JAMA Ophthalmology | Original Investigation

Minimal Disseminated Disease in Nonmetastatic Retinoblastoma With High-Risk Pathologic Features and Association With Disease-Free Survival

Viviana E. Laurent, MS; Ana Vanesa Torbidoni, PhD; Claudia Sampor, MD; Daniela Ottaviani, BS; Valeria Vazquez, PhD; Mariano R. Gabri, PhD; Maria Teresa Garcia de Davila, MD, PhD; Marco A. Ramirez-Ortiz, MD, MPH; Cristina Noemi Alonso, PhD; Jorge Rossi, PhD; Daniel F. Alonso, MD, PhD; Guillermo L. Chantada, MD, PhD

IMPORTANCE Fatal metastatic relapse may occur in children with retinoblastoma and high-risk pathologic features (HRPFs). Minimal dissemination (MD) may be an additional tool for risk estimation. The use of cone-rod homeobox (CRX) transcription factor messenger RNA for MD evaluation in metastatic retinoblastoma was previously reported, but no data in nonmetastatic cases with HRPFs are available.

OBJECTIVES To evaluate whether MD is detectable in patients with nonmetastatic retinoblastoma and to assess its prognostic effect on disease-free survival (DFS).

DESIGN, SETTING, AND PARTICIPANTS This single-institution cohort study of patients with nonmetastatic retinoblastoma and HRPFs used prospectively defined inclusion criteria and a sampling strategy to procure bone marrow (BM) and cerebrospinal fluid (CSF) samples from May 1, 2007, through October 31, 2013. Median follow-up was 38 months (range, 8-89 months). Survival analysis was closed in December 2015, and no further updates were made after that point.

INTERVENTIONS The study evaluated CRX messenger RNA by quantitative polymerase chain reaction in BM and CSF at diagnosis and follow-up. In 14 patients, GD2 synthase was used instead of CRX for CSF evaluation. Patients were treated under uniform guidelines.

MAIN OUTCOMES AND MEASURES Metastatic relapse.

RESULTS The study included 96 children (median age at study inclusion, 26 months; range, 1-168 months; 46 male [47.9%]; 50 female [52.1%]) with nonmetastatic retinoblastoma and HRPFs (isolated massive choroidal invasion in 14, postlaminar optic nerve invasion in 51 [26 with concomitant massive choroidal and 13 with scleral invasion], 12 with scleral invasion without postlaminar optic nerve invasion, and 7 with tumor at the resection margin of the optic nerve) were evaluated at the time of primary or secondary enucleation. Minimal dissemination was detected in 9 patients (7 BM samples and 2 CSF samples) and was associated with extension beyond the resection margin of the optic nerve and scleral involvement, but only the former was independently associated (adjusted odds ratio, 57.0; 95% CI, 4.8-678.2; $P = .001$). In addition, MD occurred in 8 of the 43 International Intraocular Retinoblastoma Classification group E eyes with glaucoma (18.6%) and in 8 of 80 (10%) and 1 of 16 children (6.3%) who underwent primary or secondary enucleation, respectively. Children with MD had a 3-year DFS of 0.78 compared with 0.98 in those without MD (95% CI for the difference in DFS, 0.17-0.23; $P = .004$).

CONCLUSIONS AND RELEVANCE These findings identified a high-risk population of children with retinoblastoma and HRPFs with MD. Because the number of events was small, these results, which suggest that children with International Intraocular Retinoblastoma Classification group E retinoblastoma and glaucoma have a higher risk of MD at diagnosis, should not be considered definitive at this time.

JAMA Ophthalmol. doi:10.1001/jamaophthalmol.2016.4158
Published online October 27, 2016.

Invited Commentary

Supplemental content

Author Affiliations: Hematology-Oncology Service, Pediatric Hospital S.A.M.I.C. Prof. Dr. Juan P. Garrahan, Buenos Aires, Argentina (Laurent, Torbidoni, Sampor, Ottaviani, C. N. Alonso, Chantada); Pathology Service, Pediatric Hospital S.A.M.I.C. Prof. Dr. Juan P. Garrahan, Buenos Aires, Argentina (Vazquez, Garcia de Davila); Molecular Oncology Laboratory, Quilmes National University, Bernal, Buenos Aires, Argentina (Gabri, D. F. Alonso); Hospital Infantil de México Federico Gomez, Ciudad de México, Distrito Federal, México (Ramirez-Ortiz); Immunology Service, Pediatric Hospital S.A.M.I.C. Prof. Dr. Juan P. Garrahan, Buenos Aires, Argentina (Rossi).

