Malignant Germ Cell International Consortium

2023 Annual Meeting Rally Foundation Abstracts

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Deciphering molecular and (epi-)genetic mechanisms and kinetics of yolk-sac tumor formation from embryonal carcinoma

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Introduction: Yolk-sac tumors (YST), a subtype of germ cell tumors (GCT), particularly affect young adults aged 14-45 years. Relapsing GCT patients often develop therapy-resistant YST components, suggesting that formation of YST represents an escape mechanism under therapy. Despite the clinical need, the molecular mechanisms triggering YST development remain widely unexplored.

Methods: To identify the molecular factors driving the differentiation of embryonal carcinomas (EC) to YST, high-throughput data of the transcriptome (including microRNAs) and DNA methylome of EC and YST tissues was analysed. Results were validated in GCT cell lines and YST tissues by qRT-qPCR analyses and IHC stainings using >300 YST containing GCT. By mimicking conditions driving human embryonic stem cells into endodermal yolk-sac-like cells, EC cells were driven into YST-like (YSTL) cells by forced overexpression of the YST associated transcription factor SOX17 using the CRISPR/dCas9-SAM system in combination with stimulation of various signalling pathways. The molecular signature of YSTL cells was further deciphered on single cell level by sequencing.

Results: The pioneer and differentiation factors FOXA2 and SOX17 were identified as putative key drivers of YST development, inducing the expression of YST-associated genes, like *AFP*, *GPC3*, *GATA3/4/6* and *GPC3*. Additionally, during YST formation, the BMP, WNT, and MAPK signalling pathways play a major role. Thus, in EC cells, SOX17 induction in combination with a temporal orchestrated stimulation of ActivinA, FGF and WNT signalling, induced YST formation. Single cell sequencing identified the molecular profiles of early, intermediate and mature YSTL cells during differentiation and further detected that mature YSTL cells acquired a cisplatin-resistance phenotype, which we confirmed by demonstrating a reduced apoptosis induction in cisplatin treated YSTL compared to the parental cells.

Conclusion: This study highlighted FOXA2 and SOX17 as key factors of YST development and deciphered the molecular signaling processes of YST development in detail. Cisplatin resistance seems to be concomitant along YST development depicting an escape mechanism of YST formation upon standard therapy. Furthermore, our findings allow to deduce novel YST-specific therapy options by targeting YSTL cells directly or preventing differentiation of EC into therapy-resistant aggressive YST.

2

An exploration of experiences of survivors of testicular cancer experiencing ejaculatory dysfunction following retroperitoneal lymph node dissection – a sub-study of the PREPARE clinical trial.

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Background: Ejaculatory dysfunction is a complication of retroperitoneal lymph node dissection (RPLND) in testicular cancer survivors. Whilst common immediately after surgery, the prevalence of persistent symptoms, and impact on health-related quality-of-life (HRQoL) has not been reported. We aimed to explore testicular cancer survivors' experiences of ejaculatory dysfunction following RPLND.

Methods: In a sub-study of a single-arm phase 2 clinical trial, PREPARE (ACTRN12622000542796), we invited participants reporting ejaculatory dysfunction at least 6 months following RPLND to participate in optional semi-structured interviews. Purposive sampling was used to sample from a range of participant characteristics. Interviews continued until thematic saturation was reached. Codebook thematic analysis was performed.

Results: 15 participants participated in an optional interview; median age 34 years (range 24-66), median time from surgery 41 months (range 17-113), 12/15 (80%) in a long-term relationship.
We identified three overarching themes. The first identified the worth of RPLND and being alive despite development of ejaculatory dysfunction. The second illuminated the impact of ejaculatory dysfunction as closely related to the individual's stage of life. These factors influenced the impact of ejaculatory dysfunction across five areas: fertility, sex, information needs, communication, and psychological wellbeing (including masculinity and body image). The third reflected information needs.

Fertility was a substantial source of stress and concern for younger participants. Ejaculatory dysfunction had no effect on sex for some participants while for others, sex was less pleasurable and was rarely reported to cause pain. A few participants reported ejaculatory dysfunction as challenging their masculinity, confidence, and self-esteem. Participants wanted information about ejaculatory dysfunction to be included in early conversations regarding RPLND.

Conclusions: Most participants considered ejaculatory dysfunction to have little impact on their sexual function and intimate relationships. However, for those who reported difficulties, these varied depending on age, stage of life and relationship status. Future research should examine interventions to reduce distress related to fertility, challenged masculinity and body image.

3

Transcriptomics and mouse model of Sacrococcygeal Teratomas

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Background: Sacrococcygeal teratomas (SCTs) are the most common tumor in newborns, have significant perinatal morbidity and mortality, a 35% rate of recurrence, and lack good biomarkers to predict recurrence risk. SCTs are believed to originate from apoptosis-resistant embryonic gamete-precursors known as primordial germ cells (PGCs).

Aims: We seek to understand the unique tumor environment of SCTs and to model the formation of these tumors *in vivo* using mouse models.

Methods: We performed whole-genome sequencing and bulk RNA-sequencing of 7 banked SCT specimens and matched uninvolved skin from each patient. We performed single nuclear RNA sequencing (snRNA-seq) and spatial transcriptomics of SCT tissues to better understand their heterogenous cell type composition. We also developed an *in utero* transplant model to inject PGCs into the sacrum of mouse embryos to model the growth of SCTs.

Results: SCT sequencing revealed few high confidence somatic mutations, no recurrent somatic mutations, and no evidence of chromosome amplifications unique to SCT tissue in any patient. With snRNA-seq and spatial transcriptomics we found that the tumor is made up of populations of fibroblasts, macrophages, T-lymphocytes, and endothelial cells, with an overrepresentation of several ligand-receptor pairs, including those involved in EGF signaling, which has been implicated in PGC maintenance. Our *in utero* model revealed excellent survival of PGCs transplanted to the sacrum of E13.5 mouse embryos, with continued expression of pluripotency markers.

Conclusions: These studies are the first to identify the important tumor microenvironment that occurs with SCT maintenance. The specific ligand/receptor interactions we found in the scRNA-seq dataset can also be modulated to study the tumorigenic potential of *in utero* transplanted PGCs. Improved understanding of the molecular underpinnings of SCTs can lead to improved therapies in patients with rapidly growing fetal tumors and for predicting recurrences.

4

Outcome of Children with Extracranial Malignant Germ Cell Tumors by Response Status at the End of Induction Chemotherapy: A Report from the Children's Oncology Group

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Background: The management of pediatric malignant germ cell tumors (MGCTs) commonly employs 2-3 additional cycles of cisplatin, etoposide and bleomycin (PEb) given as consolidation therapy if a complete response (CR) is not achieved at the end of induction. However, there is no evidence supporting the addition of a consolidation phase in pediatric patients with MGCTs.

Methods: We retrospectively reviewed all patients enrolled in a phase III, single-arm trial for low-risk and intermediate-risk MGCTs (AGCT0132). All patients received 3 cycles of PEb
and underwent response assessment at the end of induction. Patients not in CR were prescribed 3 additional cycles of PEb as consolidation. We compared event-free survival (EFS) and overall survival (OS) for patients who did and did not receive consolidation.

Results: Among 210 patients enrolled, 193 patients had CR after 3 cycles of induction chemotherapy, and their post-induction 4y-EFS and OS was 93% and 99%. Fifteen patients were not in CR at the end of the first 3 cycles and received additional chemotherapy, and their 4y-EFS and OS was 51% and 60%.

Conclusion: Children with MGCTs who have less than CR after the first 3 cycles of chemotherapy had an inferior outcome compared to those with a CR, despite receiving additional cycles of PEb chemotherapy. We conclude that consolidation is of unclear benefit. Although our results are limited by small sample size and lack of comparator, we suggest that pediatric MGCT patients who fail to achieve a CR after standard induction chemotherapy should receive a salvage regimen with different agents rather than consolidation with more cycles of the same chemotherapy.

5

Toward Recapitulating Carcinogenesis of Type II Germ Cell Tumors in Cell Culture Model

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Background: Carcinogenesis of Type II testicular cancers initiates before birth and is affected by both genetic predisposition factors and environmental risk factors, whose mechanistic roles need to be experimentally demonstrated.

Aims: To establish and characterize novel testicular cancer cell lines and patient-derived iPSCs, from which long-term culture primordial germ cell-like cells (PGCLCs) are generated.

Methods: Cancer cell lines and Sertoli cells were established from surgically excised testicular cancer specimens. Patient-derived iPSCs were derivated from Sertoli cells or peripheral blood mononucleated cells using a footprint-free method.

Results and Conclusion:
[MGH-T548 Embryonal Carcinoma (EC) Set] A novel EC cell line derived from a mixed tumor (MGH-T548: ~90% EC and ~10% yolk sac tumor) has a pseudo-triploid genome with four isochromosome 12p and amplified wild-type c-KIT. A short region around BAK1 remains diploid in a largely triploid background. A hypomorph CHEK2 is present as LOH in T548-EC and heterozygously in T548-iPSCs. T548-ECs grow in the mTeSR serum-free medium, and their TRA-1-60+ stem cells produce both stem and differentiated cell populations in vitro, recapitulating the mixed tumor histology in the CDX model. T548-PGCLCs grow in our novel, chemically defined PGCLC medium with initial signs of apoptosis resistance.

[MGH-T836 Seminoma (SEM) Set] A novel SEM cell line derived from the MGH-T836 pure seminoma has a pseudo-triploid genome with three isochromosome 12p and amplified c-KIT loci harboring an imatinib-resistant gain-of-function mutation. T836-iPSCs were generated from the patient's blood and subjected to PGCLC derivation. T836-SEM can grow only in our novel seminoma maintenance medium. Expression of CD24 and CD38 classifies T836-SEMs into four subpopulations, which are largely inter-convertible except for the terminal CD24+/CD38- cells whose c-KIT amplification is lost.

[Conclusion] We have established two novel cell lines – including a novel SEM line – that harbor known genetics characteristics of testicular cancers. The heterogeneous aspects of these cell lines suggest the existence of intra-tumor subpopulations of testicular cancer cells that are functionally distinct but potentially inter-convertible. These cancer cell lines, along with the associating patients-derived iPSCs and PGCLCs, would be useful for functional assessments of genetic and environmental risk factors of Type II testicular germ cell tumors.

6

A Phase II Trial of Durvalumab and Tremelimumab for Relapsed/Refractory Germ Cell Tumors.

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Background: Patients (pts) with GCT and progressive disease (PD) after salvage high-dose chemotherapy (HDCT) have dismal outcomes with median progression free survival (PFS) and overall survival (OS) of 1.0 and 4.7 months (mo), respectively (Feldman et al. Cancer 2012). Immune checkpoint blockade (ICB) has resulted in durable benefit in multiple tumor types. We tested D (anti-PD-L1) + T (anti-CTLA-4) for pts with relapsed/refractory GCTs in a phase II trial (NCT03158064).

Methods: Eligible pts were ≥ 18 years old with GCT of any primary site who had PD after first-line chemotherapy and HDCT or were not candidates for HDCT or other curative options. Pts received D (1500 mg) and T (75 mg) every 4 weeks (wks) for 4 cycles followed by D (1500 mg) every 4 wks for up to 1 year (schedule 1). The study was later amended to one cycle of D (1500 mg) and T (300 mg) followed by D (1500 mg) every 4 wks for up to 1 year (schedule 2). The primary endpoint was initially overall response rate (ORR) in a Simon's two-stage design with null and alternate rates of 5% and 20%, respectively. The study was then amended to use a primary endpoint of 16-wk PFS in a new Simon's two-stage design with null and alternate rates of 5% and 25%. The regimen would be considered promising if 16-wk PFS was achieved in ≥ 3 of 20 pts. Secondary endpoints included PFS, OS, safety, and association of PD-L1 staining with outcome.

