

Phase 1 Study of Intratumoral Poly-ICLC plus Low Dose Local Radiation in Low Grade Recurrent B -and T-Cell Lymphoma

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CLL Topics

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CO-INVESTIGATORS:

PROTOCOL SCHEMA

An accessible site of disease (lymph node, cutaneous, subcutaneous, etc.) will be selected by the principal investigator. Patients will then receive two doses of low dose irradiation (2 Gy per day) to that single site on days 1 and 2. Six patients will begin treatment at 0.25 mg of poly-ICLC with dose escalation for each group of six patients at 0.5 mg, 1 mg, and 2 mg maximum dose if the maximum tolerated dose has not been reached. Intratumorally or peritumorally Poly-ICLC will be dosed on days 3 and 4 and will be performed by the physician.

Day 1&2	Day 3	Day 4	Wk 2	Wk 3	Wk 4	Wk 8
Local XRT 2 GY/day	PICLC	PICLC	PICLC X 2	PICLC X 2	PICLC X 2	PICLC X 2

1.0 OBJECTIVES

The primary objective of this study is to evaluate the safety of intratumoral Polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) (Hiltonol®) in addition to low-dose local radiotherapy for adult patients with low grade lymphomas, including follicular lymphoma, marginal zone lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, and cutaneous T-cell lymphoma.

The secondary endpoints are response rate, immune responses, and durability of responses as well as generation of antiinflammatory response at sites of tumor involvement.

2.0 BACKGROUND AND RATIONALE

Current Status of Treatment of Patients with Low-Grade T- and B-Cell

Lymphomas

Over the past 10 years, the treatment of low-grade B-cell lymphomas including marginal cell lymphomas, follicular lymphomas, and small lymphocytic lymphoma/chronic lymphocytic leukemia has seen promising agents introduced into the clinic including rituximab, an antibody targeting the CD20 antigen, alemtuzumab, an antibody targeting CD52, radio immunotherapeutics (Bexxar and Zevalin), and nucleosides such as pentostatin, cladribine, and fludarabine. Despite the high incidence of complete clinical responses with combinations of chemotherapy typically combined with rituximab, there is no evidence to date that any of these treatments cure these diseases. Newer agents for the treatment of mycosis fungoides/cutaneous T-cell lymphoma include denileukin diftitox, alemtuzumab, and Suberoylanilide Hydroxamic Acid (SAHA). Similar to B-cell lymphoma, while there are numerous effective therapies for mycosis fungoides, there are no curative therapies. Following recurrence of disease with further chemotherapy or biological therapies, disease-free intervals tend to be shorter with each treatment.

Stem cell transplantation with either autologous or allogeneic stem cells is another therapeutic option. Allogeneic stem cell transplantation is extremely toxic and most patients are not eligible. Autologous transplantation is widely used for follicular lymphoma patients but with high dose chemotherapy and exposure to previous alkylating agents and anthracyclines there exists a higher incidence of myelodysplastic syndrome and acute myelogenous leukemia. Relatively nontoxic options, such as immunotherapies with vaccines are being explored in a variety of clinical settings.

Hypothesis

Local low-dose irradiation of lymphomas followed by intratumoral and then intermittent subcutaneous injection of Poly-ICLC will induce an efficient anti-tumor immune response through antigen cross presentation and priming by:

- 1) inducing tumor necrosis and antigen release

- 2) activating and maturing local dendritic cells to cross present tumor antigens
- 3) Priming the resulting antigen-specific cytotoxic T-cells to recognize those tumor antigens as 'non-self,' and
- 4) Enhancing the longevity and targeting of antigen-specific memory T cells.

Poly-ICLC Background

General Description

Polyinosinic-Polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC, also known as Hiltonol®), is a double-stranded RNA (dsRNA) which was used as an interferon inducer at high doses (up to 300 mcg/kg IV) in short-term cancer trials some years ago. [1] This gave mixed results with moderate toxicity, and the use of poly-ICLC was generally abandoned when recombinant interferons became available. However, lower dose (10 to 50 mcg/kg) poly-ICLC results in a broader host defense stimulation, including Dendritic cell (TLR3), T-cell and natural killer cell activation, cytokine release (interferons alpha, beta, and gamma, interleukins, corticosteroids, and TNF), a potent adjuvant effect, and a specific antiproliferative and antiviral effect mediated by the 2'5'oligoadenylate synthetase (OAS) and P68 protein kinase (PKR) enzyme systems. Poly-IC also preferentially decreases tumor protein synthesis *in vivo*.

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 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 1,000-2,000 IU of interferon per mL of serum in monkeys given 1 mg/kg intravenously.

Mechanisms of Action

While initially developed as an interferon inducer, poly-ICLC also has much broader biological effects in man, including specific antiviral and antitumor actions. One pilot trial has suggested a beneficial effect in high-grade gliomas, possibly on the basis of a tumorigenic action. [2] There are several closely interrelated mechanisms of action of poly-ICLC that, alone or in combination could explain these clinical findings to date:

Interferon and cytokine induction: Induction of interferons was classically considered as the primary mechanism of action of exogenously administered dsRNAs such as poly-ICLC. However, interferons alone do not appear to be sufficient treatment for many viral and neoplastic conditions, perhaps because of the multiple evasive mechanisms that have evolved in viruses and tumors. Nevertheless, we now recognized that the 'natural mix' of interferons plus various other cytokines and chemokines induced by poly-ICLC may be one of the important mechanisms for its broad immunomodulatory and antiviral actions described below. These include not only type I and II interferons, but also TNF α , TGF β , IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, IP-10, MCP-1, and RANTES (CCL-5). Cytokine induction can be very rapid, peaking as early as 1 to 4 hours after ip injection in mice, and most will generally return to baseline within 24-48 hours. [3]

Immune modulation: Low dose Poly-ICLC also has a direct immune enhancing action that can be relatively independent of IFN, including, T-cell and natural killer cell activation,

dendritic cell activation, cytokine and chemokine release (e.g. interferons alpha, beta, and gamma, interleukins, corticosteroids, and TNF), and a potent adjuvant effect with increased antibody response to antigen that appears to be mediated by activation of dendritic cells through TLR3.

