Neurofibromatosis Clinical Trials Consortium

# NF111 PROTOCOL:
# *A Phase II Trial of Poly-ICLC for Progressive, Previously Treated Low-Grade Gliomas in Children and Young Adults with Neurofibromatosis Type 1*

Sponsored by:

Department of Defense
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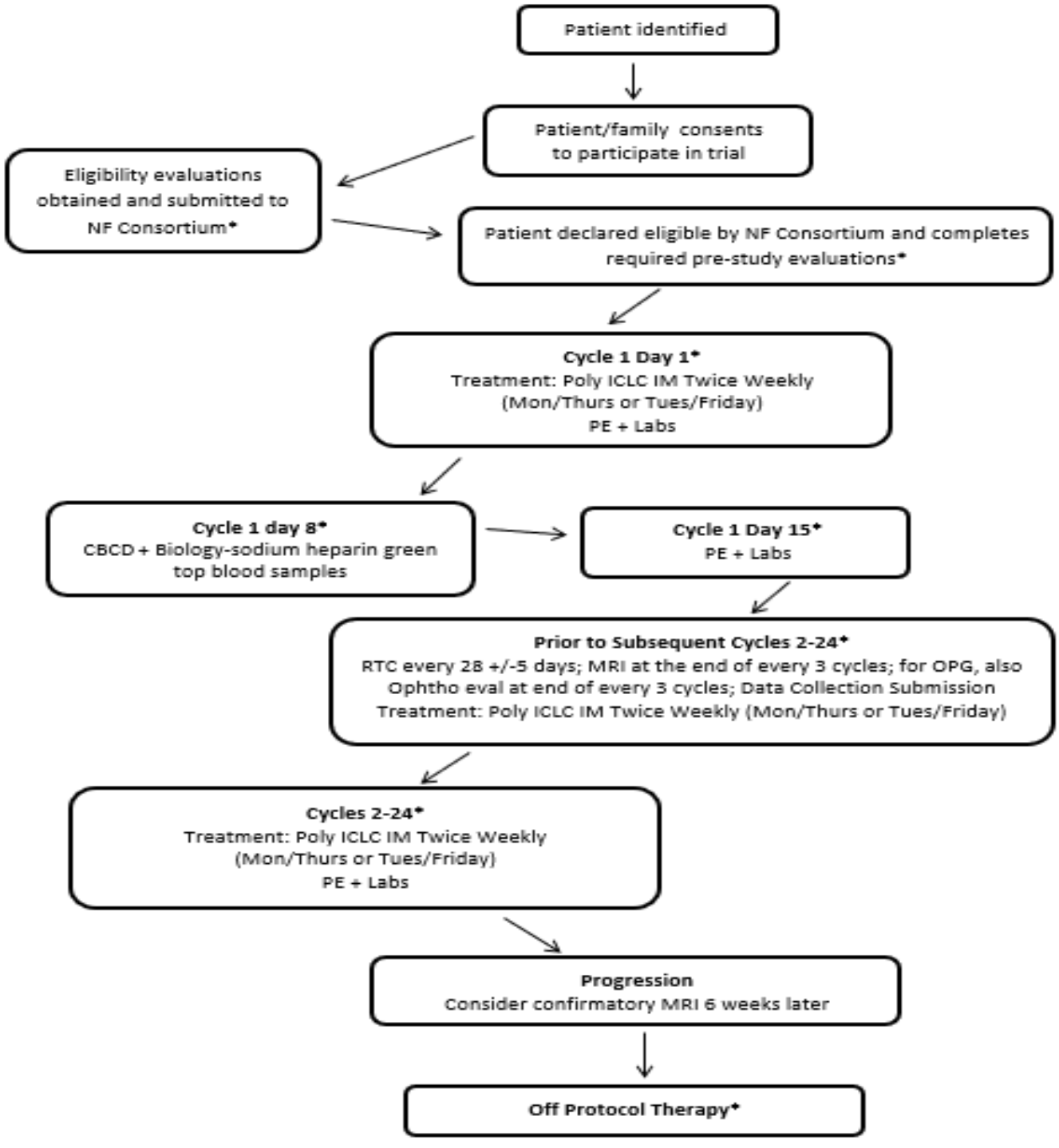
## Schema




*See study calendar for required observations

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## List of Abbreviations

| | |
|---|---|
| AE | Adverse event |
| ALT | Alanine aminotransferase/serum glutamic pyruvic transaminase/SGPT |
| AST | Aspartate aminotransferase/serum glutamic oxaloacetic transaminase/SGOT |
| BID | *bis in diem*/twice a day |
| CNS | Central nervous system |
| CR | Complete response |
| CRO | Contract Research Organization |
| CSF | Cerebral spinal fluid |
| CTO | Clinical Trials Office |
| CV | Carboplatin-vincristine |
| DOD | Department of Defense |
| DLT | Dose limiting toxicity |
| DSMC | Data and Safety Monitoring Committee |
| IB | Investigator's Brochure |
| IHC | Immunohistochemical |
| IRB | Institutional Review Board |
| JPA | Juvenile Pilocytic Astrocytoma |
| LGG | Low-grade glioma |
| MR | Minor response |
| MRI | Magnetic resonance imaging |
| MRS | Magnetic resonance spectroscopy |
| NF1 | Neurofibromatosis type 1 |
| OPG | Optic pathway glioma |
| PD | Progressive disease |
| PCP | Pneumocystis Carinii Prophalaxis |
| PJP | Pneumocystis Jeroveci Prophalaxis |
| PK | Pharmacokinetics |
| Poly-ICLC | Polyinosinic-Polycytidylic acid – poly-L-lysine carboxymethylcellulose |
| PR | Partial response |
| PRO | Patient reported outcomes |
| RANO | Response Assessment in Neuro-Oncology |
| RAPNO | Response Assessment in Pediatric Neuro-Oncology |
| RSI | Reference Safety Information |
| RTC | Return to clinic |
| SAE | Serious Adverse Event |
| SD | Stable disease |
| TMZ | Temozolomide |
| ULN | Upper limit of institutional normal |

## Glossary of Terms

| | |
|---|---|
| Assessment | A procedure used to generate data required by the study. |
| Cycle | Number and timing or recommended repetitions of therapy are usually expressed as number of days (e.g.: q28 days). |
| Enrollment/Enrolled | Point/time of patient entry into the study; the point at which all eligibility assessments have been completed and approval to enroll has been confirmed by study chairs. |
| Investigational Drug | The study treatment whose properties are being tested in the study; this definition is consistent with US CFR 21 Section 312.3 and is synonymous with "investigational new drug." |
| Investigational Treatment | Drug whose properties are being tested in the study as well as its associated placebo and active treatment controls (when applicable). This also includes approved drugs used outside of their indication/approved dosage, or that are tested in a fixed combination. Investigational treatment generally does not include other study treatments administered as concomitant background therapy required or allowed by the protocol when used in approved indication/dosage. |
| Other Study Treatment | Any drug administered to the patient as part of the required study procedures that was not included in the investigational treatment. |
| PID1 (Screening ID) | A unique participant identifying number generated when initially entered into the electronic Data Collection System (eDCS). |
| PID2 (Enrollment ID) | A second unique participant identifying number generated when entered into the electronic Data Collection System (eDCS) after enrollment has been approved by Protocol Chair. |
| Phase | A subdivision of the study timeline; divides stages into smaller functional segments such as screening, baseline, titration, washout, etc. |
| Premature Patient Withdrawal | Point/time when the patient exits from the study prior to the planned completion of all study treatment administration and/or assessments; at this time all study treatment administration is discontinued and no further assessments are planned, unless the patient will be followed for progression and/or survival. |
| Study Treatment | Includes any drug or combination of drugs in any study arm administered to the patient (participant) as part of the required study procedures. |
| Study Treatment Discontinuation | Point/time when patient permanently stops taking study treatment for any reason; may or may not also be the point/time of premature patient withdrawal. |

## Protocol Summary

| | |
|---|---|
| **Study Design:** | This is a phase II, prospective, longitudinal, multi-center trial of poly-ICLC (Hiltonol®) for progressive low-grade gliomas in pediatric and young adult patients with NF1. |
| **Primary Objective:** | Evaluate the efficacy of poly-ICLC in pediatric and young adult NF1 patients with progressive LGG as measured by objective tumor response rate (CR+ PR) within the first 48 weeks (12 cycles) of therapy. |
| **Secondary Objectives:** | Secondary objectives are to:<br>a) Determine progression free survival (PFS) at 12, 24 and 60 months in pediatric NF1 patients with progressive LGG treated with poly-ICLC<br>b) Evaluate the efficacy of poly-ICLC in pediatric NF1 patients with progressive LGG as measured by best objective tumor response rate (CR+PR) within 24 cycles of therapy<br>c) Evaluate the clinical benefit rate (CR + PR + MR+ SD) of poly-ICLC in pediatric NF1 patients with progressive LGG after 12 and 24 cycles of therapy<br>d) Assess the toxicity associated with treatment with poly-ICLC in pediatric patients with NF1 and LGG |
| **Exploratory Objectives:** | Exploratory objectives are to:<br>a) Evaluate the visual outcome in children with progressive optic pathway gliomas (OPG) treated with poly-ICLC.<br>b) Evaluate patient reported outcomes and quality of life measures<br>c) Evaluate biological correlates<br>d) Compare RANO and RAPNO response determinations in this population |
| **Treatment Description:** | Eligible patients will receive therapy with poly-ICLC at a dose of 20 mcg/kg/dose IM injections twice a week. Protocol treatment will last 24 cycles from the start of poly-ICLC in the absence of disease progression or significant toxicity. A cycle will consist of 28 days.<br><br>Participants will be seen and monitored at their treating center at the start of each cycle.<br><br>**Response Assessment**<br>Imaging to assess response will be performed at the end of cycle 3 (+/- 2 weeks) and after every three cycles (+/-2 weeks) until after cycle 24. All MRIs completed on study patients should be submitted for central |

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|---|---|
| | review. Response Assessment in Pediatric Neuro-Oncology (RAPNO) criteria will be used to determine radiographic response. |
| **Endpoints:** | **Primary endpoint**<br>• Objective response rate (CR+PR) within the first 48 weeks (12 cycles) of therapy, using RAPNO criteria<br><br>**Secondary endpoints:**<br>• PFS at 12, 24 and 60 months<br>• Best response rate (CR+PR) within 24 cycles of therapy<br>• Clinical benefit response rate (CR+PR+MR+SD) after 12 and 24 cycles of therapy<br>• Poly-ICLC-related toxicities<br><br>**Exploratory endpoints**<br>• Vision response after 12 and 24 cycles of therapy defined as 0.2 logMAR or greater in acuity improvement<br>• Patient reported outcomes and quality of life measures<br>• Biological correlates<br>• Comparison of RANO and RAPNO response determinations |
| **Accrual Objectives:** | Up to 20 participants will be enrolled to obtain a minimum of 16 evaluable participants on this study. |
| **Study Duration:** | Participants will be followed for a minimum of 5 years from treatment. Overall survival outcomes will continue to be measured up to 5 years from treatment. |
| **Interim Analysis:** | There will not be an interim analysis for efficacy. |

## 1. STUDY OBJECTIVES

This is a phase II, prospective, longitudinal, multi-center trial of poly-ICLC (Hiltonol ®) treatment for progressive low-grade gliomas in pediatric patients with NF1. The primary objective is to evaluate the efficacy of poly-ICLC in pediatric NF1 patients with progressive low-grade glioma (LGG) as measured by objective tumor response rate (CR+PR) within the first 48 weeks (12 cycles) of therapy.

The secondary objectives are to:

a) Determine 12, 24 and 60 month progression free survival (PFS) in pediatric NF1 patients with progressive LGG treated with poly-ICLC.
b) Evaluate the efficacy of poly-ICLC in pediatric NF1 patients with progressive LGG as measured by best objective tumor response rate (CR+PR) within 24 cycles of therapy.
c) Evaluate the efficacy of poly-ICLC in pediatric NF1 patients with progressive LGG as measured by clinical benefit response rate (CR+PR+MR+SD) after 12 and 24 cycles of therapy.
d) Assess the toxicity associated with poly-ICLC treatment in pediatric NF1 patients with LGG.

Exploratory objectives are to:

a) Evaluate the visual outcome measures in children with progressive optic pathway gliomas treated with poly-ICLC. Visual response is defined as 0.2 logMAR or greater in acuity improvement.
b) Evaluate patient reported outcomes and quality of life measures.
c) Evaluate biological correlates.
d) Compare RANO and RAPNO response determinations in this population.

# 2. SIGNIFICANCE

## 2.1. Background

Low-grade gliomas are the most common brain tumor of childhood (36% of childhood brain tumors). These tumors encompass a heterogeneous assortment of histological subtypes; the most common is pilocytic astrocytoma.

Neurofibromatosis type 1 (NF1) is a common neurocutaneous disorder (incidence 1:3,000 births). Pilocytic astrocytomas can be seen in up to 20% of children with NF1, commonly localized in the optic pathway. Most are diagnosed before age 7 years.[1] These tumors are most often diagnosed based on radiologic findings alone; biopsy is uncommon. Most of these tumors are indolent and do not require treatment. Factors deciding treatment initiation include imaging progression, decline in visual acuity or other functional impact.

Surgical resection is not always a possibility due to the location of the tumor. Radiation therapy is avoided in patients with NF1 given the increased risk of treatment-induced secondary neoplasm. Chemotherapy is the mainstay of treatment for NF1 patients with progressive or symptomatic tumors.

One commonly accepted chemotherapy regimen for NF1 LGG is carboplatin and vincristine. As reported by the Children's Oncology Group CCG9952, the 5-year EFS rate was 69% ± 4% for the NF1 group. Forty-two NF1 patients (33%) had adverse events, and 6 (4.7%) died. [2]

Alternative regimens have also shown efficacy in treating NF1 LGG. Patients with NF1 treated with vinblastine at a dose of 6 mg/m$^2$ IV x 70 weeks had a higher 5-year EFS than patients without NF1 (75.0%+/-15% vs 35.7%+/-7.6%, respectively). While quality of life was not affected, treatment was associated with a high incidence of bone marrow suppression, and the majority of patients required dose reductions.[3] Bevacizumab and irinotecan have activity based on imaging responses and improvement of visual acuity in patients with progressive LGG, though limited data is available for NF1 patients as compared to non-NF1 patients.[4] Initial data for MEK inhibition in NF1-associated pediatric low-grade glioma are promising: selumetinib 25 mg/m$^2$ twice daily resulted in 2-year PFS of 96%. Sixty-four percent completed 26 courses and had stable or responsive disease. Eighty-nine percent of evaluable patients had stability in visual acuity.[5] Other MEK inhibitors such as binimetinib (MEK162) and trametinib (MEK116540) are currently undergoing evaluation in clinical trials. Although the preliminary data are promising, the toxicities associated with MEK inhibitor therapy (rash, nailbed infection, decreased ejection fraction, among others) are not negligible and have significant quality of life implications in some cases. Newer forms of effective treatments with minimal side effects are much needed in children with NF1 and low-grade gliomas.

Preliminary results from a pilot study suggest that poly-ICLC may be effective in NF1 LGG, including evidence of functional improvement. We propose to study the efficacy and toxicity of poly-ICLC, a biological response modifier in children with NF1 LGG.

## 2.2. Pre-clinical Studies

Polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) is a double stranded RNA (dsRNA) that was used as an interferon inducer at high doses (up to 300 mcg/kg IV) in short-term cancer trials some years ago.[6] These trials gave mixed results with

moderate toxicity, and the use of poly-ICLC was generally abandoned when recombinant interferons became available.[7-13] However, lower dose (10 to 50 mcg/kg) poly-ICLC results in a broader host defense stimulation, including T-cell and natural killer cell activation and cytokine release (interferons alpha, beta, and gamma, interleukins, corticosteroids, and TNF). Levels of serum interferon induced by these doses of poly-ICLC are relatively low and have not been associated with anti-tumor action. Preliminary laboratory results of a pilot study showed no clear relationship between tumor response and measurable serum interferon, TNF, IL-2, IL-6, or neopterin; this agrees with prior animal studies.[14] Low dose poly-ICLC also has a direct immune enhancing action independent of interferon, including increased antibody response to antigen, and NK cell, T-cell, macrophage and cytokine activation. Recent evidence suggests that poly-ICLC is a ligand for the Toll receptor 3 (TLR3), an important component of innate immunity.

Another action of poly-ICLC is a more direct antiviral and perhaps antineoplastic effect mediated by at least two interferon inducible nuclear enzyme systems, the 2'5'oligoadenylate synthetase (OAS) and the P1/eIF2a kinase, also known as the dsRNA dependent $P_{68}$ protein kinase (PKR).[15-18] Double-stranded-RNA-dependent protein kinase PKR is a serine/threonine kinase which is activated by autophosphorylation upon binding to dsRNA. Activated PKR then phosphorylates the α-subunit of the translation initiation factor eIF-2, a modification that causes inhibition of protein synthesis. dsRNA induces an antiviral state in cells by functioning as an obligatory cofactor for OAS, which activates ribonuclease L; as well as for the PKR, which inhibits initiation of protein synthesis.[19-23] Both OAS and PKR are very sensitive to dsRNA dose and structure.[24] For example, simple, long chain dsRNA (as in poly-ICLC) is the most potent stimulator of OAS and PKR, while mismatched or irregular dsRNA can be inhibitory. Clinically, the OAS response is maximal at a dose of about 30 mcg/kg of poly-ICLC and is much diminished above a dose of 100 mcg/kg.[25] The clinical half-life of the OAS response to IM poly-ICLC is about 2.5 days, suggesting an optimum dose schedule of two or three times per week.[25]

Previously treated patients showed up to a 40-fold increase in serum OAS product in response to treatment at 10 to 20 mcg/kg, and a significant association of serum OAS with tumor response (P= .03).[26] Similarly, PKR has both high and low affinity binding sites and is inhibited by too high a dose of dsRNA.[27] Koromilas, et al, have demonstrated that expression of a functionally defective mutant of PKR results in malignant transformation in vitro, suggesting an important role for this enzyme in suppression of tumorigenesis as well.[28] They also suggest a possible relation to the p53 tumor suppressor associated with multiple malignancies (Li-Fraumeni syndrome), which includes astrocytomas, sarcomas, lung, and breast cancers.[29] Recently, the role of PKR has been implicated in signaling pathways leading to transcriptional activation of the tumor suppressor p53, possibly through the PI-3 kinase pathway.[30,31] Folkman, et al, reported that the interferons exert antiangiogenic activity against the tumor vasculature supporting a potential antiangiogenic effect of poly-ICLC.[32] Importantly, the length of therapy with interferon alpha is limited whereas the use of poly-ICLC has been extended for up to 12 years without untoward effects. This capacity for extended use further supports our clinical trial of poly-ICLC in low-grade gliomas where a more extended length of treatment (2-3 years) may be required to establish a response to therapy. These multiple effects on cellular metabolism, if sustained, may mediate a broad-spectrum antitumor effect on a wide range of tumor cell types (Fig.1). If further studies confirm the hypothesis that OAS and/or PKR may mediate the possible antitumor action of poly-ICLC, this might help explain why the high doses of poly-ICLC used in early cancer trials were relatively ineffective.

