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

Anthony P.Y. Liu*, Gang Wu*, Brent A. Orr, Tong Lin, Jason M. Ashford, Johnnie K. Bass, Daniel C. Bowers, Tim Hassall, Paul G. Fisher, Daniel J. Indelicato, Paul Klimo Jr, Frederick Boop, Heather Conklin, Arzu Onar-Thomas, Thomas E. Merchant, David W. Ellison, Amar Gajjar, and Giles W. Robinson

Department of Oncology, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA (A.P.Y.L., A.G., G.W.R.); Department of Pathology, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA (G.W., B.A.O., D.W.E.); Department of Biostatistics, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA (T.L., A.O.T.); Department of Psychology, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA (J.M.A., H.C.); Department of Rehabilitation Services, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA (J.K.B.); Division of Pediatric Hematology and Oncology, University of Texas Southwestern Medical Center, Dallas, Texas, USA (D.C.B.); Queensland Children’s Hospital, Brisbane, Queensland, Australia (T.H.); Department of Neurology, Stanford University, Palo Alto, California, USA (P.G.F.); Department of Radiation Oncology, University of Florida College of Medicine–Jacksonville, Semmes Murphey Clinic, Memphis, Tennessee, USA (D.J.I.); Department of Surgery, St. Jude Children’s Research Hospital, Semmes Murphey Clinic, Memphis, Tennessee, USA (P.K., F.B.); Department of Neurosurgery, University of Texas Health Science Center, Semmes Murphey Clinic, Memphis, Tennessee, USA (P.K., F.B.); Le Bonheur Neuroscience Institute, Le Bonheur Children’s Hospital, Semmes Murphey Clinic, Memphis, Tennessee, USA (P.K., F.B.); Semmes Murphey Clinic, Memphis, Tennessee, USA (P.K., F.B.); Department of Radiation Oncology, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA (T.E.M.)

Corresponding Author: Giles W. Robinson, MD, Department of Oncology, St. Jude Children’s Research Hospital, 262 Danny Thomas Place, Mail Stop 260, Memphis, TN 38105, USA (giles.robinson@stjude.org).

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 ± 13.5% and 68.4 ± 13.1%. Patients with TP53-wild-type tumors had a 5-year PFS of 100% as compared to 28.6 ± 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.
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. These neoplasms are clinically and molecularly distinct from their lower-grade counterparts, which include choroid plexus papilloma (CPP) and atypical choroid plexus papilloma (aCPP). 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. 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. 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. 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.
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.

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.
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 ≥ 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 probes not mapping uniquely to the human reference genome (hg19) were filtered with the cghMCR package through computing genome-wide Segment Gain Or Loss score.21,22

Results

Baseline Characteristics and Molecular Alterations

Thirteen patients with centrally confirmed CPC diagnosis were treated on SJYCY07 (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 (54 Gy, 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...
| Patient No. | Sex | Age at Diagnosis (y) | Metastasis | Risk Group | Tumor TP53 Testing | Germline TP53 Testing | Methylation Class | Treatment Phases Completed | Extent of Resection | RT | Latest Disease Status | Time to Progression (y) | Duration of Follow-up (y) | Hearing loss SIOP grade L/R |
|------------|-----|---------------------|------------|------------|-------------------|----------------------|-------------------|---------------------------|-------------------|----|---------------------|----------------------|---------------------------|----------------------|
| 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    | 9.87               | 3/3                  |                          |                        |
| 3          | F   | 0.37                | N          | IR         | -                 | -                    | CPT, Ped B        | Induction/low-risk consolidation (declined maintenance) | GTR               | Nil            | NED    | 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    | 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    | 5.11               | 1/1                  |                          |                        |
| 9          | M   | 1.61                | Y          | HR         | G245S (78%)       | G245S (43%)          | CPT, Ped B        | Induction/consolidation/maintenance | STR -> GTR       | Nil            | NED    | 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-Carboplatin-VCR) | NTR               | CSI (proton)    | PR     | 3.20               | 1/1                  |                          |                        |
| 13         | F   | 1.41                | Y          | HR         | -                 | N/A                  | N/A               | Induction/consolidation/maintenance | STR               | Nil            | SD     | 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 ± 13.5%/61.5 ± 13.5% and 84.6 ± 10.0%/68.4 ± 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
>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,
Liu et al. Outcomes of young children with CPC

