(WES) datasets. By using the full set of whole-genome sequenced (WGS) TCGTs from the Genomics England platform, we intend to fully characterise TCGTs, thereby contributing substantially to the knowledge underpinning effective genomic testing for this disease. This work will validate and facilitate the identification of genomic changes at the time of TGCT diagnosis, which may ultimately assist and influence effective clinical management.

Methods: We increase the discovery power for novel SNV, indel, copy number, and structural variant drivers of TGCTs by using a set of ~50 fresh-frozen, WGS tumours. After applying a rigorous quality control process to the provided variants, we use multiple tools separately and in combination to elucidate the various genomic aberrations present in TGCTs. This includes: copy number variants, structural variants, coding, noncoding, germline, and somatic drivers, the presence of selection, the variety of mutational signatures, the heterogeneity (subclonality) present, and the ordering of mutational events. In addition, we separate the sample set in multiple directions (seminomatous:nonseminomatous, primary:metastasis, early:late onset, etc) to explore clinical stratifiers.

Results: Early analyses have identified novel mutational drivers, copy number aberrations, and structural variants. We are exploring the subclonality present, categorising drivers, copy number aberrations, and structural variants as predominantly clonal or subclonal, alongside timing these various aberrational events. Based on this, we will categorise TGCTs into genomic groups, which may prove useful for clinical management.

GCT-22 Targeting the germline-specific gene regulatory network in testicular germ cell tumours

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Background: Germ cell neoplasia *in situ* (GCNIS), the common precursor of seminomas and non-seminomas, is thought to originate from arrested foetal germ cells. Previous studies have established that GCNIS cells shared similar morphology and gene expression pattern with human primordial germ cells (hPGCs) in the embryo. We hypothesize that hPGCs and GCNIS share a germline-specific gene regulatory network and such a common pathway could be used as a therapeutic target for testicular germ cell tumours (TGCTs).

Material and methods: We performed RNA-sequencing analysis on hPGCs, GCNIS, and the TCam-2 seminoma cell line to identify the gene regulatory networks in germ cell development and cancer. We used ex vivo foetal and adult testis culture systems to investigate the function of the common molecular pathway in hPGCs and GCNIS.

Preliminary results: We found that hPGCs, GCNIS, and TCam-2 share the expression of critical germ cell transcription factors SOX17 and OCT4. SOX17 physically interacts with OCT4 to establish and maintain the germ cell transcriptional network. Inhibition of OCT4-SOX17 interaction by a small molecular inhibitor abrogated hPGC development. Strikingly, application of the inhibitor to ex vivo cultured testicular cancer tissues completely eliminated the GCNIS cells within a week. We demonstrate that mechanistic insights into human germ cell development could lead to a new therapeutic strategy for TGCTs.

Biology – Models To Understand GCT Pathogenesis

GCT-23 DNA damage response mechanisms in the origins and therapeutic sensitivity of testicular germ cell tumours

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Background: Testicular-germ-cell-tumours (TGCTs) differ from other solid cancers in terms of their DNA damage response (DDR) features as well as their striking hypersensitivity to conventional genotoxic chemotherapy. Whereas solid cancers of somatic origins typically activate the DDR early in tumorigenesis and subsequently accumulate mutations in DDR genes, TGCTs appear to lack early-stage DDR activation and rarely acquire mutations in DDR genes like *p53* and *ATM*.

Methods: To elucidate the underlying mechanisms, we developed a mouse TGCT model featuring germ cell-specific oncogenic *Kras* activation and tumour suppressor *Pten* deletion.

Results: The resulting mice rapidly developed metastatic testicular cancers composed of both teratoma and embryonal carcinoma (EC), the latter of which exhibited stem cell characteristics, including expression of the pluripotency factor OCT4. Treatment of tumour-bearing mice with genotoxic chemotherapy prolonged survival, reduced tumour size, and selectively eliminated the OCT4-positive EC cells. Consistent with studies of human TGCTs, the murine cancers lacked early-stage DDR activation but mounted a robust DDR after chemotherapy treatment. EC cell lines were created from the murine tumours and, upon transplantation, generated teratocarcinomas that were indistinguishable from primary TGCTs. *In vitro* differentiation of EC cultures resulted in loss of tumour propagating activity and reduced genotoxin sensitivity, consistent with the findings that EC cells function as chemosensitive cancer stem cells *in vivo*. On-going studies aim to identify molecular mechanisms responsible for the differences in chemoresponsiveness between EC cells and their differentiated derivatives, which we hypothesize may be due in part to differential regulation of DNA damage repair and tolerance pathways.

