

Outcome and molecular analysis of young children with choroid plexus carcinoma treated with non-myeloablative therapy: results from the SJYC07 trial

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Abstract

Background. Choroid plexus carcinoma (CPC) is a rare and aggressive tumor of infancy without a clear treatment strategy. This study describes the outcomes of children with CPC treated on the multi-institutional phase 2 SJYC07 trial and reports on the significance of clinical and molecular characteristics.

Methods. Eligible children <3 years-old with CPC were postoperatively stratified to intermediate-risk (IR) stratum if disease was localized or high-risk (HR) stratum, if metastatic. All received high-dose methotrexate-containing induction chemotherapy. IR-stratum patients received focal irradiation as consolidation whereas HR-stratum patients received additional chemotherapy. Consolidation was followed by oral antiangiogenic maintenance regimen. Survival rates and potential prognostic factors were analyzed.

Results. Thirteen patients (median age: 1.41 years, range: 0.21–2.93) were enrolled; 5 IR, 8 HR. Gross-total resection or near-total resection was achieved in ten patients and subtotal resection in 3. Seven patients had *TP53*-mutant tumors, including 4 who were germline carriers. Five patients experienced progression and died of disease; 8 (including 5 HR) are alive without progression. The 5-year progression-free survival (PFS) and overall survival rates were $61.5 \pm 13.5\%$ and $68.4 \pm 13.1\%$. Patients with *TP53*-wild-type tumors had a 5-year PFS of 100% as compared to $28.6 \pm 17.1\%$ for *TP53*-mutant tumors ($P = .012$). Extent of resection, metastatic status, and use of radiation therapy were not significantly associated with survival.

Conclusions. Non-myeloablative high-dose methotrexate-containing therapy with maximal surgical resection resulted in long-term PFS in more than half of patients with CPC. *TP53*-mutational status was the only significant prognostic variable and should form the basis of risk-stratification in future trials.

Key Points

- Non-myeloablative chemotherapy with methotrexate benefits children with CPC even when metastatic.
- CPC with TP53 mutation has a very poor outcome.
- Adjuvant radiotherapy did not have a clear benefit in young children with CPC.

Importance of the Study

Choroid plexus carcinomas (CPCs) are rare, aggressive CNS neoplasms of infancy for which the optimal treatment is unknown. The prospective SJYC07 trial enrolled 13 patients with CPC for risk-stratified, multi-modal treatment using non-myeloablative high-dose methotrexate-containing induction therapy and consolidation with radiation therapy or further chemotherapy. SJYC07 therapy was tolerable, with outcomes that are among the best in the literature, despite inclusion of only patients younger than 3 years and exclusion

of patients with lower-grade choroid plexus tumors. *TP53* mutational status in tumors was significantly associated with outcome with all patients carrying *TP53* wild-type tumors surviving (100%; 6/6) without disease progression as compared to 28% (2/7) with *TP53* mutant tumors. Radiation use was not associated with survival. Non-myeloablative chemotherapy was effective in more than half of children with CPC and future studies should stratify patients according to *TP53* mutational status.

Choroid plexus carcinomas (CPCs) are rare, malignant central nervous system (CNS) neoplasms that commonly present during young childhood, with a median age at diagnosis of 3 years and annual incidence rate of 0.3 per million individuals.¹⁻³ These neoplasms are clinically and molecularly distinct from their lower-grade counterparts, which include choroid plexus papilloma (CPP) and atypical choroid plexus papilloma (aCPP).^{1,4-6} CPCs are voluminous and highly vascular, posing significant challenges to initial resection efforts. In addition to being locally invasive, CPCs have a propensity to metastasize along the cerebrospinal fluid pathway.⁷ Due to the rarity of the disease, treatment is frequently based only on experience from limited case series. While craniospinal irradiation (CSI) is often the mainstay of treatment for many pediatric CNS malignancies, the young age at diagnosis precludes this therapy in CPC because of its adverse effects on the immature brain. Therefore, surgery and chemotherapy constitute the bulk of therapy in an effort to avoid or, at least, defer CSI.⁸⁻¹⁰ In an attempt to maximize survival using the only available tools, myeloablative doses of chemotherapy with autologous stem cell rescue have been used to combat this disease, but the exposure to chemotherapy by this approach is considerable, the risk of acute and long-term toxicity is significant, and it is unclear if this is the optimal strategy.⁹ Between 2007 and 2017, St. Jude Children's Research Hospital led a prospective, multi-center, risk-adapted trial, SJYC07 (NCT00602667), for young children with malignant CNS tumors, inclusive of CPC. On this trial, a multimodal risk-adapted treatment approach was tested that used a non-myeloablative high-dose methotrexate-containing induction regimen combined with either focal radiation therapy for patients with

localized disease or topotecan-containing regimen for patients who had metastatic disease. Herein, we present the clinical and molecular analysis of children with CPC treated on this study.

Materials and Methods

Patients and Treatment

On SJYC07, children with CPC were treated in the intermediate-risk (IR) stratum if they had localized disease or in the high-risk (HR) stratum if they had metastasis (Figure 1). Extent of resection was evaluated based on early postoperative MRI and categorized as gross-total resection (GTR, no residual tumor), near-total resection (NTR, >90% tumor removed), subtotal resection (STR, 50–90% tumor removed) or biopsy (<50% tumor removed). After initial surgery, all patients received 4 cycles of induction chemotherapy (high-dose methotrexate [HDMTX] 5 g/m² on day 1 with folinic acid rescue; cisplatin [75 mg/m²] on day 8; cyclophosphamide [1500 mg/m²] with mesna on day 9; vincristine [1 mg/m²] on days 8 and 15; and for high-risk stratum patients only, vinblastine [1 mg/m²] on days 17, 19, 22, 24, and 26). Filgrastim (5 µg/kg/day) was given subcutaneously on day 10 until absolute neutrophil count >2000/µl on 2 consecutive days. Second-look surgery was recommended during or after induction chemotherapy if initial resection was incomplete. Consolidation for patients in the IR stratum consisted of 54 Gy of focal radiation therapy (RT), and that for patients in the HR stratum, 2 cycles of topotecan (target

