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Yelena Diarra [a], Christina Brockmeyer [a], Karen Fischhuber [b], Isabel Hülsenbeck [c], Saskia Ting [d], Madlen Reschke [e], Tobias Kiefer [c], Anna Hannbücken [a], Maren Wagemanns [a], Leyla Jabbarli [c], Selma Sirin [f], Regina Wieland [a], Gudrun Fleischhack [a], Johannes H. Schulte [e], Martin Ebinger [g], Dietmar Lohmann [h], Bert Müller [i], Daniela Süsskind [j], Christoph Schwab [k], Ines Brecht [g], Angelika Eggert [e], Stefan Schönberger [a], Petra Ritter-Sovinz [l], Nikolaos Bechrakis [c,o], Sophia Göricke [m], Beate Timmermann [n,o], Eva Biewald [c], Petra Ketteler [a,o,*]

[a] Department of Paediatric Haematology and Oncology, Paediatrics III, University Duisburg-Essen, University Hospital Essen, Germany
[b] Institute of Biostatistics and Clinical Research, University of Muenster, Germany
[c] Department of Ophthalmology, University Duisburg-Essen, University Hospital Essen, Germany
[d] Institute of Pathology, University Duisburg-Essen, University Hospital Essen, Germany
[e] Department of Pediatric Oncology and Hematology, Charité - Universitätsmedizin Berlin, Berlin, Germany
[f] Department of Diagnostic Imaging, University Children's Hospital Zürich, Zürich, Switzerland
[g] Department of Pediatric Oncology and Hematology, University Children's Hospital, Tübingen, Germany
[h] Institute of Human Genetics, University Hospital Essen, University Duisburg Essen, Essen, Germany
[i] Department of Ophthalmology, Charité, Berlin, Germany
[j] Department of Ophthalmology, University of Tübingen, Germany
[k] Department of Ophthalmology, Medical University of Graz, Graz, Austria
[l] Division of Pediatric Hematology and Oncology, Medical University of Graz, Austria
[m] Department of Diagnostic and Interventional Radiology and Neuroradiology, University Hospital Essen, Germany
[n] Department of Particle Therapy, University Hospital Essen, West German Proton Therapy Centre Essen (WPE), West German Cancer Center (WTZ), Germany
[o] German Consortium for Translational Cancer Research (DKTK), Essen, Germany

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ABSTRACT

Introduction: Advanced localized retinoblastoma can be cured by enucleation, but extraocular spread of retinoblastoma cells is associated with a high mortality. Risk-stratified adjuvant treatment with chemotherapy and radiotherapy has been shown to reduce the risk for extraocular relapse in children with histopathological risk factors.

Methods: Data of 184 patients with retinoblastoma and primary enucleation were collected in a prospective, multicenter, observational study between 2013 and 2020. The clinical characteristics were evaluated as risk factors and progression-free and overall survival rates were compared.

Results: Seventy-one percent of 184 children with retinoblastoma treated with primary enucleation were diagnosed with low risk histopathological factors (pT1/pT2a) and received no adjuvant therapy. Children with intermediate risk (pT2b,pT3; 48 children, 26.0%) and high risk for metastasis (pT4; 5 children, 2.7%) received risk-stratified adjuvant treatment. None of the children with low risk or intermediate risk (pT1-pT3) relapsed, but two of five children with high-risk retinoblastoma (pT4) developed extraocular relapses and one deceased. The 2-year progression-free survival rate and 2-year overall survival rate was 100% for children with pT1-3 retinoblastoma. However, the 2-year progression-free survival rate and 2-year overall survival rate for children with pT4 was statistically notably reduced with 2 of 5 children developing progression and 1 death among the 5 children within 2 years after diagnosis.

Conclusion: Primary enucleation alone and with additional risk-stratified adjuvant chemotherapy treatment provides high cure rates in patients with pT1-3 retinoblastoma, but children with pT4 retinoblastoma remain at high risk to develop extraocular retinoblastoma. International prospective clinical trials are required to evaluate

* Corresponding author. Department of Pediatric Haematology and Oncology Pediatrics III University Hospital Essen, Hufelandstrasse 55, 45122, Essen, Germany.
E-mail address: petra.ketteler@uk-essen.de (P. Ketteler).

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reduction of intensity of adjuvant chemotherapy in some risk groups (pT2, pT3) and intensification for pT4 retinoblastoma.

Abbreviation table

AJCC	American Joint Committee on Cancer
CNS	central nervous system
EURbG	European Retinoblastoma group
GALOP	Grupo de America Latina de Oncologia Pediatrica
HIC	high-income country
IRSS	International retinoblastoma staging system
LMIC	low- and middle-income countries
MRI	magnet resonance imaging
PFS	Progression-free survival
OS	overall survival

1. Introduction

Retinoblastoma is a malignant retinal tumor in early childhood. In high-income countries (HIC), 5-year overall survival (OS) rates of retinoblastoma are above 95% because early diagnosis and advances in multidisciplinary care prevent the spread of tumor cells beyond the natural border of the eye [1–4]. However, the prognosis of metastatic disease remains poor even with intensive multimodal therapy [3,5]. Advanced local retinoblastoma are usually treated with enucleation of the eye. If the intraocular retinoblastoma has invaded deeper ocular structures, the risk for extra-ocular relapse is increased [6–8]. Historic data demonstrated that adjuvant chemotherapy reduced the risk of extraocular relapse from 24% to less than 5% in patients with histopathological risk factors for relapse [9–11]. The potential histopathological risk factors for relapse include choroidal invasion, invasion of the anterior chamber, scleral invasion and infiltration of the optic nerve to different extents, but worldwide there is little uniformity about risk factors for metastasis [12]. Especially the need for adjuvant treatment for isolated tumor cell spread into the anterior segment of the eye and for massive choroidal invasion is debated [10,13–15].

