PNOC028: A Phase 1 Study of Intra-Tumoral Injections of *Ex Vivo* Expanded Natural Killer Cells in Children and Young Adults with Recurrent or Progressive Malignant Brain Tumors

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Protocol No.: PNOC028 **Version Date:** 02/04/2025

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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 required 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.

Study Chair

_____
Printed Name

_____ _____
Signature Date

PROTOCOL SIGNATURE PAGE – PARTICIPATING SITES

Protocol No.: PNOC028 **Version Date: 02/04/2024**

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

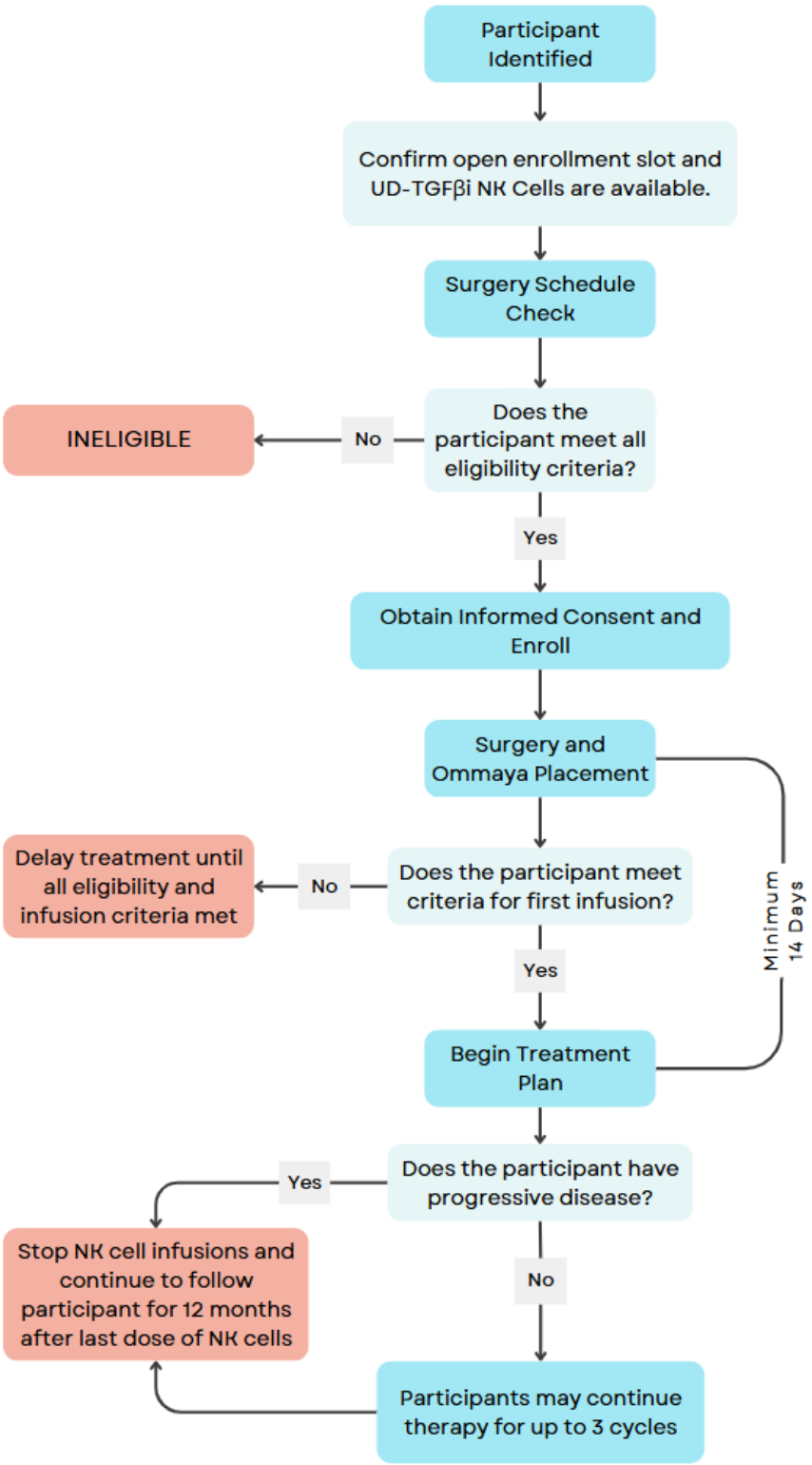
Title	Phase 1 Study of Intra-Tumoral Injections of *Ex Vivo* Expanded Natural Killer Cells in Children and Young Adults with Recurrent or Progressive Malignant Brain Tumors
Study Description	This is a phase 1 dose escalation study to evaluate the safety and efficacy of multiple infusions of universal donor (UD) -derived transforming growth factor β imprinted (TGFβi) natural killer (NK) cells *via* an Ommaya reservoir in participants with recurrent or progressive malignant brain tumors. Adoptive transfer of donor NK cells has been shown to be safe and effective in clinical trials for Acute myeloid leukemia (AML), other pediatric and adult hematologic, and solid tumor malignancies, including malignant brain tumors. TGFβi NK cellsare resistant to the immunosuppressive effects of TGFβ and secrete high levels of proinflammatory cytokines, and therefore they may be more effective than normal expanded NK cells to treat malignant brain tumors. UD-derived NK cells will eliminate many of the cost and logistics concerns of manufacturing cells on a patient-by-patient basis and make cell therapy more widely available to patients. We hypothesize that adoptive transfer of UD TGFβi NK cells to participants with recurrent or progressive malignant brain tumors will be safe and improve outcomes in this high-risk patient population.
Phase of Study	Phase 1
Participant population	Children and young adults (1 - ≤39 years) with recurrent or progressive malignant brain tumors will be eligible. The first 3 participants enrolled will be ≥ 8 years-≤ 39 years.
Rationale for Study	Over the past decade, immunotherapy has emerged as a highly effective treatment modality against malignancies. However, with the exception of cancers arising in patients with mismatch repair deficiencies, pediatric cancers in general and brain tumors specifically have extremely low mutational burden, a central requirement for responses to checkpoint inhibitors. Likewise, surface antigens that allow safe and effective targeting by chimeric antigen receptor (CAR) T cells have yet to show efficacy outside of hematologic cancers. In contrast, NK cells are critical immune effector cells which have the ability to recognize cancer in a non-antigen-dependent manner, and have been harnessed as a promising therapeutic strategy against advanced cancers. We have established Good Manufacturing Practice (GMP) infrastructure at Nationwide Children's Hospital (NCH) to efficiently generate clinical-grade patient-derived NK cells and have extensively tested their preclinical activity against several malignancies

	including brain tumors. We demonstrated that the membrane-bound IL-21 (mbIL21) -expressing feeder cells promote sustained proliferation of mature NK cells without senescence by increasing the telomere length in the expanded cells. The method enables large-scale expansion of NK cells from a small volume of peripheral blood, sufficient to deliver multiple infusions of NK cells at high cell doses. Additionally, a primary mechanism of immune escape by solid tumors is the secretion of TGFβ, which we were able to bypass by our recently modifying the expansion method to enhance NK cell function and overcome TGFβ-induced suppression [referred to as TGFβ "imprinting" (TGFβi)] by chronically stimulating the NK cells with TGFβ during the expansion process. The use of autologous cells is also logistically challenging and requires costly manufacturing for each patient product. Hence, through our collaboration with Be The Match Biotherapies (BTMB), we identify individuals with optimal NK cell characteristics who subsequently undergo donor screening, collection, and expansion of the NK cell to generate the UD NK cell bank. This study will be the first clinical trial to utilize this "off-the shelf" NK cell product in pediatric brain tumors. We hypothesize that intra-tumoral infusions of *ex vivo* expanded UD TGFβi NK cells will be safe and feasible in participants with recurrent or progressive malignant brain tumors, and may provide therapeutic benefit. The potential advantages of direct NK cell infusion into the tumor include bypassing the blood-brain barrier and maximally concentrating NK cells inside the tumor. Participants will receive 3 cycles (consisting of one NK cell infusion per week for three weeks, followed by a rest week) over a total of 12 weeks. We will also perform several correlative studies, including Next Generation Sequencing on all the recurrent tumors in order to determine their mutational landscape, and high-parameter immunophenotyping to determine the persistence and function of the adoptively-transferred expanded NK cells. Additionally, we will utilize the Nanostring platform to determine the tumor's immune profiles and to characterize the changes in T cell receptor (TCR) repertoire that result from NK cell infusions.

Primary Objective	1. To determine the safety and tolerability of natural killer (NK) cells that have been propagated *ex vivo* with genetically-modified feeder cells and administered intra-tumoral *via* an Ommaya reservoir in participants with recurrent or progressive malignant brain tumors. 2. To determine the recommended phase 2 dose (RP2D) for NK cells that have been propagated *ex vivo* with genetically-modified feeder cells and administered intra-tumoral *via* an Ommaya reservoir in participants with recurrent or progressive malignant brain tumor
Exploratory Objective	1. To determine the 6 months overall survival (OS), defined as the percentage of participants in the study who are alive at 6 months following start of treatment 2. To determine the persistence, immuno-phenotype and function of adoptively-transferred expanded NK cells, and correlate the findings with the overall response 3. To determine the immune signature-based profile of each patient's tumor 4. To determine changes in the TCR repertoire diversity before and after TGFβi NK cell treatment 5. To evaluate the effect of systemic steroids on the persistence and efficacy of TGFβi NK cells. 6. To assess Quality of Life (QOL) and cognitive measures in children and young adults with recurrent or progressive malignant brain tumors 7. To assess patient and/or proxy satisfaction with study participation via patient-reported outcome (PRO) measures in the context of race ethnicity and other health related social risks 8. To assess on therapy toxicity in the context of race, ethnicity and other health related social risks
Sample Size	The study design will be performed in up to 24 participantsin BOIN dose escalation.
Duration of Therapy	Participants will receive TGFβi NK cell infusions in 4 week cycles for a total of 3 cycles. Infusions *via* Ommaya will occur once weekly for three weeks followed by one week of rest.
Duration of Follow up	Follow-up procedures are to be captured under the PNOC COMP protocol. Participants will be followed under the PNOC COMP protocol until death or withdrawal from study.
Duration of study	The phase 1 study will reach completion approximately 3 to 4 years from the time the study opens to accrual.

Study Drug(s)	The TGFβi NK cell product on this trial will be manufactured in the Abigail Wexner Research Institute at Nationwide Children's Hospital (AWRI-NCH) Cell-Based Therapy (CBT) Core facility.
Safety Assessments	The primary endpoint for the study is dose-limiting toxicity (DLT) during the first cycle of therapy. Toxicity assessment will be evaluated using the CTCAE 5.0 and include any patient that receives at least one dose of TGFβi NK cell infusion.
Efficacy Assessments	Progression-free survival (PFS), overall survival (OS), and/or objective response rate (ORR) as appropriate.
Unique Aspects of this Study	This is the first study to evaluate the safety and tolerability of TGFβi NK cell infusions intra-tumoral *via* an Ommaya reservoir in participants with recurrent or progressive malignant brain tumors.

EXPERIMENTAL DESIGN SCHEMA



LIST OF ABREVIATIONS

AA	Anaplastic Astrocytoma
AE	Adverse event
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
AT/RT	Atypical Teratoid Rhabdoid Tumor
BSA	Body surface area
BUN	Blood urea nitrogen
CBC	Complete blood cell (count)
CNS	Central Nervous System
CR	Complete response
CRC	Clinical Research Coordinator
CRF	Case report form
CRO	Contract Research Organization
CSF	Cerebral spinal fluid
CT	Computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T lymphocytes
CTEP	Cancer Therapy Evaluation Program
DFS	Disease-free survival
DIPG	Diffuse Intrinsic Pontine Glioma
DLT	Dose limiting toxicity
DS&E	Drug Safety and Epidemiology
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
ECG	Electrocardiogram
EFS	Event-free survival
EGFR	Epidermal growth factor receptor
FCBP	Female of childbearing potential
FDA	Food and Drug Administration
GBM	Glioblastoma multiforme
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HCT	Hematocrit
HGB	Hemoglobin

HGG	High Grade Glioma
HLA	Human leukocyte antigen
Hr	Hour
IHC	Immunohistochemical
IND	Investigational new drug application
IP	Investigational product
IRB	Institutional Review Board
IV	Intravenous
LD	Longest dimension
LDH	Lactate dehydrogenase
LFT	Liver function test
LGG	Low Grade Glioma
MB	Medulloblastoma
MedDRA	Medical Dictionary for Regulatory Activities
mIL-15	Membrane-bound IL-15
mIL-21	Membrane-bound IL-21
MGMT	o^6-methylguanine-DNA-methyltransferase
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NK	Natural Killer Cell
ORR	Overall response rate
PBL	Peripheral blood lymphocytes
PBMCs	Peripheral blood mononuclear cells
PD	Progressive Disease
PD	Pharmacodynamics
PFS	Progression-free survival
PK	Pharmacokinetics
PO	*Per os* (by mouth, orally)
PR	Partial response
PRC	Protocol Review Committee (UCSF)
PT/INR	Prothrombin time/international normalized ratio
PTT	Partial thromboplastin time
PXA	Pleomorphic xanthoastrocytomas
QD	Once daily
QOL	Quality of Life
QW	Once weekly; once per week
RBC	Red blood cell (count)

RP2D	Recommended Phase II Dose
SAE	Serious Adverse Event
SD	Stable disease
TCR	T cell receptor
UD	Universal Donor
ULN	Upper limit of normal
WBC	White blood cell (count)

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1. OBJECTIVES

1.1 Primary Objectives

Primary Objectives	Endpoint(s)	Time Frame
1. To determine the safety and tolerability of natural killer (NK) cells that have been propagated *ex vivo* with genetically-modified feeder cells and administered intra-tumoral *via* an Ommaya reservoir in participants with recurrent or progressive malignant brain tumors.	Proportion of participants with Adverse Events, as graded by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE version 5.0)	From initiation of study treatment until 30 days from the end of therapy
2. To determine the recommended phase 2 dose (RP2D) for natural killer (NK) cells that have been propagated *ex vivo* with genetically-modified feeder cells and administered intra-tumoral *via* an Ommaya reservoir in participants with recurrent or progressive malignant brain tumors	RP2D, defined as the dose at which fewer than one-third of participants experience a dose limiting toxicity (DLT)	From initiation of study treatment until 30 days from the end of therapy

1.2 Exploratory Objectives

Exploratory Objective	Endpoint(s)
1. To determine the 6 months overall survival (OS), defined as the percentage of participants in the study who are alive at 6 months following start of treatment	Overall survival 6 months following start of treatment estimated using the Kaplan-Meier method
2. To determine the persistence, immuno-phenotype and function of adoptively-transferred expanded NK cells, and correlate the findings with the overall response	Participants with a tumor response or stable disease will be compared to other participants to explore whether there is an association with persistence, potency, or phenotype determinations
3. To determine the immune signature-based profile of each patient's tumor	Gene expression profile using NanoString PanCancer IO360 Panel
4. To determine changes in the TCR repertoire diversity before and after TGFβi NK cell treatment	Evaluation of TCR repertoire diversity using Nanostring custom reagent, that evaluates the VDJ sequences
5. To evaluate the effect of systemic steroids on the persistence and efficacy of TGFβi NK cells.	NK cell persistence and clinical outcomes of participants receiving low dose or high dose systemic corticosteroids will be compared to participants who are not receiving corticosteroids to explore the effect of corticosteroids on TGFβi NK cells.
6. To assess Quality of Life (QOL) and cognitive measures in children and young adults with recurrent or progressive malignant brain tumors	Evaluation of the PedsQL, PROMIS, ABAS, and BRIEF measures
7. To assess patient and/or proxy satisfaction with study participation via patient-reported outcome (PRO) measures in the context of race ethnicity and other health related social risks	Evaluation of the health-related social risk assessment.
8. To assess on therapy toxicity in the context of race, ethnicity and other health related social risks	Evaluation of the health related social risk assessment.

2. BACKGROUND

Despite the technologic advances in imaging, neurosurgery, and radiation oncology as well as the introduction of combination chemotherapy, outcomes have remained static for most of central nervous system (CNS) tumors[1], and sadly, they now represent the most common cause of cancer death in children 0–14 years in the United States[2]. Furthermore, the cumulative burden of chronic health conditions at age 50 years is highest amongst survivors of CNS malignancies when compared to other cancers[3], which illustrates the need for novel therapies to improve the survival as well as the quality of life and secondary treatment effects for pediatric brain tumor patients.

There is currently no effective standard of care for recurrent pediatric malignant brain tumors and patients are often treated with experimental therapies, which may cause limited and temporary control of the disease without any significant changes in prognosis. There is a clear and urgent need to investigate new therapies that might have benefit in patients with recurrent pediatric malignant brain tumors.

2.1 Recurrent Malignant Brain Tumors:

Curing malignant brain tumors in the recurrent setting is challenging. While some percentage of newly-diagnosed patients with atypical teratoid rhabdoid tumors (AT/RT), ependymoma, embryonal tumors, or high-grade glioma (HGG) may be cured, recurrent patients suffer from very poor outcomes. Pediatric HGG, as an example, is amongst the most common malignant CNS tumors in children with a reported age-adjusted incidence of 0.26 per 100,000 population[4], which is likely an underestimate, because poorly-differentiated HGG variants previously may have been diagnosed as primitive neuroectodermal tumors (PNET) or tumors with mixed ependymal, glial, or glioneuronal features[5]. However, no significant improvement in the outcomes for children with HGG was achieved in the last twenty years, despite the use of multimodality therapy including surgery, radiotherapy, and chemotherapy. One of the largest pediatric clinical trials that enrolled patients aged 3-22 years with non-disseminated HGGs demonstrated a 3-year event-free survival (EFS) of 0.22 (95% CI, 0.14–0.30) following maximal surgical resection and subsequent radiotherapy administration with concurrent temozolomide, and adjuvant chemotherapy consisting of lomustine. Even more striking is the uniformly-fatal. Hence, there is a significant need for newer therapies to improve the outcomes of malignant brain tumor patients in the recurrent setting, albeit more importantly, ameliorate the pronounced short- and long-term side effects of the current therapies.

2.2 Natural Killer (NK) cells

Immunotherapies along with cell and gene therapy have emerged as promising therapeutic modalities in multiple cancers. Specifically, NK cells have demonstrated tolerability in several hematological malignancies and CNS tumors with preliminary evidence of efficacy. Until recently, the CNS was considered as an immune privileged site, a necessity to strictly regulate the infiltration and local activation of immune cells that may cause irreparable damage in response to immunological insults[9]. However, in a striking study, Louveau and colleagues demonstrated novel lymphatic structures in the CNS[10]. Their data show that circulating immune cells penetrate the

blood brain barrier to perform routine immune surveillance of healthy tissue. This immune surveillance by circulating immune cells, particularly NK cells, can eliminate transformed cells as they arise, delaying the establishment of a tumor burden and the associated immunosuppressive tumor microenvironment[11]. Additionally, Haberthur has also shown that pediatric brain tumors have reduced NK cell-mediated immune surveillance, and a less immunosuppressive tumor microenvironment as compared to their adult counterparts, which indicates that NK cell therapies may have fewer obstacles to overcome in order to successfully eliminate the tumors[12].

Historically, the first description of NK cells was published in 1975[13]. They were described as a class of lymphocytes that exhibited cytotoxicity against leukemic cells in the absence of prior sensitization[13]. NK cells constitute between 5 and 15% of the peripheral blood lymphocyte population[14] and have cytotoxic and regulatory activity[15]. They participate in cancer cell recognition through antibody-mediated cellular cytotoxicity (ADCC)[16] and recognize infected cells or cancer cells that express danger signals including stress ligands, viral proteins and antibodies[15]. One main aspect that differentiates NK cells from T lymphocytes is that their recognition of targets does not depend on HLA antigen presentation. Tumor specific peptides are presented to cytotoxic T lymphocytes (CTLs) in the context of major histocompatibility complex class I molecules on the surface of tumor cells. The tumor cells modify such expression to evade immune surveillance by CTLs, employing editing and downregulation of the surface molecules[17]. The ability of NK cells to eliminate tumor cells that have such altered major histocompatibility complex expression is one reason why they seem to be such attractive candidates for the treatment of cancer. This ability has been dubbed the "missing self" hypothesis [18]. NK cells were initially christened "null cells' because of their lack of T and B cell receptor [15]. They were later defined as CD56-expressing CD3 lymphocytes and subsequently as expressing the NKp46 receptor[15,19].

In addition to their direct anti-tumor activity, NK cells release interferon-γ and other cytokines which result in several anti-cancer effects though cross-talk with the adaptive immune system, including activation of T-cells and dendritic cells, T-cell migration to the cancer and B-cell maturation[20]. In addition, the direct cytotoxicity mediated by NK cells results in tumor antigen release which is processed and presented to T cells[15]. The mechanism by which they ultimately cause cell death is not very different from that employed by CTLs, which release cytolytic granules containing granzyme and perforin that results in apoptosis of the cell[21]. The triggers for such mechanisms include cytokines binding to their receptors on NK cells as well as NK activating receptors (see below) interacting with stress-induced ligands on the surface of transformed cells[22]**.** The activating receptors on NK cells are known to crosslink, one way by which they overcome the natural "brake" on their effector function[23].

As already discussed above, NK cells differ from T cells in that they do not depend on antigen presentation for activation. In this regard, they bypass the critical requirements for therapeutic benefit of antigen-directed immunotherapies such as tumor-infiltrating lymphocytes, vaccines, and checkpoint inhibitors. Instead, they express receptors that allow for their recognition of malignant transformation but also control for self-tolerance. These receptors include killer cell immunoglobulin like receptors (KIRs), natural cytotoxicity receptors (NCRs) and C-type lectin receptors (CD94/NKG2)[20,21]. As their name implies, NCRs are activating. However, KIRs and C-type lectin receptors can be both activating and inhibitory[20]. MHC Class I positive cells provide the "self-signal" which is recognized by inhibitory receptors on NK cells[24,25]. KIRs interacting

with MHC-I on the surface of the cell stop NK cells from lethal interaction with healthy cells[26,27]. Such inhibitory receptors contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tail that activate targets which in turn interfere with activating receptor function. More specifically, they interfere with signaling from immunoreceptor tyrosine-based activating motifs (ITAMs)[26].

The NCRs include activating receptors specific to NK cells such as NKp46, NKp30 and NKp44[28]. There are also activating receptors that are present on CTLs such as NKG2D and DNAM-1[28]. The ligands activating such receptors are tumor-associated antigens (TAAs) which are in turn often present on the cell surface of many cancers[26,27,29,30].

2.3 NK cells studies in brain tumor patients

Lymphokine-activated killer, or LAK cells and NK cells have been infused in patients with brain tumors previously. The results are summarized in Table 1, which represent the composite results of four studies employing such treatment[31,32,33,34]. These studies, though few in number, show the safety of NK cells and responses in certain brain tumors. However, further development of NK therapies was hampered by the inability to produce large amounts of NK cell products, in addition to the difficulty in determining the true effector cells, since some of these NK cell products contained T cells as well.