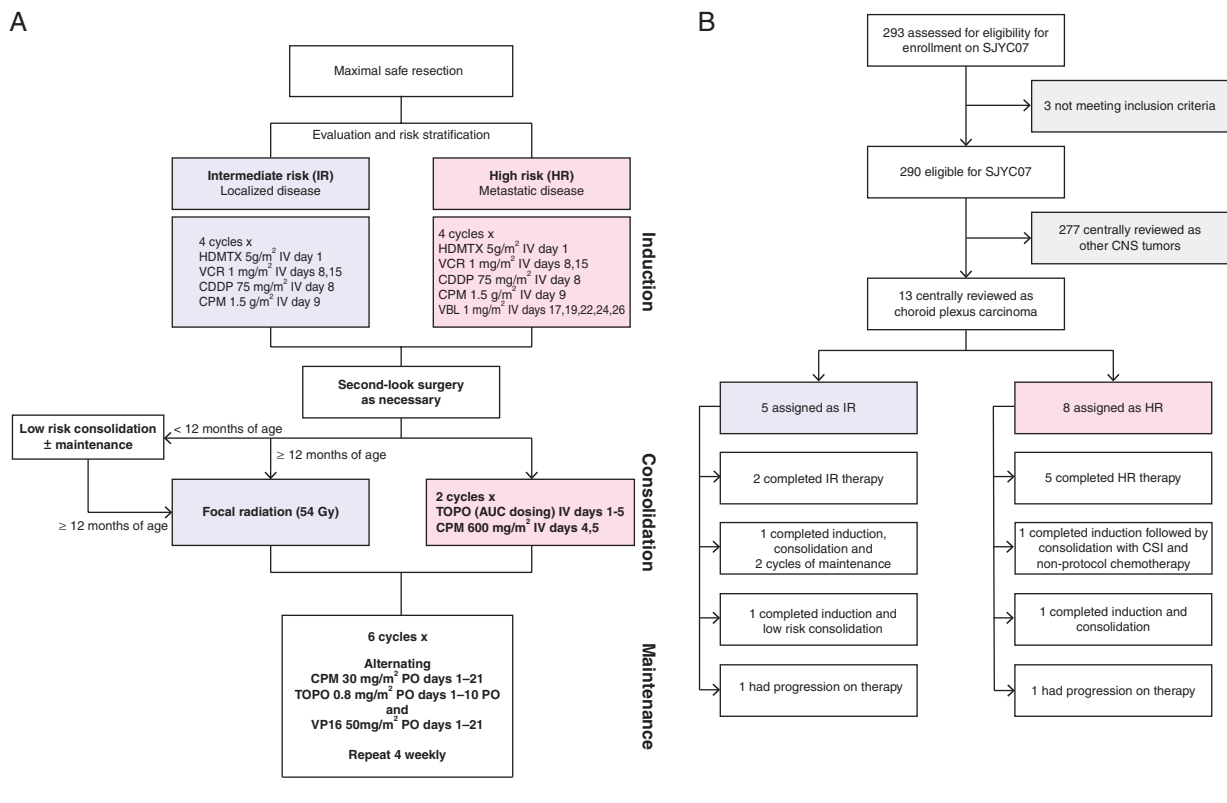


Figure 1. (A) Risk-stratified treatment approach of the SJYC07 trial for patients with CPC. (B) Patient inclusion and stratification in the current cohort. CSI, craniospinal irradiation; CDDP, cisplatin; CPM, cyclophosphamide; HDMTX, high-dose methotrexate; IV, intravenous; PO, oral; TOPO, topotecan; VP16, etoposide; VBL, vinblastine; VCR, vincristine.

AUC = 140 ± 20 ng/mL*hour on days 1–5) and cyclophosphamide (600 mg/m² on days 4 and 5). Maintenance therapy consisted of 6 alternating cycles of oral chemotherapy (cycles 1, 3, 5 – oral cyclophosphamide 30 mg/m² daily on days 1–21, topotecan 0.8 mg/m² PO daily on days 1–10; cycles 2, 4, 6 – oral etoposide 50 mg/m² daily on days 1–21). Induction, consolidation and maintenance chemotherapy cycles were repeated every 28 days. IR-stratum patients who were younger than 12 months at the time of consolidation would be given further chemotherapy according to consolidation for the low-risk stratum (2 cycles of carboplatin/cyclophosphamide/etoposide) to delay RT, and HR-stratum patients who were at least 3 years old by consolidation could be offered CSI. Patients with postoperative intracranial fluid collections received additional monitoring of MTX level, with extra doses of leucovorin.¹¹ Disease evaluations consisted of MRI of the brain and spine, with lumbar puncture for cerebrospinal fluid cytology if not contraindicated at study-defined intervals. Hearing loss was classified according to the International Society of Pediatric Oncology (SIOP) Boston ototoxicity scale, and other toxicities were reported according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0. The histopathology of all tumor samples was centrally reviewed, and *TP53* mutations were detected by paired tumor-germline sequencing. SJYC07

was approved by Institutional Review Board of St. Jude Children's Research Hospital with written informed consent obtained from patients and families.

Neurocognitive Measures

Neuropsychological studies were conducted according to the study protocol at postoperative baseline, 6 months from enrollment (during maintenance phase) and yearly following treatment up to 5 years from end of therapy. Additional evaluations according to clinical needs were also performed. Intelligence quotient (IQ) was used as a performance measure of global cognitive functioning using age appropriate tools, namely Bayley III Cognitive Composite (ages 0–3 years), Stanford Binet 5 Abbreviated IQ (ages ≥3 years) or age-appropriate Wechsler (clinical testing).^{12–16} Psychosocial adjustment and adaptive functioning were assessed by parent ratings on the Behavior Assessment System for Children-2 (BASC-2). Age-standardized scores, based on representative normative samples, were interpreted as continuous variables and considered abnormal if they were beyond 1 standard deviation (SD) from the population mean score (IQ <85, BASC-2 externalizing, internalizing, attention, executive function >60, adaptive skills <40). Results from the most recent assessments were reported.