Results: Between July 2017 and Jan 2022, 29 pts were treated, the last 7 (24%) on schedule 2. Median age was 37 (range, 21-70). Histology was non-seminoma in 25 pts (86%), and primary sites were testis in 20 (69%), mediastinum in 7 (24%), ovary in 1 (3%), and unknown in 1 (3%). Six (21%) pts had late relapse and 21 (72%) received prior HDCT. Median AFP was 310 ng/mL (range, 1–18,087) and HCG 471 mIU/mL (range, 3.3-62,302). Ten pts were treated with the ORR primary endpoint with no responses in stage 1, but 2 pts had tumor reductions of 20% and 22% with PFS of 9.9 and 10.4 mo, respectively, and 1 patient with elevated AFP but no RECIST measurable disease had a PFS of 5.7 mo. Of 19 pts treated with the 16-wk PFS primary endpoint, one had an ongoing partial response (59% tumor reduction) with 33 months follow-up; all others had PD in <16 wks (17 with PD and 1 with stable disease). Thus, the study did not meet its primary endpoint. Among 29 treated pts, the 16-wk PFS was 13.8% (range, 4.3-28.6), and median PFS and OS were 1.4 (95% CI: 4.1, 7.1) and 7.3 mo (95% CI: 3.2, 10.9), respectively. PD-L1 expression data was available for 20/29 pts - 3/13 (23%) with low/negative and 1/7 (14%) with high expression achieved PFS >16 wks. Six (21%) pts had grade 3-4 treatment-related adverse events, 3 (10%) of whom required high-dose steroids.

Conclusions: D+T had limited antitumor activity in pts with relapsed/refractory GCT, failing to meet protocol-defined success. However, some pts did appear to benefit, including one exceptional responder. Additional correlative studies are ongoing.
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Analysis of a mouse germ cell tumor model establishes pluripotency-associated miRNAs (mouse miR-290-295/human miR-371-373) as conserved serum biomarkers for germ cell cancer detection.

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Background: Malignant testicular germ cells tumors (TGCTs) are the most common solid cancers in young men. Current diagnostics for TGCTs include conventional serum protein markers, but these lack the sensitivity and specificity needed to serve as accurate markers of malignancy across all histologic TGCT subtypes. microRNAs (miRNAs) are small non-coding regulatory RNAs and can be informative as biomarkers of many different diseases. In humans, miRNAs of the miR-371-373 cluster are detectable in the serum of patients with malignant TGCTs and outperform existing serum protein markers for both initial diagnosis as well as disease monitoring.

Aims: The goal of this study was to investigate the evolutionary conservation of the production and release pluripotency-associated miRNAs as specific biomarkers of TGCTs, in part to set the stage for functional studies that are possible only in model organisms.

Methods: We previously developed a genetically engineered mouse model featuring malignant mixed TGCTs consisting of pluripotent embryonal carcinoma (EC) and differentiated teratoma. Like the corresponding human malignancies, these murine cancers originate during embryonic development and are highly sensitive to genotoxic chemotherapy. We analyzed the expression of miRNAs in the mouse miR-290-295 cluster, homologs of the human miR-371-373 cluster, in cultured murine EC cells and extracellular vesicles produced by them, as well as in mouse serum and tissues.

Results: miR-290-295 miRNAs were detectable in the serum of mice with malignant TGCTs but not in serum from mice with benign teratomas or tumor-free control mice. miR-291-293 were expressed and secreted within extracellular vesicles specifically by pluripotent EC cells, and expression was lost following differentiation induced by the drug thioridazine. Notably, miR-291-293 levels were significantly higher in the serum of pregnant dams carrying tumor-bearing fetuses compared to that of control dams.

Conclusions: These findings reveal that expression of the miR-290-295 cluster in mice and the miR-371-373 cluster in humans is a conserved feature of malignant TGCTs, further validating the mouse model as representative of the human disease. These data also suggest that serum miR-371-373 assays may be able to detect the presence of TGCTs in humans before clinical signs of the disease arise, possibly even prenatally.

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Testicular Germ Cell Tumour Cells Release MicroRNA-containing Extracellular Vesicles Resulting in Promalignant Changes in Cell of the Tumour Microenvironment.

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Background and Aims: MicroRNAs (miRNAs/miR-) are short, non-protein coding RNAs that are dysregulated in malignant germ cell tumours (GCTs), with universal over-expression of miR-371~373 and miR-302/367 clusters regardless of patient age, tumour site, or subtype (seminoma/yolk-sac-tumour/embryonal carcinoma). These miRNAs are released into the bloodstream, presumed via extracellular vesicles (EVs), and represent promising biomarkers. Here, we comprehensively examined the role of EVs, and their miRNA cargo, on (fibroblast/endothelial/macrophage) cells representative of the testicular GCT (TGCT) tumour microenvironment (TME).

Methods: Small RNA next generation sequencing was performed on 34 samples, comprising representative malignant GCT cell lines/EVs and controls [testis fibroblast (Hs1.Tes) cell-line/EVs and testis/ovary samples]. TME cells received TGCT-derived EV treatment, miRNA quantification by qRT-PCR, and a miRNA overexpression system (miR-371a-OE) to perform functional assays and assess mRNA changes.

Results: TGCT cells secreted EVs into culture media. MiR-371~373 and miR-302/367 cluster miRNAs were over-expressed in all TGCT cells/subtypes compared with control cells and were highly abundant in TGCT-derived EVs, with miR-371a-3p/miR-371a-5p the most abundant compared with normal EVs. Fluorescent labelling demonstrated TGCT-derived EVs were internalised by all TME cells. TME (fibroblast/endothelial) cell treatment with EVs derived from different TGCT subtypes resulted in increased miR-371~373 and miR-302/367 miRNA levels, and other generic (e.g., miR-205-5p/miR-148-3p), and subtype-specific (seminoma, e.g., miR-203a-3p; yolk-sac-tumour, e.g., miR-375-3p) miRNAs. MiR-371a-OE in TME cells resulted in increased collagen contraction (fibroblasts) and angiogenesis (endothelial cells), associated with dysregulation of mRNAs and relevant cellular pathways.

Conclusions: TGCT cells communicate with non-tumour stromal TME cells through release of EVs enriched in oncogenic miRNAs, likely contributing to tumour progression.

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Unraveling the Testicular Seminoma Tumor Microenvironment by Single-Cell RNA-sequencing

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Background/Aim: Seminomas is a malignant germ cell tumor (GCT) affecting male adults and is characterized by low somatic mutation burden, histological homogeneity, and high survival rate. It is currently unknown what drives tumorigenesis of this curable testicular cancer. Tumor/immune microenvironment (TME) has a vital role in cancer survival and progression with implications in cell death resistance and immune escape. Herein, we aimed to unravel the transcriptomic TME landscape of seminomas and better understand seminoma pathogenesis.

Methods: We performed single cell RNA-sequencing (scRNA-seq; 10X-Illumina platform) on surgically resected pure seminomas from four patients. A total of ~24,000 single cells were analyzed using Seurat/Bioconductor R packages and standard means for data visualization. Key findings were validated by in situ hybridization RNA analysis (RNAscope) on histological sections from these samples.

Results: T-cells and Natural-Killer cell populations were mostly predominantly enriched in this seminoma cohort, where tumor cells consisted of a distinct subpopulation with limited presence (1%). Other distinct cell populations were also identified for additional immune cell types e.g., B-cells, macrophages/monocytes and smooth muscle cells. ScRNA-seq revealed a unique set of signature marker genes (e.g., POU5F1, KHDC3L, DPPA5, NANOS3) highly expressed in seminoma cells which are associated with pivotal cellular processes e.g., developmental pluripotency and germ cell development. Notably, this gene set was also highly differentially expressed (DE) in The Cancer Genome Atlas (TGCA) seminomas (n=73) compared to TCGA non-seminomas (n=38) (FDR<0.01). Amongst top marker genes, KHDC3L, also higher expressed in seminomas compared to normal testis, was validated by RNA-scope. Notably, KHDC3L demonstrated the strongest positive correlation with DPPA5 (Spearman/Pearson; R2=0.8, p<0.0001), top #3 ranked DE gene as well as with other top 20 DE genes identified by scRNA-seq. Remarkably, similar strong correlations among other top DE genes were also found in the testicular TGCA cohort. Top DE genes including master transcription factors (e.g., POU5F1, NANOG, SOX4) are involved in the same protein pathway/networks likely suggesting synergic effect/involvement in the same regulatory circuitry.

Conclusions: Our analyses revealed novel marker genes, uniquely expressed in seminomas which consist of a homogeneous cell population across all 4 tumors analyzed, clearly distinct from other cell types identified.

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Assessing the risk to develop a growing teratoma syndrome based on molecular and epigenetic subtyping as well as novel (liquid) biomarkers

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Background: In germ cell tumors (GCT), a growing tumor during or after chemotherapy with decreased tumor markers is called the ´growing teratoma syndrome` (GTS) according to Logothetis et al. in 1982. Histologically, GTS consists of pure mature teratoma. To date, there is a lack of knowledge on its molecular pathogenesis and specific treatment options.

Aims: We aimed at updating the current understanding of GTS by molecular and epigenetic characterization. In parallel, we aimed at identifying (liquid) biomarkers for GTS.

Material & Methods: We retrospectively identified 50 GTS patients for clinical evaluation. 12 GTS tissue samples were analyzed on epigenetic (DNA methylation), transcriptional (microRNA) and proteome / secretome level. Additionally, a series of longitudinal GTS patient samples were included. Teratomas (TER) showing no features of a GTS served as controls.

Results: We calculated the tumor growth rates and stratified patients into a slow (< 0.5 cm / month), medium (0.5 - 1.5 cm / month) and rapid (> 1.5 cm / month) group. Within all subgroups, GTS patients presented mainly with metastatic disease at first diagnosis and prognosis was mostly favorable.
On a global level, the DNA methylation profiles were highly comparable between all groups, but we identified individual changes on single CpG dinucleotide level, which might serve as epigenetic biomarkers. On RNA level, we identified several microRNAs specifically expressed in the different GTS subgroups, putatively serving as liquid biomarkers. By analyzing the secretome, we identified further liquid biomarkers for the different GTS groups. A mass spectrometry-based proteome analysis revealed that proteins found enriched in all GTS groups compared to TER are mainly involved in processes like proliferation, DNA replication and the cell cycle, while proteins interacting with the immune system were depleted.

Conclusion: Our data highlights specific molecular features of GTS. Based on these findings and the tumor growth rate, GTS is clearly distinguishable from TER and is subtyped into three groups. Novel liquid biomarkers on epigenetic, transcriptional and secretome level might identify patients harboring a GTS and predict the dynamics of tumors progression. Hence, treatment and follow-up intervals can be adapted.

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Further refinement of the circulating miR-371a-3p assay for malignant germ cell tumour detection with confirmation of the utility of the indeterminate range

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Background/Aims: Circulating miR-371a-3p quantification is a highly sensitive approach to the detection/monitoring of malignant germ-cell-tumours (GCTs). We recently published an updated protocol using raw Cq miR-371a-3p values and identified an 'indeterminate' range in addition to positive/negative results, improving assay accuracy/performance from 0.84 to 0.92 (1). Here, we refined the protocol further, streamlining/optimising the test towards routine clinical use.