Recent non-randomized prospective trials demonstrated overall survival rates for children with localized advanced retinoblastoma (pT2, pT3) as high as 100% [13,16,17]. The survival rates for children with microscopic residuals after enucleation (IRSS II, pT4, N0, M0) are lower and range from 48 to 100% [16–18]. The histopathological risk factors of pT4 include infiltration of tumor cells at the resection margin of the optic nerve (N3, pT4), subarachnoidal invasion, microscopic extension of tumor cells through the sclera into the orbit (S2, pT4) and pars plana vitrectomy to the eye prior to diagnosis of undetected retinoblastoma. Retinoblastomas with pT4 histopathological features are only rarely observed in HIC, which complicates the design of prospective randomized clinical trials to improve the treatment [19,20]. Most European centers treat pT4 retinoblastoma with adjuvant chemotherapy and radiotherapy but the evidence especially for radiotherapy is scarce [21–23].

Adjuvant treatment regimens in Germany and Austria followed national guidelines since 2013 and data were collected in a prospectively, multicenter, observational study “RB-Registry”. Here, we present the data on progression-free survival rates (PFS) and OS after primary enucleation and risk-stratified adjuvant treatment.

2. Methods

2.1. Data collection

Data were collected in a prospective multicenter observational study, RB-registry (DRKS00005423) for all children with retinoblastoma diagnosed in Germany and Austria since November 4, 2013. Data were retrieved on the January 19, 2022. Data collection in RB-registry was conducted in compliance with the Declaration of Helsinki. Ethical approval was obtained from all local ethics committees of the participating centers. Inclusion criteria for RB-registry were diagnosis of retinoblastoma or other malignant eye tumors confirmed by an experienced ophthalmologist or diagnosis of a constitutional pathogenic *RB1* variant, age <18 years, no retinoblastoma-specific treatment prior to inclusion in the RB-Registry, written informed consent of the primary caregivers, and permanent residence in Germany or Austria. Central Reference histopathology was offered to all patients with enucleation.

2.2. Selection of patients in the study cohort

In RB-Registry, 403 patients with retinoblastoma were registered between November 4, 2013 and November 3, 2020. From this initial cohort, 191 individuals were excluded because of diagnosis other than retinoblastoma (n = 16), extraocular disease at diagnosis (n = 2), no primary enucleation (n = 156) or incomplete data (n = 17). In the low-risk group, 28 patients with bilateral retinoblastoma and chemoreduction for the contralateral eye were excluded. The final study cohort included 184 patients.

2.3. Risk-stratified adjuvant treatment

Retinoblastoma staging was performed by the International Retinoblastoma Staging System (IRSS) and the 8th version of American Joint Committee on Cancer Staging of Retinoblastoma classification (AJCC) [24–26]. Patients with diagnosis prior to 2017 were staged according to the 7th version AJCC at time of diagnosis and were retrospectively classified according to 8th version AJCC. According to the histopathological risk factors patients with primary enucleation were grouped into four groups (Supplementary Table S1).

2.4. Adjuvant treatment protocols

Adjuvant chemotherapy for all risk groups consisted of vincristine, etoposide, carboplatin and cyclophosphamide (CyVEC) from 2013 to 2016 and vincristine, etoposide and carboplatin (VEC) chemotherapy from 2016 to 2020 (Table 1). Radiotherapy to the orbit was applied either with proton external beam radiotherapy or with brachytherapy with 125 Iodine Seeds [27,28].

2.5. Outcome parameter

Progression-free and overall survival was assessed. Progression was defined as an extraocular relapse of the enucleated tumor. Death of any cause was assessed for overall survival.

2.6. Statistical analysis

The clinical characteristics were described by computing medians and

Table 1
Cumulative doses of chemotherapeutic agents in the VEC and CyVEC regimens.

