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LECTURES

Invited talk 1, Global burden of testicular cancer in 2022 and predictions to 2050

McGlynn K.

National Cancer Institute, NIH, Bethesda, MD, USA

Background: The burden of testicular cancer varies greatly around the world. Using recent data, the global burden of testicular cancer, incidence and mortality, will be presented, as will predictions to 2050.
Methods: Data from IARC's Globocan 2022 database were used. Age-standardized incidence (ASIR) and mortality rates (ASMR) per 100,000 were calculated for individual countries and for UN regions. Cases and deaths to 2050 were estimated based on 2022 rates and population predictions.
Results: In 2022, 72,040 men were diagnosed with, and 9068 men died from, testicular cancer. The countries with the greatest number of cases were the U.S. (n=9404), India (n=4456), Germany (n=4254), and Mexico (n=3580). The global ASIR was 1.69, but varied from a high in Western Europe (8.67) and to a low in Middle Africa (0.34). The countries with the highest ASIRs were Slovakia (13.2), Slovenia (13.0) and Hungary (11.37). In contrast to incidence, the global ASMR was 0.21, but varied from a high in Central America (0.83) to a low in Southern Africa (0.06). Countries with high ASMRs included Georgia (1.07), Slovakia (1.20) and four countries in Latin America (Mexico, Chile, Argentina, Paraguay). By 2050 the number of cases is predicted to increase 22.7% globally. There will be a decrease, however, in Eastern Asia, and in all regions of Europe except Northern Europe. Deaths are predicted to increase 40.0% globally, but will decline in Eastern (-5.2%) and Southern Europe (-3.0%). By 2050, 19.8% of the deaths are predicted to occur in Latin America, and 19.5% in South Central Asia.
Conclusions: Globally, the countries with the greatest number of testicular cancer cases are distinct from the countries with the highest ASIRs and highest ASMRs, with two exceptions. Slovakia has both a high ASIR and a high ASMR, while Mexico has a large number of cases and a high ASMR. From a global health perspective, more attention should be paid to case burden, in addition to ASIRs and ASMRs.

Invited talk 2, Environmental Risk Factors for Testicular Cancer: A Norwegian Perspective

Wojewodzic M.[1,2]

[1]*Cancer Registry of Norway, Norwegian Institute of Public Health, Oslo, Norway*
[2]*Chemical Toxicology, Norwegian Institute of Public Health, Oslo, Norway*

Studying environmental risk factors for testicular cancer (TC) poses significant challenges. Prospective cohorts, such as the Norwegian Janus Serum Bank, offer valuable opportunities to assess associations between cancer and suspected chemicals. By linking serum measurement with relevant data from registries, such as Statistics Norway, these relationships can be further explored. The Janus Serum Biobank has previously identified some associations between organochlorines and TC. Recently, IARC monograph has highlighted that high dose of per – and polyfluoroalkyl substances (PFAS), often referred to as 'forever chemicals,' may pose a risk for the development of TC. What evidence exists today regarding this group of chemicals and their link to TC?
Some occupational studies have identified certain professions associated with an increased risk of developing TC. The Nordic Occupational Cancer Study (NOCCA), which compiles 45 years of cancer incidence data by occupational category for Nordic populations, reveals patterns that could guide further research into associations between specific work-related factors and cancers, with the goal of identifying exposure-response patterns, for instance for PFAS.
I will review both old and new evidence of environmental risk factors for TC and invite collaborative efforts to further study the exposure-disease relationship. Additionally, I will highlight how we will investigate 'forever chemicals' in a new project funded by the Norwegian Cancer Society.

Invited talk 3, Identification of over forty novel testicular germ cell tumor susceptibility loci

Pluta J.[1], Nathanson K.[1], Kanetsky P.[2], Almstrup K.[3], Feldman D.[4], Cortessis V.[5], Ferlin A.[6], Gietema J.[7], Gonzalez A.[8], Hamilton R.[9], Haugen T. B.[10], Kiemeny L.[11], Krausz C.12, Lessel D.[13], McGlynn K.[14], Nead K.[15], Nsengimana J.[16], Poynter J.[17], Rafnar T.[18], Richiardi L.[19], Schwartz S.[20], Skotheim R.[21], Stewart D.[14], Turnbull C.[22], Van Allen E.[23], Wang Z.[24], Wiklund F.[25], Zheng T.[26] and The Testicular Cancer Consortium

[1]*Department of Translational Medicine and Human Genetics, University of Pennsylvania, Philadelphia, United States*
[2]*Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, United States*
[3]*Copenhagen University Hospital, Copenhagen, Denmark*
[4]*Memorial Sloan Kettering Cancer Center, New York, United States*
[5]*University of Southern California, Los Angeles, United States*
[6]*University of Padova, Padova, Italy*
[7]*University Medical Center Groningen, Groningen, The Netherlands*
[8]*Spanish National Cancer Research Center (CNIO), Madrid, Spain*
[9]*Princess Margaret Hospital, Toronto, Canada*
[10]*Oslo Metropolitan University, Oslo, Norway*
[11]*Radboud University Medical Center, Nijmegen, The Netherlands*
[12]*University of Florence, Florence, Italy*
[13]*University Cancer Center Hamburg, Hamburg, Germany*
[14]*National Cancer Institute, United States*
[15]*MD Anderson Cancer Center, Houston, United States*
[16]*Newcastle University, Newcastle upon Tyne, United Kingdom*
[17]*University of Minnesota, Minneapolis, United States*
[18]*deCODE Genetics, Reykjavik, Iceland*
[19]*University of Turin, Turin, Italy*
[20]*Fred Hutchinson Cancer Center, Seattle, United States*
[21]*Oslo University Hospital, Oslo, Norway*
[22]*The Institute of Cancer Research, London, United Kingdom*
[23]*Broad Institute, Cambridge, United States*
[24]*St. Jude Children's Research Hospital, Memphis, United States*
[25]*Karolinska Institutet, Solna, Sweden*
[26]*Brown University, Providence, United States*

Introduction

Genome-wide association studies (GWAS) of TGCT led by the Testicular Cancer Consortium (TECAC) have identified variants that explain a large proportion of the high heritability of disease. The identified loci to date implicate genes in pathways associated with male germ cell development, chromosomal segregation, sex determination, and DNA maintenance, together which help to frame the disease biology and epidemiology. We present

preliminary data from our current TECAC GWAS meta-analysis of 17,000 men with and 222,321 men without TGCT from 11 datasets.

Methods

After standard quality control, genotypes were imputed against the Haplotype Reference Consortium r1.1 backbone. Association testing was performed using SNPTEST v2.5.6. Meta-analysis was performed using METAL r2020.5.5. Markers displaying significant effect size heterogeneity across studies ($p < 1 \times 10^{-05}$) were removed.

Results

Results identify a conservative estimate of 39 novel loci (Fig. 1). The most significant locus (rs5987215; $p = 1.46 \times 10^{-15}$) is intergenic between *MECP2* and *OPN1LW*. Several loci contain genes in known TGCT susceptibility pathways, including male germ cell development and sex differentiation (*WNK1*: rs2277869, $p = 1.05 \times 10^{-9}$; *PRC1*: rs7167128, $p = 9.21 \times 10^{-11}$; *REXO1*: rs28626548, $p = 4.05 \times 10^{-9}$), chromosomal segregation (*MAPT*: rs17662403, $p = 3.50 \times 10^{-13}$), and RNA transcription (*ZNF638*: rs6725892, $p = 8.26 \times 10^{-10}$; *ZNF552*: rs140089558, $p = 2.74 \times 10^{-12}$). Three novel independent markers map to the androgen receptor region (rs4240053, $p = 7.75 \times 10^{-12}$; rs12390145, $p = 6.80 \times 10^{-14}$; rs17216906, $p = 1.96 \times 10^{-10}$).

Conclusions

Many of the loci implicate genes that encode proteins in pathways associated with susceptibility to TGCT, which explain the biological basis of disease development, link to other related conditions, and epidemiology. Multiple novel markers were identified near the androgen receptor loci, further emphasizing the role of androgen: estrogen balance in TGCT susceptibility.

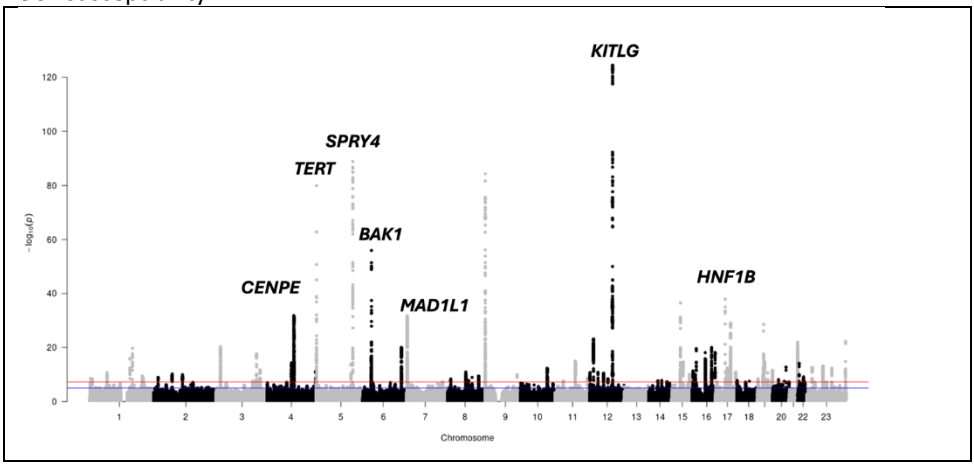

Figure 1: Manhattan plot of TGCT associations.

Invited talk 4, Using genomic ascertainment to CHEK on risk of testicular cancer

Stewart D. R.[1], Kim S. Y.[1,2], Kim J.[1], Ramos M.[1], Haley J.[3], Smelser D.[3], Shanker Rao H.[3], Mirshahi U. L.[3], Graubard, B. I.[4], Katki H. A.[4], Carey D.[3], Geisinger-Regeneron DiscovEHR Collaboration

[1]Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Rockville, MD, USA
[2]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[3]Department of Genomic Health, Geisinger, Danville, PA, USA
[4]Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Rockville, MD, USA

There is published evidence that deleterious germline variants in *CHEK2* increase the risk for testicular cancer. However, it has been difficult to replicate these findings. Here, genomic ascertainment was used to quantify cancer risk in *CHEK2* germline pathogenic variant heterozygotes.
Patients and Methods. Germline *CHEK2* variants were extracted from individuals >18 years in two exome-sequenced biobanks linked to the electronic health record (EHR): UK Biobank (n= 469,765**)** and Geisinger MyCode (n=167,050**)**. Variants were classified as per American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) criteria. Heterozygotes harbored a *CHEK2* pathogenic/likely pathogenic (P/LP) variant; controls harbored benign/likely benign *CHEK2* variation or wildtype *CHEK2*. Tumor phenotype and demographic data were retrieved; to adjust for relatedness, association analysis was performed with SAIGE-GENE+.
Results. In *CHEK2* heterozygotes in both MyCode and UK Biobank, there was a significant excess risk of all cancers tested, including breast cancer (C50; OR=1.33 and 1.41, respectively), male genital organ cancer (C60-C63; OR=1.61 and 1.77 respectively), urinary tract cancer (C64-C68; OR=1.56 and 1.73, respectively) and lymphoid, hematopoietic, and related tissue cancer (C81-C96; OR=1.42 and 2.07, respectively). Although the number of malignant neoplasm of testis (ICD-10 C62) cases in each cohort was modest ($\leq$10), there was enrichment of *CHEK2* P/LP variant frequency in MyCode and UKBB in those cases vs. controls (**Table**).
Conclusion. Relatedness-adjusted, genomic ascertainment of two population-based, exome-sequenced, EHR-linked cohorts found evidence of enrichment of testicular cancer (ICD-10 C62) cases in P/LP *CHEK2* heterozygotes compared with controls. Odds ratios were nominally significant in UK Biobank for All and pathogenic truncating variation.

Table. *CHEK2* All P/LP, pathogenic truncating (PTV) and pathogenic missense (PMV) variants counts, percentages and odds ratio/nominal p-value **(bold = signficant**) in MyCode and UK Biobank.