**Figure 1. Poly-ICLC Action**

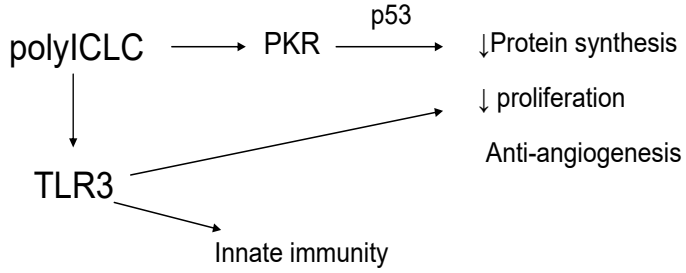



**Figure 1.** This schematic shows the molecular basis for polyICLC action on signaling pathways in glioma or stromal cells, the inhibition of protein synthesis, angiogenesis and growth arrest. PolyICLC via TLR3 also induces innate immunity.

Furthermore, there is recent evidence of a novel biological effect of poly-ICLC in dendritic cells. In vivo studies have shown cytoplasmic activation of MDA5 and lysosomal activation of TLR3 in dendritic cells to peptide antigens. This response is associated with augmented production of immune-stimulatory cytokines by the dendritic cells, e.g. IL15, type 1 interferon and increased activation of CD8+ CTL.[33]

More recently, an evaluation of poly-ICLC in healthy adult volunteers provided additional information on its mechanism of action. Profiling of whole blood samples was completed in participants exposed to poly-ICLC vs placebo. Compared to placebo, there was an upregulation of the interferon regulated genes (including TLR associated genes (TLR4 and TLG7), DDX58 and IFIH1) involved both in viral recognition and upregulation of the transcription factor genes IRF7, IRF-5 and IRF1. The upregulation of these genes leads to activation of transcription factors such as STAT1, STAT2 and STAT3. Additionally, there was upregulation of dendritic cell maturation, antigen presentation and NF-KB signaling. Another contributing mechanism of poly-ICLC is the upregulation of the complement system components including C1QB, C3AR1, SER P-ING1, and EIF2AK2. Other inflammasome associated genes that were found to be upregulated include IL-1B, IL1RN, CASP1 and CASP5.[34] This information provided clarification on the mechanism of action for poly-ICLC, however additional knowledge on the exact mechanism of action on NF-1 associated tumors is still under investigation and the completion of this trial will provide extremely valuable information.

Taken together, these data suggest that immunological profiling of serum, tumor tissue and PBMC may identify a poly-ICLC induced immune response signature associated with augmented activation of immune response against pediatric NF1 LGG.

### 2.3. Previous Clinical Trials of Poly-ICLC for Brain Tumors

A pilot trial in adults suggested a possible beneficial effect of poly-ICLC in newly diagnosed and recurrent brain tumors.[34] Thirty-eight patients with glioblastoma multiforme (GBM), anaplastic astrocytoma (AA), or recurrent GBM/AA were treated with varying doses of poly-ICLC 10-50 mcg/kg IM, 1 to 3 times per week for approximately 56 months. The drug was well-tolerated, with little or no toxicity and quality of life was preserved in most patients. The most common side effect was mild temporary discomfort at the injection site, and transient fever or malaise. Twenty of thirty patients receiving at least twice weekly poly-ICLC showed regression or stabilization of tumor on MRI. All but one patient with AA, who received poly-ICLC without interruption, remain alive, with PFS of 54 months from diagnosis. This small study demonstrated the safety and tolerability of long-term, low dose intramuscularly administered poly-ICLC at a potentially beneficial dose range of 20 mcg/kg 2-3 times weekly in patients with malignant gliomas.

Most malignant gliomas actually represent a mixture of highly malignant tumor cells and lower grade cells that eventually become malignant themselves. Chemotherapy and radiation therapy are generally more effective against rapidly dividing malignant cells but are less so against the lower grade tumor elements. Based on information available to date, agents such as poly-ICLC may be more effective in stabilizing certain of these lower grade tumor elements and could thus be useful in treatment of low-grade tumors. In one study, the patients with lower grade tumors had a much greater response to poly-ICLC than standard chemotherapy regimens, with a longer median survival in months (119 versus 59) in WHO class 1 tumors (see Table 1). One hundred percent of patients who received the poly-ICLC regimen were alive at 2 years versus only 76% who received standard chemotherapy.[34] This supports the effectiveness and less likely complications of poly-ICLC over chemotherapy.

**Table 1: Median Survival Poly-ICLC Trials**

Survival of Malignant Glioma Patients by Prognostic Class
Poly-ICLC Vs Historical Chemotherapy controls (29 patients)

| Prognostic | Median Survival (months) | | Percent 2 yr. Survival | |
|---|---|---|---|---|
| Class* | P-ICLC | Chemotherapy | P-ICLC | Chemotherapy |
| I | 119 | 59 | 100% | 76% |
| II | 104† | 37 | - | 68% |
| III | 53 | 18 | 80% | 5% |
| IV | 57 † | 11 | - | 15% |
| V | 19 | 9 | 33% | 6% |
| VI | 12 | 5 | 0 | 4% |

**Table 1.** This table demonstrates median survival (months) of patients with prognostic class I-VI tumors treated in three large chemotherapy trials versus treatment with poly-ICLC. † Represents N= only one P-ICLC patient in groups II and IV.

Two adult phase II clinical trials conducted by the NIH's North American Brain Tumor Consortium (NABTC) have been completed. The first trial evaluated long-term poly-ICLC monotherapy in 55 patients with recurrent glioblastoma, 11% had radiographic response, 6-month PFS was 24% and median survival was 43 weeks. Poly-ICLC was well tolerated but did not show improvement in 6-month PFS. The second trial included 30 patients newly diagnosed with glioblastoma. The treatment schema included a combination of radiation with poly-ICLC. The 6-month PFS was 30% and the estimated 1-year PFS was 5%. Median time to progression was 18 weeks. The 1-year OS was 69% and the median OS was 65 weeks. The combination therapy was well tolerated. This study suggests a survival advantage compared to historical studies using radiation therapy (RT) without chemotherapy but no survival advantage compared to RT with adjuvant nitrosourea or non-temozolomide chemotherapy. A trial of poly-ICLC in combination with temozolomide was conducted by the NIH's NABTT consortium.[35]

A Phase I/II study of poly-ICLC was conducted in children with multiple types of newly diagnosed or recurrent brain tumors. Four children with progressive LGG treated on this pilot study were evaluated for response. These 4 children had stable disease after documented progression for 10, 12, 22 and 24 months while receiving poly-ICLC. One of these children had leukopenia (grade 3) that resolved despite continued therapy, and another developed respiratory distress at the time of tumor

progression. Long-term responses were seen in 2 of 4 patients with LGG (unpublished data). The other cohorts of recurrent high-grade gliomas and newly diagnosed brain stem gliomas showed minimal to no response and all 8 children enrolled due to growing diffuse intrinsic pontine gliomas had progression of disease in < 8 months of therapy. Of the 11 children enrolled on the study with progressive high-grade gliomas (anaplastic astrocytoma or glioblastoma multiforme), 10 demonstrated disease progression within 1-10 months of treatment initiation. However, 1 child had radiographic and clinical stability on therapy for over 2 years. The other cohorts of recurrent high-grade gliomas and newly diagnosed brain stem gliomas showed minimal to no response. Patients with NF1 associated brain tumors, appeared to be very sensitive to poly-ICLC but demonstrated more side effects, particularly fever and erythema. Thus, it appears that poly-ICLC may have a beneficial role in children with LGG.[36]

### 2.4. Pediatric Phase II Trial of Poly-ICLC for Progressive LGG

***Published as abstract.***

A Phase II trial for progressive and refractory LGG in children was initiated in 2011 (NCT01188096). Patients were enrolled at Children's Healthcare of Atlanta and Rady Children's San Diego.[37] Preliminary results were presented at the 2018 Society for Neuro-Oncology meeting. Twenty-three patients, including 6 with refractory NF1 LGG, were enrolled and treated with poly-ICLC. In 6 patients with NF1 LGG, poly-ICLC was administered IM at 20mcg/kg/dose twice a week for 12 to 24 cycles. Each cycle was 28 days. Patients' demographic characteristics are in **Table 2**. All were pre-treated (1-7 prior regimens, including 1 patient who was previously irradiated). There were 4 patients with optic pathway gliomas, 1 with thalamic glioma and 1 with cervico-medullary junction glioma. Age ranged from 1-14 years.

**Table 2. NF1 LGG Patient Demographics**

| N | Age years | Gender | Pathology | Molecular | Location | Prior therapies | Radiation |
|---|---|---|---|---|---|---|---|
| 1 | 9 | M | JPA | N/A | Thalamic | CV vinblastine | No |
| 2 | 1 | F | N/A | N/A | OPG | CV | No |
| 3 | 14 | M | JPA | N/A | Cervico-Medullary | CV, vinblastine | Yes |
| 4 | 9 | F | N/A | N/A | OPG | CV, vinblastine | No |
| 5 | 10 | M | JPA | N/A | OPG temporal | CV, erlotinib, avastin | No |
| 6 | 10 | F | JPA | V600E+ | OPG | CV, TMZ, bevacizumab, vinblastine, everolimus, sorafenib | No |

**NF1 LGG responses at 12 months: 1 PD, 2 SD, 2 PR (PR+SD = 80%).**
**Unpublished data. Manuscript in preparation.**

Five of the 6 NF1 LGG patients are evaluable. As of September 2019, there have been 2 partial responses (PR) with a demonstrated decrease in tumor size by 67% and 68% by MRI (**Fig. 2,**

**Fig. 3, and Table 3**), 2 with prolonged stable disease (SD) with a decrease in tumor size by 15% and 7% respectively. One patient had progressive disease (PD) after 3 months on therapy. One of the patients with prolonged SD had significant improvement in visual fields after 3 months on therapy and continues to improve (**Fig. 4**). Three patients completed 24 cycles of protocol therapy All the patients with PR and SD have remained progression-free for 27+, 27+, 46+ and 102+ months.

**Table 3. NF1 LGG Patient Responses**

| N | Response at 6 months | % shrinkage | Response at 12 months | Therapy duration months | Time off therapy months | PFS | Progression | Molecular | Last follow - up |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PR | -67 | PR | 24 | 22+ | 46+ | No | n/a | Alive |
| 2 | PR | -68 | PR | 24 | 3+ | 27+ | No | n/a | Alive |
| 3 | SD | -15 | SD | 12 | 90+ | 102 + | No | n/a | Alive |
| 4 | SD | -7 | SD | 24 | 3+ | 27+ | No | n/a | Alive |
| 5 | PD | | | | | | Yes | n/a | Alive |
| 6 | NE | NE | NE | N/A | N/A | | | V600E+ | Alive |

**Figure 2. Patient 1 (Tables 2 & 3) Objective Response**

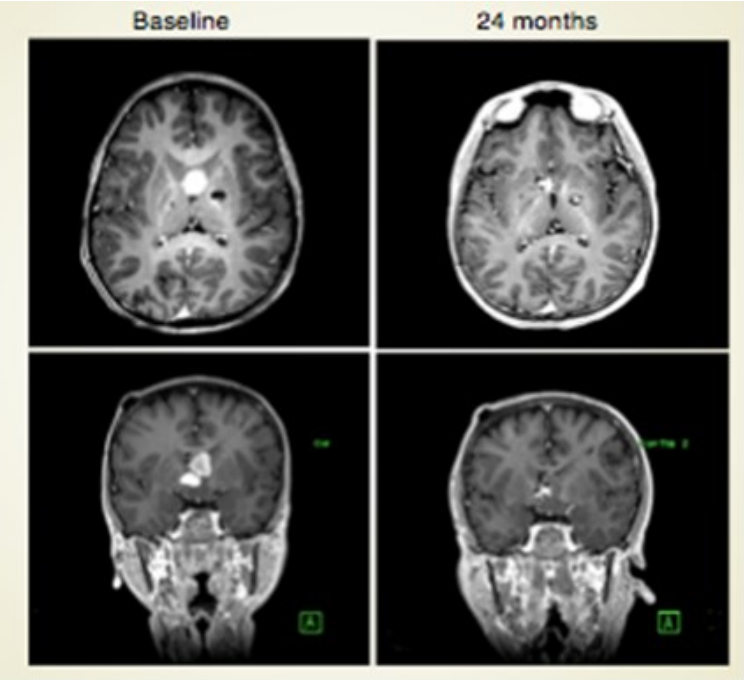


**Figure 3. Patient 2 (Tables 2 & 3) Objective Response**

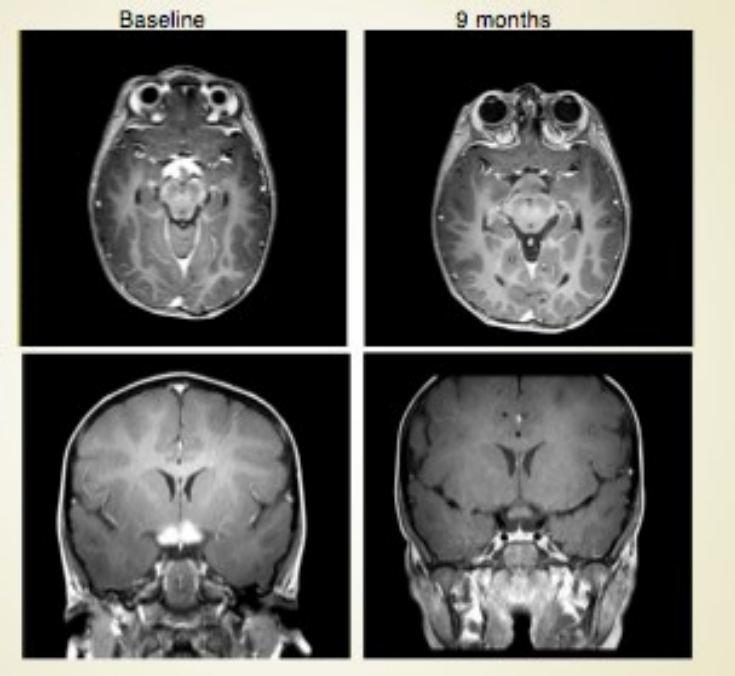
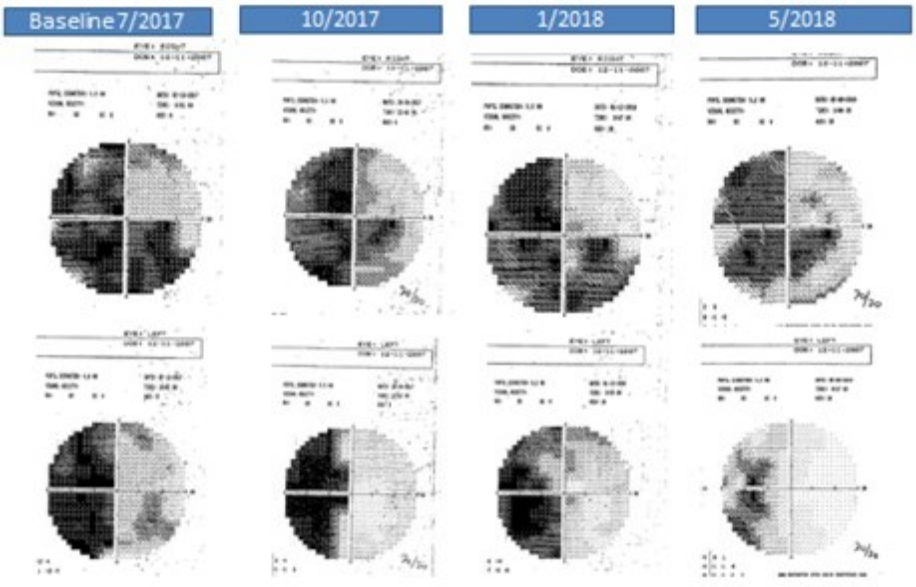



**Figure 4. Patient 4 (Tables 1 & 2) Objective Visual Response**




**Toxicities**

With one exception, only infrequent and mild low-grade toxicities were observed: erythema and pain at site of injection, fever, myalgias and ALT elevation. There was one patient with a small asymptomatic intratumoral hemorrhage likely due to tumor evolution vs response to treatment.

Platelet count and coagulation tests were normal, and this patient spontaneously recovered without further bleeding. No other risk factors were identified for bleeding. No other LGG patients (NF1 and non-NF1) had bleeding episodes and there were no Grade 3 or 4 toxicities.

Overall, in the NF1 group, compliance was greater than 90%, as verified by patient diaries.

Although the population was small, 4 of 5 evaluable NF1 patients treated with poly-ICLC had clinical responses and clinical benefit (radiographically and/or improved vision) as well as evidence of prolonged stabilization of disease after discontinuation of therapy (for up to several years) without untoward long-term effects or secondary malignancies/transformation to higher grade. Together, these pilot data support initiating a phase II clinical trial of poly-ICLC in NF1 LGG.

### 2.5. Rationale for the Biological Correlates

Current diagnostic and therapeutic monitoring for children with NF1-associated low grade gliomas are significantly hindered due to limited understanding of brain tumor biology and markers that can predict response to therapy. If available, such markers would be highly desirable and could be used to:

- Predict response to poly-ICLC therapy in children with NF-1 associated LGG.
- Predict or anticipate tumor progression in children with NF-1 associated LGG.
- Evaluate the immune profile for children with NF-1 associated low-grade glioma before and after the therapy with poly-ICLC.

**Functional assays.** We will perform Tcell proliferation as well as characterize cytokines secreted by Tcells before and after Poly-ICLC therapy. We are particularly interested in understanding the changes in antigen-specific T cell responses after exposure to poly-ICLC.

**Multiplex Luminex ELISA:** We will assess changes in plasma cytokine milieu, including circulating cytokines IL6 and TNFα at multiple time points.

**Surrogate marker for Poly-ICLC activity:** In addition, our evaluation will seek to provide a surrogate marker for sensitivity of the patient's cells to known capacity of the poly-ICLC to activate 3 pathways: TLR3, MDA5 and PKR signaling. Recent evidence from our group identified a novel biological effect of poly-ICLC not seen with poly-IC, the cytoplasmic activation of MDA5 and lysosomal activation of TLR3 in dendritic cells (DCs), a response associated with augmented production of immune-stimulatory cytokines by DCs (e.g. IL15, type 1 interferon) and increased activation of CD8+ CTL response to peptide antigens in vivo.[33] These data suggest immunological profiling of serum, tumor tissue and PBMC may identify a poly-ICLC induced immune response signature associated with augmented activation of immune response against pediatric LGG in NF1.

Leftover samples of blood and optional tissue will be banked for future studies through the NF Clinical Trials Consortium. See Appendix 7.