with either carboplatin or cyclophosphamide (CarbEV or CycEV) and RT was given only to patients aged 3 years or older. Thirty-six 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. 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. However, the added intensity did not appear to enhance patient survival and the 5-year EFS was reported as 38%. Hence, this SJYC07 study with its resultant

Figure 4. Progression-free survival (PFS) and overall survival (OS) of (A–B) the entire cohort (C–D) according to TP53 mutational status.
Table 2. Literature on Treatment Experience of Patients With CPC From Major Centers and Collaborative Trials

| Study | Duration | N | Age (range) | M+ | Treatment approach after initial surgery | CTX regimen | OS, 3-yr (%) | GTR/NTR (%) | RT given (%) | PFS/EFS, 3-yr (%) |
|-------|-----------|---|-------------|----|------------------------------------------|-------------|-------------|-------------|--------------|------------------|
| POG 8633 | 1986–1990 | 8 | 17.5 m (1.2–26 m) | 1 (12.5%) | Adjuvant CTX for 1–2 years followed by CSI | VCR-CPM-CDDP-VP16 | 5-y OS, 75% | 4 (50%) | 6 (CSI) | 3-y PFS, 50% |
| Chow E et al., 1999 | 1985–1999 | 10 | 12 m (1.5–27 m) | 7 (70%) | Adjuvant CTX with or without CSI | VCR-CPM-CDDP-VP16 | 5-y OS, 75% | 7 (70%) | 5 (CSI) | 3-y PFS, 50% |
| Wede B et al., 2009 | 2000–2008 | 34 | 18.6 m (1.1–65.3 m) | N/A | Randomization to 4 adjuvant CTX arms (standard = 6 cycles), followed by RT after cycle 2 in a subset | VCR-CPM-CDDP-VP16 | 5-y OS, 75% | 16 (47%) | N/A | 5-y EFS, 53.3% |
| Bahar M et al., 2010 (abstract) | 2009–2013 | 17 | 10 m (1–30 m) | 4 (28.6%) | Neoadjuvant/adjuvant CTX for median of 7 cycles, no RT planned | VCR-CPM-CDDP-VP16 | 5-y OS, 75% | 11 (78.6%) | N/A | 2-y EFS, 35% |
| Lafay-Cousin L et al., 2010 | 1985–2006 | 14 | 19.5 m (1–62 m) | 10 (62.5%) | Adjuvant chemotherapy only, no RT planned | VCR-CPM-CDDP-VP16 | 5-y OS, 75% | 6 (37.5%) | N/A | 5-y EFS, 53.3% |
| Lafay-Cousin L et al., 2011 | 1990–2008 | 16 | N/A | 3 (25%) | 5 cycles of induction CTX followed by consolidation myeloablative chemotherapy with autologous stem cell rescue | VCR-CPM-CDDP-VP16 | 5-y OS, 75% | 10 (83.3%) | 2 (CSI) | 2-y EFS, 35% |
| Zaky W et al., 2015 | 2009–2013 | 12 | 2.1 y (0.14–16.54 y) | 8 (62.5%) | Adjuvant chemotherapy with or without RT | VCR-CPM-CDDP-VP16 | 5-y OS, 75% | 10 (45.5%) | 0 (including 4 at recurrence) | 5-y EFS, 53.3% |
| Siegfried A et al., 2017 | 2010–2017 | 22 | 2.11 y (0.14–16.54 y) | 8 (36.3%) | Adjuvant CTX and focal RT for localized disease | VCR-CPM-CDDP-VP16 | 5-y OS, 75% | 13 (61.5%) | 5 (including 4 at recurrence) | 5-y EFS, 53.3% |

Note: PFS: progression-free survival; EFS: event-free survival; OS: overall survival; CSI: craniospinal irradiation; CTX: chemotherapy; Cl: confidence interval; CPM: cyclophosphamide; CPT: choroid plexus tumor; CS: cranial spinal irradiation; 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), 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. In vivo and in vitro studies have demonstrated radio-resistance conferred by TP53 mutations implying that TP53 mutant CPCs will not benefit from RT. 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. 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. 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. 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. 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.

