Outcome of patients with intracranial non-germinomatous germ cell tumors – lessons from the SIOP-CNS-GCT-96 trial.

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Short title: Risk-adapted CNS non-germinomatous germ cell tumor treatment

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Abstract:

**Background.** Following promising results to increase survival and reduce treatment burden in intracranial non-germinomatous germ-cell-tumors (NGGCT), we conducted a European study using dose-intense chemotherapy followed by risk-adapted radiotherapy.

**Methods.** All patients received four courses of Cisplatin/Etoposide/Ifosfamide. Non-metastatic patients then received focal radiotherapy only (54 Gy); metastatic patients received 30 Gy craniospinal radiotherapy with 24 Gy boost to primary tumor and macroscopic metastatic sites.

**Results.** Patients with localized malignant NGGCT (n=116) demonstrated five-year progression-free survival (PFS) and overall survival (OS) of 0.72±0.04 and 0.82±0.04, respectively. Primary tumor sites were: 67 pineal, 35 suprasellar, five bifocal, nine others. One patient died post-surgery in clinical remission; three patients progressed during treatment and 27 (23%) relapsed afterwards. Fourteen were local, six combined and seven distant relapses (outside radiation field). Seventeen of the 27 relapsed patients died of disease. Patients with metastatic disease (n=33) demonstrated five-year PFS and OS of 0.68±0.09 and 0.75±0.08, respectively; one patient died following progression on treatment and nine (27%) relapsed afterwards (five local, one combined, three distant). Only one metastatic patient with recurrence was salvaged. Multivariate analysis identified diagnostic alpha-fetoprotein level (serum and/or cerebrospinal fluid) (≥1000 ng/ml, 19/149 patients, of whom 11 relapsed; p<0.0003) and residual disease following treatment, including after second–look surgery (n=52/145 evaluable patients, 26 relapsed; p=0.0002), as significant prognostic indicators in this cohort.

**Conclusion.** In localized malignant NGGCT craniospinal radiotherapy could be avoided without increased relapses outside the radiotherapy field. Chemotherapy and craniospinal radiotherapy remains the gold standard for metastatic disease.
Keywords: Intracranial non-germinoma; chemotherapy; radiotherapy; relapse; toxicity.

Importance of the Study

- We report the results of the European SIOP-CNS-GCT-96 trial for patients with intracranial non-germinomatous germ-cell-tumors (NGGCT), using cisplatin-based chemotherapy followed by risk-adapted radiotherapy (54Gy focal for localized disease; 30Gy craniospinal plus 24Gy tumor-boost to primary and metastatic sites).

- The study confirms that focal radiotherapy fields are sufficient for disease control in localized intracranial NGGCT patients (five-year progression-free-survival 72%) in the context of dose-intense chemotherapy; for metastatic cases, use of craniospinal radiotherapy results in similar outcomes (five-year progression-free-survival 68%).

- Diagnostic serum or cerebrospinal fluid (CSF) alpha-fetoprotein (AFP) ≥1000ng/ml is identified as a risk factor for subsequent relapse and allows upfront identification of patients who should receive treatment intensification.

- Residual disease not resected at the end of treatment is associated with an increased relapse risk; surgical resection of residuals is therefore advocated.
Introduction

Intracranial malignant non-germinomatous germ-cell-tumors (NGGCT) are a heterogeneous group of neoplasms mostly located in pineal and/or suprasellar regions. They include yolk-sac-tumor (YST), embryonal-carciinoma (EC), choriocarcinoma (CHC) and mixed malignant tumors in various combinations, often together with teratoma or germinoma components. YST and CHC can be diagnosed based on serum and/or cerebrospinal fluid (CSF) elevation of tumor markers, namely alpha-fetoprotein (AFP) and human-chorionic-gonadotropin (HCG) respectively, with/without histological confirmation.

Compared with pure germinoma, malignant NGGCT have poor prognoses. Long-term disease-free-survival of <10% were reported in the 1970s, mostly based on treatment with radiotherapy following biopsy or small resection\(^1\). Since then, platinum-based chemotherapy has proven highly effective in disseminated extracranial malignant GCTs\(^2,3,4\) and has also been administered for intracranial GCTs\(^5,6,7,8,9\). Different approaches such as neo-adjuvant or pre-operative chemotherapy were subsequently added to conventional surgery and radiotherapy to increase remission rates. Published results for intracranial malignant NGGCT are mostly based on single institution studies with small patient numbers with limited long-term follow-up data.