Methods: Serum from malignant GCT patients (n=18) and a control group of patients without cancer (n=60) was used, comprising three age-groups (0-11y/11-18y/18-45y; n=20/age-group) and male/female sex (n=10 each per age-group). Small RNA extractions were performed using 200µl serum and Qiagen serum/plasma miRNA extraction kits, as described (1,2). PCR-related procedures used Taqman miRNA assays/reagents (1,2).

Results: Results showed that the QC and diagnostic assay steps can be successfully incorporated into a single step, without loss of sensitivity. Additionally, whilst haemolysis assessment using miR-451a levels was previously recommended (2, 3), circulating miR-371a-3p *vs.* miR-451a levels were not correlated. Consequently, visual QC inspection to exclude samples with macroscopic haemolysis is sufficient. Importantly, we further confirmed the utility of the indeterminate range (1), with no positive results recorded amongst 60 control samples when the same indeterminate thresholds [i.e., raw miR-371a-3p Cq 28-35; (1)] were utilised. Additionally, following recommended single repeat of the test for samples originally falling in the indeterminate range (1), the number of reported indeterminate control samples fell from 13/60 (22%) to 2/60 (3%). Using this adapted protocol, we showed excellent concordance between the results obtained in Cambridge-UK and UTSW-US MaGIC research laboratories, with 100% (18/18 cases) and 92% (24/26) concordance in malignant GCT cases and controls, respectively. Of note, the two discrepant control cases were indeterminate at one site and negative at the other. Finally, we showed that there is no difference in miR-371a-3p levels by age or sex in controls.

Conclusions: The circulating miR-371a-3p assay has been further optimised, retaining excellent performance, with no difference in controls by age/sex and confirmation of the indeterminate range. Such further refinements have streamlined the miR-371a-3p assay for imminent, non-biased, universal clinical adoption.

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Development and implementation of a standard of care health-related quality of life assessment tool for patients with testicular germ cell tumors

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Background: Cure can be achieved for the majority of patients with testicular germ cell tumors (GCTs). However, this often requires treatment that despite being highly effective, can have short- and long-term effects on patients' health and quality of life. Routine use of validated patient reported outcome measures (PROMs) can make the assessment of health-related quality of life (HRQoL) more systematic, objective, and clinically actionable. We report our experience developing and implementing an electronically delivered HRQoL instrument into routine clinical practice at a high-volume referral center for GCTs.

Methods: The initial iteration of our survey instrument was developed and sourced from the EORTC Testicular Cancer (TC-26) validated questionnaire and included two additional questions about general health and quality of life from the EORTC QLQ-30. An institutional governance committee comprised of patient-centered language and survey methodology experts reviewed and edited the content which was then adapted for digital delivery to patients via our patient portal (MSKEngage). The frequency and timing of delivery were selected around clinically relevant timepoints: at the time of initial diagnosis, before and after treatments with either surgery or chemotherapy, and less often during long-term surveillance. All new and return patients being seen for the management of testicular GCTs were intended to receive the survey. Education was provided to all involved clinicians and administrative staff for awareness of the implementation effort and to promote clinical integration of survey results at each visit.

Results: As of August 9, 2023 and since initial implementation on July 25, 2022, 829 assessments have been requested from 567 unique patients seen by six GCT focused physicians (3 medical oncology, 3 surgery) at three different clinic sites. In total, 389 patients have completed 522 surveys for a patient-level and survey-level completion rate of 68% and 63%, respectively. The quarterly completion rate has increased from 46% at the time of initial roll out (Q3 2022) and has been sustained at roughly 65% for all of 2023. Median time to complete the survey for patients was 3 mins.

Conclusions: The development and implementation of an electronically delivered HRQoL assessment tool is feasible for patients with testicular GCT. Exploration of differences in HRQoL among patients with varying demographics, comorbid conditions, and those given different treatments (e.g. chemotherapy vs. primary RPLND for early stage disease) will provide insight into the short and long term consequences of diagnosis and treatment. Further investigation into the underlying reasons for missed surveys and how to best intervene on patients' responses is necessary.

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NANOG and POU5F as potential molecular markers for germinomas: unveiling distinct gene expression patterns in pediatric and adult germ cell tumors.

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Background: Germ cell tumors (GCTs) are a heterogeneous group of neoplasms that arise from primordial germ cells and manifest in both pediatric and adult populations. While these tumors share similarities across age groups, distinct clinical and histological differences warrant further investigation. Advancements in molecular profiling techniques have enabled researchers to delve deeper into the genetic landscape of GCTs, and transcriptomic analyses reveal unique gene expression patterns in pediatric and adult cases.

Aim: This study aimed to evaluate the expression of *NANOG*, *POU5F*, *GATA6*, *HNF4A*, *FOXA2*, and *ERBB4* in pediatric and adult patients with GCTs done with different sequencing methodologies.

Methods: For pediatric analyses, five samples were selected including three dysgerminomas and two yolk sac tumors. Total RNA was extracted from frozen tumor tissue of pediatric GCTs stored at Barretos Cancer Hospital Biobank with Qiasymphony, library prepared using cDNA PCR barcoding, and sequencing performed on MinION flow cell. Nanopore sequencing and data analyses were performed following standardized protocols developed by the University of North Carolina and St. Jude's researchers. The gene expression was normalized into a z-score and the heatmap was created using R Studio. For adult analyses, gene expression data from 149 patients were downloaded from the Testicular Germ Cell Tumors TCGA PanCancer study at cBioPortal.

Results: In the pediatric data, there was a clear separation of histological subtypes based on gene expression, where *NANOG* and *POU5F* are highly expressed in dysgerminomas while *GATA6*, *HNF4A*, *FOXA2*, and *ERBB4* are highly expressed in yolk sac tumors. In concordance, the cBioPortal cohort showed that the seminoma/embryonal carcinoma cluster also had a higher expression of *NANOG* and *POU5F*, although, the cluster with the higher expression of *GATA6*, *HNF4A*, *FOXA2*, and *ERBB4* could not specify a histological subtype.

Conclusion: Our results suggest that NANOG and POU5F could be molecular markers for germinomas. Furthermore, nanopore sequencing is a good technology for the classification of GCTs and could be used for the diagnosis of pediatric tumors since the Nanopore is cheaper, faster, and more friendly user compared to other platforms. Therefore, more studies with a higher number of pediatric samples are essential to validate these results.

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46, XY pure gonadal dysgenesis with gonadal non-dysgerminoma malignant germ cell tumors: a report of 15 cases from Gynecologic oncology of a national medical center

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Background: The incidence and survival outcomes of 46, XY pure gonadal dysgenesis (PGD) in gonadal non-dysgerminoma malignant germ cell tumors (MGCT-NDG) remain unclear due to the extreme rarity.

Aims: To evaluate the incidence, treatment, and survival outcomes of this population in a national medical center.

Methods: A retrospective study of MGCT-NDG patients with 46, XY PGD treated in our hospital between Jan. 2011 and Dec. 2022 were conducted, and their characteristics and outcomes were investigated.

Results: A total of 15 patients (4.9%) with 46, XY PGD were identified in 307 MGCT-NDG patients. The median age at MGCT diagnoses and 46, XY PDG were 16.0 (range: 7.0 -33.0) and 15.0 years (range: 8.0 -34.0), respectively. Six cases were preoperatively diagnosed as 46, XY PDG, of which 4 received bilateral gonadectomy with or without hysterectomy while the other 2 received cystectomy and unilateral gonadectomy with hysterectomy, respectively. Of the 9 patients postoperatively diagnosed as 46, XY PDG, unilateral gonadectomy, cystectomy, and unilateral gonadectomy with hysterectomy were performed in 6, 2, and 1 patients, respectively. Mixed MGCT (10/15,66.7%), yolk sac tumor (4/15, 26.6%), and immature teratoma (1/15, 6.7%) were the pathological subtypes, in the descending order. FIGO stage I, stage II, stage III, and stage IV were noted in 7(46.7%), 4 (26.7%), 3 (20.0%), and 1 (6.6%) patients, respectively. Eleven patients received reoperation for residual gonadectomy after a median delay of 6.0 months (range: 1.0 – 19.0), of which 8 (72.7%) had MGCT-NDG and 1 (9.1%) had gonadoblastoma. Eight patients (8/15,53.3%) experienced at least one relapse, with a median event-free survival was 9.0 months (range: 2.0 -37.0), of which 2 (2/8,25.0%) were treated with surgery only at initial treatment. All the relapsed patients received surgery and chemotherapy. After a median follow-up of 24.0 months (range:11.0-85.0), 10 were free of disease, 1 was alive with the disease, 3 died of the disease, and 1 died of leukemia.

Conclusion: The crude incidence rate of 46, XY PGD in patients with MGCT-NDG was about 5%, timely diagnosis and bilateral gonadectomy should be emphasized to lower the risk of reoperation and second carcinogenesis in this population.

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Association Between Glutathione S-transferases M1 and Treatment Outcome in Germ Cell Tumor Patients

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Background: GCTs is model of curable malignancy, however, small proportion of patients experience disease recurrence and die due to disease. Cisplatin based chemotherapy is mainstay in the treatment of GCTs. Glutathione S-transferases are polymorphic enzymes that catalyze the glutathione conjugation of alkylating agents, platinum compounds, and free radicals formed by chemotherapy and thus implicated in the development of treatment resistance. The aim of this study was to assess expression level of glutathione S-transferase M1 (GSTM1) and its association with treatment outcome in GCTs patients.

Methods: This translational study included tumor specimen from 207 newly diagnosed GCTs patients as well as cisplatin-sensitive GCT cell line xenografts and their resistant variants for all histological variants of GCTs. Expression of GSTM1 was detected by quantitatitve RT-PCR and immunohistochemistry using monoclonal antibodies, scored by the multiplicative quickscore (QS) method. GSTM1 expression was correlated with patients/tumor characteristics and treatment outcome. GSTM1 expression was dichotomized to "low" or "high" based on QS score.

Results: Mean ± standard error of mean (SEM) of GSTM1 expression in GCTs was 5.48+0.54. Highest expression was observed in seminoma (7.06±0.41), choriocarcinoma (6.42±1.04), embryonal carcinoma (3.60 ± 0.28), yolk sac tumor (3.56 ± 0.64) while lowest was in teratoma (1.16±0.51) ($p < 0.0001$). Mean ± SEM of GSTM1 expression in GCTs cell line xenografts was 7.60±4.34, with no difference between cisplatin-sensitive and their resistant variants ($p=0.91$). There was no association between GSTM1 expression in tumor tissue and patients/tumor characteristics. Low GSTM1expression was associated with significantly better relapse-free survival (RFS) compared to high GSTM1 (HR=0.50, 95%CI 0.23-1.09, $p=0.03$) but not overall survival (OS) (HR=0.61, 95% 0.24-1.54, $p=0.22$). Multivariate analysis showed that prognostic value of GSTM1 was independent from IGCCCG score.

Conclusion: Our study, for the first time showed that tumor expression of GSTM1 is prognostic in GCTs, with high GSTM1 expression associated with worse outcomes, suggesting that GSTM1 could be responsible, in part, for treatment resistance in GCTs.

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Management and survival outcomes in patients with malignant ovarian germ cell tumors: 10-year experience from Gynecologic oncology of a national medical center

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Background: Management and survival outcomes in malignant ovarian germ cell tumors (MOGCT) patients from China has not been well defined in large-scale cohorts.

