SIOPEN **ANNUAL GENERAL MEETING** 2022

ATHENS

ABSTRACTBOOK & **CASE**PRESENTATIONS

Dear SIOPEN family and collaborators,
Dear friends and colleagues,

Thank you all for your participation in the SIOPEN Annual General Meeting that took place in Athens, at the Royal Olympic Hotel from 12 to 14 of October, 2022.
The scientific presentations, the interaction between colleagues and all exchanges made this face to face only meeting very productive. We made plans for collaborations and projects and took decisions about future protocols. The parents and patient advocate input was also very significant and important.
So, this year's AGM's theme: Designing the future, "From bench to bedside" was materialized.

This year and preceding the main SIOPEN AGM Program, on October 11th the Liquid Biopsy Committee had its inaugural meeting, together with the Molecular Monitoring Committee meeting that followed. In parallel, the Quality of Life- Long Term Outcome Committee had its meeting on ototoxicity issues. Additionally, the Pathology Committee reviewed cases material on multi-headed microscopes on October 12th and 13th, at the University of Athens premises.
The full program of the AGM was full of very interesting with cutting edge science on both preclinical and clinical issues.

An important aspect of the meeting this year was the submission of papers and cases for presentation. Five abstracts were presented orally and 27 had poster presentations. Additionally, five interesting and didactic cases were presented and discussed extensively.

This Abstract and Case Presentation Book is available to all participants and SIOPEN members and contains the submitted work. We expect paper and case submission as well as the publication of the Abstract and Cases Book to continue for all future SIOPEN AGMs, providing a lasting record of the SIOPEN member activities.

Thank you for all your work and efforts.
Cordially,

Maia Beck-Popovic
SIOPEN President

Vassilios Papadakis
Local Organizer

ORAL PRESENTATIONS
SIOPEN 2022

01

O_01

HIGH RESOLUTION [18F]MFBG PET-CT FOR DETECTION OF NEUROBLASTOMA LOCALISATIONS: A PROSPECTIVE PILOT STUDY.

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BACKGROUND AND AIMS

Meta-[18F]fluorobenzylguanidine ([18F]mFBG) is a positron emission tomography (PET) radiotracer allowing for fast and high-resolution imaging of norepinephrine transporter expressing tumors. This study investigates the feasibility of [18F]mFBG PET-CT in paediatric neuroblastoma patients.

METHODS

In a prospective, single-centre study, children (<18 years) with neuroblastoma referred for conventional meta-[123I]iodobenzylguanidine ([123I]mIBG) scanning were recruited. [123I]mIBG whole body planar scintigraphy in combination with single-photon emission computed tomography-CT (SPECT-CT) was performed according to standard protocol. Within two weeks from [123I]mIBG scanning, total body PET-CTs were performed at 1 h and 2 h after [18F]mFBG injection (2 MBq/kg). [18F]mFBG uptake of lesions and organs at 1h and 2h was quantified. Detection of neuroblastoma localisations on paired scans was compared. Skeletal lesions were quantified using the SIOPEN score, and soft tissue lesions were counted.

RESULTS

Twenty paired [123I]mIBG-[18F]mFBG scans were performed in 14 patients (median age 4.9 years, 13/14 with stage 4 disease). [18F]mFBG injection and scanning were well tolerated, and no related adverse events were observed. With a significantly shorter scan time for [18F]mFBG PET-CT compared with [123I]mIBG scanning (9 min vs. 84 min, respectively, $p<0.01$), sedation was only needed in 2 patients for [18F]mFBG, versus 10 patients for [123I]mIBG. More lesions were detected on the 1 h post-injection scan, despite a lower background uptake and equal lesion uptake at 2h post-injection. [18F]mFBG PET-CT scans detected more lesions than paired [123I]mIBG scanning in 80% (16/20): on average 2 additional soft tissue lesions and a 6-point higher SIOPEN score per patient.

CONCLUSIONS

Results of this pilot demonstrate that [18F]mFBG PET-CT is safe and requires less sedation in patients with neuroblastoma. In paired [18F]mFBG-[123I]mIBG scans, more lesions with clear anatomical localisation were detected on [18F]mFBG scans. [18F]mFBG PET-CT shows promise for staging and response assessment in neuroblastoma.

FUNDING

Foundation KiKa, UMC Utrecht, and Princess Máxima Centre for Paediatric Oncology.

O_02

STANDARDIZATION AND VALIDATION OF PROCEDURES FOR DETECTION OF THE ALK GENETIC STATUS IN NEUROBLATOMA SAMPLES BY THE SIOPEN BIOLOGY REFERENCE LABORATORIES.

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BACKGROUND

The collaborative study TITAN (Transatlantic Integration Targeting ALK in Neuroblastoma) will investigate the introduction of a targeted drug to frontline treatment for high-risk neuroblastoma patients with a tumour harbouring an activating alteration of the ALK gene (mutation or amplification). This study, involving the International Society of Paediatric Oncology Europe Neuroblastoma (SIOPEN) in Europe and the Children's Oncology Group (COG) in North America, will evaluate the efficacy of a third generation ALK inhibitor (Lorlatinib) in combination with the standard treatment developed by both cooperative groups, based on the survival rate of patients (EFS/OS). The European TITAN project corresponds to a soon to be launched amendment to the SIOPEN HR-NBL2 phase 3 clinical trial (NCT NCT04221035; PI Dominique Valteau-Couanet) conducted in over 20 countries. The number of European participating centres, and the complexity of molecular diagnosis in NB, highlights the need for harmonisation of molecular diagnostic techniques and standardisation of output between the SIOPEN Biology reference laboratories, with regards to the identification of ALK genetic alterations for the eligibility criteria.

AIMS

The objective of our study is to standardize procedures of molecular diagnostic techniques and reporting of results, and to demonstrate the comparability of the different techniques used by the SIOPEN reference laboratories to detect the genomic status (mutational and amplification status) of the ALK gene in neuroblastoma samples in the 21 participating SIOPEN Biology laboratories. The overall aim of the comparative analysis is to establish robust SOPs for the detection and the report of ALK genetic alterations.

RESULTS

For this interlaboratory testing, a batch of 14 genomic DNA samples (NB cell lines or NB PDX models) were shared among the 21 SIOPEN reference laboratories harbouring distinct ALK genetic alterations: a) 10 samples harbour different ALK SNVs/mutations, in a known hotspot of the tyrosine kinase domain, or

not, including also dilutions, resulting in different variant allele fractions (VAF) ranging from 91% to 1%; b) 3 samples with ALK genomic amplifications, over extended or more restricted genomic regions; c) 1 DNA sample without any ALK alteration and/or ALK mutation has been sent as a negative ALK control; d) slides of FFPE tumour samples from PDX were also sent upon request.

ALK amplifications were detected using either a pangenomic copy number technique (WES, WGS, CGHa, SNPa) or by FISH. ALK SNVs/mutations were characterized by NGS techniques to enable detection of mutations with lower VAF. Importantly, while respecting the minimum requirements of consensus, each SIOPEN biology laboratory employed their own detection technique for the characterisation of ALK mutations or amplifications Primary results show that all laboratories correctly identified and reported ALK mutations in a known TKD hotspot, with VAF >5% and large scale typical genomic ALK amplification.

A difference in interpretation and reporting is apparent when considering SNVs with a VAF below 5% or outside the known hotspots, which require expert discussion prior to validation as inclusion criteria within the HR-NBL2 amendment. Based on the technical contributions from all participating laboratories, SOPs for the molecular diagnosis of the ALK genomic status could be established.

CONCLUSION

Our results document the importance of the established SOPs and the robustness of ALK genetic testing in the SIOPEN biology reference laboratories. Furthermore, our findings underline the importance of expert discussions regarding atypical ALK alterations, in order to validate eligibility for ALK targeted treatment.

O_03
TARGETABLE GENETIC ALTERATIONS IN HIGH-RISK NB PATIENTS. A SIOPEN STUDY

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BACKGROUND AND AIMS

In high risk neuroblastoma (NB), new treatment strategies are urgently required to improve outcome. We sought to determine the frequency of genetic alterations (SNVs/Indels) in genes considered to be targetable and/or to play a role in oncogenesis in high risk NB at diagnosis.

METHODS

Diagnostic NB samples from 700 patients enrolled in the SIOPEN-HR-NBL1 trial were included in this study. 499 samples have been analyzed by targeted sequencing at the Institut Curie and 201 by WES in different European countries. 77 normal DNA from healthy donors were used as negative controls.

The gene panel consists of 85 genes known to play a role in NB oncogenesis, with 50 genes considered directly targetable.

A targeted sequencing approach (True-seq custom amplicon; TSCA®) was used to sequence exonic regions (target size = 0.35 MB) yielding mean 9Mio raw reads, mean read depth of 2960x per sample and >98.75% at 50x coverage. Variant (SNVs/Indels) and copy number analysis was performed using VarScan2 and HaplotypeCaller tools respectively. European population frequency (0.002) from gnomAD database and 77 normal samples were used to remove background polymorphisms.

RESULTS

Primary variant analysis resulted in a total of 2736 SNVs/Indels with minimum mutant allele frequency (MAF) of at least 5% (MAF below 20%/10% in 84%/60% of cases, respectively). Per NB sample, a mean of 7 SNVs/Indels (95% CI -5.4–8.3; range 0–143) was observed with the most frequent events in known cancer hotspot mutations in the genes ALK(6.2%), TP53(4%), HRAS(1,5%), PTPN11(1,5%) SNVs/Indels with low MAF (<20%) was observed largely in 8 genes (TENM4, CHD7, TNEM2, NF1, SMARCA2, PTPRD, PTCH1, ATM).

