Activating the Natural Host Defense
Poly-ICLC and Malignant Brain Tumors

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Introduction

Poly-ICLC is an experimental biological response modifier that has shown encouraging activity against brain tumors in a pilot trial several years ago, and has recently entered into multicenter phase II clinical trials for patients with malignant gliomas. While initially developed as an interferon inducer, poly-ICLC also has much broader biological effects in man, including specific antiviral and antitumor actions. Here, we briefly summarize the background and current knowledge of this compound in the experimental treatment of brain tumors.

Background

Over 40 years ago Isaacs and Lindeman discovered a substance produced by virally infected cells that interfered with further viral growth. They named it interferon (IFN), and it caused much excitement in the medical scientific community because of its therapeutic promise. The immunity established by interferons came to be known as immediate, non-specific, or innate immunity. This is in contrast to the slower onset but longer-term and more specific protection provided by antibodies and immune cells. Various forms of interferon were identified in many species, and it was subsequently shown that the interferons also had activity against certain tumors. However, interferons were very difficult and expensive to produce, and were not generally available. It was subsequently found that a number of compounds could induce cells to make their own interferon. Among the most potent of these were the double-stranded ribonucleic acids (dsRNA), and in particular, the synthetic dsRNA poly-IC, which consists of a pair of strands of poly-inosinic and poly-cytidylic acids. Interferon inducers such as poly-IC were thus initially seen as a way of resolving the problems posed by the shortage of interferon. DsRNAs are not normally found in mammalian cells, but they are the basic genetic material of some viruses and can also be a by-product of some viral infections. This may help explain their activity in stimulating some of the body’s basic host defenses.

Plain Poly-IC itself proved to be ineffective in primates because it is rapidly inactivated by natural enzymes in the blood. However, some 30 years ago, Dr. Hilton Levy at the NIH discovered how to stabilized poly-IC with poly-lysine. The resulting compound, Poly-ICLC, is a very stable dsRNA that is a potent interferon inducer in man. Early, short term, high dose cancer trials showed that high dose Poly-ICLC could induce very large amounts of interferon production in man, but with only modest therapeutic effects and moderate transient toxicity. Its use was then generally abandoned when interferons became widely available through the new recombinant DNA technologies.

However, it has since become apparent that low dose Poly-ICLC is a more potent activator of a variety of host defense mechanisms that go well beyond simple induction of interferons. These include much broader immune stimulation, gene regulatory and specific antiviral, and anticancer effects, with little or no toxicity. Certain of these critical effects are inhibited at the higher doses of Poly-ICLC used in early clinical cancer trials and it is now believed that these effects may be more important clinically than previously thought. This may help explain the inconsistent results of the early clinical trials with high dose Poly-ICLC. The activity of poly-ICLC and interferons against both certain viruses and certain cancers also serves to remind us how the body’s basic defenses can cut across traditional disease classifications.
Mechanism of Action of Poly-ICLC

There are at least four interrelated clinical actions of poly-ICLC, any of which (alone or in combination) might be responsible for its antitumor and antiviral activity. These are 1) its induction of interferons; 2) its broad immune enhancing effect; 3) its activation of specific enzymes, especially oligoadenylate synthetase (OAS) and the p68 protein kinase (PKR); and 4) its broad gene regulatory actions.

Interferon Induction. While induction of interferon is one of the important mechanisms for the action of poly-ICLC, interferons alone have been disappointing as clinical treatments for brain tumors. In addition, the levels of serum interferon induced by low dose Poly-ICLC are themselves relatively low and have not in the past been associated with antiviral or antitumor action.

Immune modulation: Low dose Poly-ICLC also has a direct immune-enhancing action independent of IFN, including, activation of white blood cells such as T-cell s, natural killer cells, and, dendritic cells, release of cytokines such as interferons, interleukins (IL2, IL6), corticosteroids, and tumor necrosis factor (TNF). It also has a potent vaccine-boosting or adjuvant effect, with increased antibody response to antigen. (Levy and Bever 1988) The immunostimulatory effects of Poly-ICLC and the interferons are complex. However, preliminary laboratory results in a pilot study in brain tumor patients showed no clear relationship between tumor response and measurable serum interferon, TNF, IL2, IL6, or neopterin. (Salazar, Levy et al. 1996) The adjuvant effect of poly-ICLC has also been demonstrated in several systems. For example, administration of low doses of poly-ICLC along with swine flu vaccination in monkeys dramatically accelerates and increases antibody production (Stephen, Hilmas et al. 1977). The complex interactions of the dsRNAs and the interferons in this regard are still incompletely understood, yet this seemingly paradoxical dual role of poly-ICLC as an antiviral agent and immune enhancer is consistent with its function in establishing an immediate defense system against viral attack while at the same time permitting the establishment of long term immunity.