Aims: To summarize the clinical characteristics, treatment, and prognosis in MOGCT patients from a Chinese national medical center.

Methods: A retrospective study of MOGCT patients treated in our hospital between Jan. 2013 and Jan. 2023 was conducted. The recurrence-free survival (RFS), disease-specific survival (DSS), and prognostic factors for MOGCT were evaluated.

Results: Overall, we included 321 patients from 28 provinces of China, of which 188 patients were referred to our hospital after initial surgery. The median age at diagnosis was 21 years (range: 1 – 73). Among them, 52 (16.2%), 97 (30.3%), 117 (36.4%), and 55 (17.1%) were dysgerminoma (DG), immature teratoma (IT), yolk sac tumor (YST), and mixed MOGCT, respectively. Most patients (233 cases, 69.5%) had FIGO stage I diseases at initial diagnosis, and 15 (4.7%), 67 (20.9%), and 16 (5.0%) patients had stage II, III, and IV diseases. Fertility-sparing surgery was performed in 278 (86.6%) patients and 285 (88.8%) patients received adjuvant chemotherapy at initial treatment, of which 90.5% were bleomycin, etoposide, and cisplatin regimens. Seven patients (2.2%) did not achieve complete remission after initial treatment, the other 266 (82.9%) patients were free of disease, and only 48 (15.0%) relapsed. Most patients (81.3%) received surgery with adjuvant chemotherapy after recurrence, but 9 of them still experienced at least second relapse, of which 4 died. The other 4 relapsed patients died even after salvage chemotherapy. The 1-year, 3-year, and 5-year RFS were 87.2%, 84.2%, and 83.8%, respectively. After a median follow-up of 4.9 years, the 1-year, 3-year, and 5-year DSS of 99.7%, 96.0%, and 94.0%, respectively. Survival analysis revealed that histological subtypes and FIGO stage (both $P < 0.05$) were the major prognostic factors. Patients with YST component or mixed MOGCT had significantly poor prognoses when compared with DG or IT patients.

Conclusion: Histological subtypes and FIGO stage were the major prognostic factors for MOGCT patients. The survival outcomes in MOGCT patients under standard management were excellent but less satisfactory in relapsed patients.

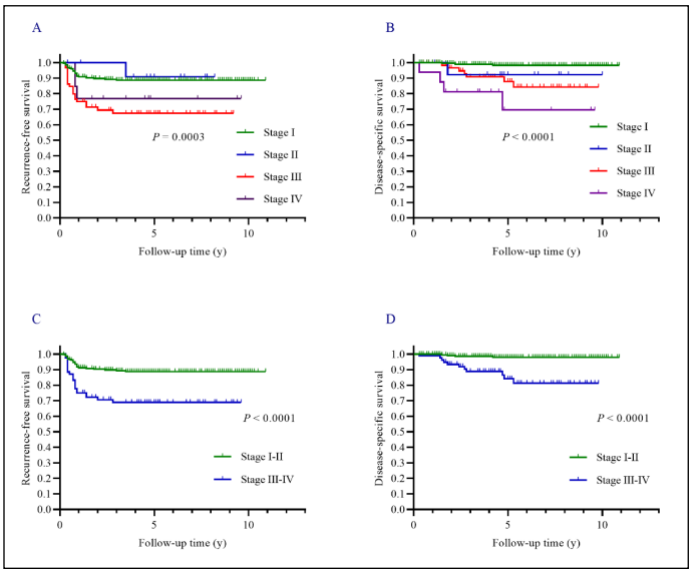

Figure 1. Survival outcomes of MOGCT patients in different FIGO stage.

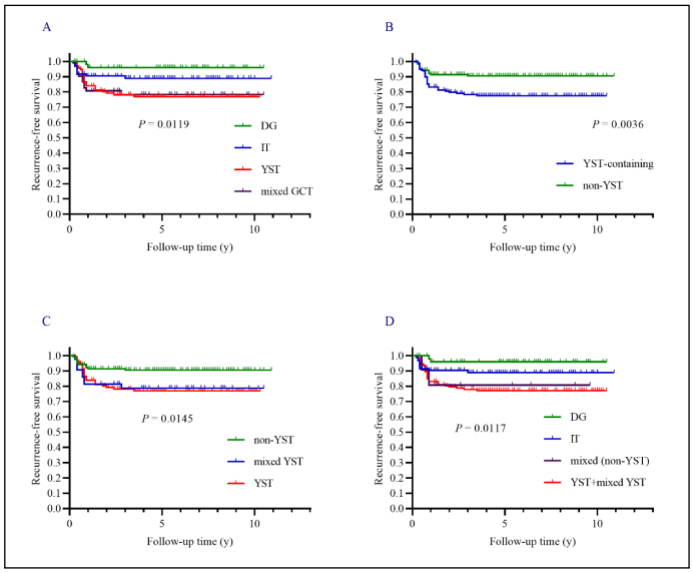

Figure 2. The RFS of MOGCT patients in different histological subtypes.

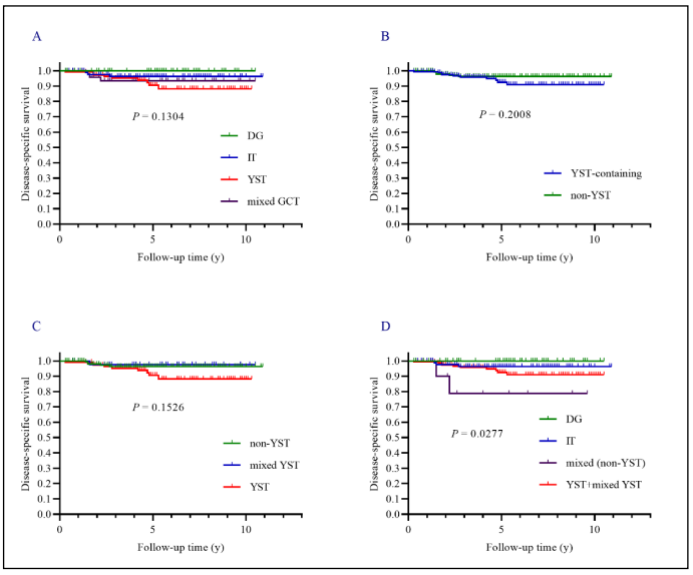

Figure 3. The DSS of MOGCT patients in different histological subtypes.

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Mitochondrial priming in human GCT cell lines is dependent on MCL1 and BCLX

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Background: Testicular germ cell tumours (TGCT) are the most common malignancy in young men, and are highly treatable by a combination of cisplatin, bleomycin, and etoposide (BEP). While the majority of patients survive, the life-long toxicity associated with treating young people with cytotoxic agents indicates a need for new therapeutic strategies. TGCTs are considered primed for apoptosis in response to DNA damage, though the mechanisms governing their hypersensitivity to BEP are not well understood.

Aims: To explore the mitochondrial priming in human GCT cell lines by pharmaceutically modulating the anti-apoptotic BCL2 family of proteins using BH3 mimetics.
Methods: Human GCT cell lines were treated with chemotherapeutic drugs in combination with broad-acting or selective BH3 mimetics. Viability was assessed using an MTT assay, with transcriptional analyses through qPCR and RNA-seq.

Results: Analysing RNA-seq. datasets, we characterised BCL2 family expression among 33 cancer types, identifying a unique signature of expression in TGCTs. Using inhibitors specific to the BCL2 family proteins, we then characterised mitochondrial priming in several GCT cell lines. We identify that the balance between cell death and survival in human GCT cell lines is tightly controlled by BCL2 proteins, and that their inhibition greatly potentiates cisplatin's effects. Upon further investigation, we show that simultaneously inhibiting MCL1 and BCLX causes synthetic lethality in all cell lines tested independent of their TP53 status, confirming their role as determinants of GCT cell line survival.

Conclusion: Our data indicate that the balance of BCL2 proteins ensures mitochondrial priming in GCT cell lines. A targeted strategy that employs BH3 mimetics in combination with conventional chemotherapeutic drugs could allow for lowering BEP dose, and may represent a new strategy for chemoresistant tumours.

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Advanced ovarian yolk sac tumor: Upfront surgery or neoadjuvant chemotherapy followed by interval debulking?

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Background: Yolk sac tumor (YST) accounts for about 20% of malignant ovarian germ cell tumors and is the second most common subtype. Approximately 30-40% patients are presented with advanced stage at initial diagnosis. Primary debulking surgery is a formidable challenge due to high tumor burden and vulnerable status of patients. The feasibility of neoadjuvant chemotherapy has been explored in advanced ovarian yolk sac tumor with promising results.

Aims: To compare the surgery and survival outcomes between neoadjuvant chemotherapy and primary debulking surgery in patients with advanced ovarian yolk sac tumor.

Methods: In this retrospective cohort analysis, patients with stage III to IV ovarian yolk sac tumor or mixed germ cell tumors containing yolk sac tumor elements and who underwent surgery at Peking Union Medical College Hospital between 2011 and 2021 were identified. Patient characteristics, treatment and survival data were analyzed between the two groups.

Results: A total of 40 patients were enrolled. Nineteen patients received neoadjuvant chemotherapy followed by interval surgery. Twenty-one patients were treated with primary debulking surgery. After neoadjuvant chemotherapy, patients experienced significant tumor shrinkage ($p = 0.006$), alpha-fetoprotein (AFP) level decrease ($p < 0.001$) and alleviation of massive ascites (100%). All the patients achieved optimal cytoreduction at interval surgery. No statistical difference was found in 3-year disease-free survival and overall survival between the neoadjuvant chemotherapy group and primary debulking surgery group (Log Rank $p=0.461$ and 0.935). Patients suffered less blood loss (328.4 vs. 1285.7 ml, $p = 0.029$), lower transfusion volume (1044.4 vs. 3066.7 ml, $p = 0.011$), shorter operation time (156.8 vs. 193.6 min, $p = 0.205$), and less peri-operative complications (15.8% vs. 47.6%, $p = 0.032$) at the interval debulking surgery after neoadjuvant chemotherapy, as compared to patients who underwent primary debulking surgery.

Conclusion: For patients with advanced-stage ovarian yolk sac tumor, neoadjuvant chemotherapy followed by interval surgery is an alternative option, especially for those who could not tolerate the primary debulking surgery because of the high tumor burden and vulnerable status.

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In vitro and in vivo models of cisplatin-resistant germ cell tumors: analysis of genes related to EMT and DNA repair

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Background: Germ cell tumors (GCTs) are benign or malignant neoplasms and the standard treatment is surgical resection and cisplatin-based chemotherapy. However, 15-20% of patients relapse due to resistance to this compound, through uncertain multifactorial mechanisms. Molecular mechanisms have been investigated in vitro and in vivo models to elucidate the resistance, including DNA repair and epithelial-mesenchymal transition (EMT), a biological process related to cancer progression, resistance, and metastasis. However, few studies have evaluated DNA repair and EMT in GCT in vitro and in vivo models. Thus, this study aimed to establish the xenograft model derived from cisplatin-resistant GCT cell lines and analyze EMT and DNA repair processes.

Methods: Cisplatin-resistant cells (NTERA-2R) were treated with incremental doses of cisplatin for 10 months until resistance phenotype. NTERA-2R or parental NTERA-2 (NTERA-2P) cells were injected subcutaneously in athymic mice (*Mus musculus*) and they were observed and weighed weekly. The tumors were collected when the volumes reached around 2000 mm3. Total RNA was extracted, quantified, and reverse transcribed into cDNA. Next, real-time PCR was performed to evaluate the genes *BRCA1*, *CDH1*, *VIMENTIN*, *CDH2*, and *GAPDH* (endogenous gene).