Geisinger MyCode	Controls/% (n=152662)	All *CHEK2* Hets/% (n=3153)	Odds Ratio (p-value)	*CHEK2* PTV Hets/% (n=913)	Odds Ratio (p-value)	*CHEK2* PMV Hets/% (n=2221)	Odds Ratio (p-value)
C62	195 (0.32%)	7 (0.58%)	1.7 (0.3)	1 (0.30%)	NA	6 (0.68%)	2.1 (0.14)
UK Biobank	Controls (n=305330)	All *CHEK2* hets/% (n=3232)	Odds Ratio (p-value)	*CHEK2* PTV Hets (n=1847)	Odds Ratio (p-value)	*CHEK2* PMV Hets (n=1290)	Fold enrichment
C62	517 (0.17%)	10 (0.31%)	**1.8 (0.03)**	7 (0.38%)	**2.2 (0.05)**	3 (0.23%)	NA

Invited talk 5, Identification of genes associated with testicular germ cell tumor susceptibility through a transcriptome-wide association study

Ugalde-Morales E.[1], Wilf R.[2], Pluta J.[2], Ploner A.[1], Yao Fan M.[2], Damra M.[2], Aben K. K.[3,4], Anson-Cartwright L.[5], Chen Ch.[6], Cortessis V. K.[7], Daneshmand S.[8], Ferlin A.[9], Gamulin M.[10], Gietema J. A.[11], Gonzalez-Niera A.[12], Grotmol T.[13], Hamilton R. J.[4], Harland M.[5], Haugen T.[14], Hauser R.[15], Hildebrandt M. A. T.[16], Karlsson R.[1], Kiemeney L. A.[4], Kim J.[17], Lessel D.[18], Lothe R. A.[19], Loveday Ch.[20,21], Chanock S. J.[17], McGlynn K. A.[17], Meijer C.[11], Nead K. T.[22], Nsengimana J.[23], Popovic M.[24], Rafnar T.[25], Richiardi L.[24], Rocca M. S.[11], Schwartz S. M.[6], Skotheim R. I.[19], Stefansson K.[25], Stewart D. R.[17], Turnbull C.[20], Vaughn D. J.[26], Winge S. B.[27], Zheng T.[28], Monteiro A. N.[29],Almstrup K.[27,30], Kanetsky P. A.[29], Nathanson K. L.[2,31], Wiklund F.[1], and the Testicular Cancer Consortium

[1]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden
[2]Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
[3]Netherlands Comprehensive Cancer Organization, Radboud university medical center, Utrecht, Netherlands
[4]Radboud University Medical Center, Nijmegen, Netherlands
[5]Department of Surgery (Urology), University of Toronto and The Princess Margaret Cancer Centre, Toronto, ON, Canada
[6]Epidemiology Program, Fred Hutchinson Cancer Center, Seattle WA; Department of Epidemiology, University of Washington, WA, USA
[7]Departments of Preventive Medicine and Obstetrics and Gynecology, Keck School of Medicine at the University of Southern California, Los Angeles, CA, USA
[8]Departments of Urology, Keck School of Medicine at the University of Southern California, Los Angeles, CA, USA
[9]Department of Medicine, University of Padova, Padua, Italy
[10]Department of Oncology, University Hospital Center Zagreb, University of Zagreb School of Medicine, Zagreb, Croatia
[11]Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
[12]Human Genotyping Core Unit, Spanish National Cancer Centre (CNIO), Madrid, Spain
[13]Cancer Registry of Norway, Oslo Metropolitan University, Oslo, Norway
[14]Department of Life Sciences and Health, Oslo Metropolitan University, Oslo, Norway
[15]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[16]Department of Lymphoma and Myeloma, University of Texas, MD Anderson Cancer Center, Houston, Texas, USA
[17]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA
[18]Institute of Human Genetics, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria

[19]*Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital-Radiumhospitalet, Oslo, Norway*
[20]*Division of Genetics & Epidemiology, The Institute of Cancer Research, London, UK*
[21]*William Harvey Research Institute, Queen Mary University, London, UK*
[22]*Department of Lymphoma and Myeloma, University of Texas, MD Anderson Cancer Center, Houston, Texas, USA*
[23]*Biostatistics Research Group, Population Health Sciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle, UK*
[24]*Cancer Epidemiology Unit, Department of Medical Sciences, University of Turin and CPO-Piemonte, Turin, Italy*
[25]*deCODE Genetics/Amgen, Reykjavik, Iceland*
[26]*Division of Hematology/Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
[27]*Department of Growth and Reproduction, Copenhagen University Hospital-Rigshospitalet, Copenhagen, Denmark*
[28]*Department of Epidemiology, Brown School of Public Health, Brown University, Providence, RI, USA*
[29]*Department of Cancer Epidemiology, Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
[30]*Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark*
[31]*Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*

Genome-wide association studies (GWAS) have significantly enhanced our understanding of the mechanisms underlying various complex traits. Following a recent meta-analysis of GWAS for testicular germ cell tumors (TGCT), a total of 78 TGCT risk loci have been identified. However, pinpointing causal genes is challenging due to the presence of multiple genes within some loci and linkage disequilibrium between genetic variants. Transcriptome-wide association studies (TWAS), which leverage expression quantitative trait loci data to associate GWAS-identified variants with changes in gene expression, present a promising approach for identifying potential causal genes. We conducted tissue-specific and multi-tissue TWAS analyses using GWAS summary statistics to assess the associations between imputed gene expression and TGCT susceptibility. We evaluated gene associations conditioned on variant-level effects from GWAS and performed fine-mapping analyses in regions exhibiting multiple TWAS signals. Furthermore, we explored the expression and protein patterns of the identified TWAS genes in relevant tissues. Among 19,839 gene-disease associations tested, we identified 165 genes associated with TGCT (with a false-discovery rate <0.01). We prioritized 57 candidate genes by accounting for GWAS-inflated signals and gene-neighbor correlations. Thirty-two of these genes overlapped with 29 GWAS loci, collectively explaining an average of 76 percent of the GWAS signal in those regions. The set of 57 genes showed significant enrichment in single-cell human gonads, germ cell neoplasia in situ, and tumor expression datasets. Additionally, we validated the protein expression of two novel candidate genes, ARID3B

and GINM1, in relevant tissues. These findings advance our understanding of the genetic predisposition to TGCT, highlighting the need for further investigation into the functional roles of these genes.

Selected abstract 6, Evaluation of familial cancer risk among testicular germ cell tumor patients

Farnetani G.[1], Vannucci M.[1], Rosta V.[1,2], Moreno-Mendoza D.[3], Riera-Escamilla A.[3], Krausz C.[1]

[1]Department of Experimental and Clinical Biomedical Sciences „Mario Serio", University of Florence, Florence, Italy
[2]National Institute of Oncology, Budapest, Hungary
[3]Andrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Barcelona, Spain

Testicular germ cell tumour (TGCT) is a multifactorial, polygenic, and complex disease, representing the most common neoplasia among men of reproductive age. It has a high heritability rate, ranging from 37% to 48.9%. Despite epidemiological studies indicating an elevated familial cancer risk among relatives of TGCT patients, the findings are heterogeneous and sometimes contradictory.
This retrospective case-control study aimed to determine whether TGCT could be part of a broader cancer predisposition by examining familial cancer risk among first-degree relatives and grandparents of TGCT patients. The study included 1407 subjects: 592 with TGCT, 352 with oncohematological (OH) malignancies, and 463 fertile, cancer-free controls.
Results showed that relatives of both TGCT and OH patients had significantly higher cancer incidence compared to controls, with no significant differences between TGCT and OH cohorts. There was a significantly higher familial incidence of TGCT and OH malignancies among patients with TGCT and OH, respectively. Specific tumor associations were also identified: TGCT patients' relatives had five different malignancies at higher frequencies, while breast cancer was significantly more common in OH patients' relatives.
The study also assessed the impact of semen phenotypes in cancer patients, finding a 1.57-fold increased risk for tumor development among relatives of patients with severe spermatogenic disturbances. Additionally, TGCT and OH patients had significantly fewer siblings compared to controls, suggesting lower fecundity rates in the parents of cancer patients.
These findings suggest a link between TGCT, increased familial cancer incidence, and sub/infertility, potentially due to genomic instability or DNA repair defects. Our data support higher morbidity rates in men with spermatogenic defects and highlight the importance of considering familial cancer risks in the clinical management of TGCT patients and their families.

Invited talk 25, New insights in early and late cardiovascular effects of cisplatin combination chemotherapy for testicular cancer. Time to act.

Gietema J.A.

Department of Medical Oncology, University Medical Center Groningen, The Netherlands

Initially, it was thought that after successful treatment testicular cancer survivors' life expectancy was comparable to that of the general male population. However, data is piling up indicating a decreased life span after testicular cancer treatment, mainly due to cardiovascular disease and second primary non-testicular cancer malignancies. The risk of myocardial infarction is increased after chemotherapy for testicular cancer and occurs at a younger age than in the general male population, which suggests accelerated vascular ageing.

We investigated development of cardiovascular disease in testicular cancer patients from five of the hospitals from the Dutch national testicular cancer cohort. Within these five expert testicular cancer treatment centers, 272 testicular cancer patients (5.7%) developed overt coronary artery disease (with or without myocardial infarction) or heart failure. During 2015 – 2017 living patients who had developed cardiovascular disease and a random sample of the cohort in the five hospitals were invited to complete a questionnaire. The questionnaire assessed lifestyle, use of medication, family history, quality of life and known adverse treatment effects. One of the main results showed that patients who experienced a cardiovascular event reported inferior quality of life on physical domains compared to testicular cancer survivors who did not develop cardiovascular disease. Patients who were obese or a smoker at diagnosis developed Raynaud's phenomenon, dyslipidemia or had a positive family history for CVD were at higher risk to develop cardiovascular disease. In the patients from the random sample of the cohort a cardiometabolic assessment was performed. Cardiovascular risk factors were frequently present. Patients in the cohort often had hypertension (50%), of which half was untreated. The prevalence of dyslipidemia among these patients was 86%, of which the majority was untreated. Furthermore, 35% of survivors had metabolic syndrome. Most cardiovascular disease risk factors were equally prevalent in patients treated with orchidectomy only compared to patients treated with chemo- or radiotherapy. In conclusion, cardiovascular disease development has a clinical relevant impact on quality of life after testicular cancer. Patients at high risk for cardiovascular disease are those with platinum-based chemotherapy, who had obesity at start of treatment, smoked and developed Raynaud's phenomenon or dyslipidemia afterwards. Many testicular cancer survivors presently have undetected and often untreated cardiovascular risk fac.

Of the patients with metastatic testicular cancer 5 – 10% develop early vascular events during and shortly after 1st-line cisplatin-based chemotherapy, which leads to serious morbidity and mortality and can cause postponed chemotherapy courses or even premature chemotherapy termination, impairing effective cancer treatment. We developed and validated, in a multicentre setting, a vascular-risk-tool, that can identify

testicular cancer patients at high-risk of developing early cardiovascular events: Testicular cancer patients with at least three out of five traditional cardiovascular risk factors: overweight, active smoking, hypertension, dyslipidemia, and impaired blood glucose at diagnosis have an increased risk to develop cardiovascular events during and after cisplatin-based chemotherapy.

A second peak of cardiovascular events occurs years after treatment in testicular cancer survivors mediated by developed of metabolic syndrome and dyslipidemia after treatment which is hypothesized to be the result of the induction of cellular senescence in healthy tissue. Prevention should be aimed at hypercoagulability, endothelial dysfunction, for which LMWHs, acetylsalicylic acid, and statins are widely available, and there is also the first data suggesting that a statin or aspirin can reduce cellular senescence: therefore we hypothesize that such an anti-coagulant intervention strategy in high-risk patients, starting simultaneously with standard cisplatin-based chemotherapy, could prevent early cardiovascular events, as well as late cardiovascular events by reducing healthy tissue damage and senescence induction.

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Invited talk 7, Genomic instability in Testicular Germ Cell Tumors

Lessel D.[1,2,3]

[1]*Institue of Human Genetics, University Hospital Salzburg, Salzburg, Austria*
[2]*Institute of Human Genetics, University of Regensburg, Regensburg, Germany*
[3]*Institute of Clinical Human Genetics, University Hospital Regensburg, Regensburg, Germany*

Cells in human body are constantly being exposed to numerous endogenous and exogenous stressors, which if not repaired in a timely manner can cause defects in a myriad of processes ultimately leading to genomic instability. The latter can range from single amino acid substitution to gross chromosomal abnormalities. The origins of genomic instability are either in alterations in one of the many DNA repair pathways or in critical factors that regulate the proper cell division during mitosis and/or meiosis. Indeed, genomic instability is one of the hallmarks of many cancers and likely the driver of its development. Testicular germ cell tumors (TGCTs) are actually tumors with one of the highest levels of genomic instability. This is exemplified by the high occurrence of isochromosome 12p (i[12p]), found in more than 80% of all TGCTs. Interestingly, TGCT´s are characterized by low somatic point-mutation rate and one of the highest aneuploidy rates among all cancers. High-throughput sequencing analyses showed that almost all TGCT´s undergo whole-genome duplications, which likely occur during the cell division. Utilizing next-generation sequencing, we recently identified in TGCTs rare variants affecting one of the key regulators of the proper metaphase-anaphase transition. Functional characterization of identified variants revealed that they disrupt the formation of spindle assembly checkpoint (SAC), which is important for the fidelity of chromosome segregation. Additionally, these variants disrupt the anaphase-promoting complex (APC), which regulates the transition from metaphase to anaphase, thus allowing the transition of the improperly assembled chromosomes. Moreover, we show that due to impaired SAC and APC formation these variants ultimately result in increased aneuploidy. Taken together, we identified a genetic cause for increased genomic instability in at least a subset of TGCTs, and provide a molecular explanation for its origin.