Corresponding Author: Guillermo L. Chantada, MD, PhD, Hematology-Oncology Service, Pediatric Hospital S.A.M.I.C. Prof. Dr Juan P. Garrahan, Combate de los Pozos 1881, Buenos Aires, Argentina C1245AAM (gchantada@garrahan.gov.ar).

Even though the disease-free survival (DFS) from retinoblastoma is higher than 95% in developed countries,[1] up to 18% of patients may present with high-risk pathologic features (HRPFs) in the enucleated eye and are therefore at a higher risk of metastatic relapse.[2] The HRPFs include invasion of the postlaminar optic nerve (PLONI) with or without tumor at the resection margin, massive choroidal invasion, and scleral invasion.[2,3] Other features, such as anterior segment, focal choroidal, or prelaminar invasion, usually in combination, are also considered HRPFs that indicate the need for adjuvant therapy by some but not all groups; however, their risk of metastatic relapse is estimated to be lower than 3% after enucleation alone.[2,4] Most groups recommend adjuvant chemotherapy to decrease the risk of extraocular relapse in patients with HRPFs, but it is not 100% effective given that some patients relapse despite adjuvant therapy.[3,5] As a result, predicting the risk of metastatic relapse only by the presence of HRPFs is imperfect because there are lower-risk children who would be cured with enucleation alone rather than with unnecessary chemotherapy, and there are children with HRPFs who still relapse after conventional adjuvant therapy who constitute a very high-risk population currently not identifiable by pathologic criteria alone.

The detection of minimal dissemination (MD) as a tool for evaluating extent of disease has only been preliminarily reported in retinoblastoma.[6,7] Theoretically, MD should be present early in the disease course for patients with retinoblastoma to relapse in a metastatic site; accordingly, if left untreated or if resistant to treatment, it would invariably lead to metastatic relapse. The use of a molecular marker, such as GD2 synthase messenger RNA (mRNA) and other markers, in small patient cohorts has been previously reported.[8] More recently, a lineage-specific biomarker, cone-rod homeobox (*CRX*) (GenBank NM_000554.4), an Otx-like homeobox transcription factor critical for photoreceptor differentiation, was found in extraocular tissues[9] and later reported for the evaluation of MD in children with metastatic retinoblastoma.[6] Quantitative polymerase chain reaction (qPCR) evaluation of CRX mRNA is highly sensitive and specific because it is only expressed by cells from a retinal origin, which makes it an attractive biomarker for this tumor.[6,10] On the basis of our data from patients with metastatic retinoblastoma, we hypothesized that MD evaluation might be a tool for a more precise estimation of the risk of metastatic relapse in children with nonmetastatic retinoblastoma and HRPFs. We undertook this study with the following aims: (1) to evaluate whether MD is detectable in extraocular sites in patients with retinoblastoma and HRPFs, (2) to detect clinical or pathologic features associated with MD, and (3) to assess the prognostic effect of MD on DFS of children with retinoblastoma and HRPFs.

Key Points

Question Is minimal dissemination (MD) an effective tool for evaluating the extent of nonmetastatic retinoblastoma using quantitative polymerase chain reaction for the cone-rod homeobox transcription factor detected in cerebrospinal fluid or bone marrow in patients with high-risk pathologic features?

