

A phase II clinical trial of poly-ICLC with radiation for adult patients with newly diagnosed supratentorial glioblastoma: a North American Brain Tumor Consortium (NABTC01-05)

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Abstract *Purpose* This phase II study was designed to determine the overall survival time of adults with supratentorial glioblastoma treated with the immune modulator, polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC), in combination with and following radiation therapy (RT). *Methods and materials* This was an open-label, single arm phase II study. Patients were treated with RT in combination with poly-ICLC followed by poly-ICLC as a single agent. Poly-ICLC was initiated 7–28 days after the surgical procedure that established the diagnosis; radiotherapy began within 7 days of the first dose of poly-ICLC and within 35 days of

surgical diagnosis. Treatment with poly-ICLC continued following the completion of RT to a maximum of 1 year or until tumor progression. *Results* 31 patients were enrolled in this study. One patient did not have a Glioblastoma multiforme and was deemed ineligible. For the 30 eligible patients, time to progression was known for 27 patients and 3 were censored. The estimated 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. *Conclusions* The combined therapy was relatively well-tolerated. This study suggests a survival advantage compared to historical studies using RT without chemotherapy but no survival advantage compared to RT with adjuvant nitrosourea or non-temozolomide chemotherapy. Our results suggest that poly-ICLC has activity against glioblastoma and may be worth further study in combination with agents such as temozolomide.

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Introduction

Glioblastoma multiforme (GBM) is challenging to treat and associated with a high degree of morbidity and mortality. Standard treatment consists of cytoreductive surgery followed by radiation therapy (RT) in combination with temozolomide (TMZ) chemotherapy followed by adjuvant TMZ which leads to a median survival of 14.6 months [1]. Based on several meta-analyses, other types of adjuvant chemotherapy, mainly nitrosoureas, seem to add some survival benefit but the gain is modest [2–4]. Continuous efforts are ongoing to develop more novel, effective agents

or combinations of agents that may improve overall survival or prolong time to progression. Such a novel agent and one that modulates the immune system is polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC).

Poly-ICLC is a double-stranded RNA (dsRNA) previously utilized as an interferon inducer and immune modulating agent at high doses (upto 300 mcg/kg IV) in clinical cancer trials. These trials were based on preclinical evidence that dsRNA possessed anti-neoplastic activity, including in glioma cell lines; this anti-tumor effect was thought due to induction of interferon as well as an interferon-independent immune enhancing effect which involved an increased antibody response to antigen, and activation of natural killer cells, T cells, macrophages, and cytokines [5–11]. These original trials showed diverse results with relatively significant toxicity and thus the use of poly-ICLC was discarded when interferons became available via recombinant DNA technology [12–17]. However, ensuing studies did continue and demonstrated that low-dose (10–50 mcg/kg) poly-ICLC resulted in less toxicity and a broader host defense stimulation, including activation of T cells, natural killer cells, and myeloid dendritic cells via Toll-like receptor 3 (TLR3) as well as induction of a mix of interferons, cytokines, and chemokines; The host defense stimulation also resulted in a antiviral and anti-proliferative effect mediated by activation of interferon-inducible dsRNA dependent enzyme systems which regulate such cell functions as protein synthesis, proliferation, and apoptosis [5, 18–24].

The enzyme systems activated by poly-ICLC include 2′5′oligoadenylate synthetase (OAS), protein kinase R (PKR), RIG-1 helicase, and melanoma differentiation associated gene MDA5, which along with the interferon and immune related responses may be responsible for its anti-neoplastic effects [25, 26]. OAS requires dsRNA as a cofactor in order to activate ribonuclease-L which then exerts anti-proliferative and proapoptotic activity in cells [27]. dsRNA also activates PKR which inhibits protein synthesis [28–31]. These enzyme systems are responsive to dsRNA dose and structure [32]. Long-chain dsRNA (as in poly-ICLC) is a potent stimulator of OAS but mismatched or irregular dsRNA may be inhibitory. Correspondingly, the OAS and PKR systems may be inhibited by a higher dose of dsRNA possibly explaining why higher doses of poly-ICLC used in early cancer trials were comparatively ineffective [33–35].