In the subset cohort of 499 patients analyzed by TSCA, Forty-one percent (206/499) of NB samples harbored at least one pathogenic genetic alteration (COSMIC and/or other predicted pathogenic alteration): 57% had SNVs/Indels in genes considered to be targetable.

Although not statistically significant, a higher number of SNVs/indels were observed in stage 4 versus non-stage 4 tumors, or MYCN-amplified versus non-MYCN-amplified tumors.

No statistically significant difference in the survival of patients with NB displaying higher versus with lower numbers of genetic alterations was observed (>/< 7 SNV/Indels, p= 0,9892).

The analysis of WES from 201 diagnostic patient received from European centers is ongoing.

CONCLUSIONS

Distinct targetable genetic alterations could be observed in 57% of high risk NB patients at diagnosis, an important information to take into account when considering introduction of targeted therapy approaches in upfront treatment strategies.

O_04

INTEGRATIVE ANALYSIS OF NEUROBLASTOMA BY SINGLE-CELL RNA SEQUENCING IDENTIFIES THE NECTIN-TIGIT AXIS AS A TARGET FOR IMMUNOTHERAPY

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BACKGROUND AND AIMS

Children with high-risk neuroblastoma have poor survival rates and urgently need more effective treatments with less side effects. Immunotherapies may fill this need, yet show limited clinical efficacy. We aimed to provide a comprehensive overview of neuroblastoma's immune environment to identify strategies for improving immunotherapy efficacy.

METHODS

25 neuroblastomas from 20 patients (17 high-risk, 6 MYCN-amplified), were collected pre-treatment (n=10) or after induction chemotherapy (n=15). Samples were enzymatically digested, FACS sorted and single-cell RNA-sequenced. Killing assays were performed with patient-derived neuroblastoma organoids and healthy donor PBMCs. Checkpoint inhibition was tested in vivo in three syngeneic models (Neuro2a, N1E-115, N18) and one chemotherapy-resistant syngeneic model (Th-ALKF1174L/MYCN 129/SvJ).

RESULTS

Neuroblastomas were infiltrated by dendritic cells, monocytes, and macrophages with an M2-like differentiation, associated with immunosuppressive and pro-tumorigenic features. Lymphoid cells in neuroblastoma consisted of NK, B, and various T cells including highly suppressive Tregs. Among two CD4+ non-Treg clusters, one likely contained tumor-reactive cells and was significantly enriched for genes associated with T cell dysfunction (TIGIT, CTLA4). CD8+ T had significantly upregulated LAG3 and PDCD1, also associated with T cell dysfunction. Overall, T cells showed signs of dysfunction/exhaustion particularly post-chemotherapy, with enhanced expression of immune checkpoint receptors. NK cells had impaired cytotoxicity (GZMB, PRF1, GNLY), particularly in pre-treatment tumors, which correlated with TGF-β1 signaling and a disbalance between inhibitory receptors TIGIT and CD96 and activating CD226. To identify functional targets for reinvigorating T/NK cell function, we constructed an unsupervised interaction network. This predicted an abundance of immunoregulatory interactions in the tumor microenvironment affecting T/NK cell function, including CLEC2D-KLRB1, PD1-PDL1 and NECTIN2-TIGIT. Since also in T cells the TIGIT/CD226 balance proved disturbed, we tested combined TIGIT/PD-L1 blockade in vitro, which significantly increased killing of neuroblastoma organoids. Moreover, TIGIT/PD-L1 blockade in vivo in three syngeneic models induced complete remissions in a subset of animals and significantly improved survival. Lastly, addition of TIGIT blockade to the standard backbone treatment for relapse/refractory neuroblastoma significantly improved survival in a chemotherapy-resistant model mimicking relapse/refractory tumors.

CONCLUSIONS

We provided a comprehensive atlas of neuroblastoma's immune environment and identified TIGIT as a promising target for (combination) immunotherapy.

O_05

CIRCULATING ADRENERGIC NEUROBLASTOMA mRNAS PREDICT OUTCOMES IN CHILDREN WITH RELAPSED AND REFRACTORY NEUROBLASTOMA; A BEACON-NEUROBLASTOMA BIOMARKER STUDY

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BACKGROUND AND AIMS

Children with relapsed and refractory neuroblastoma (RR-NBL) have poor outcomes, with overall survival (OS) less than 20%. Early identification of children at greatest risk of relapse could mean timelier modifications of treatment to improve outcomes. High levels of adrenergic neuroblastoma mRNAs in blood of children with stage M neuroblastoma receiving frontline treatment predict poor outcome (Viprey et al, 2014, JCO, 32; 1074–83). Since these markers have not been thoroughly studied in the relapsed and refractory population, we have prospectively evaluated the prognostic potential of the adrenergic neuroblastoma mRNAs paired-like homeobox 2B (PHOX2B) and tyrosine hydroxylase (TH) in blood from children with RR-NBL treated in the BEACON-Neuroblastoma trial (NCT02308527).

METHODS

Blood samples collected at baseline from 88 children were analysed by reverse transcriptase polymerase chain reaction (RTqPCR) for PHOX2B and TH mRNAs (Viprey et al, 2014). The prognostic power of these mRNAs was evaluated using Kaplan-Meier survival curves and Cox proportional hazards regression. Progression-free survival (PFS) and OS were calculated from the date that the blood sample was taken to the date of an event; progression, disease recurrence, death or censored alive at the last clinical evaluation.

RESULTS

Of the children in this cohort, 58 (66%) had relapsed and 30 (34%) had refractory disease. Twenty-three (26%) had MYC-N amplified tumours. TH and PHOX2B mRNAs were detected in 55% and 60% of blood samples respectively; the correlation coefficient between TH and PHOX2B was 0.75. Higher levels of TH, PHOX2B or TH and PHOX2B mRNAs were associated with reduced PFS and OS. For TH, median PFS for children with TH levels below the median was 12 months (95%CI, 4.6–13 months), median PFS for children with TH levels above the median was 5.5 months (95%CI, 1.8–9.4 months). For PHOX2B, median PFS for children with PHOX2B levels below the median was 11.5 months (95%CI, 7.6–34 months), compared to 5.7 months (95%CI, 1.8–10.5 months) where levels were above the median.

CONCLUSION

TH and PHOX2B mRNAs in blood collected at baseline identify children with refractory or relapsed neuroblastoma at greatest risk of progression or death.

POSTER PRESENTATIONS
SIOPEN 2022

P_06
BAF COMPLEX LINKS CHROMATIN REGULATION TO NEUROBLASTOMA METASTASIS

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BACKGROUND AND AIMS
Epigenetic programming during development is essential for determining cell lineages, and alterations in this programming contribute to the initiation of embryonal tumour development. In neuroblastoma, neural crest progenitors block their course of natural differentiation into sympathoadrenergic cells, leading to the development of aggressive and metastatic paediatric cancer. Research of the epigenetic regulators responsible for oncogenic epigenomic networks is crucial for developing new epigenetic-based therapies against these tumours. Mammalian switch/sucrose non-fermenting (mSWI/SNF) ATP-dependent chromatin remodelling complexes act genome-wide translating epigenetic signals into open chromatin states. The present study aimed to understand the contribution of mSWI/SNF to the oncogenic epigenomes of neuroblastoma and its potential as a therapeutic target.

METHODS
Functional characterisation of the mSWI/SNF complexes was performed in neuroblastoma cells using proteomic approaches, loss-of-function experiments, transcriptome and chromatin accessibility analyses, and in vitro and in vivo assays.

RESULTS
Neuroblastoma cells contain three main mSWI/SNF subtypes, but only BRG1-associated factor (BAF) complex disruption through silencing of its key structural subunits, ARID1A and ARID1B, impairs cell proliferation by promoting cell cycle blockade. Genome-wide chromatin remodelling and transcriptomic analyses revealed that BAF disruption results in the epigenetic repression of an extensive invasiveness-related expression program involving integrins, cadherins, and key mesenchymal regulators, thereby reducing adhesion to the extracellular matrix and the subsequent invasion in vitro and drastically inhibiting the initiation and growth of neuroblastoma metastasis in vivo.

CONCLUSIONS
We report a novel ATPase-independent role for the BAF complex in maintaining an epigenomic program that allows neuroblastoma invasiveness and metastasis, urging for the development of new BAF pharmacological structural disruptors for therapeutic exploitation in metastatic neuroblastoma.

P_07
DISSECTING THE ROLE OF AUTOPHAGY IN METASTATIC NEUROBLASTOMA

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BACKGROUND AND AIMS
Around 70% of patients with metastatic NB at diagnosis present bone marrow (BM) infiltration, which is considered a marker of poor outcome. Although several prognostic factors have been identified in

patients with NB, the mechanisms underlying this specific tropism to the BM have not been completely elucidated. Autophagy is a self-degradative process that plays a homeostatic role in normal cells by eliminating organelles, pathogens and protein aggregates. In cancer cells, autophagy plays a different role based on the context: suppresses tumorigenesis by inhibiting cancer-cell survival and inducing cell death, but it also facilitates tumorigenesis by promoting cancer-cell proliferation and tumor growth. Emerging evidences suggest that, bone metastasis can be supported by MSCs through the creation of metastatic niches and that the dialogue between tumor cells and the surrounding tumor microenvironment may be mediated by extracellular vesicles such as exosomes. The aim of this work is to evaluate the role of autophagy in the BM metastatic niche formation through ii) the analysis of autophagy-related proteins into NB cells-derived exosomes; iii) the study of the autophagic pathway in MSCs cells.