Table 1: Results of Studies using NK cells in Brain Tumor Patients

<table>
<tr><th colspan="6">Demographics, Pathology & Treatment in Brain Tumor Studies using NK & LAK cells</th></tr>
<tr><td>Number of patients treated/ Gender</td><td>Age Range (years)</td><td>Pathology</td><td>Route of NK cells</td><td>Number of patients receiving NK cells</td><td>Number of patients receiving LAK cells</td></tr>
<tr><td>21/
8 (M) 13 (F)</td><td>12-72</td><td>Gliomas (19)
MB (1)
Melanoma (1)</td><td>IV, IT, and intra-tumoral</td><td>9</td><td>12</td></tr>
<tr><td colspan="6">Response to Therapy & Neurologic Toxicity in Patients Treated with NK and LAK cells</td></tr>
<tr><td>Therapy</td><td>Number of Doses (patients)</td><td>Dose Range (total # of NK or LAK cells)</td><td>Neurologic Toxicity</td><td colspan="2">Response</td></tr>
<tr><td rowspan="3">NK cells only</td><td>1 dose (4)</td><td>0.6-4.3 x 10^9</td><td rowspan="3">Grade 1
(1 patient)</td><td colspan="2" rowspan="3">Response: 5 patients (initial partial)</td></tr>
<tr><td>2 doses (3)</td><td>0.7-3.2 x 10^9</td></tr>
<tr><td>3 doses (2)</td><td>0.6-6.5 x 10^9</td></tr>
<tr><td>LAK cells only</td><td>7-39 doses (12)</td><td>0.31-3.7 x 10^9</td><td>Grade 1
(1 patient)</td><td colspan="2">Response: 7 patients
CR: 3 patients (24, 30 and 90 months)</td></tr>
</table>

M, Male; F, Female; IV, Intravenous; IT, Intrathecal; CR, Complete Response

2.4 Pre-clinical data

Published data did previously demonstrate that purified and activated NK cells can lyse MB cell lines[35]. Laureano and colleagues have shown pre-clinical *in vitro* and mouse data demonstrating that genetically-modified feeder cells propagated/activated NK cells can kill tumors arising from the fourth ventricle[36]. This demonstrates that pediatric brain tumors can be very sensitive to NK cell-mediated killing, and that this killing is relatively uniform across a range of NK cell donors (Figure 1). These data also demonstrate that direct injection of NK cells into the tumor site of mice xenograft was able to mediate tumor control (Figure 2A, 2B), and more importantly that injection to the contralateral side resulted in migration of the NK cells across normal brain into the tumor site, which also provides tumor control and does so without observable damage to normal tissue (Figure 2C).

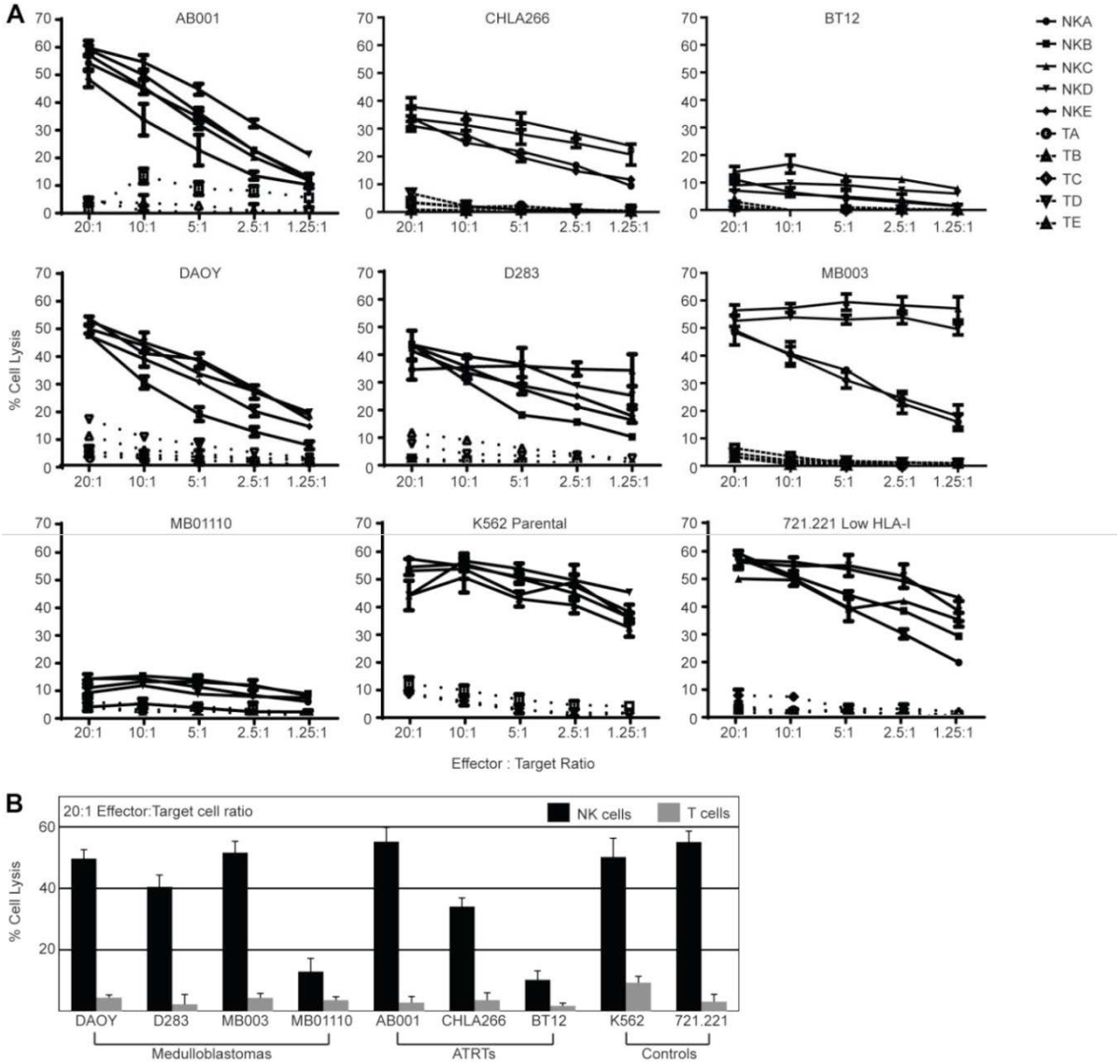
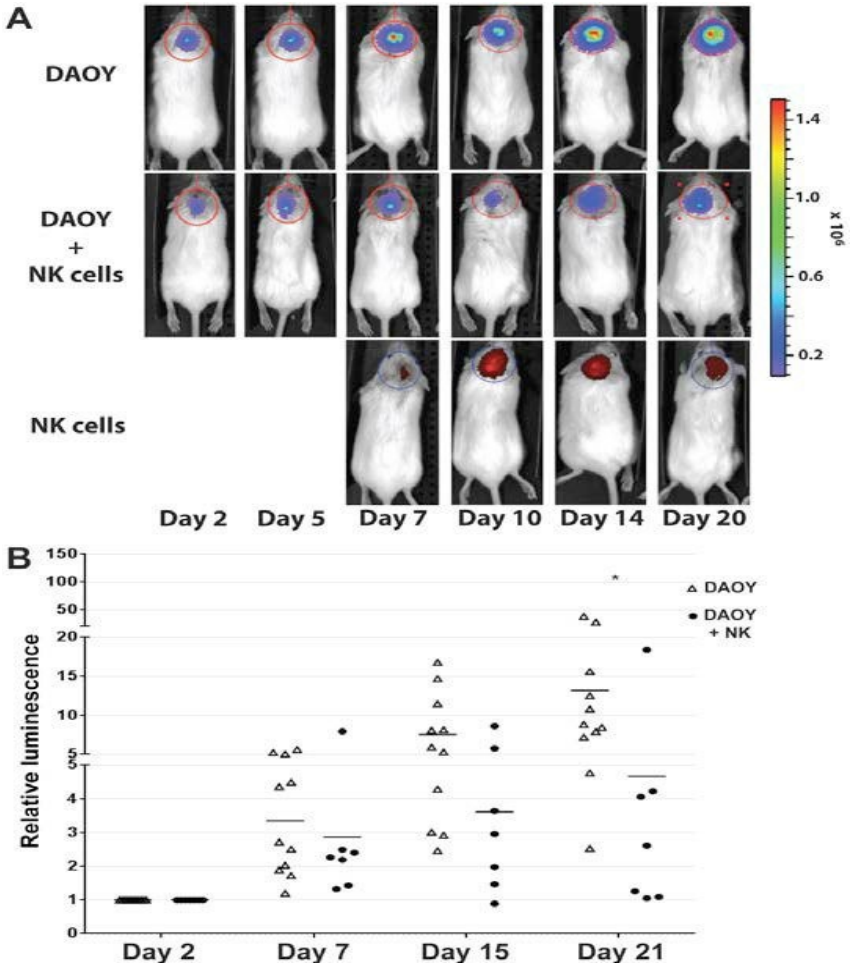


Figure 1: *Ex vivo* expanded NK cells are effective against a panel of medulloblastoma (MB) and atypical teratoid rhabdoid tumor (AT/RT) cells in vitro. The ability of *ex vivo* expanded NK cells to lyse a panel of pediatric brain tumor target cells was determined by chromium release assay. The targets included MB cell lines (DAOY and D283), primary MB cells (MB003 and MB01110), AT/RT

cell lines (CHLA266 and BT12) and primary AT/RT cells (AB001) as well as control NK sensitive cell lines (parental K562 and 721.221 Low HLA-I). The targets were loaded with Chromium 51 (51Cr) and incubated for four hours with NK and T effector cells expanded from five donors at decreasing effector: target ratios. The amount of 51Cr released from the targets was measured using a TopCount microplate scintillation counter (PerkinElmer) and used to calculate the percentage of target cell lysis. A) Line graphs showing the percentage of each target cell lysed by the NK and T effector cells at all effector: target ratios. B) Bar graph showing the average percentage of each target cell lysed by NK effector cells (black) or T effector cells (gray) at a 20:1 effector: target ratio. Error bars in A and B represent the standard deviation.



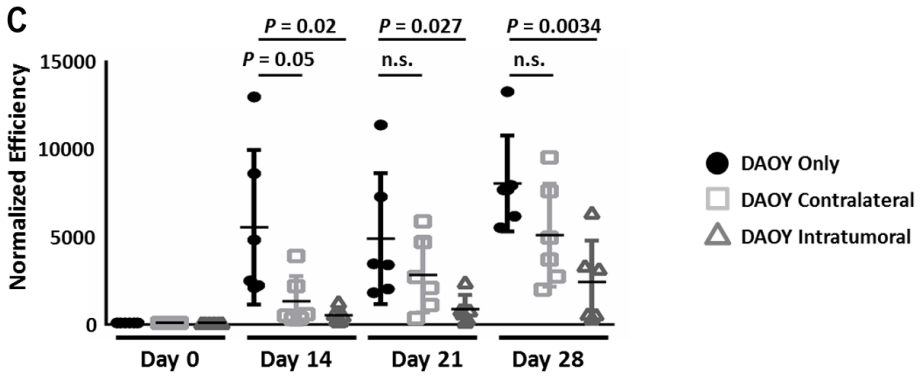


Figure 2: *Ex vivo* expanded NK cells are effective against MB tumors in vivo: To determine whether *ex vivo* expanded NK cells also have efficacy against pediatric brain tumors in vivo, DAOY cells expressing firefly luciferase were implanted in murine cerebella to establish MB xenografts. Subsequently, NK cells or media were injected intra-tumorally once a week beginning on day seven. DAOY luminescence was measured on an IVIS Spectrum (PerkinElmer) following luciferin administration to assess changes in tumor size over time. A) Representative luminescence (top and middle panels) and fluorescence (bottom panels) images of DAOY implanted mice over time. The top panel shows DAOY luminescence in a mouse that received media injections. The middle panel shows DAOY luminescence in a mouse that received NK cell injections. The bottom panel shows the fluorescence from DiR labeled NK cells in a mouse that received NK cell injections. B) Scatterplot showing the change in DAOY luminescence in mice that received NK cell injections (filled circles) compared to mice that received media injections (open triangles) over time. Black bars represent the average change in luminescence at each time point. * = ・ < 0.05. C) Scatterplot of experiment similar to that shown in B, showing tumor burden of untreated mice (closed circles) compared to those that received contralateral (open square) or intra-tumoral (open triangle) NK-cell injections.

To determine the range of pediatric cancers that might be amenable to NK cell therapy, we assessed sensitivity of the 23 cancer cell lines represented by the highly characterized and validated Pediatric Preclinical Testing Program (PPTP) for NK cell lysis by expanded NK cells. The glioblastoma multiforme (GBM) cell line SJ-GBM2 was among the most sensitive of these (Figure 3).

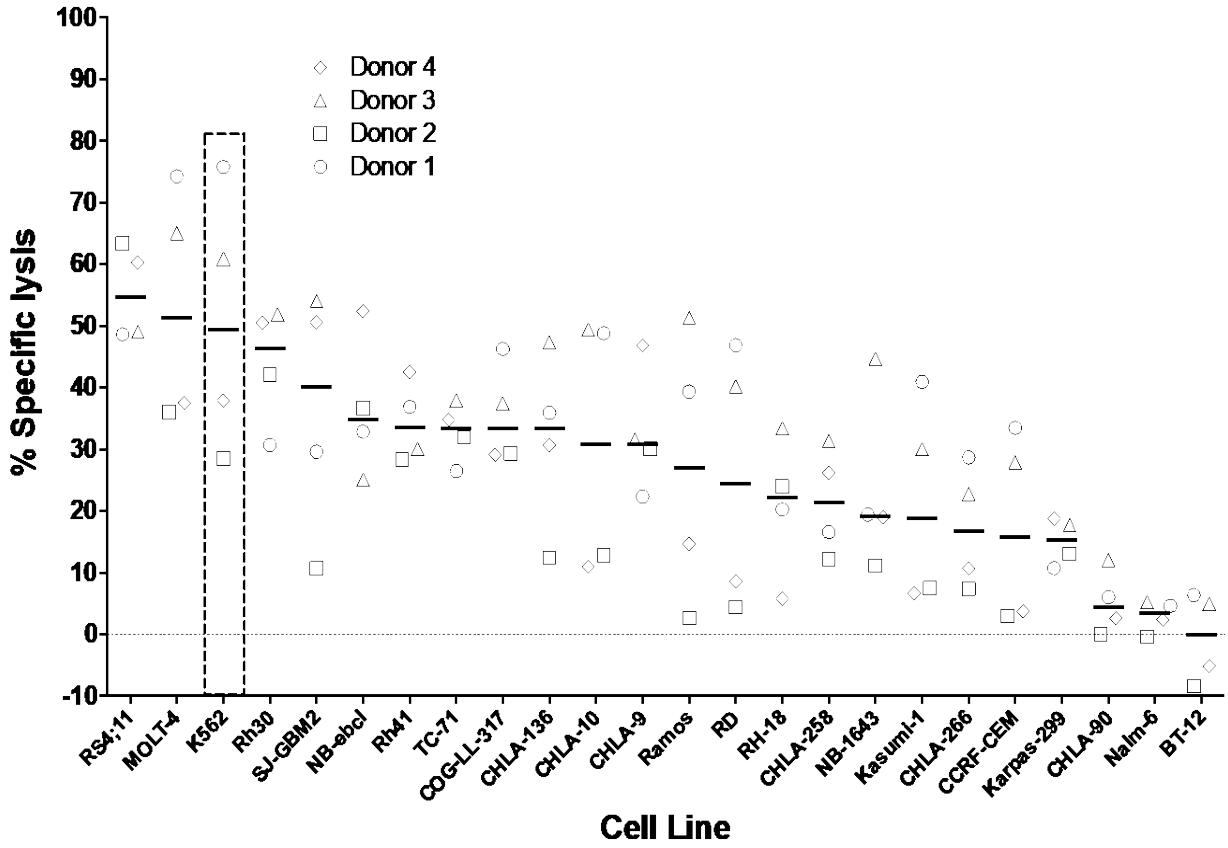


Figure 3: Waterfall plot of NK cytotoxicity against cell lines of the PPTP in vitro panel and K562. Peripheral blood NK cells from four different donors were expanded for three weeks on feeder cells. 4-hour calcein-release cytotoxicity assays were performed in triplicate at four E:T ratios for each NK donor-cell line pair. Percent specific lysis at 2.5:1 E:T ratio is plotted. K562 is shown for reference. Cell lines are ordered by median percent specific lysis (indicated by bar), Ray A *et al*.

2.5 *Ex vivo* activation/propagation of NK cells

A major obstacle for adoptive NK-cell immunotherapy is obtaining sufficient numbers of cells from the small fraction in peripheral blood. These approaches were usually limited by the high cost, donor inconvenience, and low number available from donor apheresis (~10^7 cells/kg), and low proliferation (< 200-fold in 2 weeks) under the previously-used expansion methods. Common gamma-chain cytokines are important in NK-cell activation, maturation, and proliferation. Others have described improved *ex vivo* numeric expansion with soluble cytokines, genetically-modified feeder cells, and genetically-modified feeder cells engineered with co-stimulatory molecules such as membrane-bound IL-15 (mIL-15).

We previously demonstrated that NK cells can be robustly propagated to large numbers from peripheral blood mononuclear cells (PBMCs) by co-culture with feeder cells fashioned from K562 cells and genetically modified to express co- stimulatory molecules, including membrane-bound IL-21 (mbIL21)[37]. We found that 2 weeks of expansion achieves a mean > 3,000-fold expansion, sufficient to generate enough NK cells from 3 mL/kg of peripheral blood to deliver multiple infusions of 10^8 cells/kg[37]. We also demonstrated that the mbIL21-expressing feeder cells promote sustained proliferation of mature NK cells without senescence by increasing the

telomere length in the expanded cells. The method enables large-scale expansion of NK cells from a small volume of peripheral blood, sufficient to deliver multiple infusions of NK cells at high cell doses.

We assessed whether expansion with mbIL21 might result in altered NK-cell phenotype, and assessed surface expression of the major NK-cell receptors on expanded NK cells. Although there was variation between donors, particularly in KIR expression, we found no significant difference in the KIR repertoire of expanded NK cells. Of note, the expanded NK cells had very high expression of CD16 (FcγRIIIa, the primary receptor in NK cells responsible for ADCC) and natural cytotoxicity receptors, and demonstrated high levels of cytotoxicity even after cryopreservation[37].

At Nationwide Children's Hospital (NCH), we have re-derived this genetically-modified feeder cell (CSTX002) and established master and working cell banks (MCB, WCB) that are fully tested in compliance with FDA regulation, and have validated manufacturing of clinical-grade NK cells in compliance with current Good Manufacturing Practice (cGMP).

2.6 Clinical Experience

The initial clinical experience with adoptive transfer of NK cells expanded *ex vivo* with mbIL21-expressing feeder cells was conducted in the context of haploidentical stem cell transplant (haploSCT) for myeloid leukemias. In this setting, NK cells were delivered intravenously at doses up to 10^8 NK cells/kg without infusion- or dose-related toxicities. This manufacturing approach generated NK cell products of high purity (median 98.98% NK cells, median 0.02% T cells) and viability (median 97%)[38]. When compared to 83 historic case-matched controls from the Center for International Blood and Marrow Transplantation Research (CIBMTR) who received haploSCT without NK cells, disease-free survival (DFS) increased from 50% to 85% ($p = 0.03$), which was largely mediated by improvement in relapse (35% to 8%, $p = 0.058$) (personal communication, D. Lee).

NK cells have also shown *in vitro* and *in vivo* activity against brain tumors, including GBM and medulloblastoma[39-42]. Furthermore, the group at MD Anderson Cancer Center (MDACC) initiated a Phase I clinical trial (NCT02271711) to expand and infuse autologous *ex vivo* expanded NK cells locoregionally into the fourth ventricle in children who have undergone resection of recurrent infratentorial tumors, including MB, AT/RT and ependymoma. In this trial, autologous NK cells were expanded from peripheral blood, cryopreserved, and infused three times per week (Dose level 1-2) or once a week (Dose level 3+) for three weeks, for up to three cycles. Nine patients achieved successful expansion of their NK cells, and up to 112 intraventricular infusions were administered at doses up to $3x10^8/m^2$/infusion[43]. As with the previous study, the expanded NK cell product was pure, typically containing < 1% contaminating T cells. The infusions of NK cells into the fourth

ventricle were well-tolerated, demonstrating safety with no dose-limiting toxicity attributable to the infused NK cells. Cerebrospinal fluid (CSF) samples were obtained prior to each infusion to evaluate the persistence of the infused NK cells and their effect on the local immune milieu. Significant CSF pleocytosis without evidence of infection is seen in patients receiving NK cells, representing accumulation of NK cells in some patients (Figure 4), and in some patients with $CD3^+$ T cells outnumbering NK cells, suggesting that the infused NK cells promote a pro-inflammatory environment that mediates T-cell migration to the site.

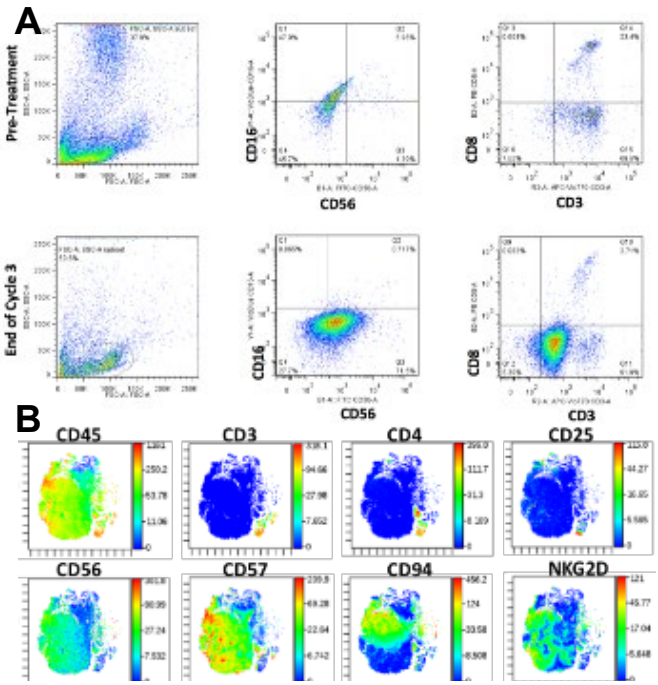


Figure 4: A) Cytometric analysis of pleocytosis in CSF samples acquired before and after 3 cycles of intra-ventricular administration of autologous expanded NK cells, demonstrating persistence and impact on T cell populations. B) CyTOF analysis of CSF after 3 cycles, showing distinct NK and T cell cell subsets with minimal Treg component.

We aim to expand on the successful results of that trial by evaluating the tolerability and feasibility of intra-tumoral infusions of expanded *ex vivo* NK cells *via* an Ommaya reservoir in patients with recurrent or progressive malignant brain tumors. Injecting NK cells directly into the tumor cavity will help enhance the therapeutic efficacy of these cells by overcoming the challenges associated with the need to cross the blood brain barrier as well as maximally concentrating the NK cells in close proximity to the tumor.

The results from the MDACC Phase I trial have demonstrated safety of intraventricular NK cells infusions in pediatric MB and AT/RT patients. Hence, we would not anticipate any differences in the feasibility or tolerability of this novel technology in other malignant brain tumors. Children with recurrent or progressive malignant brain tumors will be enrolled on our proposed Phase I trial after undergoing gross total or subtotal resection of the tumor. Participants will receive 3 cycles of NK cell infusions over 12 weeks. Each cycle will consist of three weekly injections followed by a rest week (week 4).

