Title: **A Phase II Trial of Poly-ICLC in the Management of Recurrent or Progressive Pediatric Low Grade Gliomas**

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1.0 Objectives:

1.1 Primary Objectives

1.1.1 The primary endpoint will be the progression free survival rate to poly-ICLC at 6 months, in the children with recurrent low grade gliomas; tumor assessments are to be performed per Modified McDonald's criteria. Progression free survival is defined as the time from starting study therapy to tumor progression, tumor recurrence, death from any cause, or occurrence of a second malignant neoplasm.

1.2 Secondary Objectives

1.2.1 To determine the overall response rate to poly-ICLC in the treatment of children with recurrent low grade gliomas; tumor assessments are to be performed per 3D measurements.

1.2.2 Progression free survival over the course of the study.

1.2.3 Overall survival at 6 months – the time from starting study therapy to death from any cause.

1.2.4 To assess the toxicity associated with treatment with poly-ICLC in pediatric patients with recurrent low grade gliomas.

1.2.5 To study the effect of treatment with poly-ICLC on the signaling pathways controlling apoptosis in low grade glioma tumor cells.

1.2.6 To determine if surrogate markers of tumor response and progression can be identified in the serum, PBMC and/or cerebrospinal fluid of patients with recurrent low grade gliomas treated with poly-ICLC. Proteomics analysis will be performed to obtain protein levels of these markers such as OAS, interferon α, β, γ, PKR kinase, phosphorylation state of eIF2a, and induction of $p21^{waf-1}$.

2.0 Background/Rationale

The incidence of primary pediatric brain tumors in the United States is about 1500 per year. Brain tumors are the most common solid tumor diagnosed in childhood and thus account for significant childhood mortality in the United States. Low-grade astrocytomas and gliomas are the most common type of brain tumor of childhood (36% of childhood brain tumors). These tumors encompass a heterogeneous assortment of histological subtypes including: fibrillary, protoplasmic, gemistocytic, and mixed variants. Pilocytic astrocytomas, pleomorphic xanthroastrocytomas and subependymal giant cell astrocytomas are also included. Furthermore, in young children there are some unique rare entities that behave like low-grade tumors, including infantile desmoplastic gangliogliomas, pilomyxoid variant, and desmoplastic astrocytomas.

Although children with low-grade astrocytomas often survive many years after conventional treatment with surgery and sometimes radiotherapy, there can be tremendous morbidity depending on the location of tumor and the effects of therapy. These tumors constitute a heterogeneous group because of differing locations within the brain and varying biological behavior of different subtypes. For those where total excision is possible, such as cerebellar astrocytomas, prognosis is excellent with over 90% ten-year survival rates with surgical excision alone. In contrast, survival rates in children with cerebral or diencephalic tumors are 40-70% at five years with irradiation, but decline to 11-50% at 10 years (Mundigers, 1990). Some tumors however may be unresectable/partially resectable, and radiation can have undesirable side effects in young children. While the most significant intellectual deficits occur in young children less than 5 years treated with cranial irradiation, the deficits recognized even in young adults warrant extending the age to 10 years for avoiding radiation. Chemotherapy regimens are used for high-risk patients (progressive tumor, residual tumor) as a means to avoid or delay radiation in young patients, but side effects of chemotherapy are frequently reported. Newer forms of effective treatment that will have lesser side effects are much needed in childhood brain tumors especially low-grade gliomas. We propose to study the efficacy and toxicity of poly-ICLC, a biological response modifier in children with low-grade gliomas.

PROTEOMICS

Current diagnostic and therapeutic monitoring of brain tumor patients are significantly hindered due to limited understanding of brain tumor biology and response to therapy. The majority of CNS tumors cannot be identified or followed by expression of serum or CSF markers. However, if available, such markers would be highly desirable and could be used to:

- Detect minimal residual disease
- Predict response to specific targeted therapies
- Predict or anticipate tumor progression
- Distinguish tumor recurrence from post surgical changes or post-radiation changes on neuro-imaging
- Augment current histopathologic classification systems
- Improve current clinical and pathological treatment stratification schemata
- Assess efficacy of and tumor response to specific biologic targeted therapies that may not impact tumor size as a primary tumor endpoint (e.g., small molecule inhibitors or anti-angiogenic strategies)

While such markers would be useful to prognosticate, monitor and treat all CNS tumors, its use in glial tumors including recurrent low grade astrocytomas is critical since these tumors are often biopsied at presentation, but not at recurrence. Often these tumors are not amenable complete resection or biopsy due to the eloquence of brain tissue they infiltrate (e.g., optic pathway, brainstem or hypothalamic gliomas), or the blood vessels that they encase.

CNS biologic material in CSF

Glial tumors tend to disseminate locally along white matter tracts rather than through sub-arachnoid seeding. Dissemination of low grade gliomas along the sub-arachnoid space has been reported in children with low grade gliomas. Even focal tumors are frequently adjacent to CSF pathways (e.g., intrapeduncular fossa, third and fourth ventricles) resulting in direct contact between tumor tissue and spinal fluid. Yet examination of CSF cytology for these tumors is not standard. Given limitations of identifying tumor cells in the CSF, methodologies that could improve our understanding of CNS tumors of all types are needed. This would provide a significant improvement in currently available knowledge about the biology of these tumors, and could elucidate potential therapeutic avenues.

Proteomics, a relatively new area of research whereby total protein complement of a tissue compartment is analyzed, has successfully been used to identify novel biomarkers in solid tumors (Zheng, 2003; Khwaja, 2007). Because proteins are effectors of all cellular functions, their measurement should represent the most direct means of cellular characterization and hence tumor biology. Because cells and their environment exist in an integrated state, it has been possible to interrogate the proteins of extra-cellular compartments to assess the presence and impact of tumor cells. This has been done primarily using serum or plasma to establish a method of screening for the presence of low stage tumors.

An analogous extra-cellular compartment for use in brain tumors would be cerebrospinal fluid (CSF). It circulates throughout the CNS and exchanges proteins with the extra-cellular fluid of the brain and spinal cord. CSF is continuously created and reabsorbed, providing a real time steady state proteome. Unlike serum, which contains a highly complex protein mixture ranging from very low abundance proteins in the 10-30 pg/mL range to very abundant proteins in the 35-55 mg/mL range, CSF contains a less complex protein mixture (Omenn, 2005). Therefore, the CSF is more likely to contain higher relative concentrations of tumor-specific proteins (higher signal to noise ratio) than serum. Taken together this makes CSF and attractive alternative to serum for detection of brain tumor related biomarkers.

Unlike leukemia and many solid tumors outside the CNS, where serial biopsies are readily performed, tumors of the CNS are not easily accessible other than at the time of initial or repeat resection or biopsy. While studies on these samples provide important findings regarding tumor biology, serial analyses during treatment are not reasonable. By contrast, the CSF of tumor patients can be more readily sampled in most pediatric patients. With the development of proteomic technology, investigation of tumor related signals at the time of diagnosis through treatment, and then in remission and/or at the time of recurrence or progression is possible.

While CSF for seeding tumors is readily available and routinely obtained for cytology, the systematic evaluation of the proteins within these samples could be of considerable scientific importance. In addition to identifying potential makers of disease or response to therapy, the glycosylation and phosphorylation status of many proteins can also be evaluated. Studies in tumor tissue have show that such information reveals activity of different enzymes that correlate with either treatment response (Mellinghoff, 2005; Helgi, 2005) or progression of leptomeningeal metastases (Brandsma, 2006).

Proteomics

CSF proteomics has been applied to many neurological disorders including Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, acute brain injury and Creutzfeldt-Jakob disease (Rohlff, 2001). Reports of its use in neuro-oncology are limited, but demonstrate the potential of this technology to effectively identify tumor biomarkers. One study used two dimensional polyacrylamide (2-D) gel electrophoresis to measure the relative quantities of two pre-selected markers, N-Myc and l-CaD, in the CSF of brain tumor patients (Zheng, 2003). Another used ELISA of CSF to identify Osteopontin as predictive of AT/RT and correlated with response to therapy (Kao, 2005). CSF proteomics using 2-D gel electrophoresis in combination with mass spectroscopy and cleavable isotope Coded Affinity Tag (cICAT) was used to evaluate 60 samples of CSF and tumor cyst fluid taken from adults with brain tumors and non-neoplastic controls. These techniques were used to find a panel of proteins differentially expressed in lower vs. higher-grade gliomas. Findings were confirmed using Western Blot analysis probing for eight selected proteins based on implied role in gliomagenesis and availability of antibodies. This report, which has been accepted for publication pending revisions, identified 21 potential CSF biomarkers for astrocytoma.

