(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

W.W.C. Tang[1,2], J.P. Alves-Lopes[1,2], A. C. Venzor[1,2], F.C.K. Wong[1,2], A. Kristian[3], M.A. Surani[1,2]
[1]Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, UK; [2]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK; [3]Department of Growth and Reproduction, Copenhagen University Hospital, Copenhagen, Denmark

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

A. Loehr[1], T.M. Pierpont[1], A.M. Lyndaker[1], R.S. Weiss[1]
[1]Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA

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

L. Xu[1,2,3,§], J.L. Pierce[1,4,§], A. Sanchez[1,4,§], K.S. Chen[1,4], A.A. Shukla[1,4], N.J. Fustino[1,4,#], S. Stuart[1,4], A. Bagrodia[5], M.D. Krailo[6,7], F. Shaikh[8], D. Billmire[9], F. Pashankar[10], J. Bestrashniy[11], J. Wolter Oosterhuis[12], K. Biermann[12], A.J.M. Gillis[12], J. Stoop[12], Y. Xie[2,3,13], L. Teot[14], J. Mora[15], J.N. Poynter[11], D. Rakheja[1,16], L.H.J. Looijenga[12,&], B.W. Draper[17], A. Lindsay Frazier[18], J.F. Amatruda[1,4,19,*]
[1]Departments of Pediatrics; [2]Departments of Population and Data Sciences; [3]Departments of Quantitative Biomedical Research Center; [4]Departments of Molecular Biology; [5]Departments of Urology, UT Southwestern Medical Center, Dallas, TX, USA; [6]Department of Preventative Medicine, University of Southern California, Los Angeles, CA, USA; [7]Children's Oncology Group, Monrovia, CA, USA; [8]The Hospital for Sick Children, University of Toronto, Toronto, Canada; [9]Riley Hospital for Children, Indianapolis, IN, USA; [10]Department of Pediatrics, Yale University School of Medicine, New Haven, CT, USA; [11]Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA; [12]Department of Pathology, Erasmus MC Cancer Institute—University Medical Center Rotterdam, Rotterdam, The Netherlands;