**Figure 5: Single Cell Mass Cytometry (CyTOF)**

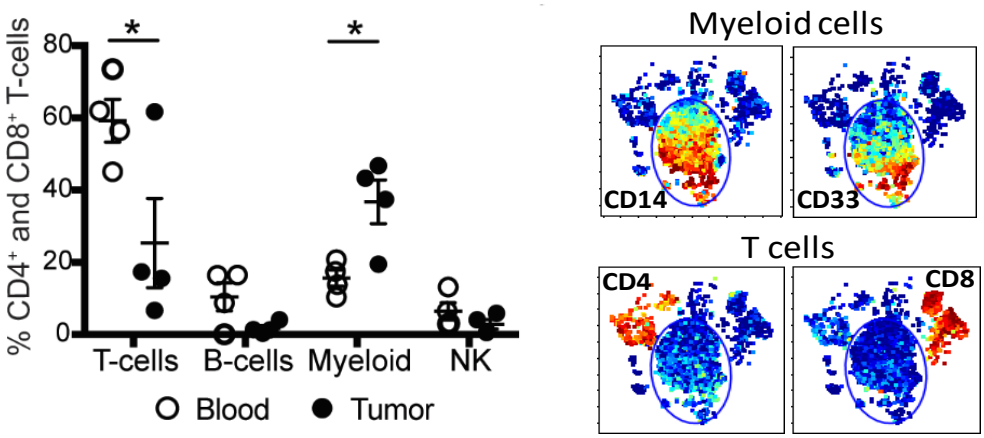



**Fig 5.** Single cell mass cytometry (CyTOF) to study the immune environment of glial tumors. CyTOF was used to examine paired blood and tumor samples from children undergoing resection of their glioma. Figure on the left shows that glioma tumor tissue is enriched for myeloid cells when compared to the peripheral blood (n=4). ViSNE analysis of the tumor tissue from a representative patient shows increased myeloid (CD14+ CD33+) cells within the tumor.

## 3. PATIENT ELIGIBILITY AND ENTRY CRITERIA

IMPORTANT NOTE: The eligibility criteria listed below are interpreted literally and cannot be waived. All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in patient's medical/research records which will serve as a source for verification at the time of the audit.

This protocol is open to both males and females of all races and ethnicities. Participants must meet the following criteria on screening examination to be eligible to participate in the study. See Inclusion Criteria regarding specific eligibility requirements for female and male patients of childbearing or child-fathering potential, respectively.

**All clinical and laboratory studies to determine eligibility must be performed within 14 days prior to enrollment unless otherwise indicated. Laboratory values used to assess eligibility must be no older than 14 days at the start of therapy. Laboratory tests do not need to be repeated if therapy starts within 14 days of obtaining labs to assess eligibility, with the exception of the pregnancy test, which must be performed within 7 days of starting treatment. Imaging studies must be obtained within 4 weeks (28 days) prior to enrollment and starting treatment. For OPG patients, functional ophthalmology assessments must be performed within 4 weeks (28 days) prior to enrollment and starting treatment.**

### 3.1. Inclusion Criteria

1) Age: Patients must be less than 22 years at the time of enrollment; there is no lower age limit.

2) All participants must have an identified pathogenetic constitutional *NF1* mutation OR the clinical diagnosis of NF1 using the NIH Consensus Conference criteria (**See Appendix 1)**.

3) Diagnosis: LGG (WHO Grade 1 and 2) of the brain and spinal cord are eligible. Histologic confirmation of tumor is not necessary in the presence of consistent clinical and radiographic findings. Biopsy for histologic diagnosis is required if there is clinical suspicion for a high-grade tumor; special attention is recommended in older adolescents or young adults to the potential for malignant transformation. Patients with metastatic disease are eligible.

4) Patients must meet at least one of the following criteria for progression or recurrence of a previously treated target tumor:

    a. Progression or recurrence on MRI.

    b. New or worsening neurologic symptoms attributable to the target tumor.

    c. For patients with OPG: visual worsening, defined as worsening of visual acuity (VA) or visual fields (VF) documented within the past year by examination or history, attributable to tumor.

5) Measurable Disease: Patients must have two-dimensional measurable tumor >1cm$^2$.

6) Prior Therapy: Patients must have had at least one prior medical treatment for the target LGG.

7) Performance Level: Patients must have a performance status of equal or > than 50 using Karnofsky for patients equal or ≥ 16 years of age and Lansky for patients < 16 years of age. **See Appendix 2 for scale.**

8) Patients must have recovered to grade ≤1 from any acute toxicities from all prior treatments. to enroll on this study and meet time restrictions from end of prior therapy as defined below:

    a. Myelosuppressive chemotherapy: must have received the last dose of myelosuppressive therapy at least 4 weeks prior to study registration, or at least 6 weeks if nitrosourea.

    b. Investigational/biological agent: Patient must have received the last dose of other investigational, immunotherapy, or biological agent > 14 days prior to study registration or at least 5 half-lives, whichever is greater. Bevacizumab last dose > 36 days prior to enrollment.

    c. Radiation therapy: Patients SHOULD NOT have received prior irradiation.

    d. Study specific limitations on prior therapy: There is no limit on the number of prior treatment regimens.

    e. Growth factor(s): Must not have received any hematopoietic growth factors within 7 days of study entry or > 14 days if pegylated GCSF is used.

    f. Prior surgery: At the time of enrollment, must be ≥ 3 weeks from prior major surgery such as craniotomy, orthopedic surgery, abdominal surgery or ≥1 week from minor surgery

and completely recovered. Port or central line placement is not considered a major surgery.

9) Organ Function Requirements:
All patients must have adequate organ function defined as:

9.1 Hematologic Function:
a. Hemoglobin: ≥ 8.0 gm/dl (may transfuse PRBCs)

b. ANC: ≥ 750/mm$^3$. Must be at least 7 days after last dose of growth factor or > 14 days since last dose of pegylated GCSF

c. Platelet Count: ≥ 75,000/mm$^3$ (transfusion independent; ≥ 7 days from last transfusion)

9.2 Renal Function: Serum creatinine which is less than 1.5 times ULN for age (as per the table below) or GFR > 70 ml/min/1.73m$^2$

**Table 3: Renal Function Normal for Age**

| **Age** | **Maximum Serum Creatinine (mg/dL)** | |
|---|---|---|
| | **Male** | **Female** |
| 1 month to < 6 months | 0.4 | 0.4 |
| 6 months to < 1 year | 0.5 | 0.5 |
| 1 to < 2 years | 0.6 | 0.6 |
| 2 to < 6 years | 0.8 | 0.8 |
| 6 to < 10 years | 1 | 1 |
| 10 to < 13 years | 1.2 | 1.2 |
| 13 to < 16 years | 1.5 | 1.4 |
| ≥ 16 years | 1.7 | 1.4 |

9.3 Liver Function:
a. Total bilirubin ≤ 1.5 x ULN (Children with diagnosis of Gilbert's Syndrome will be allowed on the study regardless of their total and indirect bilirubin levels as long as the direct bilirubin is less than 3.1 mg/dL.)
b. SGPT (ALT) ≤ 5 x ULN
c. SGOT (AST) ≤ 5 x ULN

9.4 Pulmonary Function:
No evidence of dyspnea at rest, and a pulse oximetry ≥ 92%.

9.5 Reproductive Function:
Female patients of childbearing potential must have a negative serum or urine pregnancy test within 7 days prior to the first dose of poly-ICLC. Patient must not be pregnant or breast-feeding. Patients of childbearing or child-fathering potential must be willing to use a medically acceptable form of birth control, including abstinence, while being treated on this study and for 90 days following cessation of treatment.

10) Patient is able to start treatment within 7 days after enrollment.

11) Patients with neurological deficits must be stable for a minimum of 1 week prior to enrollment.

12) Patients are only eligible if complete resection of the LGG with acceptable morbidity is not feasible or if a patient with a surgical option refuses surgery.

13) Patients and/or parents/legal guardians must provide written informed consent and agree that they will comply with the study.

### 3.2. Exclusion Criteria

1. Prior radiation treatment for the low-grade glioma.

2. Prior exposure to poly-ICLC.

3. Patients currently receiving other anti-tumor therapy or experimental therapy (targeted agents, chemotherapy radiation).

4. Patients with a current or prior diagnosis of malignant glioma (WHO grade III or IV).

5. Patients with a prior diagnosis of malignant peripheral nerve sheath tumor or other malignancy requiring treatment in the last 48 months.

6. Patients may not have fever (≥38.50 C) within 3 days of enrollment.

7. Patients who, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.

8. Active auto-immune illness.

9. Pregnant or lactating females.

10. Sexually active patients of reproductive potential who have not agreed to use an effective contraceptive method for the duration of their study participation and for 90 days after stopping study therapy are not eligible.

11. Severe unresolved infection that requires systemic IV antibiotics.

12. Patients with any significant medical illnesses that in the investigator's opinion cannot be adequately controlled with appropriate therapy or would compromise the patient's ability to tolerate this therapy.

13. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, impaired gastrointestinal function, or psychiatric illness/social situations that would limit compliance with study requirements.

14. Patients requiring high doses of steroids. Patients may not be on immunosuppressive therapy, including corticosteroids (with the exception of physiologic replacement, defined as ≤ 0.75

mg/m2/day dexamethasone or equivalent) at time of enrollment. However, patients who require intermittent use of bronchodilators or local steroid injections will not be excluded from the study.

### 3.3. Screening and Study Enrollment Procedures

The NFCTC Operations Center should be contacted to ensure availability of a treatment slot for this research. Contact the center at nfcop@uab.edu.

#### *3.3.1. Informed Consent/Assent*

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the patient or the patient's parent(s) or guardian if the patient is a child. When appropriate, pediatric patients will be included in all discussions. All patients and/or their parents or legally authorized representatives must sign a written informed consent to participate in this research. Assent, when appropriate, will be obtained according to institutional guidelines.

- A study participant should sign the institution's Release of Medical Information Waiver if applicable.

#### *3.3.2. Screening Procedures*

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial must only be done after obtaining written informed consent. Documentation of the informed consent for screening will be maintained in the participant's research chart. Studies or procedures that were performed for clinical indications (not exclusively to determine eligibility) may be used for baseline values even if the studies were done before informed consent was obtained. See section 3.4.

Before the patient can be enrolled, the responsible institutional investigator must sign and date the completed eligibility checklist. The completed eligibility checklist and redacted supporting source documents should be scanned and emailed to the NFCTC Operations Center to confirm eligibility prior to participant enrollment. Email: nfcop@uab.edu.

#### *3.3.3. Study Enrollment*

Patients may be enrolled on the study once all eligibility requirements for the study have been met, and a treatment number for participant has been confirmed by the NFCTC Operations Center. All patients who give informed consent for the protocol in order to undergo screening for eligibility will be registered but are not considered enrolled until the screening is completed, they are determined to meet all eligibility criteria, and approval for enrollment has been confirmed by the study chair. Treatment must start within 7 days of enrollment. **Participants must not receive any protocol therapy prior to enrollment.**

### 3.4. Requirements to Initiate Protocol Therapy

All studies to determine eligibility must be performed within 2 weeks (14 days) prior to enrollment except the baseline MRI study and eye exam for OPG documenting disease status, which must be obtained within 4 weeks (28 days) prior to study enrollment and starting treatment. All clinical and laboratory data required for determining eligibility of a participant enrolled on this trial must be

available in the participant's medical or research record which will serve as the source document for verification at the time of audit/monitoring.

### 3.5. Criteria for Removal from Protocol Therapy and Off Study Criteria

**Removal from Protocol Therapy**

The following are criteria for removal from protocol therapy:

a) Confirmed progressive disease (clinical or radiographic).

b) Completion of cycle 24.

c) Poly-ICLC related toxicity requiring removal.

d) Participant has not received drug (for any reason) for greater than 28 days.

e) Refusal of further protocol therapy by participant/parent/guardian.

f) Another tumor develops that requires non-surgical therapy (e.g. chemotherapy, radiation etc;).

g) Non-compliance, that in the opinion of the investigator does not allow for on-going participation.

h) Physician determines it is not in the participant's best interest.

i) Participant has complete resection of target lesion and there is no evidence of residual disease.

j) Participant becomes pregnant.

- If a participant becomes pregnant while receiving investigational therapy or within 90 days after the last dose of study drug, the participant should be followed until the outcome of the pregnancy.

**Participants who are off protocol therapy are to be followed until they meet the criteria for OFF Study (see below).**

**Off-Study Criteria:**

- Completion of their 5 year follow-up MRI scan
- Participant starts new tumor-directed therapy
- Death
- Lost to follow-up
- Withdrawal of consent for any further data submission

# 4. TREATMENT PLAN

## 4.1.Study Treatment

**Overview:**
Enrolled participants will receive poly-ICLC 20 mcg/kg/dose twice weekly IM (using Monday/Thursday or Tuesday/Friday schedule if possible). The first dose will be administered in the clinic under supervision of a nurse, advance care provider or physician. **It is recommended that the participant stay in the clinic for the 1st hour (+/- 30 min) after first dose is administered to evaluate for fever, rash, hives, or any other reactions. Premedication with acetaminophen, ibuprofen and antihistamines is strongly recommended to minimize injection related and drug related toxicities. Prophylaxis with acetaminophen and/or ibuprofen is recommended for 24 hours after injection, per treating physician discretion.**

## 4.2. Treatment Dosing Options

**Table 4: Recommended Dosing Options**

| | Option 1* | Option 2* |
|---|---|---|
| **Monday** | **X** | |
| **Tuesday** | | **X** |
| **Wednesday** | | |
| **Thursday** | **X** | |
| **Friday** | | **X** |
| **Saturday** | | |
| **Sunday** | | |

**X= drug administration. Cycles are 28 days**
*If a participant misses a dose of medication on the scheduled day, a dose can be given up to 24 hours later. Injections must be separated by at least 48 hours.

Participants will receive poly-ICLC 20 mcg/kg/dose twice weekly IM (using Monday/Thursday or Tuesday/Friday schedule if possible). The first dose will be administered in the clinic under supervision. To facilitate processing of biological samples, samples should not be obtained on a Friday.

Participant will be monitored 1 hour (+/- 30 minutes) after the first dose. For all subsequent cycle 1 doses administered at home, a log will be kept documenting the temperature of the participant before study drug administration and 12 hours (+/- 6 hours) after study drug administration. After cycle 1, temperature will only be monitored on an as needed basis, such as if the participant is not feeling well. Participants or their parents will be instructed to use a log to record the temperature monitored at home and any fever > $39^0$ Celsius should be recorded on the log.

Participant or parent will receive appropriate teaching by the medical team for intramuscular injections administration. After the first dose if family and medical team feel comfortable, then the rest of the medication will be administered at home. The study drug will be dispensed to the participant/parent/guardian by the institutional research pharmacy. The study drug will be dispensed in vials along with syringes in order to minimize errors in dosing. Needle size/gauge to be used for IM injections should follow institutional guidelines and practice.

Total length of treatment will be 24 cycles. Each cycle will be 28 days +/- 5 days. Treatment will not be continued beyond 24 cycles.

Participants will be seen and monitored at the start of each cycle. A medication supply for up to a maximum of 33 days (28 + 5 days) will be dispensed.

If a participant misses a dose of medication on the scheduled day, a dose can be given up to 24 hours later. No injections should be given closer than 48 hours.

*4.2.1 Criteria to Start Next Cycle*

a. Hemoglobin: $\geq$ 8.0 gm/dl (may transfuse PRBCs)
b. ANC: $\geq$ 750/$mm^3$
c. Platelet Count: $\geq$ 75,000/$mm^3$ (transfusion independent; ≥ 7 days from last transfusion)
d. SGPT (ALT) ≤ 5 x ULN
e. SGOT (AST) ≤ 5 x ULN

### 4.3. Surgery

Patients may undergo surgical tumor resection at any point during the protocol if it is clinically indicated per treating physician recommendation.

Study drug should be held for at least 72 hours prior to surgery. Patients who undergo resection will require a post-operative MRI of the primary tumor site, preferably within 72 hours after resection. Patients who undergo gross total resection of the target lesion with no measurable disease on post-operative imaging will permanently discontinue study drug. Patients with residual measurable tumor after resection may resume study drug when clinically cleared by medical team (usually 2-4 weeks after resection) and when they meet the criteria described in 4.2.1 to start a new cycle. Measurable tumor for this purpose is defined as tumor with a maximal diameter at least twice the slice thickness of the MRI sequence used to define tumor dimensions. Postoperative MRI will be used as the new baseline for subsequent response determinations.

## 5. REQUIRED OBSERVATIONS

### 5.1. Pre-treatment Evaluation (See Appendix 3)

**The following evaluations must be performed within 14 days prior to start treatment unless otherwise indicated. Imaging must be obtained within 4 weeks (28 days) prior to start of treatment. For OPG patients, functional ophthalmology assessment must be performed within 4 weeks (28 days) prior to start treatment.**

A. History (including concomitant medication list), vital signs (RR, HR, BP, temperature and oxygen saturation) and physical examination, including neurologic evaluation.

B. CBC, differential, platelet count.

C. CMP: including sodium, potassium, CO2, chloride, total protein, glucose, AST, ALT, alkaline phosphatase, albumin, calcium, total bilirubin, BUN, creatinine, phosphorous.

D. Disease evaluation of primary tumor with MRI. MRI must be performed within 4 weeks of starting treatment.

E. Serum or urine B-HCG in females of childbearing age (**must be repeated if not performed within 7 days of day 1 cycle 1).**

F. Lansky Play Scale assessment for patients ≤ 16 years of age; Karnofsky score for patients > 16 years old (see Appendix 2).

G. QOL Assessment after signing consent prior to study drug.

H. Ophthalmology Functional Assessments (see section 9) only for patients with optic pathway gliomas.

I. Blood biology studies after signing consent prior to study drug (see section 7).

J. Study entry form/eligibility checklist to be approved by the study chair or designee prior to starting treatment.

### 5.2. Evaluation During Treatment (See Appendix 3)

A. Disease evaluation with MRI brain (with orbital cuts for optic pathway tumors) or spine MRI depending on the primary tumor site every 3 cycles (+/-2 weeks) while on therapy (to be done at the end of the cycle). CSF cytology if clinically indicated. Tumor Evaluation forms are to be completed every 3 cycles while participant is on study. More frequent MRIs can be done at the treating physician discretion. Every MRI performed on participants on study will be submitted to the NFCTC Operations Center for central review at the conclusion of the study.

B. History, vital signs, physical and neurological examination and concomitant medication list, on cycle 1 day 1 and cycle 1 day 15 (+/-48 hours) and on day 1 (+/-5 days) of each subsequent cycle.

C. Labs:

(a) CBC/differential, platelets are to be obtained on cycle 1 day 1if not performed within 14 days, on cycle 1 day 8 (+/-48 hours) and cycle 1 day 15 (+/-48 hours) and on day 1 (+/-5 days) of each subsequent cycle.

(b) CMP (including sodium, potassium, CO2, chloride, total protein, glucose, AST, ALT, alkaline phosphatase, albumin, calcium, total bilirubin, BUN, creatinine, phosphorous) are to be obtained on cycle 1 day 1 if not performed within 14 days and on day 1 (+/-5 days) of each subsequent cycle.

**Biological correlates SHOULD NOT be obtained on a Friday.**

D. Pregnancy test if female patient is of childbearing age.

E. Toxicity monitoring and review of medication diary and temperature log at every visit after starting study treatment.

F. Lansky Play Scale assessment for participants < 16 years of age; Karnofsky score for participants ≥ 16 years old at baseline, cycle 1 day 15, and then prior to each cycle (see Appendix 3).

G. Ophthalmology Functional Assessments (see section 9) only for patients with optic pathway gliomas at the end of cycle 3 (+/- 2 weeks) then every 3 cycles (+/-2 weeks) until end of treatment.

H. QOL Assessment will be done on day 1 Cycle 6 (+/-2 weeks) and day 1 cycle 12 (+/- 2 weeks) and end of cycle 24 or end of therapy (+/- 2 weeks) (see section 8).