In Germany, patients with malignant NGGCT were treated with a combined strategy from 1986 onwards, employing platinum-based chemotherapy (Bleomycin/Etoposide/cisPlatin (BEP) or Vinblastine/Ifosfamide/cisPlatin (VIP) followed by 30Gy craniospinal radiotherapy and a 24Gy boost\(^2,3\); five-year relapse-free-survival was 67% with 110 months median follow-up\(^10\). In 1993, another European pilot study tested a four-course cisplatin-containing regimen.
(cisPlatin/Etoposide/Ifosfamide,PEI) followed by 30Gy craniospinal irradiation and 24Gy boost, leading to an event-free-survival (EFS) of 81% (median follow-up 11 months)\textsuperscript{8}.

The Japanese group published data on patients treated 1983-1995 according to risk groups.\textsuperscript{11} In the intermediate-risk group, a total tumor-free rate of 55.6% was achieved with Carboplatin/Etoposide or Cisplatin/Etoposide followed by local radiotherapy of 30Gy and additional maintenance chemotherapy. For poor-risk patients, treatment with Ifosfamide/Cisplatin/Etoposide (ICE) chemotherapy followed by 30Gy craniospinal radiotherapy and 30Gy tumor boost followed by further adjuvant chemotherapy failed to control disease. Chemotherapy-only approaches were considered inadequate for disease control, as half of patients relapsed\textsuperscript{6,12}. The recent North American trial ACNS0122 reported 102 NGGCT patients diagnosed by tumor markers, histology or both\textsuperscript{13}. Induction chemotherapy comprised six cycles of carboplatin/etoposide alternating with ifosfamide/etoposide, and second look surgery for incomplete response. Patients who did not achieve complete remission (CR) or partial remission (PR) after chemotherapy (with/without second-look surgery) received high-dose-chemotherapy and stem-cell-rescue. All patients received craniospinal irradiation (CSI; 36Gy spinal with 18Gy tumor boost, 45Gy to metastases) after chemotherapy. Five-year EFS was 0.84±0.04 and OS 0.93±0.03. Relapse occurred mainly at the primary site\textsuperscript{12}.

The European SIOP-CNS-GCT-96 trial opened in 1996, evaluating treatment in a large cohort, defining standardized approaches for diagnosis/staging. Patients with intracranial malignant NGGCTs received a combination of four courses of dose-intense cisplatin-based chemotherapy, followed by focal radiotherapy in non-metastatic cases or craniospinal if metastatic.
Methods

Patients. A total of 219 patients were enrolled into the SIOP-CNS-GCT-96 trial, of whom 35 were withdrawn because of incomplete staging and 35 who did not receive treatment according to protocol (Figure-1). A total of 149 patients (116 males, 33 females) with malignant NGGCT, confirmed by histology and/or tumor markers and complete work-up, were enrolled by 31st December 2005 and followed to 1st December 2014. The median age at diagnosis was 12 years(y) (range 4-30y). Thirty-eight (26%) were younger than 10y and 27 (18%) older than 16y at diagnosis; most of these were 16-23y; one was 30y. Patients were registered from Austria, Belgium, France, Germany, Italy, the Netherlands, Poland, Sweden, Switzerland, Spain and UK. Approval was gained from national ethics committees and all patients/parents gave written consent.

Diagnostic and staging procedures. Cranial and spinal MRI was mandatory for diagnosis and staging. For cytological detection of microdissemination, CSF sampling was required, obtained by lumbar puncture or, in cases of raised intracranial pressure, from the ventricles. Localized disease was defined as NGGCT without evidence of dissemination (negative CSF cytology and no metastatic disease on cranial/spinal imaging). Definition of bifocal disease required radiological detection of tumor in both pineal and suprasellar regions and was treated as localized disease. Metastatic NGGCT was defined as the presence of more than one intracranial focus (except bifocal disease), spinal metastases, metastases outside the central nervous system (CNS) or positive CSF cytology. Out of 149 patients, 116 (78%) had localized tumors. The most frequent primary tumor site was pineal (n=67) then suprasellar area (n=35). Five patients had bifocal disease. Other intracranial primary sites (n=9) included the ventricular system (n=3), diencephalon (n=3), frontal/parietal lobes (n=2) and...
internal capsule (n=1). Thirty-three patients (22% of the total) with metastatic disease presented with primary tumors located in the pineal (n=14), suprasellar region (n=8) and bifocal (n=5). Other intracranial sites (n=6) included the ventricular system (n=3), diencephalon (n=1) and frontal/parietal lobes (n=2). Of the 33 patients with metastatic disease, 17 (52%) showed macroscopic metastases on cranial and/or spinal MRI with negative cytology, three (9%) showed macroscopic metastases and positive CSF cytology, and 13 (39%) had positive CSF cytology alone.