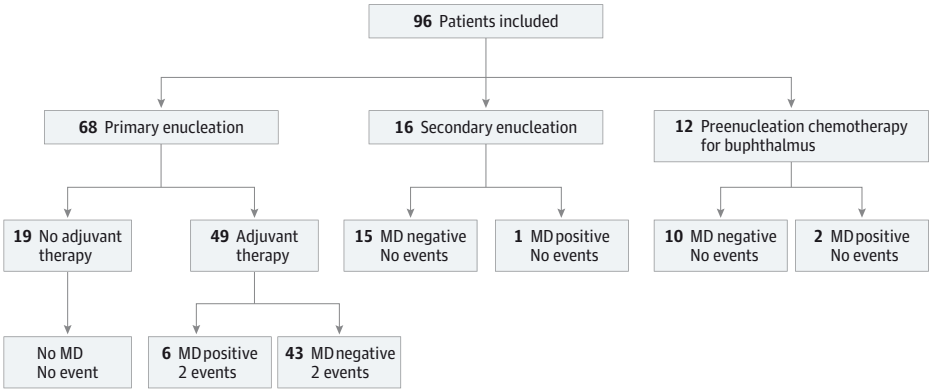
Findings In this cohort study of 96 children with retinoblastoma and high-risk pathologic features, 9 presented with MD, which was more commonly found in those with scleral compromise or invasion of the resection margin of the optic nerve and those presenting with glaucoma. Patients with MD had a lower rate of disease-free survival.

Meaning These data suggest that MD may be a tool for identifying higher-risk patients who need a more intensive approach.

Methods

This study had a prospectively designed sampling strategy for cerebrospinal fluid (CSF) and bone marrow (BM) for all consecutive children with nonmetastatic retinoblastoma and HRPFs diagnosed at our hospital from May 1, 2007, through October 31, 2013, to detect MD. Survival analysis was closed in December 2015, and no further updates were made after that point. The HRPFs were defined as massive choroidal invasion (isolated or in combination), PLONI (including those with tumor at the resection margin), or scleral involvement. Patients were divided into 3 groups for analysis (**Figure 1**): (1) patients

Figure 1. Flow of Patients Throughout the Study



MD indicates minimal dissemination.

undergoing primary enucleation, (2) patients undergoing secondary enucleation after failure of conservative therapy, and (3) patients presenting with massive buphthalmus who, according to treatment protocol guidelines, underwent planned enucleation after 2 to 3 cycles of neoadjuvant therapy.[11] In the last group, pathologic features were not considered evaluable because of the effects of chemotherapy masking pathologic findings.[12] The study was approved by the Pediatric Hospital S.A.M.I.C. Prof. Dr Juan P. Garrahan Institutional Review Board, and all procedures were performed in accordance with ethical standards and the Declaration of Helsinki.[13] On enrollment, all parents or guardians signed a written informed consent form that permitted the use of samples and clinical data for research purposes. Data were not deidentified.

The BM evaluation included 2 aspirate and 2 biopsy specimens from each posterior iliac crest,[8] which were performed when pathologic evaluation became available in enucleated eyes (usually within 2-3 weeks after enucleation) and within 7 days of diagnosis in patients with massive buphthalmus. All specimens were taken 24 to 48 hours before the initiation of adjuvant therapy and were assessed by standard cytologic and histopathologic analysis for staging purposes. Specimens for MD were preserved in a guanidinium-thiocyanate buffer at a 1:1.5 ratio of sample to buffer and frozen at −70°C until analysis, as previously reported.[7] Expression of CRX mRNA was evaluated by real-time qPCR as previously reported.[6] A 3- to 5-mL CSF sample was collected, and the collected specimen was divided into 3 vials: the first for cytologic analysis, the second for immunocytologic analysis (which was done in case the cell count exceeded 3/μL), and the third for PCR determinations as previously reported.[6,14] GD2 synthase mRNA was used for MD evaluation in 14 patients included in the initial phase of this study and was replaced by CRX mRNA in the remaining children. Patients underwent additional examinations at the end of adjuvant therapy (approximately 6 months after diagnosis) and at 12 months of diagnosis.

