Title: Paediatric Extracranial Germ Cell Tumours

Furqan Shaikh MD1*, Matthew J. Murray PhD2,3, James F. Amatruda MD4, Nicholas Coleman PhD2,5, James C. Nicholson MD3, Juliet P. Hale MD6, Farzana Pashankar MD7, Sara J. Stoneham MD8, Jenny N. Poynter PhD9, Thomas A. Olson MD10, Deborah F. Billmire MD11, Daniel Stark MD12, Carlos Rodriguez-Galindo MD13, A. Lindsay Frazier MD13

* Corresponding author

1 Division of Haematology/Oncology, The Hospital for Sick Children and the University of Toronto, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada. 1-416-813-7703. furqan.shaikh@sickkids.ca

2 Department of Pathology, University of Cambridge, Cambridge, UK

3 Department of Paediatric Haematology and Oncology, Addenbrooke’s Hospital, Cambridge, UK

4 Departments of Pediatrics, Molecular Biology and Internal Medicine, University of Texas Southwestern Medical Center; and Gill Center for Cancer and Blood Disorders, Children’s Health, Dallas, Texas, USA

5 Department of Histopathology, Addenbrooke’s Hospital, Hills Road, Cambridge, UK

6 Royal Victoria Infirmary NHS Foundation Trust, Newcastle upon Tyne, UK

7 Yale University School of Medicine, New Haven, CT, USA

8 University College London Hospitals NHS Foundation Trust, London, UK

9 Division of Pediatric Epidemiology and Clinical Research and Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA
10 Aflac Cancer and Blood Disorders Center, Emory University, Atlanta, GA, USA

11 Riley Hospital for Children, Indianapolis, IN, USA

12 Leeds Institute of Cancer and Pathology, University of Leeds, UK

13 Boston Children’s Hospital and Dana Farber Cancer Institute, Boston, MA, USA
Panel 1. Search strategy and selection criteria:

References for this Review were identified through searches of Medline with the search term “germ cell tumour” from 1990 until 2015. We included older, seminal publications that underpin understanding of the topic. Only papers published in English were reviewed. The final reference list was generated on the basis of relevance, historical impact, and opportunities for further reading.

Panel 2. Chemotherapy regimen abbreviations:

BEP: bleomycin (weekly dosing), etoposide, cisplatin

Accelerated BEP: BEP with the cisplatin/etoposide component administered every 2 weeks

CA/PVB: cyclophosphamide, actinomycin-D, cisplatin, vinblastine, bleomycin

CEB or BEC: carboplatin, etoposide, bleomycin (weekly dosing)

CEb: carboplatin, etoposide, bleomycin (paediatric trials, once per cycle bleomycin)

C-PEb: cyclophosphamide, cisplatin, etoposide, bleomycin

CBOP-BEP: carboplatin, bleomycin, vincristine, cisplatin + BEP

EP: etoposide, cisplatin

GETUG-13: BEP x1, paclitaxel/oxaliplatin/BEP x2, cisplatin/ifosfamide/bleomycin x2

HD-PE: high-dose cisplatin (150 mg/m²/cycle), etoposide

HD-PEb: high-dose cisplatin (200 mg/m²/cycle), etoposide, bleomycin

JEb: carboplatin, etoposide, bleomycin (paediatric trials; once per cycle bleomycin)

PEb: cisplatin, etoposide, bleomycin (paediatric trials; once per cycle bleomycin)

PEI: cisplatin, etoposide, ifosfamide

PVB: cisplatin, vinblastine, bleomycin
TI-CE: paclitaxel and ifosfamide followed by high-dose carboplatin and etoposide

TIP: paclitaxel, ifosfamide, cisplatin

VIP (or VeIP): vinblastine, ifosfamide, cisplatin
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SUMMARY

The management of paediatric extracranial germ cell tumours (GCTs) carries a unique set of challenges. GCTs are a heterogeneous group of neoplasms that present across a wide range of age, site, histology, and clinical behaviour. They are managed by a diverse variety of specialists. Correspondingly, their staging, risk-stratification, and treatment approaches have evolved disparately along multiple trajectories. Paediatric GCTs differ from the adolescent and adult disease in many ways, leading to complexities in applying age-appropriate evidence-based care. Suboptimal outcomes remain for several patient groups, and among survivors there are significant long-term toxicities. The challenge moving forward will be to translate new insights from molecular studies and collaborative clinical data into better patient outcomes. Future trials will be characterized by improved risk-stratification systems, biomarkers for response and toxicity, rational reduction of therapy for low-risk patients and novel approaches for high-risk patients, and improved international collaboration across paediatric and adult cooperative research groups.
INTRODUCTION