2.7 Alloreactive donor NK cells:

NK cells are regulated by KIR receptor-ligand interactions and are cytotoxic against certain HLA class I mismatched targets. Alloreactive HLA haploidentical NK cells in the hematopoietic cell transplant (HCT) setting have been reported to enhance engraftment, reduce GVHD and prevent relapse of leukemia. Based on the hypothesis that a mismatch between inhibitory KIR on NK cells and MHC molecules on tumor would lead to higher cytotoxicity, Ruggeri *et al* [44] showed that leukemia patients undergoing haploidentical T-cell depleted transplants had reduced relapse rates

when they had KIR ligand mismatch with their donors (0% *versus* 75%), a stratification that also correlated with finding anti-recipient NK cell clones in these patients. Large retrospective trials have confirmed the benefit of KIR mismatch in a variety of transplant settings.[45,46]

In a non-transplant cell therapy setting, poor-prognosis AML patients received adoptive immunotherapy with haploidentical NK cells, 3 of 4 (75%) KIR ligand mismatched patients achieved a complete response (CR) compared to only 2 of 15 (13%) KIR ligand matched patients, demonstrating an association between KIR ligand mismatch and induction of remission.[47] The NK cell products were obtained by steady-state leukapheresis followed by immunomagnetic depletion of T cells and overnight IL-2 activation. In the 36 products delivered in the final dose cohort, the final IL-2-activated product contained an NK cell dose of 8.5 x 10^6 cells/kg and a final T-cell dose of 1.75 ± 0.3 x 10^5 cells/kg. The cells were delivered after high-dose cyclophosphamide and fludarabine (Hi-Cy/Flu) lymphodepletion, and all patients received subcutaneous IL-2 after the infusions. Compared with the low-intensity Cy/Flu regimen in non-AML patients, infusions after the more intense Hi-Cy/Flu resulted in expansion of NK cells *in vivo* as shown in PCR-based chimerism assays. Donor NK cells recovered from recipient peripheral blood were functional in cytotoxicity assays.

In a similar study, Rubnitz *et al* reported the safety of infusing haploidentical KIR-mismatched NK cells as consolidation therapy for children with AML in remission.[24] In this trial patients also received a Hi-Cy/Flu regimen, NK cells, and IL-2. The cell infusion contained a median 29.2 x 10^6 NK cells (5.2 – 80.9). With a median follow up of 964 days, all 10 patients remained in remission. No GVHD was observed. Six patients with B cell NHL were treated with Hi-Cy/Flu, allogeneic NK cells, IL-2, and rituximab. The NK cell dose in this trial was 21 ± 19 x 10^6 NK cells/kg (mean ± SEM), with a final T cell dose of 8 ± 5 x 10^4 cells/kg. With a median follow up of 964 days, all 10 patients remained in remission. The NK cell infusion was associated only with Grade 1-3 fever and rigors, and no GVHD was observed. Two subjects achieved CR, two partial remissions (PR), and two had no response.[48]

Two pediatric patients were treated with two donor NK cell infusions each at approximately one month and three months after haploidentical transplant for relapsed neuroblastoma. The products contained 7.8 – 45.1 x 10^6 NK cells/kg and 7.3 – 13 x 10^3 T cells/kg.[49] No severe side effects were observed after the four infusions and no GVHD was observed. NK cells have been expanded *ex vivo* and delivered to patients for adoptive cancer immunotherapy with similar results. Barkholt *et al* [50] reported treating five patients after allogenic transplant with recipient-derived allogeneic NK/NKT cells that had been expanded for 19 days using OKT3 and IL-2. Three to six infusions were given to each patient in escalating doses for a total of 19 product infusions, with a median CD56+ cell dose of 13.2 x 10^6/kg (8.1 – 40.3) in the highest dose level. Infusion-related toxicities were limited to fever and rash, and one case of Staphylococcus epidermidis septicemia. Allogeneic NK cells expanded for 20-23 days with IL-15, and hydrocortisone were administered to 16 patients with advanced non-small cell lung cancer.[51] Each subject received 2-4 infusions of the cells for a total of 42 NK cell infusions. The median NK cell dose was 4.15 x 10^6/kg (0.2 – 29). The T cell dose was not reported, but the infusion products were reported to be 92.4% CD56+CD3- (82.7–99.6%). They reported no side effects related to the NK cells from any of the 42 infusions.

Our group performed a phase I study for myeloid malignancies, infusing escalating doses of NK

cells from an HLA haploidentical third party donor prior to HLA-matched allogenic transplant. The goal of infusing third-party alloreactive NK cells was to augment the anti-leukemic effect of the transplantation without worsening GVHD and, thus, improve the overall outcome of hematopoietic transplantation.[38] Median relapse-free, overall, and GVHD-free/relapse-free survival for all patients enrolled was 102, 233, and 89 days, respectively. There were non-significant trends toward higher survival rates in those receiving NK cells from KIR ligand–mismatched donors and KIR-B haplotype donors. This trial demonstrated a lack of major toxicity attributable to third-party NK cell infusions delivered in combination with an HLA-compatible allogeneic transplantation. The infusion of haploidentical alloreactive NK cells did not interfere with engraftment or increase the rate of GVHD after allogeneic hematopoietic transplantation. Efficacy was potentially limited by the relatively low dose of NK cells that could be obtained.

2.8 Selecting the "Ideal" Donors to Generate a Consistent and Potent "Off-The-Shelf" NK Cell Therapeutic Product

NK cells are licensed (that is, acquire enhanced killing ability) when they express inhibitory KIR for self-HLA class I molecules. This enables NK cells to recognize "self" and spare normal healthy cells from killing. Targets lacking self-HLA class I molecules (common in virus-infection and malignant transformation) are thus more likely to elicit recognition by licensed NK cells. The inhibitory KIR genes known to be relevant for NK alloreactivity are: (i) 2DL1 which binds to HLA-C group 2 alleles, (ii) 2DL2 and 2DL3 which bind to HLA-C group 1 alleles, (iii) and 3DL1 which binds to HLA-B Bw4 alleles. According to the KIR-ligand mismatch model, the potential for alloreactivity of a KIR-expressing NK cell will be increased if the corresponding ligand is present in the donor to induce licensing, and absent in the recipient such that inhibition is absent. For example, any donor possessing a Group C1 allele will be alloreactive to any individual lacking a Group C1 allele. Thus, donors who possess HLA in the C1, C2, and Bw4 families are predicted by this model to be alloreactive against the greatest number of recipients- any recipient lacking C1, or C2, or Bw4 (Table 2).

Table 2: Analysis of licensed KIR and KIR-ligand interactions for different HLA-Bw and -C group loci as predictors of donor GVL alloreactivity.

		Donor					
Recipient		**C1*C1**	**C1*C2**	**C2*C2**	**C1*C1*Bw4**	**C1*C2*Bw4**	**C2*C2*Bw4**
	C1*C1	No GvL	GvL	GvL	GvL	GvL	GvL
	C1*C2	No GvL	No GvL	No GvL	GvL	GvL	GvL
	C2*C2	GvL	GvL	No GvL	GvL	GvL	GvL
	C1*C1*Bw4	No GvL	GvL	GvL	No GvL	GvL	GvL
	C1*C2*Bw4	No GvL	No GvL	No GvL	No GvL	No GvL	No GvL
	C2*C2*Bw4	GvL	GvL	No GvL	GvL	GvL	No GvL

Whereas inhibitory KIRs prevent alloreactivity, activating KIRs (aKIR) recognize activating ligands that promote NK cell lysis.[52] Inheritance of activating KIR is widely variable- 0 to 7 aKIR are possible in any one individual. Data from patients undergoing stem cell transplantation show that patients receiving allografts from donors with more activating KIRs have a better outcome than patients receiving allograft from donors with fewer activating KIR. Similarly, we have shown that NK cells with higher numbers of activating KIR induce stronger lysis of target cells (Figure 5). In addition, the presence of activating KIR 2DS1 and 3DS1 have been associated with disease-

free survival in multivariate analysis [53].

NKG2C is an activating receptor that is expressed late in NK cell development and recognizes HLA-E rather than –B or –C [54]. NKG2C expression is induced in patients with CMV infection [55] and correlates with an adaptive NK cell phenotype [56] and improved cancer-free survival [57].

Thus, we hypothesize that the "optimal" donor will be one who has an HLA genotype carrying C1, C2, and Bw4 alleles, has a KIR genotype possessing the inhibitory KIR that bind to C1, C2, and Bw4 (leading to maximum licensing), has a high proportion of activating KIR, and has been exposed to CMV resulting in high NKG2C expression. Considering data available for Caucasian donors, C1/C2/Bw4 alleles occur in 32% of the population. Of the 23 KIR genotypes that account for 80% of the population, 25.3% meet all of these criteria. ~90% of adults will have been exposed to CMV. Thus, the "ideal" NK cell donor can be identified in approximately 1 out of 16 healthy individuals.

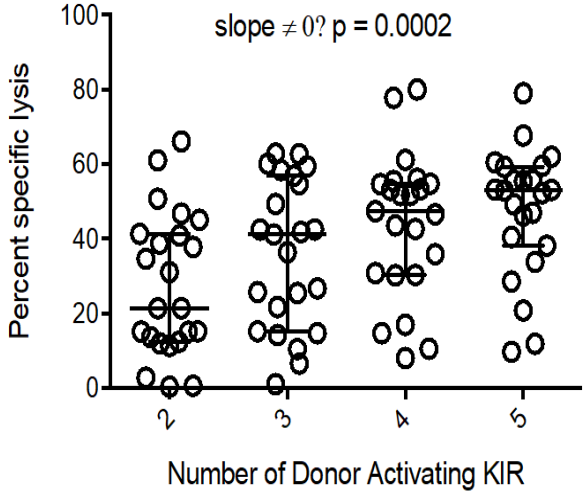
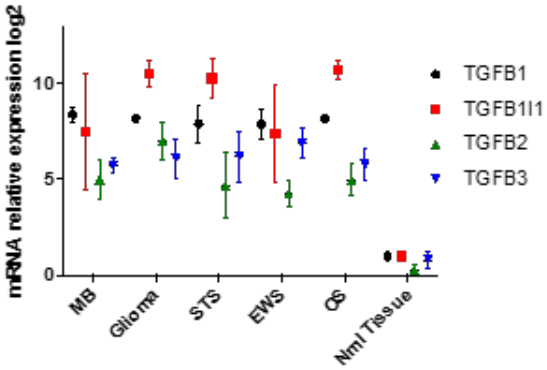


Figure 5: Correlation of NK cell determinants with sensitivity to NK cell lysis against cell lines in the PPTP panel. KIR genotyping was performed for NK cell donors and cytotoxicity determined against the PPTP cell line panel. Specific lysis for each pair is stratified donor activating content.

2.9 TGFβ induced immunosuppression of NK cells

The immunosuppressive role of the tumor microenvironment is well-described. A key contributor to this immunosuppression is transforming growth factor-beta (TFGβ) secreted by tumor cells and tumor-associated macrophages. In addition to direct pro-tumorigenic effects on cancer cell growth, TGFβ acts as an immunosuppressive cytokine that inhibits T, B, and NK cell function. Specifically, TGF-β induced phosphorylation of SMAD3 in NK cells leads to decreased IFNγ production[58] and decreased anti-tumor cytotoxicity with phenotypic down-regulation of the activating receptors NKG2D, NKp30, DNAM-1, TRAIL, and CD16[59-63].

Production of TGFβ family members by pediatric cancer cell lines is significantly higher than that of normal tissue (Figure 6), and pediatric tumors rank among the highest in TGFβ production of all cancer types tested. Levels of TGFβ are known to be higher in the serum of pediatric patients diagnosed with solid tumor malignancies when compared to age-matched, non-cancerous controls.[64] TGFβ is overexpressed by glioma cell lines, and in patients with gliomas, it is thought to contribute to tumor cell



proliferation, migration and invasion, angiogenesis and immune suppression/evasion[65-67].

While GBM is sensitive to NK cell killing *in vitro*, NK cells derived from the GBM tumor microenvironment have an altered phenotype with decreased expression of activating receptors (NKp30, NKG2D, DNAM-1) that correlates with impaired cytotoxicity against GBM.[68] Moreover, tumor-derived NK cells had increased expression of CD9 on the surface, which was previously shown to be upregulated upon exposure to TGFβ.[62] TGFβ expression is 33-fold higher in GBM tumors compared to non-tumoral samples and high expression in newly-diagnosed patients is significantly correlated with a poorer OS. [69] Epigenetic studies of AT/RT-SMARCB1 deficiency demonstrate an over-representation of TGFβ signaling pathway members, suggesting that TGFβ signaling is important in the biology of AT/RT.[70,71]

Figure 6: Expression of selected TGFB members in sarcomas and brain tumors. RNA expression data was retrieved from the CCLE database for various TGFB cytokines in tumor cell lines or normal tissues.

2.10 TGFβ imprinted NK cells

We previously described a method for propagating large numbers of clinical-grade NK cells *in vivo* with IL-2 and irradiated K562 feeder cells expressing membrane-bound IL-21 and 4-1BBL.[37,72] Subsequently, we described a modification of that method that enhances NK cell function and overcomes TGFβ-induced suppression [referred to as TGFβ "imprinting" (TGFβi)][73] by chronically stimulating the NK cells with TGFβ (10 ng/mL) during the expansion process. The addition of TGFβ during the expansion process impairs neither fold expansion nor viability of the final expanded NK cell product, but the resulting TGFβi NK cells exhibit high cytotoxicity and a pro-inflammatory hypersecretion of interferon-γ and tumor necrosis factor-alpha in response to tumor targets (Figure 7). Moreover, these cells significantly downregulate SMAD3 at the transcriptional level, resulting in resistance to suppression by TGFβ (Figure 7). Importantly, this cytokine hypersecretion persists for one month after removal of TGFβ, suggesting that the TGFβi NK cells may retain their enhanced cytokine secretion *in vivo*, where IFNγ and TNFα secretion can then stimulate adaptive immunity and sensitize tumors to NK cell killing (Figure 8).[74-76]

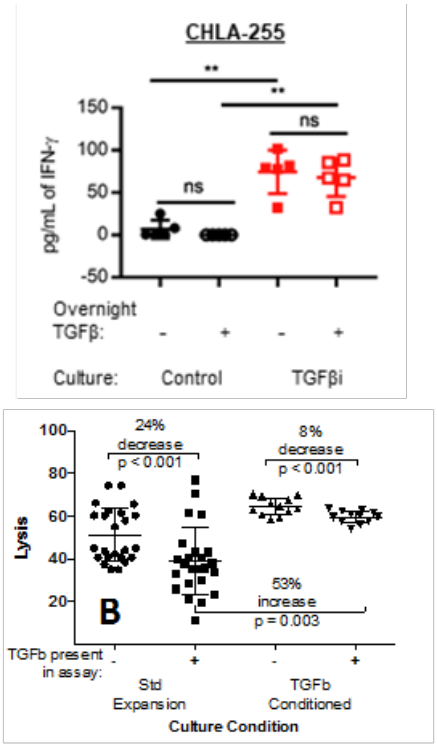


Figure 7: TGFB-imprinting enhances NK cell function and TGFB-resistance. TGFB1 imprinting increases IFNy production (top) and cytotoxicity (bottom), and reduces the suppressive effect of TGFB for both.

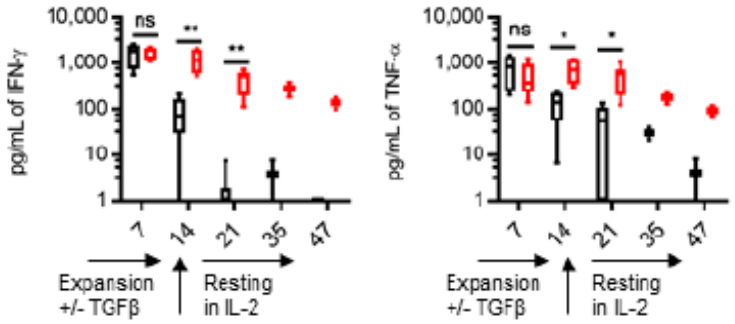


Figure 8: TGFβi (red) and control NK cell (black) anti-tumor cytokine secretion at day 7 and 14 of expansion and after removal from expansion conditions at Day 21, 35, and 47. Cells were co-cultured with DAOY (MB cell line) for 3h and supernatants were collected

2.11 TGFβ imprinted universal donor NK cells

We have collaborated with Be The Match Biotherapies (BTMB) to identify individuals with the above UD NK cell characteristics from their database of 10,000-donors with KIR genotyping and HLA typing. Donor selection, collection, and expansion of the NK cells is performed under a separate donor protocol, resulting in a UD cell bank of TGFβi NK cells. After consent and verification of donor eligibility, NK cells are collected *via* apheresis and then undergo CD3 depletion followed by a 2-week expansion. TGFβi NK cells are then generated by weekly stimulation with irradiated feeder cells (K562 expressing membrane bound IL-21 and 4-1BBL) and cultured in IL-2 and TGFβ.[73] After 2 weeks, the TGFβi NK cells are washed and cryopreserved in aliquots for future clinical use.

We propose to utilize UD TGFβi NK cells in patients with recurrent WHO Grade III/IV malignant brain tumors. Adoptive transfer of haploidentical NK cells has been shown to be safe and effective in clinical trials for AML and other pediatric and adult hematologic and solid tumor malignancies. In the hundreds of patients who have been treated in this setting, there has been no NK cell related severe GVHD reported, regardless of HLA matching. There is clear evidence that KIR ligand mismatch and high activating KIR content improves NK cell anti-tumor cytotoxicity. In addition, NK cell expansion from heavily pretreated malignant brain tumor patients leads to a low NK cell yield in our previous clinical experience (unpublished). For these reasons, having a readily available, off the shelf, optimal donor expanded NK cells is beneficial in this high-risk population. TGFβi NK are resistant to the immunosuppressive effects of TGFβ and secrete high levels of proinflammatory cytokines. *We hypothesize that adoptive transfer of universal donor TGFβi NK cells to participants with recurrent or progressive malignant brain tumors will be safe and improve outcomes.*

2.12 Correlative Studies Background

Although childhood and adult malignant brain tumors share a related histopathological appearance and comparable clinical outcomes, it is now becoming apparent that these tumors are molecularly distinct entities with differing genomic and epigenomic landscapes[77].

Due to the numbers and types of different alterations that require evaluation for correlative studies, as well as planned evaluation of the mutational and neoantigen burdens for each patient's tumor, we plan to perform next generation sequencing (NGS) assays to evaluate this complex combination of somatic profiles. The exception to this set of evaluations is the methylation status of the MGMT promoter, which will be performed as a site-specific bisulfite PCR and sequencing-based evaluation according to a standard operating procedure already in place in our CAP/CLIA laboratory. Frozen tissue from this re-resection/biopsy is required to be submitted for biomarker studies, if available.

2.12.1 **Mutational landscape studies *via* enhanced whole-exome sequencing (eWES)**

We hypothesize that specific gene alterations will correlate with differences in the response to treatment. To this end, fresh frozen tumor specimens (and cognate matched non-tumor tissue) will be processed for paired tumor/normal enhanced whole exome sequencing at our Institute for Genomic Medicine to identify somatic alterations in the form of point mutations, insertions and deletions, and copy number changes. We will isolate DNA and RNA from the tumor and DNA from the matched normal specimen. DNA from tumor and normal isolates will be evaluated by Agena genotyping assay to ensure both are derived from the same individual prior to sequencing and other assays. Whole genome libraries will be constructed from the DNA isolates of tumor and normal, wherein each library receives adapters containing universal molecular identifier (UMI) barcodes that permit library pooling and subsequent read deconvolution and sample-specific assignment based on barcode identity. Libraries will be quantitated and concentration normalized prior to equimolar pooling with an aim for 300-fold Illumina read coverage by 150 bp paired end reads. Prior to sequencing, we will perform hybrid capture on the pooled libraries using a unique exome reagent that provides optimized coverage across the coding genes (exome) as well as delivering high resolution copy number information across all chromosomes with a focus on cancer-relevant regions of the genome. In particular, this reagent combines the IDT X-Gen Lockdown Exome reagent (IDT, Coralville IA), and a cancer CNV probe set, also from IDT. The latter probe set is a specific reagent that contains a mixture of 1) hybrid capture (X-Gen Lockdown 120 bp) probes spaced evenly at approximate 30kb intervals across all chromosomes, and 2) increased hybrid capture probe density at regions known to be amplified or deleted at high frequency in human cancers (HER2, EGFR). We have extensive experience with this combined hybrid capture probe set, which we refer to as 'enhanced whole exome sequencing' (or eWES) and have determined that the copy number resolution compares favorably to analyses derived from whole genome sequencing data, in terms of the ability to delineate copy number alterations at high resolution. Once the hybridization is complete, we will process the samples by selectively binding probe:library fragments to streptavidin magnetic beads (X-Gen Lockdown probes are biotinylated), and removing excess probes and library fragments that remain in solution. After washing to reduce spurious hybridization artifacts, the captured library fragments are released by denaturation, amplified and quantitated. We produce an optimal library concentration prior to amplifying the library on the surface of an Illumina HiSeq 4000 flow cell and producing paired end, 150 bp sequencing reads.

Data analysis from the Illumina sequencing instrument data takes place in our Amazon Web Services cloud computing environment, where the reads are streamed from the sequencer as they are produced in the sequencing process, and once the run completes, are converted to fastq format sequence files. These files are binned according to the UMI adapter barcode into patient-specific

bins for tumor and normal. Each bin of reads is then aligned to the Human Reference genome version GrCh38 using the Churchill aligner (Kelly *et al*., Genome Biology 2016), and the aligned reads are evaluated by different algorithms to identify point mutations, insertion/deletion variants and for copy number alterations. The resulting variants are compared between tumor and normal datasets to identify variants of all types as tumor-specific (somatic) or as shared with both data sets (germline). The final step in this process is the interpretation of the point mutation and indel variants using VEP, which assigns the impact of each DNA variant onto the final protein sequence. The composite result of single nucleotide and indel variants and copy number altered regions for each patient is written to vcf format file and stored on local compute disks for subsequent analysis.

In addition to subtyping of each tumor based on previous definitions, described above, we will be able to evaluate the total tumor mutational burden (TMB) based on the eWES data analysis. Beyond TMB, we can identify the HLA haplotypes of each patient from the eWES data using the OptiType algorithm from the DFCI group (Cathy Wu) and input this along with the identified somatic variants to the pVACseq pipeline developed by Elaine Mardis and colleagues at Washington University School of Medicine in St. Louis[78]. The output of this pipeline is a defined list of neoantigens, corresponding calculated binding affinities for each HLA class 1 and 2 molecule, and cognate wildtype peptide binding affinities.

2.12.2 Assessment of immune gene expression patterns and TCR diversity using Nanostring panels

As above, we believe that specific gene expression signatures will correlate with treatment response. Here, we plan to compare the mRNA expression patterns of specific immune genes before and after treatment with NK cells. To this end, RNA isolated from tumor tissue will be evaluated using a novel, multiplexed gene expression assay "nCounter® PanCancer Immune Profiling Panel" (NanoString Technologies, Inc., Seattle, WA) to characterize and quantify the infiltrating immune cell type populations in each patient's tumor. The PanCancer Immune Profiling assay is run on the nCounter® analysis system, which is based on a digital color-coded barcode technology and offers many advantages, including: a) multiplex detection of the expression of hundreds of gene targets from a tumor mRNA isolate in a single assay, without need for amplification or enzymatic digestion; b) single molecule imaging with high precision and sensitivity; c) fully-automated software with complete digital detection (i.e., computationally "simple"), d) capability to assay degraded RNA from formalin fixed paraffin embedded (FFPE) tumor specimens. Specifically, the PanCancer Immune Profiling Panel measures expression of 770 immune-related genes based on known marker genes whose expression levels can identify 24 different infiltrating immune cell types. These profiles can be evaluated in the context of treatment response or non-response based on the clinical trial outcomes. In patients who progress under therapy, we may also have the opportunity to investigate changes in their immune infiltrates by studying RNA derived from subsequent biopsies.

As a secondary study, we will profile the RNA isolates from the brain cancers we have banked to identify the T-cell receptor (TCR) repertoire present in the tumor tissues. This study involves a custom assay that was devised by our collaborator, Dr. Dean Lee, and provides information about the specific identities and proportions of VDJ recombination events that lead to TCR specificity for neoantigen targets. In addition to profiling the TCR, we will attempt to correlate its diversity for each tumor with the neoantigen predictions performed for the same tumor, as described above.

If other technologies come out during the duration of the study that are applicable, they may also be considered and utilized.