Invited talk 8, RNA sequencing of germ cell neoplasia *in situ* at the single-cell level

Winge S.B.[1], Lykkegaard-Kelstrup M.[2], Almstrup K.[3]

[1]Department of Growth and Reproduction, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark
[2]International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health, Copenhagen, Denmark
[3]Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

Abnormal development of the male germline can lead to the formation of germ cell neoplasia *in situ* (GCNIS), which is the precursor lesion of testicular germ cell tumours (TGCTs). GCNIS can give rise to distinct TGCT subtypes - seminoma, non-seminoma or a combination of the two - which demonstrates its remarkable differentiation potential. Treatment regimens and disease risks differ according to TGCT subtype, but the molecular determinants underlying the progression from GCNIS to a seminoma, or a non-seminoma remain largely unknown.
The development of single-cell RNA-sequencing technologies has opened for the possibility to explore the transcriptome of individual *in situ* cells. Here, we present the first high-resolution transcriptomic atlas of GCNIS, covering 65,526 individual cells from nine independent GCNIS-containing tissues. Our data includes GCNIS adjacent to seminoma, GCNIS adjacent to non-seminoma, and GCNIS with no overt tumour, thus allowing us to explore the heterogeneity of GCNIS lesions as well as the gene expression changes associated with its differentiation.

Selected abstract 9, Release of Extracellular Vesicles Containing Pluripotency-Associated microRNAs in a Mouse Model of Malignant Testicular Germ Cell Tumor

Liu M.[1], Loehr A. R.[1], Timmerman D. M.[2], Gillis A. J. M.[2], Looijenga L. H. J.[2], Weiss R. S.[1]

[1]Department of Biomedical Sciences, Cornell University, Ithaca, NY, USA
[2]Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands

Embryonal carcinoma (EC) is a pluripotent and malignant type of testicular germ cell tumor (TGCT). EC cells resemble embryonic stem (ES) cells, and in humans both highly express the pluripotency-associated microRNA cluster, miR371-373. MicroRNAs are short, noncoding regulatory RNAs, and target gene regulation by the miR371-373 cluster is important for the maintenance of pluripotency in ES cells. Measurement of serum miR371-373 levels is approaching clinical implementation as a biomarker for malignant TGCTs that outperforms traditional serum biomarkers. We tested the evolutionary conservation of this mechanism using the germ-cell specific Pten and Kras-targeted (gPAK) mouse model that develops mixed TGCTs with EC and teratoma. miRNAs of the mouse miR290-295 cluster, the homolog of human miR371-373, were abundant in serum from TGCT-bearing gPAK mice but not in tumor-free controls, and serum miR290-295 levels were significantly reduced following differentiation treatment with thioridazine. Additionally, miR290-295 were abundant in EC cells cultured from gPAK tumors but were undetectable after differentiation, indicating that the miR290-295 miRNAs were produced by pluripotent EC cells. The stability of the miRNAs in the serum of human patients and gPAK mice suggest they are protected within extracellular vesicles (EVs). EVs are membrane-bound particles that carry bioactive cargo, including miRNAs, and mediate intercellular communication. Small RNA sequencing showed that miR290-295 cluster miRNAs were amongst the most enriched miRNAs in EC cells and purified EVs compared to their differentiated counterparts. These findings establish that release of pluripotency-associated miRNAs is a conserved feature of malignant TGCTs, further validating the mouse model as representative of the human disease. Ongoing experiments center on the functional impacts of EV-mediated miRNA transfer during TGCT pathogenesis.

Selected abstract 10, Fundamental contribution of T cells and their rare subtypes to testicular germ cell tumors: a new hope for the development of immunotherapy

Islam R.[1,2,3,4], Heyer J.[1,5], Figura M.[1,5], Wang X.[6,7], Nie X.[8], Nathaniel B.[3], Püschl D.[9], Indumathy S.[2,3,4], Hartmann K.[2], Pleuger C.[10,11], Fijak M.[10,11], Kliesch S.[12], Schorle H.[1], Dittmar F.[5], Pilatz A.[5,10], Wagenlehner F.[5,10], Hedger M.[3,4], Loveland B.[13], Hotaling J.[8], Guo J.[6,7,8], Loveland K.L.[3,4], Schuppe H-C .[5,10], Fietz D.[2.10]

[1]Department of Developmental Pathology, Institute of Pathology, University Hospital Bonn, Bonn, Germany
[2]Dept. of Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Giessen, Germany
[3]Centre for Reproductive Health, Hudson Institute of Medical Research, Clayton, VIC, Australia
[4]Department of Molecular and Translational Sciences, Monash University, Clayton, VIC, Australia
[5]Dept. of Urology, Pediatric Urology and Andrology, Justus Liebig University, Giessen, Germany
[6]State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China
[7]Beijing Institute of Stem Cell and Regenerative Medicine, Beijing, China
[8]Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA
[9]Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
[10]Hessian Centre of Reproductive Medicine, Justus-Liebig-University, Giessen, Germany
[11]Institute of Anatomy and Cell Biology, Justus Liebig University, Giessen, Germany
[12]Centre of Reproductive Medicine and Andrology, University of Muenster, Muenster, Germany
[13]Burnet Institute, Melbourne, VIC, Australia

Immunotherapy has revolutionized different cancer treatments with minimal side effects, but failed in testicular germ cell tumors (TGCTs) due to a poor understanding of the TGCT immune microenvironment. It is well-known that T cells are the major component of tumor infiltrating lymphocytes (TIL) in TGCTs; their potential role, however, has not yet been analyzed.
For the analysis of immune cells in TGCTs, immunohistochemistry (IHC) was applied on germ cell neoplasia in situ (GCNIS) +/- TIL (n=12 and 14), seminoma (SE; n=24), and embryonal carcinoma (EC; n=10) in comparison to normal spermatogenesis (NSP; n=10). Flow cytometry was performed from different locations of tumor-bearing and contralateral testes (SE n=12, EC n=6). Single-cell RNA-seq (healthy donors n=3; TGCT n=4) was used for in-depth phenotypic exploration. Despite the heterogeneity of samples, all approaches demonstrated that the immune environment in TGCTs is shifted from resident macrophages in NSP to abundant newly recruited T, B, and dendritic cells. Rare T cell

subtypes, i.e. regulatory (Treg) and follicular helper T (Tfh) cells were most abundant in SE compared to other groups. A deeper downstream analysis revealed Treg and Tfh signature molecules being highly expressed in TGCTs, where their functional involvement appears to be regulated by CXCR4-NANOG and BCL6-NANOG interactions. Initial results from a co-culture model mimicking the TGCT microenvironment indicate that TCam-2 cells directly interact with immune cells, including increased expression of TIL-supporting cytokines.
This study describes the complexity of immune landscape in TGCTs, characterizing SE and EC patterns and providing first indications of a potential importance of Treg and Tfh. However, further experiments are needed to decipher the role of "immune editing" during TGCT development, progression, and metastatic behavior. Prospective findings will help to identify novel prognostic factors and immune-therapeutic concepts in human TGCTs.

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Invited talk 11, Induction of SOX17 with stimulation of WNT, TGF-beta and FGF signaling drives embryonal carcinomas into the yolk-sac tumor lineage resulting in increased cisplatin resistance

Kotthoff M.[1], Skowron M. A.[1], Bremmer F.[2], Parmaksiz F.[1], Kretschmer P.[1], Stephan A.[1], Fichtner A.[2], Lautwein T.[3], Raba K.[4], Fuß J.[5], Köhrer K.[3], Nettersheim D.[1,6]

[1]Department of Urology, Urological Research Laboratory, Translational UroOncology, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Germany
[2]Institute of Pathology, University Medical Center Göttingen, Göttingen, Germany
[3]Genomics and Transcriptomics Laboratory (GTL), Biomedical Research Center, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany
[4]Institute for Transplantation Diagnostics and Cell Therapeutics, Core Facility Flow Cytometry, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Germany
[5]Competence Centre for Genomic Analysis, Kiel, Germany
[6]Center for Integrated Oncology Aachen Bonn Cologne Düsseldorf (CIO ABCD), Lighthouse Project Germ Cell Tumors, Germany

The overall cure rate of germ cell tumor (GCT) patients is high, except for patients suffering from the aggressive subtype, the yolk-sac tumor (YST), which accounts for a considerable number of deaths. Many relapsing GCT harbor YST components, suggesting that YST formation is an escape mechanism under therapy. So far, the molecular mechanisms inducing YST development from its stem cell-like precursor population, the embryonal carcinoma (EC), are largely unexplored.
We demonstrated that induction of the YST-associated transcription factor SOX17 in combination with stimulation of WNT, TGF-beta and FGF signalling drives EC cells into the YST lineage. As shown by single cell RNA sequencing, formation of YST-like cells is accompanied by upregulation of YST-associated factors, like AFP, ANKRD1, APOA1, CST1, FOXA2, GATA6 and GPC3 in EC cells, while pluripotency-related genes were downregulated. Additionally, the YST-like cells acquired a YST-typical microRNA expression profile. Xenografting of the YST-like cells into nude mice led to growth of mixed GCT with YST components, confirming that in vitro differentiated YST-like cells are also able to form a YST in vivo. Moreover, expression of cisplatin resistance factors was induced in a subpopulation of YST-like cells, suggesting that formation of a YST is accompanied by acquisition of cisplatin resistance. Indeed, the YST-like cells presented as less sensitive towards a cisplatin treatment than their parental cells.
In summary, we deciphered the molecular mechanisms leading to YST formation from EC, involving SOX17 activation and stimulation of WNT, TGF-beta and FGF signalling. Thus, deducing inhibitors interfering with these factors might prevent outgrowth of an aggressive YST under therapy. Additionally, we postulate that cisplatin resistance appears to be a concomitant event along the YST development, confirming that formation of YST is an escape mechanism for GCT.

Invited talk 12, Identification of Neddylation as mechanism to contribute to cisplatin resistance of testicular germ cell tumors

Funke K.[1], Einsfelder U.[2], Hansen A.[1], Liesen A.[1], Arévalo L.[1], Schneider S.[1], Nettersheim D.[3], Stein V.[2], Schorle H.[1]

[1]Department of Developmental Pathology, Institute of Pathology, University Hospital Bonn, Bonn, Germany.
[2]Institute of Physiology II, University Hospital Bonn, Bonn, Germany.
[3]Department of Urology, Urological Research Laboratory, Translational UroOncology, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

Text Type II testicular germ cell tumors (TGCT) are the most prevalent tumors in young men. Patients suffering from cisplatin-resistant TGCTs are facing very poor prognosis demanding novel therapeutic options. Neddylation is a known posttranslational modification mediating many important biological processes, including tumorigenesis. Overactivation of the neddylation pathway promotes carcinogenesis and tumor progression in various entities by inducing proteasomal degradation of tumor suppressors (e.g., p21, p27). We used a CRISPR/Cas9 activation screen to identify cisplatin resistance factors. TGCT cell lines were treated with the neddylation inhibitor (MLN4924)/cisplatin/combination and investigated for changes in viability (XTT assay), apoptosis/cell cycle (flow cytometry) as well as in the transcriptome (3'mRNA sequencing). NAE1 overexpression was detected in cisplatin-resistant colonies from the CRISPR screen. Inhibition of neddylation using MLN4924 increased cisplatin cytotoxicity in TGCT cell lines and sensitized cisplatin-resistant cells towards cisplatin. Apoptosis, G2/M-phase cell cycle arrest, γH2A.X/P27 accumulation and mesoderm/endoderm differentiation were observed in TGCT cells, while fibroblast cells were unaffected. We identified overactivation of neddylation as a factor for cisplatin resistance in TGCTs and highlighted the additive effect of NAE1 inhibition by MLN4924 in combination with cisplatin. Further to this, we started to selectively block the downstream targets of neddylation, namely the cullins in order to get an insight in the molecular mechanism of neddylation dependent cisplatin resistance. We envision, that these experiments eventually might open up new therapeutic options in TGCT treatment.

Invited talk 13, The Classification of Germ Cell Tumours: why everything you thought you knew is (probably) wrong.

Berney DM.

Centre for Cancer Biomarkers and Biotherapeutics, Barts Cancer Institute, Queen Mary University of London, London, UK

The WHO classification of germ cell tumours was published in 2022 and followed (with minor deviations) the template of WHO 2015, with the use of the pre-neoplastic lesion renamed as germ cell neoplasia in situ (GCNIS).
Ths neatly split germ cell tumours into two unequal halves: the majority derived from GCNIS and the minority a number of entities, partly paediatric, not derived from GCNIS.
However recent data looking at spermatocytic tumours has shown the existence of hybrid lesions with intermediate features between the two halves. We propose that any new classification will have to determine the significance of these hybrid lesions and the necessity for follow up.
Secondly we examine recent evidence that transformed germ cell tumours are derived not just from teratoma but also from some yolk sac subtypes. A proposal for renaming of these entities is included to attempt to engender clarity in this complex area. The risk associated with these germ cell transformations shall be re-examined.