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. 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. 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. 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. 31 Our excellent outcome for TP53 wild-type subgroup supports the use of a HD-MTX 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

Funding
This work was supported by American Lebanese Syrian Associated Charities and National Cancer Institute Cancer Center Grant (P30CA021765).

Acknowledgments
The authors thank Aksana Vasilyeva, PhD and Jana Freeman, CCRP from the St. Jude Division of Neuro-Oncology for coordination of study material, Emily Walker from the Hartwell Center for assistance with methylation profiling of our tumor samples, Ruth Tatevossian, MD, PhD, and Sujuan Jia, PhD, from the Diagnostic Biomarkers Shared Resource for providing nucleic acids extraction, Emilia Pinto, PhD, from the Department of Pathology for insightful discussions, Matthew Lear from the Biorepository for assistance in archiving and providing study material, and Cherise M. Guess, PhD, ELS, for editing the manuscript.

Conflict of interest statement. The authors declare no conflict of interest.

Authorship Statement. Data collection and analysis: all authors; central pathology review and molecular studies: B.A.O., D.W.E.; Statistical, sequencing, and methylation data analysis: A.P.Y.L., G.W., T.L., A.O.T.; manuscript drafting: A.P.Y.L., G.W., B.A.O., T.L., A.O.T., G.W.R.; all authors read and approved the final manuscript.

References
1. Dudley RW, Torok MR, Gallegos D, Liu AK, Handler MH, Hankinson TC. Pediatric choroid plexus tumors: epidemiology, treatments, and outcome analysis on 202 children from the SEER database. J Neurooncol. 2015;121(1):201–207.
2. Cannon DM, Mohindra P, Gondi V, Kruser TJ, Kozak KR. Choroid plexus tumor epidemiology and outcomes: implications for surgical and radiotherapeutic management. J Neurooncol. 2015;121(1):151–157.
3. Ostrom OT, Gittleman H, Xu J, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009-2013. Neuro Oncol. 2016;18(Suppl 5):v1–v75.
4. Thomas C, Sill M, Ruland V, et al. Methylation profiling of choroid plexus tumors reveals 3 clinically distinct subgroups. Neuro Oncol. 2016;18(6):790–796.
5. Merino DM, Shlien A, Villani A, et al. Molecular characterization of choroid plexus tumors reveals novel clinically relevant subgroups. Clin Cancer Res. 2015;21(1):184–192.
6. Cannon DM, Mohindra P, Gondi V, Kruser TJ, Kozak KR. Choroid plexus tumor epidemiology and outcomes: implications for surgical and radiotherapeutic management. J Neurooncol. 2015;121(1):151–157.
7. Wolff JE, Sajedi M, Brant R, Coppes MJ, Egeler RM. Choroid plexus tumors. Br J Cancer. 2002;87(10):1086–1091.
8. Mulhern RK, Palmer SL, Merchant TE, et al. Neurocognitive consequences of risk-adapted therapy for childhood medulloblastoma. J Clin Oncol. 2005;23(24):5511–5519.
9. Zaky W, Dhall G, Khatua S, et al. Choroid plexus carcinoma in children: the Head Start experience. Pediatr Blood Cancer. 2015;62(5):784–789.
10. Thorarinsdottir HK, Rood B, Kamani N, et al. Outcome for children <4 years of age with malignant central nervous system tumors treated with high-dose chemotherapy and autologous stem cell rescue. Pediatr Blood Cancer. 2007;48(3):278–284.
11. Wright KD, Panetta JC, Onar-Thomas A, et al. Delayed methotrexate excretion in infants and young children with primary central nervous system tumors and postoperative fluid collections. Cancer Chemother Pharmacol. 2015;75(1):27–35.
12. Bayley N. Bayley Scales of Infant and Toddler Development—third edition. San Antonio, TX: Harcourt Assessment. Journal of Psychoeducational Assessment; 2006; 25(2):180–190.
13. Roid G. Stanford-Binet Intelligence Scales—Fifth Edition. Itasca, IL: Riverside Publishing; 2003.
14. Wechsler D. Wechsler Preschool and Primary Scale of Intelligence—Fourth Edition. San Antonio, TX: The Psychological Corporation; 2012.
15. Wechsler D. Wechsler Intelligence Scale for Children-(WISC-V) Bloomington, MN: Pearson Education; 2014.
16. Reynolds CR. Behavior assessment system for children. Corsini Encycl Psychol. 2010:1–2.
17. Robinson G, Parker M, Kranenburg TA, et al. Novel mutations target distinct subgroups of medulloblastoma. Nature. 2012;488(7409):43–48.
18. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol. 2013;31(3):213–219.
19. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res.* 2012;22(2):568–576.

