Adoptive cellular immunotherapy for the treatment of malignant gliomas

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Abstract

The median survival for adults with recurrent primary malignant gliomas is 56 weeks following surgery, radiation, and chemotherapy. Generally, reoperation can extend the median survival an additional 26–32 weeks. We have developed an aggressive treatment program that utilizes low doses of interleukin-2 (IL-2) combined with ex vivo activated killer cells (LAK) infused via an indwelling catheter placed into the surgical resection cavity. Autologous leukocytes were collected during a standard 3–4 h, outpatient leukapheresis procedure, then activated ex vivo for 4–5 days with high doses of IL-2. The treatment protocol consisted of two 2-week cycles of therapy over a 6-week period. Patients with stable disease or objective response on follow-up MRI scans were retreated at 3-month intervals. Acute and cumulative IL-2-related toxicities were observed, but limited, and included fever, headache and transient neurologic irritation. Corticosteroid levels and usage were strictly controlled during immunotherapy, although higher doses were used intermittently to mitigate toxicity. Biologic changes included lymphocytic infiltration, regional eosinophilia, tumor necrosis, and the localized production of IL-2, IFN-γ and IL-12, demonstrated by in situ hybridization and immunohistochemistry. *Summary:* IL-2 plus autogeneic LAK cells can be safely administered intracavitary to treat high grade primary brain tumors with limited toxicity within the central nervous system. Six out of 28 patients had long-term survival of greater than 2 years post-reoperation plus immunotherapy with 2 patients alive over 8 years. The presence of a marked regional eosinophilia appeared to correlate with increased survival and may be predictive of a biologic and therapeutic response. Regional adoptive immune therapy was well tolerated and should be considered an option for patients with high-grade tumors refractive to standard therapeutic approaches. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

An estimated 25 000 new cases of primary central nervous system (CNS) cancer occurs each year in US, representing approximately 3% of all cancers [1]. The estimated annual number of deaths from primary brain tumors is 80%. In children, brain tumors are the third leading cause of death and second leading cause of cancer deaths. Moreover, our observations indicate that there has been a steady increase in incidence of high-grade tumors in both adults and children, that cannot be explained by more sensitive detection methods alone.

Despite advances in surgery, radiation and chemotherapy, malignant neuroectodermal tumors carry a poor prognosis with a median survival of only 51 weeks for high-grade gliomas in adults [2–4]. Aggressive second surgery and additional therapy can extend the median survival an additional 26–32 weeks [5–7]. Innovative approaches to the treatment of this disease are desperately needed. The introduction of new chemotherapeutic drugs, relevant to the CNS has been severely limited [8,9]. Only one new drug has ever been approved directly for the use against primary brain tumors, temozolomide [10]. We have developed an aggressive treatment program that utilizes low doses of interleukin-2 (IL-2) combined with ex vivo activated killer cells (LAK) infused via an indwelling catheter placed into the surgical resection cavity [11–14].

One of the most encouraging alternatives in cancer therapy has been the application of immune modulating therapies against refractory cancers. The molecular cloning of biological agents, such as recombinant interleukin-2 (rIL-2), [15,16], which are naturally produced as part of the host's immune defense mechanism, has fostered the clinical application of these factors for antitumor therapy [17–23]. In vitro, high concentrations of IL-2 can activate a subpopulation of peripheral blood leukocytes (PBL) transforming them into cytotoxic cells termed lymphokine-activated killer (LAK) cells [24,25], which are effective in many tumor models [26–32]. LAK cells can lyse natural killer (NK) cell-resistant, fresh or cultured tumor target cells in vitro including malignant gliomas [33,34]. Although unactivated PBL from brain tumor patients generally can not lyse autologous glioma cells in vitro, IL-2-activated PBL, or LAK cells, are capable of lysing autologous, as well as allogeneic glioma, but not normal host tissue including normal brain.

However, due to the tremendous toxicity associated with therapeutic levels of intravenous (IV) IL-2, regional IL-2 administration has been utilized as an alternative in order to target higher concentrations of IL-2 to a localized tumor region. Thus, regional routes of administration were likely to be more efficacious, with less toxicity [35,36].

The treatment era of the late 1980s–early 1990s saw several studies reporting the effects of regional immune therapy, including IL-2 plus LAK cells on the treatment of malignant gliomas [11,37–53]. Although partial and complete responses were observed by several groups, the overall median survivals were initially not encouraging. Protocols administering intraventricular and intrathecal IL-2 and either autologous, or allogeneic, LAK cells were designed for patients with meningeal disease; also with some limited success [13,54–56].

