Methods: This multi-institutional study pooled data of patients who died due to GCT from three academic, high-volume adult GCT referral centres (Dana-Farber Cancer Institute, Memorial Sloan Kettering Cancer Center, Indiana University) between 1997 and 2017. Additionally, we collected data of paediatric and adolescent GCT patients treated on clinical trials in the US, UK, Europe and South America from the MaGIC group. Site, stage, risk, histology, primary therapies and relapse data (including relapse histology, metastatic burden, salvage and palliative therapies) were collected, in addition to detailed cause of death.
Results: Data from approximately 900 patients (adult and paediatric) who died of GCT have been collated and currently undergoing analysis. First results will be presented at the International Extracranial Germ Cell Tumour Conference, Cambridge, 2019.

GCT-73 The features and management of late relapse of nonseminomatous germ cell tumours: The Royal Marsden Experience

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Background: Late-relapses (LR) of non-seminomatous germ-cell-tumours (NSGCT), (i.e. after disease-free-interval of ≥2 years), are increasingly recognised. We reviewed the features of LR in NSGCT within a tertiary referral testicular cancer service.
Methods: 3,064 patients were referred to the testis multi-disciplinary-team (January 2005 to Dec 2017). Patients who experienced LR where initial pathology demonstrated NSGCT were identified. Data from original and late presentation and management was reviewed.

Initial stage	n	Time to recurrence (Months) Median (95%CI)	Management at relapse		
			Surgery alone	Primary Chemo ±(surgery)	other
Stage 1	31	55 (48–108)	14	17 (9)	0
Stage 2	29	120 (84–192)	25	4(0)	0
Stage 3	41		30	8 (4)	3

Results: Of the 3,064 patients, 101 (3.3%) had LR, with 43 (43%) relapsing >10 years. 36 were symptomatic and 39 had raised markers (AFP 29, HCG 9, both 1). Table shows stage at initial presentation and time-to-relapse. 13 CS1 patients had received prior chemotherapy (8 adjuvant and 5 for early relapse). 59/60 CS2/3 patients received chemotherapy as primary treatment and 41 had post-chemotherapy retroperitoneal-lymph-node-dissection (PC-RPLND (bilateral template in 12). 20 of these 41 men who had a PC-RPLND experienced retroperitoneal LR (6 after bilateral template). Patient management at relapse – see Table. Time-to-recurrence was longer in CS2/3 patients ($p < 0.001$). 84 surgical procedures – histology was teratoma-differentiated in 44, yolk-sac 14, de-differentiated in 7 and viable GCT in 11, benign 8. To date, 22/101 (20 from NSGCT) patients have died, 13 of these patients relapsed at multiple sites. Men with symptomatic disease (13/36, 33%) and receiving chemotherapy and no surgery (10/17, 59%) tended to have worse survival. LR of NSGCT frequently occurs after an extended interval and typically occurs earlier in CS1 disease compared with higher stages. Aggressive surgery±chemotherapy can cure most patients.

GCT-74 Gene expression studies in platinum-resistant testicular germ cell tumours

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Background: Although testicular germ cell tumours (TGCT) are mostly curable using cisplatin-based chemotherapy, a small subset are platinum-resistant and have poor prognosis. To better understand platinum-resistance, we conducted gene expression studies using a case-control cohort of platinum-resistant and platinum-sensitive TGCT, comparing differences between (i) platinum-resistant and sensitive primary tumours; (ii) platinum-resistant primary and paired resistant post-chemotherapy metastases; and (iii) platinum-sensitive primary and paired post-chemotherapy teratoma.
Methods: An institutional database identified platinum-resistant (defined as recurrence/persistence following platinum-based chemotherapy) and platinum-sensitive patients (matched by IGCCCG risk). Where available, archival specimens were retrieved and macro-dissected to ensured >80% cellularity. Gene-expression analyses used Nanostring nCounter and a customized list of 30 genes selected for potential role in platinum-resistance. Differences were compared using t-tests, with $p < 0.0017$ considered significant.
Results: We identified 19 platinum-resistant and 22 platinum-sensitive patients with available primary tumour specimens. Age (median: 34 y versus 28 y), IGCCCG risk (poor: 32% versus 27%) and histology (non-seminoma: 84% versus 86%) were similar. Median time to relapse in resistant patients was 3.4 mo; median follow-up for sensitive patients was 53.8 mo. Six resistant patients had paired post-chemotherapy metastases; 5 sensitive patients had paired post-chemotherapy teratoma. Gene expression in platinum-resistant *versus* platinum-sensitive primary tumours were not significantly different. When comparing platinum-resistant primary and paired post-chemotherapy metastases, OCT4 expression was significantly reduced in metastases ($p = 0.0003$). When comparing platinum-sensitive primary and paired post-chemotherapy teratoma, expression of both XPA ($p = 0.0010$) and AKT1 ($p = 0.0005$) were significantly increased in teratoma. This hypothesis-generating study suggests OCT4 loss as a potential biomarker of platinum-resistance.

GCT-75 Evaluation of inductor factors in the epithelial-mesenchymal transition (EMT) in testicular germ cell tumours (TGCT) and their roles in cisplatin resistance

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Background: A mechanism related to the development of cancer, metastasis and drug resistance is the epithelial-mesenchymal transition (EMT), a process in which epithelial cells lose their characteristics and acquire mesenchymal cell phenotype. EMT can be induced by several transcriptional factors, including Snail, Slug, Zeb1, and Twist. However, the molecular mechanisms involved in EMT induction in the TGCTs has not been elucidated. The aim of this study is to evaluate the inducing factors of EMT in TGCTs, as well as in the cisplatin resistant treatment.