The clinical half-life of the OAS response to poly-ICLC is about 2.5 days, suggesting an optimal dose schedule of 2–3 times per week [36]. In a pilot study of poly-ICLC in patients with newly diagnosed or recurrent GBM or anaplastic astrocytoma (AA), patients were

treated with poly-ICLC at 10–50 mcg/kg, administered intramuscularly 1–3 times weekly. Twenty of 38 patients also received concurrent CCNU at 120 mg/m² once every 6 weeks while others received no chemotherapy. This study showed up to a 40-fold increase in serum OAS product and toxicity was mild [26]. About 66% of patients (including all 11 newly diagnosed AA) receiving at least twice-weekly poly-ICLC showed regression or stabilization of gadolinium enhancing tumor volume on magnetic resonance imaging (MRI) for at least 6 months from study entry. Median survival was 19 months for 18 newly diagnosed GBM patients receiving poly-ICLC at least twice a week; tumor response was associated with OAS activation but not with changes in serum interferon. In effect, this pilot study demonstrated the safety and tolerability of long-term, low-dose intramuscularly administered poly-ICLC in patients with malignant glioma and a potentially beneficial dose range of 20 mcg/kg administered 2–3 times weekly; this dose presumably activates a basic host tumor suppressor system via the mechanisms discussed above. The pilot study also provided encouraging results for both AA and GBM but was too small and the patients too heterogeneous to provide reliable evidence of efficacy.

Based on this background information, in addition to the published experience with poly-ICLC in multiple sclerosis and human immunodeficiency virus (HIV) infection, we designed this phase II trial with the primary objective of determining whether poly-ICLC with concurrent RT followed by adjuvant poly-ICLC can improve the median survival time of patients with newly diagnosed supratentorial GBM. As treatment efficacy may be related to tumor burden, the best time for treatment is presumed to be at the time of least tumor burden, after maximum surgical resection. Given this potential increased anti-tumor effect, poly-ICLC was started post-operatively prior to the initiation of radiation (RT). Secondary objectives were appraisal of the toxicity of conventional RT plus poly-ICLC followed by adjuvant poly-ICLC and assessment of progression-free survival.

Note that this clinical trial was initiated before TMZ became incorporated into the standard of care for patients with newly diagnosed GBM [1]. Given the modest efficacy of chemotherapy for GBM at the time this study was initiated and because of the possibility that chemotherapy's side effects may add to those of poly-ICLC and confound assessment of poly-ICLC, chemotherapy was withheld during treatment with poly-ICLC. Nevertheless, accrual to this study was discontinued after the results of the EORTC phase-3 study defined the standard of care for newly diagnosed patients as radiotherapy plus concomitant and adjuvant TMZ [1].

Materials and methods

Patient eligibility

Patients who were at least 18 years of age with a histologically confirmed, newly diagnosed, previously untreated GBM were eligible. Pathological material was centrally reviewed and met criteria to be classified as World Health Organization grade IV astrocytoma or GBM. Patients were required to have a Karnofsky Performance Score (KPS) of ≥ 60 and an estimated survival time of greater than 8 weeks. Patients were also required to have appropriate hematological, renal, and hepatic status. No patients were pregnant or nursing. All patients were willing to practice birth control during and for 2 months after treatment. Each patient had recovered from the effects of surgery before entry into the study. All patients or their designated surrogates signed a consent form approved by the participating institution's institutional review board (IRB).

Study design

This was a single arm, open-labeled, phase II study. All patients were evaluated for toxicity, response to therapy, time to tumor progression, and overall survival. A combination of standard neurological examination and neuroimaging was used to define overall response or progression [37]. Imaging was assessed by the treating physician. Clinical and radiological assessments of disease status were performed after RT and every 8 weeks thereafter.

Transient enlargement of contrast enhancing tumor with subsequent shrinkage has been reported during poly-ICLC treatment. Because of this possibility, if a patient had progressive disease by conventional definition [37], the treating physician and patient had the option of continuing treatment; however, for the sake of statistical analysis the official progression date was defined as the initial MRI which demonstrated an enlargement of contrast enhancing

disease. If patients continued on treatment despite an MRI with increased volume of enhancement, the protocol required removal from treatment if the MRI met the criteria listed in Table 1.