Hiltonol and Innate Immunity

The potent elements of the non-specific innate immune response and their complex interaction with the development of specific adaptive immunity are receiving increased attention. Classically, attention was focused on the interferons as being central to these defenses, and poly-ICLC was initially considered to be primarily an interferon inducer. The interferon system itself is the target of multiple viral and neoplastic evasive mechanisms, in one way confirming its critical role in innate immunity, but subsequent studies have demonstrated multiple actions of the dsRNAs that are relatively independent of interferons. [4], [5] Perhaps largely because of these evasive mechanisms, interferons alone are not sufficient treatment for many viral infections or neoplasms. One important action of dsRNAs is a more direct antiviral and antineoplastic effect mediated by the interferon-inducible, dsRNA dependent 2'5'oligoadenylate synthetase (OAS) and P68 protein kinase (PKR) enzyme systems; [4]. DsRNA induces an antiviral and antiproliferative 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 and mediates apoptosis through the p53 system and NFkB among other actions. [6] PKR is very sensitive to dsRNA dose and structure, and is also inhibited by a number of viruses, including Ebola, poxvirus, and influenza, as detailed above. This inhibition can be overcome in certain cases by addition of exogenous poly-IC. [4] The inhibition of glioma cells by poly-IC and by interferon beta is significantly associated with activation of both the OAS and PKR. Others have demonstrated that expression of a functionally defective mutant of the PKR results in malignant transformation in vitro, suggesting an important role for this enzyme in suppression of tumors.²⁶⁻²⁸ Both PKR and poly-IC are now known to regulate the p53 tumor suppressor system, which induces tumor cell apoptosis. P53 is in turn associated with the multiple malignancy Li-Fraumeni syndrome, which includes astrocytomas, sarcomas, lung, and breast cancers. Mediation of antitumor action by OAS and/or PKR activation could help further explain why the high doses of Poly-ICLC used in early cancer trials were relatively ineffective.

Another important signaling pathway of the innate immune response involves recognition of dsRNA by various cell surface pattern recognition receptors (PRR), among the most important of which now appear to be the Toll-like receptors (TLR). The Toll-like receptors (TLRs) play essential roles in the initiation of innate immunity. In mammals, the TLR family is composed of at least 11–12 members and each TLR acts as a primary sensor of conserved microbial components and drives the induction of specific biological responses. It is well established that peptidoglycan, lipopolysaccharide and flagellin are recognized by TLR2, TLR4 and TLR5, respectively. It has also been shown that the TLR3 is involved in the recognition of viral components, such as poly-IC and double-stranded (ds)RNA. In contrast, TLR7 and TLR8 appear to recognize ssRNA viruses and ssRNA. Furthermore, TLR9, which recognizes DNA unmethylated at CpG motifs, and TLR3 both appear to be involved in host defense against mouse cytomegalovirus infection. Thus the complex signaling cascade in the TLR system leads to altered expression of various interferons, cytokines, chemokines and other costimulatory molecules and transcription factors such as NFkB, cJun, IRF3, and ATF2. The TLR

system is thus currently seen as one of the critical links between innate and adaptive immunity. While some of the signaling pathways are shared, the differential activation of TLRs appears to trigger host responses that are specialized to address particular pathogen subtypes, including neoplasms. TLR-3 are found intracellularly in the early endosome of myeloid dendritic cells, which induce a Th1 or cellular immune response that is especially adapted to many viruses as well as some cancers.

Hiltonol and Adaptive Immunity

Vaccines have been used not only to prevent disease, but also in a therapeutic mode to enhance the body's defenses against existing viral or neoplastic disease. While most cancer vaccines have generally been designed to utilize one or more tumor antigens, an alternative strategy is 'autovaccination' or the use of the tumor itself as the antigen source. This strategy has included the judicious combination of chemotherapy with immunostimulants to induce maximal cross presentation and priming [7]. A variation of that approach is proposed in the present protocol, whereby tumor necrosis is induced in the tumor with low dose local radiation in order to induce cross presentation, and then an immune-priming signal is added with intratumoral injection of poly-ICLC. [8] [9]

However, it has also become apparent that many of the mechanisms that have evolved to protect the body from disease are themselves subject to inhibition by various pathogens. For example, dendritic cells are recognized to play a critical early role in immunity by processing and presenting foreign antigens to T-cells and other immune cells. Yet dendritic cells are themselves the target of inhibition by a variety of neoplastic and viral factors. It now appears that some of these effects, including Ebola, prostate cancer or influenza-induced anomalous DC maturation, can also be mitigated with poly-IC and poly-ICLC [10], [11] (Shurin, unpublished). UPMC and Oncovir are currently conducting a collaborative vaccine adjuvant study to further confirm this effect in advanced prostate cancer patients and further evaluate poly-ICLC's potential as a cancer vaccine adjuvant. Thus, DC that have been non-specifically "primed" or disinhibited through activation with a TLR agonist alone such as Hiltonol may be much more responsive to antigen and more efficient mediators of both cell mediated and humoral immunity. In vivo, this process may involve not only the direct effect of Hiltonol on DC TLRs, but also its concomitant induction of interferon and other cytokines and its induction of MHC molecules.

In addition to dendritic cell activation and maturation via Toll-like receptor 3, poly-ICLC's immune modulating action includes natural killer and T-cell activation, cytokine release (e.g. interferons alpha, beta, and gamma, interleukins, cytokines, chemokines, corticosteroids, and TNF), and a potent adjuvant effect with increased antibody response to antigen. [12]. For example, administration of low doses of Poly-ICLC along with subunit swine flu vaccination in monkeys dramatically accelerates and increases development of HAI titers. [13]. The adaptive immune response is critically influenced by the combination of pro-inflammatory genes that are activated, and the "natural" mix of cytokines and chemokines induced by Poly-ICLC may be especially relevant in this regard. On the other hand, preliminary laboratory results of a pilot study of Poly-ICLC in brain tumor patients showed no clear relationship between tumor response and measurable serum interferon, TNF, IL2, IL6, or neutering, suggesting that other mechanisms are involved in certain of Poly-ICLC's antiproliferative actions. [2]