GCT-24 Integrated genomic analysis reveals aberrations in WNT signaling in germ cell tumours of childhood and adolescence

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Background: Germ cell tumours (GCTs) are cancers of the testis, ovary and extragonadal sites that occur in infants, children, adolescents and adults. Post-pubertal (type II) malignant GCTs may present as seminoma, non-seminoma or mixed histologies. In contrast, pre-pubertal (type I) GCTs are limited to (benign) teratoma and (malignant) yolk sac tumour (YST). Epidemiological and molecular data have shown that pre- and post-pubertal GCTs arise by distinct mechanisms. Dedicated studies of the genomic landscape of type I and II GCT in children and adolescents are lacking.
Material and methods: We performed whole-exome sequencing, panel-based deep sequencing, copy-number analysis, RNASeq and methylations analysis on a clinically annotated cohort of GCTs from patients aged 0–24 years. We complemented the genomic analysis with functional studies in human cells and zebrafish models.
Results: Activation of the WNT pathway by somatic mutation, copy-number alteration, and differential promoter methylation is a prominent feature of GCTs in children and adolescents, and is associated with poor clinical outcomes. Significantly, we find that small molecule WNT inhibitors can suppress GCT cells both *in vitro* and *in vivo* in zebrafish models. These results highlight the distinctive mechanisms underlying the development of childhood and adolescent GCTs and provide a foundation for future efforts to develop targeted therapies for these cancers.

GCT-25 The avian embryo as a new patient-derived xenograft model to explore germ cell tumour aetiology, heterogeneity and therapeutic screening

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Background: Gonadal and extragonadal germ-cell-tumours (GCTs) arise from pluripotent primordial-germ-cells (PGCs). The aetiology of extragonadal GCT and metastasis is still debated, mainly because of lack of suitable models recapitulating GCT pathogenesis, including i*n vivo* patient-driven analyses of tumour migration/chemosensitivity. Our main objective was to develop an avian Patient-Derived Xenograft (PDX) system, to reproduce GCT heterogeneity/clinical features.
Methods: Based on previous experiments on neuroblastoma, we conceived an avian model of GCTs in which tumorigenesis is driven in HH25 chick embryos either in gonadal site by grafting GCTs in the migration path of PGCs, or in extragonadal brain site. We set up the technique with NCC-IT and Tera2 GCT cell lines with extension to 3 GCT patient samples, harvested from fresh surgical resections and preserved in DMSO freezing medium: one mediastinal yolk-sac tumour in a 6-year-old patient and two metastatic testicular mixed GCT (in 16- and 15-year-old patients).
Results: Cell lines grafted in the PGC migration path formed visible tumours in avian primitive gonads 5 days after engraftment while grafts in the developing brain permitted the replication of secondary foci in 2 days. Similar graft experiments on GCT patient samples confirmed a rapid and highly reproducible tumour intake in the embryo for both paradigms. Intravenous injection of 5.1 mg/kg cisplatin into embryos grafted with patient samples reduced tumour volume of these avian replicas of cisplatin-responding patients within 48 h. Our GCT avian model opens exciting possibilities ranging from the study of GCT aetiology to the evaluation of novel drug/combination efficacy on patient-derived material.

Ovarian GCTs Including Teratoma

GCT-26 Survival outcomes and long-term follow-up in children treated for ovarian nonseminomatous germ cell tumours in the French TGM-95 study

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Background: To analyze characteristics and outcomes with actualized very-long-term follow-up data from patients treated for ovarian non seminomatous germ-cell-tumours (NS-GCT).
Methods: Children (≤18 years) treated for ovarian NS-GCT in 1995–2005 in France and Belgium were included in TGM-95. Patients with localized, completely-resected tumours (FIGO-stage IA) had no adjuvant treatment (low-risk, LR). Patients with advanced-stage (FIGO-stage ≥IC) received 3–5 VBP cycles (vinblastin-bleomycin-cisplatinum) in intermediate-risk disease (IR: FIGO-stage IC-II-III and AFP<15,000 ng/ml) or 4–6 VIP (etoposide-ifosfamide-cisplatinum) in high-risk (HiR: metastatic and/or AFP ≥ 15,000 ng/ml).
Results: Seventy-seven patients were included (median age = 12 years, 43 pure yolk-sac tumour; 34 mixed NS-GCT). Primary surgery was performed in 55/77 cases. Fourteen patients were LR (12 stage IA, 2 retrospectively stage IC), 26 IR (12 stage IC, 12 stage II-III, 2 not-available) and 37 HiR (8 metastatic, 29 loco-regionally advanced). After a median follow-up of 13 years, 9 events (including 5 relapses/bilateralizations and 2 secondary acute-myeloid-leukemias) and 6 deaths occurred. All relapses/bilateralizations occurred in LR (n = 4, including the 2 retrospectively-stage-IC) and IR groups (n = 1), within 2 years post-diagnosis. Five-year EFS and OS were 89% (95%CI = 80–95%)