As mentioned above, there is evidence that gliomas disseminate through the subarachnoid space. The presence of leptomeningeal dissemination of glial tumors is significant because it demonstrates that glial tumor cells have access to the CSF space, and thus, proteins secreted from these tumors have the ability to access the CSF space and may be detected by CSF proteomics. Currently there are several consortia actively studying protein expression in the spinal fluid of children with malignant glial and embryonal tumors (Pediatric Brain Tumor Consortium and Pediatric Oncology Experimental Therapeutics Consortium). Proteins interrogated in these protocols include those involved with angiogenesis and neovascularity (EGF, VEGF and bFGF), those involved in tumor growth and migration (secreted protein with acidic and cysteine rich domains (SPARC), attractin). There is no consortium actively collecting spinal fluid sample in children with lower grade tumors. A secondary goal of this study is to examine these proteins in the CSF of children with low grade gliomas who have tumor progression. Comparison of CSF protein expression of high grade and low grade tumors is likely to help identify biological markers specific for tumor progression, or for tumor pathology.

2.1 Pre-clinical studies:

Polyinosinic-Polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) (Levy, 1985) 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. These trials gave mixed results with moderate toxicity, and the use of poly-ICLC was generally abandoned when recombinant interferons became available (Levy, 1987); (Rettenmaier, 1986); (Krown, 1985); (Theriault, 1986); (Droller, 1987); (Hawkins, 1985); (Nakamura, et al 1982). 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 in the past 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 (Black, 1992). Low dose poly-ICLC also has a direct immune enhancing action independent of IFN, including increased antibody response to antigen, and NK cell, T-cell, macrophage and cytokine activation. While discussion of the complex immunostimulatory effects of poly-ICLC and the interferons is beyond the scope of this protocol, their role in the potential anti-neoplastic effect that has been seen needs further investigation.

Recent evidence suggests that polyIC 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) (Chebath, 1992), (Samuel, 1992), (Katze, 1992) (Williams, 1999). 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 RibonucleaseL; as well as for the PKR, which inhibits initiation of protein synthesis (Levy, 1992); (Talmadge, 1985); (Levy, 1988); (Ewel, 1992); (Black, 1992). Both, OAS and PKR are very sensitive to dsRNA dose and structure (Minks, West et al, 1979). 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 (M. Kende, unpublished). 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 (Maluish, 1985) (M. Kende, unpublished). 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) (Salazar, 1996). Similarly, the PKR has both high and low affinity binding sites and is inhibited by too high a dose of dsRNA (Galabru, 1989). 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 (Koromilas, 1992). 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 (Malkin, 1990). 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 (Cuddihy, 1999); (Williams, 1999). Folkman et al reported that the interferons exert antiangiogenic activity against the tumor vasculature supporting a potential antiangiogenic effect of poly-ICLC (Folkman, 1992). 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 the 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.

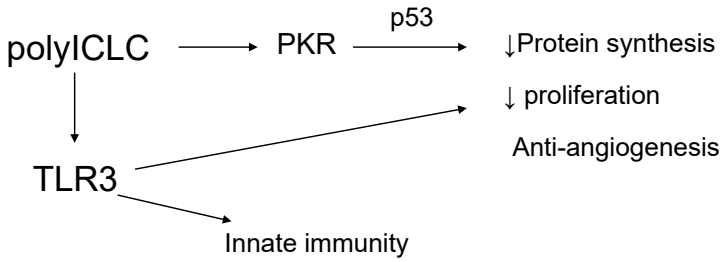

Figure 1. This schematic shows the molecular basis for polyICLC action on signaling pathways in glioma or stromal cells, the inhibition of protein synthesis, angiogenesis and growth arrest. PolyICLC via TLR3 also induces innate immunity.

2.2 Previous clinical trials:

A pilot trial in adults suggested a possible beneficial effect of poly-ICLC in newly diagnosed and recurrent brain tumors (Salazar et al, 1996). Thirty-eight patients with glioblastoma multiforme (GBM), anaplastic astrocytoma (AA), or recurrent GBM/AA were treated with varying doses of poly-ICLC of 10-50 mcg/kg IM, one to three times per week for approximately 56 months. The drug was exceptionally 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 median progression-free survival 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 nevertheless 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 Figure 2). One hundred percent of patients who received the poly-ICLC regimen were alive at 2 years versus only 76% who received standard chemotherapy (Salazar et al, 1996). This supports the effectiveness and likely less complications of poly-ICLC over chemotherapy.

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%

Figure 2. 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 patients with recurrent glioblastoma. This study included 55 patients, where 11% had radiographic response, 6 month progression-free survival (PFS) was 24% and median survival was 43 weeks. Poly-ICLC was well tolerated but did not show improvement on 6 month PFS. The second trial included patients newly diagnosed with glioblastoma. The treatment schema included a combination of radiation with poly-ICLC. 30 patients were enrolled in the trial. The 6-month progression-free survival was 30% and the estimated 1-year progression-free survival was 5%. Median time to progression was 18 weeks. The 1-year survival was 69% and the median survival 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 third trial is a combination study of poly-ICLC with temodar chemotherapy, and is being conducted by the NIH's NABTT consortium.

A Phase I/II study of poly-ICLC was conducted by the Principal investigator (Donald L. Durden), in children with multiple types of newly diagnosed or recurrent brain tumors. Long-term responses were seen in 2 of 4 patients with low-grade gliomas (unpublished data). Four children with progressive low grade gliomas treated on this pilot study were evaluated for response. These four children had stable disease after documented progression for 10, 12, 22 and 24 months while receiving poly-ICLC. One of these children suffered from reduced white count (grade 3) that resolved despite continued therapy, and another developed respiratory distress at the time of tumor progression. The other cohorts of recurrent high-grade gliomas and newly diagnosed brain stem gliomas showed minimal to no response. All eight children enrolled due to growing diffuse intrinsic pontine gliomas had progression of disease in <8 months of therapy. Eleven children enrolled on the study had progressive high grade gliomas (anaplastic astrocytoma or glioblastoma multiforme). Ten demonstrated disease progression within 1-10 months of initiation treatment, however, one child on therapy 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 neurofibromatosis 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 low-grade gliomas. However more children with low-grade gliomas need to be studied to confirm this impression.

Phase I/II data for use of poly-ICLC has also been reported in patients with renal cell carcinoma, refractory lymphomas, melanomas, and colorectal cancers (Giantonio et al, 2001, Ewel et al, 1992). Varying doses of poly-ICLC from 0.01 mg/m^2 up to 1 mg/m^2 were used either IV or IM 2 times a week. In all of these studies, poly-ICLC was well tolerated. Grade 1 or 2 fatigue, fever, nausea, vomiting and pain at injection site were seen in some patients and usually resolved by 24 hours after the injection (Ewel, 1992). In another study fever was a common side effect in 25.6% of patients, and chills in 23.1% (Giantonio, 2001). In both studies stable disease and a partial response was seen in some patients: however no complete remissions were observed. Thus it appears that poly-ICLC has been used in several types of cancers in varying doses and varying modes of administration, and is well tolerated: however best results were seen in patients with brain tumors, at doses of 20-30 micrograms/kg given 2-3 times a week as IM injections.

Based on preliminary data showing efficacy and tolerability of poly-ICLC in brain tumors, we propose to study this drug further, in children with progressive or recurrent low-grade gliomas, for clinical efficacy and tolerability. We would also simultaneously like to conduct laboratory experiments to determine the patient's cellular response to poly-ICLC, by evaluating the induction of PKR, the phosphorylation of eIF2α, and the activation of p53 specific target gene, p21^{waf-1} in an attempt to better understand the anti-tumor effects of this drug.

2.3 Drug Information and Toxicity:

Drug Information:

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 eight times that of the parent compound, and it induces peak levels of about 1000-2000 IU of interferon per ml of serum in monkeys given 1 mg/kg intravenously.

The current study will be done under IND #43984, held by Oncovir. The drug to be used in this study is prepared and packaged in the GMP facility of Bioserv, Inc, San Diego, under the direction and under contract to Oncovir. It is then tested for identity (RNAase resistance and thermal denaturation) bioactivity in primates, sterility and pyrogenicity before release for clinical experimental use.