Although often referred to as a rare paediatric cancer, malignant GCTs (MGCTs) represent 3.5% of all childhood cancers that occur before 15 years (y) of age, making them approximately as common as childhood rhabdomyosarcomas, osteosarcomas, or retinoblastomas.\(^1\) In adolescents aged 15-19y, however, MGCTs represent 13.9% of neoplasms, becoming the most common solid tumour and the second most common malignancy, after Hodgkin lymphoma, in this age-group. Based on data from the Surveillance, Epidemiology and End Results (SEER) database, the United States (US) age-adjusted incidence of extracranial GCTs is 11.7 per million in boys and 6.7 per million in girls. There are about 900 new cases of MGCT diagnosed in the US each year in patients <20y. There are two distinct peaks in incidence, one in young children (0-4y) and another from the onset of puberty through young adulthood.\(^2\)

BIOLOGY

A better understanding of the molecular basis of GCTs may allow improved risk-stratification and identification of targets for the development of novel therapies, with the aim of improving overall survival for high-risk groups and rationalising therapy reductions in low-risk groups.

Aetiology. GCTs are hypothesised to occur as a result of events \textit{in utero}, although the aetiology remains largely unknown. Strong heritability estimates suggest a genetic susceptibility.[ref4]
Potential risk factors include parental demographic characteristics, *in utero* chemical or hormone exposures, parental lifestyle factors, and congenital abnormalities. Of these, cryptorchidism and Klinefelter syndrome are associated with an increased risk of testicular and mediastinal tumours in boys. Disorders of sexual differentiation such as Frasier syndrome, Swyer syndrome, and other androgen insensitivity syndrome are associated with an increased risk of GCTs in the streak gonads, principally gonadoblastoma.

*Development.* GCTs arise from early germline progenitors known as primordial germ cells (PGCs). The totipotent nature of PGCs explains the wide variety of possible GCT histologies observed (Figure 1). A widely held hypothetical model of tumorigenesis proposed by Teilum (Figure 2) views germinomas (seminomas and dysgerminomas in testicular and ovarian sites, respectively) as arising directly from undifferentiated PGCs and therefore retaining pluripotency. Embryonal carcinomas (EC) display early embryonic differentiation. These may further differentiate into tumours containing all three germ layers (endoderm, ectoderm and mesoderm), termed teratomas. In contrast, those that follow an extra-embryonic differentiation pathway result in either yolk sac tumours (YST; formerly endodermal sinus tumours), or choriocarcinomas (CC; tumours resembling the trophoblast). Tumours that contain multiple malignant histologies are termed mixed MGCTs.

PGCs migrate from the yolk sac to the gonadal ridge during early gestation through the midline of the developing embryo. Several factors are required for the survival and migration of PGCs, including the chemokine receptor *CXCR4*, and the *KIT* ligand *KITLG*, which is expressed in an increasing gradient to the gonadal ridge. Disruption of this migration process may explain the
occurrence of extragonadal GCTs and their midline propensity. Recent genome wide association studies (GWAS) have implicated single nucleotide polymorphisms (SNPs) in the KITLG gene in the development of GCTs in adults and adolescents. More than 25 SNPs in genes at 19 independent loci have been identified. These genes are implicated through five main mechanisms, including KIT/KITLG signalling, male germ cell development, telomerase, microtubule and DNA damage repair pathways. Observed odds ratios are the highest reported for any cancer type. In the future, it may be possible to derive a polygenic risk-score to inform potential screening strategies. It remains to be determines exactly how many of these SNPs are relevant to paediatric tumours.

**Epigenetics.** Epigenetic mechanisms may also contribute to GCT development.\(^8\) Migrating PGCs undergo erasure of methylation at so-called imprinted genes, followed by gender-specific re-imprinting during gametogenesis.\(^9\) The imprinting patterns of loci such as IGF2/H19 differ in paediatric GCTs, suggesting that tumours arise from earlier stages of PGC development in children. In paediatric MGCTs, YSTs have increased methylation at many gene regulatory loci compared with germinomas, including silencing of genes associated with apoptosis and suppression of WNT signaling.\(^10\)

**Genomics.** Gain of chromosome 12p is a universal feature of adult testicular MGCTs, regardless of histological subtype, usually due to isochromosome 12p formation.\(^11\) Seminomas and EC express key stem cell genes in this 12p region including NANOG and STELLA/DPPA3, which may block differentiation and favour cell proliferation. 12p gain is present but less common in MGCTs of young children, and the frequency increases over childhood with increasing age.\(^12\)
Additionally, gains of chromosomes 1q, 11q, 20q, 22q, and loss of 1p, 6q and 16q have been described in paediatric GCTs. However, the clinical relevance of 12p gain and other genomic abnormalities in MGCTs has not yet been established. High-resolution genomic studies will likely identify copy number variations (CNVs) that are associated with clinical outcome and may be incorporated into future risk-stratifications.