20. Larson DE, Harris CC, Chen K, et al. SomaticSniper: identification of somatic point mutations in whole genome sequencing data. *Bioinformatics.* 2012;28(3):311–317.

21. Nadaf J, Majewski J, Fahiminiya S. ExomeAI: detection of recurrent allelic imbalance in tumors using whole-exome sequencing data. *Bioinformatics.* 2015;31(3):429–431.

22. Aguirre AJ, Brennan C, Bailey G, et al. High-resolution characterization of the pancreatic adenocarcinoma genome. *Proc Natl Acad Sci USA.* 2004;101(24):9067–9072.

23. R Core Team. *R: A language and environment for statistical computing.* 2018. https://www.R-project.org/.

24. Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and comprehensive bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics.* 2014;30(10):1363–1369.

25. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of normal and cancerous brain tissues. *Nature.* 2018;555(7697):469–474.

26. Maaten Lvd, Hinton G. Visualizing data using t-SNE. *J Mach Learn Res.* 2008;9(Nov):2579–2605.

27. Duffner PK, Kun LE, Burger PC, et al. Postoperative chemotherapy and delayed radiation in infants and very young children with choroid plexus carcinomas. *Pediatr Neurosurg.* 1995;22(4):189–196.

28. Chow E, Reardon DA, Shah AB, et al. Pediatric choroid plexus neoplasms. *Int J Radiat Oncol Biol Phys.* 1999;44(2):249–254.

29. Wrede B, Hasselblatt M, Peters O, et al. Atypical choroid plexus papilloma: clinical experience in the CPT-SIOP-2000 study. *J Neurooncol.* 2009;93(3):383–392.

30. Bahar M, Dhir A, Kordes U, Wolff J. PTCT-01 Intercontinental multidisciplinary data collection and treatment optimization study for patients with choroid plexus tumors. *Neuro Oncol.* 2015;17(Suppl 5):v186.

31. Lafay-Cousin L, Mabbott DJ, Halliday W, et al. Use of ifosfamide, carboplatin, and etoposide chemotherapy in choroid plexus carcinoma. *J Neurosurg Pediatr.* 2010;5(6):615–621.

32. Lafay-Cousin L, Keene D, Carret AS, et al. Choroid plexus tumors in children less than 36 months: the Canadian Pediatric Brain Tumor Consortium (CPBTC) experience. *Childs Nerv Syst.* 2011;27(2):259–264.

33. Siegfried A, Morin S, Munzer C, et al. A French retrospective study on clinical outcome in 102 choroid plexus tumors in children. *J Neurooncol.* 2017;135(1):151–160.