Toxicity:

The degree of adverse effects of poly-ICLC is dependent on three factors: (1) dose, (2) route of injection, and (3) health status of the patient. Early Phase I studies were done to determine the maximum tolerated dose (MTD) under the assumption that this was also the most effective dose. In these studies of cancer patients, it was found that the MTD was 12 mg/m2 IV in 25 adult and pediatric patients (Levine and Levy, 1978). Patients typically showed fevers of 40^{o}C, myalgia, arthralgia, malaise, nausea and vomiting, and mild elevations of liver enzymes. Fever was the primary dose-limiting factor. At this dose, the mean serum interferon level was 2000 IU/ml. While this level is rarely attained by giving exogenous interferon, levels of 100 IU/ml in response to exogenous interferon are associated with the same types and degree of adverse effects as seen with high dose poly-ICLC. Children who were terminally ill with leukemia could not tolerate 12 mg/m^2 IV, although healthier children could (Leventhal et al, 1981). In most of the early cancer trials, however, about 6 mg/m2 poly-ICLC IV was generally used. Mild (grade 1), transient (7 days) hepatic enzyme elevations were described in a trial of 100 mcg/kg poly-ICLC given intravenously in multiple sclerosis patients. In three patients this was prolonged >7 days, but in all patients the enzymes returned to normal values after temporary discontinuation of the poly-ICLC. One paralyzed multiple sclerosis patient in this group suffered a fatal pulmonary embolus which was not judged to be due to the drug. Poly-ICLC has been associated with coagulopathy in dogs, but not in other species (including primates); and there

has been no increase 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.

Low dose poly-ICLC:

It was subsequently shown that low doses were better than higher ones for enhancing immune effects, and that the higher dose actually inhibited a number of cell-associated immune functions. It was also found that intramuscular injection brought about a much milder set of side effects. The most common complaint is a mild, transient discomfort at the injection site. On occasion, 8-12 hours after doses of 10 to 50 mcg/kg IM, patients may also develop a mild flu like syndrome with fever of less than 38°C which may last for another 12 hours, but responds readily to acetaminophen or aspirin. Mild myalgia, arthralgia, sometimes nausea, and malaise are present during this period of time. This flu-like syndrome typically diminishes markedly after the first little poly-ICLC treatment. Hepatic enzyme elevations are not typically seen at these dosages. Several multiple sclerosis or malignant glioma patients at Walter Reed Army Medical Center (WRAMC) have been maintained on regular intramuscular poly-ICLC for as long as 12 years without significant adverse effects. On very rare occasions in the course of treatment patients who have been tolerating treatments uneventfully may develop a more pronounced fever with chills and malaise (typical of higher dose IV poly-ICLC) in response to a single IM injection on subsequent dosing. This will typically respond to aspirin or acetaminophen and does not recur on repeated dosing. Whether it represented inadvertent IV injection on those occasions is uncertain. In a previous pediatric study conducted by the Principal Investigator, there was no dose limiting toxicity seen over 3 years in 45 children at doses of 20-30 micrograms/kg given 2 times a week as intra-muscular injection.

Summary of Poly-ICLC Toxicity data reported to FDA, 2005:

In the two recent, ongoing multi-center NIH consortium studies in adult glioblastoma patients and in patients with multiple-recurrent anaplastic glioma, most patients (N=62) reported at least one adverse event. The majority of adverse events were classified as either grade 1 (63%) or grade 2 toxicity (28%). The reported grade 3 and grade 4 toxicities were 8% and 0.8% respectively. Only two grade 5 events were reported, which were not assessed to be related to the study drug. The most frequently reported events (toxicities) were fatigue, pain, and myalgia. Very transient elevated liver enzymes, mainly grade 1 or 2, were also reported in fourteen patients.

However, combining both trials, there were only eighteen toxicity grade 2 or higher adverse events that were definitely or probably ascribed to the study drug by the local study physician. The seventeen grade 2 and one grade 3 events occurred in only nine patients and had a median duration of five days. Two patients had six grade 2 hematologic reports with a median duration of five days. Animal studies suggest that a major portion of this leucopenia does not reflect myelotoxicity, but is related to leukocyte trafficking, with transient migration of WBC to lymphoid organs, including spleen.

3.0 Patient Eligibility Criteria and Registration

3.1 Patient Registration

This is a multi-institution study between UCSD/Rady Children's Hospital and Emory University/Children's Healthcare of Atlanta. UCSD/Rady Children's Hospital will be the coordinating site for this investigator initiated trial.

Eligible patients will be registered by contacting the Clinical Research Coordinator, Monday through Friday, 8:00 am-5:00 pm Pacific Standard Time by phone except on holidays. Calls will be returned within one business day. The research coordinator will review preliminary eligibility by phone before forwarding a registration packet to the

enrolling practitioner. The information requested in the registration packet must be completed. The signed informed consent statement along with the documentation confirming eligibility must be faxed. Upon receipt of the completed registration packet, the research coordinator will assign a unique patient registration number and study subject number. **Patients must be registered prior to starting any protocol therapy.**

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Fax: (858) 966-8966

Important note: **The eligibility criteria listed below are interpreted literally and cannot be waived.** Patients must begin treatment within two weeks of study registration. Registration will be completed only after pathologic diagnosis is confirmed by central review (please see below).

Between the two sites, we anticipate enrolling up to 10-15 subjects per year, to achieve a total of 24 evaluable subjects. Thus we are confident that accrual can be completed within 2-3 years. The estimation is reflected by the number of patients with recurrent low grade glioma tumors that are treated annually at Rady Children's Hospital and Children's Healthcare of Atlanta.

3.2 Inclusion Criteria

3.2.1 Age

Subjects must be between 0 – 21 years of age when enrolled on this protocol.

3.2.2 Diagnosis

Subjects must have pathologically confirmed low grade glioma with histologic subtypes interpreted as WHO grade I and II including:
-juvenile pilocytic astrocytoma (JPA)
-pleomorphic JPA
-diffuse astrocytoma (fibrillary, gemistocytic, giant cell, or pleomorphic xanthoastrocytoma)
-subependymal giant cell astrocytoma (SEGA)
-low grade oligoastrocytoma
-low grade oligodendroglioma
-pilomyxoid astrocytoma
-low grade glioma NOS

Tumors of all regions of the CNS, with appropriate histology are eligible for study. However patients with SEGA or tumors intrinsic to the optic nerve and involvement of the optic nerve that cannot be biopsied/resected are eligible without histological confirmation.

Subjects with neurofibromatosis type 1(NF1) are also eligible (see appendix II for NF1 diagnostic criteria).

All pathologic diagnoses of subjects from all enrolling sites will be confirmed with tissue block review of previously obtained specimens by Scott VandenBerg, M.D., Ph.D. This will be done in order to assure

uniformity in the above subtypes for these sometimes difficult to diagnose tumors. Once consent is obtained, subjects' tissue/slides and corresponding pathology report should be forwarded for central review at UCSD. These samples should be sent to:

Scott R. VandenBerg, M.D., Ph.D.
Professor of Pathology
Director of Neuropathology
University of California, San Diego
Pathology, MC 0612
BSB Room 2008
9500 Gilman Drive
La Jolla, CA 92093-0612
Phone: 858-822-2808

3.2.3 Criteria

Subjects must have demonstrated either tumor progression or recurrence by any of the radiographic criteria and/or clinical criteria as defined below:

a. Subjects with progressive non-resectable disease regardless of location in the brain or spine are eligible for this study. Patients with evidence of leptomeningeal dissemination are eligible for this study. Patients do not require biopsy/histologic confirmation at the time of progression or relapse.

b. Radiographic progression is defined as >40% increase in the product of the three perpendicular diameters of current tumor relative to the baseline measurement (defined as either the initial scan or scan at start of a previous therapy):
length (L) x width (W) x transverse (T) (current scan) > 1.4 x L x W x T (baseline scan), or the development of any new sites of disease independent of the response of the initial tumor.
See section 7.1.2 for methodology for tumor measurement.

c. Post radiation changes are often seen on post-treatment imaging studies, so that classification of a patient as having progressive disease may require several serial MRI's if the child has received radiation within the preceding 12 months.

d. Tumor volume includes the entire tumor volume seen on gadolinium enhanced T1 MR imaging plus non-enhancing abnormality seen on T2 or FLAIR. Coronal and axial images will be evaluated.

e. All tumor cysts will be included in the tumor volume

f. Clinical progression without radiographic progression includes children with optic pathway gliomas who demonstrate sustained decrease in visual fields and/or acuity in three serial vision examinations. Each of the vision examinations must be performed >2 weeks apart.

g. Children with previously negative cerebrospinal fluid (CSF) cytology who show evidence of tumor cells in fluid obtained by lumbar puncture can be designated as having progressive disease in the absence of radiographic evidence of progression.