Tumor symptoms, neurological status, endocrine function, hearing and visual status of patients were recorded at diagnosis. Fifty-nine patients (40% of total cohort) presented with diabetes insipidus at diagnosis (36 suprasellar site, 10 bifocal, seven pineal, six other site). Isolated pineal tumors with diabetes insipidus but no radiological evidence of neurohypophyseal/suprasellar involvement, were not considered bifocal in this study. Twenty-nine patients (19%) presented with panhypopituitarism (23 suprasellar, two bifocal, one pineal, three other site). Forty-five (30%) presented with visual disturbances, including oculomotor cranial nerve palsies (40 pineal, two suprasellar, three other site). In total, eighty-nine patients (60%) presented with abnormal ophthalmological examination (52 pineal, 23 suprasellar, five bifocal, nine other). Thirteen had hearing abnormalities at diagnosis (10 pineal).

Assessment of markers (AFP and HCG) in both serum and CSF was mandatory, but the site of CSF sampling (ventricular/lumbar) was not routinely recorded. A malignant intracranial NGGCT was diagnosed without need for biopsy confirmation if AFP was >25ng/ml and/or HCG >50IU/l in at least one serum/CSF compartment. The AFP and HCG cut-offs were pragmatic thresholds designed to avoid morbidity/mortality associated with upfront neurosurgical intervention. It is recognized that some HCG-secreting germinomas and AFP-secreting teratomas might consequently have been
included. Histological verification was only required for markers below these thresholds. In cases with elevated tumor markers and biopsy showing pure germinoma or teratoma, mixed-malignant GCT was diagnosed and treated as malignant NGGCT. Tumor markers were measured in all 149 patients at diagnosis. In 146, results of both markers in both compartments were available. In three patients, either AFP or HCG was measured and was elevated. AFP values were available in 148 patients (maximum 27,100ng/ml) and HCG in 147 (maximum 60,600IU/l). Patients with isolated elevated markers above threshold numbered 54 (36%) with AFP and 53 (36%) with HCG. Elevation of both markers was recorded in 32 patients (21%). Ten patients (7%) showed no marker elevation.

**Correlation of pathological analysis with tumor markers.** Pre-treatment surgical diagnosis was undertaken in 76 of 149 patients (51%). Fifty-five had a tumor resection and 21 underwent biopsy. In six of these 76, histology was either unavailable (n=3) or inconclusive (n=3) but all six had elevated tumor markers and were therefore diagnosed as malignant NGGCTs. Histology reports (local and/or reference pathology) with detection of GCTs were available in 70 patients; in two of these, the diagnosis was ‘malignant GCT’/‘teratoma with malignant transformation’ without a more precise histological classification. Among the remaining 68 patients with full histological classification, 28 showed mixed malignant GCTs (including >1 malignant component of YST, CHC, EC and germinoma).

Twenty-five patients presented with germinoma, either pure or mixed with YST (n=6), EC (n=3) or CHC (n=1). Five patients showed pure teratoma at biopsy; AFP levels in all these five patients were, however, elevated (range 115-430ng/ml), suggestive of a malignant NGGCT. Four of these five remained in CR, and one relapsed (initial suprasellar tumor with AFP of 430ng/ml).

Ten of 68 patients, with full pathology results of pre-treatment surgery available, showed no marker elevation at diagnosis. Centrally-reviewed histology showed YST component in five (all remained in
CR), YST and EC components in one (subsequent spinal relapse), pure EC in four (three CR; one combined ventricular/spinal relapse).