CYVEC[a]	cycles 1 & 4	cycles 2 & 5	cycles 3 & 6	Total cumulative dose after 6 cycles
Vincristine in mg/m²	1.5	1.5	1.5	9
Carboplatin in mg/m²	–	300	300	1200
Etoposide in mg/m²	450	450	–	1800
Cyclophosphamide in mg/m²	1200	–	1200	4800
VEC[a]	cycles 1-6			Total cumulative dose after 6 cycles
Vincristine in mg/m²	1.5			9
Carboplatin in mg/m²	560			3360
Etoposide in mg/m²	300			1800
Cyclophosphamide in mg/m²	–			–

[a] Dosages displayed here are calculated per body surface area. Dosages for children under 10 kg body weight or under 1 year of age were calculated per body weight.

ranges (quantitative variables) or absolute numbers and percentages (categorical variables). The Chi 2-test was conducted to compare categorical variables, the Kruskal-Wallis Test and the Mann-Whitney *U* Test to compare continuous variables. Kaplan-Meier estimates of progression-free and overall survival rates were calculated, and Log rank tests were performed to analyze the time-to-event and time-to-enucleation endpoints. Median follow-up times were calculated with a reverse Kaplan-Meier estimate. *P*-values <0.05 were defined as statistically noticeable. The data set was processed and statistically analyzed using IBM SPSS Statistics (version 28.0; SPSS, Chicago, IL) and RStudio (version 4.0.2; RStudio Inc.).

3. Results

3.1. Clinical characteristics of different treatment groups

The study cohort included 184 patients with primary enucleation (158 unilateral [85.9%], 26 bilateral retinoblastoma [14.1%]) resulting in an average of 26 patients per year in Germany and Austria together. Demographic characteristics are summarized in Table 2. According to histopathological risk factors, 131 patients (71.2%), 17 patients (9.2%), 31 patients (16.8%) and 5 patients (2.7%) were low risk, intermediate risk 1, intermediate risk 2 and high risk, respectively. There is no dependence between the distribution of risk groups and year of first diagnosis detected during the last 7 years (Chi^2 [18] = 20.11; p_{Chi2} = 0.33) (Supplementary Fig. S1). Comparison of the demographic characteristics between the four treatment groups showed no statistically noticeable difference between the age at diagnosis with advanced risk grouping (Low risk: median 1.9 years, intermediate risk 1: 1.6 years, intermediate risk 2: 1.9 years, high risk: 3.4 years) (Chi^2 [3] = 3.22, $p_{Kruskal-Wallis}$ = 0.36), but a trend towards a higher age in the high risk group was noted (Fig. 1). The study cohort included 26 children with bilateral disease. Children with bilateral retinoblastoma that received eye-preserving chemotherapy for the contralateral eye without indication for adjuvant chemotherapy were excluded. Three children received bilateral primary enucleation and were grouped according to the staging of the more affected eye.

3.2. Ophthalmological findings at diagnosis and details on primary enucleation

The median length of resected optic nerve was 8 mm (1–22 mm) and did not differ statistically noticeable between the risk groups (low risk: 8 mm [1–20 mm], intermediate risk 1: 6 mm [(1–19 mm], intermediate risk 2: 9 mm [3–22 mm], high risk: 14 mm [2–17 mm], (Chi 2 [3] = 3.00, $p_{Kruskal-Wallis}$ = 0.39), data missing in 14 patients). All children received an orbital implant after enucleation (1 child with dermis fat implant, 183 children with alloplastic implant). Four patients (3 low risk, 1 intermediate risk 2) presented with buphthalmos defined as an overall enlarged bulb with a corneal diameter of >12 mm in the first year of life, axial enlargement, and pressure elevation.

3.3. Risk group stratification and adjuvant chemotherapy treatment

The majority of children was classified as low risk (131 children, 71.2%). Seventeen children (9.2%) were grouped in intermediate risk 1 due to massive choroidal invasion (pT3a, C2, 13 patients) or a massive infiltration of structures in the anterior segment (pT2b, 4 patients). They received three cycles of adjuvant chemotherapy. Thirty-one children (16.8%) were grouped as intermediate risk 2 because of microscopic scleral invasion (pT3c or d, S1, 7 children) or PLONI (pT3b, N2, 22 children) or both (2 children). They received 6 cycles of adjuvant chemotherapy. Five patients with intermediate risk 2 showed N2, C2 (4 with peripapillary choroidal invasion, 1 without data) and received six cycles of adjuvant chemotherapy and did not relapse. Five children (2.7%) were considered high risk for metastasis (pT4) (Table 3). Two of the five children have had a vitrectomy in an eye with undiagnosed retinoblastoma, two showed infiltration of the cut end of the optic nerve (N3) and one patient was diagnosed with retinoblastoma and enlargement of the distal part of the optic nerve on MRI (Supplementary Fig. S2). In both children with vitrectomy and one child with N3, retinoblastoma was not diagnosed prior to surgery nor, so that the children were not referred to the retinoblastoma referral center nor preoperative magnet resonance imaging was performed prior to surgery. The second child with N3, showed no postlaminar optic nerve infiltration on MRI, but the optic nerve was only resected at 2 mm length, showing retinoblastoma cells at the cut end in histopathological examination. The patient with radiological enlargement of the optic nerve had the additional diagnosis of trisomy 21, severe neurodevelopment delay and had experienced multiple life-threatening medical complications during infancy. He received primary enucleation with deep resection of the optic nerve. The resection margin was free of tumor cells so that radiotherapy was omitted. Nonetheless we consider the staging of this patient was determined pT4, IRSS III. The vitrectomy in one child was performed with a self-sealing 25-G system resulting in low risk for tumor cell spread and radiotherapy was omitted [29].