Results: The results showed that the resistant models had a higher expression of *VIMENTIN* and *CDH2* compared to the parental, indicating an alteration that favors the mesenchymal phenotype in relation to the EMT. Although not significant, there was a trend toward an increase in *BRCA1* and a decrease in *CDH1*. These data corroborate those found in vitro models.

Conclusion: Therefore, the DNA repair and EMT processes have an important role in cisplatin-resistant GCT. The establishment of the GCT in vivo model will open a field for future studies with chemotherapeutics, as well as contribute to the development of personalized medicine.

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Infantile Choriocarcinoma – A Rare and an Unusual Disease. Case Report and Review of the Literature

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Background: Primary infantile choriocarcinoma (CC) is an extremely rare disease. The disease is rapidly fatal if the syndrome is not recognized and untreated. An infantile CC is associated with poor prognosis and high mortality. Typical symptoms at diagnosis include severe anemia, failure to thrive, hepatomegaly, and seizures. Marked elevations of β-hCG are typical (often >1 × 106 IU/L).

Case report: We present a case study of a 21-month-old girl diagnosed with infantile CC, with high β-HCG, lung, brain and liver metastases. The child in the 4th week of life was admitted to the hospital due to severe anemia, lower activity and loss of appetite. Child, delivered by caesarean section due to COVID 19 infection in the mother, 37 hbd, body weight 2900 g, Apgar score 8/8 points.
Imaging showed metastases to both lungs (several solid lesions up to 12 mm in size), significant hepatomegaly with numerous heterogeneous lesions in the liver, a polycyclic lesion measuring 1.5 x 1.1 x 1.6 cm in the right frontal lobe. The level of β-HCG was 1 023 519.6 mIU/ml, max. 2 710 759 mIU/ml.
Infantile CC was diagnosed based on the clinical data, laboratory tests and imaging results. We used chemotherapy: 6 cycles of carboplatin and etoposide, achieving stabilization of the general condition and partial response to treatment. Due to the increasing level of β-HCG we changed chemotherapy to VIP (4 cycles), and then to MTX 7 mg/m^2, ACTD 30 ug/kg, CTX 300 mg/m^2, 3 cycles of VCR (2 mg/m^2), Doksorubicin (60 mg/m^2), Bleomycin (15 mg/m^2), Cisplatin (100 mg/m^2) – based on the article "Nonseminomatous Malignant Germ Cell Tumors in Children", F. Flamant et all. 1984. The child received 5 cycles achieving the correct β-HCG. The child had resection of the lesion in the 4th segment of the liver, greater omentum and thermoablation of the focus in the 7th segment. Now the child is in the remission from 6 months.
The review of the literature was prepared.

Conclusions: Infantile CC is a very chemosensitive tumor. Early diagnosis is the most important prognostic factor.
Sometimes delayed surgery can help to achieve remission.

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PD-1 inhibitor plus platinum-based chemotherapy in squamous carcinomatosis of mature teratoma with high tumor mutation burden: two cases from gynecological oncology from a national medical center

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Background: Cancer immunotherapy has been widely used and significantly improves survival outcomes in patients with specific tumors. However, the role of immunotherapy in squamous cell carcinoma arising in mature teratoma (SCC-MT) has rarely been reported.

Aims: To evaluate the safety and efficacy of PD-1 inhibitor plus platinum-based chemotherapy in SCC-MT patients with high tumor mutation burden (TMB).

Methods: We reported two cases of SCC-MT with high TMB treated with PD-1 inhibitor plus platinum-based chemotherapy. The clinical characteristics, treatment, and outcomes were investigated.

Results: Case 1 was 44-year-old women with FIGO stage IIB SCC-MT. The preoperative SCCAg level was 11.6ng/ml. Comprehensive radical staging was performed and next-generation sequencing (NGS) of tumor showed TMB of 10Mt/Mb. Six cycles of taxol plus cisplatin (including 4 cycles of PD-1 inhibitor, Tislelizumab 200 mg every 3 weeks) followed by 12 cycles of PD-1 inhibitor was administered. After a 16-month follow-up, this patient remained free of disease and still received PD-1 inhibitor every 3 weeks for maintenance therapy. Case 2 was a 25-year-old patient who had FIGO stage IC SCC-MT, but the preoperative SCCAg was within normal value. This patient received unilateral salpingo-oophorectomy followed by two cycles of taxol plus carboplatin (including one cycle of PD-1 inhibitor, Tislelizumab 200 mg). NGS showed that the TMB was 15.95Mt/Mb. Subsequent PD-1 plus platinum-based chemotherapy was ongoing. A total of six cycles of adjuvant chemotherapy and PD-1 inhibitor every 3 weeks for 2 years was planned. Both the two patients manifested mild immunotherapy-related adverse effect (irAE of grade 2 and 1, respectively).

Conclusion: PD-1 inhibitors plus platinum-based chemotherapy showed potential promising therapeutic efficacy and safety in SCC-MT patients with high TMB.

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Histone H3K27me3 Demethylase Therapy for Testicular Germ Cell Tumors.

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We previously showed that cisplatin resistant testicular germ cell tumors (TGCTs) are exquisitely sensitive to hypomethylating agents (HMAs) decitabine and guadecitabine, linked to high expression of DNMT3B. Pretreatment with even very low doses of HMAs restored cisplatin sensitivity and cisplatin resistance was associated with DNA hypermethylation. This work provided the rationale for a Phase I trial. To better understand cisplatin resistance and HMA hypersensitivity, we utilized a pharmacogenomic approach and found that the polycomb pathway and DNA methylation are interconnected, reciprocally regulated, epigenetic drivers of cisplatin and HMA sensitivity in TGCTs. This provides a strong rationale for combining cisplatin and HMAs in future TGCT trials. We also demonstrated that at doses that do not affect tumor growth alone, pharmacologic inhibition of histone H3K27me3 demethylases with the small mw inhibitor, GSKJ4, resulted in a high degree of cisplatin sensitization of TGCT tumors both *in vitro* and *in vivo*, in both cisplatin sensitive and resistant TGCT cells, strongly suggesting that targeting of the polycomb pathway is a second effective epigenetic therapy (after HMAs) for TGCT patients. This result was validated by genetic knockdown of the H3K27me3 demethylases KDM6A and KDM6B and polycomb components EZH2 and BMI1. Further, leveraging data from TCGA we developed a 181 polycomb target gene signature that tracked with disease free survival of TGCT patients. Transcriptome data to uncover mechanisms of GSKJ4 sensitization to cisplatin are ongoing and will also be presented. Our data suggests that directly targeting H3K27 methylation with GSKJ4 or similar drugs may be highly effective in treating cisplatin resistant/refractory TGCTs and warrants clinical investigation.

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Early-Stage Ovarian Immature Teratoma: Surveillance or Chemotherapy After Surgery? Experience from Chinese national center of rare diseases.

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Background: The need of adjuvant chemotherapy after surgery in patients with stage IA grade 2-3, stage IB-IC ovarian immature teratomas (ITs) is controversial. The chemosensitivity of ITs is under debate.

Aims: To compare the survival outcomes between surveillance and adjuvant chemotherapy in patients with stage I ovarian ITs who underwent fertility-sparing surgery.

Methods: In this retrospective cohort study, patients with stage IA Grade 2-3, stage IB-IC ovarian ITs between 2011 to 2023 from Peking Union Medical College Hospital Rare Cancer Registry were identified and analyzed. A shared decision about surveillance or chemotherapy was made by gynecologic oncologists and patients or their guardians.

Results: A total of 103 patients with stage IA grade 2-3, stage IB and stage IC ovarian IMTs were identified. Among them, 40 patients adopted the surveillance after surgery, while 63 patients received chemotherapy. The median age at diagnosis was 20 years old (range 3-39). Baseline characteristics including patient age, tumor stage and grade were similar between the two groups. Forty patients without adjuvant chemotherapy were identified: 11 stage IA (27.5%), 29 stage IC (72.5%). There were 9 patients had grade 1 tumor (22.5%), 19 grade 2 (47.5%), 12 grade 3 (30%). Twenty-three patients received cystectomy, and 17 patients underwent oophorectomy. After a median follow-up period of 20 months (range 1-138 months), only one patient with stage IA grade 2 IMT who underwent cystectomy had malignant recurrence in the same ovary. In chemotherapy group, 63 patients received cisplatin-based adjuvant chemotherapy after fertility sparing surgery. There was no statistical difference of 3-year disease free survival (DFS) and overall survival (OS) between two groups (DFS 97.5% vs. 92.1%, OS 100% vs. 96.8%, Log Rank p=0.325 and 0.304, respectively, Figure 1 and 2).

Conclusion: We did not observe survival differences in recurrence between patients with stage I ovarian IMTs who underwent adjuvant chemotherapy or not. Surveillance may be safe and preferable in early stage IMT patients who underwent complete resection of tumor. Cystectomy seems to be a risk factor for recurrence which may be due to possible microscopic residual tumor in the ovary.

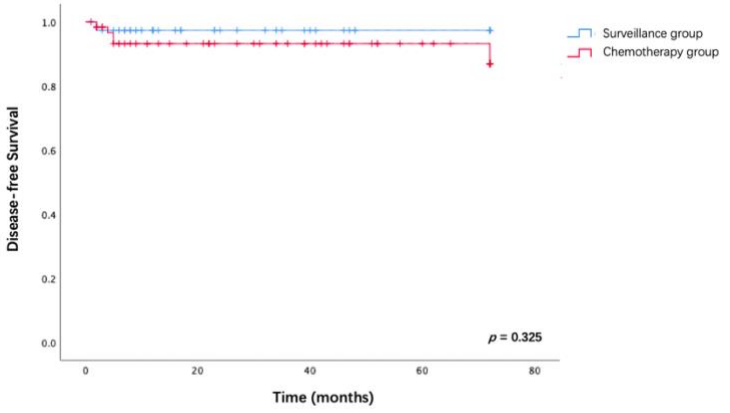

Figure 1. DFS of patients of surveillance vs chemotherapy group

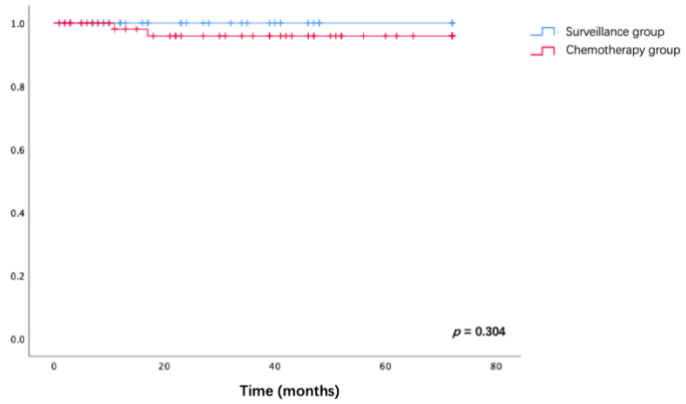

Figure 2. OS of patients of surveillance vs chemotherapy group

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High- tech radiotherapy in patients with sacral GCT: experience of a specializes service

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Introduction: Sacrococcygeal teratoma (ST) is the most common solid tumor in newborns, with an incidence of 1 / 35,000 - 40,000 live births, in a ratio of 3-4:1.5 in girls. The malignant subtype, yolk sac tumor (YST), occurs in older children and relapse cases. The treatment is based on complete resection of the tumor with removal of the coccyx, chemotherapy (when malignant), and radiotherapy. Follow up (FU) with AFP and image is required. Factors for recurrence are macroscopic disease, residual coccyx, positive microscopic disease, Spillage, and immature and malignant histology. Relapses occur in 10-15% of patients, and most relapse in YST, usually mixed.
There is no standardized therapeutic approach for patients with relapsed sacrococcygeal GCT. The most important therapy-related prognostic indicator is tumor resection. Rescue therapies include conventional chemotherapy, high-dose chemotherapy with ASCT, hyperthermia, and radiotherapy**.** There is little data about the use of radiotherapy on the relapse treatment in the literature but it is known that in some situations it is effective, so we must be familiar with them: pattern of relapse (local or regional), and insufficient local treatment. Also, patients with relapse disease should have intensified local control. RT, whose dose should be > 45 Gy, does not replace surgery, but it complements the treatment.