The systemic delivery of IL-2 in concentrations necessary to maintain LAK cell viability and recruit CTL

had inadvertently caused severe CNS toxicity when brain neoplasms were present, and most protocols included the presence of a brain metastasis as an exclusion criterion for systemic IL-2 therapy. In addition, the majority of cultured lymphoid cells injected IV into animals are sequestered in the lung, spleen and liver [31,57]. These findings suggest that local, intracavitary or regional administration of IL-2 plus LAK cells may be highly beneficial, if not essential, for a favorable antineoplastic effect, for CNS solid tumors. The systemic administration of rIL-2 and LAK cells for the treatment of CNS neoplasms was thus unlikely to generate a satisfactory anti-tumor response due to inadequate delivery with significant or unacceptable systemic and CNS toxicity.

2. Materials and methods

2.1. Phase I/II patient selection

2.1.1. Eligibility criteria

Adult patients with histologically or radiographically confirmed primary, recurrent or progressive malignant brain tumors were considered for this treatment. All patients undergoing surgical biopsy or tumor debulking had a contrast enhanced scan within 48 h of surgery to evaluate the presence of residual tumor. The post-operative Karnofsky performance status (KPS) for inclusion had to be equal to or greater than 60 (ECOG 0–2) prior to treatment. All patients had preserved cognitive function and gave signed informed consent prior to the initiation of their participation in this therapy.

Adequate organ function was defined as (a) Granulocytes $> 1500/mm^3$, platelet count $> 50\,000/mm^3$ PT and PTT within the normal range; and, (b) Bilirubin within $1.5 \times$ normal; creatinine < 1.5 mg/dl or calculated creatinine clearance greater than 60 ml/min.

Patients had adequate peripheral veins to permit leukapheresis through these sites or a femoral vein catheter was inserted just prior to leukapheresis. Patients could not have received radiotherapy, chemotherapy, or immunotherapy within the earlier 6 weeks and had to have recovered from the toxic effects of those treatments. Concurrent antineoplastic therapy was not permitted.

2.1.2. Exclusion criteria

Patients were excluded for a history of prior neurologic disease unrelated to their tumor, significant psychiatric illness, or impaired cognitive function; significant intercurrent medical problems, or evidence of active infection requiring antibiotic therapy. Patients were also excluded for positivity for hepatitis B surface antigen, human immunodeficiency virus, or a history of prior autoimmune disease.

2.2. Treatment summary

All patients had an Ommaya reservoir inserted into the tumor resection cavity during a standard neurosurgical procedure, generally performed during reoperation or tumor biopsy. The reservoir was placed with the catheter tip in the cavity of the tumor resection. In addition, the neurosurgeon removed the tip of the catheter on an angle and enlarged 2–3 openings nearest the catheter tip.

Each patient had the sutures removed following craniotomy and had no evidence of wound infection or fluid under the operative scalp flap precluding palpation of the reservoir dome. Leukapheresis was performed as a standard outpatient procedure in the Blood Bank unit for a yield of 10^9–10^{10} peripheral blood mononuclear cells (PBMC). The cells obtained were incubated, ex vivo, with IL-2 for 3–4 days to generate LAK cells. A final product of between 5×10^8 and 5×10^9 activated LAK cells were readministered with 1.2 million IU (MIU) of IL-2 via the reservoir on treatment day 1. IL-2 alone was given on days 3, 5, 8, 10 and 12 to complete one cycle of therapy. This 12-day cycle was repeated following a 2-week period for rest/recovery, evaluation, repeat leukapheresis and lymphocyte activation. Each two-cycle regimen constituted one full course of therapy.

Patients were treated with the initial doses of IL-2 plus LAK cells in the hospital at their physician's discretion, depending on the size and location of the tumor. The remaining doses of IL-2 alone were then administered during an ambulatory procedure, with 1–2 h of observation, depending on the patient's tolerance to the initial doses of IL-2.

Prior to LAK cell activation and leukapheresis, an attempt was made to reduce and eliminate corticosteroid usage, so that the optimal dose level of corticosteroids prior to and during immunotherapy would be less than 0.15 mg/kg per day dexamethasone equivalents. Patients were also evaluated for evidence of infection and were afebrile for 3 days prior to leukapheresis.