Radiation therapy

Patients were treated with conventional RT in conjunction with poly-ICLC. Radiation began no later than 7 days after starting poly-ICLC and within 35 days of the surgical procedure that established the diagnosis. Radiation was performed at either (a) the Radiation Oncology Department of the NABTC Institution, or (b) another site approved to participate in any trial of the Radiation Therapy Oncology Group (RTOG). Radiotherapy was given by external beam to a partial brain field in daily fractions of 2.0 Gy, to a planned total dose to the tumor of 60.0 Gy over 6 weeks. The site providing radiation treatment was required to have Institutional Review Board approval. Stereotactic radiosurgery and brachytherapy were not allowed. The target volume was preferably based on MRI, but computed tomography (CT) scans were acceptable for treatment planning. The target volume included the resection cavity and the contrast enhancing tissue with at least a 2 cm margin with inclusion of the presumed infiltrative volume. The margin was potentially less at natural barriers to tumor spread such as the skull, falx, or tentorium, or if necessary to protect the eye or to limit the dose to the optic apparatus to <50 Gy. The lens was shielded from the direct beam at all times. The dose to the retina was limited to <50 Gy, the dose to the optic nerve and chiasm was limited to <54 Gy, and the dose to the brain stem was limited to <60 Gy. The field arrangement was selected to maximize target coverage and to minimize dose to non-target tissues. Treatment plans included opposed lateral fields (discouraged except for central or bilateral tumors), wedge pair of fields, rotation, or multiple field techniques. Isodose distributions for the target volume were required for all patients, and the inhomogeneity within the target was kept to <5 –10%.

Table 1 Radiological criteria for unacceptable progression

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 ($\geq 25\%$ increase in the sum of products of all measurable lesions over the smallest sum observed using the same techniques as baseline or appearance of any new lesion/site) 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:

- (1) $\geq 50\%$ increase in bi-dimensional diameters for tumors starting with bi-dimensional diameters >4 cm²
- (2) $\geq 100\%$ increase in bi-dimensional diameters for tumors starting with bi-dimensional diameters of 1.0–4.0 cm²
- (3) Bi-dimensional diameters ≥ 2.0 cm² for tumors starting with no measurable disease or for bi-dimensional diameters ≤ 1.0 cm²
- (4) Unacceptable worsening of neurological symptoms that cannot be controlled with corticosteroids
- (5) 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

Poly-ICLC therapy

Poly-ICLC was given at a dose of 20 mcg/kg 3 times weekly by intramuscular injection. The days of administration were at least 2 days apart (i.e., usually a Monday–Wednesday–Friday schedule). Poly-ICLC could be administered at any time of day, but it was recommended that each patient choose a consistent time for administration. The initial dose of poly-ICLC was administered in the presence of a physician or nurse who monitored the patient for at least 30 min after injection, including a determination of blood pressure, heart rate, and respiratory rate before and after injection. Patients were trained on the proper method for storing, preparing, and administering poly-ICLC and either they or a family member administered subsequent doses. Acetaminophen was used to treat fever or flu-like side effects of poly-ICLC. Patients were pretreated with acetaminophen as warranted by side effects at the discretion of the local investigator. Such pretreatment was recorded in the treatment diary. For fever, arthralgias, or myalgias of grade 2 or greater, poly-ICLC was discontinued for at least one dose. When symptoms returned to grade 0, poly-ICLC was resumed at 50% of the original dose. If further dosing was well-tolerated, the original dose was subsequently re-instituted at the discretion of the investigator. All dosage changes were recorded in the treatment diary. Corticosteroids were used in the smallest dose to control symptoms of cerebral edema, mass effect, and fatigue, and were discontinued if possible. Anti-seizure medications were used as indicated and patients were not stratified based on the use of enzyme-inducing anti-epileptic drugs.

Statistical methods and considerations

The primary endpoint of this study was total survival from the date of surgical–pathological diagnosis. Results were compared with patients whose data were available through the database of the University of California at San Francisco (UCSF). These patients were similar to the patients in the present study insofar as they are patients seen at a tertiary center and enrolled into clinical trials early after diagnosis (see Table 3). The primary analysis used a Cox proportional hazards model including the well-accepted prognostic factors of age at diagnosis, KPS, and extent of surgical resection. Both survival and progression-free survival were estimated using Kaplan–Meier curves and medians presented are from these estimates. Time to event was measured from time of histological diagnosis. Median survival was determined using this methodology and the 95% confidence bound was calculated. Extent of resection was coded as biopsy, sub total resection, or complete resection.