Exome Sequencing and DNA Methylation Array

Tumor DNA was extracted from formalin-fixed paraffin-embedded tissue and germline DNA from peripheral blood mononuclear cells. Tumor and germline DNA exomes were captured by using the TruSeq DNA exome kit (Illumina, CA, USA) platform. Paired-end sequencing reads were mapped to the human reference genome (National Center for Biotechnology Information GRCh37). Somatic and germline single-nucleotide variations/indels were called with both public tools including mutect2, varscan2, and SomaticSniper, as well as the in-house variant caller “bambino” as previously described.^{17–20} Tumors carrying *TP53* mutations at mutant allele frequencies $\geq 20\%$ were designated *TP53* mutant tumors. Copy-number alterations (CNAs) were inferred by using *exomeAI* and summarized with the *cghMCR* package through computing genome-wide Segment Gain Or Loss score.^{21,22} Genome-wide DNA methylation array was performed with Infinium MethylationEPIC BeadChip (Illumina, CA, USA) targeting 850,000 CpG sites in accordance with the manufacturer’s instructions. Raw data files generated by the iScan array scanner were read and preprocessed by using *minfi* Bioconductor package.^{23,24} Using the *minfi* package, the same preprocessing steps as in Illumina’s GenomeStudio software were performed. As quality control, any sample with more than 20% of probes having detecting *P*-value $>.05$ was excluded. In addition, the following filtering criteria were applied: removal of probes targeting the X and Y chromosomes, removal of probes containing-nucleotide polymorphism (dbSNP132 Common) within 5 base pairs of and including the targeted CpG-site, and removal of probes not mapping uniquely to the human reference genome (hg19) after allowing for one mismatch. Methylation profiles were examined by unsupervised clustering with relevant reference classes (plexus tumor, subclasses – adult [$n = 22$], pediatric A [$n = 15$], pediatric B [$n = 46$]) from a published cohort of CNS tumors (gene expression omnibus GSE90496).²⁵ Results were visualized by using hierarchical clustering and t-SNE analysis as previously described.^{25,26} In brief, the 15,000 most variable methylated CpG probes measured by standard deviation across combined samples were selected. 1-Pearson correlation was calculated as distance measure between samples and the unsupervised hierarchical clustering was performed with average linkage agglomeration method.

Statistical Analyses

Statistical analyses were performed using R Version 3.4.3. Patient characteristics were summarized using median and range for continuous variables and via frequencies and percentages for categorical variables. Overall survival (OS) and progression-free survival (PFS) were analyzed by Kaplan–Meier methods. OS was defined as the time from the date of enrollment to the date of death for any reason or last follow-up (censored). PFS was defined as the time from the date of enrollment to the date of progression, relapse, death, or last follow-up (censored). Comparisons of survival between subgroups were made by using the log-rank test. A significant association is defined by $P < .05$.

Results

Baseline Characteristics and Molecular Alterations

Thirteen patients with centrally confirmed CPC diagnosis were treated on SJYC07 (Table 1): 5 (38%) were male, median age at diagnosis was 1.41 years (range: 0.21–2.93 years), median duration of follow-up for all patients was 3.97 years (range: 0.65–10.34 years), and median duration of follow up for surviving patients was 5.11 years (range: 3.12–10.34 years). All patients had supratentorial tumors. Eight (62%) had metastasis at diagnosis (7 with MRI features of leptomeningeal spread; 1 with malignant cerebrospinal fluid cytology); whereas, 5 (38%) had no evidence of metastatic disease. The 8 patients with metastatic disease were treated on the HR arm, and the 5 without on the IR arm. Tumor samples from 7 patients (54%) carried pathogenic *TP53* mutations (mutant allele frequency: 67–94%), among which 4 (31%) were germline in origin (Figure 2A). Widespread arm-level CNAs were observed in all tumors, including predominantly copy-number losses in 11 patients and gains in 2 patients (Figure 2B, Supplementary Figure S1). Genome-wide DNA methylation profiling classified 11 (85%) tumors as plexus tumor-pediatric B; one tumor (8%) as plexus tumor-pediatric A (#8); and one did not pass quality control (#13, Figure 2C and D).

Surgery and Adjuvant Treatment

At diagnosis, GTR was achieved in 6 patients, NTR in 1, STR in 5, and biopsy in 1 (Table 1, Figure 3). Staged surgery was performed for 3 of the 6 patients with initial STR or biopsy, resulting in GTR in 3 patients with initial STR or biopsy (#2, #5, #9). The best extent of surgery was GTR/NTR in 10 (77%) and STR in 3 (23%). Three patients required ventriculo-peritoneal shunting. For the 5 patients on the IR arm, 3 received focal RT (54Gy, proton = 2, photon = 1) as consolidation and 2 patients did not. Two patients (#1, #2) completed all protocol therapy; 1 (#4) electively stopped therapy after 2 cycles of maintenance; 1 (#3) electively stopped therapy after 2 cycles of low-risk consolidation foregoing RT and maintenance chemotherapy; and 1 (#5) stopped therapy due to disease progression. For the 8 patients on the HR arm: 5 patients (#6, #7, #8, #9, and #13) completed all protocol chemotherapy; 1 (#12) selected CSI (36 Gy with tumor boost to 54 Gy, proton) as allowed by the protocol for being >3 years old at consolidation; 1 (#11) electively stopped therapy after consolidation foregoing maintenance; and 1 (#10) stopped therapy due to disease progression (Figures 1B and 3).