Invited talk 14, New prognostic factors for relapse in patients with clinical stage I seminoma and non-seminoma: the pathologists' perspective

Wagner T.[1,2], Toft B.[2], Lauritsen J.[1], Bandak M.[1], Christensen I.[3], Engvad B.[4], Kreiberg M.[1], Agerbæk M.[5], Dysager L.[6], Rosenvilde J.[1], Berney D.[7], Daugaard G.[1]

[1]Department of Oncology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[2]Department of Pathology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[3]Department of Pathology, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark
[4]Department of Pathology, Odense University Hospital, Odense, Denmark
[5]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark
[6]Department of Oncology, Odense University Hospital, Odense, Denmark
[7]Centre of Cancer Biomarkers and Biotherapeutics, Barts Cancer Institute, Charterhouse Square, Queen Mary University of London, London, United Kingdom

Background
Risk assessment and decisions regarding adjuvant treatment for patients with clinical stage I (CSI) testicular germ cell cancer (TGCC) are based on tumor characteristics in the orchiectomy specimen. The available evidence is challenged by selection bias and variable pathology reporting, leading to ongoing controversies about the prognostic power of identified risk factors and which tumor features provide independent prognostic value. Furthermore, current risk factors for relapse lack sufficient accuracy to reliably stratify patients into high- and low-risk groups. We examined potential pathological risk factors for relapse in unselected, population-based cohorts of CSI seminoma and non-seminoma, assessed with contemporary pathology review.

Materials and Methods
Patients diagnosed with CSI disease in Denmark from 2013 to 2018 were identified in the prospective Danish Testicular cancer database. Histologic slides from the orchiectomy specimens were retrieved and reviewed, blinded to the clinical outcome. Clinical data were obtained from medical records with follow-up until 2022. The association between prespecified potential prognostic factors and relapse was assessed by Cox regression analysis. Potential prognostic factors included age, pre-orchiectomy serum tumor markers (AFP, LDH, and β-hCG), tumor size, tumor multifocality, tumor necrosis, lymphovascular invasion (LVI), pagetoid rete testis invasion, and tumor extension into the rete testis, hilar soft tissue, epididymis, tunica albuginea, tunica vaginalis, and spermatic cord, as well as the percentages of each tumor subtype in non-seminomatous tumors.

Results
The seminoma cohort included 924 patients, of whom 148 (16%) relapsed. We identified four strong predictors of relapse in this group: tumor invasion into the testicular hilum,

LVI, and elevated levels of β-hCG and LDH (Table). The estimated 5-year risk of relapse ranged from 6% to 62% depending on the combination of risk factors.
In the non-seminoma cohort, comprising 453 patients, 139 (30%) relapsed. Tumor invasion into the testicular hilum, LVI, presence of embryonal carcinoma (EC), and tumor size were strong predictors of relapse (Table). The estimated 5-year risk of relapse ranged from less than 5% to more than 85% depending on the combinations of these risk factors.

Conclusion
Our studies have clarified prognostic factors for relapse and significantly improved risk stratification of CSI TGCC patients compared to current clinical practice.

Table. Results of the final multivariable Cox models for time to relapse in the seminoma and non-seminoma cohorts, respectively.

	Variable	**Hazard ratio, 95% CI**	**p-value**
Seminoma	Testicular hilum invasion		
	Rete testis invasion -	1 [Reference]	--
	Rete testis invasion + Hilar soft tissue invasion -	1.81 (1.19-2.75)	0.0058
	Rete testis invasion + Hilar soft tissue invasion +	2.83 (1.82-4.38)	<0.0001
	LVI (present vs absent)	1.82 (1.22-2.70)	0.0032
	β-hCG (elevated vs normal)	1.89 (1.35-2.64)	0.0002
	LDH (elevated vs normal)	1.67 (1.19-2.34)	0.0031
Non-seminoma	LVI (present vs absent)	3.48 (2.38-5.10)	<0.0001
	EC		
	Absent	1 [Reference]	--
	Non-predominant	2.49 (1.20-5.15)	0.0138
	Predominant	4.06 (1.98-8.32)	0.0001
	Hilar soft tissue invasion (present vs absent)	1.70 (1.17-2.48)	0.0056
	Tumor size (log2)	1.60 (1.25-2.03)	0.0001

Selected abstract 15, Assessing the risk to develop a growing teratoma syndrome based on molecular and epigenetic subtyping as well as novel secreted biomarkers

Pongratanakul P.[1,7*], Bremmer F.[2*], Pauls S.[3], Poschmann G.[3], Kresbach C.[4], Parmaksiz F.[1], Skowron M.[1], Fuß J.[6], Stephan A.[1], Paffenholz P.[5], Stühler K.[3], Schüller U.[4], Ströbel P.[2], Heidenreich A.[5], Che Y.[7], Albers P.[7], Nettersheim D.[1]

[1]Department of Urology, Urological Research Laboratory, Translational UroOncology, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
[2]Institute of Pathology, University Medical Center Goettingen, Goettingen, Germany
[3]Molecular Proteomics Laboratory (MPL), Biological and Medical Research Center (BMFZ), Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
[4]Institute of Neuropathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany
[5]Department of Urology, University Hospital Cologne, University of Cologne, Cologne, Germany
[6]Competence Centre for Genomic Analysis Kiel, Germany
[7]Department of Urology, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
**contributed equally*

The "Growing teratoma syndrome" (GTS) is defined as a growing teratoma during chemotherapy with decreasing or normalized tumor markers. To date, its pathogenesis remains elusive and specific treatment options of GTS are limited.
We aimed at updating the GTS definition based on molecular and epigenetic features as well as identifying circulating biomarkers. We selected 50 GTS patients for clinical characterization, with 12 patients analyzed on epigenetic, transcriptional and proteome/secretome level. Moreover, we included longitudinal samples of 2 GTS patients. Teratomas (TER) showing no growth trend served as controls.
GTS were subtyped based on the growth speed over time into a slow (<0.5 cm/month), medium (0.5-1.5 cm/month) and rapid (>1.5 cm/month) group. By analyzing DNA methylation, microRNA expression and the secretome, we identified putative epigenetic and secreted biomarkers for the different GTS subgroups. We found that proteins enriched in GTS compared to TER were involved in DNA replication and repair, the cell cycle and metabolic pathways, while proteins interacting with the immune system were depleted. Additionally, GTS^{rapid} seems to metastasize more quickly and distally from the primary tumor due to a strong interaction with the surrounding microenvironment and a higher migratory capacity than GTS^{slow}. Expression of pluripotency- and yolk-sac tumor-associated genes in GTS and formation of a yolk-sac tumor or somatic-type malignancy in the longitudinal GTS samples, pointed to an additional occult non-seminomatous component after chemotherapy. Thus, updating the Logothetis definition is necessary: "The GTS describes a continuously growing teratoma that might harbor occult non-

seminomatous components considerably reduced during therapy but outgrowing over time again". Patients representing these features harbor an aggressive subtype of highly proliferative germ cell tumor and need urgent and complete resection of residual disease after chemotherapy.

Invited talk 16, Role of primary retroperitoneal lymph node dissection in patients with low volume retroperitoneal lymph node metastases

Heidenreich A.

Department of Urology, Uro-Oncology, Robot-Assisted and Specialized Urologic Surgery, University Hospital Cologne, Germany

Guideline recommended treatment of choice for clinical stage IIA/B testicular germ cell tumors is the delivery of chemotherapy with 3 cycles PEB/4 cycles PE or alternatively radiation for seminomas. Despite a high cure rate of 90 – 94% and 82 – 90% for CS IIA and CS IIB, respectively, both options are associated with a high rate of treatment-associated long-term toxicities. A significantly increased risk for the development of secondary malignancies, cardiovascular and metabolic disease as well as an increased for treatment-associated mortality has been proven in various studies. Primary nerve sparing retroperitoneal lymph node dissection (nsRPLND) has been evaluated in 5 prospective and retrospective clinical studies and it has emerged as a valid treatment alternative. The relapse-rate after a median follow-up of 25 – 33 months is in the range of 11 – 30%, so that 70 – 90% of patients are cured without being subjected to chemotherapy and potential long-term toxicities. All relapsing patients have been cured with secondary salvage chemotherapy. The frequency of significant surgery-associated complications is low with 3 – 13%.

Similar data with a high cure rate of 85 – 90% have been reported for patients with CS IIA/B marker negative nonseminomas. In men with pure testicular teratomas or malignant somatic tramsformation nsRPLND represents the therapeutic approach of choice.

Therapeutic success depends on the surgical experience of the various surgeons and the chosen template, so that this type of surgical intervention should only be performed in centers of excellence with dedicated surgeons. Preoperative evaluation of the new biomarker miR371 has been shown to predict the presence of metastatic disease with an accuracy of around 100% so that this marker might be used in daily routine prior to active treatment in CS IIA/B seminomas.

Invited talk 17, Role of post chemotherapy residual tumor resection in patients with metastatic testicular germ cell tumors

Heidenreich A.

Department of Urology, Uro-Oncology, Robot-Assisted and Specialized Urologic Surgery, University Hospital Cologne, Germany

Postchemotherapy retroperitoneal lymph node (PC-RTR) dissection or resection of extra retroperitoneal masses represents an integral part of the curative multimodality approach of patients with metastatic testicular germ cell tumors. PC-RTR can be a challenging surgical procedure with the need for the resection of adjacent visceral, vascular, or intestinal structures in about 10 – 15% of the patients. Therefore, this type of surgery should only be performed in highly experienced centers with at least 20 procedures per surgeon annually.
PC-RTR needs to be performed in nonseminomatpous TGCT with residual masses >1cm in both the horizontal and the vertical diameter. PC-RT can be omitted in patients with residual masses <1cm who started chemotherapy for good prognosis according to the IGCCCG criteria. PC-RTR should always be performed for visible lesions in patients with intermediate/poor risk at time of chemotherapy. A unilateral modified template resection can be done in patients with metastatic in the primary landing zone of the tumor bearing testicle, a mass size <5cm and no or minimal teratomatous elements in the orchiectomy specimen. A full bilateral template should always be performed in patients with large residual masses, teratoma-dominant primary testis tumors and a primary metastatic lesion located in the interaortocaval area or on the contralateral side of the tumor bearing testicle. Residual lung metastases have a discordant histology in about 30 – 40% of patients so that resection of residual masses should be done. In patients with bilateral lung disease, there is a high concordance of the histology in both lobes so that the side with the largest or most accessible lesions should be resected first. If only necrosis or fibrosis is present, the contralateral side does not need to be resected. With regard to liver metastases, there is a concordance of the histology between the retroperitoneum and the liver, so that metastasectomy is only necessary if viable cancer or teratoma is identified in the retroperitoneal residual masses.

About 3 – 5% of patients need the resection of vascular structures as the aorta or the inferior vena cava, which can be identified on the preoperative images. Skeletal surgery is indicated in 1 – 2% of the patients.

Selected abstract 18, Long-term oncological safety of robotic-assisted retroperitoneal lymph node dissection in selected testicular germ cell tumors

Pongratanakul P., Wöltjen C., Vermeulen M., Thy S., Hiester A., Albers P., Che Y.

Department of Urology, University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

Introduction and objectives

Retroperitoneal lymph node dissection (RPLND) is an essential procedure in the treatment of testicular germ cell tumors across all stages, with open RPLND being the established standard. While several studies have shown the feasibility of robotic-assisted RPLND (R-RPLND), comprehensive long-term oncological follow-up data are still lacking. This study analyzes a single-center experience regarding R-RPLND, presenting novel long-term oncological data and assessing the safety of R-RPLND.

Material and methods

We retrospectively identified and analyzed 100 patients who underwent R-RPLND between October 2010 and January 2024. A matched analysis with open RPLNDs was conducted based on matching criteria including clinical stage, tumor size, and surgical indication.

Results

All R-RPLNDs utilized the Da Vinci Si-System with modified unilateral template resection. Indication for R-RPLND included tumor sizes < 5 cm, unilateral tumor presentation, and absence of vascular infiltration. The median tumor size was 1.8 cm with a median operation time of 170 minutes and without significant blood loss. R-RPLNDs were performed in 66 primary and 34 post-chemotherapy cases. Median follow-up for primary RPLNDs in seminomas and non seminomas was 22 and 25 months, respectively. Relapse occurred in 23.8 % of seminoma cases and 16.8 % of non seminoma cases during follow-up. All post-chemotherapy patients remained relapse-free with a median follow-up of 20 months. In the matched analysis, progression-free survival was similar between the robotic and open approaches.

Conclusions

The robotic approach has proven to be a save minimal-invasive alternative to open RPLND. In terms of surgical and oncological safety, R-RPLND appears comparable to open surgery in selected patients, suggesting its potential to become the new standard of care.

Invited talk 20, Recent advances in clinical development of therapies for relapsed and refractory germ cell tumors

Alsdorf W.

Department of Oncology, Hematology and Bone Marrow Transplantation with Division of Pneumology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Despite high cure rates achieved with first-line treatment, approximately 10% – 15% of patients with germ cell tumors (GCT) relapse and require salvage chemotherapy (conventional or high-dose), which again is curative in up to 50% of patients. For patients with refractory disease or relapse after salvage therapy, overall survival is limited, and development of novel treatments in this setting remains a top research priority.

Despite multiple efforts, GCTs are yet to benefit from targeted therapy: promising preclinical activity observed with receptor tyrosine kinase inhibitors, cyclin-dependent kinase inhibitors and poly(ADP-ribose) polymerase inhibitors failed to translate into clinical efficacy. Similarly, no clinically meaningful responses were observed in single-agent trials of the CD30-directed antibody–drug conjugate (ADC) brentuximab vedotin or immune checkpoint inhibitors (pembrolizumab, avelumab, durvalumab). Multiple Phase ½ studies were stopped for futility, and ongoing clinical programs are investigating various treatment combinations.