Patients in whom CRX mRNA was detected in the BM or CSF or those in whom GD2 synthase mRNA was detected in the CSF were considered to have MD. The CRX mRNA positivity was expressed as relative expression levels according to our previous work,[6] and GD2 synthase results (positive or negative) were reported as qualitative data.[7] The presence of MD did not influence any treatment decision. Specimens were processed in a masked fashion because the pathologic and outcome results were not known to the PCR operators (V.E.L., A.V.T.) Patients received a standardized therapy as per 2 successive prospective protocols.[3]

The design and analysis of the study were adherent with the Reporting Recommendations for Tumor Marker guidelines.[15] Contingency tables were constructed, χ^2 or Fisher exact tests were used for categorical variables, and the Mann-Whitney test was used for continuous variables. Forward stepwise logistic regression analysis was performed to identify variables that were independently associated with MD. Metastatic relapse (including central nervous system or systemic metastasis) was defined as an event, and DFS curves were calculated according to Kaplan-Meier analyses. Curve comparison was performed with the log-rank test. $P < .05$ was considered to be statistically significant. Survival status was updated to December 2015.

Results

A total of 192 children with newly diagnosed intraocular retinoblastoma were evaluated at our hospital during the study period, 107 children who met the inclusion criteria were included in this study, and 11 eligible patients were excluded because they could not be evaluated for MD (lack of consent for the use of stored material in 8 and preserved material not suitable for PCR analysis in 3). The remaining 96 patients (median age at study inclusion, 26 months; range, 1-168 months; 46 male [47.9%]; 50 female [52.1%]) underwent full evaluation (Figure 1) and had a median follow-up of 38 months (range, 8-89 months). Only 4 patients had less than 12 months of follow-up. Twenty-six (27.1%) had bilateral tumors, and 70 (72.9%) had unilateral tumors. The male to female ratio was 0.89. No mRNA could be obtained from the CSF at diagnosis in 35 patients (36.5%).

Of the study patients, 68 (70.8%) were included in the primary enucleation group and 16 (16.7%) in the secondary enucleation group. They received variable regimens for eye preservation, including systemic vincristine sulfate, etoposide, and carboplatin in 12 (followed by intra-arterial or intravitreal chemotherapy in 3) and only intra-arterial chemotherapy, including a combination of topotecan hydrochloride, carboplatin, and melphalan, in 4 patients. Twelve patients (12.5%) were included in the massive buphthalmus group.

The HRPFs included isolated massive choroidal invasion in 14 children, PLONI in 51 (26 of them with concomitant massive choroidal and 13 with scleral invasion), and 12 with scleral invasion without PLONI. Seven patients had tumor at the resection margin of the optic nerve.

In addition, we could obtain material from 18 additional patients without HRPFs who were considered an observational cohort not included in the study. None received any oncologic therapy after enucleation. No child in this group had MD, and none had an event.

Sixty-one of 80 patients (76.3%) evaluated at diagnosis presented with an International Intraocular Retinoblastoma Classification (IIRC) group E eye (eyes presenting with glaucoma, anterior segment invasion, or other features suggesting advanced disease), and 18 (33.7%) had an IIRC group D eye (eyes presenting with massive vitreous or subretinal seeds or retinal detachment).[16] This information was missing in 1 patient. Forty-three patients in IIRC group E (70.5%) had glaucoma and/or buphthalmus, these findings were absent in 17 patients (27.9%), and information was not available in 2 (1.6%).

Patients With MD

Nine patients had MD (9.4% of the population) (eTable in the Supplement). Seven of them had MD evidenced by the positivity of CRX mRNA in the BM (relative expression level range, 0.00007-0.0034). In these patients, the results of additional BM examination at the end of adjuvant therapy were negative. The remaining 2 children had a positive GD2 synthase

Table. MD According to Pathologic Risk Factors[a]

Risk Factor	MD, No. (%)		*P* Value
	Negative	Positive	
Choroidal invasion (any) (n = 77)	67 (90.5)	7 (9.5)	.39
Massive choroidal invasion (n = 51)	44 (86.3)	7 (13.7)	.08
Scleral invasion (n = 25)	19 (76.0)	6 (24.0)	.002
Retrolaminar optic nerve invasion (n = 51)	50 (98.0)	1 (2.0)	.20
Tumor invasion to the resection margin of the optic nerve (n = 7)	3 (42.9)	4 (57.1)	<.001
Anterior segment invasion (n = 17)	14 (82.3)	3 (17.7)	.13

Abbreviation: MD, minimal dissemination.

[a] Children presenting with massive buphthalmus and undergoing enucleation after planned neoadjuvant chemotherapy (12 children, of whom 2 had MD) were excluded.

mRNA determination result in the CSF at diagnosis, and the BM test result was negative for CRX mRNA.