Results: Based on the literature, our service started with another therapeutic modality, radiotherapy, known to be effective for malignant sacral GCT with excellent responses, as follows:

- 3 patients, being recurrence in the spinal cord, positive margin after resection, increased tumor markers after BMT, underwent arc-modulated adjuvant RT in the tumor bed with a dose of 48.6 Gy, 50.4Gy, 50.4 Gy respectively. No rectitis or actinic cystitis, or evidence of disease. All of them are FU (5 years, almost 4 years, and about 2 years respectively.

Conclusion: It could be concluded that in case of disease with a high risk of recurrence or recurrent disease, the treatment should be intensified with radiotherapy if local control was not complete (macroscopic disease and positive margins), with or without elevated markers. In view of this, radiotherapy combined with BMT may be an alternative for cure.

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Evaluation of novel therapeutic options targeting (refractory) germ cell tumors by using NK-92-CD24-CAR cells, antibody (CLDN6)-/ nanobody (CXCR4)-drug-conjugates or multikinase inhibitors

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Background: After four decades, cisplatin-based chemotherapy still represents one of the major therapeutic pillars for the treatment of germ cell tumors (GCT). Even though this treatment approach is highly efficient, evolving resistance mechanisms deteriorate the patients' outcome. Specifically the yolk-sac tumor (YST) population often persists in refractory cases.
Aims: In this study we aimed at evaluating the cytotoxic efficacy of CD24-targeting NK-92-CAR cells and antibody / nanobody-drug-conjugates (ADC / NDC) binding CLDN6 or CXCR4, respectively, for the treatment of GCT cells. Besides, based on molecular analyses, it was anticipated to identify novel druggable signaling cascades in (refractory) YST.

Methods: The cytotoxic efficacy was measured using XTT cell viability assays, as well as propidium iodide (PI) or PI / Annexin V-staining with subsequent flow cytometry for the measurement of cell cycle distribution and apoptosis induction, respectively. The mutational profile and (phospho-)proteome was evaluated in (resistant) YST using TruSight Oncology 500 assays, mass spectrometry analyses and phospho-kinase arrays.

Results: CD24 was identified as a therapeutic target specifically for embryonal carcinoma. For seminoma, choriocarcinoma, embryonal carcinoma, and partly YST, CLDN6 was determined as a targetable protein. For the treatment of seminoma and YST, CXCR4 was identified as a potential target. Subsequently, a novel ADC targeting CLDN6 was developed, while a CXCR4-binding NDC and NK-92-CD24-CAR cells were further evaluated. Treatment with these various therapeutic strategies generally resulted in apoptosis induction and disruption of the cell cycle in the respective $CD24^+$, $CLDN6^+$ or $CXCR4^+$ GCT populations, while marker-negative tumor cells and non-cancerous controls remained rather unaffected. Profiling of mutational and (phospho)proteome status of (refractory) YST revealed FGF-, VEGF-, AURKA- and AKT/mTOR-signaling cascades as putative druggable pathways. Hence, treatment with the multikinase inhibitors AZD4547, AZD7762, danusertib, nintedanib, OSU-03012, SNS-314, sorafenib, or talazoparib was proven efficient with regard to apoptosis induction and / or cell cycle arrest in (resistant) YST cells, but not in fibroblasts.

Conclusion: This study identified CD24, CLDN6, or CXCR4 as therapeutic options for the treatment of most GCT subtypes by using NK-92-CAR cells, ADC, or NDC, respectively. Additionally, upon molecular characterization, this study offers multikinase inhibitors for the treatment of (refractory) YST patients.

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Ovarian Yolk Sac Tumor in a 13-year-old Girl with McCune-Albright Syndrome

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Background: MacCune-Albright Syndrome (MAS) is typically characterized by the triad of café-au-lait spots, fibrous dysplasia, and peripheral precocious puberty, along with variety of phenotypes including cancers. Generally, MAS is caused by mosaicism GNAS mutation arising early in embryonic development, leading to Gsα activation and constitutive cAMP stimulation.

Patients and Methods: We reported a patient who presented with classic triad of MAS with ovarian yolk sac tumor. For the molecular analysis, DNA samples were extracted from the patient's peripheral blood, yolk sac tumor and surrounding benign ovarian tissue. RNA molecules were extracted from tumor biopsy specimen to analyze the expression profile including metabolic and immune characteristics through nanostring.

Results: Beside the classic triad of MAS, the patient presented with other unique clinical conditions including ovarian yolk sac tumor and autoimmune related thrombocytopenia. Whole exosome sequencing revealed germline pathogenic missense mutation of TP53 and PRSS1. GNAS variations noticed on 5'UTR were likely benign variant. Gene expression profiling suggested the upregulated KRAS and NRAS and down-regulated PTEN in yolk-sac tumor cells as compared to normal tissue. Metabolic analysis of tumor showed weak activity of vitamins metabolism and significantly elevated activity of the tricarboxylic acid cycle.

Conclusions: This is the first report wherein a patient with MAS was found to have a yolk sac tumor with sequencing demonstrating the germline *TP53* mutation. The unusual genetic and expression findings raise the questions in understanding MAS and appropriate longtime surveillance for these patients.

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Testicular Germ Cell Tumour Cells Release MicroRNA-containing Extracellular Vesicles Resulting in Promalignant Changes in Cells of the Tumour

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Background and Aims: MicroRNAs (miRNAs/miR-) are short, non-protein coding RNAs that are dysregulated in malignant germ cell tumours (GCTs), with universal over-expression of miR-371~373 and miR-302/367 clusters regardless of patient age, tumour site, or subtype (seminoma/yolk-sac-tumour/embryonal carcinoma). These miRNAs are released into the bloodstream, presumed via extracellular vesicles (EVs), and represent promising biomarkers. Here, we comprehensively examined the role of EVs, and their miRNA cargo, on (fibroblast/endothelial/macrophage) cells representative of the testicular GCT (TGCT) tumour microenvironment (TME).

Methods: Small RNA next generation sequencing was performed on 34 samples, comprising representative malignant GCT cell lines/EVs and controls [testis fibroblast (Hs1.Tes) cell-line/EVs and testis/ovary samples]. TME cells received TGCT-derived EV treatment, miRNA quantification by qRT-PCR, and a miRNA overexpression system (miR-371a-OE) to perform functional assays and assess mRNA changes.

Results: TGCT cells secreted EVs into culture media. MiR-371~373 and miR-302/367 cluster miRNAs were over-expressed in all TGCT cells/subtypes compared with control cells and were highly abundant in TGCT-derived EVs, with miR-371a-3p/miR-371a-5p the most abundant compared with normal EVs. Fluorescent labelling demonstrated TGCT-derived EVs were internalised by all TME cells. TME (fibroblast/endothelial) cell treatment with EVs derived from different TGCT subtypes resulted in increased miR-371~373 and miR-302/367 miRNA levels, and other generic (e.g., miR-205-5p/miR-148-3p), and subtype-specific (seminoma, e.g., miR-203a-3p; yolk-sac-tumour, e.g., miR-375-3p) miRNAs. MiR-371a-OE in TME cells resulted in increased collagen contraction (fibroblasts) and angiogenesis (endothelial cells), associated with dysregulation of mRNAs and relevant cellular pathways.

Conclusions: TGCT cells communicate with non-tumour stromal TME cells through release of EVs enriched in oncogenic miRNAs, likely contributing to tumour progression.

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SLUG transcription factor is a potential target in germ cell tumors to improve the progression-free survival

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Background: Germ cell tumors (GCTs) can be classified according to location (gonadal and extragonadal) and histological type. Treatment is usually based on cisplatin-based chemotherapy; however, 15-20% of patients are resistant to this compound. Epithelial-mesenchymal transition (EMT) is one of the most mechanisms related to resistance and can be induced by several factors, including SNAIL and SLUG transcription factors. However, the relationship of transcription factors in EMT in GCTs has not yet been elucidated.

Aims: The aim of this study was to evaluate the role of transcription factors SNAIL and SLUG in GCTs and to investigate a possible treatment for cisplatin-resistant patients.

Methods: In silico analysis of EMT markers was performed using the cBioPortal database, considering data from 89 adult testicular GCT patients, including seminoma, mixed tumor, and embryonal carcinoma. Then, *SNAIL* and *SLUG* expression was evaluated in vitro in parental (NTERA-2P) and cisplatin-resistant cells (NTERA-2R), and both cells were treated with azacytidine.

Results: In silico analysis showed that patients with high levels of mRNA expression, *SNAIL* high and *SLUG* high, had lower progression-free survival (mean PFS: 29.7 and 28.0 months) compared to patients with *SNAIL* low (47.2months, p=0.67) and *SLUG* low (46.4months, p=0.022), respectively. The combinatorial analyses of *SNAIL* high *SLUG* high demonstrated a lower PFS (15.3months) when compared to *SNAIL* low *SLUG* low (PSF=16.5months, p=0.006). Next, *SNAIL* and *SLUG* expression was evaluated in vitro in parental (NTERA-2P) and cisplatin-resistant cells (NTERA-2R). NTERA-2R showed an increase in *SLUG* expression ($p<0.05$), but not in *SNAIL*. To investigate a treatment option for NTERA-2R, cells were treated with azacytidine and a sharp drop in cell viability was observed in the non-linear regression. The azacytidine treatment decreases the *SLUG* expression in NTERA-2R.

Conclusion: All results suggest that SLUG is a potential molecular target in the treatment of GCTs, in addition to azacytidine. Understanding the molecular mechanisms that induce EMT in GCTs will allow a better understanding of resistance.

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Clinical characteristics of relapsed, refractory extra-cranial malignant germ cell tumors (GCT) in children, adolescents, and young adults: A retrospective landscape report from the MaGIC consortium

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Background: Despite excellent outcomes following front-line therapy, 15-20% of children with a diagnosis of malignant germ cell tumors (MGCT) recur or have refractory disease, both of which are associated with a poor prognosis. There is limited data regarding the clinical characteristics of relapsed/refractory extra-cranial MGCT in pediatric and adolescent patients. We aim to provide a landscape of relapsed/refractory disease through compilation of global clinical trial data from the Malignant Germ Cell International Consortium (MaGIC) database.

Aims: We aim to report on the pre- and post- relapse clinical characteristics, therapies utilized, and prognostic factors predictive of post-relapse survival in pediatric, adolescent, and young adult patients with relapsed or refractory extracranial malignant germ cell tumors treated on historic consortia trials.