Historical data suggest that 12-month survival averages 50% for GBM patients in clinical trials who did not receive TMZ [38]. Since the goal of this study was to demonstrate improved survival, we considered the lower threshold for the probability of 12-month survival to be 0.50. The calculation of sample size (60 patients) was based on a binary endpoint with the goal of increasing the 1-year survival to 67%. This would represent a hazard ratio of 0.58 (experimental/historical). Sixty patients provided 90% power using a one-sided alpha of 0.1 for this comparison. One-year survival of 63%, corresponding to a hazard ratio of 0.67, would also be of interest. Using the individual patient historical data and the exact information on survival time for patients on this protocol, it was anticipated there was approximately 90% power for testing for this smaller, but meaningful difference.

Early-stopping rules for safety

The treatment strategy tested would not be considered feasible if the discontinuation rate due to toxicity was 20% or greater and the lower bound for the one-tailed 95% confidence interval was >10%. Any toxicity observed during RT that required permanent discontinuation of poly-ICLC was considered significant. The rate of toxicity, with confidence intervals, was estimated with each event. If there was a greater than 20% rate for discontinuation of poly-ICLC during RT, the study was to stop and the design reassessed.

Results

From 3/19/03 to 2/25/05 31 patients were enrolled in the study; one patient was deemed ineligible after pathology review revealed that the patient did not have GBM, leaving 30 fully eligible and evaluable patients. Enrollment was prematurely discontinued after the results of the EORTC phase-3 study defined the standard of care for newly diagnosed GBM patients as radiotherapy plus concomitant and adjuvant TMZ. Patient characteristics are shown in Table 2. Patient characteristics for the control group are in Table 3. Toxicities encountered in the study are shown in Table 4. Poly-ICLC was very well-tolerated with little toxicity or negative impact to a patients' quality of life. There were the expected mild to moderate toxicities related to fatigue and the most common toxicity encountered was mild, temporary soreness at the injection site. No patients discontinued poly-ICLC because of toxicity. At no time was there a toxicity rate high enough to trigger the early-stopping rule. No patients were continued on protocol treatment after an MRI demonstrated an enlargement of contrast enhancing disease by conventional definition; thus,

Table 2 Patient characteristics (*n* = 30)

Age (years)	
Median	53
Range	26–76
Sex	
Male	14
Female	16
KPS	
Median	90
Range	60–100
Extent of resection	
Biopsy	2
Subtotal resection	17
Gross total resection	11

Table 3 Control group patient characteristics (*n* = 552)

	No chemotherapy (<i>n</i> = 223)	Non-TMZ chemotherapy (<i>n</i> = 329)
Age (years)		
Median	57	54
Range	26–82	19–77
KPS		
Median	90	90
Range	60–100	60–100
Extent of resection		
Biopsy	55	31
Subtotal resection	147	256
Gross total resection	21	42

Table 4 Toxicity (*n* = 30 patients) using National Cancer Institute Common Toxicity Grading System 3.0

Toxicity (number of patients)	Grade				
	1	2	3	4	5
Fatigue	6	4	3	1	0
Leukopenia	5	5	4	0	0
Lymphocytopenia	2	3	2	0	0
Myalgia	2	1	1	0	0
Fever without infection	1	1	1	0	0
Thrombosis	0	0	2	0	0
Pain	4	2	1	0	0
Diarrhea	1	0	0	0	0
Rigor/chills	0	1	1	0	0

the imaging parameters for radiological progression under which the patient was definitively discontinued from treatment were not employed.

Time to progression was known for 27 of 30 patients; the remaining 3, who remained on treatment at the time of this analysis, were censored for analysis of progression. Four patients were censored for survival at 35 [patient refused further follow-up], 114, 126, and 166 weeks. Survival data are summarized in Table 5. The 6-month progression-free survival was 30% and the 1-year progression-free survival was 5%. Median time to progression was 18 weeks (95% CI: 13–24 weeks) and 1-year survival was 69%. The median survival was 65 weeks (95% CI: 54.8–74.2 weeks).