“Catalytic” Action of Poly-ICLC

The third action of Poly-ICLC is a more direct antiviral and antitumor effect mediated by at least two interferon-inducible nuclear enzyme systems, the 2’5’ oligoadenylate synthetase (OAS) and the P68 protein kinase (PKR). (Katze 1992), (Jacobs and Langland 1996). DsRNAs such as Poly-IC catalyze the interferon induced antiviral state in cells by functioning as obligatory cofactors for OAS, which activates ribonuclease-L, as well as for the PKR, which inhibits initiation of protein synthesis. This may help explain the demonstrated preferential decrease of tumor protein synthesis in vivo by poly-ICLC. The 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. Similarly, the PKR has both high and low affinity binding sites and is inhibited by too high a dose of dsRNA. (Galabru, Katze et al. 1989) Clinically, the OAS response is also maximal at a dose of about 30 mcg/kg Poly-ICLC, and is much diminished above 100 mcg/kg (M. Kende, N. Bernton, et al., Unpublished).

The inhibition of glioma cells by poly-IC and by interferon beta is also significantly associated with activation of both the OAS and PKR. (Chacko, Xiuye et al. 2002) 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. (Koromilas, Roy et al. 1992) Both PKR and poly-IC are now known to regulate the p53 tumor suppressor gene, which induces tumor cell death. (Cuddihy, Wong et al. 1999) P53 is in turn is 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.

Many viruses, including but not limited to adenovirus, pox viruses (vaccinia), foot and mouth virus, influenza, hepatitis, poliovirus, herpes simplex, SV-40, reovirus, and the human immunodeficiency virus (HIV) circumvent host defenses by down regulating OAS and/or PKR, and this effect can be reversed in vitro by exogenous dsRNA. (Jacobs and Langland 1996) A block of either PKR and/or OAS-mediated interferon action might also explain the variable response to interferons seen in both viral infections and cancer. Certain viruses as well as tumors such as malignant gliomas may use this or a similar mechanism to circumvent host defenses and cause disease. Those diseases may thus be among the prime targets for clinical Poly-ICLC therapy in a regimen that maximizes PKR activation.

Clinical Gene Regulation is a fourth mechanism by which Poly-ICLC can modify the biologic response and provide therapeutic benefit. Plain poly-IC has been shown to up-regulate or down-regulate a broad variety of over 270 genes in cell culture (Geiss, Jin et al. 2001). 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.

Antiviral Activity of Poly-ICLC

A detailed discussion of the antiviral actions of Poly-ICLC is beyond the scope of this outline. However, there is a considerable literature describing the activity of poly-ICLC in a broad variety of viral infections, including poxviruses such as vaccinia, hepatitis, influenza, herpesvirus, rabies, Japanese encephalitis, West Nile virus, ebola virus, and the human immunodeficiency virus (HIV). (Levy and Salazar 1992) For example, recent studies have shown strong protection by a single dose of poly-ICLC for as long as eight days in a mouse model of smallpox. Likewise, intranasal poly-ICLC can protect mice for as long as 3 weeks from an otherwise lethal dose of influenza virus. This broad spectrum of activity of poly-ICLC thus makes it a promising drug for containment of epidemics of certain new or emerging viruses for which positive identification or vaccine may not be immediately available, such as new strains of influenza, West Nile virus, or possibly SARS.

Clinical Pilot Studies with Poly-ICLC in Malignant Brain Tumors

Progress in the treatment of malignant gliomas has been slow. Introduction of radiation therapy a generation ago doubled median survival to about 8-9 months for patients with glioblastomas, but traditional chemotherapy has added only modestly to that. More aggressive combined chemotherapy has not provided a clear benefit to balance the increased toxicity. More recently, temozolamide, with its lowered toxicity, has improved quality of life but only modestly improved survival. Biologicals such as interferon have shown some promise, but have not lived up to original expectations. A host of new-generation, more targeted molecular therapies are also now under investigation. (Tremont-Lukats and Gilbert 2003)