3.2.4 Performance Level and Life Expectancy

Subjects must have a performance status of > 50% (Appendix I). Use Karnofsky for patients > 16 years of age and Lansky for patients ≤ 16 years of age. Subjects must have a life expectancy of ≥ 8 weeks.

3.2.5 Prior Therapy

Subjects must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study and meet time restrictions from end of prior therapy as stated below:

a. Myelosuppressive chemotherapy: Subjects must have received the last dose of myelosuppresive therapy at least 3 weeks prior to study registration or at least 6 weeks if therapy included nitrosourea.
b. Investigational / Biological agent: Subjects must have received the last dose of other investigational or biological agent >7 days prior to study registration. Subjects must not have received polyICLC in the past.
c. XRT: Subjects must be ≥ 8 weeks since the completion of radiation therapy.
d. Study specific limitations on prior therapy: There is no limit on the number of prior treatment regimens or received doses of radiation therapy.
e. Growth factor(s): Subjects must not have received any hematopoietic growth factors within 7 days of study entry or 21 days for pegfilgastrim.
f. Prior Surgery: Subjects must be ≥ 2 weeks from prior surgery.
g. Steroids: Subjects must be on a stable steroid dose for 7 days prior to study entry if they are on steroid treatment.

3.2.6 Organ Function Requirements

All subjects must have adequate organ function defined below when starting on protocol.

3.2.6.1 Hematologic Function:

a. Hemoglobin: $\geq$ 8.0 gm/dl (may transfuse PRBCs)
b. ANC: $\geq$ 750/mm^3, Must be at least 7days after last dose of growth factor
c. Platelet Count: $\geq$ 50,000 (transfusion independent; ≥ 7 days from last transfusion)

3.2.6.2. Renal Function defined as:

Creatinine clearance/GFR > 70 cc/min/1.73 m^2 or

Serum creatinine based on age/gender as follows:

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

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

3.2.6.3 Liver Function:

a. Total bilirubin ≤ 1.5 x ULN for age, AND
b. SGPT (ALT) < 2.5x ULN
c. SGOT (AST) < 2.5x ULN

3.2.6.4 Pulmonary Function:

No evidence of dyspnea at rest, no exercise intolerance, and a pulse oximetry ≥ 94% if there is clinical indication for determination.

3.2.6.5 Coagulation Function:

Normal PT and PTT at enrollment per institutional range

3.2.6.6 Reproductive Function:

Due to potential teratogenic effects of (poly-ICLC), negative serum beta-HCG in females, and agreement to the use of effective contraception in males and females of childbearing potential, **IS REQUIRED**.

3.3 Exclusion Criteria

3.3.1. Pregnant or lactating females. Women of childbearing age will agree to use contraception during the protocol.

3.3.2 Patients receiving other experimental immunotherapy.

3.3.3 Patients may not have fever (38.5^0C) within 7 days of enrollment.

3.3.4 No concurrent XRT or chemotherapy is allowed.

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

3.4 Regulatory

3.4.1 Informed Consent

All subjects and/or their parents or legal guardians must sign a written informed consent document. Written assent will be obtained from patients per institutional guidelines.

3.4.2 Protocol Approval

All institutional, FDA, and NCI requirements for human studies must be met including institutional IRB approval of the protocol.

4.0 Study Treatment

4.1 Treatment Schedule

Children will receive poly-ICLC 20 mcg/kg/dose twice weekly IM (using Monday/Thursday or Tuesday/Friday schedule if possible). The first 2 doses will be administered in the clinic under supervision.

Vital signs will be monitored every 30 minutes for 2 hours after the first dose. For all subsequent doses administered at home, a temperature log will be kept to document the temperature prior to study drug administration and at two separate time points after each of the 8 doses of study drug in cycle 1. One temperature reading will be assessed between hours 2-4 after study drug administration and one temperature reading will be assessed at 12 hours after study drug administration. After cycle 1, temperature will only be monitored on an as needed basis, such as if the subject is not feeling well. Subjects or their parents will be instructed to use a log to record the temperature monitored at home and any fever should be reported to the study team. In some cases, at the discretion of the research team, subject or parent will be allowed, after appropriate teaching, to administer the study drug at home. The study drug will be dispensed to the subject/parent by the research pharmacy. The study drug will be dispensed in vials along with labeled dose syringes in order to minimize errors in dosing.

Total length of treatment will be two years. Each cycle will be 28 days in length. If subjects demonstrate a response to therapy (see section 7.0), treatment may be continued beyond two years at the discretion of the principal investigator and pending drug availability. For statistical analysis of toxicity, subjects will be divided into two separate cohorts (those with NF1 and those without NF1). The subjects in each of these cohorts will be treated and monitored identically throughout treatment.

Subjects who travel to the coordinating or enrolling sites for protocol therapy may return home to the care of their primary pediatric oncologist after completing cycle 1 of therapy for standard of care management at the discretion of the study physician/committee. Subjects will be seen and monitored by their primary treating oncologist at the start of each cycle. For those subjects, a 3 month supply of study drug will be dispensed to the subject/family from their enrolling site. The enrolling site will continue to see the subject every 3 months for evaluation and will remain responsible for continued data capture of all required information.

4.2: Dose modifications based on Toxicity

If AST/ALT > 10x ULN while on study, Poly ICLC will be discontinued until the AST/ALT are < 5x ULN; the study drug will then be restarted at 20 mcg/kg/dose twice weekly (the initial dose).
If AST/ALT > 10x ULN for a second time, hold study drug until values < 2.5x ULN; then restart drug at the reduced dose of 10 mcg/kg/dose twice weekly.
Study drug will be stopped and subject will be off treatment for toxicity if AST/ALT do not decrease to < 5x ULN during the first episode and <2.5x ULN at second episode when drug is held for three weeks.

For the NF 1 cohort, consider restarting drug again at 5 mcg/kg/dose twice weekly if toxicities recur at the prior reduced dose of 10mcg/kg/dose.

For all other grade 3 or grade 4 toxicities the same dose modifications will be followed. The study drug will be held until resolution of these toxicities to ≤ grade 1. If the toxicity is not resolved within 3 weeks of the last dose of the study drug administration the subject will be taken off protocol therapy.

4.3: Concomitant Therapy

Corticosteroids and anticonvulsants may be given as indicated clinically. However, if the dose of steroids is increased while on therapy, MRI must be performed to rule out disease progression. Acetaminophen may be given as needed to control poly-ICLC side effects. A non-P450 inducing anticonvulsant will be used while on this study (see Appendix 3).

Radiotherapy may not be given while on study to the lesions being utilized as measurable disease in order to assess response. Patients may enter on study after completion of involved field radiation therapy, however at least 8 weeks should elapse after radiation, and scans should show residual disease prior to entry on study. Chemotherapy is not allowed.

Antibiotics, blood products and general supportive care measures may be used as clinically indicated. Appropriate antibiotics, blood products, antiemetics, fluids, electrolytes and general supportive care are to be used as necessary and as per institutional guidelines. 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.

5.0 Required Observations

All Pre-study Observations must be done within 14 days of study entry.

Observation	Pre-Study	Day 1 of Cycle 1	Day 15 of Cycle 1	Prior to subsequent cycles 2-12	Prior to subsequent cycles 13-24	End of therapy/Off Study
History, VS, Physical	x	x	x	x	x	x
Performance Status[1]	x	x	x	x	x	x
Toxicity Evaluation			x	x	x	x
Neuro Evaluation	x	x	x	x	x	x
Concomitant Meds	x	x	x	x	x	x
CBCD, Platelets	x	x	x	x	x	x
PT/PTT	x	x	x	x†	x†	x†
CMP w/Phos	x	x	x	x	x	x
Urinalysis[3]	x					
Serum Pregnancy[3]	x					
MRI-Brain/Spine**	x			Every 3 cycles starting with end of Cycle 3↑	End of Cycle 18 and 24 only↑	x
LP/CSF cytology*	x			Every 3 cycles starting with end of Cycle 3[2]	End of Cycle 18 and 24 only[2]	x[2]
Biology-red & purple top blood samples		x	x			
Data Collection Submission[4]	x			x	x	x

1:Performance Status=Karnofsky if > 16 y/o or Lansky if ≤ 16 y/o
2: repeat only if cytopathology showed tumor cells at study entry or disease progression
3: Repeat as needed for good clinical care
4: Data Collection should be submitted monthly
†: Repeat PT/PTT only if abnormal after Day 15
↑ Repeat MRI Spine only if not normal pre study

***** Lumbar Puncture/CSF cytology is not required if already done within 4 weeks of study entry.
CSF will be obtained prior to initiation of therapy with Poly ICLC. If positive, CSF will be obtained at 3, 6, 9, 12, 18, 24 months after study entry, as well as the time end of therapy/off study.