AFP elevation only, or together with HCG elevation, was seen in 37 of 68 patients with upfront surgical intervention and full histology; in 19 of these, YST was also detected histologically; in the 12 patients with combined AFP/HCG elevation, only two showed CHC histologically. Of the 21 with only HCG elevation, YST was diagnosed histologically in one and CHC components were present in four.

**Shunt procedures:** For patients who had symptomatic hydrocephalus (81 of 149; 54%), CSF diversion was performed before chemotherapy; 25 had third ventriculostomies, 15 short-term external-ventricular-drains and 41 ventriculo-peritoneal-shunts.

**Treatment:**

After diagnosis, patients received four cycles of dose-intense chemotherapy, comprising cisplatin/etoposide/ifosfamide (Figure-2). Body-surface-area cut-off was 2m². Response was evaluated with MRI head/spine and serum markers after two/four courses of chemotherapy and again at end-of-treatment. Patients in whom tumor markers had not returned to normal by the end-of-chemotherapy (n=4) were regarded as having an event, and treated off-protocol; all died of progressive disease. Patients were considered for surgery, and resection attempted, if there was evidence of growing tumor with normalized markers after the second chemotherapy cycle (usually growing-teratoma syndrome; not classed as an event) or residual disease after chemotherapy.
Radiotherapy was delivered according to initial dissemination and commenced after recovery from the fourth chemotherapy course and after second-look surgery, if undertaken. Patients without dissemination received 54Gy focal radiotherapy (30 fractions; six weeks). Bifocal disease was treated with focal radiotherapy, including both primary sites. In patients with metastases, craniospinal radiotherapy was delivered to a total dose of 30Gy (19 fractions; seven weeks) followed by a 24Gy boost to the primary tumor and macroscopic metastatic sites (Figure-2). Central review of radiotherapy plans was not part of this trial.

**End-of-treatment evaluation.** Follow-up tumor markers were obtained in serum only, unless clinical concern. End-of-treatment MRI was classified as radiological CR or residual disease (defined as any persistent enhancing visible lesions at the tumor site, regardless of treatment modality). Patients with residuals who underwent further surgery and in whom post-operative imaging confirmed disease clearance were also classified as CR.

**Follow-up.** Evaluation was performed regularly, combined with full clinical examination (including neurological status, ophthalmological, audiological and endocrine assessments), laboratory studies, measurements of serum tumor markers and imaging. MRI with contrast of brain (and spine if metastatic at diagnosis) was performed at least every six months for two years and annually thereafter. Median follow-up was 65 months (range 1 month-16y) for all patients (mean 63 months). Long-term assessment via follow-up questionnaires included the patient’s general condition, any relapse, second malignancy and persistent side-effects of therapy. Patients were followed up until 1st December 2014.
**Statistical analysis.** The survival probability was estimated according to Kaplan-Meier method. Five-year OS was calculated from date of diagnosis to date of last follow-up or death. PFS measured the proportion of patients among those treated for intracranial malignant NGGCT whose disease remained stable (without progression) for ≥five-years after treatment. EFS was calculated to measure the proportion who remain free of an event (relapse or death from any cause). Log-rank test was used to compare survival distributions (p<0.05 significant). For multivariate analysis, the variables age (<10y, ≥10y but ≤16y and >16y), gender, primary tumor site (pineal/suprasellar/bifocal/other), metastatic status, AFP levels (≤1000ng/ml, >1000ng/ml) and end-of-treatment residual disease (including after any additional surgery) were examined (p<0.05 significant). Data were recorded and monitored at University Hospital of Muenster. SAS (Version-9.2-for-Windows; SAS-Institute-Cary, NC) was used for statistical analysis.
Results

Localized malignant NGGCT

One patient with a suprasellar tumor died from surgical complications before the start of treatment but was in CR. In 30 of 116 patients (26%) with localized malignant NGGCT, progression/relapse occurred during (n=3; all died) or after treatment (n=27), giving a five-year PFS of 0.72±0.04 (Figure-3). Five-year OS was 0.82±0.04 (Figure-4). For those relapsing after the end of treatment, the most common site was local (loco-regional including ventricular; n=14), with six patients having a combined relapse (loco-regional and distant) and seven patients relapsing only outside the loco-regional tumor area (distant). Isolated ventricular relapses occurred in only two patients. The primary tumor sites of the 14 loco-regional relapses were pineal (n=6), suprasellar (n=4), bifocal (n=1), left frontomedial lobe (n=1), internal capsule (n=1) and inferior frontal lobe (n=1). Five of six patients with combined relapses outside the radiotherapy field had pineal disease at presentation. (Table-1).