No child with IIRC group D eyes enucleated at diagnosis had MD, whereas 8 of the 61 children (13.1%) with IIRC group E eyes had MD. More specifically, 8 of the 43 eyes (18.6%) of IIRC group E that had glaucoma with or without buphthalmus had MD as did 8 of 80 (10%) and 1 of 16 children (6.3%) who underwent primary or secondary enucleation, respectively. The remaining patient with MD had a bilateral IIRC group D eye at diagnosis and underwent enucleation after failure of treatment of 1 eye with intra-arterial chemotherapy. After enucleation that revealed scleral invasion, MD was found. The patient has remained disease free for 58 months after adjuvant therapy, with negative determinations for MD during follow-up. In the remaining 3 patients who underwent enucleation after failure of intra-arterial chemotherapy, MD was not found. The correlation between HRPFs and MD is given in the **Table**. Only invasion to the optic nerve beyond the resection margin was independently associated with MD (adjusted odds ratio, 57.0; 95% CI, 4.8-678.2; $P = .001$).

Survival Analysis

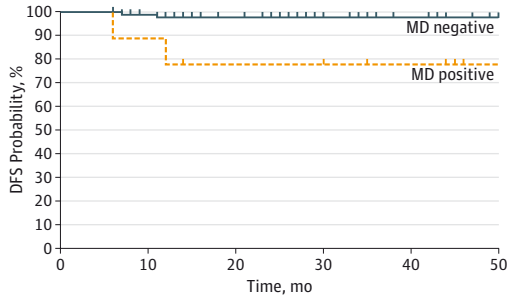
There were 4 events of metastatic relapse in the study population at a median of 9 months after diagnosis (range, 6-12 months). All occurred in children who had received intensive adjuvant therapy for initially enucleated eyes. The CSF was the only site of metastatic relapse, and this event was fatal in all cases. The probability of DFS was lower in children with MD (difference in DFS, 0.20; 95% CI, 0.17-0.23; $P = .004$) (**Figure 2**).

Discussion

Our data indicate that 9 children (9.4%) with retinoblastoma and HRPFs had MD, and this subgroup of patients have a poorer DFS. This is, to our knowledge, the first report of MD in a large cohort of children with nonmetastatic retinoblastoma. Our group reported the use of CRX mRNA for the study of MD in patients with metastatic retinoblastoma. In this study, we extended the use of qPCR for CRX mRNA[6] as a tool for the evaluation of MD in nonmetastatic disease, which is more frequent in our setting, and we were able to detect a subgroup of higher-risk patients within the group already considered as high risk because of HRPFs. Estimating the risk of metastatic relapse in these children is a challenge. Metastatic relapse occurs almost exclusively in children with retinoblastoma and HRPFs,[2] but even in this population, it is a rare event. Virtually no child without HRPFs will present with a metastatic relapse, so they are usually not given any adjuvant therapy.[17] Theoretically, a study of MD might also be helpful to identify those rare exceptions who are not recognized as high risk by pathologic evaluation. However, because BM and CSF specimens are needed for MD evaluation, this factor could not be determined in our study because only patients with HRPFs underwent these invasive procedures. However, none of the 85 patients without HRPFs whose conditions were diagnosed at our center at the same interval had a metastatic relapse, so it may be questionable to perform this invasive procedure in this lower-risk population. Despite the restrictions in invasive procedures in children without HRPFs, we were able to perform MD evaluation in 18 of such low-risk patients in whom BM and CSF samples were procured because of other clinical reasons, but none had MD and none relapsed.