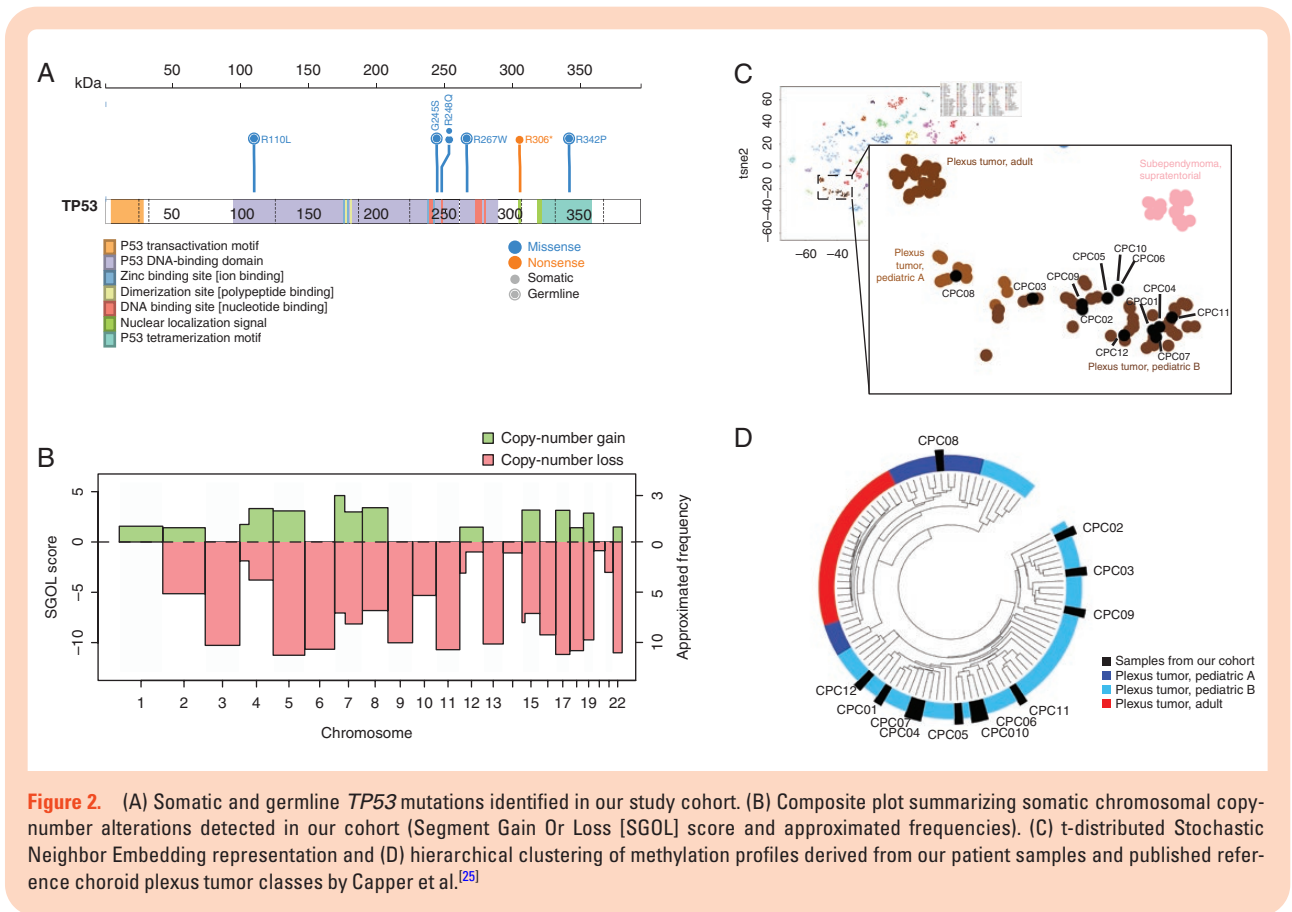
Response to Therapy

Nine of the 13 patients had evaluable disease after surgery (8 with HR/metastatic disease, 1 with IR/localized disease after STR) that could be followed over the course of the study. Other than 1 patient (#10) who had PD during

Table 1. Clinical Characteristics, Molecular Features, and Treatment Outcome of the Study Cohort

Pa-tient No.	Sex	Age at Diagnosis (y)	Me-tastasis	Risk Group	Tumor TP53 Testing	Germline TP53 Testing	Meth-ylation Class	Treatment Phases Completed	Extent of Resection	RT	Latest Disease Status	Time to Pro-gression (y)	Duration of Follow-up (y)	Hearing loss S/OP grade L/R ear
1	F	2.15	N	IR	-	-	CPT, Ped B	Induction/consolidation/maintenance	GTR	Focal RT (proton)	NED	N/A	10.34	0/0
2	M	1.96	N	IR	-	-	CPT, Ped B	Induction/consolidation/maintenance	STR -> GTR	Focal RT (IMRT)	NED	N/A	9.87	3/3
3	F	0.37	N	IR	-	-	CPT, Ped B	Induction/low-risk consolidation (declined maintenance)	GTR	Nil	NED	N/A	3.97	2/2
4	F	1.99	N	IR	R248Q (87%)	-	CPT, Ped B	Induction/consolidation/2 cycles of maintenance	STR	Focal RT (proton)	DOD	1.07	1.43	3/3
5	F	1.07	N	IR	R342P (87%)	R342P (42%)	CPT, Ped B	Induction	Bx -> GTR	Nil	DOD	0.31	0.65	2/0
6	F	0.21	Y	HR	R267W (82%)	R267W (N/A)	CPT, Ped B	Induction/consolidation/maintenance	GTR	Nil	NED	N/A	8.55	3/3
7	F	2.34	Y	HR	R306* (67%)	-	CPT, Ped B	Induction/consolidation/maintenance	GTR	Nil	DOD	1.38	5.22	1/1
8	F	0.80	Y	HR	-	-	CPT, Ped A	Induction/consolidation/maintenance	GTR	Nil	NED	N/A	5.11	1/1
9	M	1.61	Y	HR	G245S (78%)	G245S (43%)	CPT, Ped B	Induction/consolidation/maintenance	STR -> GTR	Nil	NED	N/A	5.11	3/3
10	M	0.34	Y	HR	R110L (91%)	R110L (N/A)	CPT, Ped B	1 cycle of induction	STR	Nil	DOD	0.09	3.13	0/0
11	M	0.33	Y	HR	R248Q (94%)	-	CPT, Ped B	Induction/consolidation	GTR	Nil	DOD	0.98	2.16	2/2
12	M	2.93	Y	HR	-	-	CPT, Ped B	Induction/consolidation (CSI, 2 cycles of VP16-CPM-VCR/VP16-Carbo-VCR)	NTR	CSI (proton)	PR	N/A	3.20	1/1
13	F	1.41	Y	HR	-	N/A	N/A	Induction/consolidation/maintenance	STR	Nil	SD	N/A	3.12	N/A

Bx, biopsy; carbo, carboplatin; CPM, cyclophosphamide; CPT, Ped A/B, choroid plexus tumor, subclass pediatric A/B; CSI, craniospinal irradiation; DOD, died of disease; F, female; GTR, gross total resection; HR, high risk; IMRT, intensity modulated radiotherapy; IR, intermediate risk; L, left; M, male; N, no; N/A, not available; NED, no evidence of disease; No., number; NTR, near-total resection; PR, partial response; R, right; RT, radiotherapy; SD, stable disease; STR, subtotal resection; VCR, vincristine; VP16, etoposide; y, year(s); Y, yes.



induction, 2 had complete response (#6, #8), 1 had partial response (#12), and 5 had stable disease at the end of induction (#4, #7, #9, #11, #13). With further treatment and at last follow-up, both patients who achieved complete response after induction remained disease free, and in the patient with partial response, the response was sustained. For the 5 patients who had stable disease after induction, tumor was stable in all after consolidation, and either remained stable (#11, #13) or responded completely (#4, #7, #9) in time. Two of patients who responded completely (#4, #7) relapsed while only one (#11) with stable disease progressed suggesting that “stable disease” at the end of therapy is a poor descriptor of active tumor.