Claudin 6 (CLDN6) is an oncofetal antigen: its expression in multiple solid tumor types, including testicular cancer, and absence from healthy adult tissues makes it an important target of several investigational anti-cancer therapies. Although a Phase 2 study of CLDN6-targeted monoclonal antibody (NCT03760081) was stopped due to lack of efficacy, initial positive signals were observed in Phase 1 studies of TORL-1-23, a CLDN6-targeted ADC (NCT05103683), and of CLDN6 chimeric antigen receptor (CAR) T cell therapy (NCT04503278). In this presentation, I will focus on the recent results of BNT211-01 (NCT04503278), a first-in-human, Phase 1/2 study of the investigational treatment with CLDN6 CAR T cells alone or in combination with a CAR T cell-amplifying RNA vaccine (CARVac). Initial anti-tumor activity in patients with GCTs, efficacy, safety and exploratory data will be discussed.

Invited talk 21, New prognostic factors for relapse after clinical stage I testicular cancer – impact on follow-up and clinical implications

Lauritsen J.[1], Wagner T.[1,2], Toft B.[2], Bandak M.[1], Engvad B.[3], Agerbæk M.[4], Dysager L.[5], Rosenvilde J.[1], Berney D.[6], Daugaard G.[1]

[1]Department of Oncology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[2]Department of Pathology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark. [3]Department of Pathology, Odense University Hospital, Odense, Denmark
[4]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark
[5]Department of Oncology, Odense University Hospital, Odense, Denmark
[6]Centre of Cancer Biomarkers and Biotherapeutics, Barts Cancer Institute, Barts Cancer Institute, Charterhouse Square, Queen Mary University of London, London, United Kingdom

Background
The management of patients with clinical stage I (CSI) testicular germ cell cancer (TGCC) is controversial. Follow-up varies substantially between institutions, with alternating frequency of measurement of serum tumor markers, imaging, and length of follow-up. Also, recommendations for adjuvant treatment for patients with seminoma or non-seminoma differ. Based on recently proposed new prognostic factors for relapse, we present suggestions for managing patients with CSI TGCC.

Material and Methods
Patients diagnosed with CSI disease in Denmark from 2013 to 2018 were identified. Histologic slides from the orchiectomy specimens were retrieved and reviewed, blinded to the clinical outcome. Clinical data were obtained from medical records with follow-up until 2022. The cohort was split into patients with seminoma and non-seminoma and prognostic factors based on pathological findings and serum tumor markers were identified. For patients with CSI seminoma, the risk of relapse ranged from 6% to 62%, and correspondingly, for non-seminoma, 5% to 86%, depending on the number of risk factors.

Results
Based on the identified prognostic factors, we suggest administering one cycle of adjuvant cisplatin-etoposide-bleomycin (BEP) to patients with CSI non-seminoma and a risk of relapse of more than 50%. This may result in a reduction in BEP cycles of 21% for patients with CSI non-seminoma. Adjuvant therapy for patients with seminomatous TGCC cannot yet be recommended. We suggest de-intensifying follow-up for patients with CSI seminoma and the lowest risk of relapse (<10%, 55% of patients) leading to a reduction of 26% in outpatient visits while maintaining the high cure rate.

Conclusion
The use of newly identified prognostic factors of relapse for patients for CSI TGCC can lead to a significant reduction in use of chemotherapy and outpatient visits.

Invited talk 22, Update on circulating biomarkers for testicular germ cell tumor patients: focus on microRNAs

Lobo J.[1,2,3]

[1]*Department of Pathology, Portuguese Oncology Institute of Porto (IPO Porto) / Porto Comprehensive Cancer Center Raquel Seruca (P.CCC), Porto, Portugal*
[2]*Cancer Biology and Epigenetics Group, IPO Porto Research Center (GEBC CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto) / Porto Comprehensive Cancer Center Raquel Seruca (P.CCC) & CI-IPOP@RISE (Health Research Network), Porto, Portugal*
[3]*Department of Pathology and Molecular Immunology, ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal*

The most frequent forms of testicular cancer are testicular germ cell tumors (TGCTs). Biopsy of a testicular mass is not advocated for confirming the diagnosis, due to associated risks, and so patients are referred for orchiectomy with a presumptive diagnosis of malignancy. This raises the need of circulating non-invasive biomarkers for TGCT patients.
The currently available serum biomarkers used in the clinic (AFP, HCG and LDH) are useful. However, they also have important limitations, being elevated in only 60% of patients at diagnosis and performing suboptimally for detection of minimal residual disease. TGCT patients benefit from introduction of a robust biomarker able to detect relapses early in time, allowing for giving timely salvage therapy with less toxicity.
In the last years, microRNAs of the 371~373 cluster have been the most promising biomarkers for non-invasive TGCT diagnosis and follow-up, validated in several retrospective and prospective multicenter studies and being part of ongoing clinical trials. In particular, miR-371a-3p has shown great sensitivity and specificity (outperforming the combination of classical serum tumor markers) in various settings, including diagnosis; association with tumor burden and response to treatment; detection of disease relapses in stage I patients; and identification of residual non-teratoma in post-chemotherapy metastases. This microRNA cluster is, however, negative specifically in teratoma, and currently there is no established circulating biomarker to discriminate this histological subtype.
The miR-371a-3p assay has recently been made available as an IVD CE test (M371 test), approaching clinical implementation. Still, some pre-analytical considerations need to be harmonized, in order to take the best out of this biomarker.
In this presentation, an update on circulating biomarkers available for TGCT patients will be provided, focusing on recent advances with microRNAs.

Invited talk 23, Serum levels of miR-371a-3p in testicular germ cell cancer: update on half-life, expression rates in other cancers, and utility in postchemotherapy masses

Klemke M.[1, 2], Belge G.[1], Grobelny F.[1, 3], Niemzok T.[1], Igde A.[1], Dumlupinar C.[1], Hansen B.[4], Dieckmann K.-P.[4]

[1]*Faculty of Biology and Chemistry, University of Bremen, Bremen, Germany*
[2]*mir|detect GmbH, Bremerhaven, Germany*
[3]*Faculty of Medicine, University of Hamburg, Hamburg, Germany*
[4]*Department of Urology, Asklepios Klinik Altona, Hamburg, Germany*

There is accumulating evidence for the utility of serum levels of the microRNA miR-371a-3p (M371) in the clinical management of patients with testicular germ cell tumours (GCTs). Limited data exist regarding the expression of this miR in other malignancies. We measured M371 serum levels in 139 patients with other cancers using the IVDR certified M371 test and by defining RQ=5 as upper limit of norm (ULN). Eight patients (5.8%) had slightly elevated levels. Curiously, 3 of 5 patients with multiple myeloma had elevations. This result supports the view that M371 is specific for GCTs but sporadic cases with other cancers may have elevations.

The half-life of M371 serum levels in clinical stage (CS) 1 patients after orchiectomy was suggested to be <12 hours. In 51 CS1 patients, we measured M371 levels three times during the first 24 hours after orchiectomy. Preliminary analysis of 15 patients revealed a drop of levels below ULN within 24 hours in 95% of cases with CS1a disease and 90% in CS1b cases. The estimated half-life is thus 4-8 hours. This result confirms the very rapid decay of M371 serum levels after treatment.

The histology of postchemotherapy (pc) masses can only be disclosed by surgery. However, pc surgery involves considerable perioperative morbidity. To analyse the ability of M371 serum levels of detecting viable cancer (VC) in pc masses, we preoperatively measured M371 levels in 180 GCT patients undergoing pc surgery. Surgical specimens comprised of necrosis, teratoma, or VC in 33.3%, 41.7%, and 25% of cases, respectively. M371 levels were elevated in 68.9% VC cases, 0% in teratoma and 1.7% in necrosis. Noteworthy, elevation of M371 levels was significantly associated with percentage of VC in the surgical specimen. Thus, the M371 test cannot safely identify patients who require pc surgery because only 68% of VC cases can be identified and teratoma remains undetected.

Invited talk 24, Circulating free DNA provides insights into the molecular evolution of testicular germ cell tumors

Matouskova L.[1], Bakardjieva-Mihaylova V.[1], Zwyrtkova M.[1], Skvarova Kramarzova K.[1], Stuchly J.[1], Krivonoskova S.[2], Soffrova K.[1], Rosova B.[3], Buchler T.[4], Trka J.[1], Boublikova L.[1,2]

[1]Dept. of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic
[2]Dept. of Oncology, 1st Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic
[3]Dept. of Pathology and Molecular Medicine, 3rd Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic
[4]Dept. of Oncology, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic

Testicular germ cell tumors (TGCT) possess unique molecular profiles with very low mutation rates and rare cancer gene alterations. Consequently, no specific targeted therapy has proven to be effective in TGCT so far. The molecular background is supposed to evolve during tumor progression, which may be monitored by liquid biopsy techniques. We assessed the potential of circulating free DNA (cfDNA) in TGCT management.
We have previously shown that total cfDNA concentration was significantly higher ($p < 0.0001$) in TGCT patients of all stages and various time points (n=96 pts, 173 samples) than in normal controls (n=31) but without a clear threshold that could reliably distinguish between tumor and normal samples.
Whole-exome sequencing of 15 patients with advanced and relapsing TGCT (56 samples including primary tumors, metastases persisting after platinum-based chemotherapy, and cfDNA from the disease progression) revealed somatic mutations of 6 genes with a significant role in carcinogenesis and/or testis development that were identified in more than one patient: *RBMX* (in 4 pts), *TPTE2* and *ANKRD30A* (in 3 pts), *CDC27, PRAMEF8*, and *PRDM9* (in 2 pts). The mutations were present in tumor tissue as well as cfDNA, often with an increasing frequency upon progression, except for *PRDM9* mutations which were found in cfDNA from disease progression only, and not in primary tumors. This gene encodes histone methyltransferase responsible for H3 methylation during meiosis. As epigenetic changes are supposed to play a crucial role in TGCT pathogenesis and resistance, and aberrant H3 methylation has been previously related to abnormal expression of *OCT3/4* transcription factor promoting TGCT development, *PRDM9* may be a novel candidate gene in TGCT progression and cisplatin resistance.
In conclusion, molecular studies based on cfDNA analysis bring novel insights into possible mechanisms of TGCT evolution and may uncover aberrations targetable by future therapies.
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Invited talk 26, Psychosocial issues in survivors of germ cell tumors

Chovanec M.

2nd Department of Oncology, Faculty of Medicine, Comenius Univeristy, National Cancer Institute, Bratislava, Slovakia

Germ cell tumors (GCTs) are now considered a curable cancer. Long-term cure of around 95% is achieved with surgical treatment and chemotherapy. While excellent survival rate is highly encouraging for patients and clinicians, the young age brings specific challenges in psychosocial development during the traumatic experience of cancer diagnosis. Life expectancy of more than 3 decades after treatment is sufficient to enable negative impacts in ways such as financial, body image, work plans, control over life, relationships, and plans for having children. These psychosocial disturbances lead to adverse outcomes in quality of life (QoL). Increasing number of validated tools to measure patient-reported outcomes during and after treatment allow to study QoL as a meaningful short- and long-term outcome in GCTs. Diagnosis of GCT alone may lead to anxiety, depression and cognitive impairment prior to initiation of treatment. While some studies show similar anxiety and depression levels compared to age-matched controls, others have shown significant increase in GCT survivors. Therefore, the interpretation of these studies may be challenging due to diversity of tool, cultural and regional differences. Treatment modalities lead to treatment-specific issues. Surgical management may contribute to altered body image, hypogonadism with secondary late psychological effects, retrograde ejaculation, anxiety from sexual relationships and fertility issues. Treatment with chemotherapy causes a plethora of late toxic effects in dose-depended manner creating a vulnerable milieu for psychosocial issues. Recent years brought knowledge about long-term cognitive impairment sustained longitudinally for at least 10years after treatment in survivors exposed to higher cumulative burden of chemotherapy. Concerning is an impact of chemotherapy-induced peripheral neuropathy which was shown to be significantly associated with QoL disturbances in all measured domains. Survivors of GCTs also report financial difficulties and difficulty to find jobs, while fear of recurrence is an issue for up to 45% of them. Assessing QoL and psychosocial health is an emerging field within oncology. Survivors of GCTs represent an ideal model population to study these outcomes to ultimately improve QOL during and after curative treatment for testicular cancer.

Selected abstract 27, Second malignancies in testicular cancer survivors – data from the Czech Cancer Registry

Buchler T.[1,3], Jarkovsky J.[2], Cvanova M.[2], Kucerova H.[2], Boublikova L.[3], Muzik J.[2]

[1]Department of Oncology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic
[2]Institute of Health Information and Statistics of the Czech Republic, Prague, Czech Republic
[3]Department of Oncology, First Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic

Introduction
Advancements in treatment have led to a continuous decrease in mortality rates for testicular cancer despite an increasing number of new diagnoses. Consequently, the prevalence of men with a history of malignant testicular neoplasms is rising. Long-term morbidity and mortality in patients with testicular germ cell neoplasms (TGN) are significantly influenced by oncological complications, especially second primary malignancies (SPMs).

