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A CASE OF RELAPSED REFRACTORY AUTOIMMUNE HAEMOLYTIC ANAEMIA FOLLOWED BY REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA TREATED WITH STEM CELL TRANSPLANTATION

by Zhanna Kovalova | Natalija Kurjane | Anna Valaine | Jurijs Bormotovs | University Children\'s Hospital | University Children's Hospital | University Children's Hospital

Themes: Benign Hematology
Presenter Name: Zhanna Kovalova
Keywords: autoimmune diseases, immune regulation

The patient presented to the hospital at the age of 11 months with severe warm-reactive autoimmune haemolytic anaemia, renal failure, transaminitis, mild asymptomatic thrombocytopenia, elevated D-dimer levels. Infectious causes, haemolytic uremic syndrome was excluded. Anti-DNA, anti-ds-DNA were negative, no PNH clone was detected. The boy received therapy with methylprednisolone and azathioprine. At the age of 2 year 7 months the patient presented with severe onset of cold-reactive autoimmune haemolytic anaemia, jaundice, livedo reticularis, hepatosplenomegaly, transaminitis. We treated him with methylprednisolone pulse therapy, high dose of intravenous immunoglobulins (IVIG), rituximab, azathioprine, cyclophosphamide 500mg/m2, mycophenolate mofetil and fludarabine. After adding fludarabine the haemolysis subsided. Investigations of classical and alternative complement pathways revealed no pathology. Next generation sequencing showed no primary immune deficiency. Most autoimmunity markers were negative, antibodies to beta-2-glycoprotein were temporary positive. No double negative T-cells were detected, B12 vitamin level was normal. In 3 months the patient had transient toxic hepatitis, immunosuppressive therapy (IST) was discontinued due to agranulocytosis and bone marrow stromal damage. 3 months after discontinuation of IST, the patient presented with idiopathic thrombocytopenic purpura (ITP), refractory to treatment. The patient received methylprednisolone pulse-therapy, dexamethasone, IVIG, azathioprine, rituximab, cyclophosphamide, vincristine, daily thrombocyte transfusions. Despite the broad IST and transfusions, the boy’s thrombocyte counts were continuously below 2000/μL. He had severe skin and mucosal haemorrhages with macrohematuria and epistaxis. Anti-thrombocyte antibodies were negative, no double negative T-cell population was detected. Leukocyte counts and haemoglobin levels at presentation of ITP were within normal range. Beta-2-glycoprotein, transaminases were within normal range. Slowly the patient’s condition improved. In July 2019 stem cell transplantation from HLA-identical sibling was performed, partial chimerismus 70/30 donor to recipient is detected without any clinical signs.

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A defined hydrogel for murine testicular organoid generation

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Themes: Basic Science
Presenter Name: Yanhua Cui
Keywords: Organoids, Sertoli cells, fertility preservation, germ cells, testis

Prepubertal boys subjected to oncological treatments are at a higher risk of subfertility. Currently, cryopreservation of immature testicular tissue containing spermatogonial stem cells is the only way to preserve fertility in prepubertal boys subjected to high gonadotoxic treatments. Today there is no technique available to mature these stem cells into functional sperm in humans. Hence, novel functional in vitro models, such as organoids, are required. Most protocols used for testicular organoid formation are based on Matrigel. However, Matrigel is a poorly defined xenogeneic extracellular matrix (ECM) protein with limited use for clinical applications. Therefore, artificial scaffolds and defined recombinant human ECM proteins need to be developed. This project aims to investigate synthetic Matrigel alternatives for the expansion and differentiation of murine testicular organoids.

We used a Noviogel, which is a synthetic biomimetic hydrogel based on polyisocyanopeptides, for the generation of testicular organoids. Testes of 11 days old C57BL/6 mice (n= 40) were digested into testicular single-cell suspensions and applied to the three-layer gradient culture system for testicular organoid formation. We compared the effect of combinations of Noviogel with three different types of laminins (LN121, LN521 or LN111), on testicular organoid formation for 7 days in vitro (n=4). Our results demonstrated that Noviogel supplemented with LN111, provided a supportive environment for testicular organoid formation. The effects of LN111 were superior to the effects shown for LN121 and LN521. Periodic Acid-Schiff staining showed testicular organoids cultured in Noviogel supplemented with LN111 exhibited cord-like structures similar to in vivo control tissue samples.

Our study demonstrates that primary mouse testicular cells are capable of forming testicular organoids in a synthetic defined hydrogel-based three-dimensional model. It provides first valuable knowledge for the development of testicular organoids that may further be used to develop a novel fertility restoration option for prepubertal childhood cancer patients.

Acoustically-isolated extracellular vesicles (EVs) as novel MRD markers in acute lymphoblastic leukemia

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Themes: Leukemia
Presenter Name: Ladislav Król
Keywords: ALL, Acute lymphoblastic leukemia, EVs, MRD, acoustophoretic trapping, extracellular vesicles

Acute lymphoblastic leukemia (ALL), the most common pediatric leukemia, is effectively treated using modern risk-based treatment protocols. Response to therapy is an important risk factor and is measured as minimal residual disease (MRD) by analyzing bone marrow cells. Extracellular vesicles (EVs) released by leukemic cells into the plasma have leukemia-specific properties and have therefore been proposed as possible alternative MRD markers.

We therefore aimed to develop novel ultrasound-based acoustophoretic trapping to isolate plasma extracellular vesicles (EVs) for measurement of MRD during ALL treatment. Acoustic trapping is a contact free, rapid and robust microfluidics-based ultrasound method to capture nanoparticles in small sample volumes with minimal sample preparation, which is a better alternative compared to standard EV isolation methods.

In our project, we have successfully established effective EV isolation using acoustophoretic trapping. Thus far, samples from 20 ALL patients (8 children, 1 late adolescent, 11 adults) have been collected. Analysis of selected samples showed that enough EVs could be isolated for miRNA analysis from as little as 150 ml plasma. Acoustically-isolated EVs showed a typical size distribution (NanoSight). qPCR panel analysis detected in average 73 miRNAs per sample and 19 miRNAs were identified in all samples. Preliminary PCA analysis showed that de-novo ALL diagnosis samples were located in close proximity whereas follow-up samples were located at a distance from the diagnosis samples. Interestingly, distances were highest for patients that showed a good response to therapy. Also, miRNAs that were detected in an ALL cell-line panel were identified in patient samples as well and were found to be significantly decreased by treatment (e.g. miR-26b-5p, miR-324-3p, miR-423-3p).

Based on these promising preliminary results we plan continue with our study, ideally in cooperation with other Nordic Centers, aiming to establish a MRD detection method that may add to improved risk stratification in ALL patients.

ACUTE MEGAKARYOBLASTIC LEUKEMIA IN A PHENOTYPIC NORMAL INFANT - TO TREAT OR NOT TO TREAT?

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Themes: Leukemia
Presenter Name: Sandahl, Kristian Juul
Keywords: Acute megakaryoblastic leukemia, GATA1, Infantile myeloproliferative disease, Transient abnormal myelopoiesis, Trisomy 21

Introduction

Distinguishing infantile myeloproliferative disease (IMD) from transient abnormal myelopoiesis (TAM) when neonatal leukemia presents without clinical features of Down Syndrome (DS) may be challenging and has major impact on treatment strategy.

Aim

Discuss a case presenting as acute megakaryoblastic leukemia (AMKL) within the first month of life without phenotypical features of DS to consider different treatment options.

Case presentation

A 20-day-old boy, born at term, previously thriving, presented with swelling in the legs and arms. Hepatosplenomegaly was found. White blood cells were 90 x 10^9/L, lactate dehydrogenase was 1000 U/L. Hemoglobin and platelet counts were normal. No phenotypical signs of DS were found. Mitoxantrone, etoposide, and cytarabine (MEC) was initiated based on 80% blasts in peripheral blood (PB), CD41a, CD42b, CD56, CD7, CD4dim, CD11b-neg, CD13neg and morphological AMKL.

Trisomy 21 (T21) was found in unstimulated PB (47,XY,+21 [25]), PHA stimulated PB (47, XY,+21 [8]/46,XY [2]) and FISH on PB (183/200 cells with T21).

WBC declined rapidly and remission was evident 22 days after MEC with a decrease by more than three log in WT1 expression.

Prior to planned start of the second induction, it was realized that a *GATA1* variant was present in close to 100% of the cells sampled at diagnosis. Treatment was shifted to a watch and wait strategy.

At one year of age FISH on PB was negative for T21 (0/200 cells), indicating a combination of somatic T21 and *GATA1* associated IMD although TAM caused by low-level mosaic T21

cannot be excluded. The boy remains in complete remission three years after diagnosis without any clinical signs of DS.

Conclusion

Some newborns with myeloid malignancy regress spontaneously. Decisions on therapy may be delicate. The 2022 guidelines on AMKL in infants by Bertrums, Haematologica 2022, promotes a watch and wait strategy, where investigations for T21 and *GATA1* can be performed.

ASSOCIATION BETWEEN TYPE 1 DIABETES AND CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: A POPULATION-BASED CASE-CONTROL STUDY

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Themes: Leukemia
Presenter Name: Anni Ranta
Keywords: ALL, T1D, acute lymphoblastic leukemia, type 1 diabetes

Background In the recent decades increased incidences of type 1 diabetes (T1D) and acute lymphoblastic leukemia (ALL) have been reported in the developed countries. The two diseases share several risk factors suggesting possible common causal pathways. The aim of this study was to evaluate the association between T1D and ALL in a matched case-control study based on a population with some of the highest incidences of ALL and T1D.

Methods All ALL cases aged 0-19 when diagnosed in Sweden between the years 1987-2015 were included. Patients were identified from the National Cancer Register. 25 age- and sex-matched controls were selected per patient. The odds ratio (OR) and 95% confidence interval (CI) for prevalent T1D at the time of ALL diagnosis was computed through a conditional logistic regression model. Additional analyses excluding cases with T1D diagnosed less than six or 12 months before ALL diagnosis were performed to correct for possible overestimation of the association due to increased inpatient care.

Results We observed a higher prevalence (p=0.026) of T1D in the 1870 cases of ALL (0.4%) in comparison to the controls (0.2%). A positive association was found between T1D and ALL in all analyses. Adjusted ORs for ALL were 2.23 (95% CI 1.08-4.62) for all T1D cases, 1.93 (95% CI 0.84-4.44) for T1D diagnosis more than six months before and 2.18 (0.95-5.05) for T1D diagnosis more than 12 months before ALL diagnosis.

Conclusions The results showing a positive association between T1D and ALL are in line with previous findings in literature and in support of both the common epidemiological features and possible infectious etiology. The results are based on a relatively small number of cases and more work should be done to investigate the association. Supportive findings might lead to therapeutic or preventative clinical applications.

ATYPICAL CASE OF METASTATIC EWING SARCOMA WITH DISEASE STABILISATION AND POST-CHEMOTHERAPEUTIC GANGLIONEUROBLASTOMA-LIKE DIFFERENTIATION

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Themes: Solid Tumours
Presenter Name: Agne Morkunaite
Keywords: Ewing sarcoma, chemotherapy, maturation, neural differentiation, radiotherapy

Introduction.

Ewing sarcoma is a highly aggressive malignancy, typically affecting people aged 10-20 years, with a 5-year survival rate for metastatic disease of less than 30%. Uncommonly, following chemotherapy, the disease can stabilise and even differentiate into a ganglioneuroblastoma-like tumour.

Aim.

To report a case of Ewing sarcoma with unusual disease development.

Methods.

Our patient is a 16-year-old male, diagnosed with EWSR1-ERG gene fusion Ewing sarcoma, involving a large primary tumour in the right iliac bone, along with metastases in the liver, lungs, 10th thoracic vertebra and mediastinal lymph nodes. As neoadjuvant treatment, the patient received 1 VIDE (vincristine, ifosfamide, doxorubicin, etoposide) cycle followed by 8 alternating cycles of VDC/IE (vincristine, doxorubicin, cyclophosphamide / ifosfamide, etoposide) at 14-day intervals, followed by 12 irinotecan/temozolomide (IT) cycles. During chemotherapy, the patient underwent radiotherapy, fractionated into 30 sessions of 1.8 Gy up to a total dose of 54 Gy. MRI and CT scans were used for initial and follow-up radiological evaluation and PET scan for metabolic activity evaluation.

Results.

Neoadjuvant treatment had a poor effect on both the primary tumour and the metastases (minimal regression on radiological evaluation, primary tumour remained unresectable, all lesions hypermetabolic on PET scan). On subsequent rebiopsy, the primary tumour had a very low proliferative index (Ki67 < 5%). After 12 IT cycles, albeit seemingly stable on radiological evaluation, the lesions displayed decreased metabolic activity on PET scan.

Moreover, the primary tumour revealed central necrosis on MRI (Figure 1), while the biopsy of the liver metastasis showed a ganglioneuroblastoma-like differentiation.

Conclusions.

Treatment of metastatic Ewing sarcoma remains a major challenge. Nevertheless, even with poor response to chemotherapy, metastatic Ewing sarcoma can transition to a progression-free and stable state. Further research into the treatment of Ewing sarcoma is crucial, especially given the neural maturation findings, whose prognostic significance remains unknown.