2.12.3 **Immune System Studies**
Up to 3 mL of fluid from the tumor bed/cavity will be collected from the Ommaya at the time of the first NK cell infusion in each of the 3 cycles. These samples and the expanded TGFβi NK cell product itself will be assessed for phenotype and function to estimate TGFβi NK cell persistence and anti-tumor activity. These assays will include:

- Flow or mass cytometry to assess NK cell phenotypes as feasible based on cell recovery
- The function of NK cells will be assessed by direct lysis assays or flow-based activation assay for CD107a expression in response to standardized targets.
- Luminex, Cytokine bead array (CBA), or similar assay will be used to assess the tumor inflammatory microenvironment by quantifing several cytokines, chemokines, and growth factors.

If other technologies come out during the duration of the study that are applicable, they may also be considered and utilized.

3. STUDY DESIGN

3.1 Characteristics

This is a multi-center, phase 1, single arm, open label dose escalation study. The primary outcomes are to evaluate the safety, tolerability, and recommended phase 2 dose of UD TGFβi NK cells in participants with recurrent or progressive malignant brain tumors.

3.2 Number of Participants

We anticipate enrolling 3-6 participants per dose level.

3.3 Inclusion Criteria

3.3.1 Participants must have a histologically-confirmed recurrent or progressive malignant brain tumor including, but not limited to, infant-type hemispheric glioma, gliosarcoma, intracranial sarcoma and WHO Grade II ependymoma.

3.3.2 Participants should be candidates for resection of the recurrent tumor and be deemed candidate for placement of an Ommaya reservoir placed intra-cavitary/intra-tumoral; measurable residual tumor after surgery is not required for study entry. Pre-operative imaging needs to estimate that the resection cavity will be at least 2 cm x 2 cm in two dimensions for participants to be eligible.

3.3.3 Given the lack of a standard of care treatment for children with recurrent or progressive malignant brain tumors, participants must have completed first-line treatment with radiation and/or chemotherapy prior to participating in this trial if applicable.

3.3.4 All participants must be ≥ 1 year of age and ≤ 39 years of age at the time of entry into the study. The first 3 participants must be ≥ 8 years of age and ≤ 39 years of age at the time of entry into the study.

3.3.5 Performance Score: Karnofsky ≥ 50 for participants > 16 years of age and Lansky ≥ 50 for participants ≤ 16 years of age (See Appendix A). Participants 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.

3.3.6 Must have recovered from the acute toxic effects of prior therapy (i.e., NCI-CTCAE version 5, grade 1 or less)

- An interval of at least 12 weeks must have elapsed since the completion of radiation therapy
- Chemotherapy/biologic therapy: All cytotoxic chemotherapy/biologic therapy must be discontinued ≥ 7 days prior to enrollment (except 3 weeks for temozolomide and 6 weeks from last dose of nitrosoureas)
- Immunotherapy: The last dose of anti-tumor antibody therapy must be at least 3 half-lives or 30 days, whichever is shorter, from the time of enrollment.
- For targeted agents only, patient should have recovered from any toxicity of the agent and have a minimum of 2 weeks since the last dose
- For participants who have received prior bevacizumab, at least 4 weeks is required

3.3.7 Organ Function Requirements

3.3.7.1 Adequate Bone Marrow Function Defined as:

- Peripheral absolute neutrophil count (ANC) ≥750/mm3
- Platelet count ≥75,000/mm3 (transfusion independent, defined as not receiving platelet transfusions for at least 7 days prior to registration).

3.3.7.2 Adequate Renal Function Defined as:

- A serum creatinine ≤ 1.5 x upper limit normal (ULN) based on age/gender

3.3.7.3 Adequate Liver Function Defined as:

- Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age; in presence of Gilbert's syndrome, total bilirubin ≤ 3 x ULN or direct bilirubin ≤ 1.5 x ULN
- ALT ≤ 3 x ULN
- AST ≤ 3 x ULN

3.3.7.4 Adequate Neurologic Function Defined as:

- Participants with seizure disorder may be enrolled if seizures are well-controlled. Participants on non-enzyme inducing anticonvulsants may be excluded pending interaction(s) with study drug.
- Signs and symptoms of neurologic deficit must be stable for ≥ 1 week prior to registration

3.3.8 The effects of TGFβi NK cells on the developing human fetus are unknown. For this reason and because TGFβi NK cells as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and 6 months after completion of TGFβi NK cells administration. 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.

3.3.9 Participants must enroll on PNOC COMP if PNOC COMP is open to accrual at the enrolling institution.

3.3.10 A legal parent/guardian or patient must be able to understand, and willing to sign, a written informed consent and assent document, as appropriate.

3.3.11 . Corticosteroids: Participants who are receiving dexamethasone must be on a stable or decreasing dose for at least 1 week prior to registration. The patient steroid dose should be no more than a steroid-equivalent of dexamethasone 0.1 mg/kg/day (or maximum 4mg/day; whichever is the lower dose) at time of enrollment.

3.4 Exclusion Criteria

3.4.1 Tumor involvement that would require ventricular or brainstem injection or access through a ventricle or significant risk of ventricular penetration in order to deliver the TGFβi NK cells.

3.4.2 Participants undergoing needle or open biopsy.

3.4.3 Participants who are receiving any other investigational agents.

3.4.4 Women of childbearing potential must not be pregnant or breast-feeding.

3.4.5 Evidence of active uncontrolled infection or unstable or severe intercurrent medical conditions.

3.4.6 Any medical condition that precludes surgery.

3.4.7 Prothrombin time/international normalized ratio (PT/INR) or partial thromboplastin time (PTT) > 1.5 x ULN.

3.4.8 Participants with a known disorder that affects their immune system, such as human immunodeficiency virus (HIV), or an auto- immune disorder requiring systemic cytotoxic or immunosuppressive therapy are not eligible.

3.4.9 Evidence of bleeding diathesis or use of anticoagulant medication or any medication which may increase the risk of bleeding. If the medication can be discontinued >1 week prior to NK cell infusion then the subject may be eligible following consultation with the Study Chairs.

3.4.10 Participants with significant systemic or major illnesses including but not limited to: congestive heart failure, ischemic heart disease, kidney disease or renal failure, organ transplantation, or significant psychiatric disorder.

3.4.11 History or current diagnosis of any medical or psychological condition that in the Investigator's opinion, might interfere with the participants ability to participate or inability to obtain informed consent because of psychiatric or complicating medical problems.

Important note: The eligibility criteria listed above are interpreted literally and cannot be waived.

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 written informed consent must be obtained from the patient prior to registration.

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 PNOC_Registration@ucsf.edu.

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 at PNOC_Registration@ucsf.edu. 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. AGENT ADMINISTRATION

5.1 Regimen Description

Enrolled participants must proceed to surgery for tumor resection and Ommaya placement into the resection cavity within 14 days of registration.

In the operating room, and following tumor resection and before the ommaya insertion, the neurosurgeon will measure the resection cavity to ensure that the maximum visible resection cavity dimensions are at least 2 cm x 2 cm, and confirm the lack of communication with the ventricles. Once these are confirmed, the ommaya will be inserted

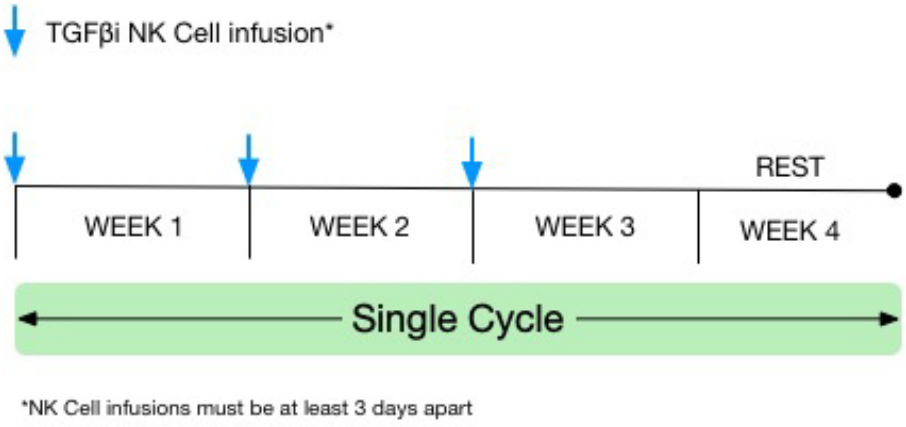
Upon conclusion of the surgery, the neurosurgeon will sign a surgical checklist (see Appendix K) to confirm:

- Tumor cavity dimensions
- Ommaya insertion
- Lack of ventricular communication

A CSF study is NOT required.

First dose of TGFβi NK cells may be administered at least 14 days after the Ommaya reservoir placement, and may not start until all acute surgical complications have resolved (maximum of 6 weeks after registration).

TGFβi NK cell infusions through the Ommaya reservoir will occur once weekly for three weeks followed by one rest week.



5.1.1 TBF βi NK Cell Infusion Procedure

- **Participant Preparation**
  - Participant will be admitted to the infusion unit or wherever ommaya can be accessed as per institutional policies, assessed by nursing and vital signs taken.
  - IV access will be established.
  - Tumor-associated fluid collection and infusion kits will be obtained for use at bedside.
  - Study provider or designee will assess participant including a focused neurological exam prior to procedure.
  - Acetaminophen should be given orally prior to infusion and may be repeated 4 to 6 hours later.

- **Preparing the TGFβi NK cells for Infusion**
  - On the day of infusion, TGFβi NK cells will be thawed at the bedside and administered within 30 minutes of thawing.

- **TGFβi NK cells infusion via the Ommaya reservoir.**
  - **The first infusion should be performed by a trained pediatric neurosurgeon and a cellular therapy certified provider or according to the**

institution's guidelines. Once cleared by neurosurgery, subsequent infusions may be administered by other trained providers, as per the institutional procedures.

- Up to 5 mL, if feasible, of tumor-associated fluid will be drawn out of the ommaya reservoir as per institutional guidelines.
- Infusion of the product should occur through a needle no smaller than 25 g in diameter.
- After tumor-associated fluid collection, TGFβi NK cells (a fixed 3 mL total volume) will be infused slowly over approximately 5 minutes.
- TGFβi NK cell infusion will be followed by approximately 1.5-2 mL preservative-free normal saline flush.

- **Patient Monitoring**
  - Monitoring of vital signs (temperature, pulse, respiratory rate, blood pressure) and neurological checks every 15 minutes (+ 5 minutes) for the first hour following infusion of TGFβi NK cell infusion. Then every 30 minutes (+ 15 minutes) for the following 1 hour, for a total of 2 hours observation following TGFβi NK cell infusion and cleared by the treating team for discharge. If the participant has tolerated the infusions, after the first cycle, the observation period can be decreased to 1 hour with vital signs and neurological checks every 15 minutes (+ 5 minutes).
  - For participants with acute infusion related reactions, including allergic reactions and anaphylaxis, follow institutional guidelines for management.
  - For participants with cytokine release syndrome (CRS), follow detailed management plan outlined in Appendix I.

5.1.2 Criteria to Start First TGFβi NK Cell Infusion:

- TGFβi NK cell product available that meets dose requirements and GMP release criteria.
- Ommaya reservoir in place, and has neurosurgical clearance to use the Ommaya.
- Patient must have no signs of serious infection or development of clinically significant co-morbid condition, which the PI determines would preclude from safe participation in the study.

5.1.3 Criteria to Continue with Subsequent NK Cell Infusions

- Patient must have tolerated prior infusions of TGFβi NK cells without occurrence of dose limiting toxicities (DLT).
- Patient must be without uncontrolled serious infection. Note: asymptomatic viremia such as CMV, HPV, BK virus, HCV, etc. is NOT considered as an exclusion for subsequent NK cell infusions.
- Any new neurological adverse events attributed to TGFβi NK cell infusion, must be resolved to $\leq$ Grade II.

5.2 Dose Escalation and De-Escalation Scheme

The dose-escalation will be performed using a Bayesian optimal interval (BOIN) design to identify the recommended phase-2 dose. To guide dose-escalation decisions, if the observed DLT rate at the current dose is ≤ 0.236, the next cohort of participants will be treated at the next higher dose level; if it is ≥ 0.359, the next cohort of participants will be treated at the next lower dose level; otherwise, stay at the current dose. The observation period for the purposes of dose escalation will be the first cycle of therapy.

We will stagger registration to allow for evaluation of DLTs according to the following:

- Within a dose level:
    - Patient 1 to patient 2: The first patient enrolled on a new dose level must be evaluated for at least 28 days from the first NK cell infusion prior to enrolling patient 2 on that dose level.
    - Patient 3 and all subsequent participants enrolled on a dose level: All subsequent registrations will be staggered by at least 1 week from the first NK cell infusion of the previous patient.
- Between dose levels:
    - Dose escalation should not occur until all the study subjects in the previous dose level have completed the 28-day safety evaluation for DLTs.

There are 4 dose levels to potentially be assessed for the BOIN:

Dose Level	**NK cell number per infusion**	**Cumulative NK cell number per cycle**	**Cumulative NK cell number per dose level after 3 cycles**	**Highest possible cumulative T cell number after 3 cycles**
1	$3x10^5$	$9x10^5$	$2.7x10^6$	$11.1x10^3$
2* (starting dose)	$3x10^6$	$9x10^6$	$2.7x10^7$	$11.1x10^4$
3	$3x10^7$	$9x10^7$	$2.7x10^8$	$11.1x10^5$
4	$3x10^8$	$9x10^8$	$2.7x10^9$	$11.1x10^6$

*starting dose level

After the trial is completed, select the RP2D based on isotonic regression as specified in Liu and Yuan (2015). Specifically, select as the RP2D the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, select the higher dose level when the isotonic estimate is lower than the target toxicity rate and select the lower dose level when the isotonic estimate is greater than or equal to the target toxicity rate.

5.3 Definition of Dose-Limiting Toxicity

A DLT will be defined as any new event possibly, probably or definitely related to the study article (including baseline neurological findings that progress), which results in:

1. Grade 3 or greater GVHD (see appendix L for GVHD grading)
2. Grade 2 GVHD by IBMTR index that requires oral or intravenous steroids and does not resolve to < Grade 2 within 7 days. (see appendix L for GVHD grading)
3. Grade 3 or greater non-neurologic, non-hematologic toxicity will be considered a DLT if it requires therapeutic intervention, hospitalization, or prolongation of current hospitalization.
4. Grade 3 neurologic toxicity that does not improve to ≤ Grade 2 within 7 days
5. Grade 3 neurologic toxicity that does not return to baseline within 28 days
6. Grade 3 neurologic toxicity that resolves, but recurs to Grade 3 again in a subsequent cycle
7. Grade 4 or greater neurologic toxicity
8. Grade 3 or 4 hematological toxicity that does not improve to ≤ Grade 2 within 14 days, with the exception of any grade 4 lymphopenia

Cerebral Edema/Pseudo-Progression toxicity exception:
NCI CTCAE 5.0 criteria categorize cerebral edema as grade 3 (New onset; worsening from baseline), grade 4 (Life-threatening consequences; urgent intervention indicated) and grade 5 (death). Cerebral edema normally presents in participants with malignant gliomas as part of the disease process and can be exacerbated by standard of care chemotherapy and radiation. Furthermore, an effective anti-tumor immune response may involve inflammatory response and edema in infiltrative tumor cells. Therefore, cerebral edema toxicity, although ranked grade 3-4 by NCI CTCAE 5.0 criteria, will be not be considered a DLT if patient is stable or improved clinically. If a cerebral edema is observed in a patient in clinical decline, the event will be considered a DLT if it is clearly attributable to the investigational drug and patient does not show improvement within 7 days of clinical management. Tumor progression will not be considered a DLT.

DLTs, or possible DLTs, must be reported to the PNOC Operations Office within 1 business day. An email notification is to be sent to PNOC028@ucsf.edu including a completed DLT Determination Form available in SharePoint.

MRI findings in the absence of clinical findings will be noted but will not be considered a DLT.

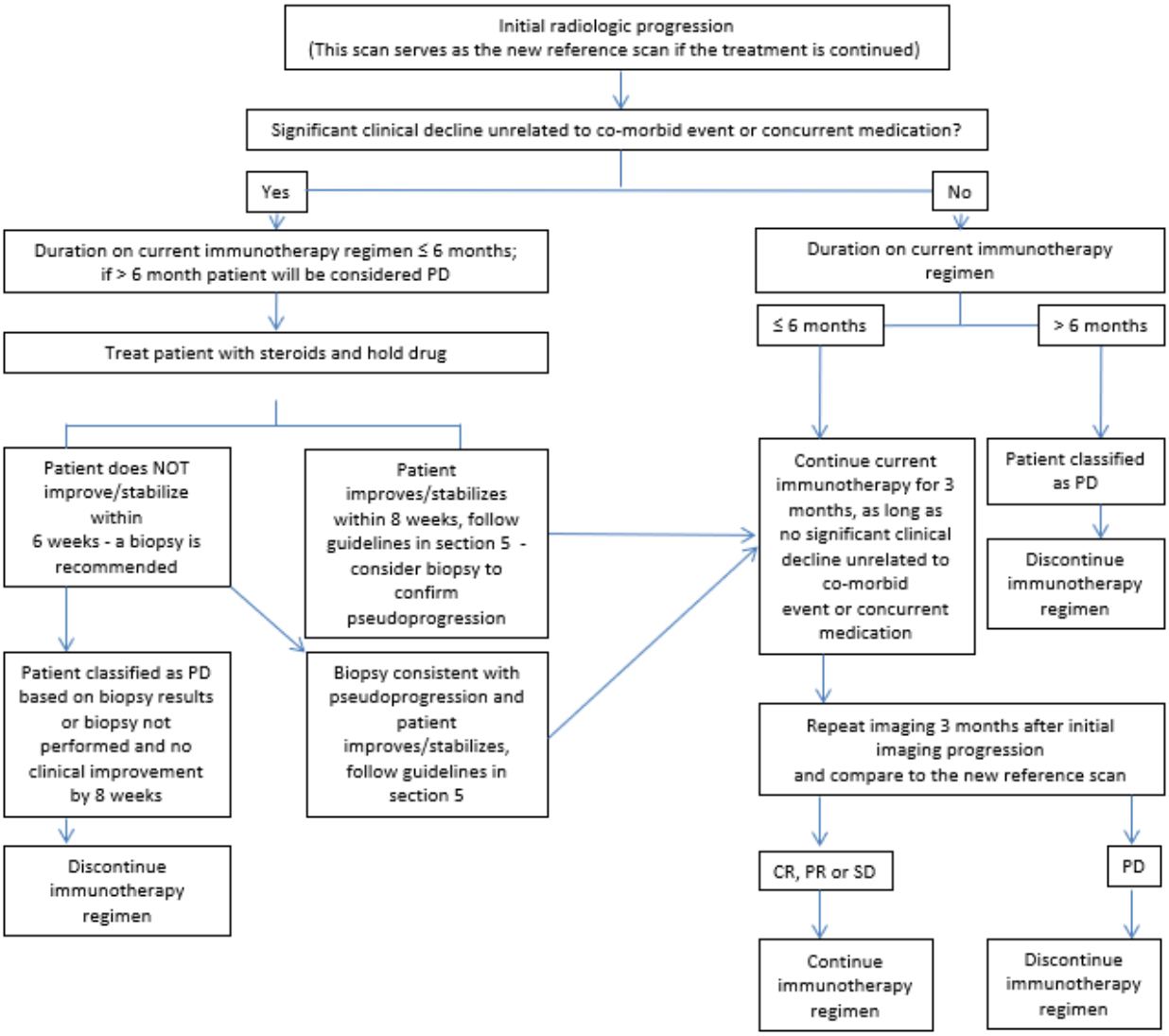
The observation period for the purposes of dose escalation will be the first cycle of therapy. **The first patient enrolled on a new dose level must be evaluated for at least 28 days from the first NK cell infusion for DLTs prior to infusing any additional participants on that dose level.**

The investigational component of this treatment plan is TGFβi NK cells administered *via* an Ommaya intra-cavitary/intra-tumoral device. The adverse events associated with surgical treatment and Ommaya placement will be considered unrelated to the TGFβi NK cell infusion. Participants may need to get tumor surgery between the time of registration and infusion of TGFβi NK cells which has risks of adverse events that are unrelated to this study. In these participants, the neurological exam after surgical recovery but before use of the investigation agent will be considered baseline. Adverse events will be attributed to the TGFβi NK cells if they are suspected to have a direct relationship with the TGFβi NK cells or the administration of TGFβi NK cells through the Ommaya.

5.4 Cerebral Edema/Pseudo-Progression

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, and/or the dose increased up to 0.3 mg/kg/day, maximum of 12 mg/day, or started on bevacicumab (10mg/kg every 2 weeks for a total of 3 doses) if clinically symptomatic. The recommendation is not to start steroids/ Bevacizumab if the patient is clinically stable and to continue the TGFβi NK cell infusions. Treatment options should be discussed with the Study Chair PRIOR to initiation of therapy. If there is evidence of clinical deterioration, MRI of the brain needs to be done and the TGFβi NK cell infusions will be discontinued and only restarted if the child is on less than max 0.1 mg/kg/day of steroids; maximum 4 mg/day and if the patient stabilizes within 8 weeks. Treating physicians must repeat the MRI of the brain before restarting the TGFβi NK cell therapy. 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, participants may remain on study and restart treatment following resolution of toxicity to grade 1 or less at the discretion of the study chair. Such participants should restart treatment at two-thirds of TGFβi NK cell dose. 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.

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.



5.5 Dosing Modifications and Delays

The PNOC Study Chair and Co-Chair and PNOC028@ucsf.edu must be notified of any dosage modifications prior to the implementation of the dose modification.

For any dosing modifications and delays according to the criteria below, participants should continue with the timing of the original treatment schedule. Please email PNOC028@ucsf.edu for questions or clarification.

Participants who have DLTs will come off study. Dose modifications for participants are not allowed on this study. Participants 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 12 months after removal from treatment.

5.6 Treatment Delays

Participants who do not meet criteria to receive their TGFβi NK cell infusion can be delayed up to 28 days then can restart the infusions as soon as the criteria are met (refer to section 5.1.3). For participants who missed TGFβi NK cell infusions mid-cycle, they may restart the infusion once the criteria are met but missed doses will not be made up.

5.7 DLTs for Participants Beyond Cycle 1

For participants receiving continuation of therapy, if any SAEs are observed, additional dosing for that patient will be halted. Furthermore, the study team will review all AEs and make determination on additional dosing.

5.8 Supportive Care Guidelines and Other Concomitant Therapy

- Concurrent cancer therapy, such as chemotherapy, radiation therapy, immunotherapy, or biologic therapy must not be administered to participants without prior approval of PI or designee(s).
- No other investigational agents may be given while the patient is on study.
- Sedatives and other medications that can alter neurological assessment should be avoided on days of TGFβi NK cell infusions except in medical emergency or prior approval of PI and/or designee.
- Participants with suspected pseudo-progression, refer to Section 5.4.
- Follow institutional guidelines for required platelet level when accessing the Ommaya reservoir.

Generally, all baseline concomitant medications should be captured at registration. Concomitant medications started after treatment due to related adverse event, which are considered anti-neoplastic, or as defined by this protocol are to be captured in the case report form (CRF).
The following medications are always to be captured in the CRF:

a. Anti-seizure medications
b. Dexamethasone or equivalent
c. Bevacizumab or equivalent
d. Supportive care medication as recommended in the protocol (e.g., anti-hyperglycemic agents, hydrocortisone ointment, antihistamines, systemic corticosteroids)

6. TREATMENT PLAN

6.1 Study Calendar

Study visits and procedures may be scheduled within +/-3 day window except for screening procedures (-14 days) prior to registration, or as otherwise indicated. One treatment cycle is defined as 4 weeks (28 days).

