Table 1.
Clinicopathological Characteristics of Patients Receiving Orchiectomy at Safety Net and University Hospital Patients with Testicular Cancer

Characteristic	No. of Patients (%)				p-Value
	Safety Net		University		
All patients	106	(100)	95	(100)	
Age: Median [IQR], years	29	[24–35]	33	[26–41]	0.005
Tobacco Use	52	(49)	28	28(29)	0.006
Marijuana Use	7	(18)	7	(7)	0.048
Presented to ED	81	(76)	8	(8)	<0.001
Insured	21	(20)	84	(88)	<0.001
Symptom onset to Diagnosis: Median [IQR], days	65	[21–183]	31	[12–89]	0.001
Diagnosis to Orchiectomy: Median [IQR], days	1	[1–3]	4	[1–9]	<0.001
Median tumour size, [IQR], mm	50	[27–87]	30	[16.5–50.5]	<0.001
Tumour Stage					0.018
T0	2	(2)	3	(3)	
T1	53	(50)	53	(56)	
T2	32	(30)	34	(36)	
T3	9	(8)	0	(0)	
T4	3	(3)	0	(0)	
TX	7	(7)	5	(5)	
AJCC Stage					0.026
Stage I	61	(58)	69	(72)	
Stage II	21	(20)	17	(18)	
Stage III	24	(23)	9	(9)	
Post-orchiectomy initial management					0.001
Chemotherapy	50	(47)	22	(23)	
RPLND	10	(9)	14	(15)	
XRT	3	(3)	7	(7)	
Surveillance	32	(30)	48	(51)	
Lost to Follow-Up (%)	11	(10)	4	(4)	
Post-Chemotherapy RPLND	19	(18)	10	(11)	0.16
CT imaging: Median [IQR]	3	[2–7]	3	[2–7]	0.77
Urology clinic visits: Median [IQR]	4	[2–8]	4	[2–6]	0.73
Oncology clinic visits: Median [IQR]	5	[1–9]	1	[0–8]	0.007
Cancer recurrence	14	(13)	9	(9)	0.51
Orchiectomy to Recurrence: Median [IQR], months	10	[5–15]	8	[2–11]	0.23
Overall mortality	4	(4)	0	(0)	0.12

Abbreviations: No., number; IQR, interquartile range; ED, emergency department; CT, computed tomography; AJCC, American Joint Committee on Cancer; RPLND, retroperitoneal lymph node dissection; XRT, radiation therapy.

Biology – Genomics and Developmental

GCT-16 Germline genomic architecture of testicular germ cell tumours: Lessons from a decade of experiments in genotyping and sequencing

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Background: As well as being a fascinating model of tumorigenesis, testicular germ cell tumours (TGCTs) have one of the highest familial relative risks of common adult-onset cancers and the advent of large-scale genotyping and sequencing technologies heralded great promise in revealing the genomic determinants thereof.
Methods and Results: I shall present how the experiments of the last decade have enlightened our understanding as to the germline genomic architecture of TGCT, how that has informed our understanding of the biology of disease and the implications for identification of individuals at elevated risk, screening and cancer prevention.

GCT-17 Developmental origins of testicular germ cell tumours (TGCT)

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Background: This review summarises evidence for the developmental origin of TGCT that are derived from germ cell neoplasia in situ (GCNIS) and discusses the main pathways implicated in the pathogenesis of this cancer.
Methods: Literature review.
Results: The GCNIS-derived TGCTs occur predominantly in young men, starting from adolescence. Epidemiological evidence (e.g. birth cohort effects) and a high risk of TGCT in patients with differences in sex development and testicular dysgenesis syndrome (TDS) are consistent with origin in the developmental period. GCNIS cells are transformed primordial germ cells/gonocytes and retain a high expression of pluripotency factors, hence maturation arrest during early development is considered the first hit in the TGCT pathogenesis. The arrest can be caused by a disruption of the cross-talk between developing germ cells and their somatic niche, which is regulated by a multitude of factors. Among the disrupted pathways, the sex differentiation signalling cascade, the androgen signalling, the TGF-beta pathway, the gonadotrophin signalling and the sex-dimorphic mitosis-meiosis switch have been identified, but other pathways likely will be discovered. The rapidly changing incidence of TGCT and other TDS components implicate mainly environmental/lifestyle factors, while ethnic differences in the incidence support a role of genetic background. Susceptibility loci identified in genome-wide and targeted genomic studies have also implicated pathways involved in sex differentiation and germ cell development (e.g. *KITLG, DMRT1, BAK1, SPRY4*). TGCT is a developmental disease, with multifactorial aetiology. The pathogenesis of TGCT is complex, and likely involves disruption of normal gonadal development, which is modulated by genetic variation.