_Gene expression._ The two most common histological subtypes of MGCTs, YST and germinoma, exhibit distinct messenger RNA (mRNA) gene expression patterns. As in adult disease, paediatric germinomas express pluripotency genes [NANOG, POU5F1 (OCT3/4), TFAP2C, and UTF], whereas paediatric YSTs express genes relevant to differentiation (KRT8, KRT19), lipid metabolism (APOA1, APOA2), and proliferation pathways. However, these profiles segregate paediatric GCTs from adult testicular GCTs of the same histological subtype (Figure 3A), suggesting that different gene expression programmes may be driven at least in part by the alterations in hormonal status that accompany puberty. At present, the differential age-related mRNA profiles in the GCTs described have not been shown to be prognostic. However, a prognostic mRNA gene expression signature predictive of overall survival has been identified and validated in adult males with MGCTs and further validation is underway. A central goal of upcoming studies is to determine whether such signatures are prognostic in children.

Non-protein-coding RNAs represent another promising area of investigation in GCTs. The pluripotency gene LIN28 is expressed in all malignant GCTs across age-groups and histologies. The best understood function of LIN28 is to prevent biogenesis of the let-7 tumour suppressor family of microRNAs (miRNAs). MiRNAs are short 18-23 nucleotide RNAs that regulate the
expression of target mRNAs. Indeed, mRNA targets of *let-7* are upregulated in MGCT cells, including known oncogenes such as *MYCN*, making the *LIN28/let-7* pathway a promising target for therapeutic intervention. The oncogenic miR-371–373 and miR-302/367 clusters\(^\text{17}\) (see Figure 3B) are over-expressed in all MGCTs, regardless of age, site, or histologic subtype. Importantly, elevated levels of these miRNAs can be detected in the serum at the time of MGCT diagnosis as well as at relapse, and decline in response to treatment.\(^\text{18}\) Measuring circulating miRNA levels provides greater sensitivity and specificity for detecting MGCTs than the conventional protein biomarkers alpha-fetoprotein (AFP) and/or human chorionic gonadotrophin (HCG) and thus may represent a universal blood-based biomarker.\(^\text{19, 20}\)

**Biochemical signaling pathways.** As embryonal tumours, GCTs are frequently enriched for the expression of genes associated with normal embryonic development. A recent integrated analysis of methylation, miRNA, and protein-coding gene data confirmed differences by GCT histology [Poynter BMC]. In addition, YSTs exhibit gene expression and biochemical evidence of *WNT* pathway signaling, in contrast to germinomas where this rarely occurs.\(^\text{21}\) Similarly, differential protein-coding gene expression leads to activation of the *TGF-beta/BMP* pathway in YSTs, whereas *BMP* pathway activity is absent in germinomas.\(^\text{22}\) However, *WNT* and *BMP* pathways currently offer few possibilities for targeted therapies. In contrast, the prominent role of the KIT tyrosine kinase in germ cell biology suggests that this kinase or its downstream targets, the *PI3K/AKT/mTOR* and *RAS/RAF* pathways, may offer a more immediate target.\(^\text{23}\) For example, *KIT* gain-of-function mutations (D816V, D816H) activate the *PI3K* pathway in seminomas, even in the absence of *KITLG*. Unfortunately, responses to the *KIT* tyrosine kinase inhibitor imatinib in clinical studies have been disappointing, with no complete or even partial remissions reported.
Other recent research advances have focused on identifying mechanisms of cisplatin resistance. A limited study, interrogating only seven genes, found somatic mutations in \textit{PIK3CA}, \textit{AKT}, \textit{KRAS} and \textit{NRAS} may contribute to cisplatin resistance in adult testicular GCTs. A recent whole exome sequencing (WES) study in adult testicular GCTs revealed a low mutation rate (43%) compared with other human cancers. Two treatment-refractory patients were shown to harbour \textit{XRCC2} mutations, which may therefore be implicated in cisplatin resistance. Recent WES studies in intracranial GCTs have also identified known (\textit{KIT}, \textit{RAS}) and novel (\textit{JMJD1C}) mutations that may improve our understanding of extracranial GCT development, given their presumed common origin. Further research in MGCTs is required to fully understand the clinical impact of these biological insights, to incorporate molecular findings into risk-stratifications, and to prioritise new therapeutic approaches. For example, further study of the molecular epidemiology of paediatric GCTs is planned in a large case-parent triad study within the Children’s Oncology Group (COG) (NCI-R01-CA151284).

