on the Greenwood's formula. If the null hypothesis is truly OS12 = 30% or OS12 = 50%, a sample size of 19 patients still achieves 80% power to detect a difference of 30%, however, the number of patients needed to reject the null changes to n = 10 or n = 14, respectively.

For the secondary endpoint for stratum C, we will assess the efficacy endpoint (OS12) in 20 evaluable patients with H3.3K27M positive DIPG or other midline gliomas, excluding primary spinal cord tumors.

In comparing outcomes to other H3K27M positive midline gliomas, OS rates similar to those for Stratum A are reported, although the reference literature is sparser for this patient population[31,32]. Thus, similar to stratum A, a sample size of 20 patients achieves 80% power to detect a difference of 30% using a one sided exact binominal test (see above)[40].

At time of data analysis, we will use the most recent published data and PNOC enrollment data in order to determine the appropriate null rate for stratum A and stratum C.

10.2.2 Accrual estimates

This study aims to enroll 19 children with H3.3K27M positive DIPG (Stratum A), 10 children with H3.3K27M positive glioma, other than DIPG, including spinal cord tumors (Stratum B) and 20 children with H3.3K27M positive DIPG or other midline gliomas, excluding primary spinal cord tumors (Stratum C). Eligible patients need to test positive for the HLA-A02:01^{+} to be eligible. Based on our previous study, we anticipate that about 40 children with DIPG and 40 children with other gliomas will need to be screened to meet accrual goals[14]. We anticipate that most of the PNOC sites will open this study and that we will be able to screen 2-3 potentially eligible patients per month which would allow us to accrue at least one patient per month. To date, we have enrolled an average of 1.4 patients/month on the phase 1 portion of PNOC007 with 14 PNOC sites actively accruing. To date, enrollment has been similar between the strata. Therefore, we anticipate that enrollment onto Strata A and B will be completed in approximately two years. We anticipate that Stratum C will follow a similar pattern, taking up to 2 years for enrollment.

10.3 Interim Analyses and Stopping Rules

The study will be monitored by Drs. Mueller and Okada and the PNOC Operation's office on a weekly basis including a review of all adverse events and deviations entered within Advarra® in concordance with the PNOC requirements for phase 1/2 clinical trials. The toxicity monitoring rules for the following events will be applied to all patients treated in the study. The regimen limiting toxicity (RLT) will be based on the tolerability observed following the vaccine doses following radiation therapy.

RLT includes any of the following events that occur during or following any vaccine given.

- Any grade 2 or greater autoimmune reaction.
- Any grade 3 or greater hematologic or non-hematologic toxicity with the exception of lymphopenia.
- Any life-threatening event.
- Any other unexpected Grade 2 or greater neurological deficit, which is possibly, probably, or definitely related to the vaccine therapy that does not respond within 21 days trial of 0.3mg/kg day dexamethasone or equivalent (max 12mg/day) or Avastin or equivalent (10mg/kg every 2 weeks for a maximum of 3 doses)

In addition to the stopping rules specific to the design detailed above, all patients will be monitored continuously for treatment-related adverse events by the study PIs Drs. Okada and Mueller.

Sequential boundaries will be used to monitor the RLT rate. The accrual will be halted if excessive numbers of RLTs are seen, that is, if the number of RLTs is equal to or exceeds b_n out of n patients with full follow-up (see table below). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.0475 when the rate of RLT is equal to the acceptable rate of 0.20.

For example, in Stratum A, the trial will be stopped if the number of RLTs is equal to or exceeds b_n out of n patients with completed follow-up.

Number of Patients, n 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Boundary, b_n - - 3 4 4 4 5 5 5 6 6 6 7 7 7 8 8 8 9

This boundary is equivalent to testing the null hypothesis, after each patient, that the event rate is equal to 0.2, using a one-sided level 0.021430 test.

θ	***φ****	***E[Y]***	***SD[Y]***	***E[N]***	***SD[N]***	***E[Y/N]***	***SD[Y/N]***
0.20	0.0475	3.70	1.62	18.51	2.39	0.21	0.13
0.30	0.2248	5.08	1.55	16.92	4.44	0.34	0.18
0.40	0.5327	5.60	1.49	14.00	5.76	0.48	0.20
0.50	0.8151	5.30	1.54	10.59	5.62	0.60	0.20

Definitions:

Y = the number of events, random, between 0 and N N = the number of patients, random, between 1 and K

φ^* = the actual probability of early stopping (hitting the boundary)

E[ ] denotes the expected value (mean) SD[ ] denotes the standard deviation

10.3.1 Analysis Population

Intent-to-Treat Population (ITT): The ITT population will include all subjects who are enrolled in the study. The ITT population will be the primary population for evaluating efficacy and subject characteristics.

As-Treated Population (AT) The AT population will include all subjects who receive at least 1 course of study therapy. The AT population will be the primary population for evaluating safety. If a patient does not receive any vaccine and poly-ICLC (strata A and B) in combination with nivolumab (stratum C), they will be replaced for evaluation of safety.

10.3.2 Analysis of Primary Endpoints

- Safety of K27M/TT vaccine and poly-ICLC in combination with nivolumab (stratum C only) will be assessed by monitoring for adverse events, scheduled laboratory assessments, vital sign measurements, and physical examinations for subjects who receive at least one course of the study therapy. The severity of toxicities will be graded according to the NCI CTCAE v5.0. Adverse events and will be summarized by maximum intensity and relationship to study drug(s).

- Safety will be assessed every 3 weeks for the first 24 weeks and then every 6 weeks. Descriptive statistics will be utilized to display the data on toxicity seen.

- OS12 in HLA-A02:01$^+$ children with H3.3K27M positive DIPG (Stratum A) will be the clinical efficacy primary endpoint. Any subject enrolled (ITT) will be considered evaluable for clinical efficacy. Patients lost to follow-up before 12 months will be considered events and the OS probability at 12 months (OS12) will be estimated from the Kaplan-Meier method. Two-sided 95% CI will be computed based on the Greenwood's formula.

10.3.3 Analysis of Secondary Endpoints

CTL response based on ELISPOT: A subject will be considered to have responded, if at any of post-vaccine time point against H3.3K27M antigen, the number of spots is double that at baseline, and there are at least 10 spots/20,000 cells, and if the number of the post-vaccine spots is at least three times the standard-deviation of the pre-vaccine value. This definition provides some protection against false positive response. We will correlate response with OS data. We will plot the time course of the magnitude of response and model it using a mixed-effects model approach.

10.3.4 Other Analyses/Assessments

The other exploratory aims associated with this study will be descriptive and will be limited to frequency tables and summary statistics.

10.4 Evaluation of Safety

Analyses will be performed for all patients having received at least one course of study therapy. The study will use the NCI CTCAE v5.0.

11 Data Reporting/Regulatory Requirements

11.1 Data Reporting

11.1.1 Method

The Principal Investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into Advarra® via standardized CRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The Clinical Research Coordinator (CRC) will complete the CRFs as soon as possible upon completion of the study visit; the Investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the subject's medical records maintained at each PNOC site. For participating sites, source documents will be maintained per institutional guidelines. All source documentation should be kept in separate research folders for each subject.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. The PI will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

All source documentation and CTMS data will be available for review/monitoring by the UCSF DSMC and regulatory agencies.