For the historical control groups, the median survival of patients treated with non-TMZ chemotherapy was 57 weeks (95% CI: 54–62 weeks) and the 1-year survival rate was 57%; these results are versus a median survival of 40 weeks (95% CI: 36–44 weeks) and a 1-year survival of 35% in the 223 patients who did not receive adjuvant chemotherapy.

Compared to the patients treated without adjuvant chemotherapy, patients on this trial did well; the hazard ratio for survival, adjusted for age, KPS, and extent of resection, was 0.71 (90% upper confidence bound of 0.93) with a one-tailed *P* value of 0.05. The corresponding hazard ratio compared to the historical controls treated with adjuvant non-TMZ chemotherapy was 1.12 (90% upper confidence bound of 1.46) with a one-tailed *P* value of 0.7.

Discussion

Given the possible activity of poly-ICLC in GBM and the intent to engage in a combined modality approach for such an aggressive disease we chose to combine RT with poly-ICLC. The hope was that this strategy would improve patient survival. However, this clinical trial was initiated before TMZ became incorporated into the standard of care for patients with newly diagnosed GBM; Accrual to this study was discontinued after the results of the EORTC phase-III study defined the standard of care for GBM patients as radiotherapy plus concomitant and adjuvant TMZ [1]. Prior to the landmark TMZ study, the benefit of chemotherapy in GBM patients was thought to be modest. Due to the indefinite efficacy of chemotherapy in GBM patients and because of the possibility that chemotherapy side effects may add to those of poly-ICLC and confound assessment of poly-ICLC, chemotherapy was withheld during treatment with poly-ICLC.

Poly-ICLC and RT were well-tolerated. As this therapy combination with radiation had not previously been administered, however, early-stopping rules were predefined. Fatigue, myalgia, and pain at the injection site were the main adverse events; these events were not substantially different from the toxicities witnessed in the previous

Table 5 Summary of results

	Study patients	Control group: treated with RT and non-TMZ chemotherapy	Control group: treated with RT alone
Estimated 1-year survival (%)	69.4	57	35
Median survival	65 weeks (95% CI: 54.8–74.2)	57 weeks (95% CI: 54–62)	40 weeks (95% CI: 36–44)

trial which utilized poly-ICLC in high-grade glioma patients. No patients went off-study because of toxicity [26].

The median survival for this study was 65 weeks (see Table 5). Compared to an appropriate matched historical group this appeared to be better than RT alone; however, there was no statistical difference in survival compared to a historical group treated with adjuvant chemotherapy (not including TMZ). However, the toxicity experienced by patients in this trial was less than that of chemotherapy agents such as nitrosoureas [3, 4].

Comparing the results of this trial to those trials involving TMZ use in newly diagnosed GBM patients, overall survival from the present study (65 weeks or 15 months) is not substantially different from that found in the original 2002 Stupp et al. [1, 39] study of RT and concurrent TMZ followed by adjuvant TMZ (16 months); overall survival is also similar to that in the landmark EORTC trial (2005) that established TMZ as part of the standard of care for newly diagnosed GBM (14.6 months) [1]. Based on these results, combining TMZ and poly-ICLC represents an intriguing possibility especially when one considers the well-tolerated side-effect profile and ease of administration of TMZ in addition to the well-tolerated toxicity profile of poly-ICLC and its possible activity in GBM. In fact, the NABTT (New Approaches to Brain Tumor Therapy) Consortium has an ongoing Phase II trial of RT plus TMZ followed by adjuvant TMZ and poly-ICLC in patients with newly diagnosed GBM (see <http://www.nabtt.org/protocols/poly.htm>). The question arises whether poly-ICLC and TMZ would augment or interfere with each other's efficacy. It has been traditionally thought that chemotherapy may counteract the cellular processes needed to produce an immune response; however, recent data suggest that chemotherapy may augment immune effects through preferential elimination of regulatory components or amplification of antigen exposure following cytotoxic cell damage. It appears that the immune system recovering from a cytotoxic insult may be acutely activated and particularly responsive due to both stimulatory cytokines and reduced regulatory elements [40–42]. Nevertheless, this area of research has been devoted to the combined use of cytotoxic agents and vaccine therapy rather than to agents such as poly-ICLC. On the other hand, it does appear that poly-ICLC may improve the efficacy of anti-CNS tumor peptide-based vaccinations by augmenting the

overall immune response to the vaccine [43]. Moreover, the pilot study discussed in the introduction, which used poly-ICLC in newly diagnosed or recurrent malignant gliomas did allow for concurrent use of CCNU; however, while the patients who took CCNU experienced no additional toxicities, they had similar outcomes to those who did not take it.