Methods: Data from seven pediatric extracranial GCT trials conducted by the Children's Oncology Group (COG) and the Children's Cancer and Leukemia Group (CCLG, United Kingdom) housed in the MaGIC clinical trial database were utilized to generate a dataset of patients aged 0-21 years with recurrent extracranial MGCTs. Disease characteristics, laboratory studies, and commonly utilized therapies were analyzed. Kaplan-Meier survival curves and Cox proportional hazards regression were used to analyze the effects of potential predictor variables on post recurrence survival (PRS).

Results: A total of 109 patients, enrolled on the seven above-referenced trials, relapsed or developed refractory disease. The median age of patients was 118 (in months) at the time of relapse/recurrence (range: 0-223 months) with a male:female distribution of 39:70. The most common site of primary disease at enrolment was gonadal and sacrococcygeal. The median time in days from enrollment to relapse was 206 days (range: 26- 303 days). Post-relapse survival at 1,2 and 5 years was 74%, 64% and 55% respectively. Additional data regarding relapse/refractory therapy delivered, prognostic factors for relapse/recurrence and post relapse death rates have also been analyzed.

Conclusion: Preliminary results from this cohort reveal patterns of relapsed/refectory disease in childhood and adolescence and support plans for additional analyses anticipated to inform data collection on frontline trials and design of future relapsed/refractory extra-cranial MGCT trials.

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Predicting outcomes in malignant ovarian germ cell tumors using the modified International Germ Cell Cancer Collaborative Group classification system: Experience from Chinese National Center of Rare Disease

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Background: Germ cell tumors (GCTs) are a heterogeneous group mainly occurring in adolescent and young adults. The International Germ Cell Cancer Collaborative Group (IGCCCG) risk classification system has been used to stratify male patients to guide treatment decisions. A modified IGCCCG risk stratification system for female patients has been proposed which needs validation for wider incorporation.

Aims: To evaluate the feasibility of the mIGCCCG risk classification system in Chinese female patients with ovarian GCTs and explore factors that may predict outcomes to further optimize the risk classification system.

Methods: In this retrospective cohort analysis, the medical record of female patients with GCTs who were treated in Peking Union Medical College Hospital between 2011 to 2020 were identified and analyzed.

Results: A total of 271 patients were enrolled. The mIGCCCG risk model classified 106 (39.1%), 84 (31%), and 81 (29.9%) patients as good-, intermediate-, and poor-risk, respectively. We observed 48 relapse or progression events and 16 deaths after a median follow-up time of 34 months. The mIGCCCG risk classification was significantly associated with 3-year disease-free survival (DFS) and overall survival (OS) (Log rank $p < 0.001$ and $p = 0.003$, respectively). In 25 patients who received neoadjuvant chemotherapy (NACT) and interval debulking surgery, patients were stratified at initial diagnosis and after NACT. The survival curves of DFS and OS separated on Kaplan-Meier analysis with different risk groups, but without statistical differences (Log rank $p = 0.772$ and 0.408, respectively). Univariate and multivariable analysis showed that tumor stage ($p = 0.033$, HR 2.05, 95%CI 1.06-3.96) was significantly associated with relapse or progression disease. Two of five patients (40%) older than 40 years had poor prognosis.

Conclusion: The mIGCCCG risk classification system was validated in Chinese female patients with GCTs and was significantly associated with DFS and OS. Classification after NACT may be more suitable for patients who received NACT compared to initial classification. Patients age and tumor stage were risk factors for prognosis, which can be used to improve the stratification systems after further analysis.

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Genomic Characterization of Germ-Cell Tumors in Childhood

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Background: Germ cell tumors (GCTs) comprise a rare and heterogeneous group of neoplasms, and unlike other solid tumors, GCTs have a low somatic mutational burden. Most molecular studies are performed among adult patients with GCT, and little is known about the molecular changes in childhood diseases. Aim: To characterize the genomic profile of pediatric GCTs through whole exome sequencing (WES).

Methods: WES was performed using Illumina paired-end sequencing strategy of 16 cases and respectively matched normal samples, including ten ovarian, five testicular, and one mediastinal tumor. Exome sequencing was performed at Sophia Genetics company. The Department of Bioinformatics of Barretos Cancer Hospital carried out data analysis. Specific mutations were confirmed by Sanger sequencing and TruSight15 panel (Illumina). The somatic alterations found were described and compared with the clinicopathological characteristics, and related to molecular databases.

Results: Somatic mutations in cancer genomes are caused by multiple mutational processes, which may generate a characteristic mutational signature. Single base substitution signature (SBS) 39 was observed in 11 samples (68.75%) and SBS22 in two ovarian samples (12.5%). Among the genes commonly involved with GCT and described as cancer drivers, copy number alterations (CNA) were identified in 4, 7, 8, 10, 12, 21, and 22 chromosomes, with amplification of CDKN1B, KRAS, CCND2, ETV6, and KDM5A genes and deletions of KIT and PTEN genes. Somatic mutations in NYAP2 and RHBDF2 genes were the most frequent among the samples (25%), in addition to mutations in MTOR (19%), KIT (12%), ATM (12%), KRAS (6%), and PIK3CA (6 %). Mutations found in the KIT gene (Asp816Val, Ala829Pro) are known for their clinical significance and therapeutic targets, and Asp816Val has a drug target (e.g., Imatinib) in gastrointestinal stromal tumors (GIST) and was validated by the TruSight15 panel. Furthermore, the mutation in KRAS (Gln61Leu) is classified with potential clinical significance and was confirmed by Sanger.

Conclusions: Our results suggest that MTOR, KIT, KRAS, and PIK3CA genes are possible therapeutic targets of pediatric GCTs. Further molecular studies are needed to determine whether these changes contribute to the arising and progression of GCTs in children.

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Oncological and endocrinological outcomes for children and adolescents with testicular and ovarian sex cord-stromal tumors Results of the TGM13 National Registry

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Rational: Sex cord-stromal tumors (SCST) are hormonally active and rare. The aim was to describe their endocrinological presentation and outcomes.

Method: Patients (<19 years) registered in the TGM13 registry between 2014 and 2021 for SCST were selected. After primary resection, patients with FIGO ≥ IC stage SCST received chemotherapy.

Results: Sixty-three ovarian SCST (juvenile granulosa tumor JGT n=34, Sertoli—Leydig cell tumor SLCT n=17, other SCST n=12) were included. The median age was 13.1 years (0.4—17.4). Germline DICER1 pathogenic variant was present in 9/17 SLCT. Sixty-one (97%) were FIGO stage I (IC n=14). Adjuvant chemotherapy was administered for 15 (FIGO IC n=12, IX n=1, III n=2). Seven had recurrence (FIGO IA n=3, IX n=2, III n=2), leading to one death. With a median follow up of 42 months (2.5—92), the 3-year PFS was 89% (95% CI 76%—95%). Among the 15 testicular SCST (Leydig cell tumor n=6, JGT n=5, Sertoli cell tumor n=3, mixed SCST n=1), the median age was 6.4 years (0.1—12.9). TNM stage was pSI in 13. Eight underwent a tumorectomy and 7 an orchiectomy. None experienced recurrence.
Among 41 patients (34 females, 7 males) with reviewed endocrinology data, 18 were prepubescent at diagnosis. Endocrine symptoms were present at diagnosis in 29 females (breast growth n=10, metrorrhagia n=14, amenorrhea n=9, hirsutism n=12, acne n=2, and voice hoarseness n=1) and 2 males (gynecomastia). After a median follow up of 11 months, 15 patients had persistent endocrine abnormalities: gynecomastia/breast growth (2 males, 2 prepubescent females), precocious/advanced puberty (4 prepubescent females), and hyperpilosity/menstruation disorders/voice hoarseness/hot flashes (8 pubescent females). The mean height at the last follow up was within normal ranges [+0.3 standard deviation].

Conclusions: SCST are associated with favorable prognoses. Tumorectomy appears safe with testicular primary. Endocrinology disorders, common at diagnosis, may persist warranting an endocrinology follow-up.

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Significance of multidisciplinary treatment and ultra-radical surgery in extensive metastatic ovarian growing teratoma syndrome: data from Gynecologic oncology of a national medical center

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Background: The significance of multidisciplinary treatment (MDT) and ultra-radical surgery in extensive metastatic ovarian growing teratoma syndrome (EMOGTS) is little known.

Aims: To share the management experience of EMOGTS based on MDT and ultra-radical surgery in a national medical center.

Methods: We performed a retrospective study of EMOGTS patients treated in our hospital between 2000 and 2022. Patients' clinical characteristics, surgical treatment, and outcomes were evaluated.

Results: Overall, 13 patients were identified and the median age at diagnosis of ovarian immature teratoma (IT) was 24 years (range: 5 – 37). The median interval between IT diagnosis and presenting GTS was 8 months (range: 2 – 60), with a median surgery delay of 5 months (range: 3 – 300). Diffuse and multiple mixed-density occupations in the abdominopelvic cavity with multiple calcified foci (13/13, 100%) and a few fat components within some mixed densities were the main CT features of the disease. Peritoneum and liver were the most commonly affected site (100%), followed by bowel (12 patients, 92.3%), diaphragm (12 patients, 92.3%), adnexa (9 patients, 69.2%), omentum (8 patients, 61.5%), uterus (7 patients, 53.8%), in the descending order. However, even the hepatic parenchyma (4 patients, 30.8%), pleural cavity (2 patients, 15.4%), and spleen parenchyma (1 patient) could also be involved. The mean operation time was 316 min (range: 180 – 625), and the mean blood loss volume was 992 ml (range: 200 – 5000). Surgical options included peritoneal metastasectomy (13 patients, 100%), diaphragmatic metastasectomy (12 patients, 92.3%), intestinal metastasectomy (8 patients, 61.5%), partial hepatectomy (4 patients, 30.8%), bowel excision and anastomosis (1 patient, 7.7%). R0 was achieved in 9 (69.2%) patients. A high rate of intraoperative blood transfusion (8 patients, 61.5%) and admission to the intensive care unit (9 patients, 69.2%) were observed, and the median postoperative hospitalization time was 8 days (range: 4 – 22). After a median follow-up of 3.3 years, 9 patients were free of disease and 4 were alive with stable residual diseases.

Conclusion: The survival outcomes in EMOGTS were satisfactory after ultra-radical surgery, while a proper therapeutic plan determined by MDT should be established due to the high perioperative risks.