Patient Outcome and Prognostic Factors

Five patients experienced progression [IR = 2 (#4, #5); HR = 3 (#7, #10, #11)] and died of disease and 8 were alive at last follow up (range: 3.12–10.34 years from enrollment). Among the survivors, 6 were alive with no evidence of disease (range: 3.97–10.34 years from diagnosis), 1 (#12) was alive with a sustained partial response at 3.2 years from diagnosis, and 1 (#13) was alive with stable disease at 3.12 years from diagnosis (Figure 3). Although recorded as partial/stable disease these persistent small radiographic lesions in the latter 2 patients are presumed to represent fibrotic or scar tissue in an area of prior disease rather than active disease since these patients have remained off therapy without tumor growth for a long time

period. However, given that the presence or absence of residual tumor cells could not be fully ascertained without histologic confirmation we stayed true to the terminology as defined in the trial. All progressions occurred within 18 months of diagnosis. The 2-year/5-year PFS and OS rates were $61.5 \pm 13.5\%/61.5 \pm 13.5\%$ and $84.6 \pm 10.0\%/68.4 \pm 13.1\%$, respectively (Figure 4). Patients who had *TP53* wild-type tumors (PFS: $P = .012$; OS: $P = .022$) had significantly better outcomes. All 6 patients with *TP53* wild-type tumors survived without progression, regardless of metastatic status (IR = 3; HR = 3). Of the 7 patients with *TP53* mutant CPC, 2 were alive at the time of this report at 8.55 and 5.11 years from enrollment, and 5 died of disease. Age, sex, risk group, resection status, metastatic disease, and use of radiation were not significantly associated with PFS or OS ($P > .05$ for all; Supplementary Figure S2). Notably, 3 patients with metastasis experienced durable disease control with GTR of primary tumor and chemotherapy alone. No second malignancies have been observed in survivors, including 2 patients with germline *TP53* mutations who did not receive radiation therapy (#6, #9).

Toxicities

Marrow suppression was common and expected during the intensive phases of chemotherapy (induction and consolidation). Patients received a median of 6 red blood cell transfusions (range: 0–11) and 5 platelet transfusions (range: 0–14) during therapy. Any hearing loss (SIOP grade

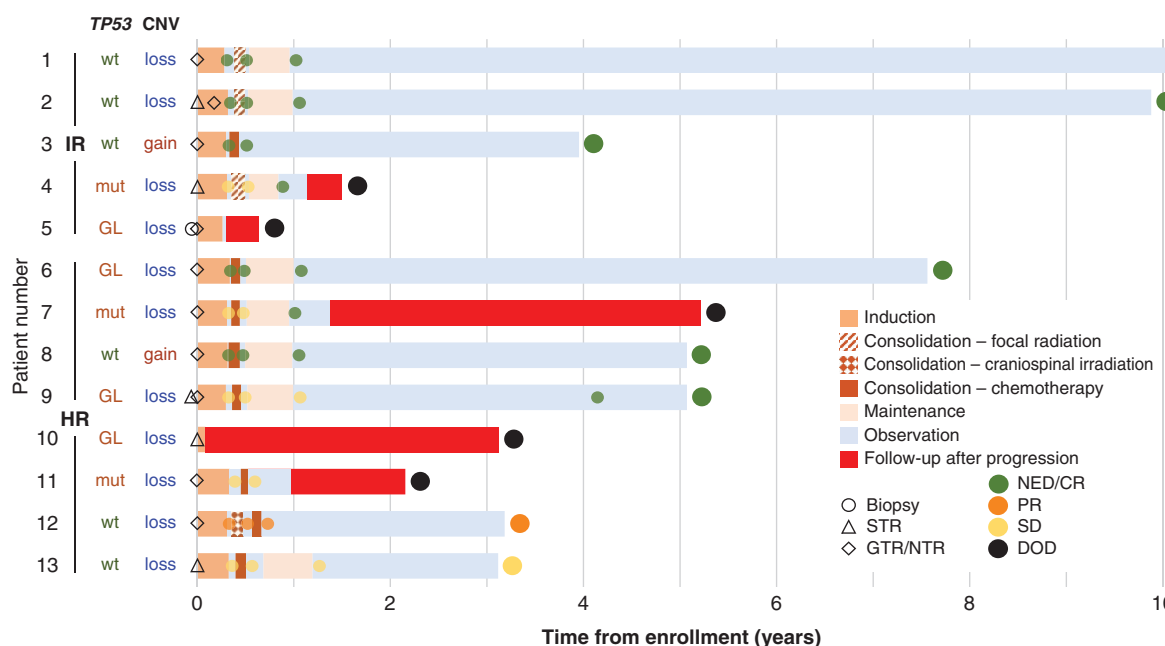


Figure 3. Swimmer's plot showing treatment profile and patient outcome in conjunction with risk strata, *TP53* mutational status, and predominant chromosomal copy-number variations (CNVs) in the respective tumors. CR, complete response; DOD, died of disease; GL, germline; GTR, gross-total resection; mut, mutant; NED, no evidence of disease; NTR, near-total resection; PR, partial response; SD, stable disease; STR, subtotal resection; wt, wild-type.