METHODS

Autophagic flux has been evaluated by western blot analysis in 8 NB cell lines (2 derived from primary tumor and 5 derived from BM metastasis) and MSCs isolated from BM of NB patients with/without BM involvement and healthy control (HC-MSCs). Exosomes of NB cell lines has been isolated, characterized and analyzed at proteomic level for the presence of proteins implicated in autophagy. Modification in autophagy flux in MSCs has been evaluated after co-culture with NB-derived exosomes.

RESULTS

Analysis of basal levels of autophagy revealed that: i) primary tumor-derived cell lines have higher basal levels of autophagy than the lines derived from BM metastases; ii) HC-MSCs have higher basal level of autophagy than MSCs derived from NB patients; iii) NB-derived exosomes contain proteins implicated in autophagy pathway regulation.

CONCLUSIONS

Our study give new insights to the characterization of the autophagy pathway in NB and in the BM niche. The understanding of this molecular process could help us to develop new therapeutic approaches in patients affected by metastatic NB.

P_08
LIN28B SHAPES ANGIOGENESIS IN NEUROBLASTOMA THROUGH SECRETION OF IGF-II

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BACKGROUND AND AIMS

Neuroblastoma (NB) is an aggressive disease of pediatric age with a high incidence of widespread metastasis in a subset of high-risk (HR) NB patients. The RNA-binding protein LIN28B is one of the molecular drivers of NB aggressiveness sustaining cell invasiveness and migration through the Src/PI3K/Akt regulatory axis. The aim of this study was to investigate the contribution of LIN28B in promoting tumor angiogenesis as a fundamental hallmark of cancer metastasis and progression, and to characterize the mechanism underlying the LIN28B-dependent vessels' sprouting.

METHODS

We assessed the effects of long-term LIN28B overexpression in promoting angiogenesis using genetically modified NB cell line SH-SY5Y (LIN28B+). Functional studies were done in vitro, in co-culture experiments with LIN28B+ and human umbilical vein endothelial cells (HUVECs), and in vivo using the zebrafish xenograft model. To determine LIN28B-driven pro-angiogenic effectors, an ELISA-based array was employed. Functional rescue experiments were performed adopting neutralizing antibody-based strategy.

RESULTS

Gene expression study and pathway analysis highlighted angiogenesis as one of the most significantly enriched hallmark gene set in LIN28B+ compared to LIN28B- cells. LIN28B+ derived conditioned medium

exhibited pro-angiogenic and pro-migratory effects on HUVECs. Moreover, injection of LIN28B+ cells into zebrafish embryos enhanced tumor-associated vessel sprouting in vivo. The insulin-like growth factor II (IGF-II) was the most abundant angiogenic factor secreted by LIN28B+ cells. The inhibition of IGF-II through a blocking antibody strategy impeded LIN28B+ conditioned HUVECs branching in vitro and shaping of the vascular network in vivo.

CONCLUSIONS

Taken together, these results support a role of LIN28B oncogene in promoting tumor angiogenesis through the increased secretion of IGF-II. These findings extend the underlying knowledge of NB pathogenesis and open new perspectives for targeted treatment of progressing disease in HR-NB patients.

P_09
MAPPING BONE MARROW DISSEMINATION IN NEUROBLASTOMA BY DEEP MULTIPLEX IMAGING AND TRANSCRIPTOMICS

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BACKGROUND

Neuroblastoma is the most common solid tumor in children below the age of one and arises from progenitor cells during sympathoadrenal development. While most primary tumor cells resemble adrenergic neurons, more undifferentiated mesenchymal or neural-crest cell-like phenotypes have been identified, especially in pretreated and high-risk cases. In the majority of high-risk neuroblastoma patients, tumor cells have disseminated to the bone marrow.

AIMS

The bone marrow, however, is a common site of dissemination not only of neuroblastoma but also of other solid cancers such as breast and prostate cancer. Still, comprehensive single-cell analyses of bone marrow metastases, i.e. disseminated tumor cells (DTCs) and cells of their microenvironment, have not yet been performed. Herein, we aimed to capture tumor heterogeneity and analyze microenvironmental changes in a solid cancer with bone marrow involvement.

METHODS

Therefore, we applied a multi-omics data mining strategy to define a multiplex imaging panel and designed DeepFLEX, a computational pipeline for subsequent multiplex image analysis. Thereby, we generated a single-cell map of over 35,000 DTCs and cells of their microenvironment in the metastatic bone marrow. Independently, we profiled the transcriptome of 38 patients with and without bone marrow dissemination.

RESULTS

We found a high level of heterogeneity among DTCs and suggest that FAIM2 can act as a complementary marker to capture DTC heterogeneity. However, DTCs in this study predominantly expressed markers of the adrenergic lineage, such as GD2, but did not present a mesenchymal phenotype. Furthermore, we demonstrate that malignant bone marrow infiltration is associated with an inflammatory response and at the same time the presence of immuno-suppressive cell types, most significantly an immature neutrophil/granulocyte-myeloid derived suppressor-like cell type.

CONCLUSIONS

Our findings demonstrate vast diversity among DTCs and suggest that the latter shape the bone marrow microenvironment, warranting deeper investigations of spatio-temporal dynamics at the single-cell level and their clinical relevance.

P_10
STRATEGIES FOR NEUROBLASTOMA MICROENVIRONMENT MIMICKING ON 3D MODELS

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BACKGROUND AND AIMS
Extracellular matrix (ECM) composition modulates the aggressiveness of Neuroblastoma (NB). Vitronectin (VN), is a glycoprotein which is highly expressed in high-risk NB samples. This glycoprotein may confer stiffness to the microenvironment promoting neuroblast malignancy. To further study VN, the building of 3D models which mimic the NB environment is essential. We expose the results of cell clusters characteristics and VN secretion in three different 3D models.

METHODS
High-risk NB cell line SK-N-BE(2) was cultured in: Model 1) Bioprinted scaffolds composed of 5% methacrylated gelatin and methacrylated alginate (between 0% and 2%). Cross-link was induced by UV exposition; Model 2) Casted scaffolds composed of 3 or 10% wt of 4-arm-poliethyleneglycol-maleimide (PEG-Mal) and 500 µg/mL of pegylated-VN. The hydrogels were cross-linked with a protease-degradable peptide (VMP) and PEG-Dithiol at 1:10 ratio. Model 3) Casted scaffolds composed of gelatin-tyramine (GTA) and silk fibroin (SF) at different proportions GTA:SF (25:75) at a final solute concentration 4%wt and VN (400µg/mL). The hydrogels were enzymatically cross-linked with horseradish peroxidase and H2O2. Afterwards, the hydrogels were processed with paraffin or OCT and H&E, Dapi and anti-VN staining were done. QuPath and Pannoramic Viewer software were used for Digital Image Analysis.

RESULTS
Bioprinting with UV-crosslinking (model 1) enables homogeneous "cluster-like" structure formation and the synthesis of homogeneously distributed within clusters. PEG-based hydrogels (model 2) enable relatively easy incorporation of biomolecules and are technically easy to build. However, their design needs deep chemistry knowledge. Regarding VN localization, it is mainly found inside the cells and pericellularly. Silk-based hydrogels (model 3) have an easy chemistry and tyrosine-containing biomolecules are linked without additional modifications. VN expression is found inside the cells and pericellularly, however, it is mainly located surrounding cell clusters in a coronary pattern.

CONCLUSIONS
Each of the approaches confers different advantages regarding neuroblastoma pathophysiological range. Specifically, these versatile models allow the fine tunning of stiffness through changing their composition while studying the effect of different molecules in NB behavior via mechanotransduction processes.

Conflict of interest: Authors declare no conflict of interest

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P_11
MINIMAL RESIDUAL DISEASE DETECTION IN THE BONE MARROW OF NEUROBLASTOMA PATIENTS BY AUTOMATIC IMMUNOFLUORESCENCE PLUS IFISH (AIPF) AND RT-QPCR - A MULTICENTRE FEASIBILITY STUDY

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BACKGROUND AND AIMS

The assessment of bone marrow (BM) disease serves to stratify neuroblastoma patients into risk groups at initial diagnosis or evaluate treatment response in high-risk patients. Cyto-morphological evaluation of BM smears is the standard, however highly sensitive methods for BM minimal residual disease (MRD) detection have been clinically validated: automated immuno-fluorescence plus iFISH (AIPF) detects GD2+CD56+ neuroblastoma cells and RT-qPCR adrenergic mRNA-markers. In SIOPEN high-risk neuroblastoma trials, both analyses are recommended, but logistics of sample collection, transport and real-time analysis in specialized labs pose challenges. We therefore performed a feasibility study and provide recommendations for preanalytics in BM disease detection.