An important element of the immune response is triggered when pattern recognition receptors (PRR) such as toll-like receptors (TLR), come in contact with PAMPS (pathogen-associated molecular patterns) such as dsRNAs that are recognized as foreign to the body. TLRs are increasingly being recognized as a one of various important links between innate and adaptive immunity. TLR ligands such as poly-ICLC initiate a cascade effect that alters gene expression in DC and other cells. Efficient priming of the adaptive immune system requires not only the presentation of antigen by DC in the context of the MHC (major histocompatibility complex) but also the induction of accessory signals (costimulators and cytokines) on antigen-presenting cells (APC). TLRs expressed on APCs such as dendritic cells have the potential to regulate some of these accessory signals through their recognition of dsRNAs, and consequently control activation of the antigen-specific adaptive immune system. There are a number of different TLRs that respond to different PAMPS. Poly-IC and poly-ICLC activate TLR-3 which are found in the early endosome of myeloid DC, respiratory epithelium and astrocytes [14] [15]. Poly-ICLC and liposomal poly-ICLC thus preferentially activate myeloid dendritic cells through TLR-3, favoring a cytotoxic T-cell response that appears critical to clearance of established virus. TLR3, but not TLR4 is induced in respiratory epithelium in response to influenza infection both in vitro and in vivo, and may be critical to the outcome of the infection. [16] [17]

Mature DC are among the most potent APC and activators of CTLs. [18], [19, 20] However the maturation process itself requires at least 24 hours and involves various cytokines, including IL-15, TNF and interferons. [21]; [22],[23] Thus, DC that have been non-specifically “primed” through activation with a TLR agonist alone such as Poly-ICLC prior to antigen exposure may be much more responsive to antigen and more efficient mediators of both cell mediated and humoral immunity.

Ichinohe and colleagues have demonstrated marked enhancement of antibody response and antiviral protection when they combined nasal influenza vaccine with nasal poly-IC. [17] Perhaps more importantly, there was also a marked enhancement of cross-strain protection, possibly related to an enhanced T cell response. This pronounced adjuvant effect was accompanied by rapid activation of the iNALT as manifested by induction of TLR3 expression. Perhaps significantly, the nasal Poly-IC/vaccine combination induced peak TLR3 induction in vivo at about 6 hours, whereas natural infection peaked at 72 hours and vaccine alone had no effect. Recently, Poly I: C and Poly-ICLC have also been demonstrated to have a marked adjuvant effect in combination with either peptide based or dendritic cell glioma and cancer vaccine. [24] [3] In vivo, this process may involve not only the direct effect of Poly-ICLC on DC TLRs, but also its concomitant induction of NK cells, interferon and other cytokines and its induction of MHC molecules. [25]

The exact interplay between dsRNA, interferon and these IFN inducible systems is not totally elucidated, but the role of dsRNAs such as Poly-ICLC may be bimodal: beginning with induction of interferons and expression of OAS, PKR, TLR3, RIG I, mda-5 and likely others; and followed by their (catalytic) activation by the dsRNA. In vivo, double dosing with Poly-ICLC at a 24-48 hour interval, or pretreatment with IFN can markedly boost activity. [26] Coincidentally this is the successful dosing regimen that we have used in our clinical glioma studies. [2] From a practical point of view in this regard,

‘slow release’, stabilized dsRNAs such as poly-ICLC or liposome-encapsulated poly-ICLC may thus offer an added therapeutic advantage

Clinical gene regulation

This is a fourth mechanism by which Poly-ICLC can independently modify the biologic response and provide therapeutic benefit. Poly-IC has been shown to independently up-regulate or down-regulate a broad variety of over 270 genes in cultures of glioma cells lacking the interferon response gene. [5] Some of these genes play critical roles in the body’s natural defenses against a variety of tumors and infections, and in controlling other cell functions, including protein synthesis, programmed (apoptotic) cell death, cell metabolism, cellular growth, the cytoskeleton and the extracellular matrix. The therapeutic implications of these actions are considerable, but have yet to be fully understood.

Safety and Tolerance of poly-ICLC

The severity of adverse effects of poly-ICLC depends 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 about 12 mg/m² IV in patients who were not terminally ill. Patients typically showed fevers of 40°C, myalgia, arthralgia, malaise, and some nausea and vomiting. Fever was the primary dose-limiting factor. At this dose, the mean serum interferon level was 2000 IU/mL. While exogenous interferon rarely attains this level, levels of 100 IU/mL after exogenous interferon are associated with the same types and degree of adverse effects as high dose poly-ICLC. In most of the early cancer trials, however, about 6 mg/m² poly-ICLC IV was generally used. It was subsequently shown that a low dose of poly-ICLC (<1mg/ m²) was better than a high dose 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 much milder side effects. [2] More specific side effects of poly-ICLC are listed below.

Discomfort at injection site:

The most common adverse effect is mild, transient discomfort at the site of intramuscular injection.

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 38°C, which may last for another 12 hours, but responds readily to acetaminophen or aspirin. Mild myalgias, arthralgias, sometimes nausea, and malaise are present during this period of time. This flu-like syndrome typically diminishes markedly after the first few poly-ICLC treatments. On very rare occasions in the course of treatment, patients who have been tolerating treatment 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. This will

typically resolve over 12 to 24 hours, responds to acetaminophen, and does not recur on subsequent dosing.

Hematologic:

Several cases of transient leukopenia have been reported. Poly-ICLC was restarted after a drug holiday in most cases, but leukopenia recurred in only one, with rapid resolution within two days after discontinuation of drug for the second time. 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 suffered a fatal pulmonary embolus which was not judged to be due to the drug.

Hepatic enzyme elevation:

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 after temporary discontinuation of the poly-ICLC. Enzyme elevation was not typically seen with doses of 10 to 50 mcg/kg three times weekly. However, one patient receiving 20 mcg/kg three times per week had to be dropped from study because of a transient enzyme elevation that persisted slightly longer than the 4-week protocol cutoff. In addition, preclinical studies have shown suppression of the p450 hepatic enzyme system by poly-ICLC, as well as by interferon, but the clinical implications of this finding are not clear.³⁶

Seizures:

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

Transient peritumoral edema:

In a pilot brain tumor trial, a few patients showed an increase in their gadolinium enhancing lesions after 3-6 months of poly-ICLC, followed by an apparent tumor response at 6-12 months and prolonged survival on continued treatment. Decadron was used as needed in first few months of treatment on that study. In a more recent follow-up open study in patients with advanced recurrent gliomas, several have shown an 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. These findings 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.

Summary of Toxicity data reported to FDA.