	Screening	Baseline	Day 1 of each cycle	Days 8 and 15 of each cycle	End of Treatment Visit (+/- 14 days)	30-day Tox (+/- 7 days)	Follow-Up
Clinical Procedures							
Informed Consent (-28 days)	X						
TGFβi NK cells (study drug administration)			X	X			
Medical History	X						
Physical Exam	X		X	X	X		
Vital signs	X		X	X	X		
Performance Status (Appendix A)	X		X	X	X		
Toxicity Assessment			X	X	X	X	
Concomitant Medications	X		X	X	X	X	
Surgical Procedure		X[7]					
Survival							X
Laboratory Procedures							
CBC with differential	X		X	X (Cycle 1 only)	X		
Serum Chemistry	X		X		X		
PTT/INR	X						
Serum or urine pregnancy test	X		X				
Imaging Procedures							
MRI of the brain and, if clinically indicated, spine MRI	X		X[1]		X		
Lumbar puncture if clinically indicated	X						

Specimen Collection							
Tumor-associated fluid collection *via* ommaya			X[2]				
Fresh tumor tissue & peripheral blood[6]		X[6]					
Health Related Quality of Life Assessments							
HRQoL (see Appendix E)[5]		X[3]			X		X
Health Related Social Risk Assessment (See Appendix J)[5]		X[4]			X		X

[1] Brain MRI, and, if clinically indicated, a spine MRI every odd cycle (Cycle 1, 3) to be performed within -28 days of Day 1 Cycle 1 and within –7 days from Day 1 Cycle 3.
[2] Tumor-associated fluid collection *via* ommaya, if feasible, should occur prior to NK Cell infusion
[3] Baseline QOL to be completed after registration and prior to first dose of TGFβi NK cells
[4] Baseline Health Related Social Risk Assessment to be completed after registration and prior to first dose of TGFβi NK cells
[5] If participant is co-enrolled on PNOC COMP, health related quality of life and health related social risk assessments are to be captured under the PNOC COMP protocol. Assessments do not need to be collected or reported under PNOC028. 2-- If participant is co-enrolled on PNOC COMP, follow-up assessments are to be captured under the PNOC COMP protocol. Assessments do not need to be collected or reported under PNOC028.
[6] Tumor tissue and peripheral blood to be obtain after registration but before 1st NK cell infusion per section 9.0 Correlative Studies. Fresh tissue and peripheral blood to be obtained during surgery for tumor resection and ommaya reservoir placement.
[7] Following eligibility and manufacturing capability confirmation, participants must proceed to surgery for tumor resection and intra-cavitary/intra-tumoral Ommaya reservoir placement within 14 days of registration

6.2 Observations and Procedures

Eligibility Screening Visit (with 14 days prior to registration unless indicated otherwise)

- Informed Consent **(-28 days)**
- Complete medical history, including baseline symptoms assessment
- Complete physical exam, to include neurological exam with testing of cranial nerves, motor and sensory function, gait and coordination.
    - Vital signs: Height, weight, blood pressure, respiration, pulse, temperature.
- Disease status
- Performance status (see **APPENDIX A**)

- Concomitant medications
- Complete blood count (CBC) with differential and platelet count
- PTT/INR
- Blood chemistry assessment, including:
    - sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, random glucose, albumin, total protein, creatinine, blood urea nitrogen (BUN), ALT, AST, total bilirubin
- Females of child-bearing potential will have a serum or urine beta-HCG pregnancy test
- Brain MRI and, if clinically indicated, spine MRI **(-28 days)**
- Lumbar puncture only if clinically indicated

Baseline after Registration

- Following eligibility and manufacturing capability confirmation, participants must proceed to surgery for tumor resection and intra-cavitary/intra-tumoral Ommaya reservoir placement within 14 days of registration.
    - At time of surgery, collect fresh tumor tissue and peripheral blood for Correlative Studies per Section 9
- If participant is co-enrolled on PNOC COMP, health related quality of life and health related social risk assessments are to be captured under the PNOC COMP protocol. Assessments do not need to be collected or reported under PNOC028.
- PNOC Health Related Quality of Life Assessments (window of administration can begin after registration until treatment start
    - PedsQL
    - PROMIS
    - ABAS
    - BRIEF
- Health Related Social Risk Assessment (see Appendix J)

The first dose of TGFβi NK cells may be administered at least 14 days after the Ommaya reservoir placement, and may not start until all surgical complications have resolved (maximum of 6 weeks after registration).

<u>Day 1 of Each Cycle (within +/- 3 days)</u>

Clinical assessment must be performed within 72 hours prior to the start of the TGFβi NK cell infusions

- NK Cell Administration (**see section 5.1.1 for administration instructions**). NK Cell infusions must be at least 3 days apart
- Complete physical exam, to include neurological exam with testing of cranial nerves, motor and sensory function, gait and coordination.
- Vital signs: Height, weight, blood pressure, respiration, pulse, temperature. (**see section 5.1.1 for required participant monitoring post-infusion**)
- Toxicity assessment
- Disease Status
- Performance status (see **APPENDIX A)**

- Concomitant medications
- Complete blood count (CBC) with differential and platelet count
    - *Must be done within 72 hours (+/-) from the first NK cell infusion of each cycle.*
- Blood chemistry assessment: Sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, random glucose, albumin, total protein, creatinine, blood urea nitrogen (BUN), ALT, AST, total bilirubin
- Females of child-bearing potential will have a serum or urine beta-HCG pregnancy test
- Tumor-associated fluid collection, if feasible, *via* ommaya (on date of NK Cell administration) (see **Section 9**)
- Brain MRI, and, if clinically indicated, a spine MRI every odd cycle (Cycle 1, 3) to be performed within -28 days of Day 1 Cycle 1 and within -7 days from Day 1 Cycle 3.

Day 8 and Day 15 of Each Cycle (within +/- 3 days)

- NK Cell Administration (**see section 5.1.1 for administration instructions**)
- Complete physical exam, to include neurological exam with testing of cranial nerves, motor and sensory function, gait and coordination.
- Vital signs: Height, weight, blood pressure, respiration, pulse, temperature. (**see section 5.1.1 for required participant monitoring post-infusion**)
- Toxicity assessment
- Performance status (see **APPENDIX A)**
- Concomitant medications
- Complete blood count (CBC) with differential and platelet count (during Cycle 1 only)

End of Treatment (within +/-14 days)

- Complete physical exam, to include neurological exam with testing of cranial nerves, motor and sensory function, gait and coordination.
- Vital signs: Height, weight, blood pressure, respiration, pulse, temperature.
- Toxicity assessment
- Disease Status
- Performance status (see **APPENDIX A)**
- Concomitant medications
- Complete blood count (CBC) with differential and platelet count
- Blood chemistry assessment: Sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, random glucose, albumin, total protein, creatinine, blood urea nitrogen (BUN), ALT, AST, total bilirubin
- Brain MRI, and, if clinically indicated, spine MRI
- PNOC Health Related Quality of Life Assessments (+/- 30 days)
    - PedsQL
    - PROMIS
    - ABAS (does not need to be completed if administered within the past 6 months)
    - BRIEF (does not need to be completed if administered within the past 6 months)
- Health Related Social Risk Assessment (see Appendix J)

30-day Toxicity Assessment (within +7 days)

- Toxicity assessment
- Concomitant medications

6.3 Long Term/ Survival Follow-up Procedures

If co-enrolled on PNOC COMP, follow-up procedures are to be captured under the PNOC COMP protocol.

Participants who are off-treatment will be followed by chart review and/or telephone/ email contact every two months or until an off-study criterion is met, to collect disease and survival status information. This information will be recorded in the eSource + EDC eCRFs due at Follow-up.

- Participants who are off-treatment will be followed for up to five years after the last day of treatment, until withdrawal of consent or until death, whichever occurs first to collect the date of progression, date of commencement of new anticancer therapy, date of last contact and date of death. Participants who expire without confirmation of disease status will be considered to have progressive disease at the time of death.

- Participants will be followed for up to one year after the last day of treatment, to collect any adverse events that are possibly, probably or definitely related to the study drug.

- Participants will be followed every 12 months after the last day of treatment until the patient starts on a new treatment, or is off study, at discretion of investigator, withdrawal of consent or until death, whichever occurs first to collect Pediatric Health Related Quality of Life & Neurocognitive measures (see Appendix E).

- Health Related Social Risk Assessment every 12 months (+/- 3 months) (see Appendix J)

6.4 Off-Treatment Criteria

Treatment may continue for 3 cycles 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.
- Protocol violation - any patient found to have entered this study in violation of the protocol might be discontinued from the study at the discretion of the Principal Investigator.
- Pregnancy

The "Off Treatment Date" and reason for discontinuation must be documented by the attending investigator in the medical record and recorded in two places within OnCore®, in the 'Follow-Up' section of OnCore® as well as in the 'PNOC End of Treatment eCRF.'

The "Off Arm Date" must be documented in the 'Treatment' section of OnCore®. The 'Off Arm Date" should correspond with the "Off Treatment Date" and is the date the participant was discontinued from protocol treatment.

The "Last Treatment Date" is recorded in two places within OnCore®, in the 'Follow-Up section of OnCore®' as well as in the 'PNOC End of Treatment eCRF'. "Last Treatment Date" is defined as the last date that the participant received protocol-based therapy.

6.5 Off Study Criteria

Participants will be considered Off Study for the following reasons:

- Participant determined to be ineligible.
- Participant, parent or legal guardian withdraws consent for continued participation.
- Participant death while on study.
- Completion of protocol specific follow up period.
- Participant has completed their 30-day toxicity visit, is co-enrolled on PNOC COMP and follow up data is being captured under the PNOC COMP protocol.

The date and reason for the participant coming off study must be documented in the 'Follow-Up' section of OnCore® as well as the 'PNOC End of Treatment eCRF'. No data will be collected after the "off study" date.

7. ADVERSE EVENTS

An adverse event (AE, 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. AEs are monitored and reported in a routine manner at scheduled times during the trial (please follow directions for routine reporting provided in the Data Reporting section).

Additionally, all serious adverse events (SAE) must be reported in an expedited manner to allow for optimal monitoring of participant safety and care. The Expedited Reporting section in this protocol (Section 7.4) provides guidelines for expedited reporting.

7.1 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All sites should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

When specific AEs 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

- **Attribution** of the AE:

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

7.1.1 **Suspected**

A suspected adverse reaction is defined as any AE for which there is a reasonable possibility that the drug caused the AE. 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.

7.1.2 **Unexpected**

An AE 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 AEs 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.

AEs 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 AEs 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 participants 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.

7.1.3 **Serious**

By definition, an adverse event is defined as a serious adverse event (SAE) according to the following criteria:

- Death,
- Life-threatening adverse event*,
- Inpatient hospitalization >24 hours or prolongation of existing hospitalization by 24 hours,
- Persistent or significant disability/incapacity,
- Congenital anomaly/birth defect, or cancer, or
- Any other experience that suggests a significant hazard, contraindication, side effect or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above,
- Event that changes the risk/benefit ratio of the study.

The following hospitalization scenarios are not considered to be SAEs:

- Hospitalization for palliative care or hospice care,
- Hospitalization for logistical reasons,
- Hospitalization due to progression of the underlying cancer,
- Planned hospitalization required by the protocol,
- Planned hospitalization for a pre-existing condition

* A life-threatening adverse experience is any AE that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

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 participant 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.

7.2 Adverse Event Monitoring

PNOC uses the web-based OnCore® Clinical Trials Management System and eSource+EDC electronic data capture (EDC) system for monitoring and recording of Adverse Events (AEs) including all adverse reactions considered "serious" (also called Serious Adverse Events, or SAEs).

All clinically significant AEs, whether or not considered expected or unexpected and whether or not considered associated with the investigational agent(s) or study procedure, will be entered into the eSource + EDC system. All Adverse Events entered into the eSource + EDC system 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.

Clinically significant AEs will be defined as the following, regardless of grade:

- AEs that the provider feels is clinically impacting to the patient *OR*
- AEs that are associated with any clinical symptoms or clinical exam findings *OR*
- AEs that require surgical or medical intervention *OR*
- AEs that include Grade 2 or greater lab values which may not be related to clinical symptoms but are a change from baseline and which require ongoing follow up on future lab assessments and could be signs of end-organ injury, such as all labs related to liver, renal, bone marrow function

In addition, all 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 Forte EDC for the purpose of the UCSF's DSMC monitoring. Please see Appendix D PNOC Data Safety and Monitoring Plan for more information.

7.3 Adverse Event Reporting

All clinically significant AEs will be entered into eSource + EDC, regardless of relationship. Appendix C includes detailed information about PNOC reporting timelines.

For participants who are enrolled onto the study, the study period during which AEs and SAEs must be reported begins after informed consent is obtained and ends 30 days following the last administration of study treatment. After this period, only SAEs that are attributable to study

treatment should be reported. Participants removed from therapy for unacceptable adverse event(s) will be followed until adverse event(s) resolves or returns to baseline status. Participants who consent to the study and experience an SAE, but do not enroll onto the study, do not need to report AEs or SAEs into OnCore/eSource + EDC.

The Investigator will assign attribution of the possible association of the event with use of the study therapy, and this information will be entered into eSource + EDC. The Investigator must also comply with all reporting requirements to their institutional Data and Safety Monitoring Committee (DSMC) and Institutional Review Board (IRB).

7.4 SAEs and Expedited Reporting

All AEs which meet the definition of 'Serious' as well as other medically significant events described below require expedited reporting to PNOC. Below are instructions for recording and reporting of these events. Please contact the PNOC Operations Office at PNOC028@ucsf.edu with any questions regarding expedited reporting requirements for this study. See 7.1.3 for the definition of an SAE.

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

- **Advarra EDC:** All SAEs must be entered into the SAE CRF in eSource + EDC. The eSource + EDC SAE record should be updated immediately as new information becomes available until the SAE is resolved.
- **OnCore:** All SAEs must be entered into the Participant Console in OnCore (https://oncore.ucsf.edu/ > Participant Console > SAE Tab on left). The OnCore SAE record should be updated immediately as new information becomes available until the SAE is resolved. (Adverse Event Details segment MUST be completed. Don't forget to click "Add" button.) Please refer to the "PNOC OnCore SAE Entry Guide: Field by Field" in SharePoint for more information.
- **Email:** Please also email PNOC028@ucsf.edu with, at minimum, the following information:

  In the participant line: "SAE: Participant PNOC ID" (e.g., "SAE: PNOCxxx-1")

  In the body of the email: Participant PNOC ID and the OnCore assigned SAE number

  Conplete and attach the SAE Reporting Form available in SharePoint
- **Site IRB:** Each PNOC site is also responsible for following their own IRB guidelines for reporting SAEs.

SAE Data Entry in AE CRF:

All SAEs must also be entered into the AE CRF for that Cycle. 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)/eSource + EDC. Please reference the "PNOC Deviation Reporting Guidelines" in SharePoint for more information.

7.4.1 Medically Significant Events

Email notification to PNOC Operations Office (PNOC028@ucsf.edu) **within one business day** of first PI awareness:

- Reports of pregnancy exposure (pregnancy encompasses the entire cycle of pregnancy and delivery, perinatal and neonatal outcomes, even if there were no abnormal findings; both maternal and paternal exposure is collected)
- Reports of lactation exposure
- Overdose (with or without an SAE)
- Abuse (use for non-clinical reasons with or without an SAE)
- Inadvertent or accidental exposure

7.4.2 Adverse Events of Special Interest (AESI)

Email notification to PNOC Operations Office (PNOC028@ucsf.edu) **within one business day** of first PI awareness:

- GVHD $\geq$ Grade 3

7.4.3 PNOC 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.

7.4.4 PNOC Reporting to UCSF Institutional Review Board (IRB)

The PNOC Operations 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.

7.4.5 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, eSource + EDC, and email (PNOC028@ucsf.edu) 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 eSource + EDC and OnCore entry: participant 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.

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 AE **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.

If the majority of the study team decides the AE **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.

8. AGENT INFORMATION

8.1 TGFβi NK cells

Product description: The TGFβi NK cell product on this trial will be manufactured in the Abigail Wexner Research Institute at Nationwide Children's Hospital (AWRI-NCH) Cell-Based Therapy (CBT) Core facility under standard operating procedures (SOP) validated according to the Chemistry and Manufacturing Control (CMC) document for TGFβi NK cell expansion.

Ordering: The expanded donor NK cell product will be manufactured prior to subjectregistration. Please see the PNOC SharePoint homepage for instructions.

Solution preparation Source PBMCs will be collected and TGFβi NK cells are propagated according to the procedures outlined in the CMC as submitted to the FDA under IND. Briefly, PBMC are depleted of CD3+ T cells and co-cultured with IFCs (or IFC-derived nanoparticles) and IL-2. At Day 7, the cultures are re-stimulated. The NK cell product will undergo lot release testing and cryopreservation on day 14, and all tests will be final prior to release of the product for infusion to participants.

Storage requirements: The doses of TGFβi NK cells will be cryopreserved in NK freeze media at a maximum cell concentration of 20 x 10^7 NC/mL according to SOP. Samples of the final formulated product will be obtained just prior to cryopreservation for repeat microbial contamination and endotoxin testing. TGFβi NK cells will be cryopreserved in cryovials and cryobags in up to 25 mL aliquots. The cryopreserved doses will be stored in vapor phase LN_2 at the Manufacturing Center until full release criteria have been met.

Route of administration: Participants may start the treatment portion of the study once GMP has confirmed that the expanded TGFβi NK cell product meets released criteria, an Ommaya reservoir is in place, and they have recovered from any neurosurgical intervention.

Accountability: The investigator, or a responsible party designated by the investigator, will be responsible for drug accountability and this should be managed per each PNOC institutions' guidelines.

9. CORRELATIVE STUDIES

Next Generation Sequencing (NGS) will be performed on all tumor samples in order to determine their mutational landscape, and any possible correlations with the outcomes. Moreover, the immune signature-based profile of each tumor will be assayed by the NanoString PanCancer IO360 panel (300ng RNA). This panel measures gene expression of 770 genes to calculate an immuno-profile based on the tumor inflammation signature (TIS) and other immune gene signatures that characterize the key immune pathways and their activation status. Additionally, the NK cell product itself will be assessed for its phenotype and function to estimate TGFβi NK cell persistence and anti-tumor activity. Lastly, changes in the TCR repertoire diversity will be

examined by using a Nanostring custom reagent that evaluates the VDJ sequences present before and after NK cell treatment (300ng RNA).

Time Point	Sample Type	Sample Amount
Screening	Fresh Tumor Tissue for NGS and NantoString assay	30-50 mg of fresh frozen tumor tissue
Screening	Germline testing from peripheral blood	5 ml blood
Beginning of Each Cycle	Tumor-associated fluid to check for NK cell phenotype and function	Minimum of 2 ml, up to 5 ml tumor-associated fluid, if feasible

9.1 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 [79]. Several studies have shown that compared to healthy controls or other cancer survivors, survivors of pediatric brain tumors have the lowest HRQoL [80,81]. For example, children with brain tumors under active therapy are frequently viewed as socially isolated and/or often absent from school by their peers [82]. Cosmetic effects of radiation or chemotherapy treatment (e.g. permanent or temporary alopecia) often occur [83], adding to social burdens and contributing to social isolation. Historically though, HRQoL measures have rarely been included as clinical trial endpoints [84-87]. 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 [79,88]. 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 [89]. 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 [85]. This approach might be particularly important in clinical trials where detecting even small changes related to an individual disease or treatment is necessary [90].

There are several cancer-centric assessment tools that satisfy the criteria above [91-98]. 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 [97].

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. New computerized assessment tools now allow for integration of cognitive assessments more regularly in clinical trials, especially as these can be performed remotely. 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 use the validated measures described in Appendix E.

9.2 Health Related Social Risk Assessment

Multiple studies have demonstrated inequities in outcomes of children and young adults with central nervous system tumors from lower socioeconomic status or identified as historically under-represented races and ethnicities[99-102]. Racial and ethnic differences in drug toxicities have also been described in the use of novel therapies[103-106] and under-representation of diverse patient populations remains a concern in pediatric clinical trials[107]. As feasible, we will investigate the patient population through self-reported data, including race, ethnicity and other health related risk factors. We will utilize descriptive statistics to assess race, ethnicity, and health-related social risks and in the context of survival outcomes, toxicities, and patient experience as per PRO responses.

10. EVALUATION CRITERIA

10.1 Response Criteria-Ependymoma

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by standard criteria as outlined by the Response Assessment in Pediatric Neuro-Oncology (RAPNO) and Immunotherapy Response Assessment in Neuro-Oncology (iRANO) international working groups.

10.1.1 Definitions

Evaluable for toxicity. All participants will be evaluable for toxicity from the time of their first treatment with expanded TGFβi NK Cells.

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

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

Evaluable for DLT period: If patient develops a DLT during cycle 1 they will be considered evaluable for estimating the MTD. Patients without DLT must complete 85% of prescribed dosing during cycle 1 to be evaluable for estimating the MTD.

10.1.2 Imaging and Disease Parameters

MRI imaging requirements for **primary brain and cord tumors**

Sequence	Comment
Brain	
Pre-gadolinium administration sequences 3D T1 GRE or TSE or 2D T1 SE[1] Axial DWI[1] SWI or GRE[3] CISS or FIESTA[3] **Post-gadolinium administration sequences** Axial T2 FSE[1] – recommended to be done first after gadolinium administration 3D T1 GRE or TSE[1] 2D T1 SE (axial or coronal)[3] 3D or 2D T2 FLAIR[2]	Perform in axial plane through posterior fossa for posterior fossa ependymomas, or through region of interest for supratentorial ependymomas. CISS or FIESTA can be replaced with Sagittal T2 weighted SPACE/CUBE/VISTA
Spine	
Pre-gadolinium administration sequences Sagittal T1 SE[1] **Post-gadolinium administration sequences** Sagittal T2 FSE or STIR[1] Axial T2 FSE[1] Sagittal T1 SE[1] 3D Axial T1 (VIBE/ FAME/LAVA/THRIVE) or Axial T1 SE[1] – (4-5mm maximal slice thickness, maximal 10% gap) Sagittal CISS or FIESTA[3]	Sagittal CISS/FIESTA can be replaced with Sagittal T2 SPACE/CUBE/VISTA

[1] Mandatory
[2] Mandatory to be post-contrast if concern for leptomeningeal disease. Can be done pre-contrast for others.
[3] Recommended

Measurable disease:

Measurable disease is defined as one or more lesions meeting a minimal size threshold. The size threshold is met if both in plane diameters are ≥10 mm or both in plane diameters are at least two times the MRI slice thickness, plus the interslice gap. These criteria apply to both CE and non-CE disease. Of note, measurements should never include cystic or necrotic portions (except for craniopharyngioma), nor the resection cavity. For instance, the rim enhancement surrounding the surgical cavity or surrounding cystic components should be categorized as non-measurable, unless presenting an enhancing nodule that meets the criteria for measurable disease.

Leptomeningeal disease can be considered measurable if focal and meeting the same size threshold.

All tumor measurements are taken using calipers on a picture archiving and communications system (PACS) and recorded in millimeters or one decimal fraction of centimeters. All baseline evaluations will be performed as closely as possible to the beginning of treatment and never more than 30 days from registration. 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.

Non-measurable disease

Non-measurable disease includes all lesions not meeting the criteria for measurable disease. Non-measurable disease can be either focal or diffuse.

Target and Non-target lesion

For most CNS tumors, only one lesion/mass is present and therefore is considered a "target" for measurement/follow up to assess for tumor progression/response. If multiple measurable lesions are present, up to 3 can be selected as "target" lesions. Target lesions should be selected on the basis of size and suitability for accurate repeated measurements. All other lesions will be followed as non-target lesions (including CSF positive for tumor cells). The lower size limit of the target lesion(s) should be per the definition of measurable disease.

Non-target lesions should be evaluated or monitored, but their size is not incorporated in the assessment of the overall tumor burden. If multiple non-target lesions are present, up to 3 can be selected as "non-target" lesions. If previously non-target lesions grow and become measurable, they can become target lesions, and their size is then incorporated into the overall tumor burden.