\textbf{CLINICAL PRESENTATION}

The diagnosis of a GCT should be considered for any midline tumour. The most common sites of extracranial presentation include the gonads (testes/ovaries), sacrococcyx, retroperitoneum, and mediastinum. Metastatic disease occurs in 20\% of cases at diagnosis and most commonly involves the lungs, but can involve bone, bone marrow, liver, or brain.
Sacrococcygeal GCTs can present around birth as a large exophytic mass. The differential diagnosis in this age-group is limited, although large haemangiomas or neuroblastic tumours may occasionally lead to diagnostic uncertainty. These sacrococcygeal GCTs are frequently detected antenatally on routine imaging and, if very large, can result in hydrops fetalis or obstruction of labour. These tumours are three times more common in girls than boys, and are usually teratomas with or without components of YST. Sacrococcygeal GCTs can also present after the neonatal period, usually <3y. In the absence of an external palpable mass, they are more likely to present as pain on sitting, buttock asymmetry, or bladder, bowel, or lower limb dysfunction. These later-diagnosed tumours are more likely to have undergone malignant transformation to include YST components.

Testicular GCTs usually present as a painless swelling of one testis. They occur either before 4y (predominantly as pure YSTs or teratomas) or after puberty (predominantly as mixed MGCTs or seminomas). The differential diagnosis includes hydrocoele, infection, torsion, sex cord stromal tumour, leukaemia, or paratesticular rhabdomyosarcoma.

Ovarian GCTs typically present with the gradual onset of abdominal distension and discomfort and a pelvic mass. Severe acute pain often indicates torsion, rupture or haemorrhage within the tumour. The peak incidence begins with the onset of thelarche around age 8y. Their histology can include mature (MT) or immature (IT) teratoma, dysgerminoma, YST, or mixed MGCT. A particular feature of ovarian IT is the propensity for peritoneal seeding as nodules of mature glial tissue, known as peritoneal gliomatosis. The differential diagnosis of an ovarian tumour in
children can include benign ovarian cysts, sex cord stromal tumours, or rarely an adult-type ovarian epithelial carcinoma.

Mediastinal GCTs present with symptoms caused by airway compression, superior vena cava obstruction, or heart failure. In prepubertal children, these tumours are usually teratomas. In adolescents, most commonly males, mediastinal GCTs typically contain a mixture of malignant components (YST, EC, CC) and teratoma, as well as possible non-germ cell components such as primitive neuroectodermal tumour. The main differential diagnosis is lymphoma, which is usually the first consideration, until the results of tumour markers become available.

**DIAGNOSTIC INVESTIGATIONS**

Elevated serum levels of the conventional protein tumour markers AFP and HCG assist in the diagnosis of YSTs and CC, respectively. Some elevation of AFP can be seen in EC or in teratomas that recapitulate endodermal elements or liver tissue, and some elevation of HCG can be seen in germinomas that contain syncytiotrophoblast (Figure 2). Consequently, AFP and HCG are not completely sensitive, as tumours without these histologies do not secrete tumour markers. It is estimated that 70% of non-germinomatous GCTs and 20% of germinomas are secreting.

Conversely, elevated tumour markers are also not specific for GCTs. The differential diagnosis of elevated AFP includes hepatoblastoma, hepatocellular carcinoma, liver surgery or inflammation, hemangioendothelioma of the liver, pancreatoblastoma, ataxia telangiectasia, and
hereditary persistence of AFP. Additionally, physiological elevation of AFP is seen during infancy. Therefore, an elevated AFP in an infant must be interpreted in the context of age-adjusted values and serial measurements. Two studies have catalogued the normal range of AFP in infants, and their results (Supplemental Table 1) provide a useful reference for clinicians. The differential diagnosis of an elevated HCG includes pregnancy, gestational trophoblastic disease, and rarely other hepatic or neuroendocrine tumours.

The level of tumour markers at diagnosis has been shown to be predictive of prognosis in adult testicular GCTs and forms part of the IGCCCG criteria. However, studies of paediatric GCTs have not consistently observed the same association. The rate of decline of AFP has also been shown to be prognostic in adult GCTs, but has not been formally evaluated in paediatric disease.

Other diagnostic investigations should include imaging of the primary tumour (ultrasound for testicular disease, cross-sectional imaging with MRI or CT scan for other sites) and potential metastatic sites (CT chest scan, bone scan, MRI head in stage IV CC).

Definitive diagnosis is based on histology. Most gonadal tumours are surgically resected upfront and therefore do not usually require pre-operative biopsy. Unresectable tumours requiring neoadjuvant chemotherapy should be biopsied in most cases. When it is unsafe to biopsy, the combination of typical radiological appearance and elevated conventional tumour markers may be sufficient to make a diagnosis and start neoadjuvant chemotherapy.
STAGING AND RISK GROUPS

Adult and paediatric cooperative groups have historically used different systems for staging and risk-stratification.