METHODS

Between 2018 and 2022, BM aspirates (n=310) from Austrian and Dutch HR neuroblastoma patients (n=124) were collected in PAXgene (0.5 ml) and EDTA tubes (3–5 ml). PAXgene tubes were stored at -80°C, shipped on dry ice and analyzed in batches by a validated RT-qPCR mRNA-marker panel for PHOX2B, TH, GAP43 and CHRNA3 in the Netherlands. EDTA-BM was subjected to density gradient separation and the mononuclear cell fraction was isolated, followed by cytospin preparation. Cytospin slides were shipped to Austria at room temperature or used directly for immuno-staining for GD2, CD56 and DAPI followed by scanning and rare cell detection with an automated microscope. An iFISH was performed in order to confirm rare tumor cells. Furthermore, data on cytomorphological evaluation was collected.

RESULTS

86% of samples were successfully analyzed with all 3 techniques. The analyzed samples showed a mean cell count of 1.5×106 cells (range 1.2×105 – 4.6×106) with 25% samples <1 million, 50% 1–2 million and 25% >2 million cells per aspiration site. iFISH analysis was performed and contributed to the clinical report in 8% of the samples. The cytospin preparation time and time to arrival at the reference lab was 1.5 days and 9 days, respectively. Time to arrival at the reference lab did not have an impact on the analytical quality.

CONCLUSIONS

This Austrian-Dutch collaborative study demonstrates the feasibility of sample logistics for BM analysis in a multi-national setting and provides recommendations for pre-analytical sample handling to ensure highest analytical quality for HR-NBL2 and other trials.

P_12

COMBINED BLOOD AND BONE MARROW CELL FREE DNA AND DISSEMINATED TUMOR CELL ANALYSIS FOR DISEASE MONITORING AND EARLY RELAPSE DETECTION

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BACKGROUND AND AIMS

NLiquid biopsy approaches have the potential to overcome current hurdles in therapy response assessment, clonal evolution of therapeutic targets and minimal residual disease (MRD)-monitoring to detect relapse early. However, tumor heterogeneity and the paucity of recurrent mutations in neuroblastoma pose a challenge in defining biomarkers for sensitive monitoring in each individual patient. Therefore this study combined circulating cell free (cf)DNA analysis in blood and bone marrow with the

detection of disseminated tumor cells (DTC) in the bone marrow and assessed their value in response and relapse evaluation.

METHODS

509 EDTA blood and 315 bone marrow (BM) aspirates from 32 patients with high-risk neuroblastoma were collected. From 16 patients whole exome sequencing of the tumor or metastasis at diagnosis was performed. Bone marrow mononuclear cells were isolated and immunostained for GD2/CD56 positive DTCs using automated immunofluorescence plus FISH (AIPF). CfDNA was isolated from plasma and subjected either to digital droplet (dd)PCR analysis for MYCN, other gene amplifications and ALK hotspot mutations or a library was prepared followed by NGS custom panel sequencing.

RESULTS

Our study showed a significant reduction of cfDNA tumor markers in response to therapy in blood and BM at mid-/end of induction therapy. Parallel DTC analysis showed similar response kinetics. All three markers were concordantly negative at 80 / 165 timepoints and at 35 equally positive, while in 21 only blood or BM cfDNA was positive and vice versa in 29 samples MRD was exclusively detected by DTC analysis. When clinical response evaluation was available, overall response was correlated with response in liquid biopsies. Remarkably, in two patients with local relapse/progressive disease without BM involvement, elevated MYCN copy numbers and tumor specific mutations were detected in blood cfDNA six weeks before clinically confirmed relapse.

CONCLUSIONS

The combined analysis of genetic markers and drug targets in blood and bone marrow plasma cfDNA and DTC enumeration is a sensitive approach for monitoring minimal disease, especially in patients with localized relapse or when clinical response evaluation fails to detect the onset of disease progression. Thus, combined liquid biopsy analysis will be an important component of neuroblastoma diagnostics.

P_13

USING ccfDNA WGS LIBRAIRIES FROM PLASMA ON ddPCR FOR ALK-HOTSPOT MUTATION DETECTIONS IN NEUROBLASTOMA PATIENTS

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BACKGROUND

Somatic ALK activating mutations are identified in approximately 10% of neuroblastoma (NB) patients. Known ALK hotspot mutations such as F1174L, R1275Q and I1170N lead to constitutive activation of the tyrosine kinase domain, justifying targeted treatment using ALK inhibitors such the 3rd generation ALK inhibitor Lorlatinib for these patients.

The study of cell free DNA (cfDNA) from liquid biopsies provides an important surrogate for the analysis of tumor cell specific genetic alterations and paves the way for important sequential analysis for patients under targeted therapies : to evaluate the content of circulating tumor DNA (ctDNA) as an indicator of disease burden and treatment response to measure treatment efficacy and to detect mechanisms of resistance. Thus, cfDNA extracted from plasma samples of HR-NB patients might be used for different analytical techniques during patient treatment and follow-up.

AIMS

cfDNA might be used for WGS Whole Genome Sequencing (WGS) libraries, for downstream approaches as (low coverage) WGS, Whole Exome Sequencing (WES) or targeted sequencing approaches. On the other hand, ddPCR is a more sensitive approach for detection of known hotspot mutations. Given the limited amount of plasma from NB patients, we thought to combine both approaches for a given sample, in order to both measure the mutated allele fraction (MAF) at the known hotspots with a high sensitivity, and to study clonal evolution, in NB patients under Lorlatinib treatment.

METHODS

In a first step, ddPCR targeting known ALK mutation hotspots (F1174L, R1275Q_, I1170N) was established for the study of cfDNA, and the MAF determined by ddPCR directly on cfDNA was compared to the MAF determined by ddPCR on WGS libraries of cfDNA, enabling the potential use of the same samples for ddPCR and cfDNA WES. For this, ALK MAF of genomic fragmented DNA was serially diluted, and then used for ddPCR analysis directly and/or for WGS library construction (using the SureSelect XT-HS low input dual index protocol from Agilent) followed by ddPCR.

In a second step, this approach by cfDNA ddPCR was applied to sequential plasma samples of NB patients under Lorlatinib treatment to follow ALK status during patient's therapy. For key time point : 1) at diagnosis, 2) at relapse after Lorlatinib treatment and 3) last known time point, we performed cfDNA WES to compare with the ALK status obtained by ddPCR, germline and tumor DNA, also to investigate the appearance of new alterations which might be involved in relapse and by extension better understand the clonal evolution's dynamic in HR-NB patient.

RESULTS

For genomic NB DNAs harboring distinct ALK mutations (R1275Q_, ALK F1174L), no statistically significant differences of ALK MAF with WGS libraries process were observed when comparing ALK MAF with no WGS libraries process by ddPCR, with a calculated detection limit of MAF at 0.04%.

In four high risk NB patients with a known ALK mutation, Lorlatinib treatment was given due to resistant/progressive disease; 3 – 10 sequential plasma samples per patient could be analyzed. MAF of ALK mutations measured by ddPCR correlated with the overall clinical disease status, with a MAF <0.1% in clinical remission, versus higher MAFs (> 30%) at disease progression. Some results also indicate that the follow-up of ALK MAF by ddPCR could detect the relapse earlier than clinical datas. The combined ddPCR/WES approach enabled analysis of cfDNA samples at progression to study clonal evolution, highlighting important cellular pathways such as gene impacting cellular growth.

SUMMARY/CONCLUSIONS

We validate the use of cfDNA WGS libraries for concomitant ddPCR and WES analysis . Our approach underlines the clinical utility of ddPCR for the follow-up of ALK MAF during treatment of HR-NB patient, and the use of WES for the study of clonal evolution, which might be integrated into clinical routine.

P_14

DINUTUXIMAB BETA COMBINED WITH CHEMOTHERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY NEUROBLASTOMA

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BACKGROUND AND AIMS

Prognosis in children with refractory and relapsed (RR) high-risk neuroblastoma (HR-NBL) is poor. Chemotherapy combined with anti-GD2 antibodies has previously been shown to increase response and survival rates.

METHODS

We carried out a retrospective review of 25 patients with relapsed or refractory (HR-NBL), who received dinutuximab beta (DB) immunotherapy combined with temozolomide and irinotecan as part of compassionate use programs at one of two centers, Krakow, Poland, and Greifswald, Germany, between December 2017 and October 2021. Observation was finished on January, 31st, 2022. DB was given as continuous long-term infusion of 10 mg/m2/day on days 2–6 of each 21-day cycle. Chemotherapy was given on days 1–5 of each cycle.

RESULTS

The median age of patients was 35.1 months (range 6.7–99.7), 11 (44%) patients had MYCN amplification. The majority of patients had metastatic disease at diagnosis. Twenty patients (80%) received chemoimmunotherapy for relapsed and five (20%) for refractory disease. Most patients were heavily pre-treated. Patients received 1–10 cycles of chemoimmunotherapy (mean 3). Complete remission was achieved in nine of 25 (36%) patients and partial remission in seven (28%), patients giving a best objective response rate of 64% (16/25). An additional five (20%) patients had stable disease giving a disease control rate of 84%. Median overall survival (OS) and progression free survival (PFS) were 10.3 months (range 0.7–43.0) and 6.3 months (range 0.2–37.0), respectively. The OS and PFS rates at 1 year were 47% and 48%, respectively. Of the 25 patients, 11 (44%) were alive at data cutoff, eight of whom completed therapy (with a median time from the beginning of therapy of 22.0 months (range 3.2–37.0), and three were still receiving therapy. No severe or unexpected toxicities were observed.

CONCLUSIONS

Our findings show that combination therapy with DB and TEMIRI in patients with relapsed or refractory neuroblastoma is feasible and well tolerated, with encouraging response rates and survival data. No severe side effects were observed in heavily pre-treated patients, including those who had previously been treated with DB. This chemoimmunotherapy combination is a promising treatment option for RR NBL and should be further explored in clinical studies.