I. Blood biology studies (see section 7) on day 8 cycle 1, day 15 cycle 1, day 1 cycle 3, 6, and 12, and at the end of treatment. Biology studies should be drawn within 24 hours prior to the next scheduled dose whenever possible. **Mandatory biological correlates SHOULD NOT be obtained on a Friday.** Samples should be at room temperature. Biological blood samples should not be obtained through a fingerstick.

### 5.3. Evaluation at Completion of Therapy or Relapsed Disease (See Appendix 3)

A. Disease evaluation: MRI brain (with orbital cuts for optic pathway tumors) with and without contrast or spine MRI with and without with contrast if primary spinal tumor (+/-2 weeks).

B. History, vital signs, physical and neurological examination, and concomitant medication list (+/- 14 days).

C. Labs (+/-14 days):

(c) CBC/differential, platelets

(d) CMP: including sodium, potassium, CO2, chloride, total protein, glucose, AST, ALT, alkaline phosphatase, albumin, calcium, total bilirubin, BUN, creatinine, phosphorous

D. Toxicity monitoring and review of medication diary and temperature log

E. Lansky Play Scale assessment for patients < 16 years of age; Karnofsky score for patients ≥ 16 years old (see Appendix 2).

F. Ophthalmology Functional Assessments (see Section 9) only for patients with optic pathway gliomas (+/-2 weeks).

G. QOL Assessment (+/-2 weeks) at cycle 24 (+/-2 weeks) (see Section 8).

H. Blood biology studies (see Section 7).

### 5.4. Evaluations Off Therapy (See Appendix 4)

A. Disease evaluation: MRI brain (with orbital cuts for optic pathway tumors) with and without contrast or spine MRI with and without contrast if spine is the primary tumor location every 3 months (+/-4 weeks) for the first 12 months, then every 6 months (+/- 4 weeks) for months 12 - 36, then every 12 months (+/-6 weeks) until month 60.

B. Ophthalmology Functional Assessments (see section 8) only for patients with optic pathway gliomas every 3 months (+/-4 weeks) for the first 12 months, then every 6 months (+/-4 weeks) for months 12-36, then every 12 months (+/-6 weeks) until month 60.

C. QOL Assessment at month 24 (+/- 4 weeks) (see section 8) or at time of post-treatment progression if applicable.

D. Blood biology studies (see section 7) at 3-month post treatment and 12-month post treatment study visits, and at the time of post-treatment progression if applicable.

E. See Section 7 for Optional Tumor Tissue and Concurrent Blood Sample.

## 6. DRUG INFORMATION

### 6.1. Poly-ICLC

**Product: Polyinosinic-Polycytidylic acid – poly-L-lysine carboxymethylcellulose**

**Name: Poly-ICLC (Hiltonol®)**

Poly-ICLC is classified as an investigational new drug. It is a synthetic complex of polyinosinic and polycytidylic acid, stabilized with polylysine and carboxymethyl cellulose. The thermal denaturation point is 89.5$^{o}$ C, about 40$^{o}$ C above that of plain polyI-polyC; the resistance to hydrolysis is several times that of the parent compound, and it induces peak levels of about 5000 IU of interferon per ml of serum in monkeys given 2 mg/kg intravenously.

The current study will be conducted under IND #43984, held by Oncovir. The drug to be used in this study is prepared and packaged in the GMP facility of Dalton Pharma Svcs, Toronto, ON, Canada, under the direction and under contract to Oncovir. It is then tested for identity (RNAase resistance and thermal denaturation) bioactivity, sterility and pyrogenicity before release for clinical experimental use.

FORMULATION: Supplied in 1 ml vials of 1.8 mg/ml opalescent solution/suspension = 1800 mcg/ml.

STORAGE: Should be refrigerated but NOT FROZEN. Stable at room temperature for 3 days.

SOLUTION PREPARATION: Solution is injected as supplied.

ADMINISTRATION: Administered as a single intramuscular injection twice per week.

Up to two doses can be obtained from the same vial. Instructions will be provided to patient/parents/guardian on how to draw, measure and administer the study medication.

SIDE EFFECTS: Discomfort at injection site, occasional malaise, myalgias, or fever. Rare transient leukopenia has been reported. At higher intravenous doses, transient liver enzyme and BUN elevations, and leukopenia have been reported. Idiosyncratic hypercoagulopathy has been reported in dogs at very high doses but has not been seen in humans. See investigator brochure.

MAXIMAL TOLERATED DOSE: As reported in cancer trials, 250-300 micrograms/kg.

### 6.2. Ordering

The drug will be supplied by Oncovir and shipped to the UAB Investigational Drug Service (IDS), which is designated as the Central Pharmacy. The UAB IDS is responsible for the transfer and transportation of shipment of poly-ICLC to study sites. Transport of poly-ICLC to study sites must be pre-approved by the sponsor and IRB before any study drug will be transported. Poly-ICLC will be transported in accordance with specific storage requirements.

When study drug is needed at a study site, an electronic ordering system is utilized for planned transport of study drug, with date and time specifications included in the request. To initiate an order, the UAB IDS ordering form will be sent to **nfcop@uab.edu.** Once received, NFCTC will approve the order and forward to UAB IDS. UAB IDS will arrange the transportation to the requesting site. Poly-ICLC will be shipped out 3-7 business days after the UAB IDS ordering form is received. Once the study site receives the shipped product, the form must be completed and sent back to **nfcop@uab.edu**.

Drug Distribution Center - UAB Research Pharmacy

- According to 21 CFR 312.6, UAB IDS will label the packaging of the investigational drug with the following statement: "Caution: New Drug - Limited by Federal law to investigational use".
- UAB IDS will maintain records showing the receipt, shipment, and other pertinent information of the investigational drug including:
    - Name of the investigator to whom the drug is shipped
    - Date, quantity, and batch number of each shipment
    - Return, destruction, or other appropriate disposition of unused study drug

UAB Research Pharmacy
Attention: Chris Chapleau, PharmD, PhD, MBA | Pharmacy Manager/IDS Pharmacy
North Pavilion | Room 3470 | 1802 6th Avenue South | Birmingham, AL, 35249
Phone: 205.934.7191 or 205.975-0376 | Fax: 205.975.6647

### 6.3. Drug Accountability

The research pharmacist at each participating site will maintain records of all dispensed vials of study drugs. All unused vials will be returned to the manufacturer (Oncovir) at the completion of the study as well as all the drug accountability forms.

### 6.4. Dose Modifications for Toxicity

Dose modifications below apply to toxicities that are at least possibly related to study drug. Laboratory results requiring drug hold must be repeated weekly or sooner if clinically indicated until they meet the criteria for restarting drug.

Liver Toxicity

If AST/ALT > 5x ULN while on study, poly-ICLC will be held until the AST/ALT ≤ 5x ULN; if resolved within 21 days, maintain the dose 20 mcg/kg/dose twice weekly.

If event resolves >22-28 days, hold study drug until values < 2.5x ULN then restart at the reduced dose of 10 mcg/kg/dose twice weekly.

If AST/ALT do not decrease to ≤ 5x ULN within 28 days (despite holding the drug for 28 days and only if elevated AST/ALT is attributable to the drug), then the study drug will be stopped, and participant will be off treatment for toxicity.

It is recommended to evaluate other medications that participant is taking for potential liver toxicity (e.g. acetaminophen, trimethoprim/sulfamethoxazole, seizure medications). It is also recommended to evaluate potential viral liver infection if there is persistent elevation of AST/ALT.
Elevations of AST/ALT are expected, and repeat elevations do not require permanent drug discontinuation unless deemed medically necessary by the treating physician after discussion with the principal investigator.

Hematological Toxicities: Thrombocytopenia

Continue drug at 20 mcg/kg/dose unless platelet count is less than 75,000/mm$^3$. Then refer to the table below for further instructions.

| | |
|---|---|
| Platelet (Plt) < 75,000/mm$^3$ | Interrupt study drug until Plt ≥75,000/mm$^3$. If the event resolves within 21 days, then study drug may be restarted at the same dose.<br>If Plt count of <75,000/mm$^3$ attributable to the study drug, and the event resolves between 22-28 days, then study drug may be restarted at lower dose of 10 mcg/kg/dose. If Plt is <75,000/mm$^3$ after 28 days and at least "possibly" attributed to study drug, the participant will be taken of protocol therapy.<br><br>Transient and repeated thrombocytopenia is expected and does not require permanent removal from study therapy unless deemed medically necessary by the treating physician after discussion with the principal investigator or study team. |

Hematological Toxicities: Neutropenia

Continue drug same dose until absolute neutrophil count (ANC) is less than 750/mm$^3$, then refer to the table below for further instructions.

| | |
|---|---|
| ANC < 750/mm$^3$ | Interrupt study drug until resolution to ≥ 750/mm$^3$. If the event resolves within 21 days, then study drug may be restarted at the same dose. If event resolves between days 22 and 28, restart at lower dose of 10 mcg/kg/dose.<br><br>If ANC is < 750/mm$^3$ greater than 28 days after dose reduction, and and at least "possibly" attributed to the study drug, the participant will be taken off protocol therapy.<br><br>Transient and repeated neutropenia is not unexpected and does not require permanent removal from study therapy unless deemed medically necessary by the treating physician after discussion with the principal investigator or study team. |

| **All other adverse events (suspected to be related to the study drug)** | |
|---|---|
| Grade 1-2 | Grade 1 AE continue drug same dose 20 mcg/kg/dose. Provide specific therapy to address symptoms.<br><br>If the event is an intolerable Grade 2 AE, not responsive to a specific therapy, consider study drug interruption until resolution to Grade ≤1. If the event is clinically significant and resolves within 21 days, then study drug may be restarted at same dose. If event resolves between days 22 and 28, restart at a lower dose of 10 mcg/kg/dose. If the patient continues to have grade 2 intolerable toxicity despite dose reduction and providing specific therapy to address symptoms, protocol therapy will be permanently discontinued. |
| Grade 3 and Grade 4 | Interrupt study drug until resolution to Grade ≤1. If the event resolves within 21 days, then study drug may be restarted at the same dose. If event resolves between days 22 and 28, or the patient has a repeat grade 3 or grade 4 event, restart at lower dose of 10 mcg/kg/dose. If toxicity returns |

| | |
|---|---|
| | to grade 3 or 4 after drug reduction, protocol therapy will be permanently discontinued. |

### 6.5. Concomitant Medications

Non-enzyme inducing anticonvulsants may be given as clinically indicated. Enzyme inducing anticonvulsant agents should be avoided if possible. See Appendix 5 for a list of the enzyme inducing anticonvulsant agents.

During the trial, corticosteroids may be given as clinically indicated in the lowest possible dose per treating physician discretion. However, if the dose of steroids is increased while on therapy due to worsening of neurological symptoms, MRI of primary tumor is recommended to evaluate for disease status.

Acetaminophen and NSAIDS (ibuprofen, naproxen) may be given as needed to control poly-ICLC side effects. Use of ibuprofen or other NSAIDS during thrombocytopenia less than 50,000 /$mm^3$ should be avoided. Other instructions on the use of NSAIDS in this setting will be per treating physician discretion.

Vitamins at the daily recommended dosage are allowed while on study treatment. Other supplements, including cannabidiol-containing compounds, are not recommended.

For additional information on concomitant medications, see Investigator Brochure.

Radiotherapy or other-directed tumor therapy are not allowed in the trial.

Appropriate antibiotics, GCSF (Granulocyte Colony Stimulating Factor), blood products, antiemetics, fluids, electrolytes and general supportive care are to be used as necessary and as per institutional guidelines.

PJP (formerly PCP) prophylaxis will be given following institutional guidelines based on absolute lymphocyte count.

Given the risk of intratumoral hemorrhage with thrombocytopenia, a platelet transfusion should be considered if the platelet count is < 25,000/$mm^3$. Higher transfusion parameters may be used if clinically indicated. All blood products will be leukocyte depleted and irradiated to prevent graft-versus-host disease.

**Influenza vaccine administration:** Inactivated Influenza vaccine can be given to patients on study, but it is recommended that it not be given the same day as study drug.

Other vaccinations must be approved by the Protocol Chairs.

### 6.6. Toxicity

See the current Investigator's Brochure for a complete list of expected toxicities.

**1. *Discomfort at IM injection site:*** The most common adverse effect is mild, transient discomfort at the site of IM injection. With subcutaneous (SC) injections, there is a transient mild to moderate grade 1 or 2 erythematous skin reaction.[3]

**2. *Flu-like symptoms:*** Approximately 8 to 12 hours after doses of 10 to 50 mcg/kg IM, patients may develop a mild flu-like syndrome with fever of less than 39 ºC, which may last for about 12 hours, but responds readily to acetaminophen or ibuprofen. Mild myalgia, arthralgia, sometimes nausea, and malaise can be present during this period. This flu-like syndrome typically diminishes markedly after the first few poly-ICLC treatments. On rare occasions in the course of treatment, patients who have been tolerating treatment uneventfully may develop an earlier, more pronounced fever with chills and malaise (typical of higher dose IV poly-ICLC) in response to an IM injection. This will typically resolve over 12 to 24 hours, responds to acetaminophen, and does not typically recur on subsequent dosing.

**3. *Hematologic abnormalities:*** Several cases of transient leukopenia have been reported at higher doses. High dose Poly-ICLC has been associated with a coagulopathy in dogs, but not in other species including primates. There has been no change in the expected incidence of deep venous thrombosis, pulmonary embolus, or coagulopathy in multiple sclerosis, AIDS or malignant glioma patients on low dose IM poly-ICLC. One paralyzed multiple sclerosis patient treated with 100 mcg/kg IV suffered a fatal pulmonary embolus, which was not judged to be due to the drug.

**4. *Hepatic enzyme elevation:*** Mild and typically transient hepatic enzyme elevation has been described in poly-ICLC when given intravenously at doses from 20 to 100 mcg/kg. Preclinical studies have shown increased hepatic NK cells, as well as suppression of the P450 hepatic enzyme system by poly-ICLC, but the clinical implications of this finding are not clear.

**5. *Seizures:*** Seizures during high febrile episodes have been reported with glioma and known seizure disorder.

**6. *Diarrhea:*** Grade 1-2 diarrhea has been reported in brain tumor patients receiving IM Poly-ICLC two to three times weekly for months.

**7. *Tuberculosis (TB) in Mice*:** Mouse studies have reported a risk of worsening of TB in mice already infected with TB that are treated with Interferon or with poly-ICLC. Temporary worsening of TB has also rarely been reported in patients treated with high doses of interferon.

**8. *Transient anaphylactic reaction:*** There have been reports of transient anaphylactic reaction to the combination of a personalized glioma vaccine, GM-CSF, and poly-ICLC in glioblastoma patients. GM-CSF is known to cause anaphylaxis, but there have been no previous such reports in patients receiving poly-ICLC alone or with other vaccines.

**9. *Transient peritumoral edema:*** In a pilot brain tumor trial, a few patients showed an increase in gadolinium enhancing lesions after 3 - 6 months of IM poly-ICLC, followed by an apparent tumor response at 6 - 12 months and prolonged survival on continued treatment. Dexamethasone was used as needed in the first few months of treatment on that study. In a more recent follow-up open study in patients with advanced recurrent gliomas, several patients have shown increased peritumoral edema after several weeks of poly-ICLC therapy. This has resolved in all cases on continued poly-ICLC, with or without concomitant steroids. Biopsy data in at least two patients treated with poly-ICLC also showed a peritumoral inflammatory response. Likewise, in a dendritic cell vaccination trial in recurrent glioma patients with twice-weekly application of poly-ICLC IM, remarkable post-vaccination immune cell infiltration into the tumor was seen. These findings are commonly reported with other immunotherapies, and they raise the possibility that poly-ICLC may at times be facilitating a relatively early immunologic response to the tumor, perhaps manifested by transient increased edema or gadolinium enhancement. In line with this, one case of post-vaccination brain edema (Grade 3) possibly related to poly-ICLC adjuvant vaccination of a multi-peptide vaccine was reported from a Phase I trial in glioblastoma.

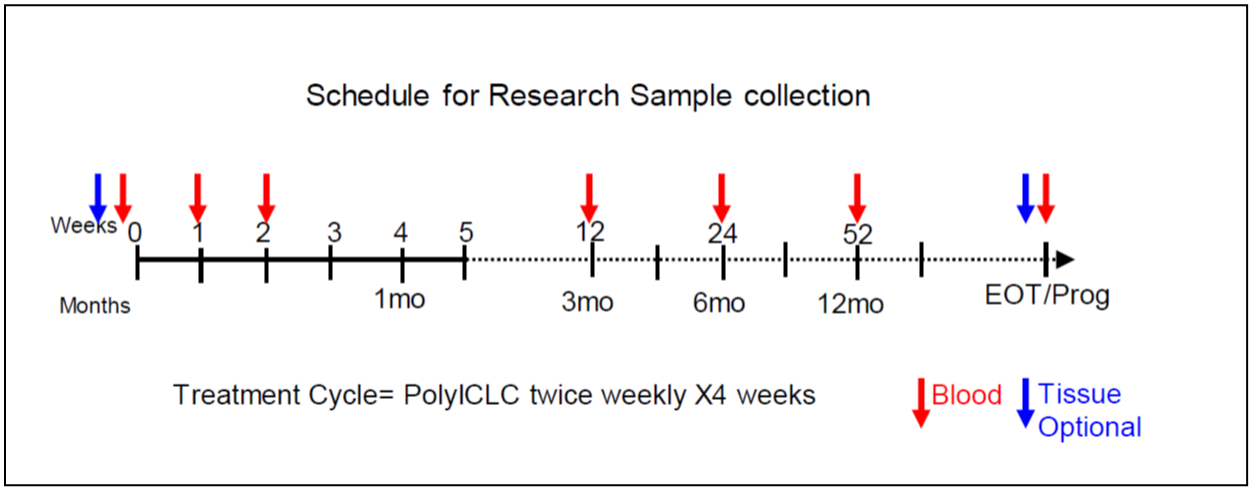
## 7. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

This protocol involves extensive correlative studies to gain fundamental insights into the effects of poly-ICLC on the circulating immune cells and whenever possible tumor infiltrating immune cells. The proposed studies may help understand correlates of response in NF1 patients with LGG who are treated with poly-ICLC.

Specimens:
Both blood and tissue specimens (if available) will be analyzed.

Time-points for mandatory biological studies:
Baseline; cycle 1 day 8 and day 15; day 1 of cycles 3, 6 and 12; and at end of therapy or progression. Additional samples will be collected at 3 time points when participant is off therapy: 3 months, 12 months and at the time of progression. See Appendix 6. We will collect 5-10ml of blood per sample in sodium heparin tubes for patients < 8 years of age. For patients that weigh less than 10 kg, collect 2 ml on cycle 1 day 8. For patients ≥ 8 years, we will collect 10-15 ml. Biological samples should be drawn within 24 hours prior to the next study dose (when applicable) whenever possible. **No biological samples should be drawn on a Friday. Samples should be shipped at room temperature. Biological samples should not be obtained through a finger stick. These samples will be processed at Emory University.**

**Figure 6: Research Sample Collection Schedule**




**Mandatory Biological Specimens**
**Single cell mass cytometry or CyTOF** will be used for phenotypic characterization of circulating as well as tumor infiltrating immune cell populations, using a 36-marker panel that we have developed. **Fig 5** shows enrichment of myeloid cells within glioma tumor tissue using multi-parameter analytic platforms such as ViSNE, which can be utilized to analyze mass cytometry data.