2.3. Generation of LAK cells

2.3.1. LAK cell activation

LAK cells were harvested and activated according to a modification of the procedures described by Muul [58] and Yannelli [59], and the FDA's Center for Biologics Evaluation and Research (CBER), 'Points to Consider in the Collection, Processing and Testing of Ex-Vivo Activated Mononuclear Leukocytes for Administration to Humans', 1989.

Leukapheresis was generally performed once for each cycle, for an estimated 3–4-h, on day 4, no earlier than 8 post-operative days. Citrate (ACD) was used as an anticoagulant. The PBMC obtained were activated ex

vivo with 6000 IU/ml IL-2 (Cetus Oncology, Chiron Corp., Emeryville, CA) in serum-free X-Vivo 10 lymphocyte activation medium (BioWhittaker, Inc., MD), which contained gentamicin as the antibiotic. This medium was further supplemented with 3 mM L-glutamine. The laminar flow biohazard hood and the incubator used to generate patient LAK cell cultures have been dedicated to this purpose.

The washed cytapheresis product was further separated by a density separation procedure using sterile Lymphocyte Separation Medium (LSM), or Accu-Prep, with centrifugation. The buffy coat was washed and resuspended in activation medium, counted, and transferred to gas-permeable, Fenwal Life-cell culture flasks, at a final cell concentration of $1.5–2.0 \times 10^6$ cells per ml. The cells were cultured for 3–4 days, without a shaker or roller apparatus, in a humidified 7% CO_2 incubator at 37°C to generate LAK cells.

2.3.2. Preparation of LAK cells for reinfusion

At the beginning of the harvest each culture bag was sampled, and an aliquot was sent for a STAT gram stain; clinical microbiological and sterility cultures including anaerobic and fungal cultures were also performed. No LAK cells were reinfused until STAT gram stains were verified to be negative.

The final LAK cell product was centrifuged to reduce its volume; the cells were recounted to confirm dose and cell viability, and aliquots were frozen for immunologic monitoring and quality control. A final microbial growth culture was performed on the injectate.

Between 1×10^8 and 1×10^9 harvested activated LAK cells were readministered over approximately 10 min together with the 1.2 MIU dose of IL-2 via the CNS reservoir with the volume of infusate not exceeding 4 cc. The infusion of LAK cells was performed on the first day of each of the two cycles.

Aliquots of activated LAK cells and baseline pre-activated PBMC were frozen, analyzed for surface marker phenotype, and tested for cytotoxic function. All cell counts, and reagent lots used were recorded.

2.4. Immune monitoring and function

2.4.1. Cytotoxicity assays

NK cells, CTL and activated LAK cells are distinguishable on the basis of target cell sensitivity to effector killing. K562 cells (NK and LAK sensitive), as well as Raji and Daudi cells (NK cell-resistant, LAK cell sensitive) were used for target cells. Glioma cell lines (U373) and autologous tumor cultures were tested for the presence of specific lysis, when available from prior surgical specimens.

2.4.2. Intracavitary fluid monitoring

Fluid was withdrawn for evaluation via the Ommaya reservoir immediately prior to each IL-2 administration. The reservoir was pumped gently 5 times over 2 min to ensure adequate mixing of the intracavitary fluid compartments.

Cell-free fluid from the Ommaya aspirates was analyzed for the presence of cytokines, including IL-2 and IFN-γ, and cytokine levels were quantitated using ELISA. Aliquots of the aspirate fluids were sent for WBC differential analysis, and the cells retrieved from the remainder of the fluid were expanded in IL-2 for 7–10 days, then tested for cytotoxicity, when adequate numbers of cells were available.

3. Results

3.1. Adult phase I/II study summary

In a Phase I/II clinical trial, adult patients received LAK cells plus IL-2 via an Ommaya reservoir implanted in the tumor cavity following surgical tumor debulking of their recurrent malignant glioma. Leukapheresis was used to obtain autologous leukocytes, which were activated with IL-2 ex vivo. These cells were readministered as LAK cells with IL-2 on the first day of treatment, via an Ommaya reservoir implanted at the time of reoperation. Additional IL-2 alone was given for a total of three times weekly for 2 weeks to complete a single cycle. A second cycle of IL-2 plus LAK cell therapy was given following a 2-week period for evaluation. Following the initial, post-operative cycles of therapy, patients showing stable disease or response to treatment, were retreated at 3 month intervals, when treatment was not limited by the development of toxicity [11].