The limitations of this single arm phase II study design include the fact that the study stopped accrual due to a change in the standard of care thereby limiting sample size and power of the study. Another limitation is the potential for selection bias (young median age, high functional status, and few patients having biopsy only) that may yield more positive results; however, these variables were accounted for in the statistical analysis, and indeed the results are comparable to those previously described for the use of adjuvant chemotherapy. Lastly, as previously stated, transient enlargement of contrast enhancing disease with subsequent shrinkage has been reported during poly-ICLC treatment. Because of this possibility and lack of central radiological review, the protocol defined criteria for radiological progression under which the patient was discontinued from this treatment; however, these parameters may have led to further variability in assessing stability of disease leading to inconsistent results. In fact, the perceptible disparity in this study between a 6-month progression-free survival of 30% and the 1-year survival of 69% may in part be due to difficulties with appropriate interpretation of imaging and raise the question of whether pseudo-progression led to premature discontinuation of the study agent.

Conclusion

We report the results of a combined strategy of RT with the novel agent poly-ICLC for patients with newly diagnosed GBM. The combined therapy was relatively well-tolerated, with the expected toxicities of fatigue, myalgia, and pain at the injection site. Survival was improved compared to patients in a historical database who were treated with radiation alone without adjuvant chemotherapy. There was no survival advantage compared to historical studies using RT with adjuvant, non-TMZ chemotherapy. Based on this study, poly-ICLC seems to add a small survival advantage when given during and after RT, similar to that associated

with non-TMZ chemotherapy. The combined use of poly-ICLC with TMZ or vaccine-based therapy presents interesting possibilities for future investigations.