CHEMOTHERAPY-RELATED TOXICITIES IN A COHORT OF CHILDREN TREATED FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Themes: Leukemia
Presenter Name: Roosa Rokkanen
Keywords: Acute lymphoblastic leukaemia, children, neuropathy, osteonecrosis, toxicity

Introduction

With the high contemporary overall survival rates in childhood acute lymphoblastic leukemia (ALL), research effort has shifted onto reducing treatment-associated toxicity. Papers generally focus only on a single toxicity or toxicity group.

Aim

To describe the full spectrum of chemotherapy-related toxicity in a cohort of children treated according to the NOPHO ALL-2008 protocol.

Subjects & methods

The cohort consisted of 73 pediatric patients treated in a single center between years 2008 and 2021. Data were captured on 19 toxicities. Data were collected from the NOPHO ALL-2008 toxicity registry and patients' medical records. The Student *t*-test, chi-square test and multivariate logistic regression were used to compare groups.

Results

All but one patient suffered from one or more toxicities (mean 1.7 [SD 0.88]). Multiple toxicities were reported in 48%. Observed toxicities were anaphylaxis, osteonecrosis, liver failure, bleeding, posterior reversible encephalopathy syndrome, stroke-like syndrome, vincristine-induced peripheral neuropathy (VIPN), pancreatitis, thrombosis, and ICU treatment. There were no cases of heart failure, kidney failure, seizures, coma, laparotomy, veno-occlusive disease, pneumocystis infection, or fungal infection. Multivariate regression showed toxicities to be more frequent in females (mean 1.9 [SD 1.0] vs. mean 1.5 [SD 0.72] toxicities, $p = 0.023$) and with increasing age (adjusted beta for one year change 0.5; 95% CI [0.0020 to 0.099], $p = 0.040$). Therapy stratification did not impact the risk of toxicities.

Most toxicities occurred during the first 3 months of treatment, following a typical pattern in relation to treatment period. The most common toxicity seen in 96% (n=70) patients, VIPN, typically developed during induction, whereas e.g. osteonecrosis was usually diagnosed during maintenance or after cessation of chemotherapy.

Conclusion

Toxic effects are common in patients treated according to NOPHO ALL-2008. Toxicities occur in a typical sequence in relation to the treatment phase. The extremely high incidence of VIPN is likely dose-related.

CHILDHOOD CANCER PATIENTS IN NEED OF SUPPORT – RETROSPECTIVE PSYCHOSOCIAL RISK GROUP IDENTIFICATION FROM ELECTRONIC HEALTH RECORDS WITH MACHINE LEARNING

by Päivi Lähteenmäki | Akseli Reunamo | Sanna Salanterä | Hans Moen | Turku University Hospital, Department of Pediatric and Adolescent Hematology-Oncology | Turku University, Computer Science | Turku University, Nursing Science | Aalto University, Department of Computer Science

Themes: Late Effects
Presenter Name: Päivi Lähteenmäki
Keywords: Childhood cancer, Natural Language Processing, Psychosocial, Survivor

Background: Due to the risk of late-effects, childhood cancer survivors require more health monitoring than the average population. They also have a higher risk for mental health disorders than their siblings. Assessing the need for psychosocial support is essential for enabeling prevention. This project aimed to investigate the use of supervised machine learning (ML) in identifying childhood cancer patients needing psychosocial support at least one year after a cancer diagnosis from nursing notes in the form of text classification.

Methods: We tested three well-known ML-based models to recognize patients who had reservations in the mental health care units from the nursing notes of 1672 patients. These patients had been diagnosed with diabetes mellitus or cancer. Notes written one week before and all after the mental health-related contact were excluded from the data to see if the models could capture hidden signals of psychosocial challenges. We used stratified five-fold nested cross-validation to evaluate the performance of the models in a binary classification task: no support or psychosocial support was needed. Patients with the latter were identified by having a reservation in a mental health care unit at least one year after a primary diagnosis. Only cancer patients were used in the evaluation. The reliability of the results was assessed with Bayesian estimation.

Results: The Random Forest classification model using the whole dataset performed best in three times repeated nested-cross validation with 0.798 mean area under the receiver operating characteristics curve (AUC) and was better with 99% probability (credibility interval - 0.2840, - 0.0422) than the neural network-based model using only cancer patients in training.

Conclusions: The use of ML for predicting childhood cancer patients needing psychosocial support was possible with good AUC. ML may help to identify patients likely needing mental health-related support later in life.

Combined GLUT1 and OXPHOS inhibition eliminates acute myeloid leukemia cells by restraining their metabolic plasticity

by Rodriguez-Zabala, Maria | Ramakrishnan, Ramprasad | Reinbach, Katrin | Ghosh, Somadri | Oburoglu, Leal | Falqués-Costa, Antoni | Bellamkonda, Kishan | Ehinger, Mats | Peña-Martínez, Pablo | Puente-Moncada, Noelia | Lilljebjörn, Henrik | Cammenga, Jörg | Pronk, Cornelis-Jan | Lazarevic, Vladimir | Fioretos, Thoas | Hagström-Andersson, Anna K. | Woods, Niels-Bjarne | Järås, Marcus | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Div. of Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Skåne University Hospital, Lund University, Dept. of Clinical Sciences, Div. of Pathology, Lund, SwedenSkåne University Hospital, Lund University, Dept. of Clinical Sciences, Div. of Pathology, Lund, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Skåne University Hospital, Dept. of Hematology, Div. Oncology and Radiation Physics, Lund, Sweden | Skåne University Hospital, Childhood Cancer Center, Lund, Sweden | Skåne University Hospital, Dept. of Hematology, Div. Oncology and Radiation Physics, Lund, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden | Lund University, Div. of Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund, Sweden | Lund University, Dept. Laboratory Medicine, Div. of Clinical Genetics, Sweden

Themes: Leukemia
Presenter Name: Rodriguez-Zabala, Maria
Keywords: AML, CRISPR screen, GLUT1, leukemia stem cell, metabolism

Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy characterized by the uncontrolled proliferation and accumulation of immature myeloid cells in the bone marrow and peripheral blood. AML is initiated and propagated by leukemia stem cells (LSCs), a self- renewing population of leukemia cells responsible fordisease initiation, perpetuation, and relapse. Hence, there is an urgent need to identify new therapeutic opportunities targeting LSCs.

Here we performed an *in vivo* CRISPR knockout screen to identify potential therapeutic targets by interrogating cell surface dependencies of LSCs. The facilitated glucose transporter type 1 (GLUT1) emerged as a critical *in vivo* metabolic dependency for LSCs in a murine *MLL::AF9*-driven model of AML. GLUT1 disruption by genetic ablation or pharmacological inhibition led to suppression of leukemia progression and improved survival of mice transplanted with LSCs. Metabolic profiling revealed that *Glut1* inhibition suppressed glycolysis, decreased levels of tricarboxylic acid (TCA) cycle intermediates, and increased the levels of amino acids. This metabolic reprogramming was accompanied by an increase in autophagic activity and apoptosis. Moreover, *Glut1* disruption caused

transcriptional, morphological and immunophenotypic changes consistent with differentiation of AML cells. Notably, dual inhibition of GLUT1 and oxidative phosphorylation (OXPHOS) exhibited synergistic anti-leukemic effects in the majority of paediatric and adult AML patient samples tested by restraining their metabolic plasticity. In particular, *RUNX1*-mutated primary leukemia cells displayed striking sensitivity to the combination treatment compared to normal $CD34^+$ bone marrow and cord blood cells.

Collectively, our study reveals a GLUT1 dependency of murine LSCs in the bone marrow microenvironment, and demonstrates that dual inhibition of GLUT1 and OXPHOS is a promising therapeutic approach for AML.

Delayed papillary thyroid cancer diagnosis in adolescent girl

by Kapitancuke Monika | Adomaitiene Irina | Beisa Virgilijus | Kemezys Robertas | Mickunaitiene Grazina | Rascon Jelena | Sendiuliene Daiva | Vidrinskaite Asta | Vaitkeviciene Elizabeta Goda | Center for Pediatric Oncology and Hematology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Center of Radiology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Center of Abdominal surgery, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Center for Pediatric, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Center for Pediatric, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Center for Pediatric Oncology and Hematology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Radiation Oncology department, National cancer institute, Vilnius, Lithuania | Radiation Oncology department, National cancer institute, Vilnius, Lithuania | Center for Pediatric Oncology and Hematology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania

Themes: Solid Tumours
Presenter Name: Kapitancuke Monika
Keywords: adolescent girl, difficulty swallowing, hoarseness, night sweats, papillary thyroid cancer, psychiatric disorder, respiratory tract infections

Introduction. Thyroid cancer is rare in childhood, however the prevalence of papillary thyroid cancer (PTC) is increasing in adolescent girls.

Case presentation. A 12-year-old girl started complaining with anxiety and fatigue. In addition, constipation, body aches and excessive night sweats appeared. In the dynamic behavioral and eating disorders developed. The patient was consulted by psychologist, commenced homeopathic medicines for nervous system, however symptoms did not regressed. According to the mother, these clinical signs were interpreted as related to puberty. In two years, the girl started complaining with difficulties in swallowing and breathing, mild hoarseness and dry cough. On the same time, respiratory tract infections (RTIs) started to recur, following often antibiotics courses with temporarily symptoms improvement, therefore the patient has not been further investigated. After suicidal episode at the age of 15 years, quetiapine and fluoxetine were prescribed. Emotions became stabilized, whereas other symptoms persisted. At the age of 16, mother took a girl to pediatric endocrinologist. Thyroid hormones level (FT3, FT4, TTH) and anti-TPO were normal. Thyroid palpation and ultrasound revealed solitary nodule in the left thyroid lobe (size 23.5 mm, TI-RADS 4). Cytology from the primary fine needle aspiration (FNA) did not show atypical cells, however the suspicion of a malignant process was high and FNA was repeated. PTC was confirmed histologically later after thyroidectomy: PTC, pT2(m) (3 tumour foci in the left thyroid lobe), pN0, LVI0. There was no evidence for distant metastasis. Thyroid hormone replacement and iodine radiotherapy were initiated.

Conclusions. The patient had delayed PTC diagnosis due to concomitant RTIs, psychiatric disorders and puberty period. It is important to be aware about malignant process in the neck and mediastinum area for adolescents when they have hoarseness, difficult to

swallowing and breath, with constitutional symptoms, especially when there is no complete recovery after treatment.

DEVELOPING NEW IMMUNOTHERAPEUTIC APPROACHES TO TREAT OSTEOSARCOMA

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Themes: Novel Methods and Therapies
Presenter Name: Sorteberg, Agnes L
Keywords: immune checkpoint inhibition, immunotherapy, osteosarcoma

Osteosarcoma (OS) is the most common malignant bone tumor in children, adolescents and young adults. The primary tumor most often occurs in the proximal limb bones, while the primary site of metastasis are the lungs. The overall survival of resectable OS has stagnated at 70% the last four decades, while the overall survival of metastatic OS is below 20%, highlighting the need for new and improved treatment strategies. OS is a cancer that historically has responded to immunostimumulatory therapies. However, in several clinical trials immune checkpoint inhibition targeting CTLA-4 or PD-1 has only had very limited efficacy. On the other hand, type and extent of immune infiltration in the osteosarcoma microenvironment have clearly been demonstrated to correlate with outcomes. Hence, our aim is to identify novel immunotherapeutic combinations. To assess the potential of immunotherapy combinations in the treatment of OS, we established a model of metastatic OS in immunocompetent mice by injecting the murine OS cell line K7M2 intravenously into syngeneic BALB/c mice. In this model, we could recapitulate anti-tumour activity of standard first-line chemotherapeutic agents alone or in combination (cisplatin, doxorubicin) against micrometastatic disease with significant prolonged survival. Currently, we evaluate the efficacy of anti-PD1 and anti-CTLA-4 alone or in combination in this model of metastatic OS to establish a base-line of efficacy for clinically used immunotherapy. Subsequently, we will combine cytotoxic T-cell activating drugs with drugs targeting the myeloid compartment of the microenvironment and other immunomodulatory strategies with and without chemotherapy. Apart from survival, we will also study systemic immune responses using mass cytometry and plasma protein quantification (O-Link). This way, we hope to identify an optimal treatment combination that could build the basis for future clinical trials.

Drugging the undruggable target: Gemcitabine and cytarabine kill Ewing sarcoma cells through inhibition of SAMHD1 and degradation of the Ewing sarcoma fusion protein

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Themes: Novel Methods and Therapies
Presenter Name: Hala Habash
Keywords: Ewing sarcoma, SAMHD1, precision medicine

Ewing sarcoma (ES) is the second most-frequent bone tumour in childhood and adolescence with a peak incidence rate in the second decade of life. Localised ES has a five-year relapse-free survival rate of only around 55% which drops to 21% for metastatic ES. Survival has largely stagnated for the last decades despite several efforts of treatment intensification.

Resulting from a chromosomal translocation, ES is driven by a fusion protein (most frequently EWS-Fli1 or EWS-ERG) with aberrant transcription factor activity that reprogrammes the cellular transcriptome. Cytarabine, widely used for treatment of haematological malignancies, was identified as a drug that can induce degradation of the fusion protein and thereby reverse its transcriptomic effects, and its anti-tumour efficacy was confirmed in animal experiments. However, a phase-II trial with relapsed and refractory ES patients failed to demonstrate meaningful clinical efficacy of cytarabine. We hypothesize that the cytarabine resistance factor SAMHD1 may explain this discrepancy.