Methods: An *in-silico* analysis of the EMT markers on TGCTs was performed using the cBioPortal software. Cisplatin-resistant cells (NTERA-2R) were derived by incubation with stepwise increasing cisplatin concentrations during the 8 months and EMT markers and stemness were analyzed by real-time PCR and Western blot. The colony formation and migration cell capacity were also evaluated.
Results: The *in-silico* analysis showed that among the transcription factors, Slug was the only one that had an impact on disease/progression-free survival. Slug showed a positive correlation with markers related to EMT, Cancer Stem cells, invasion and migration and with some signaling pathways including Notch, Wnt and TGF-β. The protein and gene expression analysis of NTERA-2R showed an increase of EMT markers (Fibronectin, Vimentin, α-SMA, Col1A1), EMT inducers (Slug and TGF-β) and CSC marker (CD44). NTERA-2R had an increase in the colony formation and migration. Understanding the molecular mechanisms that induce EMTs in TGCTs will allow a better knowledge of cancer development, metastasis and cisplatin resistance.

GCT-76 Cisplatin resistance in the ovarian yolk sac tumour cell line is associated with upregulation of adult cancer stem cell (CSC) markers

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Background: Ovarian yolk sac tumour (YST) represents a highly malignant rare neoplasm. Cisplatin resistance emerging during the treatment of ovarian YST represents a significant clinical challenge. Molecular profiling of a stable *in vitro* generated chemoresistant human ovarian YST subclone NOY-1 CisR was performed in order to unravel mechanisms of cisplatin resistance and exploring potential targeting strategies to overcome this chemoresistance.
Methods: A chemoresistant subclone of YST cell line NOY-1 was derived by continuous sublethal dose exposure to cisplatin *in vitro*. The obtained stable chemoresistant subclone NOY-1 CisR was characterized using flow cytometry, RNAseq and methylation (EPIC) profiling, targeted gene expression, protein arrays, and functional assays. Tumorigenicity *in vivo* was determined using an immunodeficient mouse model. The chemoresistant subclone was treated with inhibitors interfering with CSC properties to examine possible chemosensitization to cisplatin treatment.
Results: NOY-1 CisR subclone exhibited seven-fold higher resistance to cisplatin, cross-resistance to oxaliplatin and carboplatin, increased migratory capacity and tumorigenicity. Increased expression of genes associated with stemness such as prominin-1 (CD133), ATP binding cassette subfamily G member 2 (ABCG2), aldehyde dehydrogenase 1 isoform A3 (ALDH1A3), ALDH3A1 isoform correlating with reduced gene and promoter methylation, and higher overall ALDH activity were detected in the NOY-1 CisR subclone. The CSC targeting agents salinomycin and tunicamycin were significantly more toxic for the NOY-1 CisR subclone. Pretreatment with napabucasin resensitized the cells to cisplatin. In summary, we identified CSC markers associated with cisplatin resistance and showed that their targeting may represent a novel treatment option for chemorefractory YST.

Global Challenges in GCT Care

GCT-77 Burden of late effects and challenges faced in the long-term follow-up of paediatric germ cell tumour survivors: A report from India

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Background: Survivors of paediatric extracranial germ-cell-tumours (PEGCTs) have varying burden of late-effects, depending on host factors and treatment exposures. Long-term follow-up is especially challenging in survivors from resource-limited settings.
Material and methods: Data regarding demographics, treatment details and late toxicities (graded as per National-Cancer-Institute Common-Terminology-Criteria for Adverse Events (NCI-CTCAE) were retrieved from the prospectively maintained database of the 'After-Completion-of-Treatment' (ACT) Clinic, Tata Memorial Hospital, Mumbai, India.
Preliminary results: There were 171 5-year survivors of PEGCTs (female:male = 1.2:1), treated 1982–2013. Median-age at diagnosis was 4 years (range 6 mo–18 years), median follow-up duration 9 years (5–29 years) and median-age at last follow-up 17 years (5–42 years). Most (75%) had received chemotherapy and surgery; the rest had received combinations of surgery, chemotherapy and radiation. Around 40% had at least one documented late toxicity, with grade 2 toxicities in 7% and grade 3 toxicities in 10%. Three survivors had second malignancies: 2 gonadal adenocarcinoma and 1 Ewing sarcoma; all subsequently died. Common late-effects included (in % tested) abnormal pulmonary function, asymptomatic (25%) and symptomatic (1%), abnormal audiometry without intervention (28%), requiring hearing aid (6%), and hypogonadism (13 females, all post bilateral oophorectomy and on hormone replacement; 1 married). Of 9 documented married female survivors, 8 had normal reproductive outcomes. Only a single male survivor had documented azoospermia, post testicular radiation. Notably, 34.5% of survivors were lost to long-term follow-up. This is of concern since 20% of survivors had late effects requiring intervention. Risk-adapted treatment approaches with frequent interval-monitoring for toxicities will assist reduction of late-sequelae.

Radiotherapy

GCT-78 Role of radiotherapy for extracranial germ cell tumours: A re-visit

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Background: During the pre-cisplatin era, radiotherapy had a significant role in the management of extracranial (gonadal and extragonadal) germ cell tumours (GCTs). The evolution of highly effective primary and salvage chemotherapy regimens in the Western world made radiotherapy obsolete in the management of extracranial GCTs. Recognition of significant long-term side effects of older radiotherapy techniques has also contributed to the diminished use