Clinicians treating adult patients with testicular tumours use the AJCC/TNM system for staging and the IGCCCG for risk-stratification in metastatic disease (Supplemental Tables 2 and 3). Those treating adult patients with ovarian tumours utilise the FIGO system (Supplemental Table 4). Paediatric groups utilise various post-surgical staging systems, but generally use stage I to refer to completely resected tumours, stage II for microscopic residual disease or persistently elevated tumour markers after resection, stage III for gross disease or nodal involvement, and stage IV for distant metastases (Supplemental Table 5 and 6). There are confusing inconsistencies resulting from the multiple staging systems. For example, an ovarian tumor with positive peritoneal cytology would be stage IC by FIGO but stage III by COG staging. A metastatic testicular tumor would be stage III by AJCC but stage IV by COG staging. These differences have made conversations and collaborations between different treating groups challenging.

The combination of site and stage assigns patients to risk-groups. While risk strata have also varied across cooperative groups and over time, nearly all risk-stratification systems are based upon the concept of a trichotomous ‘functional’ classification. In this scheme, a low-risk group is defined as one where patients can be managed with resection alone, followed by active
surveillance. The research priority for this group is determining whether patients other than those with testicular stage I disease can be safely managed with this approach. An intermediate-risk group includes those patients who do require chemotherapy but who have excellent outcomes with current regimens. The research priority for these patients is maintaining the high cure-rates while reducing late-effects. Lastly, a high-risk group represents patients who have unsatisfactory outcomes with current regimens and for whom further improvements in cure-rates are still needed.

Recently, investigators from COG and the Children’s Cancer and Leukaemia Group (CCLG) assembled a large pooled database of over 1100 children with extracranial MGCTs treated across seven clinical trials, termed the Malignant Germ Cell Tumours International Collaborative (MaGIC). The database was used to develop an updated paediatric MGCT risk-stratification system (Table 2). Age ≥11 y and tumour site and stage were significant predictors of worse long-term disease-free survival. For example, those ≥11 y and either stage III/IV extragonadal or stage IV ovarian tumours had predicted survival of <70%. These results will form the basis of new paediatric risk-groups for future trials.

SURGERY

While MGCTs have historically been treated with upfront resection and adjuvant chemotherapy, there is no clear difference in cure-rates between upfront or post-chemotherapy resection.
Aggressive resections at initial presentation are not necessary if neoadjuvant chemotherapy can help reduce surgical morbidity.

*Testicular.* An inguinal approach with early vascular control of the spermatic cord is indicated in all cases of testicular tumours. A trans-scrotal approach should never be used as this disrupts lymphatic channels and upstages the patient. Pre-pubertal boys with a normal AFP can have testis-sparing surgery with enucleation of the intact tumour if possible, because the diagnosis is likely to be either a teratoma or testicular stromal tumour. Pre-pubertal boys with an elevated AFP should have radical orchiectomy without violation of the tumour capsule in the surgical field. There is no role for retroperitoneal lymph node dissection (RPLND) in pre-pubertal boys.

After puberty, all testicular GCTs, including teratomas, are associated with precursor lesions within the adjacent testicular tissue known as intratubular germ cell neoplasia (ITGCN). Hence, radical inguinal orchiectomy is required. The role of RPLND in adolescents with enlarged lymph nodes is unclear, and treating clinicians should follow adult guidelines here. Commonly, chemotherapy is utilised after orchiectomy, and RPLND is used selectively for those with residual nodal enlargement or elevated AFP/HCG at the end of chemotherapy.

*Ovarian.* The majority of ovarian masses in children are benign cysts. However, if tumours are large, solid, or associated with elevated AFP/HCG, they should be approached as a suspected GCT. The involved ovary and tumour should be resected intact, with no morcellation and no deliberate interruption of the capsule, as these will affect histopathological staging. Given the importance of stage in determining adjuvant chemotherapy use, complete surgical staging is
mandatory. The recommended components of surgical staging are collection of peritoneal fluid or washings for cytology, inspection and palpation of peritoneal surfaces, omentum, retroperitoneal lymph nodes, and the opposite ovary, with biopsies of any areas of abnormality.\(^{45}\) Peritoneal fluid or washings can have positive cytology even when inspection of all tissues is normal. If the contralateral ovary was not clearly seen on pre-operative imaging, a pre-operative karyotype and intra-operative search for a streak gonad should be pursued. In bilateral ovarian involvement, neoadjuvant chemotherapy and delayed resection may allow the possibility of fertility-sparing surgery.