Metastatic relapse occurs almost exclusively in children with HRPFs[5,9]; however, there is no general consensus on which patients should be given adjuvant therapy because the risk of metastatic relapse is variable in the different categories of patients with HRPFs. For example, this risk appears to be higher in patients with PLONI (with and without invasion at the resection margin)[5,18] or scleral involvement,[19,20] so virtually all groups use adjuvant therapy to reduce this risk.[2] Conversely, only 4% of children with isolated massive choroidal invasion present with a metastatic relapse even when no adjuvant therapy is given.[21] Hence, even though a proportion of

Figure 2. Probability of Disease-Free Survival (DFS) for Patients According to the Presence of Minimal Dissemination (MD)



No. at risk

MD negative	87	72	55	53	43	30
MD positive	9	8	7	6	5	2

The 3-year probability of DFS for patients with MD was 0.78 vs 0.98 for those without MD ($P = .004$).

children still present with an almost invariably fatal metastatic relapse despite receiving adjuvant therapy, it is important to identify these children more accurately in whom a different therapeutic approach would be warranted to improve their chance of survival. Our study identified such a population within the HRPF group because those who had MD had a DFS of 0.78 compared with a similar population without MD who had a DFS of 0.98 (P = .004). In this subgroup, MD was more common in children with tumor at the resection margin of the optic nerve and in those with scleral invasion. Therefore, a modification of adjuvant therapy is currently being considered by our group, including an intensified intrathecal treatment for children with MD in CSF to improve results. These children had received an already-intensive systemic chemotherapy regimen, and all relapses occurred in the CSF. Considering our previously reported results indicating that the CSF is usually a sanctuary site in the event of relapse,[6] the introduction of early and intensive intrathecal chemotherapy may improve the outcomes of these patients by treating the CSF compartment more effectively.[22]

Because according to our treatment philosophy not all patients with HRPFs received adjuvant therapy, we may provide some interesting observations. For example, no child of the 14 patients with isolated massive choroidal invasion had MD, none received adjuvant therapy, and none relapsed. However, 1 relapsing patient with PLONI who received adjuvant therapy had a negative MD evaluation result at diagnosis; therefore, in this case, her higher-risk status could not be identified by MD. This case highlights not only that relapse may occur even with a negative MD determination but also that adjuvant chemotherapy, even with relatively intensive regimens, is not a guarantee to prevent all cases of metastatic relapse in high-risk children.[5] The remaining patients (n = 50) with negative MD evaluation results did not relapse. It is uncertain what their outcome would be without adjuvant therapy. Hence, as seen in other malignant tumors, such as neuroblastoma, a negative MD evaluation result is not enough to guarantee a relapse-free outcome, but those higher-risk patients with MD have an additional higher risk.[23] Relapses occurred only in the CSF, and the BM evaluation result was negative for MD in all cases at the moment of relapse.[6] However, issues that affect the sensitivity of MD determinations in the CSF may be improved. The low cellularity and limited volume of the CSF sample made it impossible to obtain RNA for analysis in 36% of the cases. Differences in the expression of molecular markers that lead to false-positive results may also occur[24]; however, this result is unlikely to have happened in our cases because tumor cells tested positive for CRX mRNA in all relapsing CSF specimens, whereas MD evaluation results were persistently negative in the BM during the relapse episode.[6] Altogether, these observations indicate that CSF sampling protocol may play a critical role in the accuracy of previous PCR determinations.

As in neuroblastoma,[25] the identification of additional markers, such as the RB transcriptional corepressor 1 gene (OMIM 180200) mutation in cases of nonhereditary retinoblastoma[26] or the neuroendocrine protein gene product 9.5 (ubiquitin carboxyl-terminal esterase L1) (OMIM 191342),[14] as well as other markers, may further improve the sensitivity of the technique. Even though we found a correlation between patients with HRPFs and MD and metastatic relapse, the enucleated eye should be available for identifying these patients at risk. If MD could be identified preoperatively, higher-risk children may be more readily identified and treated accordingly. In addition, since the introduction of intra-arterial chemotherapy, most children with IIRC group D eyes and an increasingly higher number of children with IIRC group E eyes are now considered for conservative therapy in many centers.[27-29] Minimal dissemination studies may be helpful for identifying a subset of children in whom a conservative approach would not be advisable or should be stopped after intraocular disease progression. We routinely performed enucleation up front in all IIRC group E eyes in our center during the study period, and we found that MD occurred in 18.6% of children with IIRC group E eyes presenting with glaucoma, suggesting that conservative treatment in this population should not be attempted and that these patients should be treated as having potentially disseminated disease. Hence, according to our data, children with glaucoma should not be considered candidates for conservative therapy.