3.4. Adjuvant radiotherapy of the orbit for children with IRSS II retinoblastoma

Three of the five children in the high-risk group received radiotherapy (Table 3). One child with vitrectomy prior to diagnosis of retinoblastoma received orbital brachytherapy with ^{125}Iodine seeds as described by others [27,28]. Six trains with 53 seeds and an activity of 2812 MBq were implanted in the orbit for 75.5 h resulting in a total dose of 40 Gy. Two children received external beam radiotherapy (EBRT) with proton beam to the full unilateral orbit with 52.2 Gy and 39 Gy (with 1.6 and 1.8 Gy per fraction), respectively, using uniform or pencil beam scanning. The child having received 39 Gy developed a retroorbital relapse and received a second adjuvant EBRT course after R1 resection to the retroorbital local recurrence with 45 Gy using again proton beam therapy.

3.5. Progression-free and overall survival after primary enucleation and risk stratified adjuvant treatment

The 2-year PFS in primary enucleated patients IRSS I (pT1-3, low and intermediate risk) was 100% (Fig. 2), while the 2-year PFS of pT4 retinoblastoma patients was statistically notably lower ($p_{LogRank}$ <0.0001) with 2 of 5 children with progressive disease within 2 years after treatment. None of the patients with vitrectomy, but 2 of the 3 patients with cut end invasion or macroscopic enlargement of the optic nerve relapsed

Table 2
Patient characteristics.

		Total	unilateral				bilateral			
		Total [number] (%)	Low Risk [number] (%)	Intermediate risk 1 [number] (%)	Intermediate risk 2 [number] (%)	High risk [number] (%)	Low Risk [number] (%)	Intermediate risk 1 [number] (%)	Intermediate risk 2 [number] (%)	High risk [number] (%)
IRSS			IRSS I	IRSS I	IRSS I	IRSS II	IRSS I	IRSS I	IRSS I	IRSS II
AJCC TNM 8th edition			pT1, pT2a	pT2b, pT3a	pT3b,c,d	pT4	pT1, pT2a	pT2b, pT3a	pT3b,c,d	pT4
Patients		**184**	114 (87)	13 (76.5)	26 (83.9)	5 (100)	17 (13.0)	4 (23.5)	5 (16.1)	–
Age at diagnosis [years]	Median	**1.9**	2.0	2.2	2.1	3.4	1.3	1.1	1.5	–
	(range)	**0.1–9.0**	0.1–9.0	0.1–4.7	0.4–5.3	1.8–4.3	0.2–3.0	0.3–1.6	0.4–3.3	–
Sex	male	**96 (52.2)**	52 (45.6)	9 (69.2)	19 (57.7)	4 (80.0)	10 (58.8)	2 (50.0)	4 (80.0)	–
	female	**88 (47.8)**	62 (54.4)	4 (30.8)	12 (42.3)	1 (20.0)	7 (41.2)	2 (50.0)	1 (20.0)	–
Family history	familial	**6 (3.3)**	3 (2.6)	–	–	–	3 (17.6)	–	–	–
	sporadic	**177 (96.2)**	111 (97.4)	12 (92.3)	26 (100.0)	5 (100.0)	14 (82.4)	4	5	–
	Missing data	**1 (0.5)**	–	1 (5.9)	–	–	–	–	–	–
Vital status at follow-up	deceased	**2 (1.1)**	–	–	–	1 (20.0)	1 (5.9)			
	alive	**184 (98.9)**	114 (100.0)	13 (100.0)	26 (100.0)	4 (80.0)	16 (94.1)	4 (100.0)	5 (100.0)	–
Heritability[a]	Non-heritable	**141 (76.6)**	100 (87.7)	12 (92.3)	24 (92.3)	5 (100)	–	–	–	–
	heritable	**43 (23.4)**	14 (12.3)	1 (7.7)	2 (7.7)	–	17 (100.0)	4 (100.0)	5 (100.0)	–
RB1 mutation in DNA from blood	Heterozygous	**27 (14.7)**	8 (7.0)	–	–	–	12 (70.6)	4 (100.0)	3 (60.0)	–
	mosaic	**9 (4.9)**	6 (5.3)	1 (7.7)	2 (7.7)	–	–	–	–	–
	No constitutional RB1 variant detected	**128 (69.5)**	90 (79.0)	12 (92.3)	19 (73.1)	4 (80.0)	2 (11.8)	–	1 (20.0)	–
	Not analyzed	**20 (10.9)**	10 (8.8)	–	5 (19.2)	1 (20.0)	3 (17.6)	–	1 (20.0)	–
IRSS	IRSS I	**179 (97.3)**	114 (100.0)	13 (100.0)	26 (100.0)	–	17 (100.0)	4 (100.0)	5 (100.0)	–
	IRSS II	**4 (2.2)**	–	–	–	4 (80.0)	–	–	–	–
	IRSS III	**1 (0.5)**	–	–	–	1 (20.0)	–	–	–	–
Vitreous seeding	none	**134 (72.8)**	92 (80.7)	4 (30.8)	16 (61.5)	3 (60.0)	13 (76.5)	2 (50.0)	4 (80.0)	–
	local	**14 (7.6)**	5 (4.4)	4 (30.8)	3 (11.5)	–	–	1 (25.0)	1 (20.0)	–
	diffuse	**22 (12.0)**	8 (7.0)	3 (23.1)	5 (19.2)	2 (40.0)	3 (17.6)	1 (25.0)	–	–
	No data	**14 (7.6)**	9 (7.9)	2 (15.4)	2 (7.7)	–	1 (5.9)	–		
Anterior segment involvement	none	**161 (87.5)**	111 (97.4)	12 (92.3)	14 (53.8)	2 (40.0)	17 (100.0)	1 (25.0)	4 (80.0)	–
	Single cells	**9 (4.9)**	3 (2.6)	–	4 (15.4)	1 (20.0)	–	–	1 (20.0)	–
	massive	**12 (6.5)**	–	1 (7.7)	6 (23.1)	2 (40.0)	–	3 (75.0)	–	–
	No data	**2 (1.1)**	–	–	2 (7.7)	0	–	–	–	–
Buphthalmia	yes	**4 (2.2)**	1 (0.9)	–	1 (3.8)	–	2 (11.8)	0	0	–
	no	**150 (81.5)**	100 (87.7)	10 (76.9)	17 (65.4)	2 (40.0)	13 (76.5)	4 (100.0)	4 (80.0)	–
	No data	**30 (16.3)**	13 (11.4)	3 (23.1)	8 (30.8)	3 (60.0)	2 (11.8)		1 (20.0)	–
Follow-up in years	Median	**2.2**	2.0	4.0	2.5	1.6	3.0	7.1	3.6	–
	range	**0.0–7.8**	0.0–6.2	1.1–5.4	0.0–4.8	0.9–6.9	0.0–5.73	1.0–7.8	1.9–4.9	–