Patients and Methods
The aim of the study was to analyse the incidence and progression of secondary malignancies in patients with a history of TGN (n=8549). The incidence of tumors was age-standardized for comparison with the general population.

Results
The risk of SPMs was elevated in the population with a history of testicular cancer. The median latency to a SPM was 9.2 years. The most significant increase in incidence compared to the general population was observed in tumours of the (contralateral) testis, non-melanoma skin cancers, colorectal cancer, bladder cancer, non-Hodgkin's lymphomas, and melanoma. Compared to the general population, patients with antecedents of TGN had increased risk of mortality from from prostate cancer, liver tumors, brain tumors, melanoma, non-Hodgkin's lymphomas, and leukemia.

Conclusion
The population of patients with TGNs exhibits long-term increased incidence and mortality from specific types of SPMs.

Invited talk 28, Subclinical hypogonadism in testicular cancer patients: What is the evidence for testosterone substitution?

Bandak M., Lauritsen J., Wagner, T., Daugaard G.

Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark

It remains unknown if testosterone substitution is beneficial for testicular cancer (TC) patients with subclinical hypogonadism/ mild Leydig cell insufficiency.
A randomized double-blind placebo-controlled trial of testosterone substitution was performed to clarify if testosterone substitution improves metabolic health and quality of life in TC patients with mild Leydig cell insufficiency.
Patients aged 18 to 65 years, without evidence of relapse more than one year after TC treatment, were eligible for inclusion. The main inclusion criterion was presence of mild Leydig cell insufficiency defined as serum concentration of free testosterone below the age-adjusted mean and above the age-adjusted lower limit of normal in combination with serum LH above the age-adjusted upper limit of normal. Patients were randomized 1:1 to transdermal testosterone, which was titrated to a maximum dose of 40 mg pr day vs placebo. Outcomes included measures of metabolic syndrome, body composition evaluated by DXA scan, patient reported sexual function and quality of life evaluated by validated questionnaires. Outcomes were evaluated at baseline, after 6 and 12 months of treatment and 3 months after treatment cessation.
A total of 69 TC patients were included of whom 35 were allocated to testosterone and 34 to placebo. In total, 27/35 and 31/34 completed 12-months treatment in the two groups respectively. After 12 months, median free testosterone was 542 pmol/L (IQR: 410 – 715), and median LH was 4.2 IU/L (IQR: 3.5 – 6.3) in the testosterone group compared to 317 pmol/L (IQR: 274 – 347) and 7.6 IU/L (IQR: 6.3 – 8.7) in the placebo group. Testosterone substitution was associated with a statistically significant decrease in total fat mass compared to placebo at 12 months (-1.35 kg, (95% CI: -2.53, -0.18)), while there was no difference in the components of metabolic syndrome. In both groups, a statistically significant increase in sexual desire and overall satisfaction as well as a decrease in general fatigue and mental fatigue was observed, indicating a placebo effect.
In conclusion, testosterone substitution is not associated with clinically meaningful improvements of metabolic health and quality of life in TC patients with mild Leydig cell insufficiency.

Invited talk 29, Sperm DNA alterations in TGCT patients and their relevance for pre-conceptional counselling

Krausz C.

Department of Experimental and Clinical Biomedical Science "Mario Serio", University of Florence, Florence, Italy.

Antineoplastic treatments in Testicular germ cell tumor (TGCT) are highly efficient leading to a 95% 5-year survival rate. While sperm production is usually fully or partially recovered within 2 years from the end of therapy, it may induce mutagenic effects, leading to chromosomal anomalies, *de novo* mutations, aberrant DNA methylation and sperm DNA fragmentation. DNA alterations arising in the paternal genome and epigenome after cancer treatment are of concern for their consequences to the offspring's health.

The standard indication for seeking natural pregnancies in cancer survivors is 24 months after therapy. However, increased aneuploidy rates have been reported in some patients after this time interval [1]. Moreover, some children born to fathers treated for cancer before conception exhibit an hypermutated genome and specific mutational signatures that are associated with TGCT treatment regimens, such as cisplatin-based drugs 2]. Concerning the so called "selfish" mutations, only one study evaluated the rate of two *FGFR2 hotspot* mutations in cancer patients at different time intervals from cytotoxic treatments [3]. While data are reassuring for patients after several years from the treatment, an increased mean number of mutated copies per million was observed in all those men who were analyzed within the first year. Apart from genetic alterations, cancer treatment can also affect the sperm epigenome. In a recent study DNA methylation was altered 2 years post-treatment in some TGCT patients [4]. These studies raise questions about the appropriate timing for safe natural conception and warrants urgent validation in larger cohorts.

At the moment, the analysis of the above genetic/epigenetic alterations cannot be included in the routine clinical practice for their excessive cost. As an alternative, measuring sperm DNA fragmentation (SDF) is widely available and can be used as a biomarker for the evaluation of DNA integrity. Indeed, several authors proposed SDF as a biomarker to monitor the persistence of the genotoxicity of cancer treatment with a follow-up limited to 12-24 months. In our recent study, we evaluated the persistency of SDF up to three years post-treatment in the largest available longitudinal cohort of TGCT patients [5]. We performed TUNEL assay coupled with flow cytometry on fresh spermatozoa mimicking natural conception. In the cross-sectional cohort (n=115 TGCT), median SDF levels increased after 2 years from the end of treatment (T2) compared to baseline (T0). After 3 years from treatment (T3), median SDF levels returned to baseline. In order to better characterize the nature and persistence of SDF, we were interested in determining the frequency of severe DNA damage (SDD) among the patients. We defined SDD as the value which corresponds to the 95^{th} percentile in our control cohort (SDF ≥ 50%). SDD was present prior to treatment in 8.9% of patients. Post-therapy, the proportion of patients with SDD increased up to 23.4% at T2 and decreased to 4.8% at T3.

The persistent severe DNA damage after 2 years post-treatment observed in some patients suggests that there is an interindividual variation in restoring DNA integrity. We propose the use of SDF as a biomarker to monitor the treatment-induced genotoxic effects on sperm DNA in order to better personalize pre conceptional counseling on whether to use fresh or cryopreserved spermatozoa.

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Selected abstract 30, Bioenergetics of human spermatozoa in patients with testicular germ cell tumour

Simonik O.[1], Bryndova B.[1], Pratap Sur V.[1], Ded L.[1], Cockova Z.[2], Benda A.[2], Qasemi M.[1], Pecina P.[3], Pecinova A.[3], Spevakova D.[1], Hradec T.[4], Skrobanek P.[5], Ezrova Z.[6], Kratka Z.[7], Kren R.[8], Jeseta M.[9], Boublikova L.[5], Zamecnik L.[4], Büchler T.[5,10], Neuzil J.[6,11], Postlerova P.[1], Komrskova K.[1,12*]

[1]Laboratory of Reproductive Biology, Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic
[2]Imaging Methods Core Facility at BIOCEV, Faculty of Science, Charles University, Vestec, Czech Republic
[3]Laboratory of Bioenergetics, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic
[4]Department of Urology, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic
[5]Department of Oncology, First Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic
[6]Laboratory of Molecular Therapy, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic
[7]Laboratory of Immunology, IVF Clinic GENNET, Prague Czech Republic
[8]Laboratory of embryology, IVF Clinic GENNET, Prague, Czech Republic
[9]Department of Obstetrics and Gynaecology, Faculty of Medicine, Masaryk University and University Hospital Brno, Brno, Czech Republic
[10]Department of Oncology, Second Faculty of Medicine and Motol University Hospital, Prague, Czech Republic
[11]School of Pharmacy and Medical Science, Griffith University, Southport, QLD, Australia
[12]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

In patients with testicular germ cell tumors (TGCT), sperm cryopreservation prior to undergoing cancer treatment is the primary method for preserving fertility. However, this process often results in a low recovery rate of viable sperm after thawing. Since sperm cells require high energy, primarily obtained through glycolysis and oxidative phosphorylation (OXPHOS), mitochondrial dysfunction can directly cause sperm abnormalities. This study aimed to investigate the bioenergetic profile of cryopreserved sperm from TGCT patients. We utilized two advanced techniques: the Extracellular Flux Analyzer (XF Analyzer) and Two-Photon Fluorescence Lifetime Imaging (2P-FLIM), to evaluate the contributions of OXPHOS and glycolysis to energy production. We developed a novel protocol to simultaneously measure OXPHOS and glycolysis using the XF Analyzer, complemented by a custom AI-based method for semi-automated 2P-FLIM image processing. Our study introduced an optimized Low-HEPES modified Human Tubal Fluid (mHTF) media for sperm handling during both pre-analytical and analytical phases, ensuring the maintenance of physiological parameters and optimal OXPHOS. Cryopreservation negatively impacted both bioenergetic pathways, as indicated by altered

OCR and ECAR curves and derived parameters. This effect was observed in both normozoospermic and TGCT samples, when TGCT samples showing more pronounced damage in the respiratory chain compared to glycolytic activity. These findings were corroborated by 2P-FLIM analysis, which demonstrated a significant decrease in bound NADH relative to unbound NAD(P)H, reflecting reduced metabolic activity in TGCT patient samples. Our study provides new insights into the impact of TGCT on sperm bioenergetics and offers a validated protocol for assessing human sperm metabolic activity, which could be a valuable tool for further research and clinical applications in andrology.

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Invited talk 31, The Testicular Sex-cord STromal (TESST) group: report on a novel classification to refine treatment and follow-up.

Berney DM.

Centre for Cancer Biomarkers and Biotherapeutics, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom

Since 2022 there have been a large number of publications on sex cord stromal tumours of the testis which have revolutionised our thinking on these tumours. They include associations between these tumours and important germ line mutations (eg FH, APC and DICER 1) mutations as well as the identification of new specific fusion genes which appear to confer a poor prognosis.
Because of this the TESST group of 18 experts in testicular stromal tumours met in John Hopkins Hospital in March 2022.
Work included agreement on a series of consensus statements and a blinded study looking at intra-observer agreement in 44 tumours, currently under revision with 'Modern Pathology'.
The findings of the group will be presented and the ambition to create a new classification, based on morpho-molecular findings which will lead to a subset of these tumours being declared entirely benign, while others will be shown to be malignant. Renaming some entities has been suggested to aid their appropriate treatment. Further, the associations will reveal the necessity in a subset of cases to look for germ line mutations of clinical import.

Invited talk 32, Overlapping genetic susceptibility for pediatric and adult GCT

Poynter, J.

University of Minnesota, Minneapolis, MN, USA

Genetic susceptibility to adult testicular GCT is well established, with known variants explaining a substantial portion of the heritability. Similarly, a small genetic study of intracranial GCT in adolescents and young adults reported a variant near *BAK1* as a susceptibility locus for iGCT. Genomewide assocation studies (GWAS) have not been conducted to date for GCT in pediatric patients or for ovarian and extragonadal GCT. In this analysis, we present the results of a GWAS in GCT cases diagnosed between the ages of 0-19 years with tumors in all locations. We included 1,927 GCT cases identified through the Childhood Cancer Research Network and state neonatal biobanks in Michigan and California. Cases were primarily of European (N=829) or Hispanic/Latino (N=806) ancestry and had the following distribution by tumor location: 678 testicular, 441 ovarian, 435 intracranial, and 373 extragonadal. The comparison group included age and sex matched controls from state biobanks and ancestry matched controls from Geisinger Health System (phs000957.v1.p1). Genotyping was conducted using the Illumina Human Core Exome array with imputation to the TOPMed panel. Associations were evaluated using the GMMAT method for high-throughput fitting of mixed models as implemented in the GENESIS pipeline. We identified 4 loci that reached genomewide significance in our analysis of all GCTs combined (Table 1). In subgroup analyses, we noted differences by tumor location and sex. We observed the highest number of independent loci that reached genomewide significance in intracranial GCT (N=6) while no loci reached genomewide significance in extragonadal tumors. Some associations appear to be common across tumor location and age group (e.g., *BAK1*) while others are subgroup specific (e.g., *KITLG* in testicular GCT). Our results provide evidence that genetic susceptibility is important in GCT located outside the testis. Additional studies with larger sample sizes will be required to confirm differences across subgroups.

Table 1. Summary information for independent pediatric and adolescent germ cell tumor susceptibility loci.