In a recent, ongoing multi-center study in glioblastoma patients, 21 of the 24 subjects (88%) reported at least one adverse event. No adverse events were reported at the toxicity grade 5. The incidence of adverse events was reported by the worst grade for an event for an individual subject. The majority of adverse events were classified as

either grade 1 (71 of 104 or 68%) or grade 2 (28 of 104 or 27%) toxicity. There were only 3 of 104 (3%) and 2 of 104 (2%) events reported as a grade 3 or grade 4 event, respectively. The most frequently reported events (toxicities) were fatigue (15 subjects), pain-other (10 subjects), and myalgia (9 subjects). In the most recent 2005 reports, only 57 out of 380 events were definitely or probably ascribed to Poly-ICLC

In a separate ongoing trial in patients with multiply-recurrent anaplastic glioma, all 24 subjects treated (100%) reported at least one adverse event. Again, no adverse events were reported at the toxicity grade 5 and the majority of adverse events reported were classified as either grade 1 (41 of 63 or 65%) or grade 2 (14 of 63 or 22%). There were 7 of 63 (11%) grade 3 events and only 1 of 63 (2%) grade 4 event. The most frequently reported adverse event (toxicities) was fatigue (9 subjects), transient increases in SGOT, SGPT and alkaline phosphatase (4 subjects each) and pain, type not specified (4 subjects). In the 2005 report, only 19 out of 406 events were definitely or probably ascribed to the Hiltonol.

Interaction of Poly-ICLC and Chemotherapy

Poly-ICLC has been combined uneventfully with nitrosoureas, temozolomide, or cisplatin in over 50 patients with newly diagnosed or recurrent high grade gliomas.(Merchant and Young, unpublished observations and ³)

Clinical Studies of Poly-ICLC for Gliomas:

Six glioma trials have been done or are ongoing using low dose poly-ICLC:

In a pilot trial at Walter Reed Army Medical Center, poly-ICLC (10 to 50 mcg/kg IM 1 to 3 times weekly) was given for up to 56 months to patients with newly diagnosed GBM or anaplastic astrocytoma (AA), or with recurrent gliomas. Toxicity was low or absent. Twenty (20) of 30 patients (66%) receiving at least twice-weekly poly-ICLC (including all AA patients) showed regression or stabilization of gadolinium enhancing tumor volume on MRI (median = 65% volume decrease). Only 2 of the 11 AA patients subsequently showed tumor recurrence while on poly-ICLC; median progression-free follow-up was 77 months from diagnosis and overall median survival was 111 months (range 30 to 167 months). Median Kaplan-Meier survival was 19 months for GBM patients on at least twice-weekly poly-ICLC treatments. Tumor response was associated with 2'5' oligoadenylate synthetase activation ($p = .03$), but not with the minimal changes seen in serum interferon, IL2, IL6, TNF, or neopterin.

An open trial of Poly-ICLC in recurrent malignant gliomas was carried out at the Medical College of Virginia (Merchant, Broaddus, and Young, unpublished observations) 95 subjects with recurrent malignant brain tumor (glioblastoma, anaplastic glioma, and other patients) were treated with Poly-ICLC IM 2-3X weekly, with or without chemotherapy. Side effects were tolerable; most common complaints being transient pain at the injection site and fatigue. Modest increases in contrast enhancement and peritumoral edema on MRI were common for >3 cm diameter tumors in the first month. However, subsequent imaging studies improved on continued Poly-ICLC, with or without increased steroid. Patients treated with Poly-ICLC plus chemotherapy had no unexpected adverse events. Poly-ICLC was thus exceptionally well-tolerated relative to standard chemotherapy. Medical complications expected in patients with progressive malignant brain tumors were seen (including seizures, increased intracranial

pressure, and death), but no serious adverse reactions were attributed to the Poly-ICLC alone or in combination with chemotherapy.

Based on these two pilot studies, two large NCI sponsored multicenter brain tumor consortia have now completed accruals to two Phase II clinical glioma trials and are half-way through accruals for a third study. These are:

- 1) North American Brain Tumor Consortium (NABTC) Study NABTC 01-05. Poly-ICLC plus radiation therapy in newly diagnosed Glioblastoma Multiforme. (N=31)
- 2) NABTC 01-06. Poly ICLC Therapy for Recurrent Anaplastic Glioma. (N=46)
- 3) New Approaches to Brain Tumor Therapy consortium (NABTT): A Phase II Trial of Radiation plus Temozolomide followed by adjuvant Temozolomide and Poly-ICLC in Patients with Newly Diagnosed Glioblastoma Multiforme. (N=96, 56 patients accrued to date)

While efficacy data on these trials is pending completion of follow-up, registrational quality safety data has been collected and filed to Oncovir's IND confirming the relatively benign safety profile seen for this group of patients in pilot studies (please see above) Based on this limited sample, optimum dose of poly-ICLC for this indication appears to be about 20 mcg/kg two or three times weekly.

Two additional cancer vaccine trials utilizing Poly-ICLC as a vaccine adjuvant are currently ongoing. The first is in collaboration with Dr. G. Chatta at the University of Pittsburgh Cancer Center. This is entitled: "A pilot dose finding study of a MUC1 vaccine in conjunction with *Poly-ICLC* (polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose) or *Hiltonol*® in patients with recurrent and/ or advanced prostate cancer." The primary outcome measures are safety and immunologic parameters. Ten of 15 patients have been accrued to date.

The second is in collaboration with Dr. Hideho Okada, also at the University of Pittsburgh Cancer Center. This is entitled: "A phase I/II Evaluation of Vaccination with Type-1 Dendritic Cells Pulsed With Multiple Peptides and Poly-ICLC In the Treatment of HLA-A2 Positive Patients With Recurrent Malignant Gliomas (UPCI 05-115)." The primary outcome measures are safety and immunologic parameters. 1 of 15 patients have been accrued to date

Rationale for the use of intratumoral poly-ICLC and radiation in Lymphoma:

While most cancer vaccines have generally been designed to utilize one or more known or presumptive tumor antigens, an alternative strategy is 'autovaccination' or the use of the tumor itself as the antigen source, in vivo. This strategy has included the judicious combination of radiotherapy or chemotherapy with immunostimulants to induce maximal cross presentation and priming. It is based on the hypothesis that intra or peritumoral administration of a TLR ligand such as poly-ICLC will reverse DC inhibition, increase the efficiency of antigen presentation to CTL, and prevent tolerization of tumor antigen specific

CTL. In the present protocol, we propose to induce tumor necrosis and antigen release with low dose local radiation in order to induce cross presentation, and then provide an immune-priming TLR3 signal with intratumoral injection of poly-ICLC. Poly-ICLC boosters will be administered for 8 weeks intratumoral or peritumoral. Based on preclinical studies cited, the immune response generated at this one tumor site is expected to also attack tumor cells in distant metastases.