The Principal Investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the CRFs have been declared to be complete and accurate, the database will be locked. Any changes to the data entered into the CRFs after that time can only be made by joint written agreement among the Study Chair, the Trial Statistician, and the PNOC Project Leader.

Responsibility for Data Submission

Please refer to Appendix C for data submission timelines.

11.2 PNOC Oversight and Monitoring Plan

This is a multicenter trial. The UCSF Helen Diller Family Comprehensive Cancer Center Data Safety Monitoring Committee (DSMC) will be the main monitoring entity for this study. The UCSF DSMC will work together with participating member institution PIs and DSMCs to monitor each subject on study. The DSMC will routinely review all adverse events and suspected adverse reactions considered "serious". The UCSF DSMC will audit study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable. Please see Appendix D PNOC Data Safety and Monitoring Plan for more information.

11.3 Multicenter Communication

The PNOC operations office provides administration, data management, and organizational support for the participating sites in the conduct of the clinical trial. The PNOC Operations Office will coordinate, at minimum, quarterly conference calls with the PNOC member institutions to discuss risk assessment. The following items will be discussed, as appropriate:

- Enrollment information
- Adverse events (i.e. new adverse events and updates on unresolved adverse events and new safety information)
- Protocol violations
- Other issues affecting the conduct of the study

11.4 Record Keeping and Record Retention

The Principal Investigator for each PNOC institution is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects, as well as written records of the disposition of the drug when the study ends per institutional guidelines.

The site Principal Investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

In accordance with FDA regulations, the investigator shall retain records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

11.5 Coordinating Center Documentation of Distribution

It is the responsibility of the PNOC operations office to maintain adequate files documenting the distribution of study documents as well as their receipt (when possible). The HDFCCC recommends that the PNOC operations office maintain a correspondence file and log for each segment of distribution (e.g., FDA, drug manufacturer, participating sites, etc.).

Correspondence file: should contain copies (paper or electronic) of all protocol versions, cover letters, amendment outlines (summary of changes), etc., along with distribution documentation and (when available) documentation of receipt.

Correspondence log: should be a brief list of all documents distributed including the date sent, recipient(s), and (if available) a tracking number and date received.

At a minimum, the PNOC operations office must keep documentation of when and to whom the protocol, its updates and safety information are distributed.

11.6 Regulatory Documentation

Prior to implementing the protocol at each PNOC institution, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be first approved by the UCSF Institutional Review Board (IRB) and by the PNOC operations office. Prior to implementing this protocol at the participating sites, approval for the UCSF IRB approved protocol must be obtained from the participating site's IRB.

Appendix B lists the documents which must be provided to PNOC Operations Office before the participating site can be initiated and begin enrolling participants.

Upon receipt of the required documents, PNOC operations office will formally contact the site and grant permission to proceed with enrollment.

12 Protection of Human Subjects

12.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the IRB mechanism and the process of informed consent. The IRB reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The IRB also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

12.2 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign the HIPAA form and informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document. The use and disclosure of protected health information will be limited to the individuals described in the informed consent document.

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14 Appendices

Appendix A Performance Status Criteria

Karnofsky		**Lansky**	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

Appendix B PNOC Institutions Required Regulatory Documents

Before you can start enrollment at your site you must submit the following documents to PNOC:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form
- Participating Site IRB membership list
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Copy of the 1572 (Note: 1572 form is not required for FDA-exempt trials)
- Curriculum vitae and medical license for each investigator and consenting professional
- Documentation of Human Subject Research Certification training for investigators and key staff members at the Participating Site
- Participating site laboratory certifications and normals
- Signed copy of the completed delegation of authority log (found in PNOC Documents > Forms)
- Signed copy of the protocol signature page
- Signed copy of the final contract

Upon receipt of the required documents, PNOC will formally contact the site and grant permission to proceed with enrollment. All documents can be uploaded directly to SharePoint by navigating to your site's page and clicking "Add Documents"

Each PNOC site is responsible for ensuring all regulatory documents in SharePoint are up to date. Sites will upload new or revised documents as applicable to reflect any changes, including changes in staff and approved/expired documents.

Appendix C Required Data and Time Table for Submission

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 business days of registration
Baseline Assessment Forms	Within 14 business days of registration
Treatment Forms	Within 10 business days of the last day of the cycle
Adverse Event Report Forms	All AEs are due within 10 business days of the date of assessment.
Serious Adverse Event Reporting	Please refer to section on Expedited Reporting.
Response Assessment Forms	Within 10 business days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Forms	Within 14 business days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 business days of the protocol defined follow up visit date

Appendix D PNOC Data and Safety Monitoring

PNOC Data Safety and Monitoring Plan for a Phase 1 Study

It is the responsibility of each PNOC member institution to follow the National Cancer Institute (NCI) approved Data Safety and Monitoring Plan (DSMP) for their site. For PNOC trials in which the UCSF PI holds the IND, the UCSF DSMC will be responsible for monitoring all participating sites, including UCSF. Remote institutions will be electronically monitored unless there are significant findings or issues identified that warrant an in-person visit. In addition to the guidelines laid out in this document, each PNOC member institution must comply with the policies and standards put forward by their own institutional DSMC/DSMB.

The UCSF DSMC/DSMB activities for this study will include:

- Monitoring every month/six months through the DLT period (depending on subject accrual)
- Review of suspected adverse reactions considered “serious” (SAEs)
- Minimum of a biennial regulatory audit

Monitoring and reporting guidelines

All institutional Phase 0 or Phase 1 therapeutic studies are designated with a high risk assessment. The data is monitored monthly by the UCSF DSMC/DSMB as subjects are enrolled and includes all visits monitored up through the Dose Limiting Toxicity (DLT) period.

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) DSMC is responsible for monitoring data quality and patient safety for all HDFCCC institutional clinical studies. In the case of all PNOC protocols, the UCSF DSMC will work together with the non-UCSF PNOC member institution in order to ensure DSMP compliance. The UCSF DSMC/DSMB will be responsible for generating regular monitoring reports. These reports will be used by the UCSF DSMC to assess data quality, patient safety, and protocol compliance as well as to make decisions about dose escalations, where applicable.

PNOC and the UCSF DSMC reserve the right to conduct on-site monitoring at any non-UCSF member institution if DSMP requirements are not being met. If the need to perform a monitoring visit at a non-UCSF member institution arises, source documents will be provided by the member institution prior to the visit in order for the UCSF DSMC to monitor protocol compliance, patient safety, and to verify data entry.

The PNOC Operations Office provides administration, data management, and organizational support for the PNOC member institutions in the conduct of any PNOC clinical trial. The PNOC Operations Office will summarize and communicate adverse events, safety data, and other study matters to the PNOC member institutions on a quarterly basis.

The Study Chair is responsible for the overall conduct of any PNOC trial and for monitoring its safety and progress at all participating sites (as outlined in the PNOC Study Chair and Co-Chair Responsibilities SOP). The Study Chair will conduct continuous review of data and subject safety and

discuss each subject's treatment with the PNOC Operations Office. The discussions are documented in the PNOC Operations Office meeting minutes.