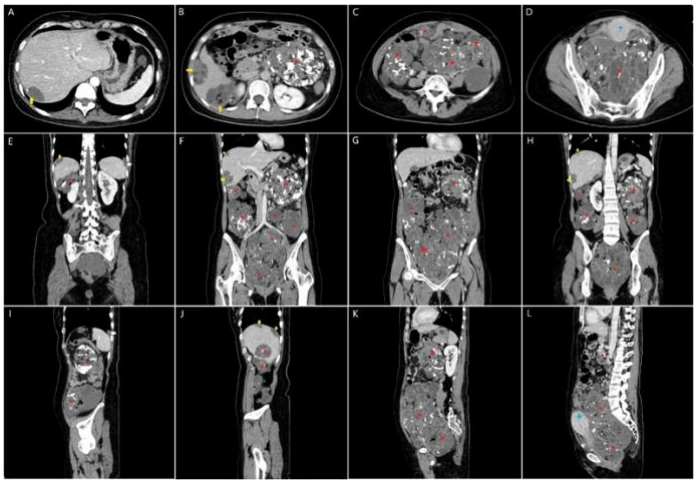

Figure 1. Representative CT images of GTS presenting extensive metastatic diseases, revealing multiple bulky seedings in the abdominopelvic cavity (enhanced scan portal phase images, A-D, axial section; E-H, coronal section; I-L, sagittal section). Non-enhancing foci of mixed density at the edge of the right lobe of the liver, with foci of fatty density, and spotty calcification (yellow arrow, A-B, E-F, H, J). The subpleural, non-enhancing hypodense lesion in the lower lobe of the left lung, without calcification (white arrow, A, I). Diffuse multifocal rounded mass in the abdominopelvic cavity with regular morphology and well-defined borders, partially indistinctly demarcated from the bowel, with irregular fatty components scattered within, mostly with foci of punctate, short streaks or irregular calcifications (red star, B-D, F-H, I-L). Intrapelvic occupancy, encircling the uterus, indistinguishable from the uterus (D, L). (White arrow, subpleural lesion; yellow arrow, perihepatic/diaphragmatic lesions; red star, abdominal or pelvic lesions; blue star, uterus).

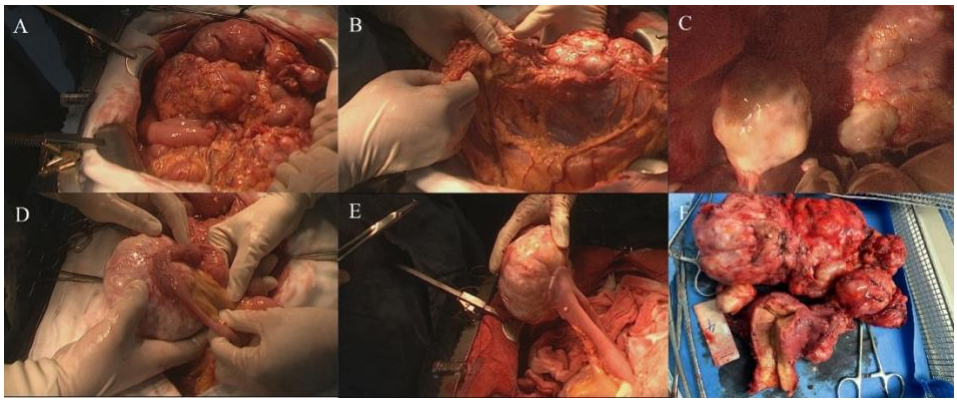

Figure 2. Intraoperative view of one patient with typical extensive metastatic GTS. (A) the overview of tumors that fully occupied the pelvic cavity; (B) diffused tumor seedings in the omentum, with a largest of about 8 cm in diameter; (C) tumors in diaphragm; (D-E) a 15-cm solid, hard tumor was observed in the mesentery of small bowel, led to severe bowel stenosis and mild blood stasis; (F) The surgical specimen showed the uterus, left adnexa, and the largest GTS tumor.

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Outcome and late effects of patients treated for childhood vaginal malignant germ cell tumors

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Purpose: Vaginal malignant germ cell tumors are rare, occurring in children <2years-old and raise the question of the optimal local treatment.

Methods: We included children treated for vaginal MGCT according to French TGM95/2013 regimen. Patients were classified as standard-risk (SR: localized disease and AFP<10 000ng/ml) or high-risk (HiR: metastatic and/or AFP>10 000ng/ml) and were treated with 3-5 VBP (vinblastine-bleomycin-cisplatin) or 4-6 VIP (etoposide-ifosfamide-cisplatin), respectively followed by conservative surgery and/or brachytherapy in case of post-chemotherapy residuum.

Results: Fourteen patients were included (median age=12 months) six of them (43%) were classified as HiR. After first-line chemotherapy AFP was normalized in all cases except one. A vaginal post-chemotherapy residuum (median size=8mm, range=1-24) was observed in 13/14 patients, treated by complete resection in 7/13 (viable cells in 3/7), incomplete resection in 4/13 (viable cells: 2/4) with adjuvant brachytherapy in 2/13 and exclusive brachytherapy in 2/13 (viable cells: 1/6). Among the six patients with viable disease, four patients received adjuvant chemotherapy. One patient (SR) had relapse immediately after surgery despite no viable residual cells and was treated with four VIP and brachytherapy. At last follow-up (median=4.6years, range=0.5-16), all patients were alive in complete remission. Five patients suffered from vaginal sequelae with synechiae and/or stenosis (four after brachytherapy).

Conclusion: Childhood vaginal MGCTs carry a very favorable prognosis with risk-adapted chemotherapy and local treatment to remove post-chemotherapy residuum preferably by conservative surgery with partial vaginectomy. Brachytherapy could be an alternative when conservative surgery is not deemed possible or in cases of incomplete resection with viable residual cells.

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MicroRNA signature for classification of each histological type from pediatric patients with Germ Cell Tumors

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Background: Germ cell tumors (GCTs) occur in children and adults, are composed of diverse histologies, and appear in gonadal and extragonadal sites. Although serum markers alpha-fetoprotein (AFP) and the beta fraction of chorionic gonadotropin (BHCG) aid in the diagnosis of GCTs, they are not tumor-specific. A group of small non-coding RNAs, microRNAs (miRNAs) is suggested as molecular biomarkers in several tumors, however, miRNAs as biomarkers in pediatric GCTs is not clear.

Aims: This study aimed to assess the miRNAs profiles in pediatric patients with GCTs to distinguish between different histological types.

Methods: We evaluated a total of 42 samples from pediatric patients with GCTs, including 31 ovarian GCTs's patients and 11 testicular GCTs's patients. The expression of miRNAs was analyzed using the Human v3 miRNA Assay CSO Panel with nCounter Analysis System technology. Data analysis was conducted using the NanoStringNorm package in the R environment.

Results: The analysis revealed distinct miRNA expression profiles for all histological types, regardless of the primary site. Mature teratomas clustered with healthy control samples, while malignant histological types (yolk sac tumor, dysgerminoma, embryonal carcinoma, and immature teratoma) formed distinct groups based on their miRNA expression patterns. We identified specific miRNA expression signatures for each histological type, including 34 miRNAs for dysgerminomas, 13 for embryonal carcinomas, 25 for yolk sac tumors, and one for immature teratoma, compared to healthy controls. Furthermore, we identified 26 miRNAs that were commonly expressed in malignant tumors, with six miRNAs (miR-302a-3p, miR-302b-3p, miR-371a-5p, miR- 372-3p, miR-373-3p, and miR-367-3p) showing significant overexpression. Notably, miR-302b-3p exhibited a significant association with all the evaluated clinical features.

Conclusion: These results provide the comprehensive description of differentially expressed miRNAs for each histological type of gonadal GCT in pediatric patients. Our findings suggest that miRNAs have the potential to aid in the diagnosis, prognosis, and management of patients with malignant GCTs.

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Guidelines and Recommendations for the use of Historical Controls in Germ Cell Tumor Trials

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Background: The use of historical controls can be beneficial in settings of rare diseases, such as in many pediatric oncology settings. By reducing the sample size requirements, the use of historical controls can help reduce study costs, and in some cases, can make studies feasible. The proper selection of the control group and the proper application of statistical methodology, however, is crucial to reducing bias in historical control trials.

Aims: The goal of this project is to discuss the appropriate selection of the historical control group and existing statistical methodology. Benefits and limitations associated with the use of historical controls and with existing methodology will be discussed. Moreover, the presenters will discuss considerations for the proper interpretation and generalizability of results.

Methods: A literature review will be conducted to guide the selection of historical controls and to provide an overview of existing statistical methodology.

Results: Knowledge obtained from past trials can be used to improve current and future studies. When selecting a control group, however, one must ensure that the population of current patients is similar to that of the control group in order to reduce bias. Existing methodology can vary in the weight that is placed on information from historical controls; however, the appropriate implementation and interpretation is necessary to understanding study results.

Conclusion: The proper selection of historical control groups and corresponding statistical methodology are crucial for the success of studies incorporating historical controls.
Without the proper control group, results could be biased and misleading. If a suitable control group is selected, however, the use of historical controls can lead to lower study costs.

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Detection of Circulating Tumor DNA and Circulating Tumor Cells in Patients with Malignant Ovarian Germ Cell Tumors

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Background: Malignant ovarian germ cell tumors (MOGCTs) are rare tumors mainly occurring in adolescent and young adults. Postoperative chemotherapy is widely used and the long-term side effects are immense. Minimally residual disease (MRD) including circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) can reflect the tumor burden and guide personalized treatment.

Aims: To explore the feasibility of ctDNA and CTC detection in ovarian malignant GCTs and preliminary analyze the correlation between the liquid biopsy results and treatment outcomes.

Methods: Plasma ctDNA was detected by tumor-informed assays. The tumor sample is sequenced by whole exome sequencing (WES) to identify somatic mutations. A personalized panel of 20 single nucleotide variants was designed to detect ctDNA in peripheral blood at certain time-points during surveillance. CTCs were enriched and separated from peripheral blood and ascites or peritoneal washings using high-throughput microfluidic Labyrinth system. For CTC detection, germ cell tumor (anti-SALL4) and epithelial cell–specific (anti-panCK) antibodies were used. Tumor cells separated from ascites or peritoneal washings were used for cell culture in vitro.

Results: Nine peripheral blood samples from 8 patients had been analyzed for ctDNA detection. The preoperative ctDNA was detected in 87.5%(7/8) of patients. One patient with stage I grade 3 immature teratoma showed ctDNA-negative at initial diagnosis. One mixed GCT patient had plasma samples available before surgery and after completion of adjuvant chemotherapy, and her ctDNA was cleared after treatment. In parallel, 13 peripheral blood samples from 11 patients were analyzed. CTCs could be detected in 90.9%(10/11) of patients, with an average of 2.7/ml. Two CTC- positive patients had plasma samples available after chemotherapy, and both of them cleared CTC when treatment finished. Tumor cells collected from peritoneal washing liquid in one patient with yolk sac tumor were cultured successfully in vitro.

Conclusion: The ctDNA and CTC can be detected in patients with malignant ovarian GCTs. The association of ctDNA and CTC with prognosis requires further research. Tumor cells collected by microfluidic system can be cultured in vitro and prepared for further investigations.

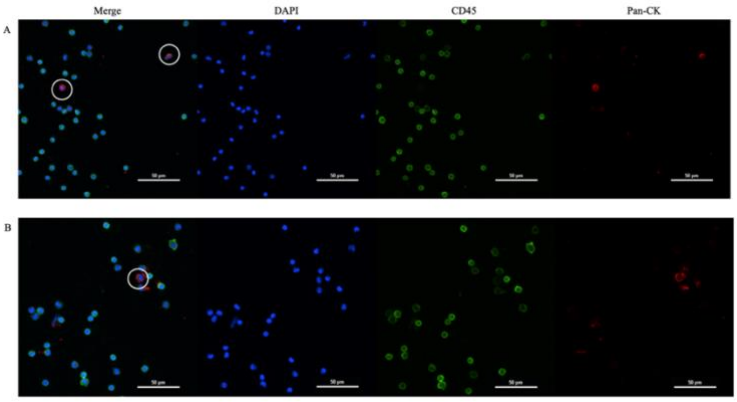

Figure 1. Representative tumor cells detected in peripheral blood of one patient with GCT. A. Representative CTCs stained with panCK (red) and white blood cells stained with CD45 (green). B. Representative CTCs stained with SALL4 (red) and white blood cells stained with CD45 (green).