Here, we demonstrated differential SAMHD1 expression in a panel of ES cell lines ranging from absent to high, and SAMHD1 levels correlated with sensitivity to cytarabine. In addition, we used immunoblotting to demonstrate the dose-dependent ability of cytarabine to deplete the ES fusion protein. More important, gemcitabine, a drug used for treatment of relapsed bone sarcomas and a substance that we have previously described as a SAMHD1 inhibitor sensitized ES cells to cytarabine in a SAMHD1-dependent manner and dramatically increased cytarabine-induced ES fusion protein depletion. In a next step, we seek to validate these findings in mouse models of ES. Giving the longstanding use of cytarabine and gemcitabine in paediatric oncology, this research promises to lay the ground for a novel treatment strategy of ES that targets its hitherto undruggable cancer-driving fusion protein which could quickly be translated into a clinical trial.

Dynamic size and association profiles of tumour-derived DNA during pancreatic cancer progression

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Themes: Novel Methods and Therapies
Presenter Name: Daniel W. Hagey
Keywords: circulating tumour DNA, diagnostics, extracellular vesicles

Circulating tumour-derived DNA (ctDNA) has become an important target in oncology since it is accessible in peripheral blood and can provide direct insight into tumour genetics and growth. Although ctDNA is often described as <200bp and associated only with histones, longer forms have also been found in extracellular vesicles (EV). Thus, increased understanding of ctDNA dynamics during disease progression would allow for improved targeting of diagnostic assays. In this work, we have isolated three types of EVs (apoptotic bodies, large and small EVs) and soluble proteins from the blood of pancreatic cancer patients at various stages of disease. We then selectively enriched for long (>400bp) or short (<200bp) fragments in each of these fractions using tagmentation- or ligation-based DNA amplification methods, respectively. We assessed the enrichment of specific blood components using electron microscopy and Western blotting for known markers, and the length and amplification of different species of DNA using chip-based electrophoresis. Finally, we judged the enrichment of ctDNA in each fraction using digital PCR targeting known patient-specific mutations in the KRAS gene. In general, we found that cancer patient blood had increased amounts of long DNA in apoptotic bodies and short DNA in the soluble protein fraction. Using digital PCR, we observed that the majority of ctDNA was found as short fragments associated with small EVs, though long fragments were also enriched in ctDNA, early in disease progression. However, this pattern changed markedly at late-stages of disease progression, such that long ctDNA fragments were relatively evenly distributed in the various fractions and short ctDNA was found primarily in soluble form. These findings suggest that targeting different species of DNA at various stages of disease would improve ctDNA enrichment. As soluble ctDNA is primarily a hallmark of late-stage disease, targeting EV-associated DNA may improve the sensitivity of clinical diagnostics.

EFFECTS OF TGF-β1 ON PREPUBERTAL TESTICULAR TISSUE SAMPLES DURING ORGANTYPIC CULTURES

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Themes: Basic Science

Presenter Name: Yifan Yang

Keywords: Cancer Treatment, Prepubertal testicular explant, Sertoli Cell, Spermatogenesis, Spermatogonia Stem Cell, TGF-β1

High gonadotoxic cancer treatments can negatively impact the numbers and/or functionality of spermatogonia stem cells (SSCs) and testicular somatic cells. Sperm cryopreservation combined with assistant reproduction technologies can restore fertility in adult patients, once they are cured of their disease. However, a method of successfully maturing sperm from immature testicular tissue is still lacking. To create a functional in vitro condition for human germ cell differentiation, the combination of novel culture conditions supplemented with different growth factors is needed. Therefore, the aim of this study was to investigate the effects of transforming growth factor-β (TGF-β1) on SSCs and somatic cells in prepubertal testicular samples in vitro. Testicular samples were obtained from 10 prepubertal boys (2.0 - 13.2 years) participating in the NORDFERTIL project. Explant tissue cultures were supplemented with different concentrations of TGF-β1 (0ng/ml, 1ng/ml, 2ng/ml, and 10ng/ml) and collected on day 0, 7, and 14. Immunohistochemistry was used to evaluate the expression of DDX4 (germ cell marker), androgen receptor, and CYP17A1 (both markers for steroidogenic active cells, such as Leydig cells), CD14 (macrophage marker), as well as SOX9 (Sertoli cell marker) to explore the effect of TGF-β1 on human testicular cells in vitro. Our preliminary results exhibit an increased CYP17a1- and CD14-expression after supplementation with TGF-β1 for 7 days compared with the control group. Interestingly, the expression of CYP17a1 and CD14 was not solely restricted to Leydig cells and macrophages, respectively, but was also observed in Sertoli cells, usually not expressing both markers. Future research will explore the reason for the altered expression profile of CYP17A1 and CD14, and might lead to an explanation of why the explant tissue culture conditions in mice

enable SSC differentiation into functional sperm, but not in humans.

Epidemiological study of paediatric liver tumors in Denmark 1985-2021

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Themes: Solid Tumours
Presenter Name: Thomas Nørrelykke Nissen
Keywords: , Epidemiology, Hepatoblastoma, Liver tumors

Background: Malignant liver tumors in children are rare and national outcomes for this group are rarely reported. This study mapped the paediatric liver tumors in Denmark from 1985 to 2021 and reports on incidence, outcome and long-term adverse events.

Methods: We identified all paediatric malignant liver tumors from the Danish Childhood Cancer and National Pathology Registries and reviewed the case records for patient and tumor characteristics, treatment and clinical outcome.

Results: We included 79 patients with malignant liver tumors in the analyses; Overall crude incidence was ~0,5 per 1 million children (<15y) per year, with 61 hepatoblastomas, 9 hepatocellular carcinomas and 9 other hepatic tumors. Overall, 5 y survival was 84%, 78% and 44% respectively. For hepatoblastomas Pretext IV, age > 8 y and metastases were all predictors of a poorer outcome. Liver transplantation was performed in 16% of the cases with an overall survival of 80% compared to 84% for patients receiving surgery.

Adverse events were: Reduced renal function in 12% (GFR<90), reduced cardiac function in 7% (EF<55) and impaired hearing function in 54% (Boston 1-4).

Conclusions: Survival for hepatoblastomas in Denmark is comparable with international results. Stratifying treatment factors in current protocols were confirmed to be significant in the Danish cohort i.e., age, pretext and metastases at diagnosis. Hearing loss is the major treatment related side effect and affects about 50% of the patients.

ETV6-RUNX1 ENHANCES THE SELF-RENEWAL OF HEMATOPOIETIC STEM CELLS AND CORRUPTS EARLY LYMPHOID DIFFERENTIATION

by Mohamed Eldeeb | Lund University

Themes: Leukemia
Presenter Name: Mohamed Eldeeb
Keywords: Acute lymphoblastic leukemia, ETV6/RUNX1, hematopoietic stem cells, mouse model

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Acute lymphoblastic leukemia (ALL) is by far the most predominant blood cancer in pediatric patients, accounting for ~80% of the cases. Of these, a large portion carries the ETV6-RUNX1 translocation, a somatic translocation that emerges in utero. By itself, the ETV6-RUNX1 fusion gene is insufficient for leukemogenesis, and secondary events/hits are always required for transformation. Previous studies have proposed hematopoietic stem cells as the potential cell of origin for ETV6-RUNX1-associated ALL; however, an appropriate experimental model to answer this question definitively has been lacking. Here, we generated a mouse model in which ETV6-RUNX1 can be conditionally activated in any primary cell type of choice upon Tetracycline treatment and confirmed that ETV6-RUNX1 accelerates B-ALL development in the context of other pediatric B-ALL associated secondary events. Without such mutations, and in line with previous studies, we observed that ETV6-RUNX1 led to an expansion of phenotypically defined HSCs and a strong block in B-cell differentiation. With the benefits offered by our model, in which we can turn off the expression of ETV6-RUNX1, we could confirm that the candidate HSCs that expand in response to ETV6-RUNX1 are phenotypically and not the least functionally similar to normal HSCs. Moreover, by comparing the impact of ETV6-RUNX1 expression in fetal versus adult HSCs, we could show that the former cells possess a higher ability to bypass the early stages of the B-cell differentiation block. Through our ongoing work, we aim to unravel the functional and molecular differences between fetal and adult HSCs in response to ETV6-RUNX1 expression. It is our anticipation that these approaches will provide new insights on the biology that underlie the most common ALL translocation found in children.

EVALUATION OF ONCOFERTILITY CARE IN CHILDHOOD CANCER PATIENTS, THE EU-HORIZON 2020 TREL PROJECT

by Egle Stukaite-Ruibiene1 | M. E. Madeleine van der Perk2 | Goda Elizabeta Vaitkeviciene1,3 | Annelies M E Bos2 | Zana Bumbuliene1,4 | Marry M. van den Heuvel-Eibrink2 | Jelena Rascon1,3 | Vilnius University, Faculty of Medicine, Vilnius, Lithuania | Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands | Vilnius University, Faculty of Medicine, Vilnius, Lithuania, Center for Pediatric Oncology and Hematology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands | Vilnius University, Faculty of Medicine, Vilnius, Lithuania, Center of Obstetrics and Gynecology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands | Vilnius University, Faculty of Medicine, Vilnius, Lithuania, Center for Pediatric Oncology and Hematology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania

Themes: Late Effects
Presenter Name: Egle Stukaite-Ruibiene
Keywords: Childhood cancer; fertility counseling; late effects; questionnaire; reproductive health

Background. While the five-year survival rate of childhood cancer exceeds 80%, many survivors develop late effects including infertility. However, most survivors perceive the information regarding fertility as insufficient. The aim of this study was to evaluate the current status of oncofertility care at Vilnius University Hospital Santaros Klinikos (VULSK) within the framework of the EU-HORIZON 2020 TREL project.

Material and methods. All parents or patients aged 12-17.9 years treated from July 1, 2021 until July 1, 2022 were invited to complete oncofertility-care-evaluation-questionnaires. After completing questionnaires a slightly amended version of the previously published tool (triage) by the Dutch Princess Máxima Center was used to stratify respondents to low (LR) or high risk (HR) of gonadal damage.

Results. Questionnaires were completed by 48 parents and 13 children. Triage resulted in 36 (59%) LR and 25 (41%) HR respondents. Six boys (4 HR, 2 LR) were counseled by a fertility specialist. Three HR boys underwent sperm cryopreservation without a delay in treatment. Only 17 (28%, 9 HR, 8 LR) respondents correctly estimated their gonadal damage risk. All counseled boys (n=6) agreed the risk for fertility impairment was mentioned compared to 49.1% (n=27) of uncounseled. All counseled respondents agreed they knew enough about fertility (vs 42%) and all knew their fertility preservation options (vs 38.8%). Only 10/55 (19.2%) of not counseled respondents received supportive material on fertility (vs 66.7%)

Conclusions. A minority of respondents correctly estimated their infertility risk. Fertility preservation was performed for a small number of male HR respondents. Results revealed a lack of information on fertility and a need for the provision of supportive material. Respondents counseled by a fertility specialist received more information than uncounseled

respondents. Based on the current experience oncofertility care at VULSK will be improved.

EVALUATION OF SPERMATOGONIAL CELL POPULATIONS IN MALE CHILDHOOD CANCER PATIENTS

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Themes: Basic Science
Presenter Name: Hajar Ba Omar
Keywords: Spermatogonia, childhood cancer, fertility preservation, stem cells, testis

Spermatogenesis is a continuous process based on the maintenance and differentiation of spermatogonial stem cells (SSCs) that enables the production of sperm. Medical treatment protocols applied in paediatric oncology include potentially gonadotoxic therapies that can affect SSCs and thus lead to the risk of subfertility. Recent single-cell RNA sequencing studies have identified SSC subpopulations (from undifferentiated (state 0) to differentiated (state 4) spermatogonia) with distinctive marker expression profiles.

In the present study, six markers (UTF1, GFRA1, ID4, PIWIL4, FGFR3, C-KIT) expressed in specific SSC states (0-4) were identified in prepubertal testicular tissue of 31 boys (0.7 – 13.1 years of age) with oncological diagnoses participating in the NORDFERTIL fertility preservation program. Immunofluorescence staining on PFA-fixed testicular tissue sections was correlated to the age of patients and treatment exposures.

Higher numbers of spermatogonia per tissue area in more advanced states were observed in older patients who were unexposed or exposed to non-alkylating agents. Decreasing spermatogonial numbers correlated with increasing exposure to cumulative cyclophosphamide equivalent doses (CED). While spermatogonia numbers were decreased, some early-state SSC reserve stem cells expressing PIWIL4 and FGFR3 were identified in 13

out of 17 (exposed to CED 2000-16000 mg/m^2) and 12 out of 17 (exposed to CED 220-16000 mg/m^2) testicular samples, respectively.

Identifying reserve stem cells may provide a tool to estimate the fertility potential of testicular tissue collected for fertility preservation. Future studies are needed to elucidate the functionality of the identified reserve stem cells.