Multicenter communication

The PNOC Operations Office will coordinate, at minimum, quarterly conference calls with the PNOC member institutions to discuss risk assessment. The following items will be discussed, as appropriate:

- Enrollment information
- Cohort updates (e.g. DLTs and dose escalations)
- Adverse Events (e.g. new AEs, unresolved AEs, and new safety information)
- Protocol violations
- Other study conduct issues

Dose level considerations

Dose level assignments for any subject scheduled to begin treatment **must be confirmed** by the PNOC Operations Office via e-mail.

If an unexpected Dose Limiting Toxicity (DLT) arises in a subject treated at any participating PNOC member institution, all member institutions must be notified of the DLT by the PNOC Operations Office within **1 business day**. Member institutions will otherwise be provided with final DLT reports as they become available.

Adverse event review and monitoring

PNOC uses the web-based OnCore® Clinical Trials Management System for all patient registrations and the Advarra EDC for data entry. The OnCore® System will also track patient level protocol compliance and safety information. The Advarra EDC system is CFR part 11 compliant. For Phase I studies, all clinically significant Adverse Events (AEs) will be entered into the Advarra EDC, regardless of relationship. All Adverse Events entered into Advarra® will be reviewed on a weekly basis by the PNOC Operations Office. The PNOC Operations Office will discuss the toxicity, grade, and relationship to study intervention for all AEs in question.

All Adverse Events must be entered into Advarra® within **10 business days** of becoming aware of the event. Member institutions will submit this information to PNOC via the Adverse Event Form within Advarra®.

In addition, all adverse reactions considered "serious" (also called Serious Adverse Events, or SAEs), regardless of relationship, must be entered in OnCore® and Advarra, and reported to the PNOC Operations Office within **1 business day**. SAEs will be reviewed and monitored by the UCSF DSMC on an ongoing basis, and will be discussed at the UCSF DSMC meeting, which take place every six (6) weeks.

If a death occurs during the treatment phase of the study, or within 30 days after the last administration of the study drug(s), and is determined to be related either to the investigational drug or to any research related procedure, the Study Chair and the PNOC Operations Office must be notified by the member institution within **1 business day.** The Study Chair or the PNOC Operations Office must then notify the

UCSF DSMC Chair, or qualified alternate, within 1 business day of this notification. The contact may be by phone or e-mail.

Increase in adverse event rates

If an increase in the frequency of Grade 3, 4, or 5 Adverse Events (above the rate reported in the Investigator Brochure or package insert), the Study Chair or the PNOC Operations Office is responsible for notifying the UCSF DSMC at the time the increased rate is identified.

If at any time the Study Chair or the PNOC Operations Office halts enrollment or ends the study due to safety issues, the UCSF DSMC Chair and Manager must be notified within **1 business day** via e-mail. The UCSF DSMC must receive a formal letter within **10 business days,** and the UCSF IRB must be notified.

UCSF data and safety monitoring committee contacts:

UCSF DSMC Chair	UCSF DSMC Manager	UCSF DSMC Monitors
Katie Kelley, MD	John McAdams, MS	UCSF-Box #0128
[redacted]	[redacted]	San Francisco, CA 94143
[redacted]	[redacted]	
[redacted]	[redacted]	
San Francisco, CA 94115	San Francisco, CA 94143	

Appendix E Specimen Collection Immuno-Monitoring

Sampling and Shipping information

- Blood for *in vitro* assays: up to 50-60 ml of blood (7 x 10 cc green top tubes: each tube should be filled as much as possible) for mononuclear cell preservation (before injection of vaccines). The volume will depend on the weight of the child based on the justification below. These samples will be sent to UCSF. Please refer to the PNOC member site SharePoint for details regarding shipping instruction and collection.

- Blood for circulating tumor DNA – we will collect 6-7 ml at baseline and with each MRI assessment (- 7 days). Please refer to the PNOC members SharePoint site for details regarding shipping instruction and collection.

The minimum age enrolling in this trial is 3 years. The mean blood volume for a child is about 80 ml/kg. Up to 5% of total blood volume over a 24 hour period and up to 10% of the whole blood volume over an 8 week period might be removed for laboratory evaluations if the subject has a normal hematocrit [41]. Based on our experience children with DIPG undergoing focal radiation have a normal hematocrit and since these children are not receiving any other therapies we anticipate that these children all have a normal hematocrit.

Based on the WHO criteria the weights based on the 50% percentile are as follows:

3 year old approximately 14 kg

4 year old approximately 16 kg

5 year old approximately 8 kg

The total blood volume for a 3 year old child is about 80x 14 kg = 1,120 ml. 5% of 56 ml; 10%112 ml.

The total blood volume for a 4 year old child is about 80x 16 kg = 1,280 ml. 5% of 64 ml, 10%128ml.

The total blood volume for a 5 year old child is about 80x 18 kg = 1,440ml. 5% of 72 ml; 10% 144 ml.

Based on these recommendations we will take 50 cc in green top tubes in children < 16 kg and 60cc in children >/= 16 kg for the immune-monitoring. We will assure that recommended dose of 5% of total volume in a 24 hour period will not be exceeded. ctDNA and other laboratory evaluations will be scheduled on different days as required by the guidelines.

Sample Shipment Instructions

Please refer to the PNOC member website SharePoint for details regarding shipping of collected specimens.

Appendix F Biospecimen Banking

Samples are collected only from patients who have agreed to have their tissues and blood banked and used for future research. Any leftover specimen samples such as tumor specimens and cell derivatives will be reserved for banking and stored at UCSF. Banked specimens may be used for further validation or, if the subject agrees, for future medical research. The laboratories have storage procedures designed to ensure that the storage process maintains the molecular and cellular integrity of the specimen.

When specimens arrive at UCSF, they will be entered in OnCore and assigned an appropriate storage location. Both of the specimen's unique identifiers will be entered into the system. If a specimen or aliquot of derivatives is shared with another project investigator, it will be recorded and tracked, which will maintain a record for reporting and audit purposes. The specimen and any other derivatives may be stored for up to 20 years to answer research scientific questions related to cancer and/or study drugs.

To obtain samples, investigators submit a request form to the Tissue Bank Manager. The request form requires an explanation of the tissue requested (type, number of samples, justification), description of the study, Institutional Review Board (IRB) approval, and Project Leader authorization. The Manager reviews each request for feasibility before presentation to the Scientific Core Committee.