3.0 PATIENT ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

1. Patients must be at least 18 years of age.
2. Patients must have biopsy confirmed low-grade B-cell lymphoma (follicular, marginal zone, or small cell/chronic lymphocytic leukemia) or mycosis fungoides. B-cell lymphoma patients must have failed at least one prior therapy (chemotherapy or immunotherapy) or mycosis fungoides patients failed at least 1 topical or systemic treatment.
3. Patients must have at least one accessible tumor site that can be injected with poly-ICLC.
4. Patients must have measurable disease other than the injection site.
5. Patients must have a Karnofsky performance status $\geq 70\%$.
6. Patients must have adequate hematologic, renal and liver function (i.e., absolute neutrophil count $\geq 1500/\text{mm}^3$, Platelets $\geq 100,000/\text{mm}^3$, creatinine ≤ 1.7 mg/dl, total bilirubin ≤ 1.5 mg/dl, transaminases ≤ 4 times above the upper limits of the institutional normal).
5. Patients must be able to provide written informed consent.
6. Patients with the potential for pregnancy or impregnating their partner must agree to follow acceptable birth control methods to avoid conception. Women of childbearing potential must have a negative pregnancy test. While animal testing has been negative, the anti-proliferative activity of this experimental drug may theoretically be harmful to the developing fetus or nursing infant.
7. Required washout period for prior therapy:
 - Topical therapy: 2 weeks.
 - Chemotherapy: 4 weeks
 - Radiotherapy: (including phototherapy): 4 weeks
 - Biological therapies: 4 weeks
 - Other investigational therapy: 4 weeks
 - Rituximab: 12 weeks

3.2 Exclusion Criteria

1. Any history of autoimmune or antibody mediated disease including: systemic lupus, erythematosis, rheumatoid arthritis, multiple sclerosis, Sjogren's syndrome, autoimmune thrombocytopenia, autoimmune hemolytic anemia, pure red cell aplasia, but excluding controlled thyroid disease, or the presence of autoantibodies without clinical autoimmune disease.
2. Off nucleoside or bendustine therapy for a minimum of 6 months
3. Prior treatment with Campath
4. Known history of human immunodeficiency virus (HIV), hepatitis B or hepatitis C (active, prior treatment, or both).

5. Patients with active infection or with a fever > 38.5°C within three days prior to the first scheduled treatment.
6. CNS metastases.
7. Prior malignancy (active within 5 years of screening) except basal cell or completely excised non-invasive squamous cell carcinoma of the skin, or in situ squamous cell carcinoma of the cervix.
8. Current anticoagulant therapy (ASA ≤ 325 mg/day allowed).
9. Significant cardiovascular disease (i.e., NYHA class 3 congestive heart failure; myocardial infarction within the past 6 months; unstable angina; coronary angioplasty within the past 6 months; uncontrolled atrial or ventricular cardiac arrhythmias).
10. Pregnant or lactating.
11. Any other medical history, including laboratory results, deemed by the investigator to be likely to interfere with their participation in the study, or to interfere with the interpretation of the results.

4.0 TREATMENT PLAN

4.1 TREATMENT

An accessible site of disease (lymph node, cutaneous, subcutaneous, etc.) will be selected by the principal investigator.

Patients will then receive two doses of low dose irradiation (2 Gy per day) to that single site on days 1 and 2.

Six patients will begin treatment at 0.25 mg of poly-ICLC with dose escalation for each group of six patients at 0.5 mg, 1 mg, and 2 mg maximum dose if the maximum tolerated dose has not been reached.

Intratumorally or peritumorally Poly-ICLC will be dosed on days 3 and 4 by the physician.

Day 1&2	Day 3	Day 4	Wk 2	Wk 3	Wk 4	Wk 8
Local XRT 2 GY/day	PICLC	PICLC	PICLC X 2	PICLC X 2	PICLC X 2	PICLC X 2

In other trials up to 50 mcg/kg were given three times weekly for up to 56 months with minimal toxicity. We are combining poly ICLC with low dose radiation therapy and will begin with a small dose of .25 mg poly ICLC for our initial dose. The doses will double up to 2 mg which we expect will be the optimal biologic dose.

4.2 Treatment Requirements

All eligible patients who consent to this study will have a baseline CT/PET up to 28 days prior to the initiation of treatment for evaluating tumor burden.

4.3 Poly-ICLC (Hiltonol®) Administration

Doses of poly-ICLC will be administered in the presence of a physician or nurse who will monitor the patient for at least 30 minutes after injection, including a determination of blood pressure, heart rate, and respiratory rate before and after injection.

4.3 Correlative Laboratory Studies

Correlative research studies will be carried out by Dr. Pawel Kalinski in the Hillman Cancer Center. These will include peripheral blood CD4+ and CD8+ T cell responses against autologous tumor cells (where possible) using IFN γ - ELISPOT readout. Additional studies are listed in the table on page 6.

5.0 PHARMACEUTICAL INFORMATION

5.1 POLY-ICLC

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. 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, Inc. (Oncovir).

Administration

The injections will be directly into the tumor site if possible or peritumoral.

How supplied

Poly-ICLC is supplied in single-use vials containing 1 mL of a 2 mg/mL opalescent solution. It is withdrawn from the vial using sterile technique and is administered as supplied.

Each vial poly-ICLC will be labeled with the following information:

Drug Name
Concentration
Storage Conditions
Lot Number
Date of Manufacture, Manufacturer
Investigational Use Statement

Storage and stability

Poly-ICLC is stable at room temperature for brief periods (days). Treatment sites are asked to store the drug at approximately 40° F. Patients should be advised to store their doses in a standard refrigerator; when necessary, vials may be kept at room temperature up to 24 hours. The vials should not be frozen.

Supplier

The drug to be used in this study is prepared and packaged under GM., under contract to the study sponsor, Oncovir, Inc. It is then tested for quality, activity and pyrogenicity by Oncovir, Inc.