P_15

OPTIMISING URINARY CATECHOLAMINE METABOLITE DIAGNOSTICS FOR NEUROBLASTOMA

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BACKGROUND AND AIMS

The analysis of urinary catecholamine metabolites is a cornerstone of neuroblastoma diagnostics. Currently, there is no consensus regarding the sampling method and variable combinations of catecholamine metabolites are used. We investigated if catecholamine metabolites in spot urine samples can be reliably used for the diagnosis of neuroblastoma.

METHODS

24-hour urine or spot urine samples were collected from patients with and without neuroblastoma at

diagnosis. Homovanillic acid (HVA), vanillylmandelic acid (VMA), dopamine, 3-methoxytyramine, norepinephrine, normetanephrine, epinephrine and metanephrine were measured by high-performance liquid chromatography coupled with fluorescence detection (HPLC-FD) and/or ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry (UPLC-MS/MS).

RESULTS

Catecholamine metabolite levels were measured in urine samples of 400 neuroblastoma patients (24-hour urine, n=234, spot urine, n=166) and 571 controls (all spot urine). Excretion levels of catecholamine metabolites and the diagnostic sensitivity for each metabolite were similar in 24-hour urine and spot urine samples ($P > 0.08$ and $P > 0.27$ for all metabolites). The area under the receiver-operating-characteristic curve (AUC) of the panel containing all eight catecholamine metabolites was significantly higher compared to the AUC of the panel containing only HVA and VMA (AUC=0.952 versus AUC=0.920, $P = 0.02$). No differences were observed between metabolite measurements by the two analysis methods.

CONCLUSIONS

Catecholamine metabolites measured in spot urine and 24-hour urine resulted in similar diagnostic sensitivities. Therefore, we recommend the implementation of spot urine as standard of care. The panel of eight catecholamine metabolites has superior diagnostic accuracy over VMA and HVA.

P_16

PRIMAGE – AN ARTIFICIAL INTELLIGENCE-BASED CLINICAL DECISION SUPPORT SYSTEM FOR OPTIMIZED CANCER DIAGNOSIS AND RISK ASSESSMENT – A PROGRESS UPDATE

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BACKGROUND AND AIMS

Patients with neuroblastoma (NB) and diffuse intrinsic pontine glioma (DIPG) suffer from relapse and death, partly due to insufficient knowledge of tumor properties and the lack of tools to integrate this knowledge into clinical decision-making. Children with NB and DIPG are enrolled in European clinical trials with standardized acquisition of patient-related imaging and molecular diagnostics, treatment and outcome data. These tumor types represent ideal use cases for the development of new integrated artificial intelligence (AI) models for medical imaging-based diagnosis and optimized initial individual risk assessment. PRIMAGE´s goal is the development and validation of the methodology and a platform to support decision making in the management of NB and DIPG.

METHODS

Patients enrolled in SIOPEN (LINES, HR-NBL1) and GPOH (NB97, NB2004, NB2004-HR, NB2016-Registry) neuroblastoma trials were included. Imaging studies (MRI, CT, PET, mIBG) obtained at time of diagnosis and at first follow-up after initial treatment were collected from local centers and analyzed using machine/deep learning methods. Clinical data and

molecular biomarker, e.g. MYCN amplification status, are provided through centralized databases (SIOPEN-R-NET) or patient-by-patient including manual curation to ensure quality controlled datasets.

RESULTS

First, a PRIMAGE repository was built holding images, molecular and clinical data. Clinical and molecular variables and corresponding e-forms were defined in line with existing trial databases. Amongst others, data was collected from approximately 50 SIOPEN centers in around 10 countries and pseudonymised using the European Patient Identifer (EUPID) service. Second, a software infrastructure for data management and radiomics analysis was established enabling quality assessment of images, segmentation and radiomics feature extraction. Third, an integrated visual analytics system allows for the interactive visual exploration of radiomics results and integration of various datatypes as well as models predicting clinical endpoints. A pilot version demonstrates feasibility, highlighting the potential of the PRIMAGE platform, but requires further refinements and integration of additional datasets to exploit its full potential.

CONCLUSIONS

PRIMAGE platform will support medical image processing and assessing imaging-derived biomarkers in the context of molecular and clinical variables, which is expected to guide diagnosis and individual risk assessment of patients with NB and DIPG in the future.

P_17

MULTIMODAL THERAPY WITH CONSOLIDATING HAPLOIDENTICAL STEM CELL TRANSPLANTATION AND DINUTUXIMAB BETA FOR PATIENTS WITH HIGH-RISK NEUROBLASTOMA AND CENTRAL NERVOUS SYSTEM RELAPSE

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BACKGROUND AND AIMS

Despite intensive multimodality treatment regimens, the prognosis of patients with high-risk neuroblastoma (HRNB) and central nervous system (CNS) relapse remains poor. Currently there is no established therapy regimen for these patients.

METHODS

We retrospectively reviewed data from 12 patients with HRNB and CNS relapse (6 patients with isolated CNS relapse, 6 with combined relapse) who received multimodal therapy with consolidating haploidentical stem cell transplantation (haplo-SCT) followed by dinutuximab beta ± subcutaneous interleukin-2 (scIL-2).

RESULTS

Following individual relapse treatment, including surgery (9/12 patients, 75%) irradiation (local/whole brain irradiation 7 patients, 58,3%, cranio-spinal irradiation 3 patients, 25%), I131-mIBG therapy (8 patients, 75%) and chemotherapy (all patients), intrathecal chemotherapy (1 patient, 8.3%), patients aged 3.6–16.8 years underwent haplo-SCT with T/B cell-depleted grafts followed by dinutuximab beta 20 mg/m²/day x 5 days, for 6 cycles (scIL2 1 × 106 IU/m² was added for 3 days in Cycles 4–6 in some patients). If response was demonstrated after Cycle 6, patients received up to nine treatment cycles. After haplo-SCT, seven patients had complete remission (CR), four had partial response (PR) and one had stable disease (SD). All 12 patients received ≥5 cycles of antibody treatment.

Overall, 5 patients (42%) with CR maintained their CR until the end of trial treatment, two patients (17%) progressed, two patients (16,7%) with PR/SD achieved CR, one patient responded partially to the antibody treatment but died of an infection after the end of treatment.

At the end of follow-up, 8/12 patients (66.7%) demonstrated complete response (one patient with a further CNS relapse achieved CR after subsequent surgery and radiotherapy). As of June 2022, all eight patients remain disease-free, with a median follow-up time of 4.9 years since relapse. 5-year event-free survival and overall survival rate estimates were 51.6% and 61.9%, respectively.

CONCLUSIONS

Consolidating Dinutuximab beta ± scIL-2 following haplo-SCT is a treatment option for patients with neuroblastoma relapsed to the CNS. Clear tumor responses have been demonstrated in three patients (combined relapses) with the combinational treatment, in one patient tumor response of the CNS metastasis to the antibody treatment was seen.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
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P_18

SINGLE AGENT ACTIVITY OF ANTI-GD2 ANTIBODY DINUTUXIMAB BETA (DB) LONG-TERM INFUSION IN HIGH-RISK NEUROBLASTOMA PATIENTS WITH RELAPSED AND REFRACTORY DISEASE. A MULTICENTER PHASE II TRIAL.

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BACKGROUND

Anti-GD2 antibodies were used in combination with isotretinoin and cytokines in previous neuroblastoma immunotherapy trials. We evaluated DB long-term infusion (LTI) as single agent in patients (pts) with high-risk relapsed/refractory neuroblastoma.

METHODS

40 pts with relapsed/refractory neuroblastoma were enrolled (EudraCT 2014–000588–42; Study 304). Pts received up to 5 cycles of 100 mg/m2 DB-LTI (d1–10). Primary endpoint was treatment response according to the International Neuroblastoma Response Criteria (INRC). Secondary endpoints were safety, pharmacodynamics and pharmacokinetics, duration of response and 3 year (y) overall and progression free survival.

RESULTS

Of 40 enrolled pts 38 remained in the full analysis set. The best overall response rate was 36.8% (4 CR and 10 PR). There were 5 pts with a minor response (MR) accounting for an INRC best response rate of 53.0%. Of 14 pts with bone marrow involvement 13 pts responded (93%) (12 CR, 1 PR). The median duration of response for patients with CR or PR was 238d [108–290d] and the 3y progression free and overall survival was 31.5% ± 7.8% and 65.5% ± 8.1%, respectively. Survival rates were significantly higher in patients with refractory compared to relapsed neuroblastoma.

Grade 3&4 adverse events with a frequency >10% were inflammation (20%), gastrointestinal disorders (12.5%) and pyrexia (10%). Pain, allergic-/hypersensitivity reaction, capillary leak were less frequent. No paraplegia occurred. Morphine was given in cycle (C) 1 (C1 100%) and decreased from cycle 2 to 5 (C2 95%; C3 12%; C4 0%; C5 0%). The median time of hospitalization decreased from 7d (C1) to 3d in (C5) and all patients received parts of the 10d LTI as outpatient.

CONCLUSIONS

Single agent use of DB as long term infusion is highly tolerable and effective in relapsed/refractory high risk neuroblastoma with a clinically meaningful response-rate and – duration.