HEPATIC LESIONS OF FOCAL NODULAR HYPERPLASIA IN PATIENTS AFTER ONCOLOGIC THERAPY FOR EWING SARCOMA

by Dominika Vasilevska | Grazina Kleinotiene | Indre Tamuliene | Goda Elizabeta Vaitkeviciene | Irina Adomaitiene | Faculty of Medicine, Vilnius University, Lithuania | Vilnius University Hospital Santaros Klinikos, Lithuania | Vilnius University Hospital Santaros Klinikos, Lithuania | Vilnius University Hospital Santaros Klinikos, Lithuania | Vilnius University Hospital Santaros Klinikos, Lithuania

Themes: Solid Tumours
Presenter Name: Dominika Vasilevska
Keywords: Ewing sarcoma; focal nodular hyperplasia; follow-up.

Introduction

Focal nodular hyperplasia (FNH) is a hepatic regenerative lesion with characteristic radiographic features. Multimodal imaging or biopsy could be used to diagnose FNH. FNH lesions have been reported in children survivors of extra-hepatic malignancies.

Aim

We report 2 cases of hepatic FNH in Ewing sarcoma survivors with 1 year follow-up.

Methods

Girl, 6 years, Ewing sarcoma (EWSR1 (22q12) translocation) of pubic bone with metastases to the abdomen, treated with the neoadjuvant chemotherapy – 6 VIDE cycles, resection of the abdominal masses, adjuvant chemotherapy 8 cycles of VAC and radiotherapy with stem cell transplantation (SCT), as the primary tumor was inoperable. 2 years after treatment suspected hepatic FNH lesion was detected on MRI scan. Contrast enhanced ultrasound (CEUS) was negative. CT scan revealed multiple hypervascular lesions. PET scan revealed no metabolic activity. During 1 year of watchful waiting some lesions grew, some had disappeared.

Boy, 10 years, Ewing sarcoma (t(11;22) EWSR1/FLI1 translocation) of the rib, treated with neoadjuvant chemotherapy (6 VIDE cycles) and 8 VAI cycles after the surgical resection. Recurrence in the diaphragm treated with 5 cycles of irinotecan, temozolomide, SCT, and radiotherapy. 2 years after the treatment suspected hepatic FNH lesion was detected on MRI and CT, characterized as a malignant on CEUS. A biopsy was performed due to the lesion’s growth on the control MRI. FNH was proved.

Results

Confirmed FNH lesions were detected 2 years after the curative treatment of Ewing sarcoma after the same onco-therapy in both patients. No malignant progression of the liver

lesions after 1 year follow-up in both patients.

Conclusions

Children can develop liver lesions after successful Ewing sarcoma treatment. Radiological modalities (MRI, PET) allows to prove the etiology of the benign lesion without intervention. However radiological diagnosis remains challenging due to atypical FNH appearance and enhanced attention to any new lesions.

HOMOZYGOUS PATHOGENIC VARIANT IN SLC19A2 GENE IN TWO UNRELATED LITHUANIANS WITH THIAMINE RESPONSIVE MEGALOBLASTIC ANEMIA

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Themes: Benign Hematology
Presenter Name: Mackus Lukas
Keywords: SLC19A2 gene mutation, Thiamine responsive megaloblastic anemia (TRMA), non-type I diabetes mellitus, sensorineural deafness

Background. Thiamine responsive megaloblastic anemia (TRMA) is a very rare autosomal recessive syndrome (prevalence <1/1.000.000), commonly presented in consanguineous families and characterized by the triad: megaloblastic anemia, progressive sensorineural deafness and non-type I diabetes mellitus. TRMA is caused by broad spectrum of *SLC19A2* gene pathogenic variants, generating defects in high affinity thiamine transporters. Treatment consists of daily significant doses of thiamine. Long-term treatment effect is generally unknown.

Results. Presenting two patients from non-consanguineous families in Lithuania diagnosed with TRMA. Both patients presented with introduced triad of symptoms and bone marrow aspirates revealed megaloblastic erythropoiesis and ringed-sideroblasts. First patient was diagnosed with TRMA at 3 years of age. The patient had poorly controlled diabetes (onset 11 months), bilateral hearing loss (onset 7 months), megaloblastic anemia (onset 2 years), decreased vision acuity and photophobia (onset 2.5 years). Novel homozygous NM_006996.3:c.205G>T, NP_008927.1:p.(Val69Phe) pathogenic variant in *SLC19A2* gene was detected. Thiamine replacement therapy improved general condition, stabilized diabetes course and resolved anemia. After nine years of follow-up – no signs of disease progression. Recently, a 17 year-old boy was diagnosed with TRMA who was previously treated for refractory megaloblastic anemia (onset in infancy), bilateral sensorineural deafness (*GJB2* gene pathogenic variants causing sensorineural deafness were excluded at 3 years old), and non-type I diabetes mellitus (onset 3 years). The same homozygous c.205G>T pathogenic variant in *SLC19A2* gene was detected. Anemia resolved within weeks after thiamine treatment was initiated.

Conclusions. Although hearing loss was irreversible, treatment with thiamine resolved

anemia and stabilized diabetes course for both patients, as well as improved general condition. The same *SLC19A2* gene pathogenic variant in two unrelated patients could speculate on common ancestor and possibly more families are affected or carriers in Lithuania. Early diagnoses and treatment initiation could considerably improve quality of life and minimize the risk of debilitating clinical conditions.

HORMONE PRODUCTION IN TESTICULAR SAMPLES CULTURED FROM BOYS ENROLLED IN THE NORDFERTIL STUDY

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Themes: Basic Science
Presenter Name: Femke Harteveld
Keywords: Sickle cell disease, alkylating agents, childhood cancer, fertility preservation, hormone production

Prior to receiving highly gonadotoxic therapies, testicular cryopreservation can be offered to paediatric patients. A cohort of 60 prepubertal boys (0.8 – 15.7 years) participated in the experimental research study program NORDFERTIL. Enrolled patients were at high risk of infertility due to facing either haematopoietic stem cell transplantation or testicular irradiation. This study aimed to investigate the functionality of somatic cells in prepubertal testicular samples obtained for fertility preservation by measuring *in vitro*-produced hormone concentrations. The samples were divided into four study groups according to diagnosis and chemotherapy exposure before biopsy: (1) non-treated/non-alkylator-exposed cancer patients, (2) sickle cell disease (SCD) patients treated with hydroxyurea, (3) non-treated patients suffering from non-malignant diseases and (4) alkylator-exposed cancer patients. Over 21 days, the testicular samples were cultured in air-liquid interface culture conditions, and media was refreshed and collected weekly. Following culture, ELISA was used to determine the levels of *in vitro* anti-Müllerian hormone (AMH), inhibin B, and testosterone in media obtained after 7, 14, and 21 days. One-way ANOVA on ranks was applied to determine if there were statistically significant differences between the four groups. Spearman's rank correlation analysis was performed to determine the association between the hormone secretions per experimental time point and age, cumulative cyclophosphamide equivalent dose (CED) and cumulative anthracycline dose (DIE). When comparing the measured hormone secretions between study groups, we observed a trend of decreased hormone secretions in SCD patients. We did neither observe a significant decline in hormone secretions in any other study group, nor observed a significant correlation between either CED or DIE exposures and hormone secretions. However, a significant negative correlation was observed between age and hormone secretions ($p<0.05$). In conclusion, we suggest that *in vitro* secretion of hormones could contribute to assessing the quality of obtained testicular tissue samples obtained for fertility preservation.

Hsa-miR-323a-3p functions as a tumor suppressor and targets STAT3 in 2 neuroblastoma cells

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Themes: Solid Tumours
Presenter Name: Trond Flægstad
Keywords: , Neuroblastoma, micro-RNA

Background: Studies conducted the last decades have revealed a role for the non-coding microRNAs (miRNAs) in cancer development and progression. Several miRNAs within the chromosome region 14q32, a region commonly deleted in cancers, are associated with poor clinical outcome in the childhood cancer - neuroblastoma. We have previously identified miR-323a-3p from this region to be downregulated in chemotherapy treated neuroblastoma cells compared to pre-treatment cells from the same patients. Furthermore, in neuroblastoma tumors, this miRNA is downregulated in advanced stage 4 disease compared to stage 1-2. In this study, we attempt to delineate the unknown functional roles of miR-323a-3p in neuroblastoma.

Methods: Synthetic miRNA mimics were used to overexpress miR-323a-3p in neuroblastoma cell lines. To investigate the functional roles of miR-323a-3p, cell viability assay, flow cytometry, reverse transcription-quantitative polymerase chain reaction, luciferase reporter assay and western blot were conducted on the neuroblastoma cell lines Kelly, SH-SY5Y and SK-N-BE(2)-C.

Results: Ectopic expression of miR-323a-3p resulted in marked reduction of cell viability in Kelly, SH-SY5Y and SK-N-BE(2)-C by causing G1-cell cycle arrest in Kelly and SH-SY5Y and apoptosis in all the cell lines tested. Furthermore, mRNA and protein levels of signal transducer and activator of transcription 3 (STAT3) were reduced upon miR-323a-3p overexpression. A direct binding of the miR-323a-3p to the 3'UTR of STAT3 was experimentally validated by luciferase reporter assay, where miR-323a-3p reduced luminescent signal from full length STAT3 3'UTR luciferase reporter, but not from a reporter with mutation in the predicted seedsequence.

Conclusions: miR-323a-3p inhibits growth of neuroblastoma cell lines through G1-cell cycle arrest and apoptosis, and the well-known oncogene STAT3 is a direct target of this miRNA.

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HUMAN PRIMORDIAL GERM CELL-LIKE CELLS: THE BEGINNING OF A PROMISING SOLUTION

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Themes: Late Effects
Presenter Name: João Pedro Alves Lopes | Jan-Bernd Stukenborg
Keywords: fertility preservation, human primordial germ cell, regenerative medicine

Advances in the treatment of childhood cancer have contributed to a growing number of cancer survivors. Children undergoing gonadotoxic cancer treatment have high risk to become infertile later in life. The opportunity to preserve or restore the fertility of these patients will create a positive impact not only in the clinical context, but also in the quality of life of patients and their families. Unfortunately, the preservation of immature ovarian and testicular tissue cannot be offered for everyone before sterilizing therapy. The only solution for these patients is to produce eggs or sperm from their own induced pluripotent stem cells (iPSCs) after sterilizing therapy.

In our research work, we are applying the cutting-edge knowledge on human primordial germ cell (hPGCs), the precursors of sperm and eggs, to engineer *in vitro* systems that more accurately model the early steps of human germline development. Recently, we published new protocols for hPGC-like cell (hPGCLCs) specification from human embryonic stem cells cultured in states in between the naïve and the primed pluripotency. Currently, we are applying these new protocols for hPGCLC specification on human iPSCs. Our preliminary results show that the translation of our protocols is robust and effective for all human iPSC lines tested so far. We detected the presence of hPGCLCs (OCT4, BLIMP1, NANOG, and SOX17 expressing cells) in all samples analysed.

To become biological parents, the generation of gametes from patient-specific iPSCs will be the only possibility for patients that face permanent sterility and were not offered the possibility to preserve their immature ovarian or testicular tissues. Moreover, iPSC-derived

somatic supportive tissues may also help to develop techniques that will contribute to *in vitro* maturation of eggs and sperm from cryo-preserved immature ovarian and testicular samples.

Identification of leukemic stem cells in infant acute leukemia

by Masafumi Seki | Hisashi Ishida | Karin Belander Strålin | Amy Hillen | Edwin Chari | Ellen Markljung | Kari Högstrand | Petter S. Woll | Sten Eirik W. Jacobsen | Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden | Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden | Department of Pediatric Oncology, Karolinska University Hospital Solna, Stockholm, Sweden | Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden | Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden | Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden | Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden | Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden | Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden

Themes: Leukemia
Presenter Name: Masafumi Seki
Keywords: Infant leukemia, MLL rearrangement, MLL-AF9, mouse model

Introduction: Oncogenic translocations involving the MLL gene are particularly prevalent in infant leukemia and associated with poor prognosis. The MLL-AF9 translocation is associated with a striking age dependence as MLL-AF9 is sufficient to establish primarily acute lymphoblastic leukemia (ALL) during fetal development, whereas acute myeloid leukemia (AML) dominates in adults with MLL-AF9. The mechanisms underlying the age-dependent features remain elusive.

Aims: The overall aim is to explore the biological and clinical significance of the normal fetal cellular targets underlying the propensity of MLL-AF9 to initiate leukemic transformation at different stages of ontogeny and in distinct cellular compartments, in order to identify the relevant leukemic stem cells and their novel therapeutic targets.

Methods: We have established a novel mouse model allowing for the conditional expression of Mll-Af9 at different stages of ontogeny and within distinct hematopoietic stem and progenitor compartments when combined with promoter-specific Cre or CreERT2 recombinase. Leukemia development following Mll-Af9 activation was monitored, and the leukemia was further characterized.

Results: Cre-mediated Mll-Af9 activation targeted to multipotent stem and progenitor cell stages using Vav1Cre, Fgd5CreERT2, Flt3Cre, Il7rCreERT2, Rag1Cre, and Mb1Cre resulted in AML development, regardless of whether Mll-Af9 expression was initiated during fetal or adult ontogeny. However, following activation of Mll-Af9 by Mb1Cre, which is first expressed in committed B cell progenitors, B lineage leukemia was observed (3/17 mice). The onset of B-ALL was first detected after 6 months of age, and we confirmed the generation of B-ALL propagating cells by regeneration of B lineage leukemia after transplantation into irradiated recipients.