Focal leptomeningeal disease can be a target lesion if meets the criteria of measurable disease as described above. If leptomeningeal disease is present, presence, type (focal vs diffuse) and location of leptomeningeal disease should be noted and change in extent/size assessed on follow up studies.

Tumor Measurements

The MRI sequence that best highlights the tumor (postcontrast T1, T2, or T2 FLAIR) will be chosen to response. The same sequence should be used for serial measurements. Response determination will be based on a comparison of product of perpendicular diameters or an area [**W** (longest diameter of the target lesion) **x T** (transverse measurement, perpendicular to W)] between the baseline assessment and the study date designated in the follow-up Report Form.

To assess response, the following ratio is calculated:

$$\frac{W \times T \text{ (current MRI)}}{W \times T \text{ (reference MRI)}}$$

Reports for the follow-up exams should reiterate the measurements obtained at baseline for each target lesion. Nontarget lesions or newly occurring lesions should also be enumerated in these reports, and changes in non-target lesions should be described.

1. The longest diameter can be measured from the axial plane or the plane in which the tumor is best seen or measured. The longest measurement of the tumor is referred to as the width (W).

2. The perpendicular measurement should be determined - transverse (T) measurement, perpendicular to the width (W) in the selected plane.

Additional considerations for cystic/necrotic lesions:

For most tumors, the cystic or necrotic components of a tumor are not considered in tumor measurements. Therefore, only the solid component of cystic/necrotic tumors should be measured. If cysts/necrosis composes the majority of the lesion, the lesion may not meet criteria for "measurable" disease. (see below bullet points)

- If the cyst/necrosis is eccentric, the W and T of the solid portion should be measured, the cyst/necrosis should be excluded from measurement.
- If the cyst/necrosis is central but represents a small portion of the tumor (< 25%), disregard and measure the whole lesion.
- If the cyst/necrosis is central but represents a large portion of the tumor, identify a solid aspect of the mass that can be reproducibly measured.

Overall Response Assessment:

The overall response assessment takes into account both the target and non-target lesions, and the appearance of new lesions, where applicable, according to the criteria described below. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant's best

response assignment will depend on the achievement of both initial measurement and subsequent confirmation criteria.

Response Criteria, per RAPNO Criteria

- **Complete Response**: Disappearance of all target and non-target lesions. There can be no appearance of new lesions. Clinical status should be stable or improved and patient off steroids. Per RAPNO-intracranial ependymoma criteria, CR requires sustained response for at least 8 weeks.

- **Partial Response**: At least a 50% decrease in target lesions, taking as reference to the baseline MRI. There can be no appearance of new lesions. Per RAPNO-intracranial ependymoma criteria, PR requires sustained response for at least 8 weeks. Clinical status should be stable or improved and patient on stable or reduced dose of steroids.

- **Stable Disease:** Does not meet criteria for complete response, partial response, or progressive disease. There can be no appearance of new lesions. Clinical status should be stable or improved and patient on stable or reduced dose of steroids.

- **Progressive Disease**: 1) At least a 25% increase in target lesions, taking as reference the baseline or best response or 2) clear increase in size of non-target lesions from baseline or best response or any new lesion or clinical deterioration.

- **Pseudoprogression**

  A 3-month confirmatory scan requirement will assure that patients are not prematurely assigned to have progressive disease while receiving immune-based therapy for high grade glioma. In addition, 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-up scan to assess for true progressive disease versus pseudoprogression. This will apply to patients that demonstrate worsening of the MRI within 6 months of start of therapy. Patients 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 use of steroid treatment, the patient will be classified as having pseudoprogression 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 patient 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 patient should be classified has having pseudoprogression and should remain on study. Patients that have tissue available will be centrally reviewed at UCSF.

In cases for which it remains difficult to differentiate between progression versus pseudoprogression, the PI should 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.

Response definitions per RAPNO for intracranial ependymoma: (patients must meet ALL criteria in each response/stable disease category, or ANY criteria in the progressive disease category) [109]

	Complete Response (must meet ALL criteria)	**Partial Response (must meet ALL criteria)**	**Stable Disease (must meet ALL criteria)**	**Progressive Disease (must meet ANY criteria)**
MRI Brain	No evidence of disease (measurable or non-measurable) for a minimum of 8 weeks; no new lesions	$\geq$ 50% decrease (compared with baseline) in the sum of the area in the axial plane of any residual primary tumor and up to 3 of the largest measurable metastatic lesions sustained for at least 8 weeks; no progression of non-measurable disease; no new lesions	Does not meet criteria for CR, PR, or PD	$\geq$25% increase (compared with the smallest measurement at any time point) in the sum of the products of 2 perpendicular diameters in the axial plain of any residual primary tumor and up to 3 of the largest metastatic lesions; clear progression of non-measurable disease; new lesions
MRI Spine	Same as MRI brain	Same as MRI brain; if positive at baseline, can be positive or negative; if negative at baseline, must remain negative	Same as MRI brain	If negative at baseline, now positive
CSF cytology	If positive at baseline, must be negative x 2 (sampling at least 2 weeks apart)	If negative at baseline, must remain negative. If positive at baseline, can be positive or negative.	If negative at baseline, must remain negative. If positive at baseline, can be positive or negative.	If negative at baseline, now positive

*Neurologic exam	Stable or improving	Stable or improving	Stable or improving	Clinical deterioration not attributable to other causes
Steroid use	Off steroids or physiologic replacement doses only	Stable or less than baseline dose	Stable or less than baseline dose	

*If it is unclear that the patient has disease progression, it may be a reasonable option to keep the patient on study until subsequent assessments (e.g., MRI, CSF cytology) confirm progression. If subsequent testing confirms progression, the date of progression should be backdated to the onset of neurologic deterioration.

10.1.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

10.1.4 Imaging Analyses and Central Review

At the end of the study, images will be evaluated by central review, as feasible and appropriate. 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.

10.2 Response Criteria – HGG

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by standard criteria as outlined by the Response Assessment in Pediatric Neuro-Oncology (RAPNO) and Immunotherapy Response Assessment in Neuro-Oncology (iRANO) international working groups.

10.2.1 Definitions

Evaluable for toxicity. All participants will be evaluable for toxicity from the time of their first treatment with TGFβi NK Cells.

Evaluable for objective response. Those participants who have measurable disease present

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

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

Evaluable for DLT period: If patient develops a DLT during cycle 1 they will be considered evaluable for estimating the MTD. Patients without DLT must complete 85% of prescribed dosing during cycle 1 to be evaluable for estimating the MTD.

10.2.2 Imaging and Disease Parameters

MRI imaging requirements for **primary brain and cord tumors**

Sequence	Comment
Brain	
Pre-gadolinium administration sequences 3D T1 GRE or TSE or 2D T1 SE[1] Axial DWI[1] SWI or GRE[3] **Post-gadolinium administration sequences** Axial T2 FSE[1] – recommended to be done first after gadolinium administration 3D T1 GRE or TSE[1] 2D T1 SE (axial or coronal)[3] 3D or 2D T2 FLAIR[2]	CISS or FIESTA can be replaced with Sagittal T2 weighted SPACE/CUBE/VISTA
Spine	
Pre-gadolinium administration sequences Sagittal T1 SE[1] **Post-gadolinium administration sequences** Sagittal T2 FSE or STIR[1] Axial T2 FSE[1] Sagittal T1 SE[1] 3D Axial T1 (VIBE/ FAME/LAVA/THRIVE) or Axial T1 SE[1] – 4-5mm maximal slice thickness, maximal 10% gap Sagittal CISS or FIESTA[3]	Sagittal CISS/FIESTA can be replaced with Sagittal T2 SPACE/CUBE/VISTA

[1] Mandatory
[2] Mandatory to be done post-contrast if concern for leptomeningeal disease. Can be done post-contrast for others
[3] Recommended

Measurable disease:

Measurable disease is defined as one or more lesions meeting a minimal size threshold. The size threshold is met if both in plane diameters are ≥10 mm or both in plane diameters are at least two times the MRI slice thickness, plus the interslice gap. These criteria apply to both CE and non-CE disease. Of note, measurements should never include cystic or necrotic portions (except for craniopharyngioma), nor the resection cavity. For instance, the rim enhancement surrounding the surgical cavity or surrounding cystic components should be categorized as non-measurable, unless presenting an enhancing nodule that meets the criteria for measurable disease.

Leptomeningeal disease can be considered measurable if focal and meeting the same size threshold.

All tumor measurements are taken using calipers on a picture archiving and communications system (PACS) and recorded in millimeters or one decimal fraction of centimeters. All baseline evaluations will be performed as closely as possible to the beginning of treatment and never more than 30 days from registration. 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.

Non-measurable disease

Non-measurable disease includes all lesions not meeting the criteria for measurable disease. Non-measurable disease can be either focal or diffuse.

Target and Non-target lesion

For most CNS tumors, only one lesion/mass is present and therefore is considered a "target" for measurement/follow up to assess for tumor progression/response. If multiple measurable lesions are present, up to 3 can be selected as "target" lesions. Target lesions should be selected on the basis of size and suitability for accurate repeated measurements. All other lesions will be followed as non-target lesions (including CSF positive for tumor cells). The lower size limit of the target lesion(s) should be per the definition of measurable disease.

Non-target lesions should be evaluated or monitored, but their size is not incorporated in the assessment of the overall tumor burden. If multiple non-target lesions are present, up to 3 can be selected as "non-target" lesions. If previously non-target lesions grow and become measurable, they can become target lesions, and their size is then incorporated into the overall tumor burden.

Focal leptomeningeal disease can be a target lesion if meets the criteria of measurable disease as described above. If leptomeningeal disease is present, presence, type (focal vs diffuse) and location of leptomeningeal disease should be noted and change in extent/size assessed on follow up studies.

Tumor Measurements

The MRI sequence that best highlights the tumor (postcontrast T1, T2, or T2 FLAIR) will be chosen to response. The same sequence should be used for serial measurements. Response determination will be based on a comparison of product of perpendicular diameters or an area [**W** (longest diameter of the target lesion) **x T** (transverse measurement, perpendicular to W)] between the baseline assessment and the study date designated in the follow-up Report Form.

To assess response, the following ratio is calculated:

$$\frac{W \times T \text{ (current MRI)}}{W \times T \text{ (reference MRI)}}$$

Reports for the follow-up exams should reiterate the measurements obtained at baseline for each target lesion. Nontarget lesions or newly occurring lesions should also be enumerated in these reports, and changes in non-target lesions should be described.

3. The longest diameter can be measured from the axial plane or the plane in which the tumor is best seen or measured. The longest measurement of the tumor is referred to as the width (W).

4. The perpendicular measurement should be determined - transverse (T) measurement, perpendicular to the width (W) in the selected plane.

Additional considerations for cystic/necrotic lesions:

For most tumors, the cystic or necrotic components of a tumor are not considered in tumor measurements. Therefore, only the solid component of cystic/necrotic tumors should be measured. If cysts/necrosis composes the majority of the lesion, the lesion may not meet criteria for "measurable" disease. (see below bullet points)

- If the cyst/necrosis is eccentric, the W and T of the solid portion should be measured, the cyst/necrosis should be excluded from measurement.
- If the cyst/necrosis is central but represents a small portion of the tumor (< 25%), disregard and measure the whole lesion.
- If the cyst/necrosis is central but represents a large portion of the tumor, identify a solid aspect of the mass that can be reproducibly measured.

Overall Response Assessment:

The overall response assessment takes into account both the target and non-target lesions, and the appearance of new lesions, where applicable, according to the criteria described below. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant's best response assignment will depend on the achievement of both initial measurement and subsequent confirmation criteria.

Response Criteria, per RAPNO Criteria [108]

- **Complete Response**: Disappearance of all target and non-target lesions. Complete resolution of previously seen reduced diffusion or reference baseline imaging sequence for high grade tumors. There can be no appearance of new lesions. Clinical status should be stable or improved and patient off steroids and antiangiogenics. Must be confirmed on at least 2 separate time points at least 8 weeks apart.

- **Partial Response**: At least a 50% decrease in target lesions, taking as reference to the baseline MRI. Decreased size of previously seen reduced diffusion or reference baseline imaging sequence for high grade tumors. There can be no appearance of new lesions. Clinical status should be stable or improved and patient on stable or reduced dose of steroids and off antiangiogenics. Must be confirmed on at least 2 separate time points at least 8 weeks apart. For pontine DMG, >=25% decrease in target lesion is considered partial response.

- **Minor Response:**>=25% but < 50% decrease in target lesions, taking as reference to the baseline MRI. Decreased size of previously seen reduced diffusion or reference baseline imaging sequence for high grade tumors. There can be no appearance of new lesions. Clinical status should be stable or improved and patient on stable or reduced dose of steroids and off antiangiogenics.

- **Stable Disease:** Does not meet criteria for complete response, partial response, minor responseor progressive disease. There can be no appearance of new lesions. Clinical status should be stable or improved and patient on stable or reduced dose of steroids and off antiangiogenics.

- **Progressive Disease**: 1) At least a 25% increase in target lesions, taking as reference the baseline or best response or 2) clear increase in size of non-target lesions from baseline or best response or increased size of reduced diffusion or any new focus of reduced diffusion not attributable to therapy or complications of therapy used in conjunction with other radiographical determinants or any new lesion or clinical deterioration.

- **Pseudoprogression**

  A 3-month confirmatory scan requirement will assure that patients are not prematurely assigned to have progressive disease while receiving immune-based therapy for high grade glioma. In addition, 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-up scan to assess for true progressive disease versus pseudoprogression. This will apply to patients that demonstrate worsening of the MRI within 6 months of start of therapy. Patients 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 use of steroid treatment, the patient will be classified as having pseudoprogression 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 patient 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 patient should be classified has having pseudoprogression and should remain on study. Patients 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 should 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.

  Response definitions per RAPNO: (patients must meet ALL criteria in each response/stable disease category, or ANY criteria in the progressive disease category) [108]

	Complete Response (must meet ALL criteria)	**Partial and Minor Response * (must meet ALL criteria)**	**Stable Disease (must meet ALL criteria)**	**Progressive Disease (must meet ANY criteria)**
MRI	No evidence of disease (measurable or non-measurable); completed resolution of	Partial response is a ≥ 50% decrease (compared with baseline) in the sum of the products of	Does not meet criteria for complete response, partial response, minor	Progressive disease is a ≥ 25% increase in the sum of the products of the 2 perpendicular diameters of target

	previously seen restricted diffusion*; no new lesions. Must be confirmed on at least 2 separate time points at least 8 weeks apart.	the 2 perpendicular diameters of target lesions; no new lesions. Minor response is a ≥ 25% but < 50% decrease (compared with baseline) in the sum of the products of the 2 perpendicular diameters of target lesions; no new lesions. Decrease in size of previously noted area of restricted diffusion* Must be confirmed on at least 2 separate time points at least 8 weeks apart.	response or progressive disease	lesions compared with (a) baseline measurement or best response or (b) a clear increase in size of non-measurable disease or non-target lesions from baseline or best response Increase size of area of restricted diffusion or any new focus of restricted diffusion not attributable to therapy or complications of therapy*
Neurologic exam	Stable or improving	Stable or improving	Stable or improving	Clinical deterioration not attributable to other causes
Antiangiogenic or steroid use	Off steroids or physiologic replacement doses only. Off antiangiogenics	Stable or less than baseline dose of steroids. Off antiangiogenics.	Stable or less than baseline dose of steroids. Off antiangiogenics.	N/A

*If diffusion-weighted imaging is not obtained at baseline, determination of tumor response or progression is acceptable with the omission of this criterion moving forward

10.2.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

10.2.4 Imaging Analyses and Central Review

At the end of the study, images will be evaluated by central review, as feasible and appropriate. 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.

10.3 Response Criteria – MB and LM Seeding Tumors

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by standard criteria as outlined by the Response Assessment in Pediatric Neuro-Oncology (RAPNO) and Immunotherapy Response Assessment in Neuro-Oncology (iRANO) international working groups.

10.3.1 Definitions

Evaluable for toxicity. All participants will be evaluable for toxicity from the time of their first treatment with TGFβi NK Cells.

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

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

Evaluable for DLT period: If patient develops a DLT during cycle 1 they will be considered evaluable for estimating the MTD. Patients without DLT must complete 85% of prescribed dosing during cycle 1 to be evaluable for estimating the MTD.

10.3.2 Imaging and Disease Parameters

MRI imaging requirements for **primary brain and cord tumors**

Brain
Pre-gadolinium administration sequences 3D T1 GRE or TSE or 2D T1 SE[1] Axial DWI[1] SWI or GRE[2]

Post-gadolinium administration sequences Axial T2 FSE[1] – recommended to be done first after gadolinium administration 3D T1 GRE or TSE[1] 3D or 2D T2 FLAIR[1] 2D T1 SE (axial or coronal)[2]
Spine
Pre-gadolinium administration sequences Sagittal T1 SE[1] **Post-gadolinium administration sequences** Sagittal T2 FSE or STIR[1] Axial T2 FSE[1] Sagittal T1 SE[1] 3D Axial T1 (VIBE/ FAME/LAVA/THRIVE) or Axial T1 SE[1] (4-5mm maximal slice thickness, maximal 10% gap) Sagittal CISS or FIESTA[2] (CISS/FIESTA can be replaced with Sagittal T2 SPACE/CUBE/VISTA)

[1] Mandatory

[2] Recommended

Measurable disease:

Measurable disease is defined as one or more lesions meeting a minimal size threshold. The size threshold is met if both in plane diameters are ≥10 mm or both in plane diameters are at least two times the MRI slice thickness, plus the interslice gap. These criteria apply to both CE and non-CE disease. Of note, measurements should never include cystic or necrotic portions (except for craniopharyngioma), nor the resection cavity. For instance, the rim enhancement surrounding the surgical cavity or surrounding cystic components should be categorized as non-measurable, unless presenting an enhancing nodule that meets the criteria for measurable disease.

Leptomeningeal disease can be considered measurable if focal and meeting the same size threshold.

All tumor measurements are taken using calipers on a picture archiving and communications system (PACS) and recorded in millimeters or one decimal fraction of centimeters. All baseline evaluations will be performed as closely as possible to the beginning of treatment and never more than 30 days from registration. 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.

Non-measurable disease

Non-measurable disease includes all lesions not meeting the criteria for measurable disease. Non-measurable disease can be either focal or diffuse.

Target and Non-target lesion

For most CNS tumors, only one lesion/mass is present and therefore is considered a "target" for measurement/follow up to assess for tumor progression/response. If multiple measurable lesions are present, up to 3 can be selected as "target" lesions. Target lesions

should be selected on the basis of size and suitability for accurate repeated measurements. All other lesions will be followed as non-target lesions (including CSF positive for tumor cells). The lower size limit of the target lesion(s) should be per the definition of measurable disease.

Non-target lesions should be evaluated or monitored, but their size is not incorporated in the assessment of the overall tumor burden. If multiple non-target lesions are present, up to 3 can be selected as "non-target" lesions. If previously non-target lesions grow and become measurable, they can become target lesions, and their size is then incorporated into the overall tumor burden.

Focal leptomeningeal disease can be a target lesion if meets the criteria of measurable disease as described above. If leptomeningeal disease is present, presence, type (focal vs diffuse) and location of leptomeningeal disease should be noted and change in extent/size assessed on follow up studies.

Tumor Measurements

The MRI sequence that best highlights the tumor (postcontrast T1, T2, or T2 FLAIR) will be chosen to response. The same sequence should be used for serial measurements. Response determination will be based on a comparison of product of perpendicular diameters or an area [**W** (longest diameter of the target lesion) **x T** (transverse measurement, perpendicular to W)] between the baseline assessment and the study date designated in the follow-up Report Form.

To assess response, the following ratio is calculated:

$$\frac{W \times T \text{ (current MRI)}}{W \times T \text{ (reference MRI)}}$$

Reports for the follow-up exams should reiterate the measurements obtained at baseline for each target lesion. Nontarget lesions or newly occurring lesions should also be enumerated in these reports, and changes in non-target lesions should be described.

5. The longest diameter can be measured from the axial plane or the plane in which the tumor is best seen or measured. The longest measurement of the tumor is referred to as the width (W).

6. The perpendicular measurement should be determined - transverse (T) measurement, perpendicular to the width (W) in the selected plane.

Additional considerations for cystic/necrotic lesions:

For most tumors, the cystic or necrotic components of a tumor are not considered in tumor measurements. Therefore, only the solid component of cystic/necrotic tumors should be measured. If cysts/necrosis composes the majority of the lesion, the lesion may not meet criteria for "measurable" disease. (see below bullet points)

- If the cyst/necrosis is eccentric, the W and T of the solid portion should be measured, the cyst/necrosis should be excluded from measurement.
- If the cyst/necrosis is central but represents a small portion of the tumor (< 25%), disregard and measure the whole lesion.
- If the cyst/necrosis is central but represents a large portion of the tumor, identify a solid aspect of the mass that can be reproducibly measured.

Overall Response Assessment:

The overall response assessment takes into account both the target and non-target lesions, and the appearance of new lesions, where applicable, according to the criteria described below. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant's best response assignment will depend on the achievement of both initial measurement and subsequent confirmation criteria. Per RAPNO-MB criteria, definition of CR and PR requires sustained response for at least 4 weeks. As such, repeat disease evaluations are encouraged at short interval (4 -8 weeks); Subsequent evaluations can then resume at study recommended intervals.

Response Criteria, per RAPNO Criteria

- **Complete Response**: Disappearance of all target and non-target lesions. There can be no appearance of new lesions. Per RAPNO-MB criteria, CR requires sustained response for at least 4 weeks. Clinical status should be stable or improved and patient off steroids.

- **Partial Response**: At least a 50% decrease in target lesions, taking as reference to the baseline MRI. There can be no appearance of new lesions. Per RAPNO-MB criteria, PR requires sustained response for at least 4 weeks. Clinical status should be stable or improved and patient on stable or reduced dose of steroids.

- **Stable Disease:** Does not meet criteria for complete response, partial response, or progressive disease. There can be no appearance of new lesions. Clinical status should be stable or improved and patient on stable or reduced dose of steroids**.**

- **Progressive Disease**: 1) At least a 25% increase in target lesions, taking as reference the baseline or best response or 2) clear increase in size of non-target lesions from baseline or best response or any new lesion or clinical deterioration.

- **Pseudoprogression**

  A 3-month confirmatory scan requirement will assure that patients are not prematurely assigned to have progressive disease while receiving immune-based therapy for high grade glioma. In addition, 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-up scan to assess for true progressive disease versus pseudoprogression. This will apply to patients that demonstrate worsening of the MRI within 6 months of start of therapy. Patients 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 use of steroid treatment, the patient will be classified as having pseudoprogression 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 patient 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 patient should be classified has having pseudoprogression and should remain on study. Patients 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 should 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.

Response definitions per RAPNO-MB: (patients must meet ALL criteria in each response/stable disease category, or ANY criteria in the progressive disease category) [110]

	Complete Response (must meet ALL criteria)	Partial Response (must meet ALL criteria)	Stable Disease (must meet ALL criteria)	Progressive Disease (must meet ANY criteria)
MRI	Complete disappearance of all disease (enhancing and non-enhancing, measurable and non-measurable) for a minimum of 4 weeks; no new lesions	≥ 50% decrease (compared with baseline) in the sum of the area of all (up to 4) measurable lesions sustained for at least 4 weeks; no progression of non-measurable disease	Does not meet criteria for CR, PR, or PD	≥25% increase (compared with the smallest measurement at any time point) in the sum of the products of perpendicular diameters of all measurable lesions; significant progression of non-measurable disease not attributed to prior therapy; any new tumor (any new lesions suspected to be treatment related should be confirmed by biopsy)

CSF cytology	If tumor cells are present at baseline, must be negative x 2 (sampling at least 2 weeks apart)	If absent (negative) at baseline, must remain absent. If present at baseline, can be present or absent	If absent at baseline, must remain absent. If present at baseline, can be present or absent	Previously absent tumor cells in CSF now present (positive)
*Neurologic exam	Stable or improving	Stable or improving	Stable or improving	Clinical deterioration not attributable to other causes
Steroid use	Off steroids or physiologic replacement doses only	Stable or less than baseline dose	Stable or less than baseline dose	
Extra-CNS disease	If positive at any time point, must be reevaluated and have no evidence of disease	No new sites of disease	No new sites of disease	New sites of disease

*If it is unclear that the patient has disease progression, it may be a reasonable option to keep the patient on study until subsequent assessments (eg, MRI, CSF cytology) confirm progression. If subsequent testing confirms progression, the date of progression should be backdated to the onset of neurologic deterioration.