Conclusions

Our data identified a higher-risk population of children within a group of retinoblastoma and HRPFs who have MD in whom DFS is lower despite intensive adjuvant therapy. However, the number of events was relatively small to achieve definitive conclusions, which may be evident with longer follow-up. An alternative approach may become needed to improve results in this population. Children presenting with IIRC group E retinoblastoma and glaucoma have a higher risk of MD at diagnosis and may not be considered for conservative therapies.

ARTICLE INFORMATION

Accepted for Publication: September 2, 2016.

Published Online: October 27, 2016. doi:10.1001/jamaophthalmol.2016.4158

Author Contributions: Drs Chantada and Torbidoni had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Laurent, Torbidoni, Gabri, D. F. Alonso, Chantada.
Acquisition, analysis, or interpretation of data: Laurent, Torbidoni, Sampor, Ottaviani, Vazquez, Garcia de Davila, Ramirez-Ortiz, C. N. Alonso, Rossi, D. F. Alonso, Chantada.
Drafting of the manuscript: Laurent, Torbidoni, Ottaviani, Ramirez-Ortiz, D. F. Alonso, Chantada.
Critical revision of the manuscript for important intellectual content: Laurent, Torbidoni, Sampor, Vazquez, Gabri, Garcia de Davila, Rossi, D. F. Alonso, Chantada.
Statistical analysis: Laurent, Torbidoni, Chantada.
Administrative, technical, or material support: Sampor, Ottaviani, Vazquez, Ramirez-Ortiz.
Study supervision: Sampor, Ottaviani, Garcia de Davila, C. N. Alonso, D. F. Alonso, Chantada.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: This study was supported by the Fund for Ophthalmic Knowledge, the Natalí Dafne Flexer Foundation (Argentina), Instituto Nacional del Cáncer (Argentina), and grant BID-PID129-

PAE37.011 from Agencia Nacional de Promoción Científica y Tecnológica (Argentina).

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

Previous Presentation: Partial results of this study were presented at the 2015 meeting of the International Society of Ocular Oncology; June 16, 2015; Paris, France.

REFERENCES

1. Broaddus E, Topham A, Singh AD. Survival with retinoblastoma in the USA: 1975-2004. *Br J Ophthalmol*. 2009;93(1):24-27.

2. Aerts I, Sastre-Garau X, Savignoni A, et al. Results of a multicenter prospective study on the postoperative treatment of unilateral retinoblastoma after primary enucleation. *J Clin Oncol*. 2013;31(11):1458-1463.

3. Chantada GL, Fandiño AC, Guitter MR, et al. Results of a prospective study for the treatment of unilateral retinoblastoma. *Pediatr Blood Cancer*. 2010;55(1):60-66.

4. Kaliki S, Shields CL, Shah SU, Eagle RC Jr, Shields JA, Leahey A. Postenucleation adjuvant chemotherapy with vincristine, etoposide, and carboplatin for the treatment of high-risk retinoblastoma. *Arch Ophthalmol*. 2011;129(11):1422-1427.

5. Kaliki S, Tahiliani P, Mishra DK, Srinivasan V, Ali MH, Reddy VA. Optic nerve infiltration by retinoblastoma: predictive clinical features and outcome. *Retina*. 2016;36(6):1177-1183.

6. Torbidoni AV, Laurent VE, Sampor C, et al. Association of cone-rod homeobox transcription factor messenger RNA with pediatric metastatic retinoblastoma. *JAMA Ophthalmol*. 2015;133(7):805-812.

7. Laurent VE, Sampor C, Solernou V, et al. Detection of minimally disseminated disease in the cerebrospinal fluid of children with high-risk retinoblastoma by reverse transcriptase-polymerase chain reaction for GD2 synthase mRNA. *Eur J Cancer*. 2013;49(13):2892-2899.

8. Chantada GL, Rossi J, Casco F, et al. An aggressive bone marrow evaluation including immunocytology with GD2 for advanced retinoblastoma. *J Pediatr Hematol Oncol*. 2006;28(6):369-373.

9. Chantada GL, Dunkel IJ, de Dávila MT, Abramson DH. Retinoblastoma patients with high risk ocular pathological features: who needs adjuvant therapy? *Br J Ophthalmol*. 2004;88(8):1069-1073.