Conclusion: We demonstrate B-ALL development in a mouse model of MLL-AF9 leukemia,

which now allows the characterization of the cellular target for MLL-AF9-mediated B-ALL transformation. In future studies, we will further extrapolate our findings also to primary samples obtained from patients with infant/childhood ALL/AML with MLL-AF9.

INTRAVENOUS IRON INFUSIONS IN PEDIATRIC PATIENTS: A SINGLE CENTER EXPERIENCE

by Bernatonytė Ernesta 1 | Šaulytė-Trakymienė Sonata 2 | 1 Faculty of Medicine, Vilnius University, Lithuania | 2 Clinic of Children's Diseases, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius University Hospital Santaros Clinics, Vilnius, Lithuania

Themes: Other
Presenter Name: Ernesta Bernatonytė
Keywords: anemia, intravenous iron, iron deficiency

Introduction: Current guidelines recommend oral iron as the first-line treatment for iron deficiency anemia (IDA), with intravenous (IV) iron mostly being used off-label in pediatric patients. However, 10-40% of patients experience adverse effects from oral iron.

Aim of the study: To evaluate effectiveness and safety of IV iron in pediatric patients treated with IV iron for absolute and functional IDA.

Material and Methods: Pediatric patients aged 1-17 who received IV iron at Vilnius University Hospital Santaros Clinics, Department of Pediatric Hematology/Oncology between Jan 2021 and Dec 2022 were reviewed for retrospective data collection. Patient demographics, pre-infusion and post-infusion laboratory values and adverse effects were analyzed using Microsoft Excel and R Studio.

Results: A total of 25 patients were included. The median age was 14 years. All 25 patients had IDA refractory to oral iron treatment. 7 out of 25 (28%) received low molecular weight dextran, 18 (72%) iron isomaltoside. The mean follow-up time for early and late post-infusion laboratory values were 4 days and 5 weeks, respectively. The early response, evaluated by hemoglobin content of reticulocytes (RET-He), was with median pre-infusion of 17.6 pg (10.9-31.3 pg) and 28.1 pg (14.22-35.1 pg) post-infusion. The median hemoglobin pre-infusion and post-infusion was 90 g/L (42-131 g/L) and 128 g/L (87-142 g/L), respectively. The mean of the mean corpuscular volume (MCV) pre-infusion was 71.4 (SD=10.7) and 81.7 (SD=7.4) post-infusion. The median serum ferritin pre-infusion was 6.1 µg/L (1.4-121.1 g/L) and 139 µg/L (24.14-448.5 µg/L) post-infusion. 23 patients (92%) experienced no adverse effects. 2 patients (8%) experienced short-term heart palpitations and pressure in the chest during infusion. There were no severe hypersensitivity reactions.

Conclusion: We deem IV iron an effective and safe alternative for pediatric patients with IDA when oral iron is infeasible or ineffective. RET-He was the most sensitive measure of response to IV iron.

METASTATIC OSTEOBLASTIC OSTEOSARCOMA IN A PATIENT WITH HEREDITARY CANCER SYNDROME, CASE REPORT

by Zhanna Kovalova | Baiba Lace | Ksenija Soldatenkova | Zane Abola | Dzintars Ozols | University Children's Hospital | University Children's Hospital | University Children's Hospital | University Children's Hospital

Themes: Solid Tumours
Presenter Name: Zhanna Kovalova | Zhanna Kovalova
Keywords: ATM gene, hereditary predisposition, osteosarcoma

Osteosarcoma is generally considered a rare and sporadic tumor, however some cases with genetical predisposition have been confirmed. We report the case of a 15-year-old adolescent with metastatic osteoblastic osteosarcoma of diaphysis of the right femur with genetical cancer predisposition syndrome. The girl presented in hospital with symptoms of right knee swelling, dynamically progressing pain and difficulties in doing daily physical activities. The patient underwent neoadjuvant chemotherapy followed by osteoblastic osteosarcoma resection and knee joint endoprosthetic reconstruction treatment, combined with adjuvant chemotherapy courses, and local therapy of pulmonary metastases, the total amount of metastatic resected lesions in right lung was 44, on left lung 56. There was no family history of malignant diseases.

Whole exome sequencing was performed using the Illumina's sequencing-by-synthesis method, which followed by analysis of the 160 genes of Comprehensive Hereditary Cancer Panel done by private company.
Pathogenic heterozygous variant c.7630-2A>C in ATM gene was identified. GnomAD frequency of variant is low (0.001%). The variant c.7630-2A>C affects a splice acceptor site in intron 51, hence results in skipping of exon 52 and introduction a premature termination of ATM protein. It has also been described as a pathogenic founder variant in individuals of Polish descent.
The girl is in remission during last 2 years. This study presents a case of surgical arthroplastic knee joint treatment manifestation in a pediatric patient with distal femur metastatic osteoblastic osteosarcoma combined with scheduled chemotherapy courses and resection of pulmonary metastases in a patient with hereditary cancer syndrome.Osteosarcoma is generally considered a rare and sporadic tumor, however some cases with genetical predisposition have been confirmed.

NIJMEGEN BREAKAGE SYNDROME IN LATVIAN PAEDIATRIC POPULATION IN 1997-2022

by Zhanna Kovalova | University Children's Hospital

Themes: Other
Presenter Name: Zhanna Kovalova
Keywords: Nijmegen breakage syndrome, Non-Hodgkin lymphoma, immunodeficiency

Nijmegen breakage syndrome (NBS) is an autosomal recessive, chromosomal instability disorder with prevalence among the Slavic population characterized by microcephaly, dysmorphic facial features, growth and mental retardation, immunodeficiency, high predisposition to malignancy and infections.

A retrospective, population-based analysis of all 5 known Latvian children with confirmed NBS are summarised.

We diagnosed 5 paediatric patients of Slavic origin with NBS, 3 males and 2 females. 2 of 5 children were siblings. The time to a genetically proved NBS diagnosis ranged from 1 to 18 years. All patients presented with typical facial features, growth retardation from -2 to -4 SD, mild mental retardation, primary immunodeficiency (PI), 2 patients had *café-au-lait* spots, experienced different infections, including cytomegalovirus. 4 of 5 known patients with NBS developed malignancies: 3 T-lymphoblastic NHL, 1 peripheral T-cell lymphoma. 3 children presented with lymphadenopathy, vena cava syndrome, pleuritis and pericarditis. The median age at NHL diagnosis was 35 months (12 to 55 months). 2 patients had bone marrow (BM) infiltration. NHL was treated according NHL-BFM 95 protocol with dosage reduction to 75%. 2 of 4 patients achieved remission. The 2 patients with BM involvement showed rapid fatal progression during therapy, in 1 case with CNS involvement. One patient with T-NHL developed T-ALL after 4 years of remission, complete remission duration for 2 years.

Since NBS was reported in 1981, 5 Latvian patients have been confirmed, 3 are alive, 2 of them are in remission for 7 and 22 years. The treatment of malignant complications remains challenging due to PI, increased toxicity of chemotherapy.

ONCOLOGISTS' KNOWLEDGE, PRACTICE AND ATTITUDE TOWARD FERTILITY PRESERVATION: A NATIONAL SURVEY

by Halima Albalushi | Sultan Qaboos University

Themes: Other
Presenter Name: Halima Albalushi
Keywords: Fertility preservation, awareness, oncofertility, oncologists

Improved chemotherapy and radiotherapy treatment protocols, fortunately, increased the rates of cancer survivors over the years. However, these treatments are accompanied by late effects and may result in infertility or subfertility in those survivors. Oncologists are often the first line of contact with this group of patients and are considered the gateway for knowledge about cancer and its treatments effects including those related to infertility. Several studies suggested that many oncologists do not discuss fertility or fertility preservation with their patients.

This study aimed to explore the perspective of oncologists treating patients with oncological diseases, in Oman, on fertility preservation.

A cross-sectional study using a standardized and validated questionnaire was used to collect data.

Thirty-four oncologists completed the questionnaire, giving a response rate of 48%. Participants reported that they are knowledgeable about sperm cryopreservation and gonadotropin-releasing hormone agonists but no other methods of fertility preservation. About 94% of the participants reported that they need more knowledge about fertility preservation.

More than half of the participants had never encountered cancer patients who used ovarian cryopreservation, testicular tissue cryopreservation, in vitro fertilization with embryo cryopreservation and oocyte cryopreservation. The majority (78 %) agreed that discussing fertility preservation with newly diagnosed cancer patients is a high priority. Most oncologists in Oman are supportive of fertility preservation and feel comfortable discussing it with their patients. However, they reported that lack of knowledge and lack of availability of well-structured fertility preservation services in Oman hinders the initiation of fertility preservation discussions with their patients.

Establishing a national training program on fertility preservation that involves all health workers deal with cancer patients will improve the care given to such patients. Moreover, it is essential to have well-structured fertility preservation services to facilitate collaboration with countries with advanced services and care provided to such patients.

PATTERN AND PREVALENCE OF LIVER INVOLVEMENT AT DIAGNOSIS IN PEDIATRIC ACUTE LYMPHOBLASTIC AND MYELOID LEUKEMIA

by Vakkila Jukka | Sandart Amelie | Arnell Henrik | Fischler Björn | Harila Arja | Clinicum, University of Helsinki | Department of Pediatrics, Karolinska University Hospital | Department of Pediatrics, Karolinska University Hospital | Clintec, Karolinska Institutet | Department of Women\\\\\\\'s and Children\\\\\\\'s Health, University of Uppsala

Themes: Leukemia
Presenter Name: Vakkila Jukka
Keywords: ALL, AML, INR, Liver, hepatomegaly

Objectives The prevalence and significance of liver involvement at diagnosis was studied in pediatric acute lymphoblastic (ALL) and myeloid leukemia (AML).

Methods A population based cohort of 189 patients (122 pre B-ALL, 22 T-ALL and 45 AML patients) was formed from the NOPHO registries (2005–2017). Hepatomegaly, elevated ALT (>41 IU/L), elevated international normalized ratio (INR) (>1.2), hypoalbuminemia (<34 g/L), low fibrinogen (<1.9 g/L) and conjugated hyperbilirubinemia (≥10 µmol/L) were used as markers for liver involvement. Minimal residual disease (MRD), time to relapse and overall survival were correlated with liver involvements.

Results Abdominal ultrasound was done for 143 patients and hepatomegaly was diagnosed in 57 patients (39.9%). The most significant risk factors were high white blood cell count (WBC) and low fibrinogen. Elevated ALT, was observed in 30 patients (16%) and its presence did not associate with hepatomegaly, WBC or any particular leukemia subset. A significant association, however, was found with low fibrinogen counts (P=0.008). Conjugated hyperbilirubinemia was present in less than 5% of patients. Signs of decreased liver function (defined as low fibrinogen, hypoalbuminemia and elevated INR) was observed in 7 patients (4%). The proportion of patients with no liver abnormalities was highest in AML and least frequent in T-ALL. Liver involvement in patients with ALL was not associated with toxicity or outcome. However, in patients with AML, liver involvement associated with lower overall survival.

Conclusions Liver involvement at diagnosis is relatively common and is more frequent in pediatric ALL than AML. Its presence associates with high WBC count and low fibrinogen. In ALL, the response to the therapy or overall survival is not affected by liver involvement at diagnosis. In AML, liver involvement associates with suboptimal prognosis.

PECULIAR CASE OF SEVERE IRON-DEFICIENCY ANAEMIA: GASTRIC TRICHOBEZOAR

by Gaudas Benediktas Trakymas | Arunas Strumila | Vilma Rutkauskaite | Faculty of Medicine, Vilnius University, Lithuania | Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania

Themes: Benign Hematology
Presenter Name: Gaudas Benediktas Trakymas
Keywords: bezoar, esophagogastroduodenoscopy, iron, iron-deficiency anaemia, trichobezoar

Introduction.

Trichobezoar is the accumulation of swallowed hair in the gastrointestinal tract. Although rare, it is an important cause of iron-deficiency anaemia that might lead to severe complications.

Aim.

To report our experience with the diagnostics and management of iron-deficiency anaemia caused by gastric trichobezoar.

Methods.

Our patient was a 13-year-old female, admitted to the hospital after experiencing a syncope episode. The patient underwent anamnesis collection, complete blood count, serum ferritin test, heart and visceral organ ultrasound and, due to suspicion of a gastrointestinal cause, flexible esophagogastroduodenoscopy (EGD). The patient received packed red blood cell (PRBC) transfusion and intravenous iron infusion, as well as underwent a laparotomic intervention.

Results.

Upon admission, the patient presented with pale appearance, koilonychia, flaky skin, complaints of falling hair and episodic epigastric pain. Laboratory tests revealed microcytic hypochromic anaemia and a lowered serum ferritin concentration. Without a notable history of anaemia, the patient reported having a regular menstrual cycle and recently following a diet, which led to her losing 10 kg of body mass. Ultrasound results were unremarkable. EGD, on the other hand, revealed a seemingly huge aggregate of undigested hair in the stomach. Correspondingly, laparotomic surgery revealed a massive stomach-shaped trichobezoar filling the whole gastric cavity. Following successful surgical removal of the trichobezoar (Figure 1) as well as the PRBC transfusion and intravenous iron infusion, the patient's ferritin and haemoglobin concentrations were successfully restored to normal, as confirmed by 2-week and 3-month follow-up evaluations.

Conclusions.