3.2. Tissue samples

Five patients were reoperated subsequent to immune therapy for tumor recurrence, and autopsies were performed in two additional patients. Viable tumor with necrosis and gliosis was present in the intracavitary area of IL-2 plus LAK cell administration with tumor recurrence tending to spread from the periphery of the cavitary area. Mononuclear cell infiltrates were present with pronounced perivascular cuffing. Eosinophils were noted in the tissues of five out of seven these patients (Fig. 1A and B). In contrast, neither mononuclear infiltrates nor eosinophils were noted in the operative specimens of these patients prior to IL-2 plus LAK cell therapy.

Although, mononuclear infiltrates may be observed in some malignant gliomas, this phenomenon is extremely variable and when noted is generally not of
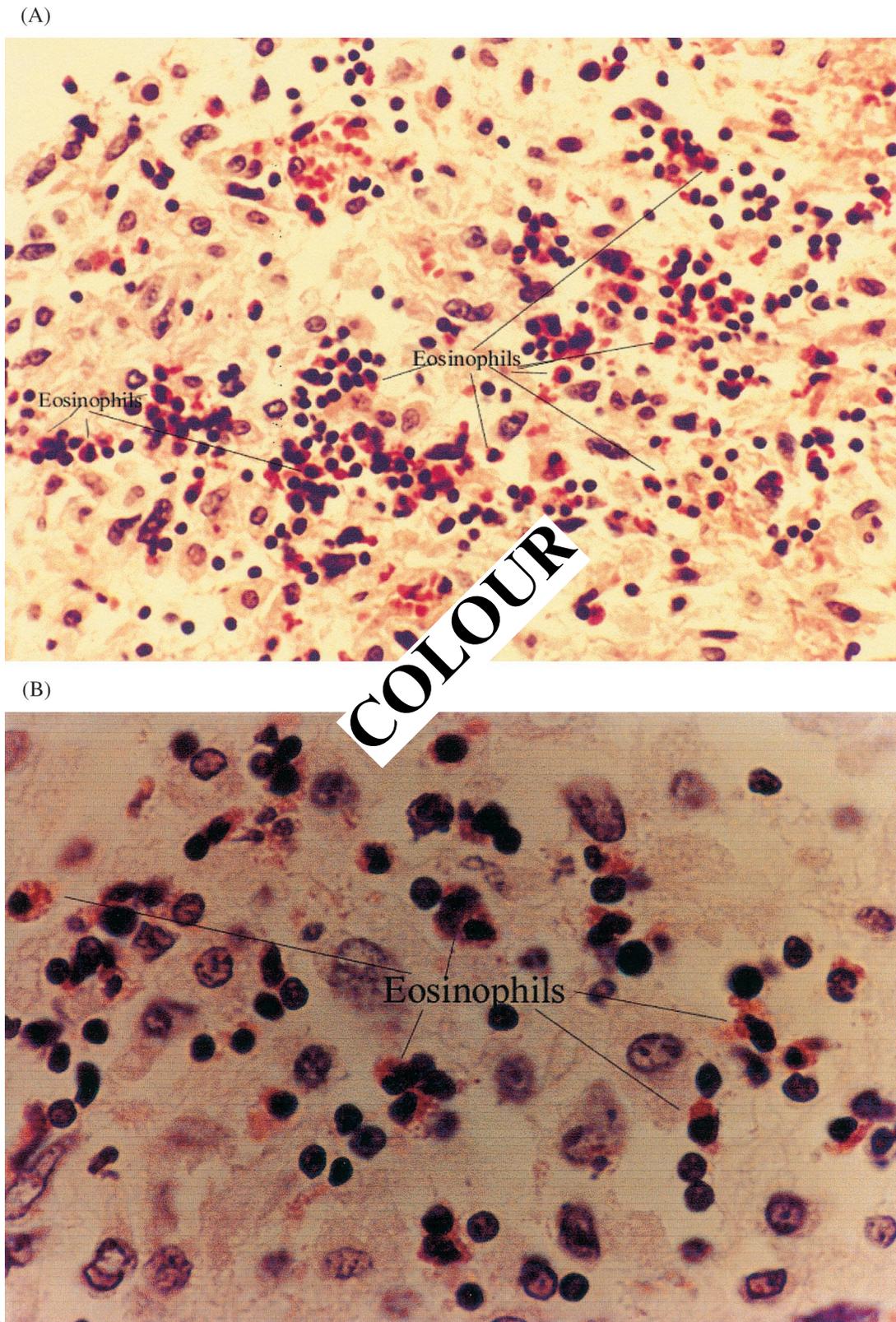
Fig. 1. Operative specimens from glioblastomas post-IL-2 plus LAK cell immunotherapy demonstrated mononuclear cell infiltrates with marked eosinophilia. Immunohistochemistry for GFAP, glial fibrillary acidic protein, with Weigert's iron hematoxylin stain for eosinophils; (A) 200X magnification, (B) 400X magnification.

great magnitude. The consistent appearance of a marked cellular infiltrate in each of these cases is of particular interest in view of the lack of such findings in tumor specimen(s) from surgeries, in the same patients, prior to IL-2 plus LAK cell therapy.

Immunohistochemical analyses of the post-immunotherapy tumor tissue sections revealed activated lymphocyte subpopulations and the local expression of cytokines, including IL-12. The expression of IL-2 and IFN-γ was confirmed by in situ hybridization using cytokine-specific probes.

3.3. Cytotoxicity assays

Although we were not able to recover cytotoxic cells from the aspirates of all patients, some treated patients generated a population of killer cells capable of lysing autologous tumor (Table 1). In three patients, cells obtained during the course of therapy from the reservoir aspirates, or from lumbar puncture (one patient), were expanded in culture in IL-2 (50 IU/ml) and assayed for cytotoxicity. In three of the patients, the aspirate cells preferentially lysed autologous tumor (Exp.1, LON; Exp.2, PFE; Exp.3, REG). In the other patients for whom autologous tumor was not available, lysis of allogeneic tumor targets was extremely low

suggesting that the cytotoxic cells were not residual LAK cells (Exp. 2, PAU, and REG).

In a similar clinical trial, ten pediatric patients with disseminated primary posterior fossa tumors were treated with intraventricular IL-2 plus LAK cells. Some patients demonstrated a substantial increase in circulating NK cell activity after the first cycle of treatment. In addition, fresh PBMC became able to lyse the NK-resistant Daudi target cell line, if 50 IU/ml of IL-2 was present during the 4 h ^{51}Chromium release assay. This IL-2 inducible killing persisted in one (clinically responding) patient for over a year (Table 2).

3.4. Aspirate differentials

Prior to each infusion of LAK cells plus IL-2 or IL-2 alone, fluid was generally recoverable from the intracavitary space via the reservoir. A saline flush was used when fluid could not be easily obtained. Although absolute numbers could not be calculated, qualitative changes in WBC differentials could be observed.

The changes in differential counts compared with the pre-treatment baseline, consisted of the appearance of eosinophils, and in some cases a progressive increase in the percentages of eosinophils during the course of therapy (Figs. 2 and 3). Of the 14 Grade, four adult

Table 1
Specific lysis of autologous glioma tumors

Experiment number	Patient code	Effector to target ratio	% Specific lysis — Target cells				
			Daudi	Raji	K562	PFE	LON
1	LON[a]	50:1		8	73	12	31
		25:1		5	55	6	20
		12.5:1		3	36	0	13
			Daudi	Raji	K562	PFE	LON
2	LON[a]	50:1	24	27	43	12	18
		25:1	13	26	39	10	8
		12.5:1	4	21	32	11	7
	PFE[a]	50:1	17	0	3	19	0
		25:1	0	0	1	15	0
		12.5:1	0	0	0	7	0
	PAU[b]	50:1	25	0	0	7	0
		25:1	18	0	0	6	0
		12.5:1	7	0	0	3	0
	REG[a]	50:1	0	1	4	8	0
		25:1	0	0	4	2	0
		12.5:1	0	0	3	0	0
			Daudi	Raji	K562	U373	REG
3	REG[a]	50:1	16	11	49	12	45
		25:1	10	8	35	6	22
		12.5:1	7	5	21	5	16

[a] Ommaya reservoir aspirate cells from three different patients cultured for 7–10 days in vitro with 50 IU/ml IL-2 tested as effector cells against NK-sensitive (K562), NK-resistant (Daudi, r Raji), a glioma cell line (U373), or autologous tumor (indicated in bold) target cells.

[b] Mononuclear cells collected by lumbar puncture from patient PAU tested for their ability to lyse tumor target cells. Autologous tumor was not available for this analysis.

Table 2
IL-2 inducible LAK-like activity in fresh peripheral blood following repetitive intraventricular, regional IL-2[a]

Effector to target ratio	Daudi		K562	
	−IL-2	+IL-2 4 h	−IL-2	+IL-2 4 h
50:1	14	52	63	69
25:1	13	35	54	64
12.5:1	9	24	47	61
6.3:1	5	15	38	49

[a] Responder cells were obtained from a patient by leukapheresis during the fourth course of therapy, 9 months after the initiation of immune therapy. Uncultured PBMC were placed directly into a 4-h ^{51}chromium release assay to assess the effect of regional IL-2 on peripheral NK cell function. In addition, low dose (50 IU/ml) IL-2 was added to replicate wells during the 4-h assay to determine, if LAK cell function was present.

patients evaluable after immunotherapy, eight showed greater than 10% eosinophils in the Ommaya aspirates at some timepoint during therapy or follow-up. One patient did not have sufficient aspirate samples to make a determination. In addition, three lumbar punctures were performed in two patients due to the development of fever and headache associated with meningeal signs; in each instance eosinophils were also present in the lumbar CSF.

Regional CSF eosinophilias were also noted in patients receiving intraventricular immune therapy. One patient presented with a WBC differential of 77% eosinophils in the aspirate fluid, which persisted throughout the treatment course. This regional

eosinophilia was confirmed in three out of five of the other patients. However, only two out of six of the patients demonstrated an increase in peripheral eosinophils (33 and 19%, respectively). This finding was in contrast to the protocol for adults, in which we noted regional intracavitary and tissue eosinophilia, but did not observe an effect on peripheral eosinophil numbers.

3.5. Survival

For the 15 adult patients with Grade 4 gliomas enrolled in our Phase I/II trial, the overall median survival as of the date of study closure, was 53 weeks (mean 70.1 ± 13.9 weeks S.E.M.), with four out of 15 of these patients still alive at the time of the published report [11]. Grade 3 (anaplastic astrocytoma) patients, who statistically live longer than Grade 4 (glioblastoma) patients, were treated but not included in the calculation of overall survival. In the Grade 4 group there was one complete response (CR), two partial responses, and one minor response with long-term stabilization of disease (greater than 3 years). In the Grade 3 group there was 1 CR.

The median survival of a contemporary group of reoperated, non-randomized patients, with similar preoperative Karnofsky scores, receiving chemotherapy, post-reoperation was 26 weeks (27.8 ± 3.6 weeks S.E.M.). This survival was similar to historical published series, as well as our institutional database [5–7]. This 'control' patient group was similar with respect to age, and histology (recurrent Grade 4 gliomas), and
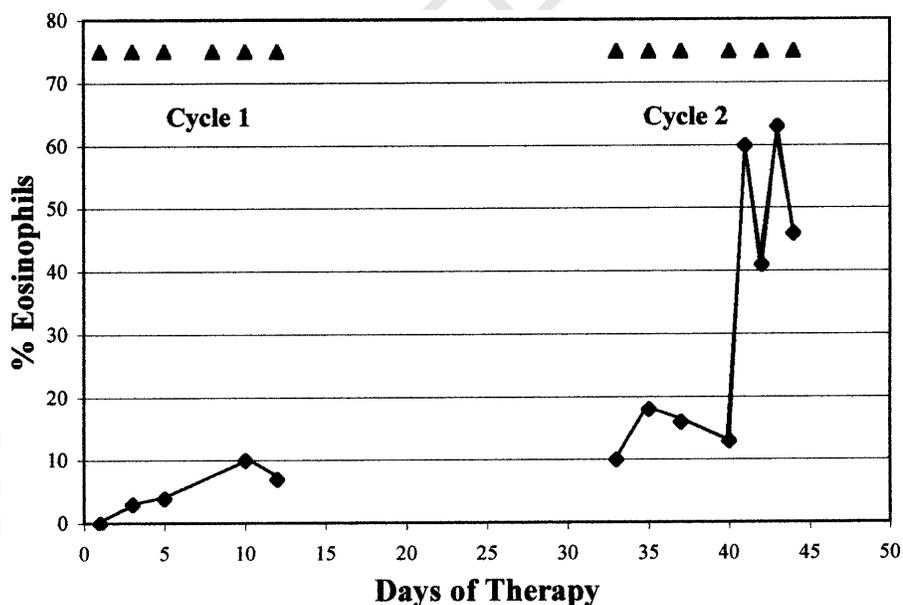

Fig. 2. WBC differentials from Ommaya reservoir aspirates removed during immunotherapy demonstrated a regional change in eosinophils over time. Fluid samples were removed prior to each IL-2 infusion. The second cycle of therapy demonstrated a marked increase in eosinophils that was not noted during the first cycle. Each IL-2 infusion is represented by the filled triangle at the top of the graph.
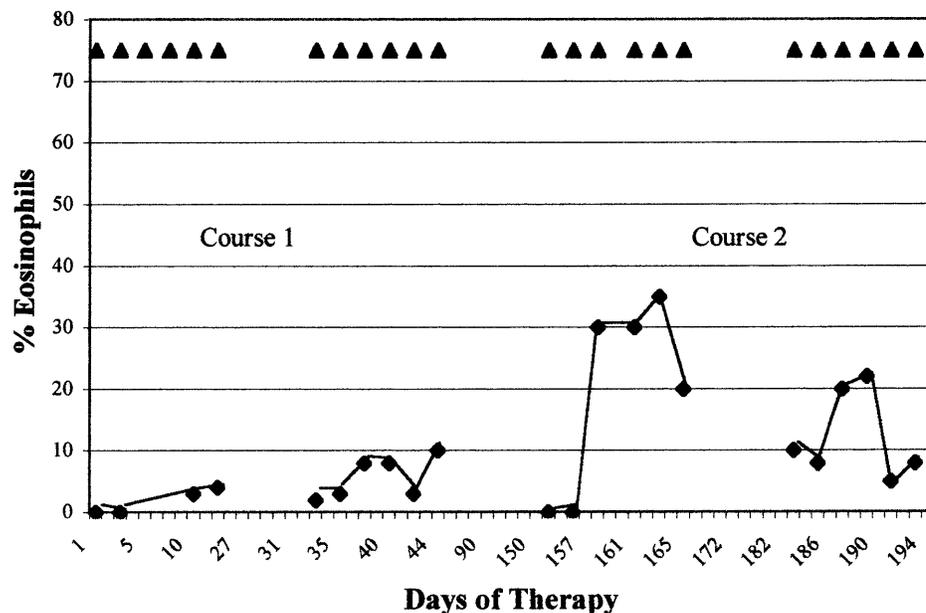
Fig. 3. WBC differentials from Ommaya reservoir aspirates removed during immunotherapy demonstrated a regional change in eosinophils over time in a different patient. Courses 1 and 2 are depicted. In this example, the eosinophil number returned to a baseline of zero between the first three cycles of treatment. However, following cycle 3 in course 2, the eosinophilia persisted and remained elevated between cycles 3 and 4. Each IL-2 infusion is represented by the filled triangle at the top of the graph.

were evaluated and entered onto study prior to re-operation, had equivalent extent of resections, and placement of an Ommaya reservoir, but did not receive immunotherapy.

There was also 1 CR in the pediatric intraventricular Phase I trial [13]. A [18-F]-deoxy-glucose positron emission tomographic (PET) scan was performed in this patient which demonstrated a hypometabolic area in the region corresponding to the residual thalamic enhancement, which was considered not to be consistent with viable tumor.

Using IL-2 plus LAK cells, a greater than 2.5-fold increase in expected survival following reoperation was demonstrated for recurrent malignant glioma patients, while preserving good quality of life. Moreover, the two complete responders from the original treatment group were still alive in 1999, with no evidence of tumor recurrence. In this journal we describe the case report of a more recent responder [14].

4. Discussion

We initially chose to evaluate the biologic effects and toxicity of local IL-2 given in conjunction with LAK cells on the presumption that the combined administration of IL-2 plus LAK cells had a greater potential therapeutic efficacy than IL-2 alone [21,28,31]. The rationale for using a multiple bolus dose schedule of IL-2 was based on therapeutic, biologic, and clinical considerations. A potentially therapeutic regimen of

IL-2 plus LAK cells for the treatment of brain tumors was likely to require continuous or periodic IL-2 administration. The requirement for such a regimen has been demonstrated in the treatment of non-CNS neoplasms, and at least, in part, reflects the necessity of sustained or repetitive bolus IL-2 administration for the maintenance of LAK cell viability and cytolytic activity. In addition, optimum therapy with this regimen appears to be enhanced by repetitive cycles of administration.

The recruitment of CTL or TIL and the in situ activation of LAK cells may be important components of the anti-tumor effect generated by IL-2 and LAK cell therapy [60–62]. Higher maximal concentrations of IL-2 are achievable following bolus doses of IL-2 than are reached using continuous infusion protocols. High concentrations of IL-2 may be required for the in situ activation of LAK, although productive CTL generation may be noted at lower IL-2 concentrations. While the in situ activation of tumor-specific cytotoxic lymphocytes or TIL was desirable, it was not clear to what extent that mechanism of anti-tumor response was going to be relevant for primary brain tumors [63].

Similar to other regional approaches like brachytherapy, IL-2 plus LAK cell immune therapy is optimally directed against partially resectable tumors. In patients with recurrent glioblastoma, interstitial radioactive implants may provide an additional 52–49 week median survival for patients with KPS of at least 70 [64,65]. However, approximately 40% of patients undergoing interstitial implants required reoperation at a median of

33 weeks post-implant due the treatment-related necrosis [64,65]. Those patients undergoing additional surgery after implant had a significantly increased survival compared with those undergoing a single reoperation. Stereotactic radiosurgery approaches, which do not require reoperative surgery, have a median survival of 8–9 months post-treatment [66,67]. Our immunotherapy survival data are as compelling as survival following advanced radiotherapy techniques, and did not require reoperation due to the development of treatment-related necrosis. Ultimately, the combination of multiple modalities of therapy, such as stereotactic radiosurgery and immune therapy, may effect a synergistic increase in survival for this almost universally fatal disease.

Furthermore, it has generally been presumed that because of the blood-brain barrier, the brain is a relatively immunologically privileged site. However, intraventricular IL-2 plus LAK cells induced an increase in LAK-like activity in the systemic circulation. This finding may indicate that lymphoid cells communicate between the brain and the periphery, and that a true 'immunologic memory' response may be induced against primary brain tumors. This may also infer that IL-2 plus LAK cells might have a role in the prevention of metastatic dissemination and/or recurrence within the CNS in an adjuvant setting.

The appearance of an eosinophilic infiltrate in the CNS is most likely to be related to the repetitive presence of IL-2 (or induced by some secondary factor, such as IL-4 or IL-5). Although transient peripheral eosinophilia has been reported during systemic IL-2-based immune therapy [68–71], this protocol was the first to demonstrate this effect following regional administration within the CNS [11]. In addition, the presence of eosinophils has been described as a good prognosis indicator in several kinds of systemic cancers [72–76].

While the mechanism and significance of eosinophil entry into the CNS is not understood, the presence of eosinophils in these aspirates and tissues indicates a biologic effect at this dose level. Eight of the ten patients who had significantly longer survivals, had greater than 10% eosinophils in the aspirate fluids, and the other patient did not have sufficient follow-up due to patient refusal; none of the non-responders showed a significant eosinophilia. Thus, the presence of eosinophils appears to correlate positively with long-term survival in primary brain tumor patients treated with immune therapy.

Patients with malignant glioma represent a distinct, well-defined patient population, with an extremely poor prognosis. In addition, the presence of the blood-brain barrier, which excludes most unactivated lymphocytes, makes brain tumors a unique model to study the potential mechanisms of immune-based therapies and their

effectiveness against solid tumors, especially using regional routes of administration.

5. Conclusions

1. IL-2 and LAK cells can be administered intracavitary into the CNS to treat primary malignant gliomas, safely with acceptable toxicity.
2. The MTD of intracavitary IL-2 given in bolus doses on a M,W,F schedule was determined to be 1.2 million IU (MIU) per dose. The MTD of intraventricular IL-2 was 0.9 MIU/dose.
3. Regional IL-2 plus LAK cell immunotherapy resulted in a significant increase in patient survival. The overall median survival for patients with Grade 4 glioma was 53 weeks (mean 70.1 ± 13.9 weeks S.E.M.); and the 1 year survival was 54% (seven out of 13), compared with two out of 15 in a similar 'control group' of chemotherapy treated patients. In addition, four objective radiographic responses were observed — two CR and two PR. One PR was followed by long-term disease stabilization of greater than 3.5 years. Moreover, the two original CR patients are still alive without recurrence greater than 8 years later. There was also one CR in a patient with glioblastoma in the pediatric patient group that lasted for 17 months prior to relapse. Of note, this patient recurred locally at the site of the original thalamic lesion, without a recurrence of the leptomeningeal disease.
4. Local infusions of IL-2 plus activated killer cells can induce biologic changes, characterized by a regional eosinophilia, lymphocytic infiltration, and the in situ production of IL-2, IFN-γ, and IL-12.

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Biographies

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