[a] Heritability was defined clinically as bilateral, trilateral or familial retinoblastoma or genetically if a constitutional RB1 variant was diagnosed. In 3 patients with bilateral retinoblastoma, a constitutional *RB1* variant was not detected. These children were considered heritable retinoblastoma based on the clinical presentation.

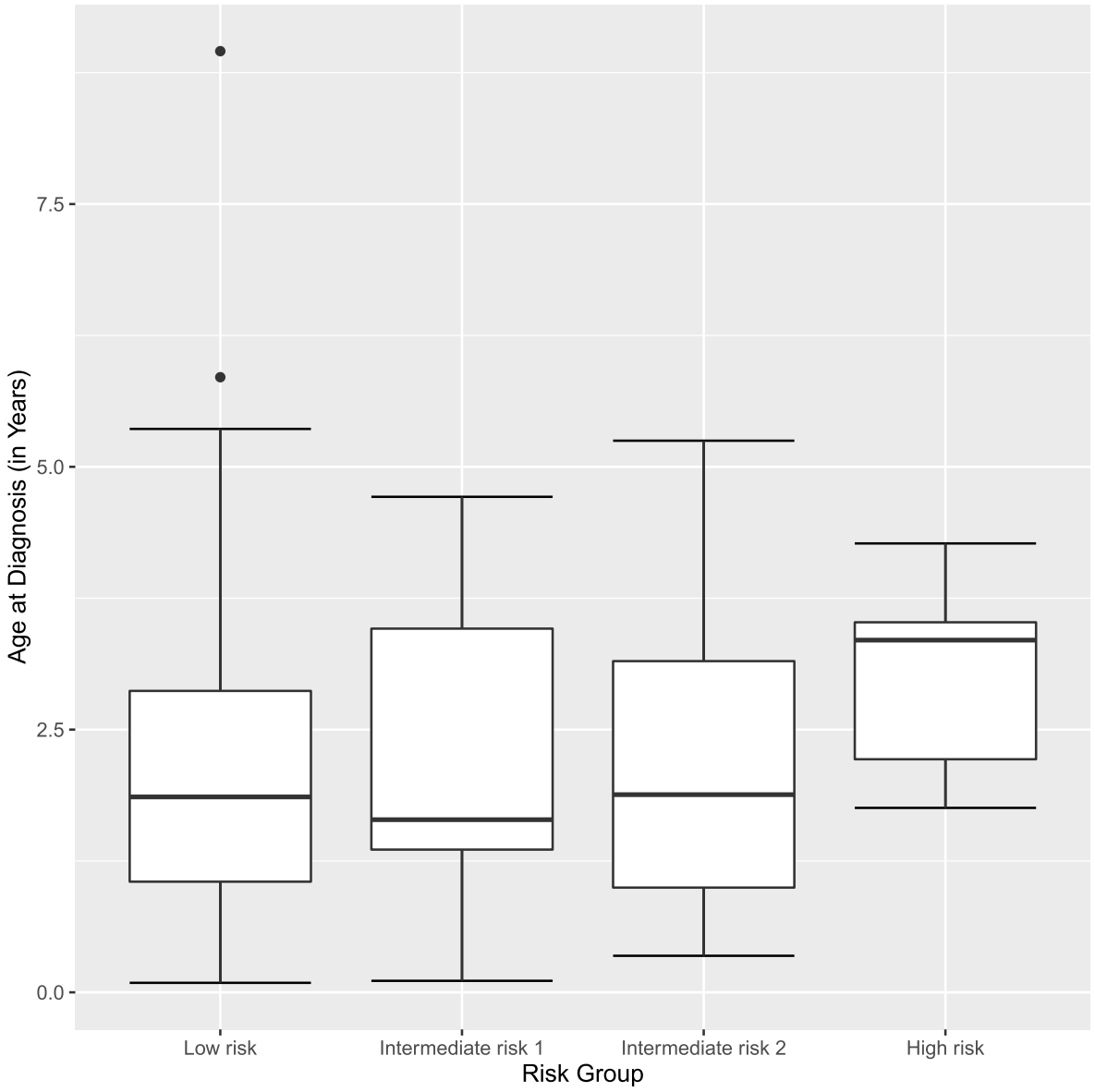

Fig. 1. The median age of diagnosis was highest in children with high risk compared to the other three risk groups. The bar graph shows the median and range of age at first diagnosis stratified per risk group.

locally and ultimately developed leptomeningeal spread of disease. Two children deceased. Causes of death were extraocular relapse (n = 1, high risk) and death due to complications of the underlying severe 13-q deletion syndrome (n = 1, low risk). The 2-year overall survival was 100% in low risk, intermediate risk 1 and intermediate risk 2 and 4 of 5 children in high-risk group survived 2 years after diagnosis ($p_{LogRank}$ = 0.001, Fig. 3). Those results did not change, when only considering the unilateral cases.

3.6. Side effects of adjuvant therapy

Data on hematotoxicity were available in 35 of 54 patients with adjuvant chemotherapy. A blood transfusion and a platelet transfusion was given in 3 of 34 children (8.8%) and 1 of 33 patients (3.0%), respectively. Most patients (88.2%) developed a neutropenia CTCAE grade 3 or 4 and 42.9% of children developed at least in one cycle fever in neutropenia. No patient died due to side effects of adjuvant chemotherapy regimen. Among the children with adjuvant chemotherapy, no ototoxicity or secondary malignancy in a median follow-up interval of 2.3 years (range 0–7.8 years) was reported. In all patients, acute side effects of EBRT were mild with only local radiodermatitis and ocular surface symptoms according to CTCAE grade 1.

4. Discussion