Appendix G Medications Associated With Prolonged QTc

Medications that prolong QTc	
Amiodarone	Flecainide
Anagrelide	Fluconazole
Arsenic trioxide	Haloperidol
Azithromycin	Ibutilide
Chloroquine	Methadone
Chlorpromazine	Moxifloxacin
Ciprofloxacin	Ondansetron
Citalopram	Pentamidine
Clarithromycin	Pimozide
Disopyramide	Procainamide
Dofetilide	Propofol
Domperidone	Quinidine
Droperidol	Sevoflurane
Dronedarone	Sotalol
Erythromycin	Thioridazine
Escitalopram	Vandetanib
Medications that <u>MAY</u> prolong QTc	
Aripiprazole	Lapatinib
Bortezomib	Lenvatinib
Bosutinib	Leuprolide
Ceritinib	Mirtazapine
Clomipramine	Nicardipine
Crizotinib	Nilotinib
Dabrafenib	Olanzapine
Dasatinib	Osimertinib
Degarelix	Pazopanib

Desipramine	Promethazine
Dolasetron	Risperidone
Eribulin mesylate	Sorafenib
Famotidine	Sunitinib
Foscarnet	Tacrolimus
Gemifloxacin	Vemurafenib
Granisetron	Venlafaxine
Isradipine	Vorinostat

Appendix H Quality of Life Measures

PNOC HEALTH RELATED QUALITY OF LIFE & NEUROCOGNITIVE MEASURES

Please note: Measures that are not available in the local language or the patient's native language should not be administered.

Pediatric Quality of Life Inventory (PedsQL):

To assess treatment and disease impact on quality of life, we will use the PedsQL 4.0 Generic Core Scales and the PedsQL 3.0 Cancer Module. These rating forms have multidimensional child *self-report* and *parent proxy report* scales to assess health-related quality of life (QOL) in children, adolescents, and young adults ages 2 – 25 years. It consists of a 23-item core measure of global QOL that has four subscales: physical functioning, emotional functioning, social functioning, and school functioning.

There are different forms for parents of patient's ages 2 – 17 years (toddler: 2 – 4; young child: 5 – 7; children: 8 – 12; teen: 13 – 17) and parallel self-report forms for patient's ages 5 – 25 years (young child: 5 – 7; child: 8 – 12; teen: 13 – 17; young adult: 18 – 25). It takes approximately 5 – 10 minutes to complete.

Patient-Reported Outcomes Measurement Information System (PROMIS):

To assess treatment and disease impact on overall health, we will use the PROMIS Pediatric/ Parent-Proxy-49. This measure consists of seven 8-item short forms to assess mental health, physical health, and social health. The specific short forms are:

- Emotional Distress – Anxiety
- Emotional Distress – Depression
- Fatigue
- Pain – Interference
- Pain – Intensity
- Physical Function – Mobility
- Peer Relationships

We will also collect information about Cognitive Function through the pediatric cognitive supplement short form 7a. To include patients greater than 17 years of age, we will utilize the PROMIS 57 for adults as well as the adult cognitive abilities short form 8a.

PROMIS is available in Spanish and selected forms are also available is other languages such as Chinese, Korean, German, etc. Self-reported measures are used for children ages 8 – 17. Parent proxy-reported measures are used for children ages 5 – 17. The test takes approximately 5 – 10 minutes to complete.

Behavior Rating Inventory of Executive Function (BRIEF):

To assess treatment and disease impact on executive functioning and self-regulation, we will use the BRIEF. The BRIEF-P Preschool will be used for preschool children and consists of 63 items, the BRIEF-2 Parent Form will be used for children and consists of 86 items, the BRIEF-2 Self Report Form will be used for adolescents, and the BRIEF-A Adult Self Report Form will be used for young adults and

consists of 75 items. All assessments are also available in Spanish and take approximately 10 – 15 minutes to complete.

***Please see PNOC QOL Guide found in PNOC SharePoint website for more information**

CogState:

To assess treatment and disease impact on domains of neurocognition, we will use CogState testing (see Table 1). The CogState test battery will take about 30 minutes and will be completed during a routine follow up visit or remotely, whatever is more convenient for the family and patient.

CogState data are automatically scored and stored on a secure server. All CogState tasks were developed as computerized adaptations of traditional neuropsychological measures. We will employ tasks assessing the following domains: executive function, visual attention, processing speed and working memory (visual and verbal).

Table 1: Summary of Individual CogState Tasks

Neurocognitive Function	Name of Task	Score	Time to Administer	Interpretation
Working Memory	Groton Maze-Learning Task	# of errors made across 5 trials	Approximately 7 minutes	Lower scores reflect better performance
Visual Attention	Identification Task	Mean log 10 reaction time for correct response	Approximately 3 minutes	Lower scores reflect better performance
Processing Speed	Detection Task	Mean log 10 reaction time for correct response	Approximately 3 minutes	Lower scores reflect better performance
Working Memory	One-Back Test	Arcsine proportion of total items correct	Approximately 3 minutes	Higher scores reflect better performance
Verbal Learning and Memory	International Shopping list International Shopping list (recall & recognition)	# of correct responses made in remembering the list on three consecutive trials or at recall at a single session.	Approximately 10 minutes	Higher scores reflect better performance

ADHD Rating Scale:

To assess treatment and disease impact on attention, we will use the ADHD Rating Scale–5: Home Version. The ADHD rating scales are based on the diagnostic criteria for ADHD as described in the Fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Both versions of

the ADHD Rating Scale–5 consist of two symptom subscales, Inattention (9 items) and Hyperactivity–Impulsivity (9 items), as well as a Total Scale (18 items). In addition, the ADHD Rating Scale–5 assesses six domains of impairment that are common among children with ADHD: relationships with significant others, peer relationships, academic functioning, behavioral functioning, homework performance, self-esteem. The ADHD Rating Scale–5 has separate forms for children (ages 5–10 years) and adolescents (ages 11–17 years). The test takes 5 – 10 minutes to administer and is available in Spanish.

Adaptive Behavior Assessment System, Third Edition (ABAS-3)

To assess treatment and disease impact on adaptive life skills, we will use the ABAS-3 questionnaire. ABAS-3 is used to measure adaptive skills important for everyday living. It has norms from birth to 89 years of age. The ABAS-3 has several versions: the Parent/Primary Caregiver Form, which consists of 232 items, the Parent Form (232 items), and the Adult Form (239 items). The ABAS-3 assesses several skill areas: communication, community use, functional academics, health and safety, home or school living, leisure, motor, self-care, self-direction, social, and work. The test takes 25 – 30 minutes to administer and is available in Spanish. [redacted]

Table 2: ADMINISTRATION SCHEDULE

	Baseline	**Every 3 months[a]**	**End of treatment[d]**	**Follow up period Every 12 months until the start of a new treatment**
PEDs-QL Generic Core Scales Cancer Module	X	X	X	X
PROMIS Parent-Proxy-49 Pediatric Profile-49 PROMIS 57 Profile Pediatric cognitive supplement short form 7a Adult cognitive abilities short form 8a.	X	X	X	X
CogState	X		X	X

	Baseline	**Every 6 months for two years then every 12 months after two years, while on treatment[b]**	**End of treatment[d, e]**	**Follow up period Every 12 months until the start of a new treatment[c]**
ABAS-3 Parent/Primary Caregiver Form Parent Form Adult Form	X	X	X	X

BRIEF® BRIEF®P Preschool BRIEF® 2 Parent Form BRIEF® 2 Self Report Form BRIEF®-A Self Report Form	X	X	X	X
ADHD Rating Scale-5; Home Version Adolescent Home Version Child	X	X	X	X
CogState	X	X	X[f]	X

[a] Every 3 months from the date of previous administration +/- 14 days

[b] Every 6 months from the date of previous administration +/- 14 days

[c] If the ABAS BRIEF or ADHD measures were not administered at EOT because they were already completed within 6 months of the EOT visit, they should be collected in follow up when the 6 month elapses from date of last administration. They should then be collected every 12 months.