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References

- Stupp R et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352(10):987–996. doi:[10.1056/NEJMoa043330](https://doi.org/10.1056/NEJMoa043330)
- Stenning SP, Freedman LS, Bleehen NM (1987) An overview of published results from randomized studies of nitrosoureas in primary high grade malignant glioma. *Br J Cancer* 56(1):89–90
- Stewart LA (2002) Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient data from 12 randomised trials. *Lancet* 359(9311):1011–1018. doi:[10.1016/S0140-6736\(02\)08091-1](https://doi.org/10.1016/S0140-6736(02)08091-1)
- Fine HA et al (1993) Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults. *Cancer* 71(8):2585–2597. doi:[10.1002/1097-0142\(19930415\)71:8<2585::AID-CNCR2820710825>3.0.CO;2-S](https://doi.org/10.1002/1097-0142(19930415)71:8<2585::AID-CNCR2820710825>3.0.CO;2-S)
- Talmadge JE et al (1985) Immunomodulatory effects in mice of polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose. *Cancer Res* 45(3):1058–1065
- Levy HB et al (1980) Immune modulating effects of poly ICLC. *Ann N Y Acad Sci* 350:33–41. doi:[10.1111/j.1749-6632.1980.tb20604.x](https://doi.org/10.1111/j.1749-6632.1980.tb20604.x)
- Hubbell HR, Liu RS, Maxwell BL (1984) Independent sensitivity of human tumor cell lines to interferon and double-stranded RNA. *Cancer Res* 44(8):3252–3257
- Black PL et al (1992) Effect of tumor burden and route of administration on the immunotherapeutic properties of polyinosinic-polycytidylic acid stabilized with poly-L-lysine in carboxymethyl cellulose [Poly(I, C)-LC]. *Int J Immunopharmacol* 14(8):1341–1353. doi:[10.1016/0192-0561\(92\)90005-6](https://doi.org/10.1016/0192-0561(92)90005-6)
- Dick RS, Hubbell HR (1987) Sensitivities of human glioma cell lines to interferons and double-stranded RNAs individually and in synergistic combinations. *J Neurooncol* 5(4):331–338. doi:[10.1007/BF00148390](https://doi.org/10.1007/BF00148390)
- Rosenblum MG et al (1990) Growth inhibitory effects of interferon-beta but not interferon-alpha on human glioma cells: correlation of receptor binding, 2',5'-oligoadenylate synthetase and protein kinase activity. *J Interferon Res* 10(2):141–151
- Strayer DR et al (1987) Growth of astrocytomas in the human tumor clonogenic assay and sensitivity to mismatched dsRNA and interferons. *Am J Clin Oncol* 10(4):281–284. doi:[10.1097/00000421-198708000-00003](https://doi.org/10.1097/00000421-198708000-00003)
- Droller MJ (1987) Immunotherapy of metastatic renal cell carcinoma with polyinosinic-polycytidylic acid. *J Urol* 137(2):202–206
- Hawkins MJ, Levin M, Borden EC (1985) An Eastern Cooperative Oncology Group phase I–II pilot study of polyriboinosinic-polyribocytidylic acid poly-L-lysine complex in patients with metastatic malignant melanoma. *J Biol Response Mod* 4(6):664–668
- Krown SE et al (1985) Phase I trials of poly(I, C) complexes in advanced cancer. *J Biol Response Mod* 4(6):640–649
- Nakamura O et al (1982) Phase I–II trials of poly(ICLC) in malignant brain tumor patients. *J Interferon Res* 2(1):1–4
- Rettenmaier MA, Berman ML, DiSaia PJ (1986) Treatment of advanced ovarian cancer with polyinosinic-polycytidylic lysine carboxymethylcellulose (poly ICLC). *Gynecol Oncol* 24(3):359–361. doi:[10.1016/0090-8258\(86\)90313-6](https://doi.org/10.1016/0090-8258(86)90313-6)
- Theriault RL et al (1986) Evaluation of polyinosinic-polycytidylic and poly-L-lysine in metastatic breast cancer. *Cancer Treat Rep* 70(11):1341–1342
- Talmadge JE et al (1985) Hyporesponsiveness to augmentation of murine natural killer cell activity in different anatomical compartments by multiple injections of various immunomodulators including recombinant interferons and interleukin 2. *J Immunol* 135(4):2483–2491
- Ewel CH et al (1992) Polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose in combination with interleukin 2 in patients with cancer: clinical and immunological effects. *Cancer Res* 52(11):3005–3010
- Black KL et al (1992) Inflammatory leukocytes associated with increased immunosuppression by glioblastoma. *J Neurosurg* 77(1):120–126
- Levy HB (1985) Historical overview of the use of polynucleotides in cancer. *J Biol Response Mod* 4(5):475–480
- Levy HB, Levine AS (1981) Antitumor effects of interferon and poly ICLC, and their possible utility as anti-neoplastic agents in man. *Tex Rep Biol Med* 41:653–662
- Matsumoto M, Seya T (2008) TLR3: interferon induction by double-stranded RNA including poly(I:C). *Adv Drug Deliv Rev* 60(7):805–812
- Kim H et al. (2008) Double-stranded RNA mediates interferon regulatory factor 3 activation and interleukin-6 production by engaging Toll-like receptor 3 in human brain astrocytes. *Immunology Immunology* 124(4):480–488
- Balachandran S, Barber GN (2007) PKR in innate immunity, cancer, and viral oncolysis. *Methods Mol Biol* 383:277–301. doi:[10.1007/978-1-59745-335-6_18](https://doi.org/10.1007/978-1-59745-335-6_18)
- Salazar AM et al (1996) Long-term treatment of malignant gliomas with intramuscularly administered polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose: an open pilot study. *Neurosurgery* 38(6):1096–1103. doi:[10.1097/00006123-199606000-00006](https://doi.org/10.1097/00006123-199606000-00006) discussion 1103–1104
- Liu W et al (2007) Tumour suppressor function of RNase L in a mouse model. *Eur J Cancer* 43(1):202–209. doi:[10.1016/j.ejca.2006.08.024](https://doi.org/10.1016/j.ejca.2006.08.024)
- Lee SB et al (1994) Activation of the double-stranded RNA (dsRNA)-activated human protein kinase in vivo in the absence of its dsRNA binding domain. *Proc Natl Acad Sci U S A* 91(22):10551–10555. doi:[10.1073/pnas.91.22.10551](https://doi.org/10.1073/pnas.91.22.10551)
- Haines GKIII et al (1998) Interferon-responsive protein kinase (p68) and proliferating cell nuclear antigen are inversely distributed in head and neck squamous cell carcinoma. *Tumour Biol* 19(1):52–59. doi:[10.1159/000029974](https://doi.org/10.1159/000029974)
- Meurs EF et al (1993) Tumor suppressor function of the interferon-induced double-stranded RNA-activated protein kinase. *Proc Natl Acad Sci U S A* 90(1):232–236. doi:[10.1073/pnas.90.1.232](https://doi.org/10.1073/pnas.90.1.232)
- Chacko MS, Ma X, Adamo ML (2002) Double-stranded ribonucleic acid decreases c6 rat glioma cell proliferation in part by activating protein kinase R and decreasing insulin-like growth factor I levels. *Endocrinology* 143(6):2144–2154. doi:[10.1210/en.143.6.2144](https://doi.org/10.1210/en.143.6.2144)
- Minks MA et al (1979) Structural requirements of double-stranded RNA for the activation of 2', 5'-oligo(A) polymerase and protein kinase of interferon-treated HeLa cells. *J Biol Chem* 254(20):10180–10183
- Galabru J et al (1989) The binding of double-stranded RNA and adenovirus VAI RNA to the interferon-induced protein kinase. *Eur J Biochem* 178(3):581–589. doi:[10.1111/j.1432-1033.1989.tb14485.x](https://doi.org/10.1111/j.1432-1033.1989.tb14485.x)
- Hovanessian AG (2007) On the discovery of interferon-inducible, double-stranded RNA activated enzymes: the 2'-5' oligoadenylate