In our national cohorts, 21% of all children diagnosed with localized retinoblastoma and primary enucleation were diagnosed with histopathological risk factors classified as pT2b, pT3 or pT4 that qualified for adjuvant therapy according to the national guidelines. This percentage is similar to other cohorts [26]. After enucleation alone, patients with retinoblastoma pT1 or pT2a had, in line with results from other groups, a 2-year PFS and OS of 100% [16,18]. After three cycles of chemotherapy, patients with pT2b or pT3a retinoblastoma also showed a 2-year PFS and OS of 100%. The high survival rates in these patients with intermediate risk 1 raise the question whether the adjuvant treatment can be further reduced or even omitted. Indeed, results from Latin America demonstrated 5-years OS of 97–100% for patients with massive choroidal invasion alone without adjuvant chemotherapy [20,30,31]. Adjuvant chemotherapy regimens for intermediate risk 2 (pT3b,c,d) comprised of six cycles of polychemotherapy with vincristine, carboplatin and etoposide (until 2016

Table 3
Details on clinical course of children with high risk factors.

Patient number	laterality	Age at diagnosis	Risk factor	CTX treatment	Radiotherapy Type	Radiotherapy dose	Radiotherapy field	Time of follow up until relapse	Relapse	Time of follow-up [a]	Vital status
HR1	Unilateral	3.35	N3 cut end infiltration	6× CyVEC	brachytherapy	40Gy	orbit	6.94	none	6.94	alive
HR2	Unilateral	3.52	vitrectomy	6× CyVEC	Proton therapy	52.2	orbit	1.61	none	1.61	alive
HR3	Unilateral	1.75	vitrectomy	6× VEC	None[b]	–	–	3.30	none	3.30	alive
HR4	Unilateral	2.22	C2, S0, N2 macroscopically enlarged optic nerve on MRI	6× VEC	None[c]	–	–	0.94	IRSS IVb	1.0	deceased
HR5	Unilateral	4.27	N3 cut end infiltration	6× CyVEC	Proton therapy	39 Gy	Orbit and optic nerve up to the chiasm	1.29	Local relapse at the remaining optic nerve	2.63	alive

[a] Calculated since diagnosis of RB until last status.
[b] The risk of tumor cell spread was considered very low in one child with vitrectomy treated with a self-sealing 25-G system with no signs of other histopathological risk factors. No radiotherapy was applied.
[c] The child with trisomy 21 and macroscopic enlargement of the optic nerve (IRSS III, Supplementary Fig. S2) and deep total resection with resection margin free of tumor cells did not receive radiotherapy.