Cytoband	rsID	Location (hg38)	REF	ALT	ALT frequency	Odds Ratio (95% CI)	P-value	Gene	Location of signal
6q13	rs3831846	33580569	TGTAA	T	0.20	1.64 (1.49, 1.82)	1.97×10^{-22}	*BAK1*	Proximal
5q14.3	rs12515244	142286728	G	A	0.49	1.38 (1.27, 1.50)	3.66×10^{-14}	*SPRY4*	
9q21.12	rs10815910	862803	T	C	0.61	1.38 (1.27, 1.51)	7.78×10^{-14}	*DMRT1*	Intronic
8q13.3	rs13277786	119893021	T	G	0.74	0.73 (0.66, 0.80)	2.11×10^{-10}	*DEPTOR*	Intronic

POSTERS

P1. Molecular and epigenetic *ex vivo* profiling of testis cancer-associated fibroblasts and their interaction with germ cell tumor cells and macrophages

Stephan A.[1], Suhrmann J. H.[1], Skowron M. A.[1], Che Y.[2], Poschmann G.[3], Petzsch P.[4], Kresbach C.[5], Wruck W.[6], Pongratanakul P.[2], Adjaye J.[6], Stühler K.[3], Köhrer K.[4], Schüller U.[5], Nettersheim D.[1,7+]

[1]*Department of Urology, Urological Research Laboratory, Translational UroOncology, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[2]*Department of Urology, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[3]*Molecular Proteomics Laboratory (MPL), Biological and Medical Research Centre (BMFZ), Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[4]*Genomics and Transcriptomics Laboratory, Biological and Medical Research Centre (BMFZ), Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[5]*Institute of Neuropathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany*
[6]*Institute for Stem cell Research and Regenerative Medicine, University Hospital Düsseldorf, Düsseldorf, Germany*
[7]*Center for Integrated Oncology Aachen, Bonn, Cologne, Düsseldorf (CIO ABCD), Lighthouse Project Germ Cell Tumors*

Germ cell tumors (GCT) type II are the most common solid tumors in young men of age 15 – 40. Earlier, we elucidated the interaction of GCT cells with cells of the tumor microenvironment (TM) *in vitro*. Thereby, the 3D interaction of fibroblasts (FB) or macrophages with GCT cells affected the tumor cell growth and cisplatin response as well as the transcriptome and secretome of the tumor cells suggesting that the crosstalk of these cells with GCT cells is crucial for tumor progression and therapy outcome.
In this study, we shed light on the mechanisms of activation of cancer-associated fibroblasts (CAF) in the GCT setting and their effects on GCT cells lines and the monocyte cell line THP-1. For the first time, we established CAF cultures *ex vivo* from seminoma (SE), embryonal carcinoma (EC), and teratoma (TER). We performed a molecular and epigenetic characterization of GCT-CAF by analyzing the DNA methylome (850k array), transcriptome (RNA sequencing), and secretome (mass spectrometry).
Here, we showed the activation state of CAF is influenced by their former prevailing TM they have resided in. We postulate that SE and EC activate CAF, while TER play only a minor role in CAF formation. Further, in GCT-CAF mostly extracellular matrix (ECM) remodeling and inflammation response related signaling pathway were induced.
In turn, CAF influenced proliferation and the expression of cisplatin sensitivity-related factors in GCT cells lines as well as the polarization status of *in vitro*-induced macrophages by the novel identified factors IGFBP1, LGALS3BP, LYVE1, and PTX3.

These findings suggest the interaction of CAF with GCT cells and macrophages has huge influence for shaping the ECM as well as for recruitment of immune cells to the TM. Conclusively, we present new targets for interfering therapeutically the CAF/ macrophages/ GCT interaction which in addition to the standard therapy might slow-down progression of GCT and re-shaping of the TM to a tumor-promoting environment.

P2. Identification of genes potentially contributing to cisplatin resistance in testicular germ cell tumors using CRISPR-KO screening

Matouskova L.[1], Soffrova K.[1], Bakardjieva-Mihaylova V.[1], Stuchly J.[1], Boublikova L.[1,2], Skvarova Kramarzova K.[1]

[1]Dept. of Pediatric Hematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic
[2]Dept. of Oncology, 1st Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic

Testicular germ cell tumors (TGCT) are the most common solid tumors in young adult men, and their incidence has been increasing globally in recent decades. Even though these tumors are highly curable due to their high sensitivity to cisplatin (cDDP), a minor proportion of patients develops a resistance to this cytostatic that often results in disease progression and death. The cause of cisplatin resistance development in TGCT has not been elucidated yet.

To address this issue, we used CRISPR-Cas9 gene editing technology that enables the investigation of functional consequences of gene alterations on cell phenotype. Since it is presumed that multiple genes of lower impact are participating in cDDP resistance in TGCT, we employed a CRISPR-KO screening in TGCT cell line that allowed us to test a large number of genes in a single experiment. In particular, we prepared Cas9-expressing NCCIT cell line transduced with a lentiviral library of sgRNAs (Addgene #101926) targeting 3015 genes which contribute to cell cycle regulation and cancer development. The resulting cell population was cultured with or without cDDP for 7 days. Samples of treated and control cells were harvested on days 0 and 7 of cDDP treatment, the sgRNA cassettes were sequenced by NGS, and the abundance of sgRNAs was determined using MAGeCK-Flute pipeline.

Analysis of the CRISPR-KO screening data identified several genes, whose targeting sgRNAs were positively selected after exposure of the cells to cDDP, such as *HDAC8* and *MAGEA2*. Inactivation of these genes potentially contributes to the development of cDDP resistance. We also identified genes that were negatively selected upon cDDP treatment, e.g. *FANCB*, *ERCC5,* and *ERCC6*. These genes represent potential therapeutic targets in cDDP-resistant tumors.

CRISPR-KO screening identified genes relevant for the development of cDDP resistance in TGCT and may suggest the ways how to overcome it. The identified hits will be validated in subsequent experiments.

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P3. Assessing an extracellular vesicles-derived miR-371a-3p detection methodology in testicular germ cell tumors

Tavares N.T.[1,2], Lourenço C.[1,2,3], Constâncio V.[1,2], Silva-Santos R.[4], Braga I.[1,5], Henrique R.[1,4,6], Bagrodia A.[7], Jerónimo C.[1,6], Lobo J.[1,4,6]

[1]Cancer Biology and Epigenetics Group, Research Center of IPO Porto CI-IPOP/RISE@CI-IPOP Health Research Network- Portuguese Oncology Institute of Porto IPO Porto/Porto Comprehensive Cancer Center Raquel Seruca Porto.CCC Raquel Seruca- 4200-072 Porto- Portugal.
[2]Doctoral Programme in Biomedical Sciences, School of Medicine and Biomedical Sciences- University of Porto (ICBAS-UP), 4050-313 Porto- Portugal.
[3]i3S-Instituto de Investigação e Inovação em Saúde- Universidade do Porto- 4200-135 Porto- Portugal, Instituto Nacional de Engenharia Biomédica, Portugal, Portugal.
[4]Department of Pathology, Portuguese Oncology Institute of Porto (IPO Porto) 4200-072 Porto- Portugal
[5]Department of Urology, Portuguese Oncology Institute of Porto IPO Porto- 4200-072 Porto- Portugal
[6]Department of Pathology and Molecular Immunology, School of Medicine & Biomedical Sciences - University of Porto (ICBAS-UP), 4050-313 Porto- Portugal
[7]Department of Urology, University of California San Diego, San Diego – CA – USA

Testicular germ cell tumors (TGCT) are the most common tumors in young-adult men. Extracellular vesicles (EVs) are membrane-derived particles with vital roles in cell communication, and EV-derived microRNAs (miRNAs) have been considered potential cancer biomarkers. For TGCT there is already a well-identified miRNA biomarker, miR-371a-3p. It is part of a germ-cell specific cluster, miR-371-373 cluster, and has been tested in different clinical settings with high specificity and sensitivity. Despite this, the cluster cell secretion dynamics are still widely unknown and it was scarcely tested in EV-derived setting. Thus, we aimed to isolate EVs from different TGCT sample types, and test the presence and levels of the 3 cluster miRNAs.
A panel of 9 TGCT cell lines, 12 patient-derived tissue explants conditioned media and matched plasmas representative of different histological types were used. Conditioned media were retrieved after cells and tissues were cultured in medium. Plasma samples were processed routinely in our Biobank. EVs were isolated by a differential ultracentrifugation protocol, divided into two populations (large EVs (lEV) and small EVs (sEV)), and characterized according to MISEV guidelines. EV-RNA was extracted and miR-371a-3p, 372-3p and 373-3p were tested by RT-qPCR for all sample types.
The cell lines results revealed a pattern of EV-miRNA levels similar to the cells intrinsic RNA, corroborating our hypothesis that these miRNAs are secreted into EVs. Additionally, we were able to isolate EVs from TGCT fresh tissue (tumor and normal) conditioned media, showing a release of a significantly higher number of particles into the medium for the tumors when compared to normal tissue, both for lEV and sEV.

So, after being able to validate the release of miR-371a-3p in EVs from TGCT cell lines, we are optimizing the measurement of miR-371-373 in EVs by RT-qPCR for clinical samples, in order to validate our findings in relevant patient samples.

P4. Expression of miR-371a-3p is slightly increased in serum of pregnant women

Klemke M.[1, 2], Hubert M.[1], Ragosch V.[3], Hansen B.[4], Belge G.[1], Dieckmann K.-P.[4]

[1]*Faculty of Biology and Chemistry, University of Bremen, Bremen, Germany*
[2]*mir|detect GmbH, Bremerhaven, Germany*
[3]*Maternity Department, Asklepios Klinik Altona, Hamburg, Germany*
[4]*Department of Urology, Asklepios Klinik Altona, Hamburg, Germany*

MicroRNA 371a-3p is strongly expressed in embryonic stem cells where this miR is involved in pluripotency control. This miR is not found in adult cells but it is strongly expressed in germ cell tumours (GCTs). This finding is consistent with the known similarities between GCTs and embryogenesis regarding morphology and biochemical features (alpha fetoprotein, beta human chorionic gonadotropin). MicroRNA-371a-3p has also been detected in seminal plasma and in tissues of the seminal tract. We wondered if this miR will also be present in the serum of pregnant women who are still bearing the developing fetus in their body.

We analysed the expression of M371 in the serum of thirty-six pregnant women using the IVDR certified qPCR-based M371-Test using a cut-off of RQ=5. The median age of the patients is 34 years (range 25 – 42 years). All of the patients were in the third trimester of pregnancy with a median duration of pregnancy of 269 (range 220 – 283) days.

Thirty patients (83.3%) had slightly elevated miR-371a-3p serum levels with a median M371 expression of RQ=10.51 (IQR 6.18 – 19.40).

The expression of miR-371a-3p in the majority of pregnant women aligns with previous detection of this miR in the tissue of reproductive organs and it represents another analogy between GCTs and physiological features of human reproduction. Further investigations are required to explore the expression of this miR and related miRs from chromosome 19 during the course of pregnancy and thereafter and to look if this miR could represent a novel marker of pregnancy.

P5. BRD9 inhibition as potential treatment option for testicular germ cell tumors

Hansen A.[1], Funke K.[1], Arévalo L.[1], Fronhoffs F.[2], Sanders C.[2], Kristiansen G.[2], Schorle H.[1]

[1]*Department of Developmental Pathology, Institute of Pathology, University Hospital Bonn, Germany*
[2]*Institute of Pathology, University Hospital Bonn, Germany*

Testicular germ cell tumors (TGCT) are the predominant tumor in younger males between the ages of 15 and 35. Cisplatin-based treatment leads to curation rates of up to 95 %, but 15-20 % of patients with metastatic non-seminomas develop resistance to chemotherapy. In prostate cancer, glioblastoma and breast cancer interfering with the epigenetic landscape by inhibiting BET proteins (BRDT, BRD2, BRD3 and BRD4) was already shown to be effective. The bromodomain-containing protein 9 (BRD9) is an epigenetic reader and part of a chromatin remodelling complex modulating gene expression by recruiting transcription factors. BRD9 showed significantly increased protein levels in acute myeloid leukemia (AML) cells and high expression in malignant rhabdoid tumor (MRT) cells.
Here, we tested the effect of the BRD9 inhibitor I-BRD9 on TGCTs. First, expression of BRD9 was investigated by using the UCSC Xena browser showing only low expression in tumor tissue compared to normal testis tissue. Nevertheless, a tissue microarray (169 patient samples and 5 normal testis tissues) showed heterogeneous expression of BRD9 with highest expression levels in germ cell neoplasia in situ (GCNIS) and embryonal carcinomas. In TGCT cell lines BRD9 inhibition led to strong decrease in viability whereas the control cell lines (MPAF and FS1) showed only slight reduction of viability. FACS analysis revealed increased apoptosis and cell cycle arrest in G1-phase in TGCT cells treated with I-BRD9. Analysis of 3'mRNA-sequencing data showed prominent downregulation of the pluripotency network including NANOG, PRDM14 and KLF4 in TGCT cell lines already after 24 hours of I-BRD9 treatment. In addition, an upregulation of genes associated with epithelial differentiation was detected.
Further analyses are aimed at further understanding the effect of BRD9 inhibition on TGCT cell lines on a molecular level. The data suggest I-BRD9 as an effective treatment option for TGCTs.

P6. G-quadruplexes (G4s): a new potential target for the treatment of testicular germ cell tumors?