10. Glubrecht DD, Kim JH, Russell L, Bamforth JS, Godbout R. Differential CRX and OTX2 expression in human retina and retinoblastoma. *J Neurochem*. 2009;111(1):250-263.

11. Bellaton E, Bertozzi AI, Behar C, et al. Neoadjuvant chemotherapy for extensive unilateral retinoblastoma. *Br J Ophthalmol*. 2003;87(3):327-329.

12. Zhao J, Dimaras H, Massey C, et al. Pre-enucleation chemotherapy for eyes severely affected by retinoblastoma masks risk of tumor extension and increases death from metastasis. *J Clin Oncol*. 2011;29(7):845-851.

13. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053

14. Yamane S, Shirai C, Arimoto A, et al. Disseminated retinoblastoma successfully treated with myeloablative chemotherapy: implication for molecular detection of minimal residual disease. *Bone Marrow Transplant*. 1999;23(9):971-974.

15. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM; Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. Reporting recommendations for tumor marker prognostic studies. *J Clin Oncol*. 2005;23(36):9067-9072.

16. Linn Murphree A. Intraocular retinoblastoma: the case for a new group classification. *Ophthalmol Clin North Am*. 2005;18(1):41-53, viii.

17. Chantada GL, de Dávila MT, Fandiño A, et al. Retinoblastoma with low risk for extraocular relapse. *Ophthalmic Genet*. 1999;20(3):133-140.

18. Antoneli CB, Steinhorst F, de Cássia Braga Ribeiro K, et al. Extraocular retinoblastoma: a 13-year experience. *Cancer*. 2003;98(6):1292-1298.

19. Antoneli CB, Ribeiro KB, Rodriguez-Galindo C, et al. The addition of ifosfamide/etoposide to cisplatin/teniposide improves the survival of children with retinoblastoma and orbital involvement. *J Pediatr Hematol Oncol*. 2007;29(10):700-704.

20. Cuenca A, Giron F, Castro D, et al. Microscopic scleral invasion in retinoblastoma: clinicopathological features and outcome. *Arch Ophthalmol*. 2009;127(8):1006-1010.

21. Bosaleh A, Sampor C, Solernou V, et al. Outcome of children with retinoblastoma and isolated choroidal invasion. *Arch Ophthalmol*. 2012;130(6):724-729.

22. Dimaras H, Héon E, Doyle J, et al. Multifaceted chemotherapy for trilateral retinoblastoma. *Arch Ophthalmol*. 2011;129(3):362-365.

23. Yáñez Y, Grau E, Oltra S, et al. Minimal disease detection in peripheral blood and bone marrow from patients with non-metastatic neuroblastoma. *J Cancer Res Clin Oncol*. 2011;137(8):1263-1272.

24. Stutterheim J, Gerritsen A, Zappeij-Kannegieter L, et al. *PHOX2B* is a novel and specific marker for minimal residual disease testing in neuroblastoma. *J Clin Oncol*. 2008;26(33):5443-5449.

25. Stutterheim J, Gerritsen A, Zappeij-Kannegieter L, et al. Detecting minimal residual disease in neuroblastoma: the superiority of a panel of real-time quantitative PCR markers. *Clin Chem*. 2009;55(7):1316-1326.

26. Dimaras H, Rushlow D, Halliday W, et al. Using *RB1* mutations to assess minimal residual disease in metastatic retinoblastoma. *Transl Res*. 2010;156(2):91-97.

27. Abramson DH, Shields CL, Munier FL, Chantada GL. Treatment of retinoblastoma in 2015: agreement and disagreement. *JAMA Ophthalmol*. 2015;133(11):1341-1347.

28. Munier FL, Gaillard MC, Balmer A, Beck-Popovic M. Intravitreal chemotherapy for vitreous seeding in retinoblastoma: recent advances and perspectives. *Saudi J Ophthalmol*. 2013;27(3):147-150.

29. Shields CL, Manjandavida FP, Lally SE, et al. Intra-arterial chemotherapy for retinoblastoma in 70 eyes: outcomes based on the International Classification of Retinoblastoma. *Ophthalmology*. 2014;121(7):1453-1460.