GCT-18 Germ cell commitment occurs after PGC colonization of the gonad in mammals

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Background: In mammalian development, primordial-germ-cells (PGCs) are induced in epiblast and later migrate to nascent gonads, undertaking gametogenesis. Despite dramatic differences in early steps of germ-line development, evidence suggests that, once formed, migratory PGCs remain developmentally uncommitted, not yet

irreversibly committed to only produce gametes. The process leading to this restriction of developmental potential of the germ-line, in any vertebrate, remains unknown.
Methods: Through genome-wide analyses of embryonic germ-line in humans and mice, we have identified a conserved developmental program, activated in PGCs after gonadal colonization, which demarcates definitive germ-cells from other somatic lineages. Through genetic studies in mice, we demonstrate that DAZL, is necessary *in vivo* for restriction of developmental potential. Germ-line cells deficient in *Dazl* migrate to nascent gonads but maintain prolonged expression of pluripotency factors (e.g. *Nanog*), and extended capacity for derivation of pluripotent cell-lines. This leads to spontaneous teratomas in both sexes of mice and in *DAZL*-deficient pigs. Further, germ-line cells failing to restrict their developmental potential usually undertake cell-death. By genetically attenuating apoptosis, *Dazl*-deficient male mice develop bilateral teratomas.
Results: We propose a revised model for germ-line lifecycle of mammals where migratory PGCs are developmentally uncommitted, and germ-line undertakes a restriction of developmental potency only after PGC colonization of gonad. Through comparative analyses, we infer that the germ-cell commitment program is likely operated in the common ancestor of all vertebrates. Finally, failure to complete this process of germ-cell commitment in the embryo, together with cell-death evasion, may account for the origin of mammalian germ-cell-tumours.

GCT-19 Generation of human primordial germ cell-like cell culture models reflecting genetic characteristics of human testicular Type II germ cell tumours for studying molecular events during early pathogenesis

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Background: The familial risk of testicular Type II germ cell tumours is among the highest in human malignancies, suggesting strong contributions of genetic factors to their pathogenesis. These tumours typically harbour iso-chromosome 12p while they have few gDNA mutations. Gain-of-function *KIT* mutations are detected in ~30% of seminomas while it is rare in non-seminomas. Whole-genome association studies identified a few linked loci, including *KITLG* and *BAK1*.
Methods: Human iPSC clones expressing gain-of-function KIT mutants (imatinib-resistant D816V and sensitive N822K) were generated using lentiviral vectors. Knockout clones lacking *BAK1*, *BAX*, or both were generated by CRISPR/Cas9. Clones harbouring trisomy 12p were generated by prolonged maintenance in the naïve pluripotency (5i/LAF) condition [1]. PGC-Like Cells (PGCLCs) mimicking migrating PGCs were generated from human iPSCs as we previously described [2].
Results: iPSCs expressing gain-of-function KIT mutants showed significant growth advantage in the primed-pluripotency culture condition. *BAK1/BAX* double-knockout hiPSCs were significantly resistant to the intrinsic-pathway apoptosis whereas single knockout showed minimal effects. Trisomy 12p did not affect growth or apoptosis of iPSCs. The KIT mutants, *BAK1/BAX* KO, or trisomy 12p – without combinations – did not cause apparent transformation of PGCLCs in embryoid bodies or cell culture conditions. Effects of combinations of these genetic manipulations are being examined.

References

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[2] Mitsunaga *et al.* (2017) Relevance of iPSC-derived human PGC-like cells at the surface of embryoid bodies to pre-chemotaxis migrating PGCs. *PNAS* 114(46):E9913.

GCT-20 The molecular and (epi)genetic mechanisms driving microenvironment-triggered reprogramming of seminomas into an embryonal carcinoma cell fate

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Background: Testicular germ-cell-tumours (GCTs) are very common in young men and are stratified into seminomas and nonseminomas. While seminomas share a similar gene expression and epigenetic profile with primordial germ cells, the stem cell population of the nonseminomas, the embryonal carcinoma (EC), resembles malignant embryonic stem cells. Thus, ECs are able to differentiate into cells of all three germ layers (teratomas) and even extra-embryonic-tissue-like cells (yolk-sac-tumour, choriocarcinoma).
Methods: We demonstrated that cellular microenvironment considerably influences the plasticity of seminomas (TCam-2 cells). Upon microenvironment-triggered inhibition of BMP signalling pathway *in vivo* (murine flank/brain), seminomatous TCam-2 cells reprogram to an EC-like cell fate. We identified SOX2 as a key factor activated upon BMP inhibition mediating the reprogramming process by regulating pluripotency, reprogramming and epigenetic factors. Indeed, CRISPR/Cas9 SOX2-deleted TCam-2 cells were able to maintain a seminoma-cell fate *in vivo* for about six weeks, but small sub-populations still initiated differentiation – potentially driven by FOXA2, since many FOXA2-interacting genes and differentiation factors like AFP/EOMES/CDX1/ALB/HAND1/DKK/DLK1/MSX1/PITX2 were upregulated. We generated TCam-2 cells double-deficient for SOX2+FOXA2 using the CRISPR/Cas9 technique and xenografted those cells into the flank of nude mice.
Results: Upon loss of SOX2 and FOXA2, TCam-2 maintained a seminoma cell fate for at least twelve weeks, demonstrating that both factors are key players in the reprogramming to an EC-like cell fate. Therefore, our studies add important pieces to the puzzle of GCT development and plasticity, providing interesting insights in what can be expected in a patient, when GCT cells are confronted with different microenvironments.

GCT-21 Testicular cancer genomics England Clinical Interpretation Partnership (GECIP): A genomic exploration of testicular germ cell tumours

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Background: The characterisation of testicular germ cell tumours (TCGTs) has to-date been limited to panel or whole-exome sequenced