- synthetases and the protein kinase PKR. *Cytokine Growth Factor Rev* 18(5–6):351–361
35. Hovanessian AG (1991) Interferon-induced and double-stranded RNA-activated enzymes: a specific protein kinase and 2',5'-oligoadenylate synthetases. *J Interferon Res* 11(4):199–205
 36. Maluish AE et al (1985) Immunomodulatory effects of poly(I, C)-LC in cancer patients. *J Biol Response Mod* 4(6):656–663
 37. Macdonald DR et al (1990) Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 8(7):1277–1280
 38. Curran WJ Jr et al (1993) Recursive partitioning analysis of prognostic factors in three radiation therapy Oncology Group malignant glioma trials. *J Natl Cancer Inst* 85(9):704–710. doi:[10.1093/jnci/85.9.704](https://doi.org/10.1093/jnci/85.9.704)
 39. Stupp R et al (2002) Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. *J Clin Oncol* 20(5):1375–1382. doi:[10.1200/JCO.20.5.1375](https://doi.org/10.1200/JCO.20.5.1375)
 40. Chong G, Morse MA (2005) Combining cancer vaccines with chemotherapy. *Expert Opin Pharmacother* 6(16):2813–2820. doi:[10.1517/14656566.6.16.2813](https://doi.org/10.1517/14656566.6.16.2813)
 41. Wheeler CJ et al (2004) Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. *Clin Cancer Res* 10(16):5316–5326. doi:[10.1158/1078-0432.CCR-04-0497](https://doi.org/10.1158/1078-0432.CCR-04-0497)
 42. van der Most RG et al (2006) Cranking the immunologic engine with chemotherapy: using context to drive tumor antigen cross-presentation towards useful antitumor immunity. *Cancer Res* 66(2):601–604. doi:[10.1158/0008-5472.CAN-05-2967](https://doi.org/10.1158/0008-5472.CAN-05-2967)
 43. Zhu X et al (2007) Toll like receptor-3 ligand poly-ICLC promotes the efficacy of peripheral vaccinations with tumor antigen-derived peptide epitopes in murine CNS tumor models. *J Transl Med* 5:10. doi:[10.1186/1479-5876-5-10](https://doi.org/10.1186/1479-5876-5-10)