Drug accountability

The intent of drug accountability is to assure that supplied agents are only used for patients enrolled on an approved trial. According to FDA guidelines the investigator is ultimately responsible for all agents shipped in his/her name. FDA regulations require investigators to establish a record of the receipt, use, and disposition of all investigational agents. The sponsor of investigational trials has the responsibility to assure the FDA that systems for drug accountability are being maintained by investigators in their clinical trial program. Investigators may delegate responsibility for drug ordering, storage, accountability and preparation to his/her designee.

Drug ordering information

Order Poly-ICLC supplies by contacting:

Andres M. Salazar, MD
Oncovir, Inc
3203 Cleveland Ave, NW

Phone 202-342-1726
FAX (202) 248-2324
email asalazar@oncovir.com

6.0 STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤ 4 weeks prior to start of therapy.

	Pre-Study	Dy 1	Dy 2	Dy 3	Dy 4	Wk 2	Wk 3	Wk 4	Wk 8	Wk 12	Wk 16	q 3 mo	Off Study
Radiation to disease site		X	X										
PICLC				X	X	X ¹	X ¹	X ¹	X ¹				
Informed consent	X												
Demographics	X												
Medical history	X												
Physical exam	X			X		X	X	X	X	X	X	X	X
Vital signs	X			X		X	X	X	X	X	X	X	X
Height	X												
Weight	X			X		X	X	X	X	X	X	X	X
Performance status	X			X		X	X	X	X	X	X	X	X
CBC & diff, electrolytes				X		X	X	X	X	X	X	X	X
Serum chemistry with LDH				X		X	X	X	X	X	X	X	X
Correlative studies 4 (6 green caps)	X									X		X	X
EKG	X												
Tumor measurement by exam	X			X ²		X ²	X ²	X ²	X ²	X ²	X ²	X	X
CT/PET	X									X	X ³	X	X

1; 2 doses one day apart

2; Tumors may become inflamed and increase in size before they respond. We will evaluate response for purposes of this study at week 12.

3; To confirm response (responders only).

4; Peripheral blood CD4+ and CD8+ T cell responses against autologous tumor cells (where possible) using IFN γ ELISPOT redout. In any HLA-A2+ patients on the protocol, we will evaluate peripheral blood CD8+ T-cell responses against the defined CLL-related peptide epitopes (including hTERT, survivin, p35, MDM2 and fibromodulin; see refs 27-33), using IFN γ and IL-5 ELISPOT).

7.0 EVALUATION OF RESPONSE (week 12)

Tumors may become inflamed and increase in size before they respond. We will evaluate response for purposes of this study at week 12.

7.1 Chronic lymphocytic leukemia/small lymphocytic leukemia

7.11 Complete remission requires all of the following for a period of at least 2 months.

7.111 Absence of lymphadenopathy by physical examination and appropriate radiographic techniques.

- 7.112 No hepatomegaly or splenomegaly.
- 7.113 Absence of constitutional symptoms.
- 7.114 Normal CBC as exhibited by:
 - 7.1141 Polymorphonuclear leukocytes $\geq 1\,500/\text{mm}^3$.
 - 7.1142 Platelets $> 100,000/\text{mm}^3$.
 - 7.1143 Hemoglobin $> 11.0\text{ gm/dl}$ (un-transfused).
 - 7.1144 Peripheral blood lymphocytes $< 4000/\text{mm}^3$.
 One marrow aspirate and biopsy should be performed 2 months after clinical and laboratory evidence of a CR to document that a complete remission has been achieved. The marrow sample must be at least normocellular with $< 30\%$ of nucleated cells being lymphocytes. If it is hypocellular, a repeat determination should be made in 1-2 months. Samples will be submitted for pathology review and the presence of nodules noted, although not included in the current definition of CR. A patient who is in CR, but has nodules, will be considered to have nodular PR & recorded separately.
- 7.115 Any other laboratory assays (e.g., quantitative immunoglobulins, PCR for unique immunoglobulin rearrangement) will not be used currently as an index for response but will be recorded for clinical correlations.
- 7.12 To be considered a PR, the patient must exhibit the features in Sections 8.121, 8.122, and 8.123 (if abnormal prior to therapy) as well as one or more of the remaining features (Sections 8.124, 8.125, 8.126) for at least 2 months. In addition to the parameters listed below, the presence or absence of constitutional symptoms will be recorded.
 - 7.121 $\geq 50\%$ decrease in peripheral blood lymphocyte count from the pretreatment baseline value.
 - 7.122 $\geq 50\%$ reduction in lymphadenopathy.
 - 7.123 $\geq 50\%$ reduction in size of liver and/or spleen.
 - 7.124 Polymorphonuclear leukocytes $\geq 1500/\text{mm}^3$ or 50% improvement over baseline
 - 7.125 Platelets $> 100,000/\text{mm}^3$ or 50% improvement over baseline
 - 7.126 Hemoglobin $> 11.0\text{ gm/dl}$ or 50% improvement over baseline without transfusions.
- 7.13 Progressive disease (PD) will be characterized by at least one of the following:

- 7.131 50% increase in the sum of the products of at least 2 lymph nodes on 2 consecutive examinations 2 weeks apart (at least 1 node must be 2 cm). Appearance of new palpable lymph nodes.
- 7.132 50% increase in the size of liver and/or spleen as determined by measurement below the respective costal margin; appearance of palpable hepatomegaly or splenomegaly which was not previously present.
- 7.133 50% increase in the absolute number of circulating lymphocytes.
- 7.134 In the absence of progression as defined above, the presence of a > 2 gm/dl decrease in hemoglobin, or > 50% decrease in platelet count and/or absolute granulocyte count will not exclude a patient from continuing on study. Bone marrow aspirate and biopsy are strongly encouraged to better define the cause of the suppressed counts.
- 7.135 Transformation to a more aggressive histology (e.g., Richter's syndrome or prolymphocytic leukemia with > 55% prolymphocytes).
- 7.14 Patients who have not achieved a CR or PR, or who have not exhibited findings consistent with Progressive Disease will be considered as having Stable Disease.
- 7.2 Follicular, marginal zone lymphoma, and small lymphocytic lymphoma without a leukemia phase.
 - 7.21 Complete Response (CR): *A complete response* requires all of the following:
 - 7.211 Complete disappearance of all detectable clinical and radiologic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of those biochemical abnormalities (e.g., lactate dehydrogenase, LDH)
 - 7.212 All lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in their greatest diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse Diameter after treatment, or by more than 75% in the Sum of the Products of the greatest diameters (SPD).
 - 7.213 The spleen, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination.
 - 7.214 If the bone marrow was involved by lymphoma before treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of the same site. The sample on which this determination is made must be adequate (≥ 20 MM biopsy core).
 - 7.22 **CR/unconfirmed (CRu)** includes those patients who fulfill criteria 1 and 3 above, but with one or more of the following features:

7.221 A residual lymph node mass > 1.5 cm in the greatest transverse diameter that has regressed by more than 75% in the SPD. Individual nodes that were previously confluent must have regressed by more than 75% in their SPD compared with the size of the original mass.