**Functional assays:** We will perform T cell proliferation as well as characterize cytokine secreted by T cells before and after poly-ICLC therapy. We are particularly interested in understanding the changes in antigen specific Tcell responses which has been shown to be important for glioma initiating/stem cells.

**Multiplex Luminex ELISA:** We will assess changes in plasma cytokine milieu, including circulating cytokines IL6 and TNFα.

**Optional Pre and post treatment tissue and blood samples:**
When available, fresh frozen and/or FFPE tumor sample is requested (either from surgical resection within 6 months prior to enrollment, during study therapy and/or progression). Blood sample will be collected at the time of surgical resection. These samples will be stored in a tissue bank located at Children's Hospital of Philadelphia for future use studies. See Appendix 7.

**Tumor and Blood samples** left over from the biological correlates drawn at any of the above time points may be banked for future research.

## 8. QUALITY OF LIFE (QOL) STUDIES AND NEURO-COGNITIVE BATTERY

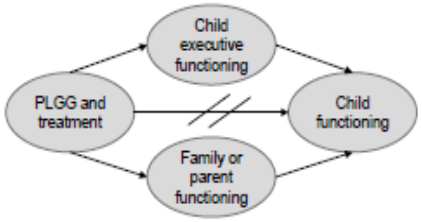
Brief Background: Mounting literature suggests that pediatric cancer survivors are negatively affected by persisting and clinically significant late-effects due to cancer and its treatment. Compared to other diagnoses, CNS-tumor survivors are at highest risk for neurocognitive, psychological, cardiopulmonary, endocrine, and musculoskeletal complications and second malignancies. Only limited data describe pediatric LGG and/or NF outcomes, despite their

prevalence.[38-41] Extant research describes HRQOL impairments for adults with a LGG[39,40] and, while not methodologically robust (i.e., small sample size, retrospective design, lack of validated PROs), preliminary research in PLGG survivors provides evidence of cognitive decline, learning disability, and adjustment difficulties following treatment.[41-45] For youth with refractory or progressive pediatric brain tumors especially, longer life may come at significant cost in morbidity and health-related quality of life (HRQOL). Use of both patient-reported outcomes (PROs), data collected "directly from the patient, without interpretation of the patient's response by a clinician[46] and objective performance-based measures to monitor potential neuro-toxicity are critical to understanding the impact of new treatments on a child's general functioning. Novel treatments that increase survival while also maintaining HRQOL are highly desirable.[47-50]

Diagnosis and subsequent treatment may result in impaired neurobehavioral functioning or, given its relatively encouraging prognosis, may not result in adverse outcomes directly. Rather, as suggested by the Human Evolutionary Response to Trauma/Stress (HEART) model,[51] insult to the child's developing nervous system (i.e. as measured by the parent proxy reported executive functioning measure) may moderate the relationship between diagnosis/treatment and long-term functioning (Figure 7). Guided by the HEART theoretical model, a complementary battery of measures was selected whereby the patient is embedded within the family and larger social systems.[52] Given a growing literature suggesting a cancer diagnosis may also result in growth/positive adjustment, measures of both negative and positive psychological functioning were selected. Factors have broad support in previous literature and are highly salient to existing interventions.[53-55] In the event the cognitive battery or self-report PRO battery cannot be completed due to intellectual disability (i.e., known IQ ≤ 70 by medical record review), informant PRO measures will still be completed. Measured constructs and their corresponding measure are briefly described in Table 6. All measures are psychometrically sound; appropriate validity, reliability, and normative data is available for all ages proposed.

### 8.1. Patient Reported Outcome Battery

**Figure 7: HEART Model, Adapted**




Children and adolescents will complete a PRO measurement battery (by patient age; Table 7) prior to first dose at treatment initiation. cycle 6, cycle 12, end of treatment (cycle 24) or progression and when applicable, at 24 months post completion of therapy (+/-5 days). Of note, safety and suicidal ideation will not be assessed; however, negative affect items will be reviewed, and each site notified of elevated scores. A trained study team member (supervised by a licensed clinical psychologist) will be available to assist younger children with PRO questionnaire completion as needed.

Time to complete the complete the PRO battery (treatment initiation, cycle 6, cycle 12, cycle 24 or time of progression, 24 months post completion of treatment) varies by age: Young Child (3-7y) = 0 minutes; Child (8-17y) = 27-37 minutes; Adult (≥18y) = 32-42 minutes, Parent/Informant = 35 minutes. The demographic questionnaire (5 minutes) will be completed at baseline only by adult patients and parents/informants.

PROs will be administered via paper form, iPad using the NIH Toolbox App and the eDCS, a secure, web-based application designed to support data capture for research studies. Similar to other data collection programs, both eDCS and the NIH Toolbox App provide a questionnaire response system designed to collect sensitive information to reduce social desirability.[58]

Participant responses are entered directly into the computer program, minimizing data entry errors and/or multiple responses to items. This method of measure administration has been used successfully in other pediatric research protocols at pediatric institutions.

**Table 6. Patient-Reported Outcome Battery by Age (years)**

| CONSTRUCT | CHILD: 8-17 | YOUNG ADULT: ≥ 18 | PARENT/INFORMANT (of all patients) |
|---|---|---|---|
| HRQOL | *Pediatric Quality of Life Inventory (PedsQL 4.0)*: physical, emotional, social, and school/work HRQOL; self- and proxy report | | |
| Executive functioning | — | — | *Behavior Rating Inventory of Executive Function (BRIEF-2)*: inhibit, plan/organize, emotional control, working memory, organization, etc. |
| Negative Affect | *PROMIS Depression, Anxiety, Anger*: fear, sadness, anger; self- and parent proxy report (CAT) | | |
| Psychological Well-Being | *PROMIS Positive Affect, Life Satisfaction*: positive affect, life satisfaction; self- and parent proxy report (CAT) | | |
| Stress & Self-Efficacy | *PROMIS Stress Experiences/Psychosocial Impact and Self-Efficacy* perceived stress, self-efficacy; self- and parent proxy report (CAT) | | |
| Social Relationships | *PROMIS Peer and Family Relationships/Companionship and Emotional Support*social support, companionship, social distress; self- and parent proxy report (CAT) | | |
| Fatigue | *PedsQL Multidimensional Fatigue Scale*: general fatigue, sleep and rest fatigue, cognitive fatigue; self- and proxy report | | |
| Adverse Symptoms | *Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE™):* NCI patient-reported outcome measure of adverse events in patients on clinical trials | | |
| Social Attainment | — | *Demographic Questionnaire*: will collect information re. socio-demographic status, education/vocational attainment, independent living, Supplemental Security Income (SSI), supportive services, etc. Baseline only | |

*Note. Items for PROMIS scales will vary; computer adaptive testing (CAT) will be utilized to minimize the number of items required to be completed.*

*IMPORTANT ADMINISTRATION GUIDELINES: English and Spanish translations of all measures below will be utilized. There are different PRO forms based on patient age as described below. The participant will complete the questionnaire according to the age they are on the date of survey completion.

*PedsQL 4.0 Core Scale and PedsQL Multidimensional Fatigue Scale*. For this study, patients 8-25 years old will complete the PedsQL *self-report* measures. Parents or informants of patients 2-25 years of age will complete the PedsQL *parent proxy report* measures. Parents/informants will complete proxy forms by the following patient age: toddler: 2 – 4 years; young child: 5 – 7 years; child: 8 – 12 years; adolescent: 13 – 17 years; young adult: 18 - 25 years. Patients will complete parallel self-report forms by patient's age: child: 8 – 12 years; adolescent: 13 – 17 years; young adult: 18 – 25 years.

*NIH Toolbox PROMIS Measures*. Patients 8-25 years old will complete the PROMIS *self-report* measures. Parents or informants of patients 2-12 years of age will complete the PROMIS *parent proxy report* measures. Parents/informants will complete proxy forms by the following patient age: young child: 3–7 years (Anger, Fear Sadness, Positive Affect, General Life Satisfaction, Positive Peer Interaction), child: 8-12 years (Anger, Fear Sadness, Positive Affect, General Life Satisfaction, Perceived Stress, Self-Efficacy, Positive Peer Interaction). Patients will complete parallel self-report forms by patient's age: child 8-17 years (Anger, Fear Sadness, Positive Affect, General Life Satisfaction, Perceived Stress, Self-Efficacy, Emotional Support,

Friendship) and adult 18-25 years (Anger (Affect), Fear (Affect), Sadness, Positive Affect, General Life Satisfaction, Perceived Stress, Self-Efficacy, Emotional Support, Friendship).

*Behavior Rating Inventory of Executive Function*. Parents or informants of patients 5-25 years will complete the BRIEF by patient age: 5-18 BRIEF2 Parent Report, 19-25 BRIEFA Adult Informant Report.

*Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE)*. Patients will complete the following forms by patient age: Peds PRO-CTCAE: 8-17 years, PRO-CTCAE: 18-25 years. Parents or informants of patients 3-7 years of age will complete the Peds PRO-CTCAE Caregiver *parent proxy report* measure.

## 9. OPHTHALMOLOGIC STUDIES

For patients with tumors involving the optic pathway (including suprasellar/hypothalamic tumors) all of the assessments in this section are mandatory. For patients with tumors not involving the optic pathway, ophthalmologic examinations are at the discretion of the treating physician.

For patients with optic pathway gliomas, the study examinations should occur at baseline, after cycle 3 (+/-2 weeks) then every 3 months while on therapy (+/- 2 weeks), and every 3 months for the first year off therapy (+/-4 weeks), and every 6 months (+/-4 weeks) thereafter for the following 2 years, then yearly (+/-6 weeks) for years 4 and 5.

The following functional assessments should be performed (see Appendix 11):

- Teller Acuity Cards (TAC, all participants)
- HOTV (in participants developmentally able to perform)
- Visual fields to confrontation
- Optic disc appearance

### 9.1. Visual Acuity (best corrected)

IMPORTANT NOTE: if the VA data at a particular visit is felt to be unreliable due to poor cooperation, testing should be repeated within 4 weeks. Only the visit believed to have yielded the most reliable data should be reported.

Teller acuity testing using TAC II cards will be attempted on all participants at all visits. All VA testing will be performed by study personnel who have undergone TAC teaching by the study team. VA testing will be performed in each eye separately at a distance of 55 cm in all participants. The tester will utilize the “Two down, one up” protocol to achieve the best visual acuity. Acuity will be reported in cycles/cm by the site and converted by the operations center to logMAR. Reasons for VA testing not being completed will be recorded.

**Table 8: Visual acuity in feet and logMAR equivalents for acuity in cycles/cm**

| Card @ 55 cm (Cycles/cm) | VA equivalent | logMAR |
|---|---|---|
| 26 | 20/24 | 0.06 |
| 19 | 20/32 | 0.20 |
| 13 | 20/47 | 0.36 |
| 9.8 | 20/63 | 0.49 |

| | | |
|---|---|---|
| 6.5 | 20/94 | 0.66 |
| 4.8 | 20/130 | 0.80 |
| 3.2 | 20/190 | 0.97 |
| 2.4 | 20/260 | 1.10 |
| 1.6 | 20/380 | 1.27 |
| 1.3 | 20/470 | 1.36 |
| 0.86 | 20/710 | 1.54 |
| 0.64 | 20/960 | 1.67 |
| 0.43 | 20/1400 | 1.84 |
| 0.32 | 20/1900 | 1.97 |

HOTV will be attempted on participants who are old enough. HOTV will be performed using the PEDIG ATS-HOTV computerized protocol in each eye separately at a recommended testing distance of 3 meters in all participants. Acuity will be reported in logMAR. Reasons for VA testing not being completed will be recorded.

For primary analysis, the VA endpoint will be the difference between best-corrected VA at baseline and at approximately 1 year (i.e., at the post-cycle 12 assessment).

### 9.2. VA Norms

Note: The rate of visual development varies across patients. The table is presented as a reference only.

**Table 9: VA Norms**

| Age | Grating acuity by TACII | | | Recognition Acuity | |
|---|---|---|---|---|---|
| | Normal VA (cy/cm) | logMAR | Snellen Equivalent § | Normal VA (feet) | logMAR |
| 6m | 2.4 | 1.097 | 20/260 | | |
| 12m | 3.2 | 0.972 | 20/190 | | |
| 18m | 4.8 | 0.796 | 20/130 | | |
| 24m | 4.8 | 0.796 | 20/130 | | |
| 30m | 6.5 | 0.664 | 20/94 | | |
| 36m | 9.8 | 0.486 | 20/63 | | |
| 42m | 13.0 | 0.363 | 20/47 | | |
| 48m | | | | 20/32 | 0.2 |
| 5yo | | | | 20/25 | 0.10 |
| ≥6yo | | | | 20/20 | 0.00 |

¶ Normal VA with TAC [low limit of normal at 95% CI]
§ Conversion of grating acuity to Snellen equivalents is not recommended and is only provided for purposes of communication.

### 9.3. Visual Fields

Visual field testing will be performed by confrontation and reported as the number of quadrants (0, 1, 2, 3, or 4) with VF deficits.

### 9.4. Optic Disc Appearance

Optic discs will be assessed for the presence or absence of pallor and edema.

### 9.5. Visual Response

Primary functional outcome is based on visual acuity (VA) and is defined as the difference in in VA after approximately 12 cycles of treatment. Eyes above the visual "ceiling" (0.2 logMAR below normal for age or 0.2 logMAR below a previous reliably documented VA) at baseline cannot improve and will be excluded from the analysis of visual response. A significant improvement in VA will be defined as a decrease of ≥ 0.2 logMAR (corrected for age) from baseline (pre-treatment baseline) to end of ~12 cycles of treatment. The analysis will be based on per participant outcome (rather than per eye).

A significant improvement in VA will be defined as a decrease of ≥ 0.2 logMAR from baseline (pre-treatment baseline) to end of ~12 cycles of treatment.

**Ophthalmologic (Visual Acuity) Response Criteria**
· Visual Responsive ≥ 0.2 logMAR improvement in VA compared to baseline
· Stable VA = does not meet criteria for Improved or Worsening VA compared to baseline
· Visual Progressive Disease ≥ 0.2 logMAR worsening compared to baseline*

* 0.2 logMAR worsening (or up to a 2-card drop in Teller Acuity) will not be considered visual progressive disease at the first on-therapy staging evaluation (at 12 weeks), if the tumor is stable or smaller on MRI. In these cases, a repeat visual acuity assessment will be required 6-12 weeks later to confirm that further visual progression is not occurring. If the repeat visual acuity is stable or improved (0.2 logMAR or less [or a 2 card drop in Teller Acuity or less] from pre-treatment baseline), the patient will remain on protocol therapy. If the visual acuity is now > 0.2 logMAR (or > 2 card drop in Teller acuity) worse from pre-treatment confirmed by a second exam, then the participant will be removed from the protocol.

**Visual worsening ≥ 0.2 logMAR in the setting of possible pseudoprogression should be discussed on a case-by-case basis with the study chair. In most cases, patient may continue on study until pseudoprogression vs progression is confirmed.** Corticosteroids may be considered, and a short interval repeat eye exam is recommended. If a repeat vision exam shows continued deterioration of visual function, then patient will be permanently removed from therapy and the time of progression will be the date of the initial MRI or vision exam showing progression. See section 10.4 for additional information regarding possible pseudoprogression.

## 10. IMAGING PARAMETERS AND RESPONSE CRITERIA

### 10.1. General Considerations

Patients with NF1-associated LGG may have multiple lesions; typically, however, decision to treat is based on a clinical or radiographic progression of a single lesion. If multiple measurable lesions are present, ONE should be selected as the "target" lesion. The overall response assessment takes into account response in the **target lesion only.** The target lesion should be the same as the target tumor identified for eligibility confirmation (section 3.1), and should be selected on the basis of clinical relevance, size, and suitability for accurate repeated measurements. The lower

size limit of the target lesion should be at least twice the thickness of the slices showing the tumor to decrease the partial volume effect (e.g., 8 mm lesion for a 4 mm slice).

Up to 2 additional lesions may be followed as non-target lesions. Any change in size of non-target lesions should be noted. The response to non-target lesions will be evaluated independently of the response of the target lesion but will not be included in the primary response assessment.

Development of new lesions is not considered progressive disease for this study.

### 10.2. Measurement of Effect

Methods for Evaluation of Measurable Disease: Whole Brain MRI With and Without Contrast.
For patients with optic pathway gliomas, orbital cuts are necessary. For target tumors outside the brain (i.e. spinal cord), clinically appropriate imaging modalities may be added or substituted.

The following section describes the methodology.

1. For MRI imaging, the longest diameter can be measured from the axial plane or the plane in which the tumor is best seen or measured, provided the same plane is used in follow-up. This longest measurement of the tumor is referred to as the width (W).

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

3. The cystic components of a tumor are considered in tumor measurements.

4. Leptomeningeal tumor spread is usually not a target lesion, and usually cannot be measured accurately. Presence and location of leptomeningeal tumor spread should be noted and change in extent/thickness assessed on follow up studies.

***Imaging Response Criteria for Target Lesion***

Response criteria are assessed in 2 dimensions – the product of W x T.
Baseline measurements should include the longest diameter and the longest perpendicular diameter. These same 2 measurements should then be followed throughout therapy.

For example, if anterior-posterior (AP) is the longest diameter and cranio-caudal (CC) is the longest perpendicular diameter, and then AP and CC should be followed throughout therapy for that lesion.

To assess response/progression/relapse, the ratio is calculated:

**W x T (current scan) / W x T (reference scan)**

Imaging: Standard MRI evaluation is required for disease evaluation as per institutional imaging parameters. Both 1.5T and 3.0 T magnets are acceptable.

Additional Imaging Studies: MR diffusion-weighted imaging (DWI), dynamic contrast-enhanced (DCE), and T1 permeability perfusion MRI, and MR spectroscopy (MRS) are recommended as

part of standard of care at each evaluation period. The data obtained from these studies will be correlated with patient response and outcome.

The following sequences incorporate the standard CNS imaging protocol. Institutional brain tumor or solid tumor imaging protocols can be substituted as long as similar disease evaluation is possible.