10.3.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

10.3.4 Imaging Analyses and Central Review

At the end of the study, images will be evaluated by central review, as feasible and appropriate. 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.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design

We will employ the Bayesian optimal interval (BOIN) design (Liu and Yuan, 2015; Yuan et al., 2016) to find the RP2D. The BOIN design is implemented in a simple way similar to the traditional 3+3 design, but is more flexible and possesses superior operating characteristics that are comparable to those of the more complex model-based designs, such as the continual reassessment method (CRM) (Zhou et al., 2018).

The target toxicity rate for the RP2D is $\phi = 0.3$ and the maximum sample size is 24. We will enroll and treat participants in cohorts of size 3. DLTs are defined in **Section 5.3**, and only those DLTs that occur within **the first cycle** will be used for dose finding. As shown in Figure 9, the BOIN design uses the following rule, optimized to minimize the probability of incorrect dose assignment, to guide dose escalation/de-escalation:

- if the observed DLT rate at the current dose is ≤ 0.236, escalate the dose to the next higher dose level;
- if the observed DLT rate at the current dose is > 0.359, de-escalate the dose to the next lower dose level;
- otherwise, stay at the current dose.

For the purpose of overdose control, doses j and higher levels will be eliminated from further examination if $\Pr(p_j > 0.3 \mid \text{data}) > 0.95$ and at least 3 evaluable participants have been treated at dose level j, where p_j is the true DLT rate of dose level $j, j = 1,\cdots, 4$. This posterior probability is evaluated based on the beta-binomial model $y_j \mid p_j \sim \text{binomial}(p_j)$ with $p_j \sim \text{uniform}(0,1)$, where y_j is the number of participants experienced DLT at dose level j. When the lowest dose is eliminated, stop the trial for safety. The probability cutoff 0.95 is chosen to be consistent with the common practice that when the target DLT rate <=1/6, a dose with 2/3 participants experienced DLT is eliminated. The above dose escalation/de-escalation and elimination rule can be equivalently presented in Table 3, which will be used to conduct the trial.
The steps to implement the BOIN design are described as follows:

1. Participants in the first cohort are treated at dose level 2.
2. To assign a dose to the next cohort of participants, conduct dose escalation/de-escalation according to the rule displayed in Table 3. When using Table 3, please note the following:
    a. "Eliminate" means eliminate the current and higher doses from the trial to prevent treating any future participants at these doses because they are overly toxic.
    b. When we eliminate a dose, automatically de-escalate the dose to the next lower level. When the lowest dose is eliminated, stop the trial for safety. In this case, no dose should be selected as the RP2D.

c. If none of the actions (i.e., escalation, de-escalation or elimination) is triggered, treat the new participants at the current dose.

d. If the current dose is the lowest dose and the rule indicates dose de-escalation, treat the new participants at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point terminate the trial for safety.

e. If the current dose is the highest dose and the rule indicates dose escalation, treat the new participants at the highest dose.

3. Repeat step 2 until the maximum sample size of 24 is reached, or stop the trial if the number of evaluable participants treated at the current dose reaches 12 and the decision according to Table 3 is to stay at the current dose.

Table 3. Dose escalation/de-escalation rule for the BOIN design

	1	2	3	4	5	6	7	8	9	10	11	12
Number of evaluable participants treated at current dose	1	2	3	4	5	6	7	8	9	10	11	12
Escalate if # of DLT <=	0	0	0	0	1	1	1	1	2	2	2	2
Deescalate if # of DLT >=	1	1	2	2	2	3	3	3	4	4	4	5
Eliminate if # of DLT >=	NA	NA	3	3	4	4	5	5	5	6	6	7

Note. "# of DLT" is the number of participants with at least 1 DLT. When none of the actions (i.e., escalate, de-escalate or eliminate) is triggered, stay at the current dose for treating the next cohort of participants. "NA" means that a dose cannot be eliminated before treating 3 evaluable participants.

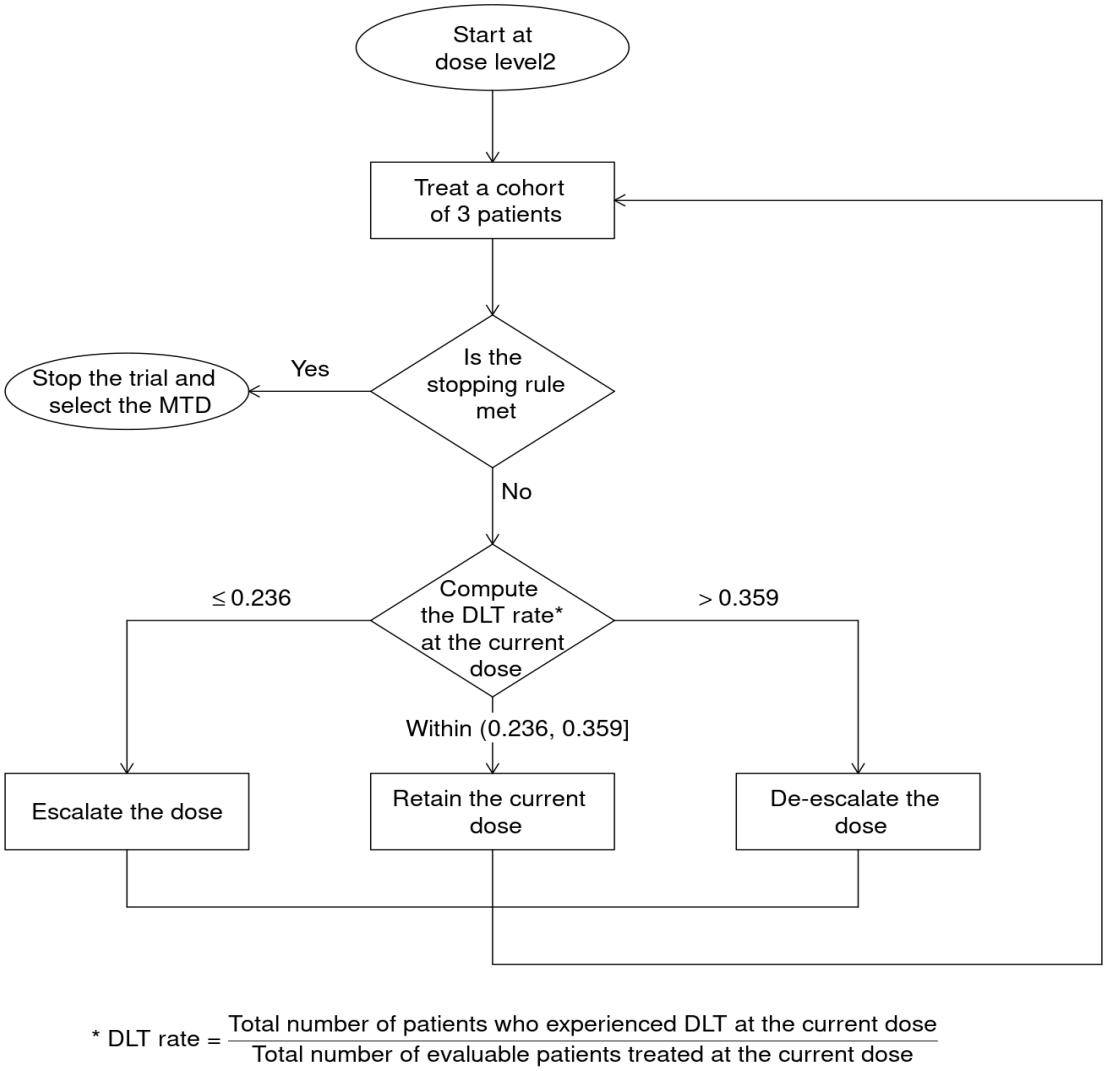


Figure 9. Flowchart for trial conduct using the BOIN design

After the trial is completed, select the RP2D based on isotonic regression as specified in Liu and Yuan (2015). This computation is implemented by the shiny app "BOIN" (Zhou et al., 2020) available at http://www.trialdesign.org. Specifically, select as the RP2D the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, select the higher dose level when the isotonic estimate is lower than the target toxicity rate and select the lower dose level when the isotonic estimate is greater than or equal to the target toxicity rate.

Operation Characteristics

Table 4 shows the operating characteristics of the trial design based on 1000 simulations of the trial using shiny app "BOIN" (BOIN V2.6.4.0) available at http://www.trialdesign.org. The operating characteristics show that the design selects the true RP2D, if any, with high probability and allocates more participants to the dose levels with the DLT rate closest to the target of 0.3.

Table 4. Operating characteristics of the BOIN design

	1	2	3	4	Number of Participants	% Early Stopping
Scenario 1						
True DLT Rate	0.3	0.47	0.55	0.64		
Selection %	61.1	26.3	2.7	0		9.9
% Pts Treated	45.8	44.7	8.8	0.7	18.4	
Scenario 2						
True DLT Rate	0.11	0.3	0.45	0.67		
Selection %	17.3	60.9	21.5	0.3		0
% Pts Treated	17.3	51.6	26.6	4.5	19.6	
Scenario 3						
True DLT Rate	0.02	0.13	0.3	0.47		
Selection %	0.2	21.1	59.5	19.2		0
% Pts Treated	1.5	32.8	43.2	22.5	20.9	
Scenario 4						
True DLT Rate	0.05	0.1	0.15	0.3		
Selection %	0.2	4	27.5	68.3		0
% Pts Treated	0.9	21.4	33.1	44.6	20.7	

Note: "% Early Stopping"" refers to early stopping due to excessive DLT.

11.2 Sample Size and Accrual Rate

The study design will be performed in up to 24 participants in BOIN dose escalation. The target accrual expected would be 6 to 8 participants per year. With this projected accrual it is expected that this study will be completed in 3-4 years.

The secondary study endpoints include the activation status and the persistence of TGFβi NK cells, the immunophenotype and function of TGFβi NK cells, as well as the response to TGFβi NK cells. Tumor-associated fluid samples will be collected at the indicated time points for laboratory evaluation of *in vivo* activation of the expanded TGFβi NK cells to study the effect of this therapy on the immune system.

11.3 Analysis of Primary Endpoints

Safety of TGFβi NK cell infusions will be assessed by monitoring for adverse events, scheduled laboratory assessments, vital sign measurements, and physical examinations for participants who receive at least one dose of the study drug. The severity of toxicities will be graded according to the NCI CTCEA v5.0. Adverse events and clinically significant laboratory abnormalities (meeting Grade 3, 4, or 5 criteria according to CTCAE) will be summarized by maximum intensity and relationship to study drug(s). Grade 1 and 2 adverse events will be summarized if related to study therapy. Safety will be assessed at the end of cycle 1 (28 days). Descriptive statistics will be utilized to display the data on toxicity seen. Toxicities will be summarized by tabulation in terms of type, grade and attribution for each dose level of each group of participants studied at the end of the trial. Antitumor activity will be described for each group of participants based on imaging and cytology.

11.4 Analysis of Exploratory Endpoints

OS in children with recurrent or progressive malignant brain tumors will be the clinical efficacy secondary endpoint. Any eligible subject that receives at least one dose of the study drug will be considered evaluable for clinical efficacy. OS will be estimated using the Kaplan-Meier method. 95% confidence intervals will be provided for OS estimates.

Fluid from the tumor cavity will be obtained for quantitative analysis by standard laboratory techniques before therapy, during the TGFβi NK cell treatment period, and after TGFβi NK cell treatment as described in Section 8. Data derived from these samples will be summarized by dose level with simple summary statistics: means (possibly after transformation) or medians, ranges, and standard deviations (if numbers and distribution permit). Scatterplots will be used to explore possible associations between the dose and estimates of the persistence, potency, or these determinations and toxicity (as reflected in the maximum grade of toxicity experienced or in clinical measurements). Participants with a tumor response or stable disease will be compared to other participants to explore whether there is an association with persistence, potency, or phenotype determinations. Statistical manipulations on complex phenotypic datasets may utilize SPADE, ViSNE, or tSNE to construct relatedness cloud mapping and identify phenotypic subsets that are similar or distinct between expanded TGFβi NK cells product and NK cells recovered from the tumor site.

Quality of life assessments, cognitive measures, and health related social risk assessment will be collected for descriptive purposes. No formal hypothesis will be performed on these assessments. Results will potentially be used as reference for future clinical trials.

11.5 Stopping Rules

- More than 1 participant with grade 3 or higher toxicity that does not improve to grade 1 or baseline after withholding protocol therapy for 28 days.
- More than 1 participant who experiences irreversible neurological deficits that are grade 3 or higher and definitely related to adjuvant therapy with study drug.
- More than 1 participant who experiences a serious, unexpected adverse event possibly related to protocol therapy.
- Any participant death at any time determined to be at least possibly related to protocol study agent.
- A death occurring within 30 days from administration of the study agent unless clearly due to disease progression

11.6 Analysis Population

11.6.1 Intent-to-Treat Population (ITT)

The ITT population will include all participants who are enrolled in the study. The ITT population will be the primary population for evaluating efficacy and subject characteristics.

11.6.2 **As-Treated Population (AT)**

The AT population will include all participants who receive at least 1 dose of study drug. The AT population will be the primary population for evaluating safety. If a patient does not receive any vaccine they will be replaced.

12. DATA REPORTING / REGULATORY REQUIREMENTS

12.1 Data Reporting

12.1.1 Method

The PNOC site Principal Investigator (PNOC PI) 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 eSource + EDC *via* standardized CRFs in accordance with the eSource + EDC study calendar, using single data entry with a secure access account. Study personnel at each site will complete the CRFs as soon as possible upon completion of the study visit.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the participant'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 participant.

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

All source documentation and CTMS/eSource + EDC data will be available for review/monitoring by the UCSF DSMC and regulatory agencies. The DSMC performs remote review/monitoring for non-UCSF PNOC sites. Study personnel will upload redacted source documents per guidance in SharePoint.

The PNOC PI 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.

12.1.2 Responsibility for Data Submission

Please refer to Appendix C for data submission timelines.

12.2 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 DSMCs to monitor the study in accordance with the available NCI approved Data Safety and Monitoring Plans (DSMPs). For member institutions that do not follow an NCI approved DSMP, the UCSF DSMC will be considered the institutional DSMC. 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.

12.3 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 registration information, risk assessment, and other issues affecting the conduct of the study, as appropriate.

12.4 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 participants, 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 participant 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.

12.5 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.

12.6 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 registration.

12.7 Protection of human subjects

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.

12.8 Protection of privacy

Subjects 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 subject's medical records, and each subject 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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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

Please check SharePoint for any updates or relative additional information for this list.

Prior to opening a study at any member institution, the following regulatory documents must be submitted to the PNOC Operations Office:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form and HIPPA form (if separated)
- Participating Site IRB membership list
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Copy of the 1572
- 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
- Completed and signed financial disclosure forms (FDFs) for all staff listed on participating site's 1572
- Participating site laboratory certifications and normals
- Signed copy of the completed delegation of authority log (found in PNOC Documents > Forms)
- Signed copy of the site initiation visit log
- Signed copy of the protocol signature page
- Signed copy of the final contract

Upon receipt of the required documents, the PNOC Operations Office will formally contact the site and grant permission to proceed with registration. 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 days of registration
Baseline Assessment Forms	Within 14 days of registration
Treatment Forms	Within 10 days of the last day of each cycle
Adverse Event Report Forms	All AEs are due within 10 business days of the date of assessment.
Serious Adverse Event Reporting	Within 1 business day of PI first awareness
Response Assessment Forms	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Forms	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 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 institutional DSMC/DSMB activities for this study will include:

- Participant monitoring prior to dose escalation.
- Review of participant data in each cohort
- Approval of dose escalation by DSMC Chair or Vice Chair
- Review of serious adverse events
- Minimum of biennial regulatory auditing

Monitoring and reporting guidelines

The UCSF HDFCCC Data and Safety Monitoring Committee (DSMC) is responsible for participant safety for all domestic sites for HDFCCC Multicenter and Consortium institutional clinical trials. The International sites must be monitored by a Clinical Research Organization (CRO) that is formally approved by the HDFCCC Cancer Center Clinical Research Oncology Committee (CCCROC) and the HDFCCC DSMC via the HDFCCC Policy of Minimum Standards for Partnership with International CROs.

All multicenter phase 1 dose escalation trials are monitored prior to the requested dose escalation of the dosing cohort. All participants are monitored through the Dose Limiting Cohort until the Recommended Phase-2 Dose (RP2D) is determined. Once the RP2D is determined, then the trial is audited on a semiannual basis with twenty percent of the participants enrolled in this expansion cohort that are audited through their first five cycles of treatment. Scheduled auditing of participant source documents is complete after all files have been reviewed for 2 cycles of treatment (20% of participants). For Phase 1 high risk therapeutic trials that are not dose finding, all participants are monitored on a quarterly basis (depending on accrual) through the first cycle of therapy.

DSMC Monitor/Auditors will send a follow-up report to the study team within 20 business days after the monitoring visit is complete for the PI and the study team to resolve all action items from this report within 20 business days. An abbreviated regulatory review (i.e., reviewing protocol and consent versions, SAEs, PVs, DOA logs, 1572 forms, etc.) will occur at each participant monitoring review; however, a full regulatory review will occur on a biennially basis by the DSMC for regulatory compliance

Monitoring of enrolled participants in the dose expansion portion of the trial will be complete after 20% of enrolled participants have been monitored through five cycles of treatment. However, regulatory reviews of the trial, safety reviews (i.e., Serious Adverse Event (SAE) reviews and

Protocol Violation (PV) reviews), as well as audit/inspection preparation (as applicable) will continue until the trial is closed by the IRB.

The UCSF HDFCCC Data and Safety Monitoring Committee (DSMC) is responsible for participant safety for all domestic sites for HDFCCC Multicenter and Consortium institutional clinical trials. The International sites must be monitored by a Clinical Research Organization (CRO) that is formally approved by the HDFCCC Cancer Center Clinical Research Oncology Committee (CCCROC) and the HDFCCC DSMC via the HDFCCC Policy of Minimum Standards for Partnership with International CROs. In the case of all PNOC protocols, the UCSF DSMC will work together with PNOC member institution DSMC/DSMBs in order to ensure DSMP compliance.

PNOC and the UCSF DSMC reserve the right to conduct on-site monitoring at any non-UCSF member institution. 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 PNOC and the UCSF DSMC to monitor protocol compliance, participant 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 participant safety and discuss each participant'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. The following items will be discussed, as appropriate:

- Registration information
- Cohort updates (i.e. DLTs and dose escalations)
- 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

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

If a participant experiences a Dose Limiting Toxicity (DLT), the PNOC Operations Office will notify all sites within one business day of awareness. If the DLT occurs at a participating site, the local investigator must report the DLT to the PNOC Operations Office within one business day. The team has one business day in which to report the DLT information to all participating sites.

Dose Escalations

At the time of dose escalation, a written and signed Dose Escalation Report will be submitted to the DSMC Chair (or Vice Chair) and DSMC Director describing the cohorts, dose levels, adverse events, safety reports, and any Dose Limiting Toxicities (DLTs) observed, in accordance with the protocol. The report will be reviewed by the DSMC Chair or Vice Chair and written authorization to proceed or a request for more information will be issued within two business days of the request. The report is then reviewed at the subsequent DSMC Committee meeting. In the event that the committee does not concur with the DSMC Chair's (or Vice Chair's decision, study accrual is held while further investigation takes place.

Adverse event review and monitoring
PNOC uses the web-based OnCore® Clinical Trials Management System for all participant registrations and eSource + EDC for data entry. The OnCore® System will also track participant level protocol compliance and safety information. The eSource + EDC system is CFR part 11 compliant.

For Phase 1 studies, all Adverse Events (AEs) will be entered into OnCore®/the eSource + EDC, regardless of relationship. All AEs entered into The Advarra EDC 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 AEs must be entered into eSource + EDC within **10 business days** of becoming aware of the event. Member institutions will submit this information to PNOC via the Adverse Event Form within eSource + EDC.

In addition, all adverse reactions considered "serious" (also called Serious Adverse Events, or SAEs), regardless of relationship, must be entered in eSource + EDC, OnCore®, 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 meetings, 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, Vice Chair, and the DSMC Director within 1 business day of this notification, and the sponsor within 1 business day.

The data (i.e., redacted copies of source documents) from the participating sites will be downloaded into the PC console of OnCore prior to the monitoring visits or the DSMC will be provided with access to the participating site's electronic medical record (EMR) access in order for the DSMC to perform remote monitoring of the participating site's compliance with the protocol and applicable FDA regulations (for global sites, see site-specific approved monitoring plan).

Review of Adverse Event Rates
In the event of an increase in the frequency of Grade 3 or 4 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. The report will indicate if the incidence of adverse events observed in the study is above the range stated in the Investigator's Brochure or package insert.

If at any time the Study Chair or the PNOC Operations Office halts enrollment or stops the study due to safety issues, the DSMC Chair (or Vice Chair) and the DSMC Director must be notified within one business day via e-mail and the IRB must be notified their reporting requirements.

Data and Safety Monitoring Board (DSMB) Reports

Data and Safety Monitoring Board (DSMB) Reports which provide information on trial accrual, participant safety, and data integrity will be provided to all sites, including the domestic and international sites, on an annual basis. The DSMB Report will be signed by the DSMC Chair (or Vice Chair) and provided to the DSMC Committee for formal review at the next scheduled DSMC Committee meeting.

UCSF Data and Safety Monitoring Committee contacts:

UCSF DSMC Chair	UCSF DSMC Director
Katie Kelley, MD	John McAdams, MS
(415) 353-9888	(415) 476-8496
Katie.Kelley@ucsf.edu	John.Mcadams@ucsf.edu
UCSF-Box #3211	UCSF-Box #0981
San Francisco, CA 94158	San Francisco, CA 94158

APPENDIX E 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 participant's native language should not be administered. If participant is co-enrolled on PNOC COMP, health related quality of life assessments are to be captured under the PNOC COMP protocol. Assessments do not need to be collected or reported under PNOC028.

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, the PedsQL 3.0 Cancer Module, and the PedsQL 3.0 Multidimensional Fatigue 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.

Regarding ages for the PedsQL:

- Use the Toddler Parent Form for all participants ages 2-4
- Use the Young Child Form (self- and parent versions) for all participants ages 5-7
- Use the Child Form (self- and parent versions) for all participants ages 8-12
- Use the Teen Form (self- and parent versions) for all participants ages 13-17
- Use the Young Adult Form (self-report only) for all participants ages 18-25

PedsQL is available in several languages such as Spanish, German, Hebrew, French, etc. The test 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 participants greater than 17 years of age, we will utilize the PROMIS 57 for adults as well as the adult cognitive abilities short form 8a.

Regarding ages for the PROMIS:

- Parent Report Forms to be administered
  - Use Parent-Proxy 49 for all participants 5-17 years old
- Self-report Forms to be administered
  - For participants ages 8-17 use:
    - Pediatric-49
    - Cognitive Function supplemental short form 7a
  - For participants ages 18 and older use:
    - PROMIS-57
    - 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. 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.