In a recent pilot trial at Walter Reed Army Medical Center, low dose Poly-ICLC (about 1-2 mg) was given intramuscularly two to three times weekly for up to 56 months to 38 malignant brain tumor patients who had a life expectancy of only 1-2 years. (Salazar, Levy et al. 1996) Patients tolerated the
regimen well, with little or no toxicity and a preserved quality of life. Twenty of 30 adequately treated patients (including all anaplastic astrocytoma patients) showed regression or stabilization of tumor. Only two of the 11 anaplastic astrocytoma patients subsequently showed tumor recurrence while on Poly-ICLC, and their median progression-free follow-up is over 6.5 years from diagnosis (range 2-13+ years). Median overall survival is now 8 years, in contrast to an expected survival of 2 years on conventional chemotherapy. Median survival for glioblastoma patients was 19 months, only one of that group remains alive and well over 10 years from diagnosis. (Please see Tables 1 and 2 below) Two additional “compassionate use” open protocols in over 150 patients with advanced recurrent brain tumors have confirmed the safety of Poly-ICLC, alone or combined with chemotherapy.

Most malignant gliomas actually represent a mixture of highly malignant tumor cells and lower grade or “benign” 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 or in maintaining remission after more aggressive chemotherapy or radiotherapy in higher grade tumors. However encouraging these results may have been, these pilot trials were not designed to definitively demonstrate efficacy, and Poly-ICLC remains an experimental drug for brain tumors.

Based on these data, the prestigious, multicenter North American Brain Tumor Consortium (NABTC) is conducting and cosponsoring two separate Phase II open studies of Poly-ICLC in about 110 patients with either: 1) recurrent malignant anaplastic astrocytoma, or 2) newly diagnosed, grade IV glioblastoma.

**Conclusions**
The therapeutic expectations raised in the medical –scientific community with the discovery of the interferons some 40 years ago have so far been only partially realized. Interferons are now in widespread clinical use for such disparate conditions as certain cancers, certain viral infections, and multiple sclerosis. However, much has also been learned about the mechanisms by which certain other viruses and cancers evade the natural host defenses mounted by the interferon system. It now appears that some of these evasive mechanisms can be circumvented by treatment with dsRNAs such as poly-ICLC. Experimental agents such as Poly-ICLC can thus be expected to show activity in situations in which interferons are inactive or only marginally active. DsRNAs are now also recognized to have multiple biological effects that go well beyond the interferons, including multiple gene regulation, and activation of certain basic antiviral and antitumor host defenses. The full clinical therapeutic implications of these findings, however, will only be elucidated through properly designed clinical trials.

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**References**


### Table 1
Survival of Malignant Glioma Patients by Prognostic Class
Poly-ICLC Vs Historical Chemotherapy controls (29 patients)

<table>
<thead>
<tr>
<th>Prognostic Class*</th>
<th>Median Survival (months) P-ICLC</th>
<th>Percent 2 yr. Survival P-ICLC</th>
<th>Percent 2 yr. Survival Chemotherapy</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>119</td>
<td>100%</td>
<td>76%</td>
</tr>
<tr>
<td>II</td>
<td>104†</td>
<td>80%</td>
<td>5%</td>
</tr>
<tr>
<td>III</td>
<td>53</td>
<td>80%</td>
<td>5%</td>
</tr>
<tr>
<td>IV</td>
<td>57 †</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>19</td>
<td>33%</td>
<td>6%</td>
</tr>
<tr>
<td>VI</td>
<td>12</td>
<td>0</td>
<td>4%</td>
</tr>
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* = Prognostic class based on various risk factors, and reflecting survival of 1578 patients participating in three large chemotherapy trials (Curran, Scott et al. 1993), † N = only one PICLC patient in groups II and IV. (Updated from (Salazar, Levy et al. 1996))

### Table 2
Percent Survival of Malignant Glioma Patients on Poly-ICLC

<table>
<thead>
<tr>
<th>Survival</th>
<th>GBM</th>
<th>AA</th>
<th>AA (pf)</th>
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<tbody>
<tr>
<td></td>
<td>12 patients</td>
<td>11 patients</td>
<td>11 patients</td>
</tr>
<tr>
<td>1 yr</td>
<td>92% (50%†)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>2 yr</td>
<td>50%</td>
<td>100% (50%†)</td>
<td>91%</td>
</tr>
<tr>
<td>3 yr</td>
<td>25% (2.2%†)</td>
<td>91%</td>
<td>82%</td>
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<td>4 yr</td>
<td>17%</td>
<td>91%</td>
<td>82%</td>
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<tr>
<td>5 yr</td>
<td>8%</td>
<td>91%</td>
<td>73%</td>
</tr>
<tr>
<td>8 yr</td>
<td>8%</td>
<td>82%</td>
<td>36%</td>
</tr>
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</table>

GBM = glioblastoma, AA = anaplastic astrocytoma, pf = progression free survival, † = Expected survival on standard treatment with or without chemotherapy.