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. MRI will be assessed at 3, 6, 9, 12, 18, 24 months after starting the therapy (at the end of those cycles), as well as at the time end of therapy/off study or if subject is going off study prematurely.
******MRI spine does not have to be repeated during the study if it was normal pre-study. It should be repeated at the time end of therapy/off study or if subject is going off study prematurely.
A copy of the MRI images on a CD should be sent to the coordinating site.

5.1: Pretreatment Evaluation (must be performed within 14 days of study entry)

A. History, vital signs and physical examination, including neurologic evaluation and concomitant medication list
B. CBC, differential, platelet count, PT/PTT
C. AST, ALT, alkaline phosphatase, albumin, calcium, phosphorus, total bilirubin
D. BUN, Cr, urinalysis
E. Disease evaluation with either MRI of brain/spine and CSF cytology. (LP is not required if already done within 4 weeks of study entry).
F. Serum B-HCG in females of childbearing age
G. Lansky Play Scale assessment for patients ≤ 16 years of age; Karnofsky score for patients >16 years old. (see Appendix I).
H. Study entry form/eligibility check list to be filled out prior to starting treatment.

5.2: Evaluation during treatment

A. Disease evaluation (including MRI), if positive at study entry, every 3 cycles for the first 12 cycles (to be done at the end of the cycle), then every 6 cycles for as long as the subject remains on study. MRI spine does not have to be repeated if it was normal pre-study. Disease status and events form is to be filled every 3 months for the first 12 months and then every 6 months while subject is on study.
B. History, vital signs, physical examination and concomitant medication list at day 1 and day 15 of cycle 1, and then prior to each cycle (monthly for as long as the patient remains on study).
C. Labs are to be obtained at day 1 and day 15 of cycle 1, and then prior to each cycle (monthly for as long as the subject remains on study).
   - CBC/differential, platelets
   - Chemistry panel including: AST, ALT, alkaline phosphatase, albumin, calcium, phosphorus, total bilirubin, BUN, Cr
   - PT/PTT at Cycle 1 Day 1 and 15, repeat further only if abnormal
D. Neurologic assessment at each visit with scoring of overall exam compared to previous exam as follows: No change from previous exam; worsening from previous exam; improvement from previous exam.
E. Record at each visit if steroids are being given and if dose is same, lower, higher than at previous visit. MRI required if steroid dose increased.
F. Toxicity monitoring form and Lansky Play Scale assessment for subjects ≤ 16 years of age; Karnofsky score for subjects >16 years old. (see Appendix I) at week 2 and then prior to each cycle.

5.3: Evaluation at the end of therapy or relapsed disease

A. Disease evaluation (including MRI brain and spine) and lumbar puncture for CSF cytology (required only at time of disease progression) with all studies that were initially positive at study entry

B. History, vital signs, physical examination, and concomitant medication list

C. Labs:

- CBC/differential, platelets
- Chemistry panel including AST, ALT, alkaline phosphatase, albumin, calcium, phosphorus, total bilirubin, BUN, Cr
- PT/PTT, if still abnormal

D. Neurologic assessment with scoring of overall exam compared to previous exam as follows: No change from previous exam; worsening from previous exam; improvement from previous exam.

E. Record if steroids are being given and if dose is same, lower, or higher than at previous visit.

F. Toxicity monitoring form and Lansky Play Scale assessment for patients ≤ 16 years of age; Karnofsky score for patients >16 years old. (see Appendix I).

G. If patient death occurs, fill out death report form.

5.4: Special laboratory specimens

A. Biologic correlates

1. Blood Specimens

Obtain 5ml of peripheral blood in a red top tube at study entry and at day 15 prior to the scheduled dose of the study drug. Separate serum and freeze at -70C in four equal aliquots. Label each specimen with subject's research identifier and date drawn. These specimens will be sent to the lab identified on the next page.

Obtain 10ml of peripheral blood in purple top tube, at study entry and at day 15 prior to the scheduled dose of the study drug. Label each specimen with subject's research identifier and date drawn. These specimens will be sent to Dr. Donald L. Durden's laboratory for isolation of peripheral blood mononuclear cells (PBMC). Samples will be shipped to the address listed below.

Serum OAS and interferon α, β, γ levels will be measured by standard ELISA assays. Peripheral blood mononuclear cells (PBMC) will be isolated by ficoll-hypaque density gradient separation and stored as pellets at –80 degrees C. Levels of PKR kinase, phosphorylation state of eIF2α, and the induction of $p21^{waf\text{-}1}$ will be determined in cell lysates by standard in vitro kinase and Western blot methods using phospho-specific antibodies as described by Williams (1999). This evaluation will provide a surrogate marker for sensitivity of patient's cells to known capacity of poly-ICLC to activate this serine/threonine kinase and to activate the p53 tumor suppressor gene activity.

Total RNA for RNA sequencing or microarray analysis of PBMCs before and after treatment with poly-ICLC will be collected to determine differences in gene expression induced by poly-ICLC as index of somatic response to this agent. This will be compared to patient's clinical response to poly-ICLC.

Peripheral mononuclear cells will also be studied by Fluorescence Activated Cell Sorting to establish an immunophenotypic profile for each sample pre and post treatment with poly-ICLC. Regulatory T cells (Tregs), dendritic cells, mononuclear cells, macrophages, B/T cell subtypes, and pro-anti-inflammatory cytokines can also be analyzed. This will also be tested to determine if these markers can serve as feasible additional biomarkers of disease and response.

2. CSF Specimens (optional)

CSF will be obtained prior to initiation of therapy with Poly ICLC. CSF will be obtained via lumbar puncture as part of clinical staging prior to study enrollment, if not already done within 4 weeks of study entry. CSF cytology will be evaluated for presence of tumor cells by an institutional pathologist using standard methods. Approximately 3 ml of CSF will be requested for proteomic analysis. The CSF will be spun at 400-500 g for 10 minutes to remove cellular contaminants. The first 2 ml of CSF supernatant will be aliquoted into four .500 ml units and frozen at -20 to -80 degrees Celsius to be used for proteomic analysis. Each tube must be labeled with the subject's research identifier, the sample type and the time it was drawn.

The remaining 1 ml of CSF will be aliquoted in 0.2 ml units and frozen at -20 to -80 degrees Celsius to be evaluated for cytokines. Each tube must be labeled with the subject's research identifier, the sample type, site from where the sample was obtained, and the time it was drawn.

All blood and CSF biologic samples should be sent on dry ice, along with a completed biologic specimen transmittal form to Dr. Donald Durden's laboratory located at the Moores Cancer Center at UCSD. Enrolling practitioners should contact Donald Durden at 858-534-3355 to notify him of specimens ready for shipment or pickup and delivery:

Donald L. Durden, MD PhD
Moores UCSD Cancer Center RM 3312
3855 Health Sciences Drive #0819
University of California, San Diego
La Jolla, CA 92093-0819
ddurden@ucsd.edu
858-534-3355

Samples will only be <u>received</u> Monday through Thursday.

B. Laboratory analysis

1. Proteomic Biomarker Evaluation

Criteria for a successful proteomic methodology for identification of a usable biomarker include high throughput, reproducibility and toleration of real world collection and shipping conditions. In addition, such methods must be able to identify differentially expressed proteins in an unsupervised manner as well as provide information about specific proteins of interest. Currently, there is not a single mature technology available to meet all these criteria. Therefore, a combined approach will be employed in this exploratory study to attempt to fit all of these characteristics and improve the body of knowledge about the utility of these strategies.

All these approaches will require concentration of the samples using Centricon 3kD filtration and partial depletion of IgG and albumin (Proteoprep Blue Albumin Depletion kit). Proteins can be precipitated using 15% trichloracetic acid, re-suspended in denaturing buffer and stored at -20 to -80 degrees Celsius until the time of analysis.

The first component of the proteomic approach will employ 2-D gel electrophoresis to separated proteins in two dimensions according to their size and charge. The gels are then scanned and relative positions and intensities are quantitated by pixel reading software. Individual spots are normalized to total signal and proteins that are found to be differentially expressed can be identified by in-gel digestion and MALDI-TOF-TOF mass spectrometry [Clin Chem 2005; 51:2031-2042].

The second approach to employ is LC-MS/MS in combination with stable isotope labeling. The entire sample is subjected to mass spectrometry after trypsin digestion. Individual proteins can be quantitated and identified from the peptides present in the digestion reaction. The protein complements contained in CSF from different disease states can then be compared.