Metastatic malignant NGGCT

One patient of 33 with metastatic disease progressed on treatment (rising markers) and died; nine relapsed after treatment, resulting in five-year PFS of 0.68±0.09 (Figure-3). Five-year OS was 0.75±0.08 (Figure-4). Seven progressions/relapses occurred in the 20 patients (35%) with macroscopic metastatic disease and three in the 13 (23%) with micrometastatic (cytological) disease only. There was no significant difference in relapse rates between these two groups (p=0.51). The primary tumor sites of metastatic patients comprised: 14 pineal region, eight suprasellar, five bifocal and six other (three ventricles, two frontoparietal, one diencephalon). Of the nine patients relapsing following treatment, five had local relapses, one combined and three distant (of which only one was
outside the radiation field). Only one of the 10 patients with progression/relapse could be salvaged (Figure-3). The primary tumor sites of these five local relapse patients were pineal (n=3), suprasellar (n=1) and bifocal (n=1) (Table-1).

**Prognostic factors**

**Diagnostic tumor markers.** Markers were below the defined thresholds in only 10 patients (7%) and two of these patients relapsed. HCG levels in HCG marker-elevated patients were 51-32,500IU/l (median 321) in serum and 53-60,600IU/l (median 306) in CSF. AFP levels in marker-positive patients were 29-27,100ng/ml (median 314) in serum and 26-15,700 ng/ml (median 97) in CSF. Isolated elevation of HCG did not affect prognosis (7/53 patients relapsed; 13%), whereas 31 of 86 (36%) with isolated AFP elevation or combined elevation with HCG relapsed. These figures compare with 40 progression/relapses from the total cohort of 149 patients (27%). AFP levels were divided into four groups for initial comparison (ng/ml): ≤25, ‘low’ group; >25 but ≤100, ‘mild’ elevation group; >100 but ≤1000, ‘moderate’ group; and >1000, ‘high’ group). There was a significantly worse PFS for patients in the ‘high’ AFP group (>1000ng/ml) compared with each of the three other groups individually by log-rank-test (p<0.0001 vs. low AFP group; p=0.04 vs. mild AFP group; p=0.0015 vs. moderate AFP group). None of the other AFP group comparisons were significant. Having identified an AFP value of >1000ng/ml as a risk-factor for worse PFS, we undertook a comparison of just two groups using this cut-off. This confirmed a worse PFS for the high AFP group (>1000ng/ml), where 11/19 patients relapsed (five-year PFS 0.32±0.12), compared with all other AFP levels (≤1000ng/ml), where only 29/130 patients relapsed (five-year PFS 0.76±0.04) (p<0.0001) (Figure-5). Analysis of HCG levels did not reveal any additional adverse prognostic information.
**Impact of residual disease.** The presence of residual disease on imaging at the end-of-treatment was also assessed as a potential risk-factor for relapse. Of 145 evaluable patients (end-of-treatment MRI centrally-reviewed), 52 had residual disease and 93 were in CR. Of these 93, 41 were completely resected. Consequently, there were 93 patients (65%) in CR with 52 remaining patients (35%) with residual. Only 13 of 93 CR patients relapsed compared with 26 of 52 with residual, resulting in five-year PFS of 0.85±0.04 and 0.48±0.07, respectively ($p<0.0001$) (Figure-6). Pathological findings at resection after chemotherapy could not be correlated with survival as they were not collected in case-report-forms, although those with information (n=12) had either teratoma (n=5) or necrotic/scar tissue (n=7).

**Multivariate analysis.** Multivariate analysis identified high diagnostic serum or CSF AFP (>1000ng/ml) and residual disease (after treatment and any second-look surgery) as conferring a worse PFS. Frequency of relapse differed significantly between patients with and without high AFP level at diagnosis (58% vs. 22%, respectively; $p=0.0003$) as well as with and without residual disease at the end-of-treatment (50% vs. 14%, respectively; $p=0.0002$). No significant differences in outcome were identified by patient age at diagnosis, gender, primary tumor site or metastatic status.