[d] +/- 14 days

[e] If measures have been administered within the past 6 months of EOT visit, they do not need to be repeated

[f] If participant is at EOT and CogState was administered less than 6 months prior, please contact PNOC Operations Office for specific guidance.

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Appendix I Management Algorithms

These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the Medical Monitor representing the Sponsor. The guidance applies to all immuno-oncology agents and regimens.

A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated.

Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended.

The frequency and severity of the related adverse events covered by these algorithms will depend on the immuno-oncology agent or regimen being used.

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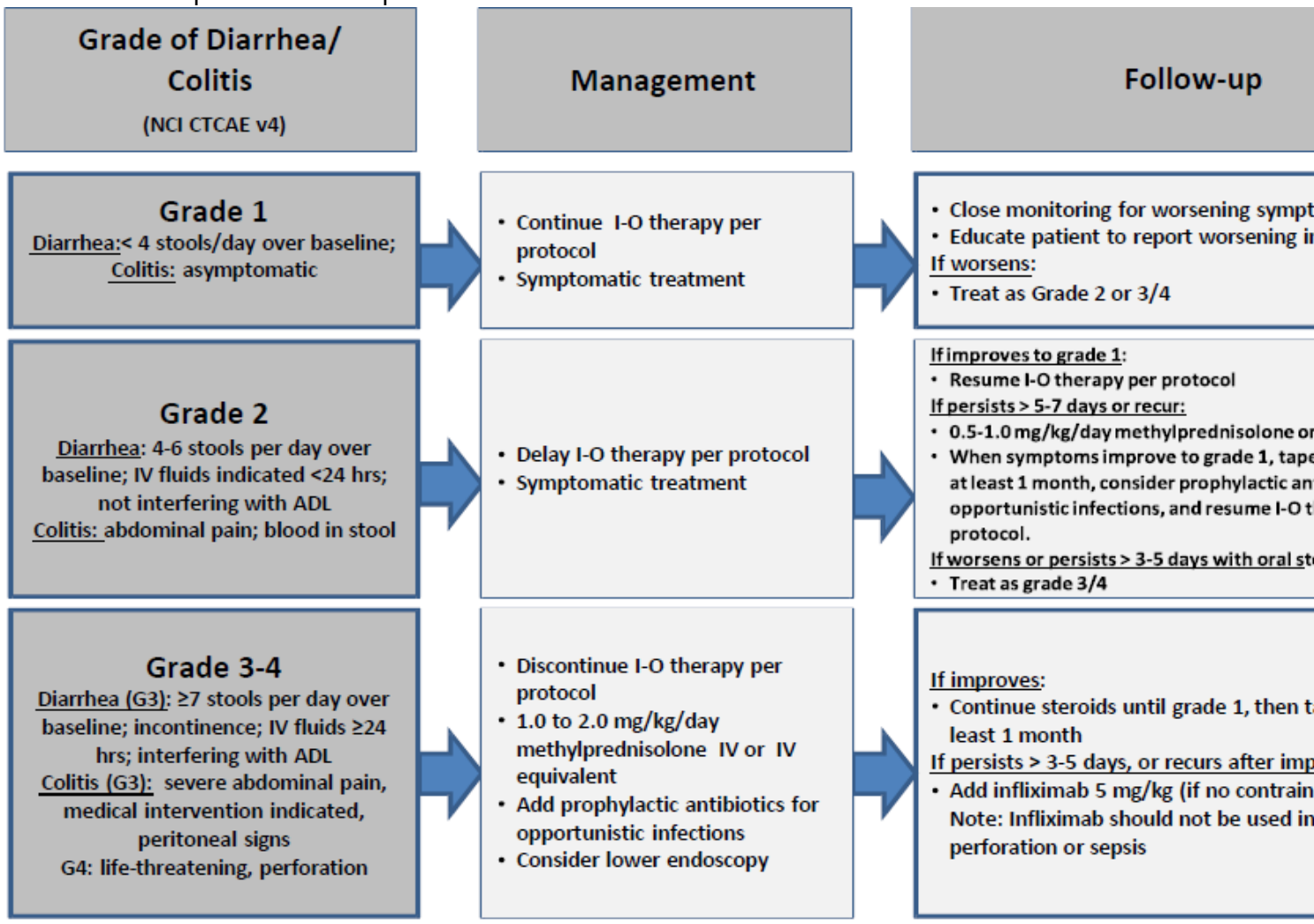
GI Adverse Event Management Algorithm

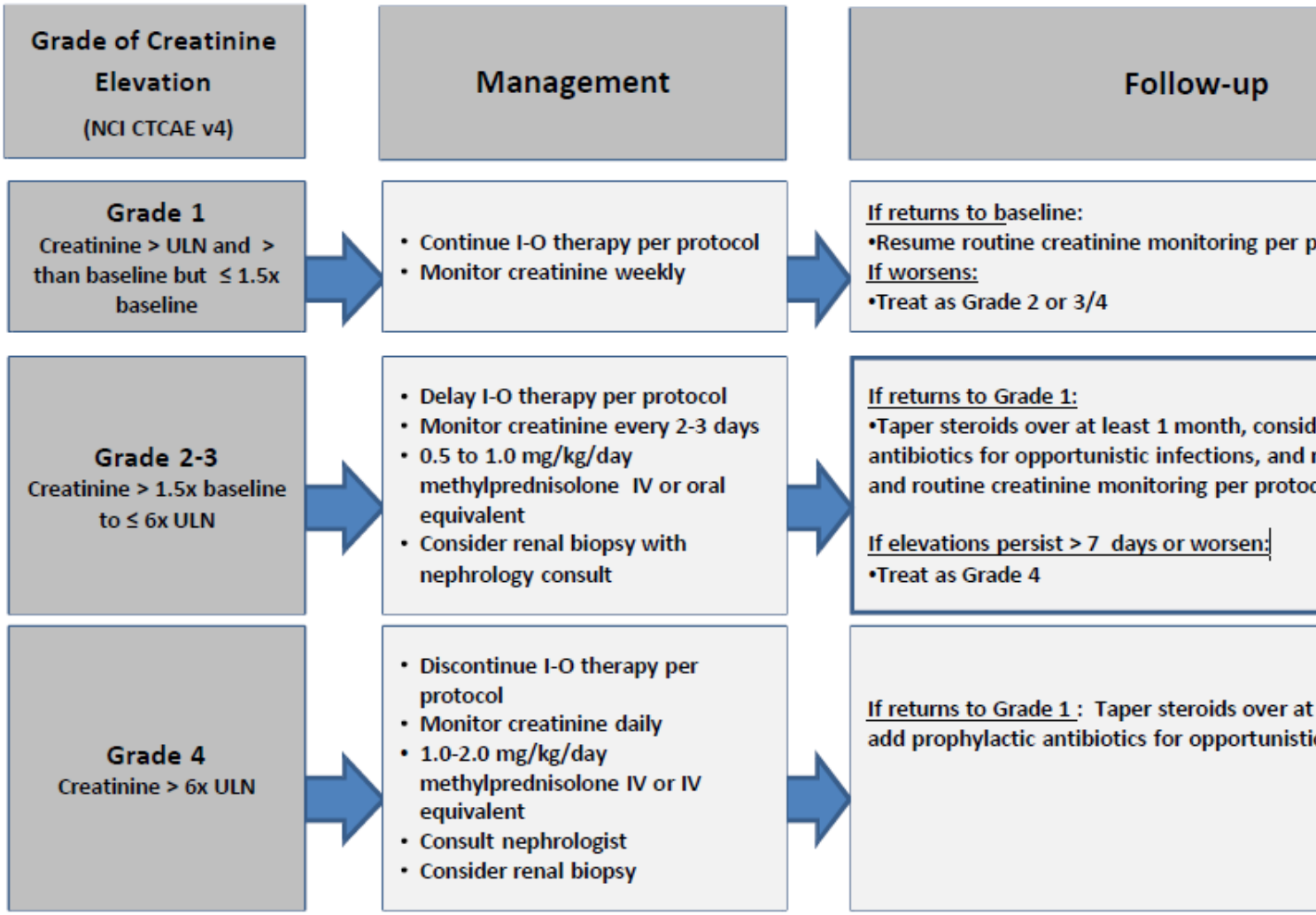
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