>0 in either ear) was detected in 10 patients (Table 1). Four patients had SIOP Grade 3 hearing loss in either ear. Other reported adverse effects during the induction or consolidation phases that were CTCAE Grade 3 or higher were febrile neutropenia ($n = 7$), non-neutropenic fever ($n = 2$), rash ($n = 2$), anorexia ($n = 3$), diarrhea/enteritis ($n = 3$), vomiting ($n = 2$), mucositis ($n = 2$), and subdural bleeding ($n = 1$). Reported toxicities of CTCAE Grade 3 or higher during maintenance therapy were neutropenia ($n = 5$); elevation of alkaline phosphatase ($n = 1$); and radiation necrosis ($n = 1$; Grade 3 after protons and treated with hyperbaric oxygen).

Neurocognitive Performance

IQ scores were available for 7 at follow-up (range: 6 months–5 years from diagnosis) (Supplementary Table 1). Four patients (57%) had IQ lower than 1 SD below normative mean, representative of performance significantly below age expectations. Parent-reported assessments on behavior and adaptive function were collected for 8 patients at follow-up (range: 6 months–6 years from diagnosis). Four patients (50%) displayed impairment in at least one domain with attention being most commonly affected.

Discussion

SJYC07 treatment resulted in outcomes that are among the best reported to date for young children with CPC

(Table 2).^{9,27–33} Importantly, these outcomes were achieved on a treatment regimen that was less intensive and, therefore likely to be less toxic, than those involving myeloablative chemotherapy with autologous stem cell rescue therapy. Remarkable outcomes were achieved in children whose tumors did not harbor *TP53* mutations (PFS and OS = 100%) and, somewhat surprisingly, metastatic status was not associated with a worse outcome. While extent of resection and receipt of radiation therapy were also not associated with outcome these results should be interpreted with caution since only 3 (23%) patients received STR and 4 (31%) received radiation. With the caveat that our cohort was small, these data support the use of chemotherapy in this population but also highlight the significant challenge facing patients with *TP53* mutant tumors.

Chemotherapy in the treatment of CPC has long been explored but the optimum regimen remains elusive. Efficacy of adjuvant chemotherapeutics in young children with CPC was first reported through the Pediatric Oncology Group 8633 protocol, in which vincristine, cyclophosphamide, cisplatin, and etoposide were given to delay RT in children younger than 3 years with CNS tumors.²⁷ Since most patients ultimately received planned RT, the long-term response to this chemotherapeutic regimen was not measurable. However, the favorable outcomes did suggest that CPC was a chemo-sensitive tumor and this led to more attempts to avoid or reduce RT. A combination of ifosfamide, carboplatin, and etoposide (ICE) over a median of 7 cycles with no RT planned resulted in a 5-year PFS of 53%.³¹ In CPT-SIOP-2000, patients with CPCs were randomized between 6 cycles of etoposide and vincristine,

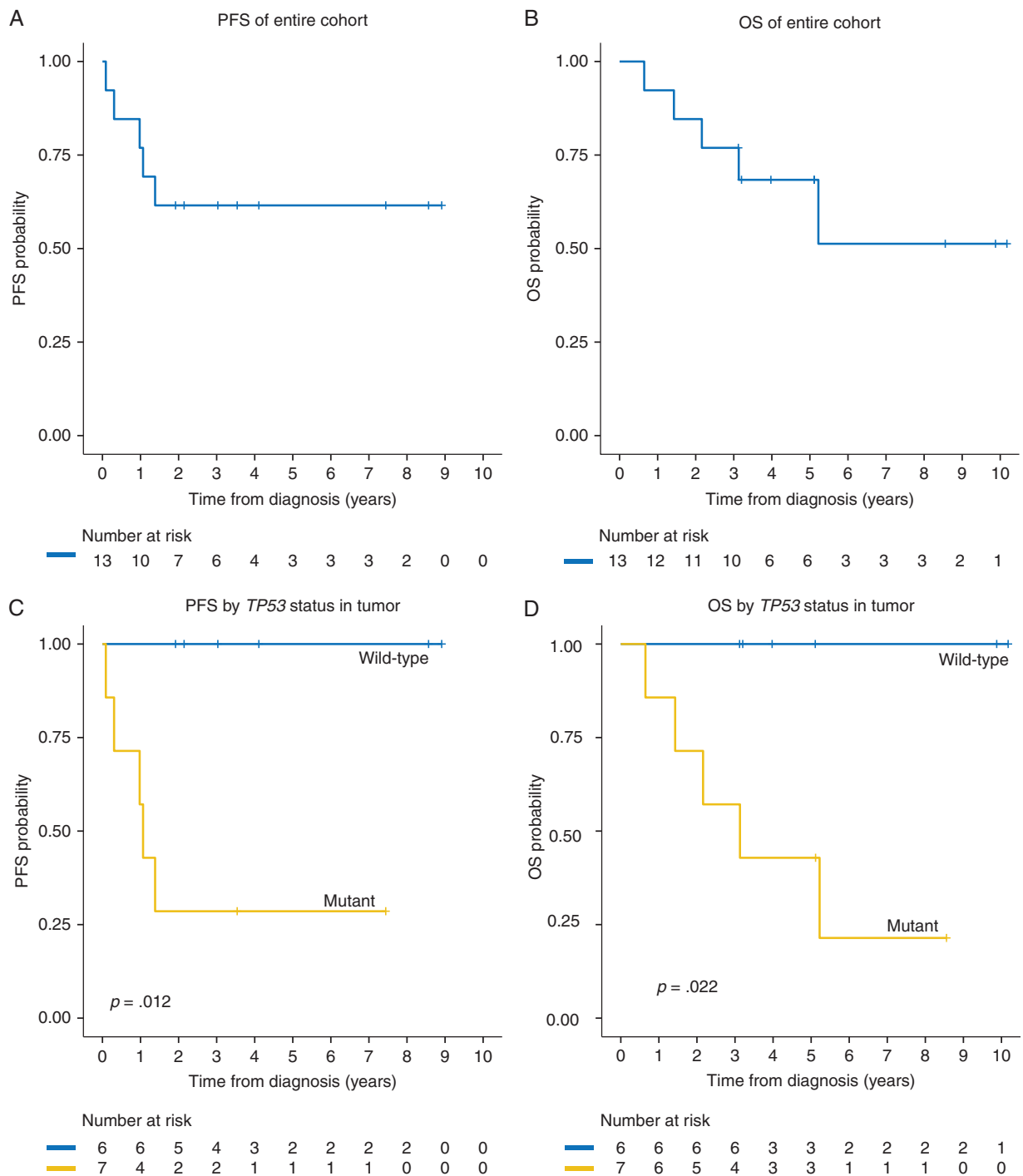


Figure 4. Progression-free survival (PFS) and overall survival (OS) of (A–B) the entire cohort (C–D) according to *TP53* mutational status.

