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