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

Renal Adverse Event Management Algorithm

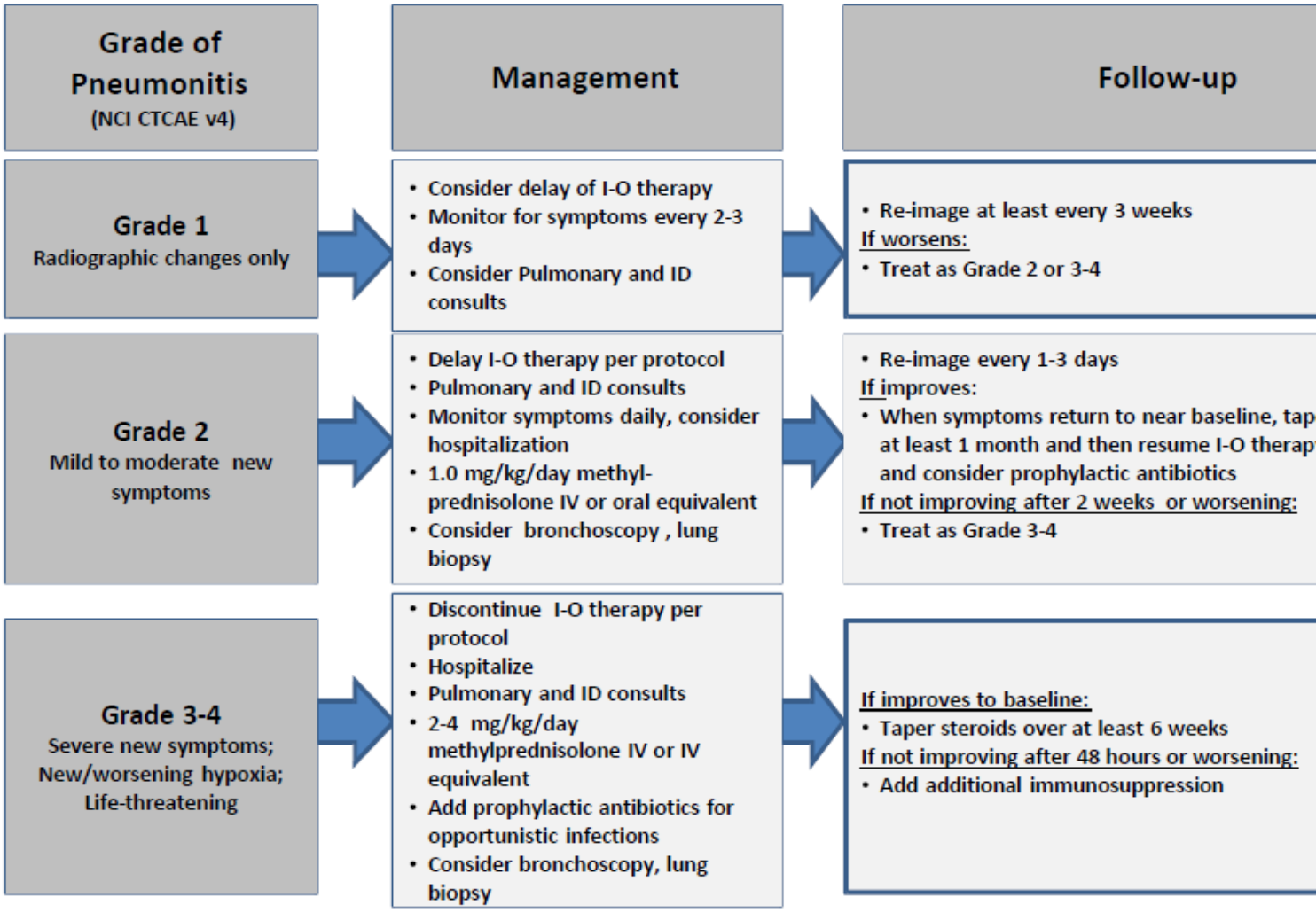
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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