### 10.3. Imaging Sequences

**Table 10: Imaging Sequences 1.5 T**

| Sequence | Slice Thickness | TR (ms) | TE (ms) | TI (ms) | Directions | $B_0$ | FOV (cm) | Matrix |
|---|---|---|---|---|---|---|---|---|
| Sagittal T1 | 5 mm skip 1 | 400 | 9 | | | | 22 | 256 x 256 |
| Axial FSE T2 | 2 mm skip 0 | 3800 | 102 | | | | 20 | 256 x 256 |
| Axial T2 FLAIR | 4 mm skip 0 | 10000 | 160 | 2200 | | | 20 | 256 x 224 |
| Axial diffusion | 2.5 mm skip 0 | | | | 12-30 | 1000 | 26 | 128 x 128 single shot |
| Sagittal T2 | 3 mm skip 1 | 3800 | 102 | | | | 22 | 256 x 256 |
| Axial MPGR | 4 mm skip 0 | | | | | | | |
| Axial T1 | 4 mm skip 0 | 500 | min | | | | 20 | 320 x 224 |
| Axial T1 with Contrast | 4 mm skip 0 | 500 | min | | | | 20 | 320 x 224 |
| Sagittal T1 FSPGR with contrast | 1.5 mm skip 0 | | min, flip angle 15 | | | | 24 | 256 x 256 |
| T1 permeability perfusion sequence | 5 mm skip 0 (See below) | | | | | | | |
| Single-voxel MR Spectroscopy PRESS | Volume: 15x15x15 $mm^3$ | 1500 ms | 35ms | | | | | |

**Table 11: Imaging Sequences 3.0 T**

| Sequence | Slice Thickness | TR (ms) | TE (ms) | Directions |
|---|---|---|---|---|
| Sagittal T1 MPRAGE | 1.0 mm skip 0 | 2530 | | |
| Sagittal T2 | 3.0 mm skip 1.0 mm | | | |
| Axial T2 | 2.5 mm no skip | 11730 | 91 | |
| Axial T2 FLAIR | 4 mm, no skip | 9000 | 137 | |
| Axial diffusion | 2 skip 0 | 9100 | 88 | 30 |
| Sagittal MPRAGE | 1.0 mm no skip | | | |
| Axial T1 post contrast | 4.0 mm skip 0 | 650 | 9.7 | |
| Axial SWI | 1.25 mm no skip | | | |

| | | | | |
|---|---|---|---|---|
| T1 permeability perfusion sequence | 5 mm skip 0 (See Below) | | | |
| Single-voxel MR Spectroscopy PRESS | Volume: 15x15x15 $mm^3$ | 2000 ms | 35ms | |

**T1 Permeability Imaging**
T1 permeability should begin with a set of T1 maps with flip angles of 2, 5, 10, and 15 followed by the T1 permeability sequence. After the T1 map sequence with 15-degree flip angle, start the T1 permeability series and 20 seconds into it inject 0.1 mmol/kg of intravenous contrast. See below.

**Table 12: 3D T1W Specifications for T1 Maps and Dynamic Series**

| Sequence Type | Spoiled Gradient Echo |
|---|---|
| Imaging mode | 3D |
| Slice orientation | Axial |
| Frequency direction | A/P |
| Phase direction | R/L |
| FOV - frequency | 220 mm |
| FOV - phase | 220 mm |
| Matrix - frequency | 256 |
| Matrix - phase | 160-192 |
| In-plane resolution | ≤ 1 mm |
| Fat-suppression | No Fat Sat |
| TR | ~4 msec |
| TE | Less than 2 msec or min full |
| TI (STIR sequence) | N/A |
| Flip Angle | DCE -15 degrees; T1 maps - 2, 5, 10 and 15 |
| Slice thickness (acquired, not interpolated) | 5mm, maximum 6mm |
| Number of slices | Minimum 10 prior to zero fill |
| Slice Gap | No gap |
| Parallel imaging factor | ≤ 2 |
| Number of averages | 1 for DCE, 2 for T1 maps |
| k-space ordering | standard, non-centric |
| Temporal Resolution of “T1 DCE”: (seconds per phase/measurement) | ≤ 6 seconds |
| “T1 DCE” imaging duration | ≥ 5 minutes |

**Table 13: T1 Maps/DCE**

| Series Name | Sequence | Flip Angle | Notes |
|---|---|---|---|
| T1 map15 | 3D fast GRE | 15 degrees | Axial, 2 NEX |
| T1 map10 | 3D fast GRE | 10 degrees | Axial, 2 NEX |
| T1 map05 | 3D fast GRE | 5 degrees | Axial, 2 NEX |
| T1 map02 | 3D fast GRE | 2 degrees | Axial, 2 NEX |
| T1 DCE | Dynamic Series, 3D fast GRE | 15 degrees | Axial, 1 NEX, inject 20 sec into this |

### 10.4. Imaging Response Assessment

The overall response assessment takes into account response in the target lesion ONLY.
The response in the non-target lesions will be evaluated independently from the response for the TARGET lesion.

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

**Evaluation of Target Lesion**

• **Complete Response (CR)** Complete disappearance of the target lesion on T2 weighted and T2 weighted fluid attenuated inversion recovery (FLAIR) imaging and contrast imaging using baseline MRI or best recorded response for comparison. Overall, the patient must be clinically stable or have improved on physical examination and functional or neurological assessment.

• **Partial Response (PR):** a 50% or greater reduction in the target lesion on T2 weighted and T2 weighted-FLAIR imaging using the baseline MRI for comparison. Overall, the patient must be clinically stable or have improved on physical examination and functional or neurological assessment.

• **Minor Response (MR):** a 25-49% reduction in the target lesion on T2-weighted and T2-weighted-fluid-attenuated inversion recovery imaging using the baseline MRI or best recorded response for comparison. Overall, the patient must be clinically stable or have improved on physical examination and functional or neurological assessment.

• **Stable Disease (SD):** An increase or a decrease in the target lesion that is not sufficient to qualify as progressive disease or responsive disease (partial response or minor response), respectively. Overall, the patient must be clinically stable or have improved on physical examination and functional or neurological assessment.

• **Progressive Disease (PD):** A greater than 25% increase in the target lesion, usually assessed on T2-weighted and T2-weighted FLAIR using the baseline MRI or best recorded response for comparison. A determination of PD may be deferred if pseudoprogression is suspected (see "**Consideration for possible pseudoprogression / treatment-related effect"** below). Given the possibility of pseudoprogression with immunotherapy, a short interval MRI at the discretion of the treating physician is recommended for participants with PD.
Note that the appearance of new discrete lesions should be noted, but is not considered progressive disease. See section 3.5 regarding off therapy and off study criteria for patients requiring therapy for new or progressive non-target lesions.

See section 9.5 for definition of visual progressive disease. For progressive disease by clinical criteria, confirmatory imaging studies should be obtained when feasible.

• **Unevaluable (UN):** Assessment of target lesion cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

***Consideration for possible pseudoprogression / treatment-related effect**

Immunotherapy may be associated with transient inflammatory-mediated increase in tumor size, or "pseudoprogression." In many cases, it may not be initially possible to distinguish true progression from pseudoprogression.

Where pseudoprogression is suspected in a patient who meets criteria for PD, the patient may continue on study therapy. A shorter interval repeat MRI may be performed if clinically indicated but is not required. Corticosteroids may be considered for symptomatic patients. See section 9.5 for additional information regarding visual symptoms due to suspected pseudoprogression.

If the tumor size is stable or smaller on repeat imaging, and any signs and symptoms related to progression vs pseudoprogression are stable or have improved, patient may remain on study and continue protocol therapy. If the repeat MRI shows continued disease progression, then patient will be permanently removed from therapy and the time of progression will be the date of the initial MRI showing progression.

### 10.5. Duration of Response

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

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

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

Progression-Free and Overall Survival: Progression-Free Survival (PFS) is defined as the duration of time from patient enrollment to time of objective disease progression or death from any cause.

Overall Survival (OS): Overall survival is defined as the duration of time from patient enrollment to time of death from any cause.

### 10.6. Central Review

Central imaging review for response evaluation will be performed at the conclusion of the trial. Optional imaging studies will also be reviewed. Real time response determination will be made by the site investigator based on the local MRI read. For assistance with response determinations, a real-time MRI review may be requested in consultation with the study chair. In addition to the primary RAPNO response determinations, response assessments will also be made using RANO criteria. RANO response assessments will be used to satisfy the exploratory response comparison objective only, and will not be used for the primary response determination. RANO criteria are as described by Wen et al. [56]

### 10.7. Ophthalmologic (Visual Acuity) Response Criteria

See section 9.5 regarding visual response criteria.
See "Consideration for possible pseudoprogress/treatment-related effect" in section 10.4 regarding management of pseudoprogression.

## 11. ADVERSE REPORTING REQUIREMENTS

### 11.1. Definitions

**Adverse Events**: An adverse event is any new, undesirable medical occurrence or change (worsening) of an existing condition in a participant that occurs from study enrollment until 30 days after drug discontinuation, whether or not considered to be product related. Therefore, adverse events are treatment emergent signs or symptoms. Elective hospitalizations for pretreatment conditions (e.g., elective cosmetic procedures) are not adverse events. Non-clinically significant abnormal laboratory values should not be reported as adverse events; however, any clinical consequences of the abnormality should be reported as adverse events. All adverse events must be noted on the electronic case report forms and submitted via eDCS within 2 weeks of completion of every treatment cycle.

For all adverse events, the investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification (within 24 hours) to the site PI and clinical coordinator, as well as the NFCTC Operations Center. Follow-up of the adverse event, even after the date of therapy discontinuation, is required if the adverse event or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator and sponsor.

**Serious Adverse Events**: A serious adverse event is defined by regulatory agencies as one that suggests a significant hazard or side effect, regardless of the investigator's or sponsor's opinion on the relationship to investigational product.

This includes, but may not be limited to, any event that (at any dose):

is FATAL;

is LIFE THREATENING (places the participant at immediate risk of death);

requires HOSPITALIZATION or prolongation of existing hospitalization;

is a persistent or significant DISABILITY/INCAPACITY; or

is a CONGENITAL ANOMALY/BIRTH DEFECT

Important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the participant or require intervention to prevent one of the outcomes listed above, or result in urgent investigation, may be considered serious. Examples include allergic bronchospasm, convulsions, and blood dyscrasias.

### 11.2. Grading of Adverse Events using Common Terminology Criteria (CTCAE)

Adverse events (toxicities) will be graded according to the National Cancer Institute CTCAE version 5.0 for reporting of adverse events. A copy of the current version of the CTCAE version 5.0 can be downloaded from the CTEP home page: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

### 11.3. Attribution

Definitions of relationship to study medication are as follows:

- UNRELATED: bears no relation to timing of medication, similar to symptoms or signs expected in the disease process, does not recur on rechallenge.
- UNLIKELY: does not have temporal relationship to intervention, could readily have been produced by the participant's clinical state, environmental, or other interventions, does not reappear or worsen with reintroduction of intervention.
- POSSIBLY: bears relation to timing of medication, similar to symptoms or signs expected in the disease process, does not recur on rechallenge.
- PROBABLY: bears clear relation to timing of medication, distinct from symptoms or signs expected in the disease process, does not recur on rechallenge.
- DEFINITELY: bears clear relation to timing of medication, distinct from symptoms or signs expected in the disease process, occurs on rechallenge.

Adverse events can be "Expected" or "Unexpected".

***Expected adverse event***

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator's Brochure (IB).

***Unexpected adverse event***

For the purposes of this study, unexpected adverse events are those not listed in the current Investigator Brochure or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the package insert or IB.

### 11.4. Reporting Procedures for All Adverse Events

All observed or volunteered adverse events, regardless of suspected causal relationship to study drug, will be recorded as Adverse Events in the electronic case report forms and submitted via eDCS within 14 days of completion of each treatment cycle. Events involving adverse drug reactions, illnesses with onset during the study, or exacerbation of pre-existing illnesses should be recorded. Objective test findings (e.g. abnormal laboratory test results) should also be recorded if considered to be clinically significant by the treating investigator.

It will be left to the investigator's clinical judgment whether or not an adverse event is of sufficient severity to require that the participant should be removed from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable adverse

event. If either of these occurs, the participant will be given appropriate care under medical supervision until symptoms cease or until the condition becomes stable.

The severity of toxicities will be graded in accordance with the Common Terminology Criteria for Adverse Event (CTCAE) version 5.0 (CTCAE_v5_Quick_Reference).

The relationship of adverse events to the study medications will be assessed by means of the question: "Is there a reasonable possibility that the event may have been caused by the study medication?", Please answer Yes or No.

### 11.5. Expedited Reporting Guidelines

**The following adverse events require expedited reporting:**

- All adverse events that are both serious and unexpected regardless of grade
- Adverse events that might influence the benefit-risk assessment of administration of poly-ICLC as outlined in the protocol
- All grade 5 adverse events
- A serious adverse event that occurs within 30 days of the last dose of the investigational agent
- Pregnancy

  If a participant or a participant's partner becomes pregnant while receiving investigational therapy or within 30 days after the last dose of study drug, a report should be completed and expeditiously submitted as an SAE. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female participant exposed to the poly-ICLC should be reported as an SAE.
- Expedited AE reporting timelines defined:

  "24 hours; 3 business days" – The investigator must initially report the AE within 24 hours (or immediately if the event is fatal or life-threatening) of learning of the event to the Study Chair (dolly.aguilera@choa.org) and NF Operations Center, followed by a complete written report within 3 business days of the initial 24-hour report.

Adverse events requiring expedited reporting will be reported and documented on **Form FDA 3500A MedWatch Form** http://www.fda.gov/medwatch/getforms.htm) and forwarded to:

NFCTC Operations Center
Clinical Research Manager: Lauren Ashley Baldwin, BS
Program Director: Karen Cole – Plourde, BS
Research Compliance Manager: Juliette Southworth, BS, CCRP
Direct Email: nfcop@uab.edu

The clinical research manager from the NFCTC Operations Center will forward all related adverse events that are both serious and unexpected to the FDA on **Form FDA 3500A MedWatch Form** https://www.fda.gov/media/69876/download:

MedWatch
Fax: 1-800-FDA-0178

The final MedWatch Form or CIOMS-1 form will be submitted by the Clinical Research Manager to Oncovir within one to two business days of submission to the FDA or applicable regulatory agency (including confirmation of date that the report was submitted) to allow Oncovir time to cross-report to Oncovir's IND. E-mail: asalazar@oncovir.com; Fax 202-248-2324.

### 11.6. Reporting of Protocol Violations/Deviations and Unanticipated Problems

Site reporting to NFCTC Operations Center
Sites will report unanticipated problems and/or protocol deviations that impact participant safety or the scientific integrity of the study to the Operations Center promptly to nfcop@uab.edu and entered into eDCS. Other protocol violations and deviations should be reported to the NF Operations Center in eDCS within 14 days of the visit.

NFCTC Operations Center reporting requirements
The Operations Center will report unanticipated problems that impact participant safety or the scientific integrity of the study to the USAMRMC Office of Human and Animal Research Oversight (OHARO) OHRO promptly. Unanticipated problems will also be reported to the protocol team, Research Monitor, and DSMB.

All unanticipated problems involving risk to participants or others must be promptly reported by the NFCTC Operations Center via phone (301-619-2165), email (usarmy.detrick.medcom-usamrmc.other.hrpo@health.mil), or facsimile (301-619-7803) to the OHRO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the Sponsor, or regulatory agencies will be promptly reported to the USAMRMC OHARO OHRO.

Other protocol violations and deviations that do not impact participant safety or affect scientific integrity of the study will be provided annually to the Sponsor.

## 12. RESEARCH MONITORING AND DATA SAFETY MONITORING PLAN

The trial PI and clinical coordinator will review the study progress regularly. Participants entered on the trial and adverse events will be reviewed to ensure that the study is implemented as outlined in the protocol. Monthly reports will be generated by the NFCTC to assess completeness of data. There will be monthly phone conferences between the NFCTC and the Principal Investigator to address QA issues. A Data Safety Monitoring Board has been established for the purpose of ensuring data compliance and regular monitoring of this trial.

Additionally, there will be a designated research monitor for this study. This person is a qualified physician and is not associated with this particular protocol. The designated research monitor will work closely with the Principal Investigator to monitor the participants' treatment while on this study.

The research monitor's duties should be based on specific risks or concerns about the research. The research monitor may perform oversight functions and report their observations and findings to the IRB or a designated official. The research monitor may be identified from within or outside the PI's institution.

Research monitor functions may include:

- observing recruitment and enrollment procedures and the consent process for individuals, groups or units,
- overseeing study interventions and interactions,
- reviewing monitoring plans and UPIRTSO reports;
- overseeing data matching, data collection, and analysis

There may be more than one research monitor (e.g., if different skills or experiences are necessary). The monitor may be an ombudsman or a member of the data safety monitoring board.

At a minimum, the research monitor:

- may discuss the research protocol with the investigators, interview human participants, and consult with others outside of the study about the research;
- shall have authority to stop a research protocol in progress, remove individual human participants from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human participants until the IRB can assess the monitor's report;
- shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the OHRO.

In addition, on this study, the research monitor is specifically required to review all unanticipated problems involving risk to participants or others, serious adverse events and all participant deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor should comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The research monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator.

## Safety Monitoring Criteria

Table 14 below defines the maximum allowed number of Grade 4 adverse events that are at least possibly attributable to the study drug.

If the number of events exceeds the number in the second column, accrual to the study should be placed on hold for safety evaluation, pending review of toxicities by the study committee in consultation with the DSMB and the research monitor, to determine whether the study should continue, be amended, or be terminated. For example, if 2 or more patients experience an event before the sixth patient begins therapy, the trial will be on hold.

**Table 14. Safety Rules for Grade 4 Adverse Events**

| Number of Patients | Maximum Number Permitted | Probability to Stop | Cumulative Probability to Stop |
|---|---|---|---|
| 5 | 1 | 0.081 | 0.081 |
| 10 | 2 | 0.070 | 0.151 |
| 16 | 3 | 0.068 | 0.219 |

**Statistical Justification:** The trial should hold enrollment and have a DSMB evaluation for safety if the probability that a Grade 4 adverse event (defined above) exceeds 10% is equal to or higher than 0.95, i.e., stop if $Pr\{\Theta>0.10|data\}\geq0.95$. The table above gives the safety rule for each set of 5 participants that enrolls in the study, with column 2 indicating the maximum number of cases of events that are permitted. The third column gives the probability of holding enrollment on the trial when in fact, the true $\Theta = 0.10$. Safety rules were generated using a binomial distribution.

# 13. REGULATORY CONSIDERATIONS

## 13.1. Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed, and approved by a properly constituted IRB. All decisions of the IRB concerning the conduct of the study must be made in writing.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB.

Additionally, the DoD (Department of Defense)/OHRO (Office of Human Research Oversight) must approve ALL substantive modifications to the protocol and any modifications that could potentially increase risk to participants prior to implementation. OHRO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change in the IRB of Record, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc.), significant change in study design (i.e. would prompt additional scientific review), or a change that could potentially increase risks to participants.

The NF Clinical Trials Consortium (NFCTC) Operation Center will disseminate protocol amendment information to all participating investigators.

## 13.2. Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study.

The formal consent of a participant, using the IRB-approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by

the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

### 13.3. Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
  https://www.fda.gov/media/93884/download

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

    - Title 21 Part 11 – Electronic Records; Electronic Signatures
      https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=11

    - Title 21 Part 50 – Protection of Human Subjects
      https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50

    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

    - Title 21 Part 56 – Institutional Review Boards
      https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=56

    - Title 21 Part 312 – Investigational New Drug Application
      https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRsearch.cfm?CFRPart=312

- State laws

- It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

### 13.4. Data Collection

The trial is being conducted by the NFCT Consortium. Case report forms developed by the NFCTC Operations Center and Protocol Chair will be used for submitting clinical data to the Operations Center. Data must be submitted to the Operations Center within two weeks of completing each required evaluation while the subject is on study via eDCS. Delinquent data delays will be reported to the NFCT Consortium's Site Evaluation Committee and the NFCTC Operations Center.

Completed QOL and pain evaluations will be submitted in real-time via an iPad and NIH Toolbox to the NFCTC Data Coordinating Center.