Our case seeks to show that a less typical phenomenon, a trichobezoar, can be the culprit for iron-deficiency anaemia. Accurate diagnosis as well as timely and appropriate management can prevent unnecessary complications and preserve patient’s health. Psychiatric referral is important, as patients with this condition may have underlying psychiatric problems.

PILOT FEASIBILITY STUDY FOR DEVELOPMENT AND VALIDATION OF INTEGRATED ELECTRONIC INSTRUMENT TO ASSESS PATIENT REPORTED MEASUREMENTS

by Goda Vaitkeviciene | Roma Puronaite | Justas Trinkunas | Danguole Jankauskiene | Clinic of Pediatrics, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania | Vilnius University Hospital Santaros klinikos, Vilnius, Lithuania | Vilnius University Hospital Santaros klinikos, Vilnius, Lithuania | Mykolas Romeris University, Vilnius, Lithuania

Themes: Other
Presenter Name: Goda Vaitkeviciene
Keywords: Children, cancer, health-related quality of life, patient reported experience, patient reported outcomes

Background. Feedback of patients' experiences at the different stages of healthcare system and their reported outcomes in health-related quality of life (HRQoL) is crucial to improve the healthcare and quality of life for children with cancer and severe hematological illnesses. In view of modern patient-centered approach, involvement of the patients into treatment process and healthcare management is an important, however, difficult process. **Aim.** To develop and test the feasibility of a combined e-health system-integrated instrument for the assessment of patients reported outcomes (PROMs) in HRQoL and experience measures (PREMs) during the journey throughout the different parts of healthcare system. **Methods.** PedsQL generic core scale was used for HRQoL evaluation and an original PREM instrument was developed to measure patients reported experiences in different stages of patients journey including diagnostic approach within primary care, inpatient and outpatient treatment in tertiary center, reabilitation and social integration. Consented parents and ≥10 year-old patients received the e-mails with the links to on-line questionnairs that were integrated into the hospital e-system. Responses were automatically pseudoanonymized and sent to the study investigators for analysis. **Results.** Two hundred questionnaire links were sent out to parents of 94 patiens and ≥10 year-old patients with 87 responses received (43.5%). Mean time to fill-in the questionnair was 3.5 min., most quickly filled-in by children ≥10 years (mean: 1.9 min) and most slowly by parents of 13-18 year-old patients (mean: 5.7 min). Analysis of associations between reported HRQoL scores and experiences in healthcare system revealed significant relationship ($p < 0.05$) between better social dimensions evaluation scores and better experiences in all levels of healthcare system. **Conclusions.** Integrated e-instrument is feasible with a short filling time and convenient retrieval of pseudoanonymized data for analysis. A better option for providing with the link to e-questionnaire should be suggested to improve response rates.

Prepubertal patient-derived testicular organoids

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Themes: Basic Science
Presenter Name: Cui, Yanhua
Keywords: SOX9, Sertoli cells, Testicular organoid, childhood cancer, fertility preservation, spermatogenesis

Prepubertal boys subjected to high gonadotoxic treatments are at high risk of suffering from fertility-related problems in adult age. Modelling human testicular function *in vitro* paves the road for novel strategies to restore the fertility of these boys. However, such efficient experimental models are still under development. Therefore, we generated testicular organoids from human prepubertal testicular tissue collected for clinical fertility preservation and studied the impact of patient age and previous chemotherapy exposure on organoid assembly.

Testicular samples obtained from 12 boys (0.8–13.3 years) participating in the NORDFERTIL fertility preservation project, were enzymatically digested and applied to the three-layer gradient culture system. Immunofluorescence analysis was carried out on testicular organoids after seven days to evaluate the presence of germ and somatic cells, such as Sertoli, Leydig and peritubular myoid cells as well as extracellular matrix proteins. Statistical significance between the two groups was evaluated by the Mann-Whitney test. Pearson correlation coefficient (r) was used to study correlations between SOX9 expression and patient age, treatment regime and reaggregation pattern.

We observed that primary testicular cells derived from prepubertal boys assembled in two different patterns, single aggregates (testicular organoids (TOs)) and multiple aggregates. In contrast to the multiple aggregates (n=7), the TOs (n=5) displayed compartmentalized tubular structures and interstitial areas. We observed that testicular samples expressing a higher ($p<0.05$) proportion of SOX9-expressing Sertoli cells (n=5, 80.1 ± 15.8%) were able to form TOs, while samples exhibiting a lower proportion of SOX9-expressing Sertoli cells (n=7, 31.8 ± 32.0%) failed to form TOs. In addition, the capacity to assemble TOs is negatively correlating with the dose of exposure to alkylating agents before biopsy.

This study indicates that SOX9 expression may serve as a quality assessment for the potential options for testicular engineering for childhood cancer patients.

PRESCRIPTION DRUG PURCHASES AND SPECIAL REIMBURSEMENTS FOR CHRONIC CONDITIONS IN ADULT MALE LONG-TERM SURVIVORS OF CHILDHOOD CANCER

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Themes: Late Effects
Presenter Name: Koskela, Mikael
Keywords: chronic morbidity, health condition, long-term outcome, register study

Background: Treatment of childhood cancer predispose to several late effects and premature death. Cardiovascular risk factors potentiate the risk of cardiac events among survivors exposed to anthracyclines and chest radiotherapy. Male hypogonadism may further potentiate these effects. Our aim was to study therapy exposures and their association to adult chronic conditions and prescription drug purchases in male childhood cancer survivors (CCS).

Methods: The study comprised 252 males with childhood cancer (diagnosed in 1964–2000, survival >5 years) and 1257 population controls. The study cohort did not contain brain tumor patients. Analyses involved registry-based data on prescription drug purchases and special reimbursements for chronic conditions that occurred >5 years after the cancer diagnosis. Subdistributional hazard models (age at onset known) or age-adjusted logistic regression models (age at onset unknown) served as statistical methods. Influence of therapy exposures was assessed with cause-specific hazard models.

Results: Mean age of CCS was 43.1 years. Compared to controls, CCS had more reimbursements for hypogonadism, hypopituitarism, and hypothyroidism. Testicular radiation >6 Gy was associated with hypogonadism. Hypogonadism developed typically during pediatric follow-up, but in some patients after age >35 years. Occurrence rate was higher in CCS also in cardiac insufficiency [hazard ratio (HR) 12, 95% confidence interval (CI) 3.6–38], hypertension (HR 2.3, CI 1.3–4.2), diabetes (HR 2.2, CI 1.4–3.7), and epilepsy (HR 4.1, CI 1.9–8.7). Occurrence of reimbursement for any cardiovascular disease was associated with cardiac radiation >10 Gy. CCS bought more often lipid-lowering drugs [odds ratio (OR) 2.5, CI 1.7–3.6] and antihypertensives (OR 2.4, CI 1.8–3.2). No differences between CCS and controls occurred in purchases of opioids and antidepressants.

Conclusions: Endocrine and cardiovascular diseases and drug purchases related to cardiovascular risk factors were more common in male CCS. Cardiac radiotherapy

potentiated the risk for any cardiovascular disease. Further associations to therapy exposures will be analyzed and presented.

PROGRESSION OF CONCEALED MDS TO ALL AND AML IN A TATTON-BROWN-RAHMAN SYNDROME PATIENT, ACHIEVING REMISSION AFTER SECOND TRANSPLANT

by Pohlak, Triin | Paabo, Triin | Avvo, Elis Maria | Kõrgvee, Lenne-Triin | Tartu University Hospital, Estonia | Tartu University Hospital, Estonia | University of Tartu, Estonia | Tartu University Hospital, Estonia

Themes: Leukemia
Presenter Name: Pohlak, Triin
Keywords: genetic predisposition, leukaemia, transplantation

Background.

Tatton-Brown-Rahman syndrome (TBRS) is a congenital overgrowth syndrome caused by constitutional mutations in the epigenetic regulator DNA-methyltransferase 3A (DNMT3A) that can also play a role in haematologic cancer development. We hereby present a patient case with TBRS who, during the treatment for acute lymphoblastic leukaemia (ALL), presented with a progression of concurrent myelodysplastic changes into acute myeloid leukaemia (AML).

Case description.

A 6-year-old boy was diagnosed with pre-B-ALL with monosomy 7 including IKZF1 deletion, chromosome 5p terminal duplication, and 10q terminal deletion. After induction therapy according to NOPHO ALL-2008 protocol remission was achieved. 14 months later, during maintenance therapy, secondary AML with myelodysplastic changes was diagnosed. BM cytogenetic studies showed monosomy 7 with 85% mosaicism as a sole chromosomal abnormality.

As the patient presented with developmental delay combined with dysmorphic changes and haematological malignancies, he was referred to genetic counselling. Previously, at the age of 3, cytogenetic studies had shown mosaic trisomy 8 and mosaic uniparental disomy 7 without any haematological abnormalities. Now, based on molecular analyses, diagnosis of TBRS with a *de novo* germline heterozygous missense mutation p.(Arg882Cys) in the DNMT3A gene was made. Other features, like obesity and cryptorchidism, were also associated with the syndrome.

The treatment of AML followed NOPHO-DBH AML 2012 protocol, including haematopoietic stem cell transplantation (HSCT). A year later, disease relapse was diagnosed in myelodysplastic phase. After one cycle of chemotherapy succeeded by azacitidine, second HSCT was performed. The disease has remained in remission two years after the second

HSCT.

Conclusion.

Haematopoietic malignancies are known features of TBRS. However, to our best knowledge, this is the first case with more than one haematological disease, receiving azacitidine and two HSCTs. It also highlights the need for thorough diagnostic work-up in case of chromosomal abnormalities typical of myelodysplastic syndrome, preceding overt haematological disease.

QUALITY OF LIFE IN INDIVIDUALS DIAGNOSED WITH LANGERHANS CELL HISTIOCYTOSIS IN CHILDHOOD

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Themes: Other
Presenter Name: Malin Sveijer
Keywords: Langerhans cell histiocytosis, fatigue, follow-up, pain, quality of life

Purpose: Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplastic disorder with variable clinical presentation, ranging from self-healing single lesions to multisystemic potentially fatal disease. Years after assumed remission there is risk of reactivation, and long-term consequences are common, including dreaded progressive neurodegeneration. How LCH affects long-term everyday life is not well known, which this study aimed to investigate.

Methods: 28/51 eligible individuals ≥10 years old, treated for LCH in childhood (<18 years) at Karolinska University Hospital during 1990-2014, completed ≥5 years later questionnaires to examine quality-of-life, fatigue, pain, depression and attention deficit.

Results: 11/28 (39%) had had multisystem disease, including 3/28 (11%) with risk organ involvement (RO+). A third (7/22) of the patients now ≥15 years reported symptoms of depression. 25% (5/20) of the now adult (≥18 years) patients' Adult ADHD Self-Report Scales (ASRS) scores indicated symptoms of inattention, and 15% (3/20) reported a neuropsychiatric diagnosis. 45% (10/22) of the patients now ≥15 years reported chronic pain. Patients with RO+ rated their pain during the last year at a mean of 7.3 out of 10 [standard deviation (SD) 0.6]; markedly higher than patients without risk organ involvement (RO-) (mean 3.2, SD 3.1). The mean total health-related quality-of-life (HRQOL) score of all patients was 77.7 (SD 16.9). RO+ patients reported a markedly lower total HRQOL score (mean 53.2, SD 12.6) and PedsQL fatigue score (mean 39.8, SD 14.9) than RO- patients (mean 80.7, SD 14.9, and mean 70.0, SD 17.7, respectively). Higher scores (0-100) indicate better HRQOL and less fatigue.

Conclusions: At follow-up, patients with LCH in childhood reported high frequencies of

depressive symptoms and pain, and their scores indicate a suboptimal quality of life including problems with attention deficit and fatigue. Moreover, RO+ patients described more pain and fatigue and poorer quality of life than other LCH patients.

REFRACTORY NEUROBLASTOMA IN A PATIENT WITH FANCONI ANEMIA

by Zhanna Kovalova | Gita Taurina | Elizabete Cebura | University Children\'s Hospital | University Children\'s Hospital | University Children\'s Hospital

Themes: Solid Tumours
Presenter Name: Zhanna Kovalova
Keywords: Fanconi anaemia, dinutuximab, neuroblastoma

3 years 6 months old girl (11.3 kg, 91 cm) with previously diagnosed Fanconi anemia developed metastatic neuroblastoma in July 2022. The girl was born in term with birth weight 2 400 g and congenital hypothyreosis,visible multiple cafe-au-lait spots at spine and gluteus, clinodactyly, referred to gastroenterologist due to poor weight and height gain (both -3 SD) in the age of 1 year 3 months, genetical investigation was started. The diagnosis of Fanconi anaemia was confirmed with compound heterozygous condition (BRCA2 c658_659del, p.(Val220Ilefs*4 and BRCA2 c.5268dup, p.(Asn 1747*).

In July 2022 high grade fever started followed by pain in both knees and abdomen, the child refused to walk, osteomyelitis was suspected, MRI performed, and multiple diffuse lesions in both femoral and pelvic bones were described, trephine biopsy results showed neuroblastoma cells in bone marrow. Femoral bone biopsy supported undifferentiated neuroblastoma metastases in bones. Abdominal MRI discovered neuroblastoma of left adrenal gland with multiple metastasis in bones, bone marrow, paraaortal lymphnodes.