P_19

BONE MARROW INFILTRATION IN HIGH-RISK NEUROBLASTOMA: CHARACTERISTICS OF INFILTRATING CELLS AND PROGNOSTIC IMPACT

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BACKGROUND AND AIMS

Neuroblastoma (NB) is the most common extracranial solid tumour in childhood. The bone marrow (BM) represents the most frequent NB metastatic sites (70% at diagnosis in high-risk patients) and is a place where disease recurrence often takes place. NB cells dissemination in BM is a negative prognostic marker in high-risk (HR) patients. Although long-term survival has improved over the past 25 years, particularly with the introduction of the immunotherapy, 50% of patients still relapse or have refractory disease. Therefore, it is essential to identify new prognostic factors for metastatic and recurrent NB. In this study we will evaluate the morphological pattern of BM involvement from NB cells at diagnosis evaluating the prognostic role in HR patients.

METHODS

Retrospective analysis of high risk neuroblastoma BM samples from 49 patients treated in Oncology-Hematology Unit of Padua University from January 2004 to December 2018. The main objective of the project encompassed the comprehensive characterization of BM specimens at morphology evaluation with optical microscopy. We described and characterized two different morphological pattern of BM invasion at diagnosis: clumps pattern (A pattern) and diffuse-type pattern with replacement of the normal hematopoietic component (B pattern). We assessed the correlation between morphological pattern of BM invasion and the clinical disease characteristics.

RESULTS

BM involvement from NB cells was present at diagnosis in 43 of 49 patients (88%). Twenty-six (60%) of these presented with an A pattern, 17/43 (40%) with a B pattern. 5-year OS and PFS of the cohort were 48% and 38%, respectively. Considering the different pattern of BM invasion, B pattern appears to be associated with poor outcome (OS=35%; PFS=18%) in comparison to A pattern (OS=50%; PFS=42%) or to patients without BM infiltration (OS=80%; PFS=83%). In fact, patients with B pattern invasion relapsed more frequently (82%) than patients with A pattern (54%) or with negative BM (17%).

CONCLUSIONS

These are the first data on the prognostic role of the different BM infiltration in HR NB. B pattern invasion is associated with a less favourable outcome than A pattern, due to the higher relapse rate.

P_20

RATIONAL FOR IRRADIATION OF PERSISTING OLIGO-SKELETAL METASTASES TO IMPROVE SURVIVAL OF METASTATIC NEUROBLASTOMA PATIENTS WITH A POOR RESPONSE TO CHEMOTHERAPY? A RETROSPECTIVE STUDY

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BACKGROUND AND AIMS

Most High-Risk Neuroblastoma are metastatic at diagnosis with positive skeletal uptakes on MIBG scintigraphy. Persistent MIBG-positive skeletal metas-

tases at the end of induction correlate with a poor outcome and lead to treatment intensification. Radiotherapy of residual bone metastases may be a way for improving outcome of these patients.
Our aim was to investigate if there is a rational for a prospective randomized study evaluating the impact of radiotherapy of oligo residual bone metastatic sites with the aim of improving survival of patients.

METHODS

Patients over one year at diagnosis treated between 2000 and 2020 at Gustave Roussy for a stage M neuroblastoma were included. Patients had to have a positive MIBG scan at diagnosis and persistent skeletal metastases after high-dose chemotherapy (HDC). Patient's disease and treatment characteristics were retrospectively collected. MIBG scans were reviewed by two nuclear physicians.

RESULTS

202 patients were identified, 78 (38.6%) had progressive disease before the end of treatment.
Only 32 patients (15.8%) had persistent skeletal MIBG-positive scans post HDC. Two of them relapsed during maintenance. Five had negative MIBG scan at the end of treatment and one became negative during follow-up; none of them relapsed (median FU 63 months [2–176]).
17/24 patients with persistent skeletal uptakes at the end of the treatment had a disease progression within a median time of 14 months [2–43]. They had a higher SIOPEN score at each evaluation and received a lower treatment intensity.
97% of persistent skeletal uptakes after HDC and end of treatment were also present at relapse but represent only 31% and 23% respectively of the 139 sites of relapse in all our patients. 85% of metastatic sites at relapse were present at diagnosis.

CONCLUSIONS

Our study underlines that radiotherapy of persisting oligo-skeletal metastases would concern a minority of patients. Recurrence mainly occurred in disease sites present at diagnosis that cleared with chemotherapy. On-therapy control of the disease is the main issue. In addition, persisting MIBG sites cannot be by itself a criterion to include patients in innovative treatment study. A larger retrospective study is needed to help define future strategy.

P_21
INTERIM ANALYSIS OF THE UK RELAPSED NEUROBLASTOMA STUDY

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BACKGROUND

Despite advances in neuroblastoma cases treatment, relapse still occurs in 50% of high risk (HR) cases and in most cure is no longer possible. Some clinical and genetic factors associated with length of survival following relapse have been identified. However, many other genetic factors may be important in predicting response to Phase I and II treatments given at relapse. Our previous study in 2016, showed the 5-year post relapse overall survival (PROS) for relapsed HR cases diagnosed from 2000–2010 with MYCN amplified (MYCNA) disease was 7.7% (95% CI 1.3–21.7%) vs 12.8% (95% CI 4.7–25.2%) for MYCN non-amplified cases.

AIMS

to investigate clinical and genetic factors associated with neuroblastoma relapse and length of survival following relapse.

METHODS

Retrospective study including all relapsed identified from 2000–2021 (aged 0–40 years) from UK paediatric oncology treatment centres. Relapse was defined as recurrence or progression following an initial response (including partial) to any neuroblastoma therapy.

RESULTS

128 cases identified from 11 centres so far are included in this interim analysis. 93% of cases were stage 4(M), 44% MYCNA with a median age at diagnosis of 3.4 years (IQR=1.9–4.6). At diagnosis 105/128, were treated on the HR neuroblastoma (HRNBL1) trial. Median progression free survival time from diagnosis to relapse was 1.2 years (IQR=0.8–1.9 year). Median overall survival time (OS) was 27months (95% CI 24–41), for MYCNA disease was 16 months (95% CI 12–31) and for MYCN non-amplified disease was 34months (95% CI 22–65); 5-year OS was 18% (95% CI 12–26%); 10% (95% CI 3–21%) for MYCNA disease vs 28% (95% CI 17-39%) for MYCN non-amplified cases (P<0.001). Median PROS time was 9.5 months (95% CI 7.7–11.3); for MYCNA disease it was 5.7 months (95% CI 2.3–8.1) and for MYCN non-amplified disease it was 12.6 months (95% CI 10.2–18.1) (P<0.001). The 5-year PROS was 14% (95% CI 9–22%); 9% (95% CI 2.7–20.7%) for MYCNA vs 20% (95% CI 11–32%) for MYCN non-amplified.

CONCLUSIONS

Results from this interim analysis show the 5-year survival for relapsed HR patients has improved slightly compared with our previous study especially for the MYCN non-amplified cases.

P_22
NEUROBLASTOMA AND CNS DISEASE: A PANHELLENIC 20-YEAR RETROSPECTIVE STUDY

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BACKGROUND AND AIMS

Presentation of patients with neuroblastoma and CNS disease at diagnosis or at relapse. The percentage of CNS disease in neuroblastoma patients is less than 3% and with dismal prognosis. Currently, no need for CNS prophylaxis is established.

MATERIAL AND METHODS

Neuroblastoma patients with CNS disease were studied retrospectively during 2000–2021 in Greek Paediatric Oncology Departments nationwide.

RESULTS

Fifteen patients were diagnosed with CNS disease, 13% (2/15) at diagnosis and 87% (13/15) at relapse. Male/Female ratio: 1,1. Median age at diagnosis was 3,6 years (range 4 months-14 years). All patients

were stage 4/M at diagnosis (100%, 15/15). Nmyc amplification was detected in 53% (8/15) of the patients. Initial treatment was based on the current SIOPEN-HR-1 protocol. Most of the patients with CNS disease at relapse were at first relapse (77%, 10/13). Neuroblastoma CNS metastases were detected in variable anatomic areas such as cerebellum, frontal lobe, lenticular nucleus and temporoparietal area. CNS neuroblastoma involvement for 2 patients at diagnosis were located at the temporal-occipital area and intra-spinal cord, respectively. Treatment included surgery in 53% (8/15), CNS radiotherapy 60% (9/15) and conventional chemotherapy including TemIri, ICE and TVD. Two patients received MIBG treatment according to the VERITAS protocol. No patient received innovative marker-directed targeted therapy, apart from intravenous dinutuximab beta. Only one patient received Omburtamab, a radiolabeled monoclonal antibody 8H9 (131I-8H9) targeting B7-H3, experimental intraventricular administration in the US and he is alive. Most patients (80%, 12/15) have died, at a median time of 2 years from initial diagnosis (range 3 months-4,5 years). Post detection of CNS disease, they died in a median time of 10 months (range 2–26 months). The 3 patients that are alive had second relapse at 4, 32 and 55 months post-diagnosis, retrospectively.

CONCLUSIONS

In this nation-wide cohort, neuroblastoma CNS disease is rare at relapse and even more at diagnosis. Prognosis remains poor, but the use of new treatment approaches may improve survival of this subgroup of paediatric patients with historically dismal prognosis.