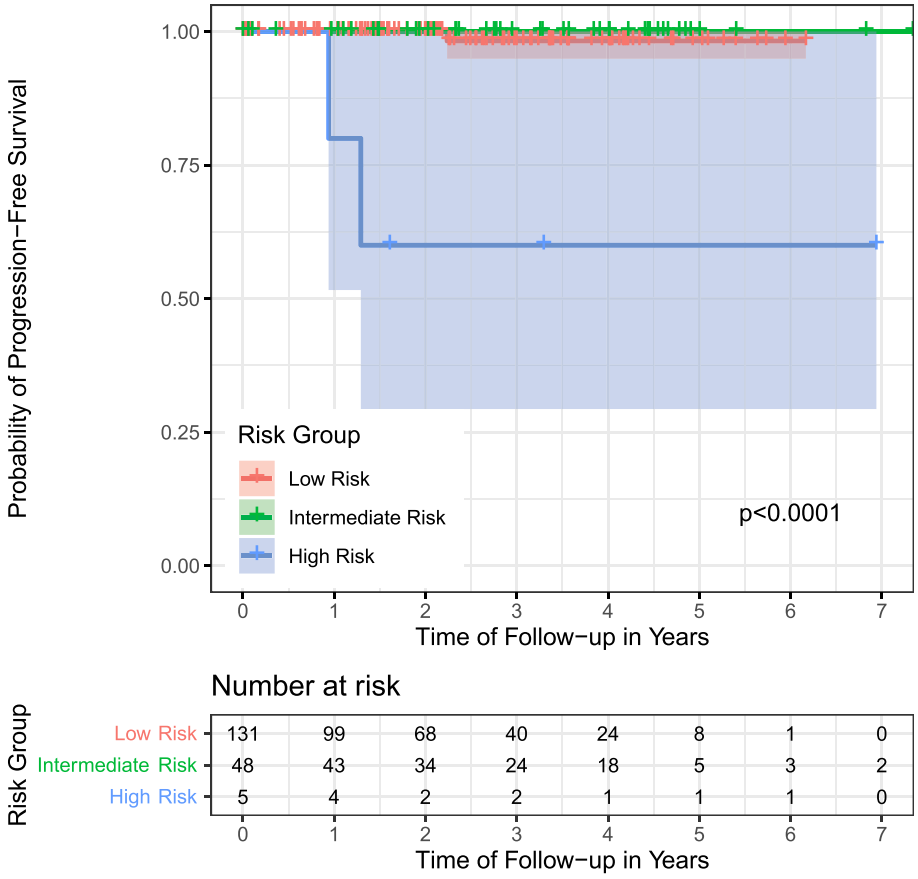

Fig. 2. Progression-free survival of children with retinoblastoma and primary enucleation stratified per histopathological risk factors. Two children in the high risk group developed extraocular relapse. No extraocular relapses were observed in the low and intermediate risk groups.

CyVEC). None of our 31 patients with PLONI without infiltration of the cut end or scleral invasion (S1) developed metastasis [13]. Similar survival rates have also been reported after a reduced number of four cycles of adjuvant chemotherapy [16]. Based on these results, further treatment reduction for the intermediate risk 1 and 2 retinoblastoma patients will be discussed for future national treatment guidelines. Some groups have identified N2, C2 as high-risk factors for extraocular relapse [13,31] and recommend intensification for this group of patients. Limited by a small number of patients, our data do not confirm this observation as the four children with peripapillary choroidal invasion were grouped in intermediate risk 2, received 6 cycles of CyVEC or VEC and did not relapse.