The third component is low mass fingerprinting. Peptides are isolated from the CSF by ultra-filtration. These peptides are interrogated by MALDI-TOF-TOF mass spectrometry and profiles of peaks are generated. Peptide profiles are analyzed by bioinformatics software after signal normalization and informative peaks that distinguish between known disease states are identified. Combinations of informative peaks are compiled and tested against “unknowns” to validate their predictive power.

2. ELISA for confirmation/correlation of target proteins

CSF samples will be evaluated for expression of specific secreted proteins implicated in tumor progression, migration and response to therapy. These target proteins, described above, have well-characterized, commercially available antibodies including VEGF, dFGF, attractin, SPARC and EGF.

3. Interrogation of spinal fluid samples will be performed after investigators have obtained extramural funding to pay for the studies. If compelling evidence for biomarkers other than those described about becomes available as proteomics studies are completed in CSF of children with malignant tumors, inspection for these markers will be performed.

5.5: Pathology specimens (optional)

If the subject has available fixed, frozen and/or cryopreserved tumor tissue from a prior tumor resection upon study entry it will be evaluated for genomics and other biomarkers by study pathologist Scott VandenBerg. Samples should be shipped or picked up and delivered Monday-Friday to:

Scott R. VandenBerg, M.D., Ph.D.
Professor of Pathology, Director of Neuropathology
University of California, San Diego
9500 Gilman Drive, MC 0612
La Jolla, CA 92093-0612
Phone: 858-822-2808

Additional Consideration
If a patient relapses or second look surgery and/or lumbar puncture is required while the subject is on study, the tissue and/or CSF may be obtained for additional analysis post treatment with Poly ICLC.

6.0 DRUG INFORMATION

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° C, about 40° 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 done under IND #43984, held by Oncovir. The drug to be used in this study is prepared and packaged in the GMP facility of Bioserv, Inc, San Diego, under the direction and under contract to Oncovir. It is then tested for identity (RNAase resistance and thermal denaturation) bioactivity in primates, sterility and pyrogenicity before release for clinical experimental use.

FORMULATION: Supplied in 1 ml vials of 2 mg/ml opalescent solution

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.

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.

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

The drug will be obtained from Oncovir.

To obtain drug, call:

Andres M Salazar, MD
Oncovir, Inc
3203 Cleveland Ave, NW
Washington, DC, 20008
Tel: 202-342-1726
Fax: 202-248-2324
Email: asalazar@oncovir.com; asalazar@starpower.net

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. A copy of these records will be kept in the study binder at the coordinating site.

7.0 RESPONSE CRITERIA

MRI Response Criteria: Comparison of objective assessments are based on major changes in tumor size compared to the baseline scan. Determination of progressive disease is based on tumor measurements described below.

Interpretation of anatomic (MRI) response rates in gliomas can be difficult. Tumors may be non-enhancing, and certain area of enhancement may represent post-operative or radiation change. Shrinkage of enhancing lesions can also be seen for months after radiation alone. These factors may confound the MRI interpretation of tumor response to poly-ICLC. Also in the Principal investigator's previous experience, tumor regression or stabilization on MRI was seen at 12 and 52 months in some patients on poly ICLC. Hence in the absence of clearly progressive disease (worsening neurological status, significant increase in tumor size on MRI), poly-ICLC should be continued for possible late benefits.

7.1 EVALUATION CRITERIA

7.1.1 Methodology to Determine Tumor Measurement

All measureable tumors will be measured at each evaluation and will be used in the response assessment.

Measurable Disease: Bi-dimensionally measurable lesions with clearly defined margins by MRI or CT scan.

Evaluable Disease: Uni-dimensionally measurable lesions, masses with margins not clearly defined.

Non-Evaluable Disease: Not Applicable for response evaluation

For all measurable tumors, the 3 dimensional measurements: width (W), transverse (T), and length (L) measurements need to be recorded (if length L can be measured), using either T1 or T2 MR weighted images (which ever gives the best estimate of tumor size). The following section describes the methodology.

1. Longest diameter of the tumor should be selected in the axial plane or the plane in which the tumor is best seen or measured, provided the same plane is used in follow ups.
2. The longest measurement of the tumor (or width, W) should be determined.
3. The 2 perpendicular measurements should be determined (transverse (T) measurement-perpendicular to the width in the selected plane, and the length (L) – tumor extent in the plane perpendicular to the selected plane).

The primary tumor response rate endpoint will be based on the 2 dimensional measurements (TxW) as in modified MacDonald criteria (section 7.1.2), which, in addition to radiographic scans, comprise elements of the steroid use. The response rate based on the 3 dimensional measurements (TxWxL) will be a secondary endpoint (section 7.1.3).

All lesions are to be measured at each evaluation; however, if there are too many measurable lesions to measure at each evaluation (i.e. more than 10), choose the largest two to be followed before a subject is entered on study. The remaining lesions will be considered evaluable for the purpose of objective status determination.

7.1.2 **Tumor Response Criteria on 2 Dimensional Measurements (per Modified MacDonald Criteria)**

a. Complete Response (CR): Complete disappearance of all measurable and evaluable disease. There are no new lesions and no evidence of non-evaluable disease. All measurable, evaluable and non-evaluable lesions and sites must be assessed using the same techniques as baseline. Patients must not be on any steroids.

b. Partial Response (PR): ≥ 50% decrease over baseline in the sum of products of perpendicular diameters of all measurable lesions. There is no progression of evaluable disease and no new lesions. All measurable and evaluable lesions and sites must be assessed using the same techniques as baseline. The steroid dose at the time of the scan should be no greater than the maximum dose during the first 8 weeks since initiation of therapy.

c. Stable Disease (SD): Does not qualify for CR, PR, or progression. All measurable and evaluable sites must be assessed using the same techniques as baseline. The steroid dose at the time of the scan should be no greater than the maximum dose used during the first 8 weeks since initiation of therapy.

d. Progressive Disease (PD): ≥ 25% increase in the sum of products of all measurable lesions over baseline using the same techniques as baseline, **or** clear worsening of any evaluable disease, **or** appearance of any new lesion/site, **or** clear clinical worsening **or** failure to return for evaluation due to death or deteriorating condition (unless clearly unrelated to this cancer).

7.1.3 Tumor Response Criteria Based on 3 Dimensional Measurements

a. Complete Response (CR): Disappearance of all measurable and evaluable tumors. There are no new lesions and no evidence of non-evaluable disease.

b. Partial Response (PR): > 65% decrease in the sum of the products of the three perpendicular diameters of the measurable tumors relative to the sum of products of initial baseline measurements - LxWxT (current scan) < 0.65 * LxWxT (initial scan). There is no progression of evaluable disease and no new lesions.

c. Stable disease (SD): Neither sufficient decrease in the product of the three perpendicular diameters to qualify for partial response nor sufficient increase to qualify for progressive disease.

d. Progressive Disease (PD): >25% increase in the sum of products of the three perpendicular diameters of the measurable tumors relative to the initial baseline measurement - LxWxT (current scan) > 1.25* LxWxT (initial

scan) **or** the development of any new sites of disease. As post-radiation changes are often seen on post-treatment imaging studies, classification of a patient as having progressive disease may require several serial MRIs.

7.1.4 Response Assessment

Tumor response will be assessed at 3, 6, 9, 12, 18, 24 months after starting the therapy, as well as at the time of going off study if prematurely. The overall response rate (ORR), defined as the percent of evaluable subjects who experience a complete response or partial response or stable disease, will be determined. The primary endpoint will be progression free survival rate (per MacDonald Criteria) at 6 months after starting therapy. Secondary endpoints also include tumor ORR at 6 months and 2 years after starting therapy and also the duration of the 'good' response. The traditional overall response rate (CR's and PR's only) will also be summarized.

Each subject will be classified according to their "best response" for the purposes of analysis of treatment effect. Best response is determined from the sequence of the objective statuses described above.

Any subject removed from study protocol because of unacceptable progression (see Section 8.1) will be counted as progressive disease in the efficacy analysis.

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a. Progressive disease

> **Note:** Transient enlargement of enhancing disease with subsequent shrinkage has been reported during poly-ICLC treatment. Because of this, if the patient has progressive disease by the conventional definition above but does not have unacceptable progression by the definitions below, the treating physician and patient have the options of continuing poly-ICLC treatment on this protocol or of discontinuing treatment.

b. Criteria for unacceptable progression: See definitions of tumor progression outlined in section 7.1.3

1. >50% increase in the product of the three perpendicular diameters of initial tumor relative to the initial baseline measurement – LxWxT (current can)> 1.5 x LxWxT (initial scan), or the development of any new sites of disease independent of the response of the initial tumor. Post radiation changes are often seen on post-treatment imaging studies, so that classification of a patient as having progressive disease may require several serial MRI's if the child has received radiation within the preceding 12 months.