Regarding ages for the BRIEF:

- Use the BRIEF-P parent report for all participants ages 2-4
- For participants age 5:
  - If the participant has **not** yet started Kindergarten, administer the BRIEF-P
  - If the participant has started Kindergarten, administer the BRIEF-2.
- Use the BRIEF-2 parent report for all participant ages 6-10.
- Use the BRIEF-2 parent report and BRIEF-2 self-report for all participants 11-17.
- Use the BRIEF-A self-report for all participants aged 18 and above.

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

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.

Regarding ages for the ABAS-3:

- Parent Report Forms to be administered:
  - 0-5 Parent/Primary Caregiver Form for all participants ages 0-4
  - For all participants age 5:
    - 0-5 Parent/Primary Caregiver Form for participants **not** yet in kindergarten
    - 5-21 Parent/Primary Caregiver Form for participants who have started Kindergarten
  - 5-21 Parent/Primary Caregiver Form all participants ages 6-21
- Self-report Forms to be administered:
  - Adult Self-Report Form for all participants aged 22 and older
  - Adult Self-Report Form for all participants aged 18-21 **only if** they live independently and attend medical appointments alone (i.e., without a parent/guardian)

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

ADMINISTRATION GUIDELINES

Please refer to SharePoint for the tests' administration guidelines.

APPENDIX F Biospecimen Banking

Biospecimen banking is an optional research activity. Samples are collected only from participants who have agreed to have their left-over tissue, CSF, 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 participant 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 indefinitely 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 Imaging Guidelines for PNOC Studies

Pre-Study Imaging Qualification

The most critical aspect of the advanced imaging being performed in this study is to match quantitative exam protocols prior to the initial treatment and at follow-up studies, so that direct comparisons of intra-patient parameters can be made. Each PNOC site must be satisfied that the anatomic imaging sequences being used at these times satisfy clinical criteria for evaluating their participants. Hence, while there should be an attempt to make the protocols as similar as possible between institutions, it may not be feasible for them to be identical, and so any comparisons that are being made will focus on changes within the patient rather than differences among individuals. Please note that the radiologist at each PNOC site should interpret the anatomic images for clinical purposes and then send them to UCSF for quantitative analysis.

All images generated for each patient should be uploaded centrally to Ambra so that they can be evaluated and confirmed that the protocol satisfies the requirements of the study. Sites should upload all images pertaining to each patient in real time.

Guidelines for Imaging Protocols

Serial exams should be performed on the same 3T MR system using the commercial 8-channel or other multi-channel head coil. The sequences may either be performed in a pure axial orientation or lined up with the AC-PC line, as is the default in many institutions.

Recommended outline of MR imaging protocol:

1. 3-plane localizer
2. T1-weighted pre-Gadolinium images: used as a reference for comparing with the post-Gadolinium images and to identify any sign of hemorrhage.
3. T2-weighted images: used in conjunction with the FLAIR images to define the spatial extent of the T2 lesion.
4. FLAIR images: required for defining treatment response using the RANO criteria or iRANO criteria as indicated per each study protocol.
5. Diffusion weighted images: the entire brain should be covered with at least 6 different gradient directions at b=1000 and with one acquisition having b=0. The slice thickness and spatial resolution should be chosen to allow calculation of maps of apparent diffusion coefficient and fractional anisotropy.
6. Echo planar gradient echo dynamic susceptibility contrast (DSC) images: A series of images should be acquired during the injection of a bolus of 0.1mmol/kg of Gadolinium contrast agent that is delivered at a rate of 3-5ml/s using a power injector and with a 15-20ml flush of normal saline delivered at the same rate. The dose and timing of Gadolinium should be kept consistent to facilitate clinical interpretation. Slice thickness (3-5mm) and location should be chosen to cover as much of the T2 lesion as possible. The injector delay should be set at 15-30s to allow a good definition of baseline intensities from the pre-bolus images.
7. Post-Gadolinium T1-weighted volumetric images: this high-resolution image is used to define the spatial extent of the enhancing volume and for registration between examinations.

8. Post-Gadolinium T1-weighted images: these should match the pre-Gadolinium images are used to define the extent of the enhancing lesion.

Any of the above sequences or a combination thereof may be used for quantitative analysis of disease response and/or treatment effect. Decisions regarding which sequences will be utilized will be determined as based on the specific study intervention and anticipated imaging findings that accompany the intervention (e.g. immunotherapy vs. targeted small molecules), as well as individual characteristics of tumor subtypes. In addition to assessing disease response and treatment effect, sequences may be used for pre-surgery exams, clinical evaluation of the patient, and volumetric analysis of regions of interest.

De-Identification and Labeling

De-Identification of Digital Images

Sites will utilize Ambra for the de-identification process. It is the responsibility of the PNOC sites to de-identify images within Ambra according to HIPAA, Institutional Review Board (IRB) guidelines, GCPs and local regulatory requirements, with the following considerations:

- Do not remove the date of the exam or the technical information (eg, slice location, kVP, echo time, etc).
- Do not modify time or date information before or during the de-identification process.
- Do not include MRI reports or secondary captures as part of the dicom images to upload

Labeling of Digital Images

Use the patient ID, the exam date (ddmmyy) and scan number (01 or 02 for the two advanced imaging exams) to label the data as follows: PatientID_exam date_xx

Checklist for Media Submission

- De-identified DICOM images.
- Completed Ambra Imaging Log (maintained at the site level)

Uploading Digital Images via Ambra:

- PNOC sites should upload images pertaining to each patient as close to the imaging timepoint as possible.
- PNOC sites should follow the process as outlined in the Ambra SOP on the PNOC SharePoint website

Example of Data Analysis Performed by PNOC Central Review

The anatomic images will be used to manually define the contrast enhancing lesion (CEL) and the T2 lesion (T2L), as well as T2/FLAIR changes. The T1 weighted pre-contrast image will be used to define a brain mask so that intensity values can be normalized. The diffusion images are processed to generate maps of apparent diffusion coefficient (ADC) and fractional anisotropy (FA). The perfusion data are processed to calculate maps of relative cerebral blood volume (rCBV), peak height (PH) and percentage recovery (RECOV).

APPENDIX H: Maximum Allowable Total Blood Draw Volumes for Research Purposes in Children

Body Weight (Kg)	Maximum volume (mL) drawn for research purposes in a 28-day period	Maximum volume (mL) drawn for research purposes for any single draw†
1	5	2.5
2	10	5
3	12	6
4	16	8
5	20	10
6	24	12
7	28	14
8	32	16
9	36	18
10	40	20
11-15	44-60	22-30
16-20	64-80	32-40
21-25	84-100	42-50
26-30	104-120	52-60
31-35	124-140	62-70
36-40	144-160	72-80
41-45	164-180	82-90
46-50	184-200	92-100
51-55	204-220	102-110
56-60	224-240	112-120
61-65	244-260	122-130
66-70	264-275	132-138
Greater than 70	275	138

† **Maximum allowable volume in one blood draw is limited to 2.5% of total blood volume, or one-half of the 28-day maximum allowable volume.**

APPENDIX I: Guidelines for Management of CRS

Definition: Cytokine release syndrome (CRS) associated with immune effector cell therapy is defined as a supraphysiologic response following any immune therapy that results in the activation or engagement of endogenous or infused T cells and/or other immune effector cells. Symptoms can be progressive, must include fever at the onset, and may include hypotension, capillary leak (hypoxia) and end organ dysfunction. The common symptoms of CRS are often not unique to CRS. Investigators must be cautious and exclude other causes of fever, hypotension, hemodynamic instability, and/or respiratory distress, such as an overwhelming infection. Bacteremia and other infections have been reported concurrent with, and even mistaken, for CRS. A reasonable temporal relationship to the cell therapy must be present.

Timeline: Typically within 14 days of effector cell infusion. Although immune effector cell-associated CRS may have a delayed onset, it rarely presents beyond 14 days after initiation of therapy.

Grading: Cytokine release syndrome grading is detailed in Appendix I Table 1. Organ toxicities associated with CRS may be graded according to National Cancer Institute (NCI) Common Terminology for Adverse Events (CTCAE) v5.0 but they do not influence CRS grading.

Appendix I Table 1: Grading of CRS

CRS parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever[1]	≥ 38ºC	≥ 38ºC	≥ 38ºC	≥ 38ºC
		With		
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
		And/or[2]		
Hypoxia	None	Requiring low-flow nasal cannula[3] or blow-by	Requiring high-flow nasal cannula, facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (eg, continuous positive airway pressure, bilevel positive airway pressure, intubation and mechanical ventilation)

Source: Lee et al., 2019

[1] Fever is defined as temperature ≥ 38°C not attributable to any other cause. In participants who have CRS then receive antipyretic or anti-cytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

[2] Cytokine release syndrome grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a participant with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as Grade 3 CRS.

[3] Low-flow nasal cannula is defined as oxygen delivered at 6 L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at > 6 L/minute.

Management: Management guideline is detailed in Appendix I Table 2. Upon developing the prodrome of high-persistent fevers following NK-cell infusion, participants should then be followed closely. Infection work-up should be immediately undertaken. The pharmacy should be notified of the potential need for tocilizumab and dexamethasone. Participant management in an intensive care unit may be required and the timing is dependent upon local institutional practice. In addition to supportive care, tocilizumab and dexamethasone may be administered in cases of moderate to severe CRS, especially if the participant exhibits any of the following:

- Hemodynamic instability despite IV fluid challenges and moderate stable vasopressor support.
- Worsening respiratory distress, including pulmonary infiltrates, increasing oxygen requirement including high-flow oxygen, and/or need for mechanical ventilation.
- Any other signs or symptoms of rapid deterioration despite medical management, such as neurological signs.

Appendix I Table 2: Management guideline for CRS

CRS Grade	Management
Grade 1	• Antipyretics and IV hydration • Diagnostic work-up to rule out infection • Consider growth factors and antibiotics if neutropenic
Grade 2	• Supportive care as in Grade 1 • IV fluid boluses and/or supplemental oxygen • Tocilizumab ± dexamethasone (0.5 mg/kg [maximum 10-20 mg per dose] IV divided every 6–8 hours) or its equivalent of methylprednisolone
Grade 3	• Supportive care as in Grade 1 • Vasopressor support and/or supplemental oxygen • Tocilizumab + dexamethasone (0.5 mg/kg [maximum 10-20 mg per dose] IV divided every 6–8 hours) or its equivalent of methylprednisolone
Grade 4	• Supportive care as in Grade 1 • Monitoring in intensive care unit • Vasopressor support and/or supplemental oxygen via positive pressure ventilation • Tocilizumab + dexamethasone (consider 1-2 mg/kg per dose, IV divided every 6–8 hours) or its equivalent of methylprednisolone

*Adapted from Neelapu 2019

While tocilizumab is effective in systemic CRS, it is known not to penetrate CNS rapidly. If the CNS signs are the predominant presentation, dexamethasone may be necessary to alleviate CNS toxicities. Because dexamethasone would interfere with NK cell function and efficacy, if used, it should be rapidly tapered.

Siltuximab, an anti-IL-6 therapy, may be administered beginning 2 to 24 hours after the first dose of tocilizumab, at the Investigator's discretion. Other anti-cytokine therapies, such as repeat administration of tocilizumab or siltuximab or etanercept, may also be considered if the participant does not respond to initial dose therapy. If the participant experiences ongoing CRS despite administration of anti-cytokine directed therapies, anti-lymphocyte (T or NK) therapies such as cyclophosphamide, anti-thymocyte globulin, or alemtuzumab may also be considered and as discussed by the study team.

APPENDIX J: Health Related Social Risk Assessment

The Health Related Social Risk Assessment is only applicable to study participants who reside in the United States. The assessment included below is only to be used as an example. Only administer the IRB approved assessment. Please refer to SharePoint for detailed administration instructions.

Health-Related Social Risk Questionnaire

The goal of this questionnaire is to better understand your life outside of the hospital. Please answer these questions to the best of your ability. There are no right or wrong answers. Please choose the answers that best reflect your current circumstances.

These survey answers are confidential and will not be uploaded into your medical record or shared with your providers and treatment team. If there are any items you would like to discuss further, please reach out to your local social worker or provider who can help connect you with a social worker. Additionally, providers may ask you if they can reach a social worker on your behalf.

We hope that you will answer all the questions, however we do understand that some of these questions might be difficult to answer. You are free to skip any questions that you do not feel comfortable answering. You are also free to stop completing the survey at any time.

The survey will take about 15 minutes to complete.

Thank you so much for your time.

Health-Related Social Risk Questionnaire

Name: _____

DOB: _____

Age: _____

Diagnosis: _____

Date of Diagnosis: _____

A. Self-Reported Racial/Ethnic Identity
[Pew Research Center/Office of Management and Budget definitions of race and ethnicity]

1. Are you of Hispanic, Latinx, or Spanish origin, such as Mexican, Puerto Rican, or Cuban? You can select as many as apply to you.
- ☐ No, not of Hispanic, Latinx, or Spanish Origin
- ☐ Yes, Mexican, Mexican American, Chicano
- ☐ Yes, Puerto Rican
- ☐ Yes, Cuban
- ☐ Yes, another Hispanic, Latino, or Spanish origin

2. Which of the following describes your race? You can select as many as apply to you.
- ☐ American Indian, Alaska Native, Aleutian, First Nations
- ☐ Asian or Asian-American
- ☐ Black or African-American (having origins in any of the Black racial groups of Africa)
- ☐ Middle Eastern or North African
- ☐ Native Hawaiian or Pacific Islander
- ☐ White (having origins in Europe, the Middle East, or North Africa)

B. Preferred Language
[Patient Demographic Questionnaire-Staff Administered HCUP]
[US Census 2020]

3. Do you speak a language other than English at home?
- ☐ Yes: _____
- ☐ No

3a. What language do you speak at home primarily?
_____

3b. How well do you speak English?
- ☐ Very well
- ☐ Well
- ☐ Not well
- ☐ Not at all

3c. How well do you understand English?
- ☐ Very well
- ☐ Well
- ☐ Not well
- ☐ Not at all

3d. What language do you feel most comfortable using when speaking to a doctor or nurse?
- ☐ English
- ☐ French
- ☐ Spanish
- ☐ Other: _____

3e. What language do you prefer receiving written medical information?
- ☐ English
- ☐ French
- ☐ Spanish
- ☐ Other: _____

3f. Would it help you to have an interpreter when you speak with a doctor or nurse?
- ☐ Yes
- ☐ No
- ☐ Don't know

C. Insurance and Medical Care
[Children's Health Watch and Children's Health Survey]

4. What type of health insurance does your child have? [If child has more than one type of health insurance, including public insurance, mark relevant public insurance]

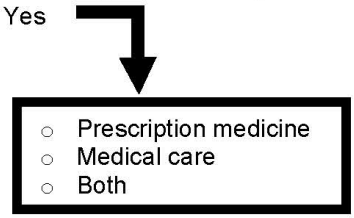
- ☐ Medicaid
- ☐ Other public insurance/Free Care
- ☐ No insurance/Pay out of pocket
- ☐ Private insurance
- ☐ Tricare/military insurance
- ☐ Other: _____

5. In the past year, was there ever a time when your child did not have health insurance?

- ☐ Yes
- ☐ No

6. Was there any time when you needed a prescription medicine or medical care, but were unable to get it because you couldn't afford it?

- ☐ Yes



- ☐ No

7. Is there a place you USUALLY take your child when they are sick or you need advice about their health?

- ☐ Yes
- ☐ No

8. If yes, where does this child USUALLY go first?

- ☐ Doctor's office
- ☐ Hospital Emergency Room
- ☐ Clinic or Health Center
- ☐ Retail Store Clinic or "Minute Clinic"
- ☐ School (Nurse's Office, Athletic Trainer's Office)
- ☐ Other

9. Is there a place you USUALLY take your child when they need routine preventive care, such as a physical examination or well-child check-up?

- ☐ Yes
- ☐ No

10. If yes, is this the same place this child goes when they are sick?
- ☐ Yes
- ☐ No

D. Education, Income, and Employment
[Children's Health Watch]
11. Which of the following best describes the highest level of education in the household?
- ☐ Some high school or less
- ☐ High school graduate or GED
- ☐ Technical school or some college
- ☐ College graduate
- ☐ Master's level or higher

12. Are you employed?
- ☐ Yes
- ☐ No

12a. If so, how many jobs do you have? _____

12b. How many hours do you work per week? _____

13. How many people are employed in the household? _____

14. What is your approximate household monthly income from all sources (employment, child support, alimony, TANF, SSI/SSDI, food stamps, all other sources)? $ _____

E. Housing Insecurity
[Children's Health Watch]

15. During the last 12 months, was there a time when you were not able to pay the mortgage or rent on time?
- ☐ Yes
- ☐ No

16. At any time were you homeless or living in a shelter (including now)?
- ☐ Yes
- ☐ No

F. Food Insecurity
[Children's Health Watch- Hunger Vital Signs]

17. Within the past 12 months, we worried whether our food would run out before we got money to buy more.
- □ Often true
- □ Sometimes true
- □ Never true
- □ Don't know

18. Within the past 12 months, the food we bought just didn't last and we didn't have money to get more.
- □ Often true
- □ Sometimes true
- □ Never true
- □ Don't know

G. Access to Utilities and Transportation
[Children's Health Watch, American Community Survey, NHIS]

19. Within the past 12 months, has the gas, electric, or oil company sent you a letter threatening to shut off the gas or electricity in the house for not paying bills?
- □ Yes
- □ No

20. Within the past 12 months, has the gas, electric, or oil company shut off or refused to deliver the gas, electricity, or oil for not paying bills?
- □ Yes
- □ No

21. In the last 12 months, have you ever used a cooking stove to heat the room, house, or apartment?
- □ Yes
- □ No

22. In the past 12 months, were there any days that the home was not heated because you could not pay the bills?
- □ Yes
- □ No

23. In the past 12 months, were there any days that the home was not cooled because you could not pay the bills?
- □ Yes
- □ No

24. In the past 12 months, were there any days that the water was shut off because you could not pay the bills?
- □ Yes

□ No

25. Can you or any member of this household both make and receive phone calls when in your residence?
□ Yes
□ No

26. At your residence, do you or any member of your household own or use any of the following types of computers:
26a. Desktop or laptop
□ Yes
□ No
26b. Smartphone
□ Yes
□ No
26c. Tablet or other portable wireless computer
□ Yes
□ No
26d. Some other type of computer (please specify)
□ Yes _____
□ No
□

27. At your residence, do you or any members of this household have access to the Internet?
□ Yes, by paying a cell phone company or Internet service provider
□ Yes, without paying a cell phone company or internet service provider
□ No access to the Internet at this residence

28. Do you or any members of this household have access to the Internet using:
28a. Cellular data plan for a smartphone or other mobile device?
□ Yes
□ No
28b. Broadband (high speed) Internet service such as cable, fiber optic, or DSL service installed in this household?
□ Yes
□ No
28c. Satellite Internet service installed in this household?
□ Yes
□ No
28d. Dial-up Internet service installed in this household?
□ Yes
□ No
28e. Some other service?
□ Yes _____
□ No

29. There are many reasons people delay getting medical care. Have you delayed getting care in the past 12 months because you did not have transportation?
- □ Yes
- □ No

H. Experiences of Discrimination
[Williams Everyday Discrimination Scale- Short Version]

In your day-to-day life, how often do any of the following things happen to you?

30. You are treated with less courtesy than other people are.
- □ Almost everyday
- □ At least once a week
- □ A few times a month
- □ A few times a year
- □ Less than once a year
- □ Never

31. You receive poorer service than other people at restaurants or stores.
- □ Almost everyday
- □ At least once a week
- □ A few times a month
- □ A few times a year
- □ Less than once a year
- □ Never

32. People act as if they think you are not smart.
- □ Almost everyday
- □ At least once a week
- □ A few times a month
- □ A few times a year
- □ Less than once a year
- □ Never

33. People acts as if they are afraid of you.
- □ Almost everyday
- □ At least once a week
- □ A few times a month
- □ A few times a year
- □ Less than once a year
- □ Never

34. You are threatened or harassed.
- □ Almost everyday

- At least once a week
- A few times a month
- A few times a year
- Less than once a year
- Never

35. If answering "A few times a year" or more frequently to at least one question: What do you think is the main reason for these experiences?

- Your ancestry or national origins
- Your gender
- Your race/ethnicity
- Your age
- Your religion
- Your height
- Your weight
- Some other aspect of your physical appearance
- Your education or income level
- Your disability
- Your shade of skin color
- Your tribe

I. Health Knowledge
[Cancer Health Literacy Test-6, Chew et al 2004]

36. How often do you have problems learning about your medical condition because of difficulty understanding written information?

- Always
- Often
- Sometimes
- Occasionally
- Never

37. How often do you have someone help you read hospital materials?

- Always
- Often
- Sometimes
- Occasionally
- Never

38. How confident are you filling out medical forms by yourself?

- Extremely
- Quite a bit
- Somewhat
- A little bit
- Not at all

APPENDIX K: Neurosurgical Checklist

PNOC028 Neurosurgical Checklist

Patient Name:
PNOC Screening ID (PNOC028-XX):
Date of Birth:
Date of Surgery:

This form stands to document neurosurgical procedures post-surgery. This form must be completed upon conclusion of the surgery.

Resection Cavity Dimensions:

Distance (in centimeters). *Please ensure the maximum visible resection cavity dimensions are at least 2 cm x 2 cm.*

Resection cavity: _____ cm **X** _____ cm

Lack of Ventricular Communication:

I confirm there is a lack of ventricular communication. _____ (surgeon initials)

Ommaya Insertion:

Once the maximum visible resection cavity dimensions and lack of ventricular communications are confirmed, the Ommaya may be inserted.

I confirm the Ommaya reservoir has been inserted. _____ (surgeon initials)

Surgeon Name: _____

Surgeon Signature: _____ **Date:** _____

PNOC028 Neurosurgical Checklist
v. 1.0 (6 Oct 2023)

APPENDIX L: STAGING AND GRADING OF ACUTE GRAFT VERSUS HOST DISEASE (AGVHD)

Extent of Organ Involvement			
Stage	**Skin[1]**	**Liver[2]**	**Gut[3,4]**
0	No GVHD rash	Total bilirubin <2 mg/dL	Diarrhea <500 mL/day or Pediatric <10 ml/kg/d
1	Maculopapular rash <25% BSA	Total bilirubin 2-3mg/dL	Diarrhea: >500 mL/day or Pediatric 10-19.9 mL/kg/day OR persistent nausea, vomiting, or anorexia with a positive upper GI biopsy
2	Maculopapular rash 25-50% BSA	Total bilirubin 3.1-6 mg/dL	Diarrhea: >1000 mL/day or Pediatric 20-30 mL/kg/day
3	Maculopapular rash >50% BSA	Total bilirubin 6.1-15mg/dL	Diarrhea: >1500 mL/day or Pediatric > 30 mL/kg/day
4	Generalized erythroderma (>50% BSA) with bullous formation or desquamation >5% BSA	Total bilirubin > 15mg/dL	Severe abdominal pain, with or without ileus, and/or grossly bloody stool

Overall Grade			
Grade	Skin	Liver	Gut
I	Stages 1-2	None	None
II	Stage 3	Stage 1	Stage 1
III	Stage 0-3	Stages 2-3	Stages 2 – 3
IV	Stage 4	Stage 4	Stage 4

"Rule of Nines"		
Body Area	**Percent**	**Total Percentage**
Single Upper Limb	9%	18%
Single Lower Limb	18%	36%
Chest & Abdomen	18%	18%
Back	18%	18%
Head	9%	9%
Perineum	1%	1%

[1]Use "Rule of Nines" to estimate extent

[2]Total bilirubin. Downgrade 1 stage if additional etiology of elevated bilirubin has been identified

[3]Includes diarrhea and/or persistent nausea. Downgrade 1 stage if additional cause of diarrhea is identified.

[4]Use adult values if patient is ≥50kg