Extragonadal. Most neonatal sacrococcygeal tumours are benign teratomas, and complete surgical resection including the coccyx is necessary to reduce the recurrence risk.\(^{27}\) Continued follow-up with serial AFP levels, rectal exams and/or ultrasound imaging is required, due to a YST recurrence rate of up to 14%.\(^{46}\) For sacrococcygeal tumours diagnosed beyond the neonatal period, data from the German MAKEI trials demonstrated improved outcome with neoadjuvant chemotherapy followed by delayed tumour resection.\(^{27}\)

The surgical approach to a mediastinal primary tumour may be through a thoracotomy or sternotomy. Retroperitoneal primary tumours require generous trans-abdominal exposure. Complete resection of these tumours is challenging but is generally required for cure, despite associated morbidities.\(^{47}\)

CHEMOTHERAPY
ADULT TRIALS

Due to the epidemiology of GCTs, much of our understanding of the role of chemotherapy is based on investigations in the population of adult men with testicular cancer.

Prior to the 1970s, testicular cancers were treated with sarcoma regimens, with poor responses. The major breakthrough came in 1977, when Einhorn and Donahue used the cisplatin, vinblastine, and bleomycin (PVB) regimen, obtaining 100% response and 64% survival in men with disseminated testicular GCTs.48 This discovery was recently named as one of the top five advances in 50 years of modern oncology by the American Society of Clinical Oncology (ASCO).49 Etoposide was introduced in the 1980s, and a subsequent randomised trial showed the superiority of bleomycin, etoposide, and cisplatin (BEP) over PVB.50

In the 1990s, the IGCCCG developed a risk-stratification system for metastatic GCTs (Supplemental Table 3), categorising them as good-, intermediate- or poor-risk based on the combination of disease sites and AFP, HCG and lactate dehydrogenase (LDH) levels.35 In the good-risk group, clinical trials attempted to reduce the late-effects of BEP while maintaining excellent cure-rates through various strategies. Five randomised controlled trials (RCTs) investigated substituting carboplatin for cisplatin,51, 52 and another five RCTs investigated reducing or eliminating bleomycin,53 but BEP produced superior outcomes and remained the standard regimen.54 Two RCTs found that, in men with good-prognosis GCTs, three cycles of BEP were non-inferior to four.55, 56 In the intermediate- and high-risk groups, multiple intensification strategies were tested against four cycles of BEP, but none have shown improved survival. The GETUG-13 trial recently showed a small improvement in EFS after intensification
for men with poor tumour marker decline, but utilising a complex regimen whose generalisability and adoption remain to be established.  

PAEDIATRIC TRIALS

While building on the results of studies in adult MGCTs, paediatric oncology collaborative groups have modified and tested these approaches. The results of paediatric trials for MGCTs are summarised in Table 2.

In the 1990s, the North American Pediatric Oncology Group (POG) and the Children’s Cancer Group (CCG) conducted two intergroup studies to determine the optimal management of children with MGCTs.  

These studies incorporated the cisplatin, etoposide and bleomycin combination as developed in adult studies. However, due to the fear of excessive pulmonary toxicity in the developing lungs of children, the frequency of bleomycin was reduced from once every week to once every three weeks per cycle. Of note, the reduced frequency of bleomycin was not studied in a comparative manner against the weekly administration. This modified regimen is often referred to as PEb, to distinguish it from the adult regimen BEP. In contrast, the German MAKEI 96 study eliminated bleomycin entirely and substituted ifosfamide for advanced tumors.

The POG9048/CCG8882 study (INT-0106) successfully treated pre-pubertal boys with stage I testicular MGCTs with surgical resection and surveillance alone, as had been done for
Secondly, it showed excellent outcomes in children with intermediate-risk MGCTs treated with PEB. The second intergroup study, POG9049/CCG9981 (INT-0097) investigated whether a two-fold cisplatin dose escalation could improve survival in high-risk patients. While EFS improved, the utility of the high-dose strategy was limited by its significant ototoxicity. In the high-dose arm, 67% of children required hearing aids, compared to 10% in the standard-dose arm.

The intergroup studies were followed by the next generation of studies by COG, AGCT0132 and AGCT01P1. For low-risk patients, AGCT0132 attempted to extend the strategy of surgery and active surveillance to stage I ovarian tumours, with PEB chemotherapy reserved for recurrences. The 4-year EFS was 52% and OS was 96%. Thus, half of all patients could be spared the morbidity of chemotherapy, and almost all patients with recurrence could be rescued. The single patient who died in this study had chemoresistant disease from the outset of therapy.