Pulmonary Adverse Event Management Algorithm

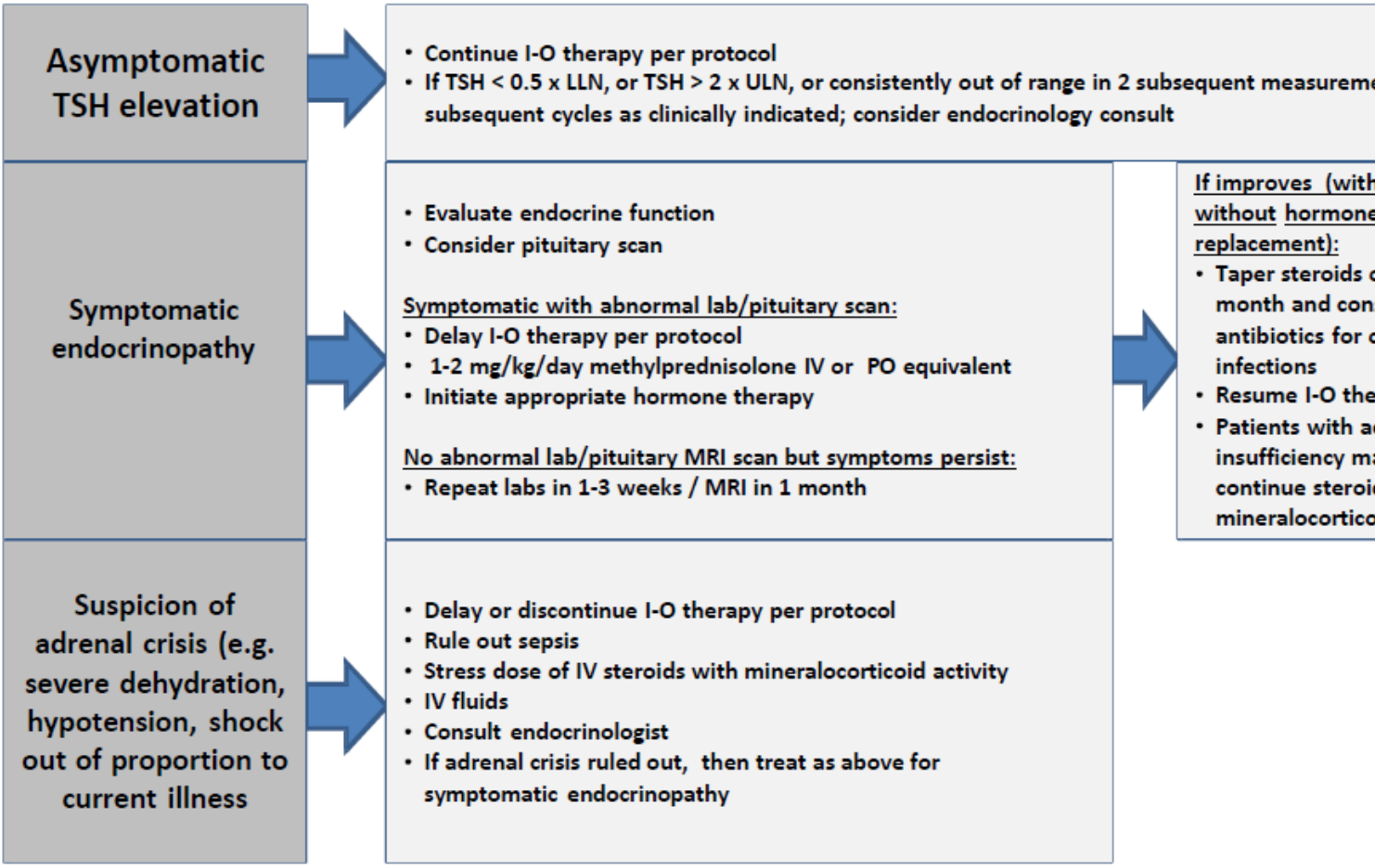
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

Hepatic Adverse Event Management Algorithm

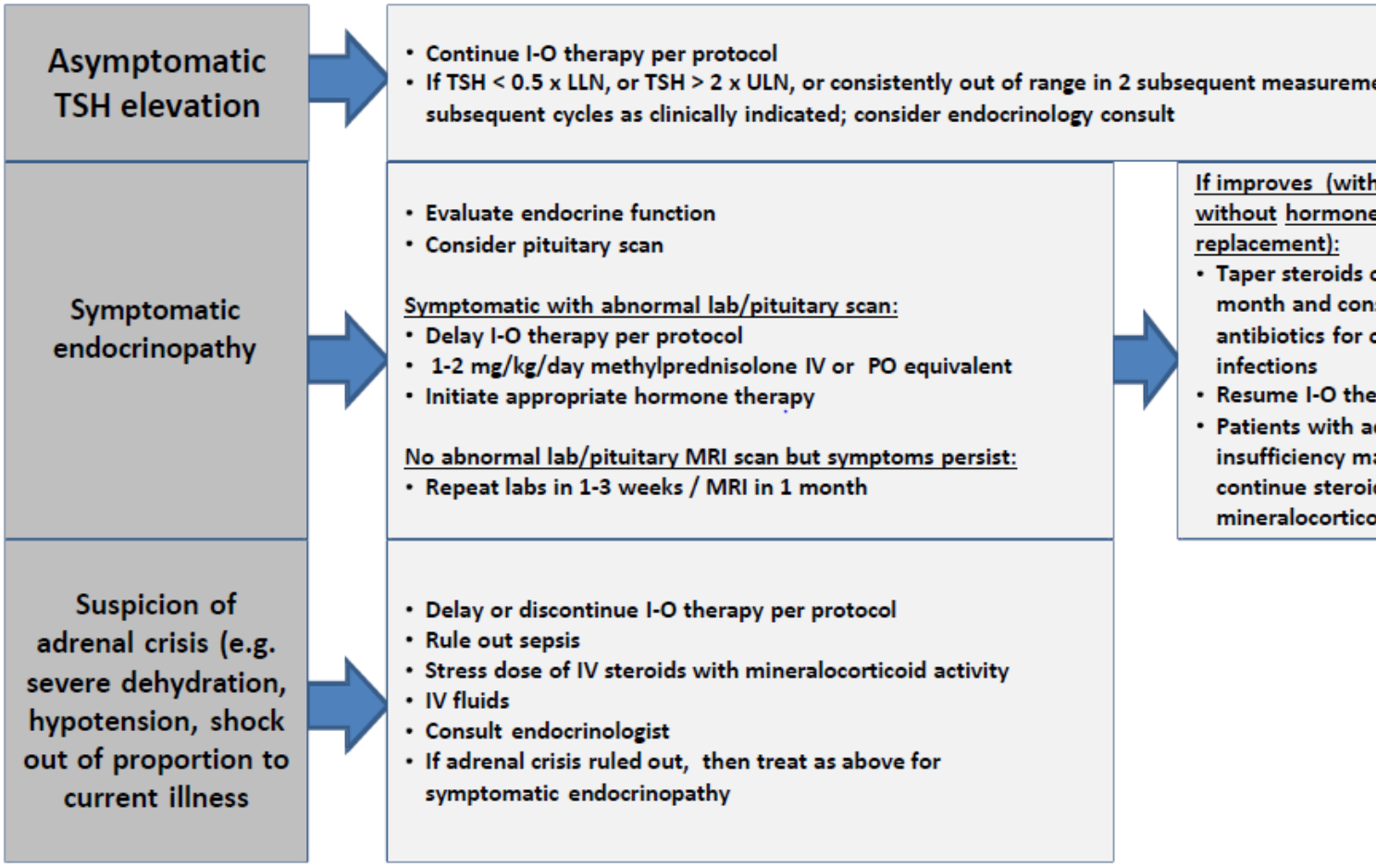
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