The 2-year overall survival rate of 4 of 5 children in the high-risk

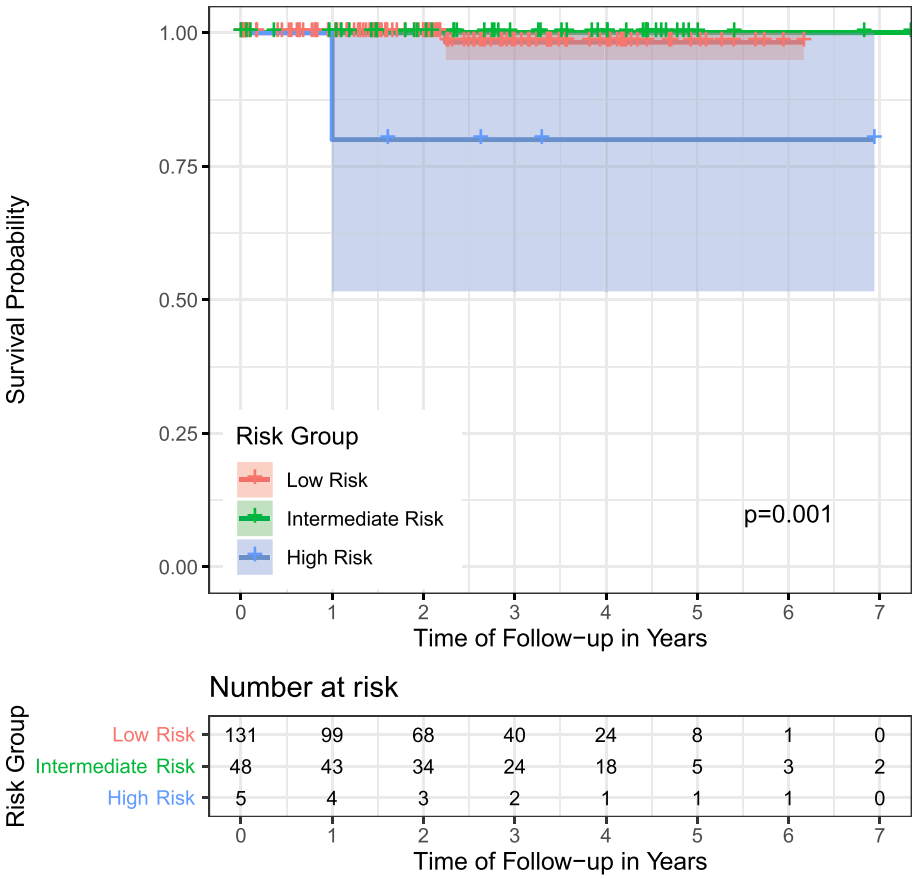

Fig. 3. Overall survival of children with retinoblastoma and primary enucleation stratified per histopathological risk factors. Three children in the cohort deceased. Causes of death were extraocular relapse (n = 1, high risk), trilateral retinoblastoma (n = 1, low risk) and death due to complications of the underlying 13-q deletion syndrome (n = 1, low risk).

group (pT4) was statistically noticeable lower compared with 100% in the low and intermediate risk group despite small number of patients. This is in line with reported survival rates for pT4 retinoblastoma patients of 48–100% [16,18,26]. The recommended treatment in our study was six cycles of adjuvant chemotherapy and radiotherapy of the orbit. Internationally recommended radiotherapy doses range from 40 to 52 Gy [22,28]. As an intensification of therapy, some centers use high-dose chemotherapy followed by autologous stem cell transplant as consolidation treatment for pT4 retinoblastoma [16]. The risk factors of the five children in the high-risk group are very heterogeneous and may differ from the risk for metastasis in pT4 retinoblastoma presenting in LMIC countries. The study findings emphasize the importance of preoperative imaging and enucleation with resection of a long optic nerve by an experienced surgeon. We did not include any patients with *trans*-scleral invasion but two patients with vitrectomy prior to enucleation. It is important to highlight, that retinoblastoma always needs to be excluded prior to vitrectomy in children. Both children are long-term survivors after 6 cycles of adjuvant chemotherapy and orbital radiotherapy in one of these two patients. However, the risk for metastasis in children with vitrectomy is most likely lower than that for children with *trans*-scleral invasion. Children with retinoblastoma and isolated *trans*-scleral invasion have been cured without radiotherapy but using a more intensive chemotherapy regimen than VEC [32]. In conclusion, high risk patients in this study and most likely in HIC in general are nearly always patients who are not diagnosed or treated according to current standards of care for retinoblastoma. All children with a suspected retinoblastoma have to be referred to a specialized retinoblastoma center to reduce the risk of high-risk retinoblastoma by interventions such as vitrectomy prior to diagnosis of retinoblastoma. Standards of care include a preoperative MRI and in case of enucleation the resection of a long segment of the optic nerve by a retinoblastoma-experienced ophthalmologist.

Adjuvant therapy is very effective but the benefit in reducing the risk for metastasis has to be balanced with long-term late effects. Reported short-term side effects of adjuvant chemotherapy regimens include transient bone marrow suppression and fever in neutropenia. A treatment-related mortality of 4% was reported in Central America after VEC chemotherapy [33]. However, in Europe and in North America, treatment-related mortality after conventional chemotherapy for retinoblastoma is nearly 0% and this was confirmed in the here presented study [11,18]. Ototoxicity seems to be rare in most cohorts [34–37], but remains an important potential side effect to be considered for patients who already have a visual handicap. Adjuvant treatment also prolongs the treatment for retinoblastoma and may increase the psychosocial burden for patients and their families. There is evidence that chemotherapy with alkylating agents or topoisomerase inhibitors increase the risk for second malignancies, especially in patients with heritable retinoblastoma, but the number of second malignancies after adjuvant therapy alone is low [11,38,39]. In summary, side effects of adjuvant treatment are tolerable but not neglectable. Adjuvant treatment has therefore to be restricted to patients with a significant risk of metastatic disease.

5. Conclusion

Primary enucleation alone and with additional risk-stratified adjuvant chemotherapy treatment provides high cure rates in patients with pT1-3 retinoblastoma, but OS is still significantly reduced in children with localized pT4 retinoblastoma. In HIC, the diagnosis of pT4

retinoblastoma is very rare and often associated with misdiagnosis of the clinical signs and lack of referral to a specialized retinoblastoma care center. Intraocular tumors need to be carefully excluded prior to any ophthalmic surgery in children. Prospective clinical trials are required to evaluate reduction of adjuvant chemotherapy in pT2,3 retinoblastoma and intensification for patients with pT4 retinoblastoma. Precise molecular genetic biomarkers such as circulating tumor DNA [40–42] for risk stratification could contribute to improve risk stratification and ultimately reduce side effects and improve survival. Especially increasing number of patients treated with eye-preserving therapies requires molecular and radiological biomarkers in addition to histopathological risk factors for treatment stratification.

Author's contribution

YD and PK analyzed the clinical data and wrote the manuscript. PK designed the study. KF performed the statistical analysis. AH analyzed the data on toxicity in the cohort. All authors contributed to data collection, validation, writing and reviewing of the article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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