2. Unacceptable worsening of neurological symptoms that cannot be controlled with corticosteroids.

3. Any other radiological or clinical evidence of worsening to the extent that the treating physician feels it is not in the patient's best interest to continue poly-ICLC.

c. Physician determines it is in patient's best interest.

d. Patient and/or parent/guardian refusal to continue or inability to comply with protocol

e. Grade 3 or 4 toxicity that does not resolve to Grade 1 within three weeks after interruption of therapy, or recurrent grade 3 or 4 toxicity at a reduced dose level (see section 4.2).

Note: If final analysis confirms a beneficial effect of poly-ICLC, and/or if tumor recurs after withdrawal from poly-ICLC at the end of the 2-year prescribed treatment period, participating subjects may be offered continued treatment with poly-ICLC (subject to drug availability at that time).

f. Completion of planned therapy.

Subjects who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Follow-up data will be required, unless consent is withdrawn, every 3 months for the first year, every 4 months for the second year, every 6 months for the third year, and then yearly thereafter

8.2 Off Study Criteria

a. Death.
b. Lost to follow-up.
c. Entry into another therapeutic study.
d. Withdrawal of consent for any further data submission.

9.0 STATISTICAL CONSIDERATIONS

a. The primary goal of this trial is to obtain preliminary estimates of the ability of poly-ICLC to prevent or delay disease progression in subjects with recurrent or newly diagnosed low-grade gliomas. The primary endpoint will be progression free survival rate at 6 months (PFS6) after starting therapy. Progression free survival is defined as the time from starting study therapy to tumor progression, tumor recurrence, death from any cause, or occurrence of a second malignant neoplasm. Tumor assessment for the primary endpoint will be based on modified McDonald's criteria (section 7.1.2). Tumor assessment results obtained based on the 3D measurements will be considered secondary. The overall response rate (ORR) at 6 months will be a secondary endpoint. The ORR will include complete response, partial response, and stable disease (see Section 7.1.4). Time to progression will be documented for all patients who progress.

b. The second goal is to further define the side effects and toxicity of this regimen in these subjects. Secondary endpoints also include tumor ORR at 2 years after starting therapy and also the duration of the good response. Tumor response will be assessed at 3, 6, 9, 12, 18, 24 months after starting the therapy, as well as at the time of going off study if prematurely. Other secondary endpoints also include tumor response rates based on 3 dimension measurements as in section 7.1.3 at 3, 6, 9, 12, 18, 24 months after starting the therapy, progression free survival and overall survival over the course of the study.

c. The third goal will be to examine a series of biochemical determinations (e.g. serum OAS and interferon levels, PKR, phosphorylation state of eIF2a, $p21^{waf-1}$) as surrogate markers for sensitivity of patients to poly-ICLC. These will be compared to clinical response data. A subset of the proteins involved in apoptosis and signaling pathways will be looked into specifically to assess the effect of the study treatment on these signaling pathway changes. The specific endpoints are:

- Association of baseline levels of proteins (such as OAS, interferon, PKR, phosphorylation state of eIF2a, $p21^{waf-1}$) in serum, PBMC and/or cerebrospinal fluid with tumor response status at 6 months after treatment with Poly-ICLC.
- Association of baseline levels of proteins (such as OAS, interferon, PKR, phosphorylation state of eIF2a, $p21^{waf-1}$) in serum, PBMC and/or cerebrospinal fluid with progression free survival.
- Association of changes in levels of proteins (such as OAS, interferon, PKR, phosphorylation state of eIF2a, $p21^{waf-1}$) serum, PBMC and/or cerebrospinal fluid from baseline with tumor response status at 6 months after treatment with Poly-ICLC.
- Association of changes in levels of proteins (such as OAS, interferon, PKR, phosphorylation state of eIF2a, $p21^{waf-1}$) in serum, PBMC and/or cerebrospinal fluid from baseline with progression free survival.

9.1: Study Design and Justification of Sample size

9.1.1 This is a single arm study, to determine the radiographic progression free survival (PFS) rate to poly-ICLC in the treatment of children with recurrent or progressive low grade gliomas. The primary endpoint is the progression free survival status at 6 months (PFS6) after starting therapy. The tumor response categories will include complete response, partial response, stable disease or progressive disease, as assessed according to section 7.1.2.

Simon's optimal two-stage design will be used for this study (Simon, 1989). Suppose 50% is the uninteresting level, i.e., if the PFS6 rate is ≤ 50%, we will consider the new regimen as not promising. This 50% baseline response rate in the absence of treatment is from historical experience (this rate was also used by the Everolimus study which is a phase II study for recurrent or progressive LLG in children), also taking into account that this study patient population are more refractory. We will test the null hypothesis H0: p≤50% against the alternative hypothesis H1: p>75%, where p is the PFS6 rate. The two stage design proposed below will have 80% power to reject the null and conclude that the true response rate is above 50%, if the true response rate is ≥75%, at 5% significance level.

The study design can be described in detail as follows:

Stage 1: Fourteen subjects will be accrued; accrual will be held up until the PFS6 results for all the 14 subjects are known. The trial will be terminated at Stage 1 if ≤7 subjects have a PFS6 response; otherwise it continues to Stage 2. Note that any subject who goes off study early due to toxicity, will be counted as a non-response (an intent to treat analysis).

Stage 2: Nine more patients will be accrued. We will reject the therapy if among all the 23 (14+9) subjects, the number of patients who have a defined response is ≤15.

Early stopping probability: Under this design, if the null hypothesis is true, the probability of stopping the trial early will be 61%.

9.1.2 Interim analysis and early stopping for efficacy:

The interim analysis will be conducted as soon as the first 14 patients have accrued and are evaluable for the primary outcome. If ≤7 subjects had a defined response, the trial will be stopped early for lack of efficacy.

9.1.3 Early stopping for safety and feasibility

The study will also be stopped any time for safety. If 3 or more subjects have a grade 4 or 1 subject has grade 5 toxicity probably or definitely treatment related toxicity while on therapy or within 3 months off the therapy, the trial will be stopped early for lack of safety.

Interim Monitoring for Feasibility
Primary early monitoring for feasibility will be based on the short-term feasibility endpoint that at least 60% of doses are administered to the subject, as assessed at 16 weeks. Less than 60% of doses administered for any reason constitutes an unacceptable level of dosing. An acceptable feasible outcome would be the successful administration of the study therapy to 80% or more of subjects at 16 weeks. Treatment compliance will be assessed in each cohort (children with or without NF1) when 4 patients have been accrued and completed the first 16 weeks of treatment. Failure to administer $\geq$ 60% of doses in this interval in three out of the four patients will result in suspension of accrual to that cohort.

9.1.4 Final analysis for the primary endpoint
If the study continues, after all 23 subjects are evaluable for efficacy and safety the study data set will be locked. Out of the total of 23 subjects, if the number of subjects with PFS6 is 16 or more, the conclusion will be that the trial has demonstrated a statistically significant improvement above a 50% PFS6 rate. The final estimated PFS6 rate will also be presented, along with a lower 90% confidence interval.

9.1.5 Definition of study population for analysis
All subjects who undergo at least one dose of the study treatment will be included in the analysis population. If applicable, “Intention-to-treat” analyses will be performed for an endpoint. If the endpoints for a subject cannot be assessed (for example, a subject drops early prior to the 6 months assessments due to toxicity), it will be considered to be an event that does not favor the study therapy (e.g., disease progression for the primary outcome).

9.1.6 Accrual and study duration
All the 23 patients are expected to be accrued in 3 years and the total study duration will be about 5 to 5.5 years.

9.2: Analysis of Results

9.2.1: Analysis of anti-tumor activity
The "best response" rate is defined as the percent of evaluable subjects who experience a CR or PR or SD (see Section 7.1.2). The best response rates as well as overall response rates at 6 and 24 months will be estimated and exact lower one sided confidence intervals for the true response rates will be presented (Jung, 2004). The duration of response for each subject that has tumor response will be summarized in a table. The same analysis will be done for the tumor response rate based on 3 dimension measurements as in section 7.1.3.

9.2.2: Summary of toxicities and side effects
All observed toxicities and side effects will be recorded on toxicity sheets and the data collection forms. Toxicity information including type, severity, time of onset and resolution, and probable association with the study drugs will be recorded. Toxicities will also be analyzed by NF1 status (NF1 vs. non NF1 subject cohorts). Tables will be constructed to summarize the observed incidence by type of toxicity and severity.