For intermediate-risk patients, AGCT0132 investigated whether a reduction in therapy to three cycles of PEB could achieve equivalent outcomes to a matched historical cohort that received four cycles. In the overall analysis, EFS was significantly lower with three cycles. Post-hoc analyses showed that three cycles could be associated with excellent outcomes in lower-stage patients, but the study was not specifically powered for subgroup analyses. (Shaikh et al., unpublished). Therefore, four cycles of PEB remain the current standard. For high-risk patients, AGCT01P1 investigated combining cyclophosphamide with PEB but no clear improvement in EFS was evident.
In the United Kingdom (UK), a series of single-arm trials (GCI to GCIII) conducted by the CCLG substituted carboplatin for cisplatin with the goal of reducing the rate of long-term toxicities while maintaining high cure-rates. While adult trials had shown carboplatin to be inferior to cisplatin, the adult trials had generally used carboplatin at a lower dose, intensity, or frequency than the CCLG. The GCII trial used carboplatin 600mg/m2 every three weeks, corresponding to a median area-under-the-curve (AUC) of 7.9 mg/ml/min. For 137 children treated with JEB in this trial, the 5-year EFS was 88% and OS was 91%, which was comparable to the outcomes using PEb, but with no reports of sensorineural hearing loss. These results suggested that carboplatin could be a potential alternative to cisplatin in children when used in sufficient doses. This hypothesis forms the basis of an upcoming collaborative trial.

Several important clinical trials of paediatric GCTs have been conducted by cooperative groups in Germany, France, Brazil, and other centres. Their characteristics and results are summarised in Table 1, and readers are referred to the relevant references for further reading.

SPECIAL SCENARIOS

Teratomas. Teratomas are classified as MT (containing only well-differentiated tissues) or IT (containing less differentiated tissues including neuroectoderm). IT is graded from 0 to 3 based on the amount of immature neuroectodermal elements seen per microscopy field. MTs are managed by surgical resection alone. The management of ITs, particularly the need for adjuvant
chemotherapy, has been controversial. Based on a historic study, which observed a 70% relapse rate in adult women with grade 3 ovarian IT, adjuvant chemotherapy has commonly been used by gynaecological oncologists\textsuperscript{68}. However, paediatric trials did not confirm this observation. In a study by the CCLG,\textsuperscript{46} the EFS of 124 IT patients treated with surgery alone, regardless of grade or stage, was 86%. In a parallel COG trial,\textsuperscript{69} the EFS of 73 patients also observed after surgery without adjuvant chemotherapy was 93\%. In a recent pooled analysis, the risk of recurrence was seen primarily in patients with grade 3 stage III tumours. At present, most paediatric oncology groups do not utilise chemotherapy for IT. Teratomas with an MGCT component should receive chemotherapy directed to the malignancy. The MaGIC group is working on next steps for a joint paediatric-adult clinical trial for IT.

Adolescent and young adults (AYA). As paediatric oncologists have historically managed most patients <18y in North America or <16y in the UK, some adolescents have been treated with approaches developed for young children. Therefore, they may not be risk-stratified using the IGCCCG criteria, receive the added intensity of weekly bleomycin, or have the opportunity to receive a lower cumulative dose of cisplatin in the IGCCCG good-risk group. However, adolescent MGCTs more closely resemble the epidemiological and clinical characteristics of adults. Recently, the outcome for adolescents was shown to be worse than for young children and adults with testicular tumours.\textsuperscript{70} We have also validated this observation within the MaGIC database (Frazier \textit{et al.}, unpublished). Compounding the poorer AYA outcomes is the observation that adolescents with MGCTs are under-represented in clinical trials, frequently
missing the age inclusion criteria of both paediatric and adult studies. This represents a potent example of the ‘AYA gap’ in cancer care and research.\textsuperscript{71}

It is generally accepted that age-appropriate therapy is best delivered in an age-appropriate environment. The approach to AYA cancer care has started to change with the recognition of the specific medical and psychological needs of AYA patients, e.g. national UK referral pathways have been developed to ensure access to cancer care in specialist AYA treatment centres.\textsuperscript{72}

FUTURE TRIALS AND COLLABORATIONS

There are several planned and upcoming clinical trials for paediatric GCTs. A hallmark of each of these trials is international and transdisciplinary collaboration.

The AGCT1531 trial will be a collaborative effort involving the COG and national paediatric oncology centres in UK, Brazil, India, and Japan. As well, it will be co-sponsored by the National Research Group (NRG) Oncology and will enroll adult patients through the National Clinical Trials Network (NCTN) mechanism in the US.

The trial will include a low-risk and a standard-risk arm. For the low-risk group of patients, the trial will evaluate whether a strategy of complete surgical resection followed by surveillance can maintain an OS rate of $\geq 95\%$ for paediatric, adolescent and adult patients with stage I MGCT at
any site (testicular, ovarian, or extragonadal). The low-risk group will thus be expanded to include stage I extragonadal disease and will include patients up to age 50.

For the standard-risk group, the trial will compare the EFS of a carboplatin- versus a cisplatin-based regimen for paediatric, adolescent and young adult patients with MGCTs of all primary sites. Patients <11y will be randomised to CEb or PEb, while patients 11-25y will be randomised to BEC or BEP.