Blood and tissue samples for banking will be handled and stored in the biorepository located in the Colket Translational Research Building at the Children's Hospital of Philadelphia (CHOP), Philadelphia, PA. The specimens will be under the management of the Center for Data Driven Discovery in Biomedicine (D3b) and the Biorepository Core (BioRC), according to their standard operating procedures (SOPs). Samples will be stored at -80°C. Biological specimens will be coded with a randomly generated study number prior to shipping to the biorepository in order to prevent identification of subjects and protect confidentiality. The study number will not be derived from or related to information about the subject and will not contain any elements of PHI. The specimen number will be entered into the NFCTC Operations Center eDCS, thus providing a link between the specimen and the study data. Specimens may be shared with outside investigators/laboratories, who may analyze (and store, if applicable) the samples. Shared specimens will be labeled only with a study number. Requests for samples will be reviewed by the NF111 Study Chairs and the NFCTC Biology Committee. If a subject withdraws consent to store samples after collection, existing samples in the biorepository will be destroyed; however, any samples already shared to an outside laboratory or other entity will not be destroyed and any data generated already from the samples will not be destroyed.

### 13.5. Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

### 13.6. Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies. Study-related documents will be stored in a secured location accessible only to the investigators and formally designated study personnel.

### 13.7. Multi-Center Guidelines

This protocol will adhere to the policies and requirements of NFCTC IRB, DoD and the Sponsor. The specific responsibilities of the Protocol Chair, NFCTC Operations Center, and Participating Institutions are presented in the Multi-Center Data and Safety Monitoring Plan.

- The Protocol Chair and NFCTC Operations Center are responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.
- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

### 13.8. Publication of Data and Protection of Trade Secrets

The Principal Investigator (Protocol Chair) holds the primary responsibility for publication of the study results; provided that the PI will provide any such publication to Oncovir, Inc. for review at least sixty (60) days prior to submission and also comply with any provisions regarding publication as are agreed to between the Neurofibromatosis Clinical Trials Consortium and Oncovir, Inc. in the Clinical Trial Agreement related to this study. The results will be made public within 13 months of the end of data collection.

However, if a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. In any event, a full report of the outcomes should be made public no later than three (3) years after the end of data collection. Authorship for abstracts and manuscripts resulting from this study will be determined according to guidelines established by the International Committee of Medical Journal Editors.

## 14. STATISTICAL CONSIDERATIONS

**Primary Endpoints**

The primary endpoint of the study is objective response rate (defined as CR or PR) during the first 12 months of treatment. Response will be assessed through the time of the post-12$^{th}$ cycle tumor assessment, or through the last tumor assessment time during this nominal period for patients in follow-up but who have withdrawn from protocol therapy, or through the last available tumor assessment for patients who are lost to follow-up during this time.

The percent change in tumor sizes compared to baseline will be recorded at each evaluation time and will be used to define the categorical response as described below, per the definition of CR, PR, MR, SD, and PD in section 10.4.

• For tumors that regress compared to baseline during the assessment period without first satisfying the criteria for progressive disease (PD), response will be defined at the nadir of the tumor size compared to baseline provided that the percent reduction at the nadir satisfies the definition of CR, PR, or MR per section 10, with the response value of CR, PR, or MR determined by the percent reduction at this time.

The associated time of response will also be recorded.

• For tumors that progress compared to baseline and satisfy the definition of PD without first satisfying the criterion at least for minor response (MR), response will be defined at the first time at which the percent increase in tumor size compared to baseline satisfies the criterion for PD. The response value will be PD. The associated time of response will also be recorded. Progressive disease could be confirmed with a short follow-up MRI (+/- 6 weeks) per treating physician decision, otherwise continue with MRI interval per protocol.

• For tumors that neither regress sufficiently to satisfy the criterion for MR nor progress sufficiently to satisfy the criterion for PD during the assessment period, the response value will be determined to be SD.

Patients will be considered evaluable for response if they have received at least one dose of treatment and have at least one post-baseline tumor assessment.

Patients who undergo gross total resection while on study will be excluded from further response at the time of surgery. Patients who have measurable residual disease (at least twice the thickness of the MRI slide) after resection will be able to continue study drug and the post-op MRI will be used as the new baseline.

**Secondary Endpoints**

• Progression free survival (PFS), defined as the time from start of treatment to the first occurrence of tumor recurrence, tumor progression, occurrence of a second malignancy, or death from any cause. Patients event-free at last follow-up will be censored in analysis. PFS will be determined at 12, 24 and 60 months.

• Objective response rate (defined as CR or PR) after 24 cycles of treatment. Response will be assessed through the time of the post-24$^{th}$ cycle tumor assessment, or through the last tumor assessment time during this nominal period for patients in follow-up, but who have withdrawn from protocol therapy, or through the last available tumor assessment for patients who are lost to follow-up during this time.

• Clinical benefit defined as (CR+PR+MR+SD). Clinical benefit will be assessed through the time of the post-12 and 24$^{th}$ cycle tumor assessment, or through the last tumor assessment time during this nominal period for patients in follow-up but who have withdrawn from protocol therapy, or through the last available tumor assessment for patients who are lost to follow-up during this time.

• The grade and duration of CTCAE toxicities observed during treatment using CTAE version 5.0.

• Evaluation for visual acuity for patients with optic pathways gliomas will be assessed at 12 months and 24 months from starting therapy. Patient visual outcome will be assessed through the time of the post-12$^{th}$ and 24$^{th}$ cycle visual assessment, or through the last visual assessment time during this nominal period for patients in follow-up but who have withdrawn from protocol therapy, or through the last available tumor assessment for patients who are lost to follow-up during this time.

**Exploratory Endpoint**

The RANO criteria for response assessment will be determined and descriptively compared to the primary response using RAPNO.

### 14.1. Participant Accrual

Participants of both genders, from all racial and ethnic groups are eligible for this trial if they meet the criteria outlined in Section 3.0. To date, there is no information that suggests differences in drug metabolism or disease response among racial or ethnic groups or between the genders, indicating that results of the trial will be applicable to all groups. Efforts will be made to extend the accrual to a representative population, but in a phase II study with limited accrual, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand, and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that

correlate to gender, age, racial, or ethnic identity are noted, accrual may be expanded, or additional studies may be performed to investigate those differences more fully.

### 14.2. Sample Size

Target is 20 patients for 16 evaluable participants.

To allow for 25% unevaluable participants, a maximum of up to 20 participants will be enrolled. We anticipate enrollment to be completed in 36 months with primary outcome measure defined in 12 months. The sample size for this trial is based on 3 primary factors: safety based on risk versus benefit. Sample size estimates were based on a single stage phase II clinical trial with the goal of demonstrating that the objective response rate of poly-ICLC is better than an objective response rate of a *poor* drug. For the sample size calculation, we assumed that the response rate of a poor drug (P0) was 5% (i.e., P0 = 5%) and that the objective response rate poly-ICLC (P) would be at least 25% (i.e., P = 25%).

In addition, the type I error rate (alpha) was set to 0.05 (5%) and the type II error rate (beta = 1 – power) was set to 0.20 (20%). Using a single stage design, a sample size of 16 participants achieves at least 80% power to demonstrate that the objective response rate of poly-ICLC is better than a poor objective response rate of 5% and at least 25%. If the number of responders is 3 or more, the hypothesis that P ≤ 5% is rejected with an actual error rate of 0.043. If the number of responses is 2 or less, the hypothesis that P ≥ 25% is rejected with an actual error rate of 0.197. Sample size estimates were determined using PASS v. 19.0.2 (NCSS, LLC, Kaysville, UT).

**Table 15: PASS Output Single-Stage Phase II Clinical Trials Analysis**

**Design for Testing H0: P ≤ P0 versus H1: P ≥ P1**

| P0 | P1 | Alpha | Beta | Cut-Off R + 1 | N | Actual Alpha | Actual Beta |
|---|---|---|---|---|---|---|---|
| 0.05 | 0.25 | 0.050 | 0.2 | 3 | 16 | 0.04 | 0.197 |

**Report Definitions**

- P0 is the maximum response proportion of a poor drug.
- P1 is the minimum response proportion of a good drug.
- N is the sample size.
- If the number of responses ≥ R+1, P0 is rejected.
- If the number of responses ≤ R, P1 is rejected.
- Alpha is the probability of rejecting that P ≤ P0 when this is true.
- Beta is the probability of rejecting that P ≥ P1 when this is true.

### 14.3. Statistical Analysis Plan

The primary goal of the study is to evaluate the effectiveness of the poly-ICLC. Based on the single stage design, response of the drug will be determined after 16 participants are evaluable for the primary endpoint assessment. Participants will be classified based on response (CR/PR vs. MR/SD/PD). If at least 3 of 16 participants respond to treatment, the hypothesis that P ≤ 5% is rejected at alpha = 0.05 and accept that the higher rate of P greater or equal to 25% is more likely; otherwise, the drug will be deemed ineffective based on having an objective response rate

not significantly different than the objective response rate of a poor drug. Comparison between RANO and RAPNO response criteria will be descriptive only.

Key secondary outcomes will be examined at baseline including clinical, quality of life and visual function. Following complete accrual, study enrollment and completion will be summarized as per a Consort Diagram with reasons for withdrawal or unable to complete the study. Appropriate data analysis sets will be defined as follows. The full-analysis set will include data from all participants who receive ≥ 1 dose of therapy on this study; a safety analysis set will comprise data from participants in the full-analysis set with any treatment doses. Other data sets (responding, evaluable) will be defined and will include data from participants who have the necessary baseline and on-study measurements to provide interpretable results for specific parameters of interest.

Descriptive summaries will be prepared to show sample size, mean, standard deviation, 95% confidence intervals (CIs) median, minimum, and maximum for continuous variables and counts, percentages, and 95% CIs for categorical variables.

For endpoints relating to tumor control, participant well-being, and biomarkers, analyses will be done based on the full analysis, responding, evaluable data sets, as appropriate. Time-to-event analyses will be performed with reference to the date of first treatment on this study. Analyses will focus on evaluation of outcomes and will be largely descriptive. Changes in tumor volumes will be summarized by medians, ranges, and the corresponding 95% CI.

Based on the safety analysis set, information regarding study treatment administration, drug dosing and cycle compliance, safety variables, will be described and summarized. Data from the biological analysis will also be described and summarized. Safety will be assessed via tabulations of AEs and SAEs as well as Diary recordings following treatment dose/cycle of therapy.

All analyses for outcome results will be based on evaluable participants, as defined in section 14.

At the end of the trial, we will report poor performance if the treatment is rejected when not meeting the criteria of at least 3 responders' cases. If there is no success, we will report a failure to reject the null hypothesis. If the null hypothesis is rejected, the probability of the estimated success rate will be reported and 95% confidence intervals as well as the distribution of changes in tumor size.

Dose-limiting toxicity (DLT) is not an objective in this trial. Toxicity will be continuously monitored.

## 15. PUBLICATION PLAN

Timely publication will be the responsibility of the principal investigator. No data should be submitted for presentation at public meetings or in abstract form without approval of the Principal Investigator and the NF Clinical Trials Consortium. Guidelines will follow the rules for publication for the NF Clinical Trials Consortium.

The results should be made public within 13 months of the end of data collection, sooner if possible. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of data collection.

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## APPENDIX 1: NIH Consensus Conference Criteria for NF1

• Six or more café-au-lait spots (≥ 0.5 cm in prepubertal participants or ≥ 1.5 cm in post pubertal participants)

• Two or more neurofibromas of any type or one plexiform neurofibroma

• Freckling in the axilla or groin

• Optic glioma

• Two or more Lisch nodules

• A distinctive bony lesion (dysplasia of the sphenoid bone or dysplasia or thinning of long bone cortex)

• A first-degree relative with NF1

## APPENDIX 2: Karnofsky and Lansky Performance Status Criteria

| **PERFORMANCE STATUS CRITERIA**<br>Karnofsky and Lansky performance scores are intended to be multiples of 10 | | | |
|---|---|---|---|
| **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 3: Study Calendar and Required Observations (through end of therapy)

| Observation | Pre-Study | Day 1 Cycle 1 | Day 8 Cycle 1 | Day 15 Cycle 1 | Prior to subsequent cycles 2-24 | End of Therapy/Off Study |
|---|---|---|---|---|---|---|
| History, VS: BP,HR, temperature, oxygen saturation, RR, height, weight, Physical and neurological exam[1] | x | x | | x | x | x |
| Performance Status[1] | x | x | | x | x | x |
| Toxicity Evaluation | | x | x | x | x | x |
| Review Medication diary/temperature log | | | x | x | x | x |
| Concomitant Meds | x | x | | x | x | x |
| CBC Diff[7] | x | x | x | x | x | x |
| CMP/ Phosphorous[6] | x | x | | | x | x |
| Serum or Urine Pregnancy[2] | x | Prior to initiation of each cycle | | | | |
| MRI-Brain with and without contrast[3] (with orbital cuts for optic pathway tumors) | x | | | | Every 3 cycles starting with end of Cycle 3 | x |
| MRI Spine with/without contrast[3] | x | | | | Every 3 cycles starting with end of Cycle 3 | x |
| Ophthalmology Functional Assessments[4] | x | | | | Every 3 cycles starting with end of Cycle 3 | x |
| Biology-sodium heparin green top blood samples | x | | x[8] | x | Day1 cycle 3<br>Day 1 cycle 6<br>Day 1 cycle 12 | x |
| Quality of Life Studies | x | | | | Cycle 6 day 1<br>Cycle 12 day 1 | x |
| Tissue (with concurrent blood sample) submission if available[5] | | x | At the time surgery is performed | | | |

1: Performance Status = Karnofsky if ≥ 16 y/o or Lansky if < 16 y/o; All evaluations for Day 8 and Day 15 of Cycle 1 must be done +/-48 hours, then +/-5 days for subsequent cycles.
2: Required for female patients of childbearing potential; urine or serum pregnancy test must be negative within 7 days of starting each cycle
3: Brain and/or spine MRI should be obtained depending on tumor location. MRI will be obtained at the end of cycle 3(+/-2 weeks), then every 3 cycles (+/-2 weeks), at the end of therapy/off study or at progression.
4: **Ophthalmology evaluation only for patients with optic pathway gliomas** at the end of cycle 3 (+/-2 weeks), then every 3 cycles (+/-2 weeks)
5: Tissue submission with concurrent blood sample if available 6 months prior to enrollment and at any point during protocol treatment (optional).
6: CMP: including sodium, potassium, CO2, chloride, total protein, glucose, AST, ALT, alkaline phosphatase, albumin, calcium, total bilirubin, BUN, Creatinine including phosphorous. Labs must be completed within 14 days of cycle 1 day 1 and on day 1 +/-5 days of subsequent cycles.
7. CBC/Differential/Platelet Count: Labs must be completed within 14 days of cycle 1 day 1, +/- 48 hours of cycle 1 day 8 and day 15, and +/-5 days of subsequent cycles.
8. Collect 5-10ml of blood per sample in sodium heparin tubes for patients < 8 years of age. For patients that weigh less than 10 kg, collect 2 ml on cycle 1 day 8. For patients ≥ 8 years, collect 10-15 ml.

**Note:** MRI Brain/Spine should be obtained with and without gadolinium including pre T1, post T1, T2 and flair sequences. Both axial and either coronal or sagittal pre-post gadolinium images should be obtained. A copy of the MRI de-identified images will be submitted to the coordinating site.

## APPENDIX 4: Off Therapy Observations

| Observation | 1-12 Months Off Therapy | 13-36 Months Off Therapy | 37-60 Months Off Therapy |
|---|---|---|---|
| MRI-Brain with and without contrast[1,2] (with orbital cuts for optic pathway tumors) | Every 3 months | Every 6 months | Every 12 months |
| MRI Spine with and without contrast[1,2] | Every 3 months | Every 6 months | Every 12 months |
| Ophthalmology Functional Assessments[3] | Every 3 months | Every 6 months | Every 12 months |
| Quality of Life Studies | | At month 24[6] | |
| Biology-sodium heparin green top blood samples | 3 month and 12 month off therapy visit[5] | X[5] | |
| Tissue (with concurrent blood sample) submission if available[4] | X | X | X |

1: MRI should be done +/-4 weeks for 1-12 months, +/-4 weeks for 12-36 months, +/-6 weeks for 36-60 months
2: Brain and/or spine MRI should be obtained depending on tumor location.
3: Ophthalmology evaluation only for patients with optic pathway gliomas (+/- 4 weeks for 1-12 months, +/-4 weeks for 12-36 months, +/-6 weeks for 36-60 months)
4: Tissue submission with concurrent blood sample if available at the time of progression if surgery performed (optional)
5: Biology samples should be drawn at the 3 months off therapy visit, 12 months off therapy visit and if MRI shows progression. Collect 5-10ml of blood per sample in sodium heparin tubes for patients < 8 years of age. For patients ≥ 8 years, collect 10-15 ml.
6: QOL assessment to be done at 24 months post therapy +/- 4 weeks or at progression if sooner.

**Clinical evaluations including laboratory evaluations should be performed as per standard of care.**

## APPENDIX 5: List of Enzyme Inducing Anticonvulsants

**Enzyme inducing anticonvulsants**

-Carbamazepine
-Phenytoin
-Phenobarbital
-Primidone
-Oxcarbazepine
-Topiramate
-Fosphenytoin

## APPENDIX 6: Mandatory Biological Specimens

The following type of bio-specimens will be collected for the performance of correlative studies.

The samples obtained will be processed in the receiving lab. Aliquots of mononuclear cells will be utilized for the performance of several correlative studies (depending on sample availability and quality) as described. Plasma will be used for detection of circulating cytokines and chemokines.

| Sample Type | Collection Times |
|---|---|
| Peripheral Blood | • Baseline (prior to Cycle 1 Day 1)<br>• Cycle 1 Day 8<br>• Cycle 1 Day 15<br>• Cycle 3 Day 1<br>• Cycle 6 Day 1<br>• Cycle 12 Day 1<br>• End of treatment/time of progression<br>• 3 months post treatment<br>• 12 months post treatment<br>• Post treatment progression |

**Procedure:**

- For children age 8 or less, obtain 5- 10 ml of peripheral blood. For patients that weigh less than 10 kg, collect 2 ml on cycle 1 day 8.
- For children older than 8 years of age, obtain 10-15 mlof peripheral blood.
- Draw in sodium heparin green top tubes and shipped at room temperature the same day the sample is drawn. Do not draw from a finger stick.
- No processing is required prior to shipping blood. DO NOT REFRIGERATE OR FREEZE THE SAMPLE. Label each specimen with participant's research identifier, date drawn, and sample collection time point (Cycle 1 Day 8, etc.).

**Shipping and Handling:**

Blood samples must be shipped at room temperature the same day they are collected (Monday-Thursday only). They should be shipped first priority if possible, otherwise by priority overnight, taking care to avoid Friday collection and shipping. Shipping of samples on Fridays is not allowed. Accordingly, in the event that a sample collection date falls on a Friday, sample may be collected a day earlier.
Please notify the receiving laboratory by emailing: Ava Horvat (ava.alyse.horvat@emory.edu and Mahesh Shrestha (mahesh.shrestha@emory.edu when samples are shipped.