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

Endocrinopathy Adverse Event Management Algorithm

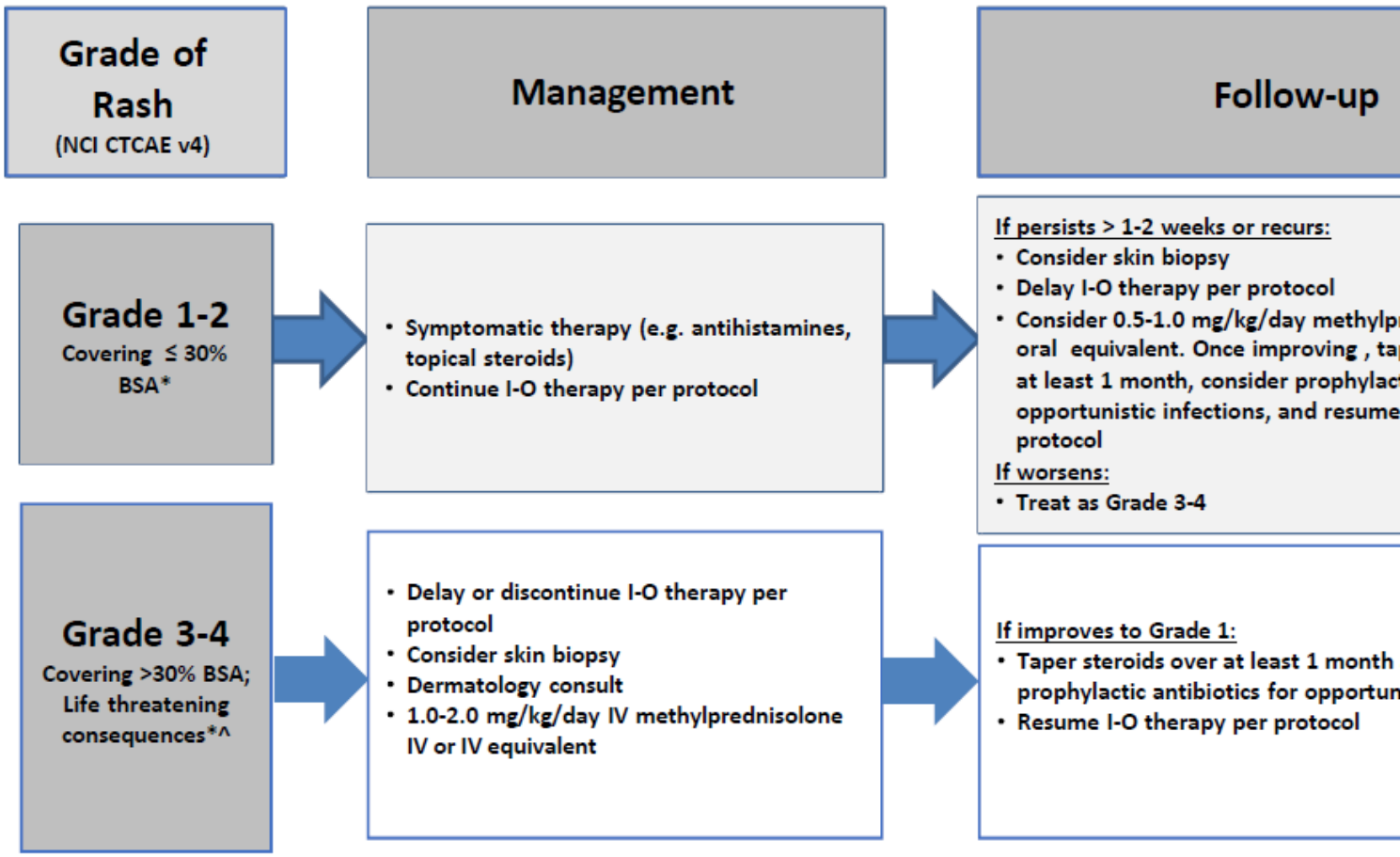
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

Skin Adverse Event Management Algorithm

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



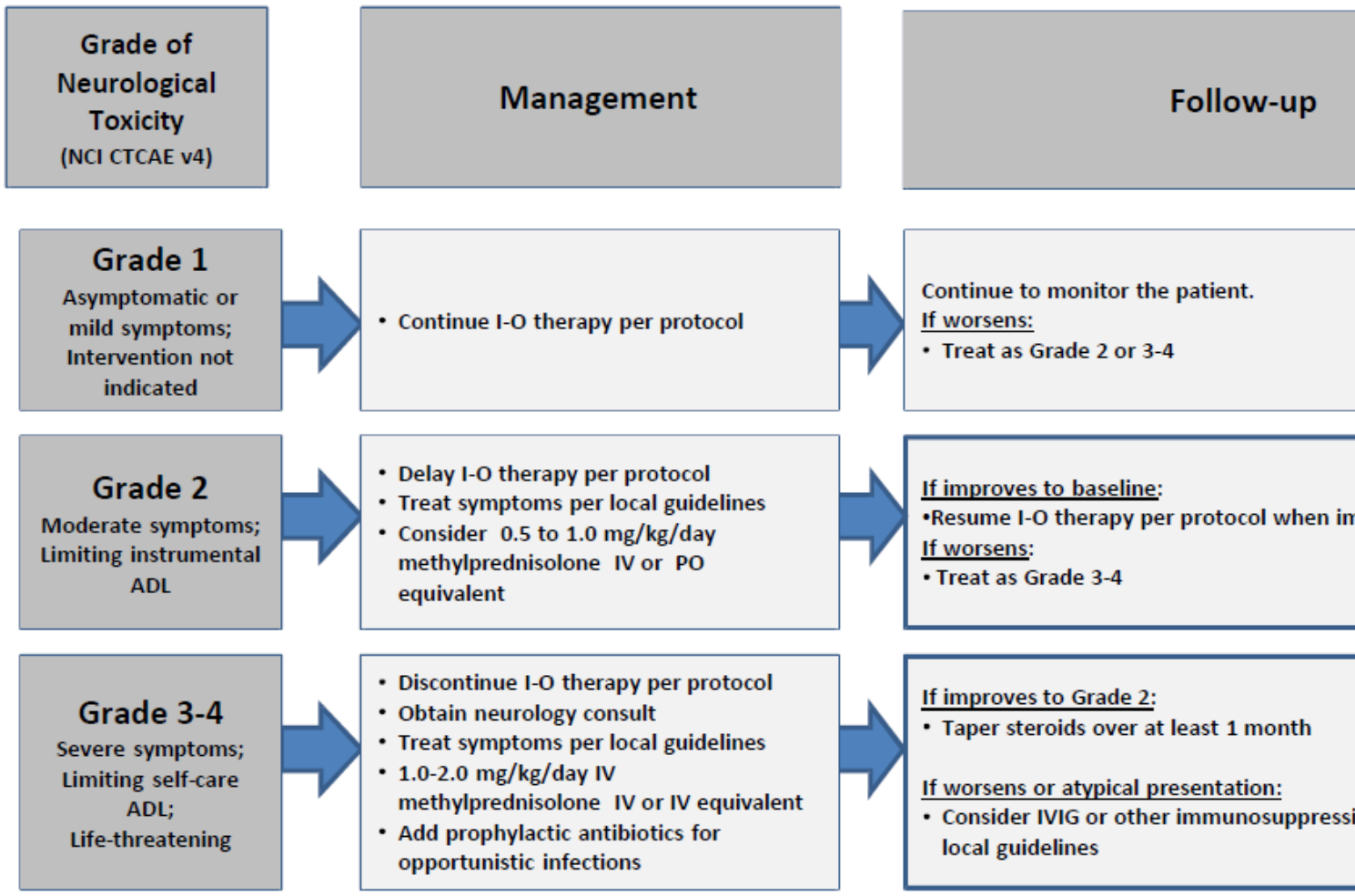
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

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

^If SJS/TEN is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

Neurological Adverse Event Management Algorithm

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



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