**Acute side effects of treatment**

Acute grade III or IV toxicities (NCI Criteria) occurred in 93/116 (80%) patients with localized disease and in 28/33 (85%) with metastatic disease; as toxicity rates were similar in both groups, they were
evaluated together. Grade III and IV chemotherapy toxicities consisted predominantly of leucopenia (n=109), thrombocytopenia (n=62) and vomiting/nausea (n=45). Nineteen patients developed grade III or IV infection. Renal function was impaired in 16 patients (low GFR in 13 and elevated serum creatinine in three). Fourteen patients experienced metabolic disturbances with severe fluid imbalance, all attributed to diabetes insipidus. Nine patients developed central neurotoxicity and nine subjective or objective hearing disturbance. In another ten patients, liver enzyme disturbances were seen. Other reported grade III or IV toxicities were rare and included oral toxicity (n=4), diarrhoea (n=5), elevated bilirubin (n=3), and peripheral neurotoxicity (n=2). One patient had an asystolic arrest after the first course of PEI requiring cardiopulmonary resuscitation and survived but the relationship to chemotherapy remained unclear. All reported acute toxicities related to chemotherapy resolved.

Grade III or IV toxicities related to radiotherapy were rarely reported in localized and metastatic disease, and included, mainly in metastatic disease, nausea/vomiting/diarrhoea (n=9), myelosuppression (leucopenia n=5, thrombocytopenia n=3) and skin toxicity (n=1). One patient died of surgical complications. Other toxicities after surgery (initial or during treatment) were confined to central neurotoxicity and infection. Four developed neurological deficits (hemiplegia, two 3rd cranial-nerve-palsy, disturbance of sensation and gait disorder of left leg). Infections were observed in three patients (meningitis, two with osteomyelitis of bone flap).
Discussion

We describe the largest prospective series of patients with intracranial malignant NGGCT, treated in the multinational European protocol SIOP-CNS-GCT-96, with follow-up outcome data. Previously, our group reported excellent outcomes for patients with pure germinoma treated on this study, using a combined chemo-radiation approach in localized cases to reduce radiotherapy volumes. 

Intracranial malignant NGGCT diagnosis on this protocol was based on typical radiology and then predominantly on AFP and HCG tumor markers in both serum and CSF. Biopsy was only mandated when both markers were below threshold values in both body fluid compartments, in particular to distinguish germinoma from EC cases, as treatment schedules were different for these two malignant subtypes. This approach was recommended to minimise avoidable morbidity and mortality from unnecessary neurosurgical interventions. Consequently, tumor tissue collection on this trial for molecular studies was limited. Patients were only included in the trial if they had full diagnostic work-up, including craniospinal MRI imaging, marker analysis, and CSF cytological assessment.

Our trial approach was to optimize overall outcomes, given the relatively poor prognosis for malignant NGGCTs when compared with their pure germinoma counterparts. Previous studies had confirmed the inadequacy of radiotherapy-only and chemotherapy-only strategies for intracranial NGGCTs. Other reports showed early promise for a combined chemo-radiation strategy for intracranial NGGCTs, albeit in single institution studies and/or with small cohorts and limited follow-up. These included the combined BEP/VIP, pilot-PEI and VP16/cisplatin studies. The pilot-PEI study was important in showing that the proposed strategy for SIOP-CNS-GCT-96 was both
safe and well-tolerated \(^8\), and the trial itself contained detailed guidance for managing complex patients, e.g. those with diabetes insipidus, to minimize potential acute treatment-related toxicities.

The SIOP-CNS-GCT-96 study reported here shows that combination chemotherapy and radiotherapy for all intracranial NGGCT patients, with risk-adapted radiotherapy tailored according to initial dissemination (focal for those with localized disease and craniospinal plus focal boost for metastatic cases), was effective at producing long-term durable treatment responses. Five-year PFS of 72% and 68% for localized and metastatic disease respectively, reflect this achievement. The results from this large multinational study were similar to previous and more limited reports on chemo-radiation delivery.