34. Kordes UR, Hartung S, Löbel U, et al. EMBR-08. Choroid plexus tumors in 2018: the CPT-SIOP experience and long-term outcome. *Neuro Oncol.* 2018;20(suppl 2):i70.

35. Bahar M, Kordes U, Tekautz T, Wolff J. Radiation therapy for choroid plexus carcinoma patients with Li-Fraumeni syndrome: advantageous or detrimental? *Anticancer Res.* 2015;35(5):3013–3017.

36. Boyle JM, Mitchell EL, Greaves MJ, et al. Chromosome instability is a predominant trait of fibroblasts from Li-Fraumeni families. *Br J Cancer.* 1998;77(12):2181–2192.

37. Lee JM, Bernstein A. p53 mutations increase resistance to ionizing radiation. *Proc Natl Acad Sci USA.* 1993;90(12):5742–5746.

38. Meadows AT, Friedman DL, Naglia JP, et al. Second neoplasms in survivors of childhood cancer: findings from the Childhood Cancer Survivor Study cohort. *J Clin Oncol.* 2009;27(14):2358–2362.

39. Armstrong GT, Liu G, Yasui Y, et al. Long-term outcomes among adult survivors of childhood central nervous system malignancies in the Childhood Cancer Survivor Study. *J Natl Cancer Inst.* 2009;101(13):946–958.

40. Tsui K, Gajjar A, Li C, et al. Subsequent neoplasms in survivors of childhood central nervous system tumors: risk after modern multimodal therapy. *Neuro Oncol.* 2015;17(3):448–456.

41. Sun MZ, Ivan ME, Clark AJ, et al. Gross total resection improves overall survival in children with choroid plexus carcinoma. *J Neurooncol.* 2014;118(1):179–185.

42. Wrede B, Liu P, Wolff JE. Chemotherapy improves the survival of patients with choroid plexus carcinoma: a meta-analysis of individual cases with choroid plexus tumors. *J Neurooncol.* 2007;85(3):345–351.

43. Tabori U, Shlien A, Baskin B, et al. TP53 alterations determine clinical subgroups and survival of patients with choroid plexus tumors. *J Clin Oncol.* 2010;28(12):1995–2001.

44. Sengupta S, Weeraratne SD, Sun H, et al. α5-GABAA receptors negatively regulate MYC-amplified medulloblastoma growth. *Acta Neuropathol.* 2014;127(4):593–603.

45. Renaux-Petel M, Charbonnier F, Thyé JC, et al. Contribution of de novo and mosaic TP53 mutations to Li-Fraumeni syndrome. *J Med Genet.* 2018;55(3):173–180.

46. Chompret A, Abel A, Stoppa-Lyonnet D, et al. Sensitivity and predictive value of criteria for p53 germline mutation screening. *J Med Genet.* 2001;38(1):43–47.

47. Vazquez A, Bond EE, Levine AJ, Bond GL. The genetics of the p53 pathway, apoptosis and cancer therapy. *Nat Rev Drug Discov.* 2008;7(12):979–987.

48. Sanz G, Singh M, Peugeot S, Selivanova G. Inhibition of p53 inhibitors: progress, challenges and perspectives. *J Mol Cell Biol.* 2019;11(7):586–599.

49. Pienkowska M, Choufani S, Turinsky AL, et al. DNA methylation signature is prognostic of choroid plexus tumor aggressiveness. *Clin Epigenetics.* 2019;11(1):117.

50. Thomas C, Metrock K, Kordes U, Hasselblatt M, Dhall G. Epigenetics impacts upon prognosis and clinical management of choroid plexus tumors. *J Neurooncol.* 2020;148(1):39–45.

51. Mosleh O, Tabori U, Bartels U, Huang A, Schechter T, Bouffet E. Successful treatment of a recurrent choroid plexus carcinoma with surgery followed by high-dose chemotherapy and stem cell rescue. *Pediatr Hematol Oncol.* 2013;30(5):386–391.