For the high-risk group, current plans are for paediatric groups to join ongoing exploratory trials of promising regimens, such as the Australian and New Zealand Urogenital and Prostrate (ANZUP) group trial of compressed BEP, where the cisplatin and etoposide components are administered every two weeks instead of three. Thereafter, the combined adult gynaecological oncology, adult testicular and paediatric groups plan to launch an international multi-arm randomised trial comparing among the most promising regimens for high-risk MGCTs.

For relapsed patients, the Alliance-sponsored study AO31102, referred to as the TIGER trial, will compare survival of male patients randomised to conventional chemotherapy with paclitaxel, ifosfamide and cisplatin (TIP) versus a regimen consisting of two cycles of paclitaxel and ifosfamide followed by three cycles of high-dose carboplatin and etoposide with stem-cell rescue (TI-CE).\textsuperscript{73} It will also prospectively evaluate the properties of the International Prognostic Factor Scoring Group (IPFSG) system\textsuperscript{74} as a predictor of outcome after relapse. The trial will be made available to adolescent patients >14y through a co-sponsorship with the COG.
Biologic aims of these upcoming trials include defining robust biomarkers and molecular signatures that predict risk of disease progression or chemoresistance, evaluating the potential for serum miRNAs as sensitive and specific tumour markers for malignant disease response and recurrence, investigating the pharmacogenomics of chemotherapy and late-effects, and identifying targets for novel therapeutic agents. It is likely that further progress for the high-risk group of patients will be achieved primarily through novel approaches, including the identification of molecular targets.

LATE-EFFECTS

Because most children with GCTs are cured, treating clinicians need to be aware of, and try to mitigate, late-effects of treatment. However, GCTs were not included in the Childhood Cancer Survivor Study, and hence knowledge of late-effects is largely extrapolated from the experience of adult patients treated for testicular cancer.

The most common toxicities of cisplatin are ototoxicity, nephrotoxicity, and neurotoxicity. Cisplatin ototoxicity is caused by damage to the hair cells in the cochlea resulting first in high frequency hearing loss, although loss in lower frequencies are also observed in children with either prolonged exposure or an inherent susceptibility to cisplatin ototoxicity.\(^{75}\) High frequency tones are important for language development in young children, and cisplatin ototoxicity is more severe at younger ages of treatment. Several studies demonstrate that cisplatin ototoxicity is not static but worsens over time, and hearing loss may first be diagnosed as late as two years
after therapy completion. Moreover, even children without overt ototoxicity have an advanced ear age and may be prone to early onset age-related hearing loss.

Men with testicular cancer treated with cisplatin have a 15% decrease in glomerular function that is immediate and irreversible. Although this decrease is initially subclinical, similar decrements in renal function have been associated with increased cardiovascular and all-cause mortality. Neurotoxicity such as paraesthesia are also caused by exposure to cisplatin, although these are more commonly seen in adults than in children.

Cisplatin is a heavy metal and circulating levels of cisplatin adducts can be detected in the serum of patients more than ten years after treatment. The degree of circulating platinum has been shown to correlate with the severity of neurotoxicity in adults.

Up to half of patients develop evidence of pulmonary toxicity upon exposure to bleomycin. Although this is reversible in most patients, recent studies in testicular cancer survivors have found an 8% prevalence of restrictive lung disease, and a 2.5-fold elevated risk of death from pulmonary disease compared to the general population.

A two-fold increased risk of cardiovascular disease and second malignancy exists in men treated for testicular cancer. Of note, the increased second cancer risk occurs at a rate of approximately 1% per year, with no plateau. By 75y, a seminoma patient would have a cumulative risk of 28% if treated at 50y, 36% if treated at 35y, and 47% if treated at 20y. Although the relative risk for younger age at diagnosis is not known, this trend is concerning if
extrapolated to paediatric patients, who may be treated with the same regimen as early as infancy.

CONCLUSIONS

We allude to the challenges in the management of paediatric GCTs and highlight ways in which these have been, or can be, overcome. Although significant challenges remain, the way forward has been charted. A new era of collaboration is underway, building bridges between paediatric and adult cooperative groups as well as across international borders. These collaborative efforts will allow for the development of a standardised vocabulary for staging, risk-stratification, and treatment approaches and for new clinical and biological insights. Options for reduction of therapy for those with excellent probability of cure and intensification or novel approaches for those with poor-risk disease will be explored. Together, these advances will allow us to approach the goal of curing all patients with MGCTs, and to do so with the least possible late-effects.
CONFLICT OF INTEREST STATEMENT

Dr. Stark received grants from Teenage Cancer Trust during the conduct of the study. Dr. Frazier has served on the Germ Cell Tumor Advisory Board for Seattle Genetics and has provided consulting to Decibel Therapeutics. All other authors declare no conflict of interest.

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