Tumor tissues showed 1p deletion, 11q deletion, 3p deletion, 2p gain, 17q gain. MYCN was negative, but MYCN amp-SCA+ was found.The treatment according COJEC courses was started , reducing dosage by 30%. Despite dosage reduction the girl demonstrated prominent myelotoxicity, several infections , typhlitis and Cl.difficile infection during first 5 courses of chemotherapy. After 3 courses of chemotherapy the abdominal lesion was decreased in size, infiltration in bone marrow disappeared, bone lesions persisted.

Infiltration with neuroblastoma cells appeared again in bone marrow biopsies in December 2022. Due to refractory disease the therapy with dinutuximab was started in January 2023, with good tolerability.

Reliability and validity of a self-report questionnaire for assessing levels of physical activity and sedentary time according to guidelines in adult childhood cancer survivors

by Laura Jess | Marianne Jarfelt | Maria Bäck | 1)Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. 2)Närhälsan Bollebygd Rehabilitation Center, Bollebygd, Sweden. | 1)Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. 2) The Long-term Follow-up Clinic for Adult Childhood Cancer Survivors and Cancer Rehabilitation, Sahlgrenska University Hospital, Gothenburg, Sweden. | 1) Department of Occupational Therapy and Physiotherapy, Sahlgrenska University Hospital, Gothenburg, Sweden. 2)Institute of Medicine, Department of Molecular and Clinical Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden.

Themes: Late Effects
Presenter Name: Laura Jess
Keywords: GT3x, accelerometer, actigraph, measurement, paediatric cancer survivorship, surveys

Background There is no available valid and reliable questionnaire that can evaluate physical activity level and sedentary time in adult childhood cancer survivors. The Swedish National Board of Health and Welfare designed two questions to assess levels of physical activity (NBHW-PA questions), one is assessing exercise and one everyday physical activity. Furthermore, a single-item question was developed to measure sedentary time (SED-GIH). These questions have been found valid for the general population.

Aim To determine test–retest reliability and criterion-related validity of the NBHW-PA questions and the SED-GIH question in adult childhood cancer survivors.

Method non-experimental methodological study. In total 60 participants (50% women), age 28 years (min-max 18-54) were included at the Long-term follow up clinic at Sahlgrenska University Hospital. Participants were instructed to wear an accelerometer for seven days, to report the wear times in an activity diary and to answer the NBHW-PA questions and the SED-GIH question before and after the seven days. Agreement was calculated with weighted kappa (k).

Results Test-retest reliability regarding the SED-GIH's question showed a high agreement (k=0.88), while the NBHW-PA questions showed a moderate agreement, NBHW-PA exercise (k=0.50), NBHW-PA everyday physical activity (k=0.47). The criterion-related validity, comparing self-reported data with accelerometer data, was interpreted as fair for the NBHW-PA exercise (k=0.30) and the SED-GIH (k=0.24), while the question about NBHW-PA everyday physical activity was interpreted as low (k=0.11).

Conclusion The NBHW-PA questions and SED-GIH have shown moderate to high reliability, while the validity for the SED-GIH and NBHW-PA exercise were fair. This simple questionnaire assessing physical activity and sedentary time can be helpful in clinical practice to identify individuals in need for support to increase physical activity level.

SEROPREVALENCE OF ANTI-SARS-COV-2 IGA AND IGG IN PEDIATRIC HEMATOLOGY AND ONCOLOGY PATIENTS

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Themes: Infections
Presenter Name: Emil Sundberg
Keywords: IgA, Immunology, Oncology, Pediatrics, SARS-CoV-2

Background

Immunoglobulin A (IgA) is a class of antibodies existing both in the mucosa and in blood. Mucosal IgA plays a key role in protection against many infections, and recent studies have confirmed its protective effects against SARS-CoV-2, the virus causing COVID-19. The role of serum IgA is less studied, however, although serum IgA is known to have immunoregulatory and anti-inflammatory effects. Here, we investigated the seroprevalence of anti-SARS-CoV-2 IgA in pediatric oncology and hematology patients, and correlated IgA and IgG seroprevalence.

Procedure

Patients (n=149) were sampled up to eight times between June 2020 and March 2022 at Uppsala University Children's Hospital, Sweden. Anti-SARS-CoV-2 IgG and IgA antibodies targeting the wild-type S1 protein were then detected in serum samples, using a COVID-19 suspension immunoassay. Patient data were collected from medical journals.

Preliminary results

Analysis on samples collected until January 2023 is ongoing, as is analysis on total serum IgA, IgM, and IgG levels. Additional results will be available for presentation at the NOPHO Annual Meeting in May, and the following results are preliminary. A cumulative anti-SARS-CoV-2 IgG seroprevalence of 43% (64/149 patients, 30 girls) was observed in this patient cohort. Twenty of these patients (20/64, 31%, 9 girls, mean age 14 [IQR 11.5] years) were also IgA seropositive (IgA+), while the other 44 patients (21 girls, mean age 8 [IQR 8.75] years) were only ever IgG seropositive (IgG+). No IgA+ samples were IgG negative (IgG-), however, seven of the IgA+ patients were in one or more blood samples IgA seronegative (IgA-), while still being IgG+.

Conclusions

In this ongoing study, anti-SARS-CoV-2 seropositive pediatric oncology and hematology patients were more often IgA-/IgG+ than IgA+/IgG+. Antibody dynamics also varied between IgA and IgG, with previously IgA+/IgG+ patients becoming IgA-/IgG+, suggesting a lower IgA than IgG durability.

SWEDISH PAEDIATRIC ONCOLOGISTS' EXPERIENCES OF EARLY PHASE STUDIES; A NATIONAL MIXED-METHODS SURVEY

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Themes: Other
Presenter Name: Anna Schröder Håkansson
Keywords: Early phase clinical trials, mixed methods, paediatric oncology, palliative care

Background: There are increasing numbers of early clinical trials with novel therapies available for children with relapsed or refractory cancer in Sweden. Two of the six Swedish paediatric oncology departments are part of the consortium for Innovative therapies for children with cancer. However, all six departments refer patients to early clinical trials. There is limited knowledge regarding how physicians in Sweden handle decisions related to the child's well-being and chance to cure in relation to novel therapies. Purpose: The aim was to study Swedish paediatric oncologists' (POs) practical and emotional experiences when enrolling children in early phase clinical trials. Method: A nationwide survey including a structured interview with a study-specific questionnaire and options to make comments was conducted with 29 POs (16 females,13 males). The majority of POs (N=19) had more than 10 years' experience of paediatric oncology (range 4 to 32 years). Quantitative and qualitative data were analysed according to a convergent mixed methods approach and compared, interpreted, and integrated in the result. Result: The POs highlighted the role of well-grounded decisions at the local department level, as well as discussions at national and Nordic treatment conferences in providing them professional and emotional support in the work with these rare patients. The analysis revealed three themes: Optimally-based approach, family-based approach and team-based approach. These themes reflect the balance between treatment decisions, preservation of hope, the role of information, family wishes, and palliative care. Conclusion: The prime important issue for the POs was the child's well-being. Perceived challenges were how to select the best treatment options, including novel therapy and palliative care, while maintaining hope for the family. More structured training initiatives and organised arenas where the paediatric oncology community could discuss ethical implications in early phase studies were requested.

SYSTEMATIC ANALYSIS OF NON-CODING VARIANTS REVEALS NOVEL CIS-REGULATORY MECHANISMS IN PEDIATRIC B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

by Efe Aydın | Eleanor Woodward | Rebeqa Gunnarsson | Bertil Johansson | Minjun Yang | Kajsa Paulsson | Lund University | Lund University | Lund University | Lund University | Lund University | Lund University

Themes: Leukemia
Presenter Name: Efe Aydın
Keywords: acute lymphoblastic leukemia, cis regulation, gene regulation, non coding variants, regulatory network

Acute Lymphoblastic Leukemia (ALL) is the most common pediatric cancer in the world and despite overall survival rates of more than 90% for several genetic subtypes, dose intensity on conventional chemotherapy has reached its limit. Thereby, ALL has a disease profile that can benefit most from precision medicine and a regulatory network perspective. Non-coding regions account for ~98% of the human genome and harbor the majority of variants. Our knowledge of these regions is still limited compared to their protein-coding counterparts and there is a huge room for discovery from a functional standpoint. Focusing on the somatic non-coding alterations occurring in diagnostic samples from childhood B-cell precursor ALL patients using two cohorts, comprising a total of 353 cases subjected to whole genome sequencing, we identified a total of 406 mutational hotspots, where three or more cases had somatic single nucleotide variants (SNVs) occurring within 200 base pairs. Further analyses using RNA-sequencing combined with the integration of multi-dimensional regulatory network data in these hotspots revealed gene expression changes associated with mutational hotspots close to the well-known ALL-associated genes *NRAS*, *KRAS*, *PBX1* and *STAT6*. Experimental validation of these findings is ongoing. Here, we used an integrated multi-omics framework combined with manual curation to demonstrate the implications of non-coding somatic variants on leukemogenesis through the alteration of cis-regulatory architecture.

Targeting nuclear transmembrane proteins to rectify nuclear size: would this new therapeutic approach to reduce metastasis be relevant also to hematological malignancies?

by Andrea Rizzotto | Eveliina Koponen | Sonja Koivukoski | Leena Latonen | Eric C. Schirmer | University of Edinburgh | University of Eastern Finland | University of Eastern Finland | University of Eastern Finland | University of Edinburgh

Themes: Novel Methods and Therapies
Presenter Name: Sylvain Tollis
Keywords: cell migration nuclear envelope, lamina, metastasis, nuclear envelope proteins, nuclear mechanics, nuclear size, systemic toxicity

For many cancers, lower survival correlates with nuclear size alterations in a tumor-type/tissue-specific manner. We showed that such changes might confer an advantage to tumor cells when migrating through tissues to establish metastasis sites[1,2], and that Nuclear Envelope Transmembrane (NET) proteins play a role in the tissue-specificity of those nuclear size changes. Strikingly, genetic alterations in leucocyte-specific NETs correlate with bad prognosis in childhood leukemia; furthermore, ALL/AML cells have a larger nucleus than healthy mature leucocytes, leading us to revisit our hypothesis: would nuclear size alterations help ALL/AML cells to squeeze through tissue barriers and spread leukemia like in “standard” metastasis? This poster proposes to confront this idea to the expertise of the pediatric hematology community.

We screened for FDA/EMA-approved compounds that reverse characteristic tumor nuclear size changes in cell lines representing (solid) tumors from different tissues. We found distinct, largely nonoverlapping sets of compounds that rectify nuclear size changes for each line specifically. Strikingly, selected nuclear size-rectifying (NSR) compounds all inhibited cell migration/invasion, in the same conditions (cell line/concentration/treatment duration) where they corrected nuclear size defects[1,2]. We next investigated the mechanisms that underlie the tissue-specificity of NSRs. NET genes have tissue-specific expression, influence nuclear mechanics and are frequently altered in metastatic cancers, making them ideal candidates to influence tissue-specific metastatic nuclear size changes. We screened for size phenotypes upon overexpression (OE) or deletion of 35 NETs, and assessed the nuclear size-regulating hit NETs against NSRs, focusing on NETs which are lost/amplified during cancer evolution. This chemogenomic approach established NET expression requirements for *tissue*- and *cancer stage-specific* nuclear size rectification[3]. Hence, NET expression-dependent NSRs may selectively affect (pro-)metastatic cancer cells, limiting systemic toxicity of treatments that could be personalized to patients’ NET status. Yet, so far NETs have been overlooked in blood cancer research, including paediatric malignancies. Why?

Temporal Dynamics of TNF-Mediated Changes in Hematopoietic Stem Cell Function and Recovery

by Alexandra Rundberg Nilsson | Isabel Hidalgo | David Bryder | Cornelis Jan Pronk | Lund University: Division of Molecular Hematology, Division of Molecular Medicine and Gene Therapy, Lund Stem Cell Center | Lund University: Division of Molecular Hematology, Lund Stem Cell Center, Wallenberg Center for Molecular Medicine | Lund University: Division of Molecular Hematology, Lund Stem Cell Center | Lund University: Division of Molecular Hematology, Lund Stem Cell Center, Wallenberg Center for Molecular Medicine, Skåne University Hospital: Childhood Cancer Centre

Themes: Other
Presenter Name: Alexandra Rundberg Nilsson
Keywords: Hematopoietic stem cells, Stem cell recovery, TNF, transplantation

Tumor necrosis factor (TNF) is a critical mediator of immune response and tissue repair. However, dysregulation of TNF has been linked to a variety of conditions, including cancer, autoimmunity, bone marrow failure and aging. In oncology, TNF levels are increased during treatment-related infections and cancer treatment, including conditioning therapy before hematopoietic cell transplantation (HCT). A comprehensive understanding of the role of TNF in different contexts is essential for deciphering normal and pathogenic hematopoiesis and advancing clinical therapies.

In this study, we focus on hematopoietic stem cells (HSCs), as these cells are required for autologous and allogeneic reconstituting of the blood system following myelotoxic chemotherapy and HCT, respectively. Previous studies suggested that TNF restricts the self-renewal capacity of HSCs, but its long-term effects on HSCs remain unclear. Using mouse mode, we show that *in vivo* administration of TNF leads to a transient exit of HSCs from quiescence, resulting in a compromised repopulation capacity. However, this functional impairment is fully reversible, even following prolonged/chronic transient exposure to TNF, demonstrating the remarkable recovery potential of HSCs. Notably, antagonizing TNF signaling in HCT recipients enhances donor HSC contribution, suggesting that TNF inhibitors during transplantation may be beneficial for improving transplantation outcomes.