P_23

FIVE YEARS' EXPERIENCE OF THE UK NATIONAL NEUROBLASTOMA ADVISORY PANEL

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BACKGROUND

The National Neuroblastoma Advisory Panel (NNAP) UK was established in 2017 by a multidisciplinary group with expertise and an interest in multimodal management of neuroblastoma. The aims were: to provide a national forum to enable discussions and provide guidance on the management of complex neuroblastoma patients, and to support families through shared decision, providing an opportunity for second opinions following referral by their oncologist. Here we review our five years' experience.

METHODOLOGY

A monthly virtual meeting of 2 hours was established with the support from Children's Cancer and Leukaemia Group. The panel comprised of 15 medical members, with expertise in the biology and molecular characterisation, clinical trials and management, surgery, radiology and radiotherapy including molecular radiotherapy. The referring paediatric oncologist submitted an anonymised online proforma, with the clinical details to the panel. The referring team presented the patient, including any parental wishes, and received the written outcomes to be shared with the family afterwards. The panel's role was mainly advisory; the responsibility for final decisions rested with the referring team.

RESULTS

A total of 60 meetings over 5 years discussed over 250 patients across 22 centres with a 40% increase in the referrals over the 5 years. Median of 5 (range 2–7) cases were discussed per meeting. Most were paediatric but 25 adults were also discussed. The disease distribution was across the various risk groups and various time points in the treatment. Queries were from low-intermediate risk including relapses (26%), high-risk primary management (18%), high-risk primary refractory (29%) and high-risk relapses (27%) and surgical opinions (35–40%). The time commitment per meeting was 6–8 hours for the chair, 3 hours radiology, 1 hour surgical and 2 hours for everyone on the panel for discussions. This time was voluntary with no administration support.

CONCLUSIONS

A national expert advisory panel was able to contribute to complex neuroblastoma cases with the voluntary commitment from a panel of dedicated experts. The local oncology teams (and the families) were very grateful for the opinions given. Commissioning for this service to continue is needed. Adults with neuroblastoma benefit from paediatric specialist care guidance.

P_24

THYROID BLOCKADE DURING 123 I MIBG SCAN: COMPARISON OF STANDARD METHODS TO LAST HOURS, ON SITE IODINE ADMINISTRATION

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BACKGROUND AND AIMS

Thyroid gland is sensitive to radioactivity. When radioactive iodine isotope loaded tracers are used for diagnostic tests, like metaiodobenzylguanidine (123I-MIBG) for neuroblastoma imaging, administration of potassium iodide aims to limit this uptake by the thyroid parenchyma .

Until 2015, in our center we have used the EANM-recommendations (EJNMMI (2010)37:2436–2446) for iodine-prophylaxis for pediatric patients, according to age (<1month, 1month-2years, 3–12years, <12years) with the Lugol's solution premedication at 48–24 hours prior 123I-MIBG administration (Group A). Starting from 2016 the same age-related doses premedication were delivered on the Oncologic Department within 3–1 hours prior the radio-tracer administration (Group B), according to the WHO Iodine Thyroid Blocking GL (2017 ISBN 9789241550185), that suggests better thyroid protection. This study was performed as a pilot for the SIOPEN Quality Of Life&Long Term Outcome Committee to compare thyroid blockade effectiveness.

The aim was to evaluate whether there is difference in thyroid blockade in Group B patients compared to the historical Group A patients by Fisher's exact test, Pearson Correlation, T-test (SPSStatistics).

METHODS

There were 319 123I-MIBG scintiscans performed form 2010–2021, 107 GroupA, 211 GroupB. Scans were grouped by patient-age on the basis of administration schedule : <1y (N=10 GroupA; 23 GroupB) and 1–3y (N=32 GroupA; 52 Group B), 3–12y (N=57 GroupA; 116 GroupB), >12y (N=8 GroupA; 20 GroupB). All scans were read by 2 physicians as having or no thyroid uptake.

RESULTS

There is no statistically significant difference in the thyroid blockade between Group A and Group B patients overall (p=0,1344). A significant difference was found only in males 3–12y old: Group A positive= 52% vs Group B positives= 29% (p<0.04). A close positive correlation (p=0.0001) was found between mean age and thyroid visualization percent into the groups : r= 0,9953 for Group A and r= 0,9951 for Group B, and a better thyroid blockade for younger children <1y (87%) respect the elders 13–18Y (4%). Mean age in positive cohort was higher than in negative one (p< .00001; see graph1).

CONCLUSIONS

These data suggest that 1–3h Lugol premedication before 123I-MIBG, within healthcare setting, results in an unaffected or even better thyroid suppression efficiency according to WHO-GL.

P_25

IMMUNOTHERAPY IN CHILDREN WITH HIGH RISK NEUROBLASTOMA- EXPERIENCE FROM A SINGLE CENTER IN CROATIA

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AIMS

Comparision in outcome of patients who receive immunotherapy and others who receive only retinoic acid in the maintenance therapy.

METHODS

A retrospective review of the clinical chart of all patients with HR-NB treated between 2012 and 2021.
Patients had HR-NB if they were at least 12 months of age with INSS stage 4 neuroblastoma (primary tumor with dissemination or <12 months of age with INSS stage 4 disease and MYCN amplification.

RESULTS

In total, 23 pts received treatment for HR-NB. 11 patients received maintance therapy with dinutuximab beta and 12 pts didn't receive immunotherapy (before May 2017 when dinutuximab beta became available in Croatia).
All pts received intensive induction chemotherapy. Most pts underwent tumor resection with or without lymphadenectomy. Most pts received MAT (87%) with either Busulphan Melphalan or Carboplatin, Etoposide and Melphalan followed by a peripheral blood ASCT.
All 11 pts in the DB group received up to five cycle dinutuximab maintance therapy as continuous infusion over 10 days 10 mg/m2 per day, one patient relapsed after receiving only one cycle and another stopped after 4 cycle due to adverse event.
All patient in the no immunotherapy group received maintance therapy with isotretinoin alone.
In the no immunotherapy group, one of 12 pts had complete remission (follow up- 9 yrs and 2 months and other 11 pts died to tumor progression between 4 m and 6 y 4 m after diagnosis.
In pts in the DB group, CR was achieved in the 8 of the 11 pts (73%), median duration of remission 5 y and 2 months, 2 patient died due to disease both 1 y and 10 m after diagnosis.
All pts in the DB group had achieved CR following MAT and ASCT. Patients with HRNB who received DB in addition to the multimodal therapy had a higher median EFS.
DB was generaly well tolerated and all adverse event were manageable.

P_26

BIODOSIMETRY ROLE IN THE USE OF ^{131}I-METAIODOBENZYLGUANIDINE ^{131}I-MIBG IN THE TREATMENT OF HIGH-RISK NEUROBLASTOMA

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BACKGROUND AND AIMS

^{131}I-mIBG is a targeted radiopharmaceutical that has shown efficacy in patients with neuroblastoma, with better response being observed when reaching a dose of 4 Gy. Our objective was to analyze dicentric chromosomes (DCs) resulting from the erroneous repair of genetic damage produced by the interaction between ionizing radiation and genetic material, as complementary tool to standard body dosimetry (DCTfis), using a personalized approach.

METHODS

Physical dosimetry (n=3):

Whole body (WB) dosimetry was accomplished according to the MIRD schema [Buckley].

The time-activity curves follow a bi-, tri-, and penta-exponential curves for fitting the measurements.

Biodosimetry (n=1):

Procedures were made to carry out the dicentric chromosome (DC) assay, obtaining blood samples d0 pre ^{131}I-mIBG infusion and d7.

In vitro irradiation of blood samples was performed to prepare a dose-effect curve.

The frequency of dicentrics was assessed from the analysis of 100 first-division metaphases.

Dwb -biol was calculated using the frequency of dicentric and the Chromosomal Aberration Calculation Software (CABAS) and using the procedure for calibration [Montoro].

RESULTS

Dicentrics frequency for d0 and d7 were 0.03 ± 0.017 and 0.44 ± 0.066, respectively. Therefore, the DCT-bio was 0.47 (0.12–1.01) Gy for d0 and 2.71 (2.25–3.21) Gy for d7. For the second phase of treatment, the frequency of dicentric for d21 was 0.37 ± 0.097 and a whole body dose of 2.38 Gy was reached (1.63–3.24) and for d28, a dicentric frequency of 0.75 ± 0.194 with an estimated dose of 3, 67 (2.64–4.83) Gy was observed. The DCTfis after fitting the dose rate measurements to a function of three exponentials was 1.44 ± 0.15 Gy for d7.

CONCLUSIONS

The variability of the estimated Dwb ranged from 4 % to 15% when comparing different fitting expressions. The tri-exponential curve presented the best correlation coefficient in all the cases.

Although we do not expect the same value for the physical and biological dosimetry, there is a good concordance.

Further studies with larger population should be performed to evaluate the correlation between both methods and to elucidate whether biological dosimetry can be considered a complementary tool for WB-dosimetry.

P_27

NEUROBLASTIC TUMORS IN INFANTS AND CHILDREN: A SINGLE INSTITUTIONS EXPERIENCE

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BACKGROUND AND AIMS

Neuroblastoma comprises 7–11% of pediatric cancer. Prognosis varies from spontaneous regression to metastatic disease with high mortality. Our aim is the documentation of all patients with neuroblastic tumors, treated in our unit the last decade.

METHODS

Age, gender, tumor location, treatment, histologic/molecular characteristics and outcome were documented. Therapy and stratification were according to SIOPEN guidelines.

RESULTS

Sixty-three patients (pts) were included (25 girls), mean age: 2.3 years (0–15 years).