7.222 Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia)

7.23 Partial response (PR) requires the following:

7.231 $\geq 50\%$ decrease in SPD of the six largest dominant nodes or nodal masses. These nodes or masses should be selected according to the following features: (a) they should be clearly measurable in at least two perpendicular dimensions, (b) they should be from as disparate regions of the body as possible, and (c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.

7.232 No increase in the size of the other nodes, liver, or spleen.

7.233 Splenic and hepatic nodules must regress by at least 50% in the SPD

7.234 With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease.

7.235 No new sites of disease.

7.24 **Stable disease** (SD) is defined as less than a PR but is not progressive disease (PD)

Relapsed/Progressive disease (after CR and CRu) requires the following:

7.241 Appearance of any new lesion or increase by $\geq 50\%$ in the SPD of previously involved sites

7.242 $\geq 50\%$ increase in greatest diameter of any previously identified node greater than 1 cm in its short axis or in the SPD of more than one node

7.25 Progressive disease (PD) require the following:

7.251 $\geq 50\%$ increase from nadir in the SPD of any previously identified abnormal node for PRs or non-responders

7.242 Appearance of any new lesion during or at the end of therapy.

7.3 Mycosis Fungoides – Cutaneous T-Cell Lymphoma

7.31 Criteria for response:

7.311 Complete Response (CR) – 100% clearance of all skin lesions,

7.312 Partial Response (PR) – 50 to 99% improvement in baseline skin score,

7.313 Minimal Response (MR) – 25 to 49% improvement in baseline skin score,

- 7.314 Stable Disease (SD) – 0 to 25% improvement in baseline skin score,
- 7.315 Progressive Disease (PD) - > 25% increase in baseline skin score,
- 7.316 Positive response defined as at least 25% improvement in baseline.

7.32 Tumor Burden in the Skin (physical examination and assessment):

- 7.321 Severity-Weighted Assessment Tool (SWAT) Calculation: $SWAT = (\text{patch \%TBSA} \times 1) + (\text{plaque \%TBSA} \times 2) + \text{tumor or ulcer \%TBSA} \times 3$. The subject's lesions will be drawn on the chart with patches (flat, erythematous, scaly areas) shaded by single-hatched markings, plaques (elevated areas) by cross-hatching, and tumors (dome-shaped lesions with 1 cm elevation) and ulcers by solid color. Erythroderma with mild infiltration will be mapped as patch disease, with moderate infiltration will be mapped as plaques, and with tumorous infiltration will be mapped as tumors. Questionable lesions will be biopsied to clarify its nature. SWAT will be calculated after pathology results are available. Calculation of the percentage of body area affected by a lesion will be calculated by dividing the number of grid intersections overlying each lesion by the maximum number of possible number of intersections for the body diagram times 100%.
- 7.322 Total Body Photography (TBP): Photographic documentation of the affected skin.
- 7.323 Total Body Surface Calculation: Percentage of skin affected by the disease; as part of the evaluation of T status of TNM staging.
- 7.323 Lymph Node Evaluation: Physical examination and CT scan of chest, abdomen, and pelvis for lymph node assessment. Lymph node biopsies may be performed if clinically indicated as per discretion of PI.

8.0 Definition of Dose Limiting Toxicity

Toxicities are to be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Effects (NCI CTCAE, Version 3.0). A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (<http://ctep.cancer.gov>).

Dose Limiting Toxicity (DLT) is defined as any of the following:

Hematologic

- Grade 4 thrombocytopenia (platelet count $\leq 10,000/\text{mm}^3$), or grade 3 thrombocytopenia (platelet count $\leq 50,000/\text{mm}^3$) lasting greater than seven days.
- Grade 4 neutropenia ($\text{ANC} < 0.5 \times 10^9/\text{L}$) of ≥ 7 days duration despite use of growth factors.
- Febrile neutropenia only if it occurs after seven days of neutropenia.

Non-hematologic

- Any other $\geq$ grade 3 non-hematologic toxicity considered by the investigator to be related to study drug, with the exception of alopecia, inadequately treated nausea, vomiting and/or diarrhea and fatigue.

Any delay in therapy of greater than 4 weeks from last treatment due to investigational agent-

associated toxicity will be considered dose limiting.

9.0 ADVERSE EVENT AND ADVERSE EVENT EXPEDITED REPORTING

Patients will be evaluated for toxicity if they have received at least one dose of poly-ICLC.

The timely reporting of adverse events (including deaths) is required by the Food and Drug Administration. The reporting of adverse events is part of the data reporting for this study.

All adverse events will be reported to Oncovir by the investigative site in the timely manner described and University of Pittsburgh's Institutional Review Board.

Definition - Adverse Event

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).

All adverse events should be followed up in accordance with good medical practice. Abnormalities of laboratory events which, in the opinion of the Investigator, constitute adverse events (even if not serious) should be followed.

Relationship

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

- *Unrelated*
The adverse event is clearly not related to the investigational agent(s).
- *Unlikely*
The adverse event is doubtfully related to the investigational agent(s).
- *Possible*
The adverse event may be related to the investigational agent(s).
- *Probable*
The adverse event is most likely related to the investigational agent(s).
- *Definite*
The adverse event is clearly related to the investigational agent

Serious Adverse Events

Definition – Serious Adverse Event (SAE)

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.

- All SAEs, regardless of expectedness, must be documented.
- Oncovir, Inc. will be notified automatically when a serious adverse event (SAE) is reported. All SAEs will be documented and tracked by Oncovir, Inc. When an expedited report is required (15 days), a speedy resolution of queries will be expected in order to allow for on time reporting to the FDA. Oncovir, Inc. is responsible for reporting **all** SAEs to the FDA.

10.0 OFF TREATMENT/OFF STUDY CRITERIA

Each subject has the right to withdraw from the study at any time without prejudice. The investigator may discontinue any subject's participation for any reason, including adverse event or failure to comply with the protocol.