with either carboplatin or cyclophosphamide (CarbEV or CycEV) and RT was given only to patients aged 3 years or older.^{29,34} Sixty patients were randomized and the 5-year EFS was 35% with no significant difference between the 2 arms. The subsequent CPT-SIOP-2009 trial ventured to randomize patients between 4 chemotherapy arms with alternating cycles of CarbEV/CycEV as the backbone but

was closed prematurely due to insufficient accrual.³⁰ Owing to the still less than satisfactory outcomes, the use of myeloablative chemotherapy and autologous stem cell rescue as consolidation was explored in the Head Start series.⁹ However, the added intensity did not appear to enhance patient survival and the 5-year EFS was reported as 38%.⁹ Hence, this SJYC07 study with its resultant

Table 2. Literature on Treatment Experience of Patients With CPC From Major Centers and Collaborative Trials

POG 8633	St. Jude (retrospective)	SIOP-CPT 2000	SIOP-CPT-2009	Sickkids	Canadian Pediatric Brain Tumor Consortium	Head Start I-III	French Pediatric Cancer Society	Our Study (SJYC07)
Duffner PK et al., 1995	Chow E et al., 1999	Wrede B et al., 2009	Bahar M et al., 2010 (abstract)	Lafay-Cousin L et al., 2010	Lafay-Cousin L et al., 2011	Zaky W et al., 2015	Siegfried A et al., 2017	
1986–1990	1985–1999	2000–2008	2009–2013	1985–2006	1990–2005	1991–2009	2000–2012	2007–2017
N = 8	N = 10	N = 34	N/A	N = 14	N = 16	N = 12	N = 22	N = 13
Age median (range)	12 m (1.5–27 m)	2.3 y (0.3–17.1y)	N/A	18.6 m (1.1–65.3 m)	10 m (1–30 m)	19.5 m (1–62 m)	2.11 y (0.14–16.54 y)	1.41 y (0.21–2.93 y)
M+	7 (70%)	7 (21%)	N/A	4 (28.6%)	10 (62.5%)	3 (25%)	8 (36.3%)	8 (61.5%)
Treatment approach after initial surgery	Adjuvant CTX with or without CSI	6 cycles of CTX, RT (CSI or focal RT) for patients >3 y old after cycle 2	Randomization to 4 adjuvant CTX arms (standard = 6 cycles), followed by RT after cycle 2 in a subset	Neoadjuvant/adjuvant CTX for median of 7 cycles, no RT planned	Adjuvant chemotherapy only, no RT planned	5 cycles of induction CTX followed by consolidation myeloablative chemotherapy with autologous stem cell rescue	Adjuvant chemotherapy with or without RT	Adjuvant CTX and focal RT for localized disease; Adjuvant CTX for metastatic disease; Maintenance chemotherapy for all
CTX regimen	Mainly combinations of VCR-CPM-CDDP/Carbo-VP16	VCR-VP16-CPM/Carbo (randomization)	Alternating VCR-VP. 16-CPM/Carbo cycles (standard arm N = 12) Insufficient accrual for randomization	ICE	“baby brain” protocols, the SIOP 2000 CPT protocol, or ICE chemotherapy (N = 14)	HS-I: VCR-CDDP-CPM-VP16HS-II: HDMTX-VCR-CDDP-CPM-VP16HS-III: alternating HDMTX-VCR-CDDP-CPM-VP16-TMZ-VP16-VCR-CPM	BB-SFOP (Carboplatin/procarbazine/VP16-CDDP/VCR-CPM, every 21 days, total 21 courses) and a variety of other regimens	Induction: HDMTX-VCR-CDDP-CPM ± VBLConsolidation: CPM-TopoMaintenance: Oral CPM-Topo-VP16
GTR/NTR	4 (50%)	16 (47%)	N/A	11 (78.6%)	6 (37.5%)	10 (83.3%)	10 (45.5%)	10 (76.9%)
RT given	6 (CSI)	N/A	N/A	2 (CSI)	0	5 (including 4 at recurrence)	9	4 (1 with CSI)
PFS/EFS	3-y PFS, 50%	5-y EFS, 28% (N = 29)	2-y EFS, 35%	5-y PFS, 53.3%	N/A	3-y and 5-y PFS, 58% and 38%	5-y EFS 25.2%	2-y and 5-y PFS, 61.5% and 61.5%
OS	3-y OS, 75%	1-y OS, 100%; 2-y OS, 87.5%; 3-y OS, 72.9%	2-y OS, 78%	5-y OS, 74.1%	2-y and 5-y OS, 50% and 31.3%	3-y and 5-y OS, 83% and 62%	5-y OS 64.7%	2-y and 5-y OS, 84.6% and 68.4%

carbo, carboplatin; CDDP, cisplatin; CI, confidence interval; CPM, cyclophosphamide; CPT, choroid plexus tumor; CSI, craniospinal irradiation; CTX, chemotherapy; EFS, event-free survival; GTR, gross total resection; HDMTX, high-dose methotrexate; HS-I/II/III, Head Start I/II/III; ICE, ifosfamide/carboplatin/etoposide; m, month(s); M+, metastatic disease; N, number; N/A, not available; NTR, near total resection; OS, overall survival; PFS, progression-free survival; POG, Pediatric Oncology Group; RT, radiotherapy; SFOP, French Society of Pediatric Oncology; SIOP, International Society of Paediatric Oncology; TMZ, temozolomide; topo, topotecan; VCR, vincristine; VP16, etoposide; y, year(s).

5-year PFS and OS of about 60%, shows that this non-myeloablative methotrexate-containing chemotherapy is, at least, an equivalent, if not better, and potentially less toxic, treatment option.