Risk factors

**AFP.** For many years, serum AFP levels have been used for risk stratifying both pediatric \(^{21}\) and adult extracranial GCT management. In the former study, serum AFP >10,000ng/ml identified patients at greater risk of relapse and thus many classification systems have resulted in such patients receiving additional chemotherapy cycles. For adults, the IGCCC system used serum AFP to assist classification in good-, intermediate- and poor-risk groups, which influenced prognosis and also the number of chemotherapy cycles delivered. Previous studies of intracranial disease involved too few patients to reliably identify high-risk groups warranting treatment intensification. In this study, 11 of 19 patients (58%) with diagnostic serum or CSF AFP levels >1000ng/ml relapsed, identifying them as a group with significantly worse outcome. Diagnostic HCG levels were, however, not associated with increased relapse-risk; indeed only 13% of patients with an isolated elevation of HCG relapsed, compared with
36% of cases with an AFP increase or an elevation of both markers. This may reflect that fact that the HCG-only group may have included some HCG-secreting germinomas.

**Residual disease.** The frequency of surgery to resect residual disease either after the end of induction chemotherapy, or at the end-of-treatment, was variable in patients treated on the SIOP-CNS-GCT-96 study. However, this allowed the assessment of the presence of residual disease on the likelihood of relapse. We previously demonstrated that residual disease in intracranial pure germinoma was not associated with inferior outcomes. In contrast, here we identified that survival for patients with intracranial malignant NGGCT who had end-of-treatment residual disease (including after second-look surgery), was significantly worse. Based on these results, we now recommend surgical resection of any residual lesions following completion of chemotherapy, to maximise the chance of achieving local tumor control.

**Relapses.** Twenty-seven of 116 localized NGGCT patients (23%) experienced a relapse following treatment, although importantly, only seven of these 27 (26%) experienced an isolated distant relapse. It should also be noted that only one of 13 patients with diabetes insipidus and non-suprasellar primary tumors relapsed in the suprasellar region, outside the radiotherapy field. These data support the use of focal radiotherapy fields for this population, particularly in the context of a dose-intense chemotherapy induction regimen. Nine of 33 metastatic patients (27%) had a relapse of their disease following treatment. These events were comparable between those with macroscopic and those with microscopic (cytological) metastatic disease. The similar rates of relapse between patients with localized and metastatic disease treated on the SIOP-CNS-GCT-96 protocol underscores the benefit of the risk-adapted radiotherapy strategy. These data also serve to further emphasise the importance of full diagnostic work-up for all intracranial GCT patients, including assessment of tumor
marker levels in both serum and CSF compartments, whole neuroaxis MRI and CSF cytology, to allow appropriate risk-adapted treatment to be delivered. Outcomes of a subgroup of patients who relapsed following treatment according to the SIOP-CNS-GCT-96 protocol are described elsewhere.  

Radiotherapy fields for localized NGGCT. It should be noted that for patients with localized NGGCTs, the volumes and doses of radiotherapy have historically varied. Prior to SIOP-CNS-GCT-96, the French group reported their chemo-radiation approach for NGGCT cases with focal fields, with EFS of 67% (24). Other national groups have used wider radiotherapy fields 13,25. The North American study reported excellent results using six courses of chemotherapy (alternating carboplatin/etoposide with ifosfamide/etoposide), prior to craniospinal irradiation 13. The most recent North American trial (ACNS 1123) asked whether in localized intracranial NGGCT cases radiotherapy volumes can be reduced to include just the whole ventricular system, and results of this trial are awaited. As noted above however, the focal radiotherapy fields employed for such cases in the SIOP-CNS-GCT-96 study were not associated with an excess of relapses in the ventricles or at distant intracranial sites, likely to be due to the dose-intensive chemotherapy regimen and short time to radiotherapy.

Late-effects. With improving survival for patients with intracranial NGGCT, minimizing potential long-term treatment-related effects assumes greater importance. SIOP-CNS-GCT-96 late-effects data will be reported separately.

Limitations. The SIOP-CNS-GCT-96 trial had a number of limitations. Firstly, it was not randomized, but merely recommended risk-adapted treatment based on extent-of-disease. Secondly, there was no real-time central review to confirm eligibility for trial inclusion based on adequate diagnostic and staging work-up, and consequently, a substantial number of enrolled patients had to be excluded.
from formal trial analysis due to missing data. Furthermore, reviewed pathology reports from second-look surgery at the end-of-treatment were not routinely available. That notwithstanding, this description of the largest prospective series of intracranial NGGCT patients treated in a uniform manner has allowed key risk factors for recurrence to be identified.