Should a subject withdraw from the study, the reason(s) must be stated on the case report form, and a final evaluation of the subject should be performed. (See Section 9.1)

OFF TREATMENT CRITERIA

1. Extraordinary Medical Circumstance: If at any time the treating physician feels constraints of this protocol are detrimental to the patient's health remove the patient from protocol therapy.
2. Patient's refusal to continue treatment: In this event, document the reason(s) for withdrawal.
3. Failure to comply with protocol (as judged by the investigator such as compliance below 80%, failure to maintain appointments, etc.).
4. Patients who experience unacceptable toxicity.

11.0 STATISTICAL CONSIDERATIONS

11.1 Overview:

This study is designed to determine the safety and phase II recommended dose of poly-ICLC in addition to low-dose local radiotherapy for adult patients with low grade lymphomas. Response rate and duration of response will be analyzed as the secondary endpoints.

11.11 Analysis population:

Safety population: All enrolled patients who received at least one dose of poly-ICLC will be included in the safety population.

Evaluable population: All enrolled patients who complete the 10 poly-ICLC treatments and follow-up examinations and evaluations will be included in the analysis of clinical response.

- #### **11.12 Primary endpoint:** The primary endpoint is the toxicity rate at each of the four dose levels of poly-ICLC: 0.25 mg, 0.5 mg, 1 mg and 2 mg. Based on other trials with poly-ICLC, these doses are expected to be lower than the maximum tolerated

dose (MTD) and there is no scientific data to suggest a higher dose will be any more effective.

11.2 Study design/Sample size

11.21 Decision rule for finding phase II recommended dose

The phase II recommended dose is defined to be the highest dose level at which no more than 1 of 6 treated patient experiences a DLT. The DLT is defined as any grade 3 or 4 toxicity. As per the NCI Common Terminology Criteria for Adverse Events Version 3.0e (CTCAE v3.0e), the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment.

The phase II recommended dose will be determined with dose escalation scheme using the dose levels defined in Section 10.12. Only toxicities observed during the 12-week observation period following the first administration of poly-ICLC will affect dose escalation. Cohorts of 6 patients will be treated at a dose level, starting from the lowest dose. If 0 or 1 DLT is observed in the 6 patients, then treat the next cohort with the next higher dose level, or declare the dose at this level to be phase II recommended dose if at the highest dose level. If 2 DLTs are observed, then stop the dose escalation and declare the previous dose level to be the phase II recommended dose. Cohorts need not be completed if decisions can be made on the basis of an incomplete cohort. For example, if the first two patients in an intended cohort of six experience DLTs, escalation can be stopped without accruing the remaining members of this cohort.

11.22 Sample size and accrual: The number of patients accrued to the study will depend on the phase II recommended dose. Up to 24 evaluable patients will be treated in the trial, with no more than 6 patients per dose level. Accrual is expected to occur at a rate of 1-2 patients per month.

11.3 Statistical analysis

11.31 Analysis of the Primary Endpoint: The toxicity rate and its 90% confidence intervals will be estimated at each of treated dose levels. With 6 patients per dose group, these estimates corresponding to the observed number of patients with toxicity is presented in the following table.

Number of patients with toxicity in a dose group	toxicity rate estimate (90% Confidence interval)
0	0 (0, 0.39)
1	0.17 (0.01, 0.58)
2	0.33 (0.06, 0.73)
3	0.50 (0.15, 0.85)
4	0.67 (0.27, 0.94)
5	0.83 (0.42, 0.99)
6	1 (0.61, 1.00)

The toxicity profile (i.e., the dose response curve for toxicity) will be estimated with a logistic regression model, using log(dose) as the explanatory variable. Baseline descriptive statistics on all evaluable patients will be provided for demographic variables (age, sex, race/ethnicity), ECOG performance status, disease stage and status at the time of enrollment and treatment regimens previously used. The NCI CTCAE v3.0e will be used to evaluate toxicity. We will consider a toxicity to be an adverse event that is possibly, probably or definitely related to treatment. The maximum grade of toxicity for each category of interest will be recorded for each patient and the summary results will be tabulated by category and grade. We will describe all DLTs and other serious ($\geq$ Grade 3) AEs on a patient-by-patient basis; descriptions will include dose level and any relevant baseline data. Statistics on the number of injections received by patients and any dose reductions will be tabulated.

- 11.32 Analysis of the Secondary Endpoint: Response rates will be estimated among the response evaluable patients by the proportion of patients with a best response of CR, PR, or SD defined in Section 7.0, with the corresponding 90% confidence intervals being reported. The distribution of response duration (time to progression among patients achieving CR, PR or SD) will be characterized by median or quartiles, with the corresponding Kaplan-Meier estimate being made. To the extent possible from the available data, proportional hazards regression will be used to assess whether the rate of disease progression and overall survival varies materially among the three subgroups of tumor response: CR, PR, and SD.

11.4 Data and safety monitoring plan

A data and safety monitoring plan for this study consists of monitoring of the adverse events and data to evaluate the efficacy of the study. The emphasis of this review is on subject safety. The investigators and clinical research coordinator meet monthly to review and discuss study data to review subject safety issues. Decisions to continue or close the trial to accrual are discussed during these meetings. Accrual of subjects and the progress of accrual is also discussed. Any modifications necessary to ensure patient safety are discussed and modifications will be submitted to the IRB. Any changes to the risk/benefit ratio, which show the study should be closed, will be forwarded to the IRB. All serious adverse events will be reported to the IRB according to the established guidelines in the IRB reference manual Chapter 3.0, 3.4 and 3.5. Serious adverse events will also be reported to the sponsor and /or other regulatory agency as per their requirements. All study data reviewed and discussed during these meetings will be kept confidential. Any breeches in confidentiality regarding the trial data will be forwarded to the IRB and sponsor.

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13.0 INFORMED CONSENT

13.1 Ethical and Legal Considerations

This study will be conducted in accordance with the Declaration of Helsinki and according to the guidelines in the attached appendices and in compliance with all applicable laws and regulations of the locale where the study is conducted.

It is the responsibility of the investigator that the patient is made aware and consent is given that personal information may be scrutinized during audits by competent authorities and properly authorized persons, but that personal information will be treated as strictly confidential and not be publicly available. The investigator is responsible for the retention of the patient log and patient records.