Our findings provide molecular and functional insight into HSC regulation, with implications for both acute and chronic inflammatory conditions. Our study highlights the importance of TNF signaling in HSCs and suggests that TNF inhibitors could have therapeutic potential for various hematopoietic disorders.

TESTOSTERONE SUBSTITUTION IN MALE LONG-TERM SURVIVORS OF CHILDHOOD CANCER – A POPULATION-BASED STUDY (THE FEX-CAN STUDY)

by Haavisto, Anu | Wettergren, Lena | Lampic, Claudia | Lähteenmäki, Päivi M. | Jahnukainen, Kirsi | Department of Psychology and Logopedics, University of Helsinki, Helsinki, Finland; Department of Women's and Children's Health, Karolinska Institutet, Sweden | Department of Women's and Children's Health, Karolinska Institutet, Sweden; Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden | Department of Women's and Children's Health, Karolinska Institutet, Sweden; Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden; Department of Psychology, Umeå University, Umeå, Sweden | Department of Women's and Children's Health, Karolinska Institutet, Sweden; Department of Pediatric and Adolescent Medicine, Turku University Hospital, Turku, Finland; FICANWEST, University of Turku, Turku, Finland | Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; NORDFERTIL Research Lab Stockholm, Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

Themes: Late Effects
Presenter Name: Haavisto, Anu
Keywords: cardiovascular risk, childhood cancer, late effects, registry study, testosterone substitution

In postpubertal males, testosterone deficiency leads to reduced sexual function, fatigue, and increased cardiovascular risk. Our aim was to assess the prevalence of testosterone substitution and its association to treatment variables and late sequelae in male long-term survivors of childhood cancer.

The study is part of the Fex-Can Childhood project, combining registry and survey data for adult survivors of childhood cancer in Sweden. A national survivor cohort was identified through the National Quality Registry for Childhood Cancer. In all, 1213 men replied (52% response rate). Median age at diagnosis was 7 (range 0-17), and age at survey 29 (range 19-40) years. The outcome measure was ongoing testosterone substitution by self-report. Logistic regression analysis included clinical variables (chemotherapy, cranial radiotherapy, abdominal radiotherapy, hematopoietic stem cell transplantation [HSCT]), age at diagnosis and at study.

In total, 72 (6.1%) men indicated ongoing testosterone substitution. The odds for testosterone substitution were 13-fold after HSCT and 4-fold after cranial radiotherapy (both $p<.001$). Survivors on testosterone substitution were less likely to ever have had a sex partner (18.1% vs. 10.5%, $p=.047$), cohabitated with a partner (36.1% vs. 61.6%, $p<.001$), conceived a biological child (9.7% vs. 29.6%, $p<.001$), or to be currently working/studying (81.9% vs. 91.1%, $p=.010$) compared to the non-substituted. They were also more likely to have other hormone deficiencies (growth hormone 58.3% vs. 6.8%, $p<.001$; thyroid 54.2% vs. 7.2%, $p<.001$), cholesterol (8.5% vs. 1.5%, $p<.001$) or hypertension medication (8.5% vs. 4.0%, $p=.068$), and depressive symptoms (29.6% vs. 14.5%, $p<.001$).

The need of testosterone substitution in male long-term childhood cancer survivors was associated with other hormonal and sociodemographic late effects and cardiovascular risk factors. Because androgen insufficiency associated with depressive symptoms, which can reduce help seeking, adult surveillance after HSCT and cranial radiotherapy might facilitate detection of testosterone deficiency.

The Impact of body weight, consumption of supplements and infection rate on Childhood Cancer

by Igne Kairiene | Jelena Rascon | 1-Vilnius University, Faculty of Medicine, Lithuania; 2-Center for Pediatric Oncology and Hematology, Vilnius University Hospital Santaros Klinikos, Lithuania | 1-Vilnius University, Faculty of Medicine, Lithuania; 2-Center for Pediatric Oncology and Hematology, Vilnius University Hospital Santaros Klinikos, Lithuania

Themes: Other
Presenter Name: Jelena Rascon
Keywords: body weight, childhood cancer, infections, nutrition, supplements

Background: Disbalance of nutritional factors may lead to genome damage and cancer development[1]. Some nutrients have anticancer effect, such as vitamin D[2]. Malnutrition, expressed as underweight or obesity, may reflect the risk of cancer[3, 4] Association between early infections and childhood cancer is also highly debated[5, 6]

Objective: to determine if body weight, consumption of supplements and infection rate may be associated with childhood cancer.

Methods: from December 2021 till December 2022 parents of childhood cancer patients and those of healthy children were asked to answer an anonymous electronic questionnaire. Variables (child's weight, consumption of supplements, frequency of infections and antibacterial treatment) were compared between the groups. Respondents were divided into three age groups: 0-6, 7-11, 12-17 years old. To analyze the association, chi-squared and Fisher's exact test were used.

Results: The questionnaire was fulfilled by 126 parents (72 childhood cancer, 54 healthy controls). In the youngest group, cancer was less frequent in children who took vitamin D (p=0.009), but more common in those who took vitamin C (p=0.009) during the first year of life. Within 7-12 years old group there was an association between cancer and infections in infancy (p=0.036), vitamin complex (p=0.026) and herbal medicine (p=0.022) consumption during the first 6 years of life. Children who used vitamin D during the first 6 years of life were diagnosed with cancer less frequently (p=0.040). In the eldest group, consumption of vitamin complex during the 7-11 years of age had a negative association with cancer development (p=0.029).

Child weight and antibacterial treatment were not associated with cancer.

Conclusions: Childhood cancer might be associated with a higher frequency of infections during infancy and unjustifiable consumption of vitamin complex. Vitamin D may appear to have a protective effect. Larger studies are necessary to confirm the results.

THE IMPACT OF THE HORIZON 2020 TWINNING PROJECT ON RESEARCH AND CARE OF CHILDHOOD SOLID TUMOURS IN LITHUANIA

by Jelena Rascon | Renata Blackute | Alma Cerkauskiene | Ruth Ladenstein | Sabine Taschner-Mandl | Nuno Anrade | Adriana Planinic | Stefan Rutkowski | Ulrich Schuller | Karsten Nysom | Ruta Tuckuviene | Jesper Brok | Kjeld Schmiegelow | Marry M. van den Heuvel-Eibrink | M. E. Madeleine van der Perk | Riccardo Haupt | Monica Muraca | Davide Saraceno | Birgit Geoerger | Anne Blondeel | Giorgia Manuzi | Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania | Children's Cancer Research Institute, Vienna, Austria | Children's Cancer Research Institute, Vienna, Austria | Children's Cancer Research Institute, Vienna, Austria | Children's Cancer Research Institute, Vienna, Austria | University Medical Center Hamburg-Eppendorf, Hamburg, Germany | University Medical Center Hamburg-Eppendorf, Hamburg, Germany | Rigshospitalet, Copenhagen, Denmark | Rigshospitalet, Copenhagen, Denmark | Rigshospitalet, Copenhagen, Denmark | Rigshospitalet, Copenhagen, Denmark | Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands | Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands | Istituto Giannina Gaslini, Genova, Italy | Istituto Giannina Gaslini, Genova, Italy | Consorzio Interuniversitario, Bologna, Italy | Institute Gustave Roussy, Paris, France | European Society for Paediatric Oncology, Brussels, Belgium | European Society for Paediatric Oncology, Brussels, Belgium

Themes: Other
Presenter Name: Jelena Rascon
Keywords: Horizon 2020, paediatric solid tumours, research, twinning

Background. Inequality in research across European countries affects pediatric cancer survival rates. Due to the historical gap between Lithuania and research-intensive countries, a nine-partner consortium was formed to pursue twinning activities supported through a Horizon 2020 project TREL "**T**winning in **R**esearch and **E**ducation to improve survival in Childhood Solid Tumours in **L**ithuania". Our project aims to foster and scale up translational, clinical, and observational research with the final goal to increase the survival of Lithuanian children with solid tumours.

Methods. The most frequent paediatric solid tumours – central nervous system tumours, neuroblastoma, and renal tumours – were selected for the twinning activities in Translational research and modern diagnostics (WP3), Treatment Innovation (WP4), Clinical trial preparedness and Cross-disciplinary training (WP5), Quality of survival and late-effect research (WP6), and Research Methodology and Management (WP7).

Results. Since January 2021 VULSK researchers have joined SIOP-BTG, SIOPEN, RTSG-SIOP international research groups. Two Survivorship Passports were issued. Five agreements were signed to initiate three international academic multicentre clinical trials and two observational studies. Twenty-seven patients benefited from multidisciplinary discussions with experts from partner institutions and received individual treatment recommendations. 18 rare genetic variants were discussed and classified by the twinning

bioinformatician teams with direct consequences on patient management. Ten multidisciplinary VULKS teams were seconded to partner premises. Seven workshops, including a series of four workshops on research methodology and early-phase clinical trials were organized. Basic and clinical research on oncofertility and fertility care plan implementation has been initiated at VULSK.

Conclusions. The Horizon 2020 Twinning programme provides a powerful platform for expanding scientific collaboration in a less research-intensive country. The activities initiated within the TREL project time frame (2021-2023) will have a sustainable impact on the roadmap of paediatric oncology in Lithuania.

TOWARDS A MECHANISTIC UNDERSTANDING OF CELL CYCLE REWIRING DURING HAEMATOPOIETIC DIFFERENTIATION: INSIGHT FROM SINGLE CELL TRANSCRIPTOMICS AND QUANTITATIVE LIVE-CELL IMAGING.

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Themes: Leukemia
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Keywords: Leukaemia, bio-imaging, quantitative, single-cell

Leukaemic cells leave the bone marrow with high proliferative potential, reflecting a de-regulated cell division cycle. It is therefore crucial to understand how haematopoietic cells decide to divide. Evidence is accumulating that cell cycle commitment is governed by quantitative changes in core cell cycle protein levels.

Using live yeast and quantitative bio-imaging, we measured absolute concentrations of cell cycle proteins. We found that key G1/S transcription complexes that coordinate division are limiting with respect to their DNA target sites in small daughter cells. Those division activators gradually accumulated with growth until saturating target promoters for commitment to division. G1/S TF levels were modulated by external nutrient pools, thereby coupling environmental signals to division. Nutrient-dependent modulation of G1/S TF expression appeared critical for proliferation in poor conditions, providing a paradigm for how quantitative modulations of rate-limiting factors dictate a cell state transition.

To understand how haematopoietic cells commit to division and identify the core gene network that integrates proliferation/differentiation signals prior to commitment, we analysed the expression of cell cycle genes with scRNA-seq in different haematopoietic cells. We found that several cyclins, CDKs, CDK inhibitors and G1/S transcription regulators were differentially expressed across cell types, and between healthy and leukaemic cells. Moreover, the cell-to-cell variability of those key cell cycle activators/inhibitors expression within subpopulations was often cell type-dependent, suggesting cell cycle network re-wiring along the differentiation path and upon disease. We’re now measuring their protein levels in order to establish quantitative determinants of the commitment to division along haematopoietic lineages. This will improve our understanding of how core cell cycle activators/inhibitors integrate external stimuli in different haematopoietic cells, in order to quantify how particular leukaemia-associated mutations bias the coordination between differentiation and proliferation towards the latter. We ascertain this is a key pre-requisite

to the design of personalized treatment against leukaemia.

UPDATE OF FERTILITY PRESERVATION RELATING TO CANCER TREATMENT OF PEDIATRIC CANCER PATIENTS IN NORDIC COUNTRIES

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Keywords: Fertility preservation, cancer treatment, infertility, late effect, pediatric

Exposure to gonadotoxic agents are the major factors for development of fertility preservation technologies. Since the first Nordic IVF-baby was born in 1982 in Gothenburg, the Nordic countries have been in the forefront of assisted reproduction technology development through research contributions.

Fertility preservation is the only option for pediatric cancer survivors to save their fertility following gonadotoxic treatments. The aim of this study was to update the status of fertility preservation options for pediatric cancer patients in Nordic countries.

A questionnaire was sent to 18 main pediatric oncology centers in Nordic countries. We received information regarding indications, guidelines, counseling, and available fertility preservation options from 16 centers in 2010 and 13 centers in 2021.

The results show an increase in providing a national guideline on fertility preservation for cancer patients from 25% to 75%. Counseling on fertility preservation options for patients who fulfill indications for fertility preservation has increased from 25% to 83%.

Sperm cryopreservation is still the most available fertility preservation option for adult male patients in Nordic countries, while there is a significantly increase in the offering of testicular tissue preservation (13% to 58% after puberty/ 0% to 33% before puberty).

There is also an increase in offering the most relevant fertility preservation options for female patients including oocyte fertility preservation (56% to 83%) and ovarian tissue preservation (18% to 83% after puberty/ 0% to 92% before puberty).

During the past decades there is a progress in establishing national guidelines on fertility preservation for pediatric cancer patients in Nordic countries. There is also more focus on counseling for fertility preservation options for patients and their parents. The fertility preservation options for cancer patients in pre-puberty age offers more to female pediatric cancer patients compared to male pediatric cancer patients.