**Emory University - Winship Cancer Institute**
**Dhodapkar' s Lab**
Attn.: Ava Horvat (ava.alyse.horvat@emory.edu
Mahesh Shrestha (mahesh.shrestha@emory.edu)
HSRB, Room E369
1760 Haygood Drive, Atlanta, GA 30322
Work phone: 404-778-4724

## APPENDIX 7: Optional Tumor Tissue with Concurrent Blood Sample

If a patient has surgery 6 months prior to enrollment or surgery is indicated during therapy or at the time of progession, tissue and concurrent blood sample may be obtained for banking to store in the biorepository located at the Children's Hospital of Philadelphia (CHOP), Philadelphia, PA.

### 1) Blood

Prior to the day of blood draw, the site will have arranged for a sample collection kit supplied by the biorepository to be shipped to the site. The Center for Data Driven Discovery in Biomedicine (D3b) at CHOP will be providing kits and labels. The kit will contain tubes and bar-coded labels (which include a specimen number) for blood collection. It will also contain shipping materials and instructions for sending collected blood samples to the biorepository, as well as a pre-paid shipping label.

Samples

- Streck tube (for plasma): ~ 2 mL.
- EDTA tube (for DNA): ~ 5 mL.
- PAXGene tube (for RNA) ~ 2.5 mL.

Tubes should be inverted at least 10 times after being drawn.

The samples will only be obtained if the total blood volume does not exceed 5 mL/kg. If not enough blood can be drawn for all tubes, the priority order for obtaining the specimens is: 1) Streck tube, 2) EDTA tube, 3) PAXGene tube.

Label the specimens with the specimen labels provided with the specimen kit.

Blood samples must be shipped (unprocessed) same day at ambient temperature to the biorepository by overnight delivery using shipping container provided by the biorepository. Samples must be shipped Monday through Thursday.

Enter the specimen numbers into the NFCTC Data Coordinating Center eDCS.

For questions about shipping, kits, tubes, etc., and to confirm the shipping address prior to sending samples, please contact: Catherine Sullivan (sullivanca@email.chop.edu) and BioRc (biorc@email.chop.edu).

### 2) Tumor Tissue

Prior to the day of surgery, the site will have arranged for a tissue sample collection kit supplied by the biorepository to be shipped to the site. The Center for Data Driven Discovery in Biomedicine (D3b) at CHOP will be providing kits and labels. The kit will contain bar-coded cryovials (which include a specimen number) for tissue collection. It will also contain shipping materials and instructions for sending collected tissue samples to the biorepository, as well as a pre-paid shipping label.

Samples

- Fresh frozen tissue: 1-2 pea sized aliquots (~ 0.3 $cm^3$, equivalent to 0.8 cm in diameter) of excess (left over) tumor tissue. Place each aliquot in a separate cryovial and label the specimen with subject's study number. Snap freeze the aliquots in liquid nitrogen

(preferred) or dry ice and then store in a freezer at -80°C or below until they can be sent to the biorepository.
- 1 H&E stained slide cut from each submitted piece to confirm tumor is present.
- If fresh frozen tissue is unavailable, please submit processed DNA (at least 3 micrograms) and RNA (at least 400 nanograms with optimal RIN > 7) if available.

Label the specimen manifest with the subject's study number.
Fresh frozen tissue or DNA/RNA must be shipped on dry ice via overnight delivery using shipping materials provided by the biorepository. The H&E slide should be shipped at ambient temperature. Samples must be shipped Monday through Thursday.

Enter the specimen numbers into the NFCTC Data Coordinating Center eDCS.

For questions about shipping, kits, tubes, etc., and to confirm the shipping address prior to sending samples, please contact: Catherine Sullivan (sullivanca@email.chop.edu) and BioRc (biorc@email.chop.edu).

**APPENDIX 8: Poly-ICLC Diaries**

## Cycle 1: Monday/Thursday Dosing
## Cycle 1: Tuesday/Friday Dosing
## Cycle __:  Monday/Thursday Dosing
## Cycle __: Tuesday/Friday Dosing

**A: NF Protocol 111 Medication Diary Cycle 1** Patient Initials_____
**Monday/Thursday Dosing** PID#_____

Administer Poly-ICLC as an intramuscular injection two days a week on Mondays and Thursdays. Each dose must be given at least 2 days apart. If a dose is missed, it may be given within 24 hours of when the initial dose is due. After 8 doses, do not administer any more doses until you return to clinic.

Write down the date, time, and site of administration of Poly-ICLC. For Cycle 1 record the temperature prior to each dose and 12 hours (+/- 6 hours) after each dose. After Cycle 1 temperature is only monitored on an as needed basis (if patient is not feeling well), and should be recorded if greater than 102.2° Fahrenheit (39.0° Celsius).

| | Date | Time | Site of Administration | Temperature | Other Problems |
|---|---|---|---|---|---|
| **Week 1 Dose 1** (Monday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 1 Dose 2** (Thursday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 2 Dose 1** (Monday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 2 Dose 2** (Thursday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 3 Dose 1** (Monday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 3 Dose 2** (Thursday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 4 Dose 1** (Monday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 4 Dose 2** (Thursday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |

**B: NF Protocol 111 Medication Diary Cycle 1** Patient Initials_____
**Tuesday/Friday Dosing** PID#_____

Administer Poly-ICLC as an intramuscular injection two days a week on Mondays and Thursdays. Each dose must be given at least 2 days apart. If a dose is missed, it may be given within 24 hours of when the initial dose is due. After 8 doses, do not administer any more doses until you return to clinic.

Write down the date, time, and site of administration of Poly-ICLC. For Cycle 1 record the temperature prior to each dose and 12 hours (+/- 6 hours) after each dose. After Cycle 1 temperature is only monitored on an as needed basis (if patient is not feeling well), and should be recorded if greater than 102.2° Fahrenheit (39.0° Celsius).

| | Date | Time | Site of Administration | Temperature | Other Problems |
|---|---|---|---|---|---|
| **Week 1 Dose 1** (Tuesday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 1 Dose 2** (Friday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 2 Dose 1** (Tuesday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 2 Dose 2** (Friday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 3 Dose 1** (Tuesday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 3 Dose 2** (Friday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 4 Dose 1** (Tuesday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |
| **Week 4 Dose 2** (Friday) | | | | Pre-Dose Temp_____ Time_____<br>12 Hour Temp _____ Time_____ | |

**C: NF Protocol 111 Medication Diary Cycle ____** Patient Initials_____
**Monday/Thursday Dosing** PID#_____

Administer Poly-ICLC as an intramuscular injection two days a week on Mondays and Thursdays. Each dose must be given at least 2 days apart. If a dose is missed, it may be given within 24 hours of when the initial dose is due. After 8 doses, do not administer any more doses until you return to clinic.

Write down the date, time, and site of administration of Poly-ICLC. For Cycle 1 record the temperature prior to each dose and 12 hours (+/- 6 hours) after each dose. After Cycle 1 temperature is only monitored on an as needed basis (if patient is not feeling well), and should be recorded if greater than 102.2° Fahrenheit (39.0° Celsius).

| | **Date** | **Time** | **Site of Administration** | **Temperature (after Cycle 1 only required if patient not feeling well)** | **Other Problems** |
|---|---|---|---|---|---|
| **Week 1 Dose 1** (Monday) | | | | | |
| **Week 1 Dose 2** (Thursday) | | | | | |
| **Week 2 Dose 1** (Monday) | | | | | |
| **Week 2 Dose 2** (Thursday) | | | | | |
| **Week 3 Dose 1** (Monday) | | | | | |
| **Week 3 Dose 2** (Thursday) | | | | | |
| **Week 4 Dose 1** (Monday) | | | | | |
| **Week 4 Dose 2** (Thursday) | | | | | |

**D: NF Protocol 111 Medication Diary Cycle ____** Patient Initials_____
**Tuesday/Friday Dosing** PID#_____

Administer Poly-ICLC as an intramuscular injection two days a week on Mondays and Thursdays. Each dose must be given at least 2 days apart. If a dose is missed, it may be given within 24 hours of when the initial dose is due. After 8 doses, do not administer any more doses until you return to clinic.

Write down the date, time, and site of administration of Poly-ICLC. For Cycle 1 record the temperature prior to each dose and 12 hours (+/- 6 hours) after each dose. After Cycle 1 temperature is only monitored on an as needed basis (if patient is not feeling well), and should be recorded if greater than 102.2° Fahrenheit (39.0° Celsius).

| | **Date** | **Time** | **Site of Administration** | **Temperature (after Cycle 1 only required if patient not feeling well)** | **Other Problems** |
|---|---|---|---|---|---|
| **Week 1 Dose 1** (Tuesday) | | | | | |
| **Week 1 Dose 2** (Friday) | | | | | |
| **Week 2 Dose 1** (Tuesday) | | | | | |
| **Week 2 Dose 2** (Friday) | | | | | |
| **Week 3 Dose 1** (Tuesday) | | | | | |
| **Week 3 Dose 2** (Friday) | | | | | |
| **Week 4 Dose 1** (Tuesday) | | | | | |
| **Week 4 Dose 2** (Friday) | | | | | |

## APPENDIX 9: Poly-ICLC Roadmap for Cycle 1

Start cycle when:

- ANC ≥ 750/mm$^3$
- Hgb ≥ 8gm/dL (may transfuse PRBCs)
- Platelets ≥ 75,000 (transfusion independent; > 7 days from last transfusion)
- SGPT (ALT) and SGOT (AST)= or < 5x ULN

| Drug | Route | Dose | Days | Comments |
|---|---|---|---|---|
| Poly-ICLC | IM | 20mcg/kg/dose | Twice weekly on Mon/Thurs OR Tues/Friday | Injections must be given at least 48 hrs apart; a missed dose can be made up within 24 hrs Premedication with acetaminophen or ibubrofen and continued dosing every 6 hrs for 24 hrs is recommended |

Cycle: __1__ Height: _____ cm Weight: _____kg BSA: _____m$^2$

| Date Due | Date Given | Day | Poly-ICLC | Site Given | Observations |
|---|---|---|---|---|---|
| | | 1 | _____mcg | | A, B, C, D, E, F |
| | | 4 | _____mcg | | |
| | | 8 | _____mcg | | B, D |
| | | 11 | _____mcg | | |
| | | 15 | _____mcg | | A, B, D, E |
| | | 18 | _____mcg | | |
| | | 22 | _____mcg | | |
| | | 25 | _____mcg | | |
| | | 28 | | | |

A. History, VS, Height, weight, PE with neuro evaluation and Performance Status
B. CBCD
C. CMP, Phos
D. Biology Blood Samples:. Collect 5-10ml of blood per sample in sodium heparin tubes for patients < 8 years of age. For patients that weigh less than 10 kg, collect 2 ml on cycle 1 day 8. For patients ≥ 8 years, collect 10-15 ml.
E. Pregnancy Test (urine or serum BHCG) – only in females of childbearing potential (all menstruating females and any female ≥ 12 years of age)
F. QOL
G. MRI brain with and without contrast – at the end of Cycles 3, 6, 9, 12, 15, 18, 21 and 24 (end of treatment). MRI orbits with and without contrast only for OPG.
H. MRI spine with contrast – only if primary tumor is on the spine or clinically indicated
I. Ophthalmology Exam – for any tumor involving the optic pathway ophthalmology exams are required at the end of Cycles 3, 6, 9, 12, 15, 18, 21, and 24 (end of treatment)

MD Signature/Date: _____

PID1/PID2:_____

## APPENDIX 10: Poly-ICLC Roadmap for Cycles 2 – 24

Start each cycle when:

- ANC ≥ 750/mm$^3$, Hgb ≥ 8gm/dL (may transfuse PRBCs), Platelets ≥ 75,000 (transfusion independent; > 7 days from last transfusion)
- SGPT (ALT) and SGOT (AST) = or < 5x ULN

| Drug | Route | Dose | Days | Comments |
|---|---|---|---|---|
| Poly-ICLC | IM | 20mcg/kg/dose | Twice weekly on Mon/Thurs OR Tues/Friday | Injections must be given at least 48 hrs apart; a missed dose can be made up within 24 hrs Premedication with acetaminophen or ibubrofen and continued dosing every 6 hrs for 24 hrs is recommended |

Cycle: _____ Height: _____ cm Weight: _____kg BSA: _____m$^2$

| Date Due | Date Given | Day | Poly-ICLC | Site Given | Observations |
|---|---|---|---|---|---|
| | | 1 | _____mcg | | A, B, C, D, E, F |
| | | 4 | _____mcg | | |
| | | 8 | _____mcg | | |
| | | 11 | _____mcg | | |
| | | 15 | _____mcg | | |
| | | 18 | _____mcg | | |
| | | 22 | _____mcg | | |
| | | 25 | _____mcg | | |
| | | 28 | | | G, H, I |

A. History, VS, height, weight, PE with neuro evaluation and Performance Status
B. CBCD
C. CMP, Phos
D. Biology Blood Samples required at C3D1, C6D1, and C12 D1. 5 -10 ml of blood per sample in sodium heparin tubes for patients < 8 years of age. For patients ≥ 8 years, we will collect 15 ml of blood.
E. Pregnancy Test (urine or serum BHCG) – only in females of childbearing potential (all menstruating females and any female ≥ 12 years of age)
F. QOL – required at beginning of Cycle 6 and 12 and end of therapy
G. MRI brain with and without contrast – at the end of Cycles 3, 6, 9, 12, 15, 18, 21 and 24 (end of treatment)
H. MRI spine with contrast – only if primary tumor is on the spine or clinically indicated
I. Ophthalmology Exam – for any tumor involving the optic pathway ophthalmology exams are required at the end of Cycles 3, 6, 9, 12, 15, 18, 21, and 24 (end of treatment)

MD Signature/Date: _____

PID1/PID2:_____

## APPENDIX 11: Poly-ICLC Ophthalmology Worksheet

| PID1/PID2 | NF111– | Date of Visit: |
|---|---|---|

**Best Corrected Teller Grating Acuity measured at 55cm (Cycles/cm, choose best card achieved):**

Right Eye:
- ☐ 38
- ☐ 26
- ☐ 19
- ☐ 13
- ☐ 9.8
- ☐ 6.5
- ☐ 4.8
- ☐ 3.2
- ☐ 2.4
- ☐ 1.6
- ☐ 1.3
- ☐ 0.86
- ☐ 0.64
- ☐ 0.43
- ☐ 0.32
- ☐ 0.23Low vision/HM/LP
- ☐ 0.16 No light perception
- ☐ Not Done (please select the reason):
  - ☐ Test not attempted
  - ☐ Subject unable to cooperate
  - ☐ Unevaluable Eye / Other

  Please specify: _____

Left Eye:
- ☐ 38
- ☐ 26
- ☐ 19
- ☐ 13
- ☐ 9.8
- ☐ 6.5
- ☐ 4.8
- ☐ 3.2
- ☐ 2.4
- ☐ 1.6
- ☐ 1.3
- ☐ 0.86
- ☐ 0.64
- ☐ 0.43
- ☐ 0.32
- ☐ 0.23 Low vision/HM/LP
- ☐ 0.16 No light perception
- ☐ Not Done (please select the reason):
  - ☐ Test not attempted
  - ☐ Subject unable to cooperate
  - ☐ Unevaluable Eye / Other

  Please specify: _____

**Best Corrected HOTV Visual Acuity using ATS protocol (in logMAR):**

Right Eye:
- ☐ -0.1
- ☐ 0.0
- ☐ 0.1
- ☐ 0.2
- ☐ 0.3
- ☐ 0.4
- ☐ 0.5
- ☐ 0.6
- ☐ 0.7
- ☐ 0.8
- ☐ 0.9
- ☐ 1.0
- ☐ 1.1
- ☐ 1.2
- ☐ 1.3
- ☐ 1.4
- ☐ 1.5
- ☐ 1.6
- ☐ 1.7(>20/800, hand motions or light perception)
- ☐ 1.8(No Light Perception)
- ☐ Not Done (please select the reason):
  - ☐ Test not attempted
  - ☐ Subject unable to cooperate
  - ☐ Unevaluable Eye / Other

  Please specify: _____

Left Eye:
- ☐ -0.1
- ☐ 0.0
- ☐ 0.1
- ☐ 0.2
- ☐ 0.3
- ☐ 0.4
- ☐ 0.5
- ☐ 0.6
- ☐ 0.7
- ☐ 0.8
- ☐ 0.9
- ☐ 1.0
- ☐ 1.1
- ☐ 1.2
- ☐ 1.3
- ☐ 1.4
- ☐ 1.5
- ☐ 1.6
- ☐ 1.7(>20/800, hand motions or light perception)
- ☐ 1.8(No Light Perception)
- ☐ Not Done (please select the reason):
  - ☐ Test not attempted
  - ☐ Subject unable to cooperate
  - ☐ Unevaluable Eye / Other

  Please specify: _____

**Visual Field by Confrontation Method (number of abnormal quadrants per eye):**

Right Eye:
- ☐ 0 - normal visual field
- ☐ 1 - abnormal quadrant
- ☐ 2 - abnormal quadrants
- ☐ 3 - abnormal quadrants
- ☐ 4 - abnormal quadrants
- ☐ Not Done (please select the reason):
  - ☐ Test not attempted
  - ☐ Subject unable to cooperate
  - ☐ Unevaluable Eye / Other

  Please specify:_____

Left Eye:
- ☐ 0 - normal visual field
- ☐ 1 - abnormal quadrant
- ☐ 2 - abnormal quadrants
- ☐ 3 - abnormal quadrants
- ☐ 4 - abnormal quadrants
- ☐ Not Done (please select the reason):
  - ☐ Test not attempted
  - ☐ Subject unable to cooperate
  - ☐ Unevaluable Eye / Other

  Please specify: _____

**Optic Nerve Swelling:**

Right Eye:
- ☐ Swelling
- ☐ Normal
- ☐ Did not perform

Left Eye:
- ☐ Swelling
- ☐ Normal
- ☐ Did not perform

**Optic Pallor:**

Right Eye:
- ☐ Pallor
- ☐ Normal
- ☐ Did not perform

Left Eye:
- ☐ Pallor
- ☐ Normal
- ☐ Did not perform

**In the ophthalmologist's opinion has overall visual acuity declined since last visit?**

Right Eye:
- ☐ Yes, Due to:
  - ☐ Optic Pathway Glioma
  - ☐ Amblyopia
  - ☐ Unsure (OPG and/or suspected amblyopia)
  - ☐ Other

  Please specify: _____
- ☐ No
- ☐ N/A - Baseline

Left Eye:
- ☐ Yes, Due to:
  - ☐ Optic Pathway Glioma
  - ☐ Amblyopia
  - ☐ Unsure (OPG and/or suspected amblyopia)
  - ☐ Other

  Please specify: _____
- ☐ No
- ☐ N/A - Baseline

**In ophthalmologist's opinion is the overall visual acuity NORMAL?**

Right Eye:
- ☐ Yes
- ☐ No, Due to:
  - ☐ Optic Pathway Glioma
  - ☐ Amblyopia
  - ☐ Unsure (OPG and/or suspected amblyopia)
  - ☐ Other

  Please specify: _____

Left Eye:
- ☐ Yes
- ☐ No, Due to:
  - ☐ Optic Pathway Glioma
  - ☐ Amblyopia
  - ☐ Unsure (OPG and/or suspected amblyopia)
  - ☐ Other

  Please specify: _____