The role of radiotherapy in managing CPC, especially for young children, has been controversial. Review of data from the SEER database indicated no survival benefit with the use of radiotherapy for pediatric CPC (<20 years of age),^{1,6} and a meta-analysis of 28 children with CPC and Li-Fraumeni syndrome demonstrated a trend, albeit nonsignificant, towards inferior survival when treated with radiation.³⁵ In vivo and in vitro studies have demonstrated radio-resistance conferred by *TP53* mutations implying that *TP53* mutant CPCs will not benefit from RT.^{36,37} On the contrary, the risk of radiation-induced subsequent neoplasm is genuine and such a risk is exaggerated in children younger than 5 years at diagnosis and in those with germline *TP53* mutation; a clinical profile enriched in patients with CPC.^{35,38–40} In this study the effect of radiation therapy was hard to ascertain since only 4 (31%) patients received radiation and, when received, radiation was not uniform since 3 received focal therapy while 1 received CSI. Nonetheless, 5 out of the 8 surviving patients did so without radiation therapy and our analysis revealed no statistical survival difference between those who did and did not receive radiation. Furthermore, only one patient with *TP53* mutated CPC received radiation therapy and this patient died of disease. Thus, our data are too sparse to make a statement about the utility of RT in this population. However, with no appreciable benefit and a well-known high adverse event profile in this young population who already display a high prevalence of neurocognitive and behavioral impairment, we recommend against a RT-containing regimen for frontline treatment of children with CPC unless convincing evidence to the contrary becomes available.

The previously observed survival advantage of patients undergoing GTR/NTR could not be reproduced in our cohort, however, owing to the small number of patients in our cohort who did not achieve a GTR/NTR, it seems unwise to argue against aggressive surgical management in this disease.^{41,42} Our surgical approach was influenced by these prior findings and our patient population was managed accordingly. If GTR/NTR was not achieved after the first surgery, a staged approach was taken to allow for repeat surgery after 2–4 cycles of induction chemotherapy. In this way 77% of the population achieved a GTR/NTR before completion of therapy. Consequently, owing to our favorable outcomes, our recommendation is to continue this approach in future management of CPC.

Similar to radiation therapy, the benefit of the maintenance chemotherapy was hard to ascertain in this small population whose outcome was so heavily predicated by *TP53* mutational status. Eight patients received maintenance and six survived while two, both with *TP53* mutations, died. Thus, the utility of maintenance remains questionable and with no appreciable benefit our impression is that this additional therapy is not necessary.

Perhaps the most important, but unsatisfactory, finding of this study is that *TP53* mutant CPC harbor a very poor prognosis. The poor survival observed herein, confirms previous findings and helps to define a high-risk group

within CPC.⁴³ The unsatisfactory aspect of this finding lies in the knowledge that, at present, a better, more curative, therapeutic alternative is not available for these patients. Nonetheless, determining the *TP53* mutational status in CPC at the time of diagnosis is paramount for 3 reasons: (1) this knowledge allows oncologists to better prepare and guide parents through therapeutic decisions, (2) this feature, when positive, identifies a very high risk population in whom alternative novel therapeutic options should be explored and, (3) this identifies a population for whom germline testing should be offered. As seen in our cohort, about half of the patients had *TP53* mutated CPC and one-half of these carried the *TP53* mutation in their germline. This translates to approximately one-third of CPC patients having Li Fraumeni syndrome which predisposes them and any affected family member to almost 100% lifetime risk of cancer.⁴⁴ Therefore identification of patients with Li Fraumeni syndrome is important not just for the patient and their prognosis but also for the family as it informs on other affected family members and the need for additional surveillance.^{45–48}

Finally, we showed, through extensive molecular analysis, that our histological diagnosis of CPC was compatible with the DNA methylation and copy-number profiling performed on this study. Prior studies have shown that DNA methylation profiling can discriminate between the histological grades of CPTs (ie CPP, aCPP, CPC) often allowing for a more accurate and reproducible means of diagnosis.^{4,5,49,50} The methylation class Choroid Plexus Tumor, Pediatric A comprised of mostly histologic CPP or aCPP while class Choroid Plexus Tumor, Pediatric B was enriched with histologic CPCs.⁵⁰ In our study, 11 of 12 patients had tumors that clustered with reference class Choroid Plexus Tumor, Pediatric B. The one sample that clustered with subclass A was histologically described to display features of aCPP but judged more compatible with CPC due to mitotic figure counts, Ki-67 and metastatic status. In addition, all our study samples harbored genomic instability as reflected by frequent and multiple chromosomal copy-number alterations; a feature that is known to characterize CPC.^{4,5} In all, these data affirm that our cohort represents an accurate histologic and molecular depiction of CPC.

Despite the multi-institutional effort over a 10-year period, our study was limited by its small sample size. Our cohort of only 13 subjects restricted statistical power, prevented multivariable analyses, and may have kept us from detecting possible associations between clinical variables and outcome. Larger clinical-molecular cohorts will also be required to confirm characteristics such as prevalence of metastasis observed in molecularly verified choroid plexus tumors. Nonetheless, our analysis indicates that *TP53* mutational status is an important biomarker for the disease. As such, we propose that patients with newly-diagnosed CPC be stratified into *TP53* mutant and *TP53* wild-type subgroups for purposes of classification in diagnosis and risk-group assignment in upcoming clinical trials. Yet with regard to these subsequent trials, given the rarity of this condition and the need to subdivide this disease into these categories, we strongly advocate for these to be born out of international collaborations that can accrue the numbers necessary to demonstrate an improvement in outcomes. Therein, the *TP53*

wild-type subgroup could be managed with surgery and chemotherapy while novel approaches that incorporate intensified therapy or much needed experimental agents should be explored for the *TP53* mutant subgroup.⁵¹ Our excellent outcome for *TP53* wild-type subgroup supports the use of a HDMTX containing non-myeloablative regimen but exploring outcomes of *TP53* wild-type patients on other chemotherapy regimens could also yield alternative options for these studies. Moreover, our data suggest that avoidance of radiation in the front-line setting is the best current practice and reinforces the importance of germline *TP53* testing in children with CPC to determine their future cancer risk.

Supplementary Data

Supplementary data are available at *Neuro-Oncology Advances* online.

Keywords

choroid plexus carcinoma | clinical trial | high-dose methotrexate | infant | *TP53*

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