Islam R.[1], Hansen A.[1], Roque CSR.[2], Liesen A.[1], Paeschke K.[2], Schorle H.[1]

[1]Department of Developmental Pathology, Institute of Pathology, University Hospital Bonn, Bonn, Germany
[2]Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany

Testicular germ cell tumors (TGCTs) are the most common solid tumor in young men (aged 15 – 45 years). Despite being considered as one of the most curable cancers, TGCTs have several issues including long-term sequelae after chemotherapy, development of resistance (10 – 15%), tumor recurrence (20 – 30%) following initial therapy etc., suggesting the need to develop alternative therapeutic approaches with minimal adverse effects. Recent studies discovered that G-quadruplexes (G4s) could be a therapeutic target for cancer therapy. G4s are four-stranded secondary structures of DNA that form in guanine-rich sequences in the genome. G4s are predominantly found in promoters, enhancers, telomeres, and transcription factor binding sites of transcribed genes and impinge on replication, gene expression, epigenetics, genomic stability, telomere maintenance. It was demonstrated that G4 ligand-assisted formation and/or stabilization of G4s could be a therapeutic target for breast lung and pancreatic cancer. The role of G4s in germ cells and TGCTs has not yet been studied. Here we investigated G4 structures in TGCTs. First, the presence of G4s and the influence of G4 ligand PDS on the formation/stabilization of G4s in different TGCT cells were analyzed by BG4immunofluorescence and BG4-flow cytometry. Subsequently, an XTT cell viability assay revealed a significant dose- and time-dependent cytotoxic effect of various G4 ligands on different TGCT cells. Furthermore, G4 ligands induce apoptosis of TGCT cells. Next, RNA-seq and mass spectrometry will identify the molecular cascades triggered by G4 ligand- mediated stabilization of G4s. G4 mapping using CUT&Tag will be performed to explore the structure-function relationship of G4s in TGCT cells. These studies will not only lay the groundwork to understand the role of G4 structures in TCGTs but will further shed light on the efficacy of G4 ligands for TGCT treatment.

P7. Novel drug combinations for the treatment of refractory testicular germ cell tumors identified by multiplexed drug screening

Kucerova L.[1,2], Udvorkova N.[1,2], Majtanova K.[2], Jochova-Schmidtova S.[2], Kalavska K.[1], Mego M.[1]

[1]*Translational Research Unit of the 2nd Oncology Clinic, Faculty of Medicine, Comenius University, and of the National Cancer Institute, Bratislava Slovakia*
[2]*Department of Molecular Oncology, Cancer Research Institue, Biomedical Research Center, Bratislava, Slovakia*

Introduction
Testicular germ cell tumors (TGCTs) develop resistance to cisplatin treatment driven by multiple mechanisms. Therefore, we assessed antiproliferative efficacy of 22 different clinical stage inhibitors on 15 different cisplatin-resistant TGCT cell lines in order to identify novel strategies for clinical application in TGCTs patients.

Results and Discussion
We developed chemoresistant embryonal carcinoma (EC) model cell lines derived from established TERA2, NCCIT, NTERA2 and NEC8 cell lines. Moreover, we used previously derived platinum-resistant NCCIT, 2102Ep and TERA2 cells kindly and inherently resistant TERA1 cells provided as a gift from other laboratories. Seminoma TCAM2 and teratocarcinoma SuSa cell lines were also included. Following compounds were tested (target kinases indicated in brackets): Adavosertib (Wee1), Tazemetostat (EZH2), Selinexor (CRM1), RO4929097 (gamma-secretase, Notch, Aβ40), Ganetespib (HSP90), Simvastatin (HMG-CoA reductase), Entrectinib (TrkA, TrkB, TrkC, Ros1, ALK), Enasidenib (mutIDH2), Curcumin (Nrf2, Ferroptosis, HDAC), Roxadustad (HIF propylhydrolase), Cyclosporin A (calcineurin), Dasatinib (Abl, Src, c-Kit(D816V), c-Kit(wt)), Ruxolitinib (JAK1, JAK2), Copanlisib (PI3K αβγ),ABBV-744 (BDII), Devimistat (PDH, α-KGDH), Fenofibrate (PPAR-α, CZP2C19, CYP2B6), Vismodegib (Hedgehog), Crizotinib (ROS1,C-Met), Tozasertib (Aurora A, Aurora B, Aurora C, FLT3, Bcr-Abl), Galunisertib (TβRI), Plurodemstatbesylat (LSD1). We established 3D culture conditions to grow TGCT cells in spheroid structures and recapitulate the features of small avascular tumors. We confirmed that platinum-resistant variants retained the tumorigenicity or exhibited more aggressive tumor growth on immunodeficient NSG mouse model *in vivo*. In both types of models (3D spheroids and xenografts), cisplatin-resistant cell lines retained their refractoriness to cisplatin, confirming their suitability for preclinical testing.
We attempted to circumvent the sensitivity to cisplatin in the chemoresistant cells by using small molecule inhibitors of intracellular signaling either as a single agent or in combination with cisplatin. We identified high antiproliferative activity of Ganetespib, HSP90 inhibitor, exhibiting IC50 at a concentration of 10 nM – 100 nM in monotherapy. Similarly, we identified potent antiproliferative activity of Adavosertib, WEE1 inhibitor, exhibiting IC50 at a concentration of 110 nM – 330 nM in monotherapy. Selinexor as a selective inhibitor of nuclear export, that inhibits exportin-1 protein (XPO1), was also

effective at nanomolar concentrations. Compounds with IC50 below 1μM in cisplatin-resistant TGCT cells (adavosertib, Selinexor, ganetespib, simvastatin, entrectinib, cyclosporin A, dasatinib, copanlisib, ABBV-744 and tozasertib) were tested for synergistic effect in combination with cisplatin. The data confirmed synergy for ganetespib in cisplatin-resistant EC cell lines NT2, NCCIT, 2102Ep and NEC8. Interestingly, antagonistic effect was observed for ganetespib and cisplatin in seminoma TCAM2 cells and choriocarcinoma JEG3 cells. Adding cisplatin to adavosertib or selinexor did not improve the inhibitory effect. Other inhibitors did not show additive or synergistic effect with cisplatin on the panel of 15different platinum refractory TGCT cell lines. The data were validated by three independent methods of cytotoxicity measurement based on luminescence, live-cell kinetic imaging and kinetic label-free impedance platform. In the presentation we will discuss the method of multiplexing cytotoxicity measurements and present preclinical data from ongoing experiments assessing the effect of ganetespib monotherapy in TGCT xenograft mouse model.

Conclusion

New molecules emerge as potential agents to treat cisplatin-resistant germ cell tumors either as single treatment or in combination with cisplatin in synergistic fashion. Our broad panel of chemoresistant TGCT cell lines enables high-throughput screening of compounds that could rapidly enter clinical testing.

P8. Investigating novel treatment options: Inhibition of Cullin 1, 4 and 5 reduces viability of testicular germ cell tumour cell lines

Liesen A.[1], Hansen A.[1], Funke K.[1], Islam R.[1], Kristiansen G.[2], Schorle H.[1]

[1]*Department of Developmental Pathology, Institute of Pathology, University Hospital Bonn, Germany*
[2]*Institute of Pathology, University Hospital Bonn, Germany*

Testicular germ cell tumors (TGCT) are one of the most common tumors in young men. The treatment consists of surgery and chemotherapy. However, in 10-30% of patients diagnosed with metastatic non-seminoma, survival rates are low due to cisplatin resistance, so new treatment approaches are needed. Recently, our lab applied a CRISPR-activation screen in TGCT cell lines and identified neddylation to contribute to cisplatin resistance. We further showed that the NAE1 inhibitor MLN4924 effectively reduced the viability of TGCT cells and re-sensitzed them to cisplatin treatment.
Neddylation contributes to the regulation of protein content in the cell by binding the ubiquitin-like NEDD8 to target proteins, which impinges on their stability, function or cellular localization. During neddylation, NEDD8 is activated by NAE1 (NEDD8-activating enzyme) and covalently bound to a substrate via a three-step enzymatic cascade. Since the substrates of neddylation are non-Cullins and Cullins. Inhibition of selected Cullin ring ligases (CRLs) blocks only parts of the Neddylation cascade, opening the possibility of reduced side effects compared to the inhibition of the complete Neddylation by blocking NAE1 by MLN4924.
A meta-analysis of published expression datasets revealed, that in TGCT tissue and cell lines aside of Cullin 3, Cullin 1,2 and 4-7 are expressed. Next, we tested the effect of Cullin inhibitors using an XTT viability assay on embryonal carcinoma cell lines (2102EP, NCCIT, NT2/D1), a choriocarcinoma cell line (JAR), and a seminoma cell line (TCam2). We show that inhibition of Cullin4A & 4B using the inhibitor 33-11 and inhibition of Cullin1 & 5 using Gossypol displayed the strongest cytotoxic effect on TGCT cells.
Additionally, a combination of Gossypol and cisplatin resensitized cisplatin-resistant (NCCIT-R, NT2/D1-R) cells to cisplatin. Moreover, non-resistant TGCT cells (2102EP, NCCIT, NT2/D1, JAR and TCam2) sensitivity to cisplatin increased when treated with Gossypol. The combination of 33-11 and cisplatin resulted in an increased decrease in viability to TGCT cell lines (2102EP, JAR and TCam2). The data suggest that inhibition of Cullin4A & 4B using 33-1 and Cullin1 and 5 using Gossypol appears promising. It suggests that the effect inhibition using MLN4924 was primarily caused by inhibition of Cullin1, 4A, 4B and 5. Proteome analyses will shed light on the molecular cascades deregulated in germ cell tumor cell lines vs controls and will contribute to further understanding of germ cell tumor biology.

P9. Sacituzumab Govitecan: A Promising Antibody-Drug Conjugate to Treat Recurrent Refractory Testicular Germ Cell Tumors.

Udvorková N.[1,2], Kučerová L.[1,3], Mego M.[1,3]

[1]*Cancer Research Institute, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava*
[2]*Faculty of Medicine, Comenius University, Bratislava*
[3]*Translational Research Unit, National Cancer Institute and the 2nd Oncology Clinic of Medical Faculty, Comenius University, Bratislava*

Testicular germ cell tumors are highly curable with cisplatin-based chemotherapy but present significant problems when they becomes refractory to standard therapy. Recent advances in oncology have introduced antibody-drug conjugates (ADCs) as a therapeutic option for patients which are unresponsive to standard therapy. ADCs combine the specificity of monoclonal antibodies with the potent cytotoxic effects of chemotherapeutic agents, enabling targeted delivery directly to cancer cells. One of the ADCs approved by the FDA for the treatment of solid tumors is Sacituzumab govitecan. This conjugate targets the tumor cell surface marker TROP-2. In our work, we detected this surface marker on several types of GCT (germ cell tumors) cell lines, and pilot studies demonstrate cytotoxic and antitumor effects. We also observed a cytotoxic effect of this ADC on a GCT cell line that does not express the target marker. In this particular case, we are also investigating the possible mechanism of action. Further research and clinical validation are needed to fully establish the efficacy and safety of ADCs in this context, but initial findings are encouraging and suggest that the Sacituzumab govitecan ADC could become an important part of the therapeutic arsenal against recurrent refractory testicular cancer.

P10. The role of public health nurses, who are the pioneers of health education, in increasing the rate of men who perform testicular self-examination

Korkmaz M.

Yozgat Bozok University, Yozgat, Turkey

Problem Definition and Objective: Testicular cancer (TC), the most common cancer in young adults, causes morbidity and mortality. The aim of this review was to systematically analyse the studies conducted in the last 10-14 years on the level of knowledge about TC and TSE, sources of information on these topics, status and frequency of TSE performing, TC health belief levels and fatalistic tendencies of adult men living in Turkey.

Material-Methods: Review method was used in this study. Between 2010 and 2024, studies on attitudes towards TC, knowledge level, health belief models and fatalism tendency in Turkey were reviewed.

Results: In studies conducted in Turkey 10-15 years ago, it was found that 88% – 93.8% of men had not heard of TC, 79.8% did not know TSE, 9% of those who knew about TC and TSE accessed this information from the internet, social media and friends, 12% – 17.7% of men performed TSE, and only 1% of men regularly performed TSE once a month. When the TC screening health beliefs of men at that time were evaluated, the score of sub-dimensions ranges from 9,36 ± 2. 68 (min: 3 - max: 15) to 20,69 ± 6.94 (min: 7 - max: 35). The current data in Turkey show that 54.5% of men do not know TC, 66.2% of men do not know TSE, 10% of those who know about TC and TSE access this information from the internet, social media and friends, 23.5% of men perform TSE, only 1.3% of men regularly perform TSE once a month. TC health beliefs of men were evaluated, the score of sub-imensions ranges from 8,96±3,28 (min: 3 - max: 15) to 19,31±7,05 (min: 7 - max: 35. Men's TC health beliefs are at moderate level. Testicular cancer fatalism tendency of men is at moderate level (67,61±11,35 (min: 24 - max: 96)).

Conclusıon: Although there are positive developments in TC knowledge, TSE knowledge and testicular cancer self-examination, it is not at the desired level compared to 10% – 14 years ago when testicular cancer data in Turkey are analysed. Public health nurses, who are pioneers in health education, should inform young men about testicular cancer, teach men how to prevent testicular cancer, and encourage repeated testicular self-examination and behavior as part of their educational role.

Keyword: Testicular cancer, Testicular cancer self-examination, Testicular cancer screening health beliefs, Fatalizm tendency.