9.2.3: Analysis of Survival
Overall survival and progression-free survival rates will be estimated using the Kaplan-Meier method (Kaplan, 1958).

9.2.4: Analysis of biochemical measures.
Laboratory studies will be done on subject's serum and peripheral blood drawn prior to and at two weeks after starting treatment with Poly ICLC. Levels and changes in the measurement of serum markers and proteins such as OAS, interferon α, β, γ, PKR kinase, eIF2a, and $p21^{waf-1}$ will be determined via proteomic analysis. For each marker, a Fisher's exact test or a Wilcoxon rank sum test will be used to assess the association of a baseline protein level or changes from baseline against tumor response status at 6 months. A log rank test will be used if the outcome is progression free survival.
All the p-values will be adjusted using the Benjamini & Hochberg method to control the false discovery rate (FDR) due to the fact that there may be thousands of proteins produced by proteomics. A marker will be claimed significant at FDR≤ 0.05. To assess the effect of the study treatment on signaling pathways such as the one controlling apoptosis, paired t-tests will be used to compare if there are significant changes from baseline for the proteins involved in these pathways. To assess differential effects on these pathways between the subjects who had a response and those who did not, gene set enrichment analysis (GSEA) can be performed to see if a pathway is significantly up- or down-regulated in the patients with a response. These analyses will help to understand how poly-ICLC limits tumor growth and ultimately to identify patients who will benefit most from this form of therapy if it is effective.

10.0: ADVERSE REACTION REPORTING AND TOXICITY CRITERIA

The timely reporting of adverse drug reactions (including toxic deaths) is required by the Food and Drug Administration. Reactions definitely felt not to be treatment related should not be reported. However, a report should be submitted if there is any suspicion of drug effect or in the case of Serious Adverse Events. The reporting of adverse reactions is in addition to and does not supplant the reporting of toxicities as part of the data reporting for this study.

Serious Adverse Events (SAEs) Timely reporting of all serious adverse events or drug reactions (including toxic deaths) is required by the Food and Drug Administration, regardless of attribution. The reporting of serious adverse reactions is in addition to and does not supplant the reporting of toxicities as part of the data reporting for this study.

Adverse event is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable or definite).

A Serious Adverse event is any adverse event, occurring at any dose that is fatal or life-threatening, results in persistent or significant disability/incapacity, requires in-patient hospitalization or prolongation of existing hospitalization is a congenital anomaly/birth defect, or is an important medical event.

Relationship/attribution: The Investigator will be asked to document his/her opinion of the relationship of the event to study medication as follows:

1) *Unrelated*: The adverse event is clearly not related to the investigational agent(s).
2) *Unlikely*: The adverse event is doubtfully related to the investigational agent(s).

3) *Possible*: The adverse event may be related to the investigational agent(s).

4) *Probable*: The adverse event is most likely related to the investigational agent(s).

5) *Definite*: The adverse event is clearly related to the investigational agent(s).

10.1: Reporting Adverse Drug Reactions Occurring with Investigational Agents

Within 24 hours of recognition, contact the Principal Investigator or his designee by telephone to verify the identification process and the existence of an adverse drug reaction or toxic death.

The following data **must** be reported to the study PI:

- Subject identification number
- Presenting diagnosis, Description of event
- Laboratory data supporting event
- Total and daily doses of suspected drug
- Time of event
- Type and grade of adverse event
- Methods used to recognize and characterize the effect
- Physician attribution/relationship of event to study drug (see above)

The participating site must then submit a completed adverse event case report form to the coordinating site.

Within 10 days of the adverse event, a completed NCI Adverse Reaction Form for Investigational Agents with the NCI protocol number should be submitted to the IRB for life threatening (Grade 4) and fatal known reactions (except Grade 4 myelosuppression) or any unexpected, unanticipated Grade 2 and 3 reactions that are possibly, probably, or definitely attributed to the study drug.

A completed (NCI) Adverse Reaction Form for Investigational Agents should be sent electronically or by FAX to the FDA (see Appendix IV for the electronic links). Oncovir will be notified as soon as an adverse event form has been submitted.

10.2: Toxicity Criteria.

The NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be used and referenced. The site, measure, grade, relationship, action taken, and outcome for all toxicities (grades 3 and 4 as well as any unexpected, unanticipated Grade 2 reactions that are possibly, probably, or definitely attributed to the study drug) are to be reported on the appropriate toxicity monitoring and adverse event forms.

10.3: Data Safety Monitoring

10.3.1 Data and Safety Monitoring Board

This study will be monitored in accordance with the UCSD/Rady Children's Hospital plan for data and safety monitoring of Phase 1 and 2 studies. This study will use the UCSD Moores Cancer Center DSMB. In brief, the role of the Data and Safety Monitoring Board is to protect the interests of patients and the scientific integrity for all Phase 1 and 2 studies. The DSMB consists of 7 members including a chair, a statistician, and a pharmacist. The DSMB meets on a regular basis to review current study results, as well as data available to the DSMB

from other related studies. The DSMB will provide recommendations for each study reviewed to change the study or to continue the study unchanged. Data and Safety Board reports for institutional review boards will be prepared.

10.3.2: Study Monitoring
The Principal Investigator will monitor the study regularly to validate subjects' eligibility, evaluability, and any toxicities that are entered in the study database. In addition, the study committee comprised of the coordinating site members and at least 2 members of the participating site via phone conference will discuss and review once a month the enrolled subjects, toxicities, and integrity of data collection.

Monitoring of Source Documents:
The participating site will submit case report forms and copies of necessary supporting clinical source documents to the coordinating site for review of data accuracy prior to data submission. This will occur at a monthly interval from the time of subject's enrollment into the study.

Additionally a member of the DSMB at the coordinating site will monitor the data collection and the source documentation of all subjects enrolled at the enrolling sites. This monitoring will review protocol compliance, drug accountability, and adverse event reporting as well as regulatory compliance annually.

10.4: FDA Annual Reporting

Oncovir, the IND holder of the study drug, will submit an annual report to the FDA. This report will include de-indentified aggregate data from this study as well as compiled NCI adverse event reports.

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APPENDIX I

PERFORMANCE STATUS SCALES/SCORES

PERFORMANCE STATUS CRITERIA

Karnofsky and Lansky performance scores are intended to be multiples of 10

ECOG (Zubrod)		Karnofsky		Lansky*	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	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.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	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.
2	Ambulatory and capable of all self-care but unable to carry out about more than 50% of waking hours	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.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	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.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	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.

**The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.*

APPENDIX II: Criteria for Type 1 Neurofibromatosis:

- Clinical diagnosis requires the presence of at least 2 of 7 criteria to confirm the presence of NF1. Many of these signs do not appear until later childhood or adolescence, and thus confirming the diagnosis often is delayed despite a suspicion of NF1. The 7 clinical criteria used to diagnose NF1 are as follows:
    - Six or more café-au-lait spots or hyperpigmented macules greater than or equal to 5 mm in diameter in children younger than 10 years and to 15 mm in adults
    - Axillary or inguinal freckles
    - Two or more typical neurofibromas or one plexiform neurofibroma
    - Optic nerve glioma
    - Two or more iris hamartomas (Lisch nodules), often identified only through slit-lamp examination by an ophthalmologist
    - Sphenoid dysplasia or typical long-bone abnormalities such as pseudarthrosis
    - First-degree relative (eg, mother, father, sister, brother) with NF1

http://www.emedicine.com/neuro/topic248.htm

APPENDIX III: List of Anticonvulsants Based on CYP3A/4/5 Enzyme Induction

Anticonvulsant drugs with little or no enzyme induction:
ELIGIBLE for concomitant use in study.

Generic Name	*Trade Name*
Gabapentin	Neurontin
Lacosamide	Vimpat
Lamotrigine	Lamictal
Levetiracetam	Keppra
Tigabine	Gabitril
Topiramate	Topamax
Valproic Acid	Depakote, Depakene
Zonisamide	Zonegran

Enzyme inducting anticonvulsant drugs (EIACD):
NOT ELIGIBLE for concomitant use in study.

Generic Name	*Trade Name*
Carbamazepine	Tegretol
Felbamate	Felbatol
Phenobarbital	Phenobarbital
Phenytoin	Dilantin
Primidone	Mysoline
Oxcarbazepine	Trileptal
Rufinamide	Banzel

APPENDIX IV: MED Watch Links

Use the following links to download the MED Watch forms and instructions for adverse event reporting to the NCI.

http://www.fda.gov/downloads/Safety/MedWatch/HowToReport/DownloadForms/ucm082728.pdf

http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm -