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Detection of the *bcl-2* t(14;18) Translocation and Proto-Oncogene Expression in Primary Intraocular Lymphoma

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Abstract

PURPOSE—Primary intraocular lymphoma (PIOL) is a diffuse large B cell lymphoma that initially infiltrates the retina, vitreous, or optic nerve head, with or without central nervous system involvement. This study examined the expression of the *bcl-2* t(14;18) translocation, the *bcl-10* gene, and high expression of *bcl-6* mRNA in PIOL cells.

METHODS—Microdissection and PCR analysis were used to examine vitreous specimens in patients with PIOL for the presence of *bcl-2* t(14;18) translocations, the *bcl-10* gene, and expression of *bcl-6* mRNA. A medical record review was also conducted to determine whether the *bcl-2* t(14;18) translocation correlated with prognosis.

RESULTS—Forty of 72 (55%) PIOL patients expressed the *bcl-2* t(14;18) translocation at the major breakpoint region. Fifteen of 68 (22%) patients expressed the translocation at the minor cluster region. The *bcl-10* gene was detected in 6 of 26 (23%) patients, whereas 4 of 4 (100%) PIOL patients expressed higher levels of *bcl-6* mRNA compared with inflammatory lymphocytes. An analysis of clinical outcome in 23 PIOL patients revealed no significant association between *bcl-2* t(14;18) translocations and survival or relapse. However, patients with the translocation were significantly younger.

CONCLUSIONS—PIOL has unique molecular patterns of *bcl-2*, *bcl-10*, and *bcl-6* when compared with other systemic lymphomas. This study lays the foundation for future studies aimed at exploring the genotypic classification of PIOL based on the quantitative molecular framework of gene expression profiling, with the goal of providing useful adjuncts to the pathologic diagnosis of this complex disease.

Primary intraocular lymphoma (PIOL) is a subset of central nervous system lymphoma (PCNSL) in which malignant lymphoid cells invade the retina, vitreous, or optic nerve head.^{1,2} PIOL is usually a diffuse large B cell lymphoma (DLBCL) but occasionally presents as a T cell lymphoma.^{3,4} Sixty percent to 80% of patients with PIOL eventually have brain involvement, whereas 15% to 25% of PCNSL patients have ocular involvement.^{5–9} Because the clinical presentation often mimics uveitis, PIOL is considered a masquerade syndrome, and diagnosis requires pathologic confirmation, usually by vitrectomy.^{4,8–13}

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Although cancer prognosis has historically been based on clinical and laboratory findings, analysis of the expression of various gene translocations and proteins has burgeoned as a method for determining prognosis. This study examined the involvement of three genes—*bcl-2*, *bcl-6*, and *bcl-10*—in PIOL cells. Bcl-2 is an antiapoptotic molecule whose gene is located on chromosome 18.¹⁴ The immunoglobulin heavy chain (*IgH*) gene is located on chromosome 14. The *bcl-2* t(14;18) translocation brings the *bcl-2* gene under the control of the *IgH* enhancer, resulting in deregulated bcl-2 expression.¹⁵ Most (60%) translocations occur at the major breakpoint region (Mbr) located within exon 3, whereas 10% to 25% of translocations occur at the minor cluster region (mcr) located 20 kb downstream of the Mbr.^{16–18} On chromosome 14, the translocation occurs within the joining region of the *IgH* gene.^{16,19,20} Bcl-10 is a proapoptotic molecule with a caspase-recruitment domain. It is located at chromosome 1p22 and is involved in the t(1;14)(p22;q32) translocation typically associated with mucosa-associated lymphoid tissue (MALT) lymphoma.²¹ However, Bcl-10 is also seen in 24.6% to 49.2% of patients with DLBCL.²² Bcl-6, whose gene is located on chromosome 3q27, is a zinc finger transcriptional repressor and is involved in apoptosis and cell growth and differentiation.²³

This study also examined the role of the *bcl-2* t(14;18) translocation in PIOL survival and relapse. Because PIOL typically presents as a subtype of PCNSL, we hypothesized that PIOL cells express the *bcl-2* t(14;18) translocation and the *bcl-6* and *bcl-10* genes in a pattern similar to that seen in DLBCL and PCNSL. However, we did not predict that the *bcl-2* t(14;18) translocation correlates with PIOL prognosis, which is more likely related to age, CNS involvement, treatment selection, and genotypic subtypes.

MATERIALS AND METHODS

This study followed the tenets of the Declaration of Helsinki and was approved by the National Eye Institute Institutional Review Boards for human subjects. Informed consent was obtained from all patients.

Ocular Specimens

Vitrectomy specimens from 72 patients with diagnoses of PIOL were used for the microdissection and PCR analyses. Diagnosis was based on clinical findings and cytopathology identification of B lymphoma cells, as described previously.²⁴ In addition, infiltrating lymphocytes from four uveitic eyes were collected.

Microdissection of Ocular Specimens

Microdissection was performed on the Giemsa-stained cytology slides of the vitreous specimens either manually or with the use of laser capture microscopy (PixCell II; Arcturus, Mountain View, CA), as described previously.^{25,26} For PCR analysis, at least 15 atypical cells were required. All captured PIOL cells were immediately placed in a single-step DNA extraction buffer containing 0.5 mg/mL proteinase K for 24 hours, which provides the starting point for PCR amplification.

Detection of the *bcl-2* t(14;18) Translocation

To detect the *bcl-2* t(14;18) translocation, the PCR-amplifiable mixture contained 1 μ L microdissected DNA; 4 pmol ³²P-labeled sense primer for the Mbr (5'-TTA GAG AGT TGC TTT ACG TGG CCT-3') or for the mcr (5'-GAC TCC TTT ACG TGC TGG TAC C-3'); 4 pmol antisense primer CFW1 (5'-ACC TGA GGA GAC GGT GAC CAG GGT-3'); 4 nmol dNTP; 25 nmol MgCl₂; and 0.5 U AmpliTaq Gold DNA polymerase (AmpliTaq Gold Enzyme; Applied Biosystems, Foster City, CA) in a final volume of 10 μ L.²⁷ The cycling parameter for Mbr was as follows: initial incubation at 94°C for 9 minutes; 40 cycles of 94°C for 45

seconds, 55°C for 2 minutes, and 72°C for 2 minutes; and a final incubation at 72°C for 7 minutes. The cycling parameter for mcr was as follows: initial incubation at 94°C for 9 minutes; 35 cycles of 94°C for 1 minute, 55°C for 2 minutes, and 72°C for 2 minutes; and a final incubation of 72°C for 7 minutes. PCR products for the *bcl-2* Mbr and mcr analyses were separated on 3% agarose gels and visualized with autoradiography. Mbr was analyzed in 72 samples, and mcr was analyzed in 68 samples. Limited sample availability resulted in the difference in sample size for the mcr analysis.

Detection of the *bcl-10* Gene

To detect the *bcl-10* gene, the PCR-amplifiable mixture contained 1 μ L microdissected DNA; 32 P-labeled primers for the second exon of the *bcl-10* gene (sense, 5'-AGA CTG CCA ACT AAT AGT CAC GT-3'; antisense, 5'-AAG TAG TCT AAC AAT TTT CCA GCC C-3'); 4 nmol dNTP; 25 nmol MgCl₂; and 0.5 U AmpliTaq Gold Enzyme in a final volume of 10 μ L. The cycling parameter was as follows: initial incubation at 94°C for 9 minutes; 35 cycles of 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 45 seconds; and a final incubation at 72°C for 7 minutes. The PCR product was separated on 15% polyacrylamide gels and visualized with phospho imaging. The *bcl-10* analysis was performed on 26 samples.

Detection of *bcl-6* mRNA Expression

RNA was extracted from microdissected PIOL and uveitis cells (Pico-Pure RNA isolation kit; Arcturus). PCR was performed on the cDNA complement (SuperScript RNase H⁻ Reverse Transcriptase kit; Invitrogen, Carlsbad, CA). Primers were used for *bcl-6* and *18S*, a housekeeping gene. The PCR-amplifiable mixture contained cDNA, 4 pmol 32 P-labeled *bcl-6* primer pairs (sense, 5'-AGC AAG GCA TTG GTG AAG ACA-3'; antisense, 5'-ATG GCG GGT GAA CTG GAT AC-3') or 4 pmol 32 P-labeled *18S* primer pairs (sense, 5'-AGG AAT TGA CGG AAG GGC AC-3'; antisense, 5'-GGA CAT CTA AGG GCA TCA CA-3'), 4 nmol dNTP, 25 nmol MgCl₂, and 0.5 U AmpliTaq Gold Enzyme in a final volume of 10 μ L. The cycling parameter for *bcl-6* was as follows: initial incubation at 94°C for 9 minutes; 40 cycles of 94°C for 1 minute, 60.5°C for 1 minute, and 72°C for 2 minutes; and a final incubation at 72°C for 7 minutes. The cycling parameter for *18S* was as follows: initial incubation at 94°C for 9 minutes; 40 cycles of 94°C for 1 minute, 59°C for 1 minute, and 72°C for 2 minutes; and a final incubation at 72°C for 10 minutes. PCR products were separated on 15% polyacrylamide gels and semiquantitated with phospho imaging. Expression of *bcl-6* was determined by taking the ratio of *bcl-6:18S* expression with 1.0 used as the cutoff for normal *bcl-6* expression. Only four PIOL samples could be studied for RNA analysis.

Controls

To ensure the reliability of the PCR assays, we included a positive control (1 μ L positive DNA) and a negative control (diethylpyrocarbonate-treated water) in all reactions.

Medical Record Collection and Chart Review

From 1991 to 2003, the National Eye Institute received ocular specimens from 72 patients with diagnoses of PIOL. Patients were from the United States and other countries. The following information was obtained: demographics (age, sex, race); clinical findings at initial presentation (visual acuity, anterior chamber cell and flare, vitreous cells and haze, retinal examination, and fluorescein angiogram); results of diagnostic tests (neuroimaging, cerebrospinal fluid analysis, bone marrow biopsy, and ocular tissue biopsy); date of diagnosis; basis of diagnosis (tissue specimen); initial treatment regimens (chemotherapy and radiation); length of remission, if any; time to recurrence, if any; therapeutic regimens used for recurrence; and date and cause of death. Complete clinical data were available for 23 patients.

Statistical Analysis of the *bcl-2* t(14;18) Translocation and Disease Prognosis

To determine the relationship between detection of the *bcl-2* t(14;18) translocation and PIOL prognosis, several statistical analyses were performed. Outcome variables of interest included duration of survival and relapse. Duration of survival was defined as the time interval from the date of diagnosis to the date of death. Censoring was used for patients still alive at the time this analysis was performed. Relapse was defined as the requirement of more than one course of treatment (chemotherapy, radiation therapy) for disease remission. First, univariate analysis was performed. Age was examined with a two-sided *t* test, and survival was examined with the log-rank test. Because of censoring in the survival data, which biases the results of univariate analysis, it was necessary to use Cox regression analysis. After adjusting for age, analysis of survival was made from two-sided Wald statistics from Cox regression with age as a covariate. Relapse was determined by two-sided Wald statistics from the logistic regression analysis with age as a covariate.

RESULTS

Lymphoma cells were identified in the vitreous specimens of all 72 patients with PIOL. PIOL cells (Fig. 1), characterized by scanty basophilic cytoplasm, large nuclei, and prominent nucleoli, were selectively microdissected from the slides of vitreous specimens. DNA was then extracted from these cells. PCR analysis was performed to detect the presence of the *bcl-2* t(14;18) translocation and the *bcl-10* gene. Seventy-two vitreous specimens were examined for expression of the *bcl-2* t(14;18) translocation at the Mbr, and 68 specimens were examined for the *bcl-2* t(14;18) translocation at the mcr. The Mbr translocation was detected in 40 of 72 (55%) specimens, and the mcr was detected in 15 of 68 (22%) specimens (Fig. 2). It should be noted that 14 of 15 of these specimens were also positive at the mbr region, indicating overlap of the breakpoint regions.

Because of the quantity of material available of each sample, sufficient DNA was available in only 26 patients for further molecular evaluation. The *bcl-10* gene was detected in 6 of 26 (23%) patients (Fig. 3). Four of these six patients were also positive for the *bcl-2* t(14;18) translocation.

To assess *bcl-6* mRNA expression, PIOL and normal lymphocytes from ocular specimens of uveitic eyes were also microdissected. Only four patients had sufficient material for *bcl-6* gene analysis. In all four patients with PIOL, higher expression of *Bcl-6* mRNA was observed compared with mRNA samples isolated from the infiltrating lymphocytes of the four uveitic eyes or from mRNA isolated from the white blood cells of a healthy subject (Fig. 4). Three of these four patients also had the *bcl-2* t(14;18) translocation at the Mbr site.

The detailed clinical course was available for 23 immunocompetent patients; mean follow-up was 29 months (Table 1). The countries of origin of the patients included the United States, France, Puerto Rico, Brazil, Japan, and Canada. Sixty-one percent expressed the *bcl-2* t(14;18) translocation. Forty-eight percent of patients were older than 65. Sixty-one percent of patients had CNS involvement. Of the 14 patients who had CNS involvement, 12 had PCNSL at the time of diagnosis of PIOL, whereas in two patients CNS lesions developed during the course of the illness. The timing of the development of CNS disease was unclear from the final patient's record. Sixty-three percent of patients received radiation therapy (10–60 Gy), and 48% received methotrexate-based therapy (including systemic high-dose, intravitreal, and intrathecal therapy). Other systemic chemotherapies included vincristine, thiopeta, procarbazine, dexamethasone, acepromazine, and cyclophosphamide, hydroxydaunomycin, Oncovin, prednisone (CHOP). Relapse occurred in 50% of patients. Therapeutic regimens after relapse included systemic bischloroethylnitrosourea (BCNU), temozolomide, cisplatin, thiopeta, cytarabine (Ara-C), methotrexate, and stem cell transplantation; intrathecal

methotrexate, BCNU, Ara-C; intravitreal methotrexate; and radiation (Table 1). Too few patients received each therapy for adequate statistical analyses and conclusions about the specific therapeutic regimens. Survival ranged from 1 month to more than 84 months at the time of follow-up.

Patients who were positive for the *bcl-2* t(14;18) translocation were significantly younger (mean age, 58.9 years) than those who lacked the translocation (mean age, 73.9 years) ($P = 0.001$). After adjusting for age, no statistically significant difference was found in survival (hazard ratio, 2.33; $P = 0.478$) or relapse (odds ratio, 1.28; $P = 0.840$) between patients who did or did not have the *bcl-2* t(14;18) translocation.

DISCUSSION

This study used microdissection and PCR techniques to examine the expression of *bcl-2* t(14;18) translocation and the *bcl-10* and *bcl-6* genes. The *bcl-2* t(14;18) translocation occurs in 57% to 90% of patients with follicular lymphoma (FL) and in 9% to 30% of patients with DLBCL; 55% to 60% of translocations occur at the Mbr, whereas 10% to 20% occur at the mcr.^{16,28–35} There is no known correlation between the *bcl-2* t(14;18) translocation and *bcl-2* expression.^{28,29} We found that 55% of samples expressed the translocation at the Mbr and that 22% of samples expressed the translocation at the mcr. Fourteen of 15 (93%) of the samples that were positive for the mcr translocation were also positive for the Mbr translocation. This finding is expected given the proximity of these two regions, which leads to overlap among the primer pairs.

Studies have reported conflicting roles for the *bcl-2* t(14;18) translocation in patients with FL.^{33,36–40} However, a recent report of ¹³¹I-tositumomab therapy (an anti-CD20 antibody) for advanced stages of FL found that though there was no difference in outcome between patients with or without the translocation at baseline, this therapy resulted in the conversion of translocation status from positive to negative in 34 of 36 patients. Furthermore, negative translocation status correlated with clinical remission and predicted a durable complete remission.⁴¹ The only study involving PCNSL examined *bcl-2* expression as opposed to the translocation and found no effect on prognosis.⁴²

Conflicting reports have also been published on the role of the *bcl-2* t(14;18) translocation in DLBCL prognosis. The recent development of DNA microarray technology has led to a genomewide approach to the study of DLBCL and has resulted in three distinct molecular subtypes of DLBCL indicative of different functional stages of B cell differentiation.^{43–46} The first type expresses genes characteristic of germinal center B cells (GCB-DLBCLs), whereas the second type expresses genes normally seen during in vitro activation of peripheral blood B cells (ABC-DLBCLs). The third type, primary mediastinal B cell lymphoma (PMBL), is more heterogeneous and not well defined. The GCB group has a significantly better survival than the ABC and PMBL groups. In two recent articles, expression of *bcl-2* translocation and *bcl-2* protein was reported to be associated with a significantly worse outcome in the non-GCB groups but not in the GCB group.^{47,48} However, another report demonstrated overall survival was significantly worse for *bcl-2*-positive patients in the GCB group but not in the non-GCB groups.⁴⁹ Our study did not distinguish the genotypic classification or expression of the *bcl-2* protein of the PIOL samples.

Although there are known prognostic factors in PCNSL, such as age, immune status, initial performance status, and CSF protein levels, there are no known prognostic factors for PIOL. Our study did not find a role for the *bcl-2* t(14;18) translocation in determining length of survival or likelihood of disease relapse, though it is interesting to note that patients with the *bcl-2* t(14;18) translocation were significantly younger, a finding similar to that observed by

Gascoyne et al.²⁸ in patients with DLBCL. This may suggest a role for the translocation in accelerating disease presentation. In addition, this may suggest a need for aggressive therapy for younger patients with the translocation.

It is not surprising that there was no significant effect of the translocation on survival or occurrence of relapse. Because of the small number of patients affected by this disease, the numbers available for analysis are also small and may be inadequate. In addition, some of these patients received diagnoses in the early 1990s, when radiotherapy was considered the treatment of choice for PIOL and PCNSL.^{7,50} However, since that time, the benefits of systemic, intrathecal, and intravitreal chemotherapy and high-dose methotrexate have become more evident. These treatments have led to improved prognosis and survival of patients with PIOL. Furthermore, it is likely that the length of time to diagnosis and the extent of CNS involvement may play a more significant role in determining prognosis for patients with PIOL. Early systemic treatment of PIOL, before the onset of CNS involvement, results in better disease control and longer survival (39 months for isolated PIOL versus 24 months for PCNSL with ocular involvement).¹¹

Bcl-10 is a molecule that regulates apoptosis.²¹ Mutations resulting in truncated proteins (that have subsequently lost their apoptosis-inducing function) have been detected at low frequencies in patients with DLBCL.^{51,52} With the use of immunohistochemistry studies, Ohshima et al.²² detected bcl-10 in 24% to 50% of patients with DLBCL. They did not detect any significant effect of bcl-10 expression on DLBCL prognosis. No reports have been published of bcl-10 expression in patients with FL or PCNSL. Our study detected the *bcl-10* gene in 23% of PIOL samples. The relatively low expression could be related to the observation that *bcl-10* is most closely related to mucosa-associated lymphoid tissue (MALT) lymphoma, a low-growth fraction lymphoma usually related to chronic inflammation.⁵³ On the other hand, PCNSL and DLBCL each follow a more aggressive clinical course.²³ Thus, it is not surprising that *bcl-10* was detected at a low frequency in PIOL, a subset of PCNSL.

Although the International Prognostic Index is usually used to stratify DLBCL patients by level of risk, a number of cellular factors have been found to have prognostic significance in DLBCL. A phenotype similar to that seen in germinal center cells has been linked to a more favorable outcome. Lymphomas that are thought to have a germinal center origin express bcl-6 along with CD10.^{43,54}

Bcl-6 is required for the formation of normal germinal center B cells and T cell-dependent antigen responses.²³ It is also considered a proto-oncogene because of its involvement in translocations repeatedly found in DLBCL. *Bcl-6* rearrangements are found in 23% to 37% of patients with DLBCL.^{29,55–57} Lossos et al.⁵⁸ examined bcl-6 expression and found that overexpression was linked to better overall survival in patients with DLBCL. In DNA microarray analysis, the overexpression of *bcl-6* correlates with GCB-DLBCL, which is associated with better prognosis.^{43–45} Similar findings were also found in PC-NSL.⁵⁹ However, Chang et al.⁴² found that bcl-6-positive patients had poorer overall survival. In one PIOL study,⁶⁰ immunohistochemistry found that PIOL cells were positive for Bcl-6. Our analysis looked at mRNA and found higher expression of *bcl-6* mRNA in the PIOL cells of four selected patients who had sufficient samples for analysis compared with control and uveitis patients. This may support that PIOL cells have a germinal center origin, as also suggested by other studies.⁶¹

The current study provides important information about the expression pattern of several different apoptotic and cell differentiation genes in PIOL. In addition, it lays the foundation for the examination of p53, c-Myc, and other oncogenes in PIOL. However, it is important to recognize one important barrier that impeded this study. Working with vitreous specimens from PIOL patients provided a limited sample size (as few as 15 lymphoma cells per sample),

which made microarray analysis and other techniques such as quantitative PCR extremely difficult. Indeed, as evidenced by the few samples analyzed for *bcl-6* expression, mRNA from these lymphoma cells was extremely difficult to isolate. On the other hand, in studies of systemic and CNS lymphomas, larger amounts of tissue from biopsy specimens had been used. Thus, while great advances have been made in gene expression profiling for DLBCL, it is less likely that such a technique will be readily available for PIOL in the near future.

In summary, PIOL has unique molecular patterns of *bcl-2*, *bcl-6*, and *bcl-10* compared with other types of lymphomas, such as follicular lymphoma, DLBCL, or PCNSL. Gene expression seems more closely related to GCB-DLBCL. The *bcl-2* t(14;18) translocation is found significantly more often in younger patients with PIOL.

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FIGURE 1. Typical cytology of a PIOL cell

A characteristic PIOL cell (*arrow*) from the vitreous with basophilic cytoplasm and large prominent irregular nuclei.

FIGURE 2.

(A) Sample gel of PCR analysis for the *bcl-2* t(14;18) translocation at the Mbr. PCR amplification of DNA from microdissected cells from vitreous specimens of PIOL patients using primers for the *bcl-2* t(14;18) translocation at the Mbr. Lanes 1 to 5, PIOL samples; +, positive control; -, negative control. Seventy-two vitreous specimens were examined; 40 (55%) were positive. (B) Sample gel of PCR analysis for the *bcl-2* t(14;18) translocation at the mcr. PCR amplification of DNA from microdissected cells from vitreous specimens of PIOL patients using primers for the *bcl-2* t(14;18) translocation at the mcr. Lanes 1 to 3, PIOL samples; +, positive control; -, negative control. Sixty-eight vitreous specimens were examined; 15 (22%) were positive. In both (A) and (B), differences in migration through the gel were caused by variability where the translocation could occur in the J_H region of the *IgH* gene, resulting in different sizes of amplified product.

FIGURE 3. Sample gel of PCR analysis for the *bcl-10* gene

PCR amplification of DNA from microdissected cells from vitreous specimens of PIOL patients using primers for the *bcl-10* gene. *Lanes 1 to 5*, PIOL samples; *-*, negative control. Twenty-six vitreous specimens were examined; 6 (23%) were positive.

FIGURE 4.

Analysis of expression of *bcl-6* mRNA in PIOL cells compared with non-PIOL cells. Expression of *bcl-6* was examined by PCR amplification of the cDNA complement of mRNA from four PIOL samples, four uveitis samples, and one healthy control sample. Autoradiography was used to compare relative amounts of *bcl-6* and *18S* mRNA. All four PIOL patients had higher expression of *bcl-6*, but none of the non-PIOL patients had high *bcl-6* expression.

Clinical Outcomes of PIOL Patients

TABLE 1

Patient	Age (y)	Country	CNS Involvement	Initial Therapy			Relapse	Subsequent Treatment	Survival (mo)	<i>bcl-2</i> t(14; 18) Translocation
				Radiation	MTX	Other Chemotherapy				
1	76	France	+	—	+ (systemic)	—	—	N/A	1	—
2	63	United States	—	—	+ (intravitreal)	—	+	Intrathecal: MTX	56+	+
3	60	Puerto Rico	+	+	—	—	—	Intravitreal: MTX N/A	6	+
4	69	Japan	+	+ (30 Gy)	—	+ Systemic: CHOP	+	Radiation: 40 Gy	19	+
5	46	Brazil	+	+ (10 Gy)	—	—	—	N/A	64+	+
6	51	United States	+	—	+ (intrathecal)	+ Systemic: dexamethasone; Intrathecal: Ara-C	+	Systemic: BCNU, cisplatin, temozolomide, Ara-C	67+	+
7	77	Japan	—	+ (30 Gy)	—	—	—	Intrathecal: MTX, BCNU	11+	—
8	63	Japan	—	+ (26 Gy)	—	—	—	Intravitreal: MTX	19+	—
9	80	Japan	—	+ (35 Gy)	—	—	—	N/A	23+	+
10	48	United States	+	+ (54 Gy)	+ (intrathecal)	+ Systemic: Promace/ Cytabom	+	None	9	+
11	73	Japan	—	+ (30 Gy)	—	Intrathecal: Ara-C	—	N/A	34+	—
12	80	Japan	—	+ (39 Gy)	—	+ Systemic: CHOP	—	N/A	48+	+
13	40	Israel	+	U	U	—	U	U	20+	+
14	38	United States	+	+ (40 Gy)	+ (systemic high dose; intravitreal)	+	+	Systemic: MTX, stem cell transplantation; Radiation: dose unknown; Intravitreal: MTX	29+	+
15	67	United States	—	—	+ (systemic high dose)	—	—	N/A	12+	+
16	57	United States	—	—	+ (systemic high dose; intrathecal)	+ Systemic: vincristine, procarbazine	+	Radiation: dose unknown	33+	+
17	78	United States	+	+ (45 Gy)	U	—	U	Intravitreal: MTX	5+	—
18	58	United States	+	—	+ (systemic high dose)	+ Systemic: thiotepa, vincristine	+	Systemic: MTX	84+	+
19	79	United States	+	+ (60 Gy)	—	Intrathecal: Ara-C	+	Intrathecal: MTX, Ara-C Radiation × 2; 30 and 49 Gy	33+	—
20	47	India	+	+ (45 Gy)	+ (systemic high dose)	+ Systemic: vincristine, thiotepa, dexamethasone, Intrathecal: Ara-C	+	None	67+	+
21	74	United States	+	+ (unknown dose)	—	—	+	Systemic: thiotepa, vincristine, MTX, dexamethasone, bone marrow transplantation	12+	—
22	73	United States	—	—	—	—	—	Intrathecal: MTX and Ara-C	8	—
23	57	Canada	+	—	+ (systemic high dose)	—	—	N/A	6+	+

U, information unavailable; MTX, methotrexate; N/A, not applicable.