**Future directions and biology.** In future trials, incorporation of prospective biological studies will be important. Much recent progress has been made in understanding the molecular changes underlying intracranial GCT pathogenesis, e.g. the GCT mutational landscape through whole-exome-sequencing. Given the limited intracranial NGGCT tissue specimens available to study in Europe and North America however, collection of serum/plasma and CSF may in future allow both non-invasive diagnosis using microRNA expression levels and the identification of the presence of tumor mutations through circulating-tumor-DNA (ctDNA) analysis, which may inform novel treatment strategies.

**Conclusion.** In summary, we report the outcomes of the largest prospective series of patients with intracranial malignant NGGCT, treated in a multinational European protocol with a successful combined, risk-adapted, chemo-radiation approach. Following dose-intense chemotherapy, focal radiotherapy fields were sufficient for the treatment of localized disease whereas patients with metastatic disease had similar outcomes using craniospinal radiotherapy. The trial identified two key risk factors for recurrence. Firstly, patients with diagnostic serum or CSF AFP levels >1000ng/ml warrant treatment intensification. Secondly, those with end-of-treatment residual disease, including after second-look surgery, have worse outcomes. Resection of residual disease is therefore strongly recommended. These additional refinements aim to further improve OS for this patient cohort,
whilst continuing to spare patients with localized disease the long-term sequelae of CSI, through the successful use of focal radiotherapy.
Conflicts-of-interest

We have no conflicts-of-interest.

Role of the funding source

The supporting association of the study (German Cancer Aid and Elisabeth Dreves Stiftung) had no role in study design, data collection, analysis and interpretation, or report writing. CA/RK/JCN/MLG/CP/UR/FS/DF/DK/JRM/TP/AV/UG/MJM had access to national-level raw data. The corresponding author (GC) had full access to all study data and had final responsibility for publication.

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Figure and Table legends

**Figure-1.** CONSORT diagram of the 219 patients enrolled on the SIOP-CNS-GCT-96 trial; 149 patients were eligible.

**Figure-2.** Overview of the treatment regimen for the treatment of patients with intracranial malignant non-germinomatous germ-cell-tumors (NGGCTs) on the SIOP-CNS-GCT-96 trial protocol.

**Figure-3.** Progression-free-survival (PFS) for the 149 eligible patients on the SIOP-CNS-GCT-96 protocol (116 localized versus 33 metastatic patients).

**Figure-4.** Overall-survival (OS) for the 149 eligible patients on the SIOP-CNS-GCT-96 protocol (116 localized versus 33 metastatic patients).

**Figure-5.** Progression-free-survival (PFS) for the 149 eligible patients on the SIOP-CNS-GCT-96 protocol by diagnostic AFP level (130 patients with AFP ≤1000ng/ml versus 19 patients with AFP >1000ng/ml).

**Figure-6.** Progression-free-survival (PFS) for the 145 evaluable patients on the SIOP-CNS-GCT-96 protocol by end-of-treatment status (93 patients without residual tumor versus 52 with residual).
Table-1. Pattern-of-relapses following treatment for the 27 localized patients and nine metastatic patients on the SIOP-CNS-GCT-96 trial.

References


### Table 1: Pattern of relapses following treatment for the 27 localized patients and nine metastatic patients on the SIOP-CNS-GCT-96 trial

<table>
<thead>
<tr>
<th>Localized malignant NGGCT (n=27)</th>
<th>Metastatic malignant NGGCT (n=9)</th>
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</table>

**Key**

- **micrometastatic** = positive CSF cytology only
- **macrometastatic** = radiological metastases present on full neuroaxis imaging (MRI head and spine)
localized relapse = locoregional
combined relapse = locoregional and distant
Figure 1.
Cumulative dose of PEI:
CisPlatin (0.4 g/m²), Etoposide (1.2 g/m²), Ifosfamide (30 g/m²)

Figure 2.
Figure 3.
Figure 4.

- localized alive 97/116 OS = 0.82±0.04
- metastatic alive 25/33 OS = 0.75±0.08

p(log-rank) = 0.37
Figure 5.
Figure 6.

without residual tumor CR 80/93 PFS= 0.85±0.04  
with residual tumor CR 26/52 PFS= 0.48±0.07  
\( p_{\text{log-rank}} < 0.001 \)