Fourty-nine pts (77.7%) had neuroblastomas of low differentiation, 12 (19%) ganglioneuroblastomas and 2 (3.1%) ganglioneuromas.

Unfavorable histologic/molecular characteristics had 19% of patients. Intraspinal tumors had favorable histologic/molecular characteristics, despite their severe clinical presentation. Nine patients (14.2%) had NMYC amplification

Patients were stratified into low (39 pts), intermediate (5) and high risk (HR) groups (19).

Primary locations included: adrenals (41 pts), mediastinum (9 pts), paraspinal (8 pts) and intraspinal (5 pts). Six patients (all infants, mean age 3.8 months, dumbbell primary 5 pts) presented with neurologic deficits due to spinal cord compression. In 5 patients, in whom therapy started promptly, symptoms resolved after completion of therapy. One patient with delayed diagnosis, still has neurological deficits

(paresis, neurogenic bladder). Three patients had opsoclonus-myoclonus, which resolved. Metastatic disease was documented in 32 patients (50.7%).
All therapies were included; 12 (19%) only observation, 53 (84.1%) surgery, 34 (53.9%) chemotherapy ,14 high-dose chemotherapy, 9 (14.2%) immunotherapy. Second line therapies included MIBG treatment (3 pts) and targeted therapy (ALK inhibitors, 2 pts). Nine patients from HR group had progressive/refractory disease and were treated as very high risk patients according to VERITAS protocol. Eight patients from HR group are alive. Four still under treatment, 2 in first and 2 in second remission with ALK inhibitors. Mean follow-up was 3.2 years. Overall survival (OS) for low and intermediate risk groups was 100%, for high risk 55.5% and for very high risk 14.2%. OS for patients with NMYC amplification was 37.5%.

CONCLUSIONS

Stratification of patients has improved prognosis and helped in personalizing treatment. Despite progress made, treatment of high-risk patients poses many challenges. Prompt institution of therapy may prevent sequelae while targeted therapy may induce remission.

P_28

NEUROBLASTOMA STAGE MS IN INFANTS UNDER 1 YEAR OF AGE: A NATIONAL REPORT FROM GREECE

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5. Department of Pathology Oslo University Hospital Rikshospitalet Sognsvannsveien Oslo

BACKGROUND AND AIMS

Neuroblastoma, an embryonal tumour arising from the sympathetic nervous system, is the most common malignancy of infancy. The prognosis of stage Ms neuroblastoma has traditionally been reported as excellent with 97% O.S. The purpose of the study was to evaluate the outcome of infants diagnosed with neuroblastoma stage Ms between 2005–2022.

METHODS

4 pediatric hematology-oncology centers in Greece participated in this retrospective study that covered the period between 2005–2022. Demographics, tumor characteristics and outcome were analyzed. 21 patients (13 boys) were reviewed with a median age of 2 months. According to the LINES SIOPEN protocol patients separated into 4 groups.

- **Group 4:** Patients with NCA genomic profile without life threatening symptoms
- **Group 5:** Patients with NCA genomic profile with life threatening symptoms
- **Group 6:** Patients with SCA genomic profile without or with life threatening symptoms
- **High Risk Group:** Patients with N-myc amplification

RESULTS

The adrenal gland was the primary tumor site for 16 patients, while the other 5 had paraspinal site. All patients had liver metastasis, 10 bone marrow and 2 skin metastasis. 9 patients were on Group 4, 6 underwent observation, 2 underwent surgical resection of primary tumor and one received chemotherapy due to progressive disease. 7 patients were on Group 5, all of them received chemotherapy while 6 underwent surgical resection of primary tumor. Two of seven patients died due to progressive disease and organ compression. 3 patients were on Group 6, all of them received chemotherapy and underwent surgical resection of primary tumor. 2 patients with amplified MYCN gene received more aggressive chemotherapy. One patient died due to progressive disease and the other relapsed after 4 year.

CONCLUSIONS

A very high rate of survival is achievable in patients with intermediate risk neuroblastoma by chemotherapy or chemotherapy and surgery. In our study the overall survival rates were 100% for Group 4 and 6 and 72% for Group 5. N-myc amplification is significantly correlated with poor prognosis. More-effective treatment strategies still are needed for infants with N-myc amplification.

P_29

PATIENT-SPECIFIC ASSAYS FOR LIQUID BIOPSIES IN PEDIATRIC SOLID TUMORS REFLECT TUMOR BURDEN

Lieke M.J. Van Zogchel[*1,2], Nathalie S.M. Lak[*1,2], Nina U. Gelineau[1,2], Irina Sergeeva[3], Ellen Stelloo[3], Joost Swennenhuis[3], Harma Feitsma[3], Max van Min[3], Erik Splinter[3], Margit Bleijs[1], Marian Groot Koerkamp[1], Willemijn Breunis1[1], Michael Meister[1], Waleed Hassan Kholossy[1], Jan Molenaar[1], Wendy de Leng[4], Janine Stutterheim[1], C.E. Ellen vd Schoot[2], G.Lieve A.M. Tytgat[1]

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2. Sanquin Research Department, Amsterdam, the Netherlands
3. Cergentis B.V., Utrecht, the Netherlands
4. Department of Pathology, UMC Utrecht, Utrecht, the Netherlands

BACKGROUND AND AIMS

Liquid biopsies combine minimally invasive sample collection for sensitive detection of residual disease. However, pediatric malignancies often harbor tumor-driving fusion genes or copy number aberrations. These are tumor-specific DNA sequences, which can be determined by targeted locus amplification (TLA) on formalin-fixed, paraffin-embedded material (FFPE-TLC). In a pilot study we investigated whether these patient-specific targets can be used for detection of tumor-derived cell-free DNA (cfDNA) in plasma from patients with different pediatric solid tumors.

METHODS

Regions of interest for breakpoint analysis were identified through standard clinical diagnostic pipelines, using SNP array or whole-exome sequencing (WES) for copy number aberrations and Shallow whole-genome sequencing (sWGS) was performed on the TLC template. A corresponding patient-specific droplet digital PCR (ddPCR) was designed and tested on cfDNA isolated from plasma samples from the patients. Methylation-specific RASSF1A, ALK and MYCN ddPCR were also performed, if applicable. As well as RT-qPCR for detection of mRNA-markers.

RESULTS

TLA was performed on 6 organoid cells derived from primary tumor, from 2 rhabdomyosarcoma patients, 1 Ewing sarcoma and 3 neuroblastoma patients. FFPE-TLC was performed for 7 neuroblastoma patients. For 1 neuroblastoma patient, both primary tumor cells and FFPE material were available and both yielded the same patient-specific sequence. For all patients, at least one patient-specific ddPCR was designed successfully. The patient-specific targets were detected in all plasma samples taken at initial diagnosis for the different tumor entities. In the rhabdomyosarcoma and Ewing sarcoma patients, all samples after start of therapy were negative. In neuroblastoma patients, presence of the patient-specific targets in the cfDNA tracked the tumor burden, decreasing during induction therapy, disappearing at complete remission and re-appearing at relapse. Performance of patient-specific and RASSF1A-M ddPCR were comparable.

CONCLUSIONS

In this study, we demonstrate that patient-specific targets can be identified in different pediatric solid tumors using TLA. Furthermore, we show that these targets can be detected in cfDNA from plasma and their presence correlates to disease burden in neuroblastoma. This approach holds promise for use in several types of pediatric solid tumors and will be investigated further in a multicenter setting with Institute Curie.

CASE PRESENTATIONS SIOPEN 2022

CP_30
INFANT WITH EXTENSIVE INTRATHORACIC AND INTRASPINAL MS NEUROBLASTOMA: CONSIDERATION FOR SURGICAL DEBULKING VS. OBSERVATION

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Department of Pediatric Hematology – Oncology (TAO), Agia Sofia Children's Hospital, Athens, GREECE

CASE

A term one month old baby presented with floppiness, respiratory problems and seizures and was eventually found to have neuroblastoma of the neck and chest (C4 to T4) with significant >80% symptomatic spinal cord invasion (impaired reflexes and movement of the lower extremities and questionable urinary and bowel function). Upon admission (45 days/old) he immediately underwent laminoplasty and intraspinal-mass biopsy. Following registration to the LINES Protocol, VP-Carbo chemotherapy was commenced.

Initial staging documented 6.0 × 3.5 × 3.0 cm mass, multiple liver metastases, BM smears and biopsies x2 were negative, no other metastases were evident on tomographies and the MIBG scan was positive at the primary mass area only (no uptake at the liver metastases, CNS clear). Repeated re-evaluation following 2 VP-Carbo cycles found the patient in excellent condition, gaining developmental milestones, breast feeding and growing. He has been weaned off anti-epileptic medications uneventfully. MRI did not reveal any significant change of the masses: 5.0 x 3.5 × 3.3 cm, still with intracanal invasion and spinal cord compression, the multiple liver metastases being rather unchanged. No repeat MIBG scan was done at the end of chemotherapy. As per protocol, LTS are not present and he has stopped further treatment, as he was found to have Numerical Chromosomal Aberrations and no MYCN amplification (LINES Group 105).

Upon follow-up, he had persistent elevated urine catecholamines, that prompted re-staging 1.5 months after the second chemotherapy course, that documented the mass measuring 3.0×2.8×4.0 cm, with decreased diffusion, still with contrast enhancement, and decreased spinal cord displacement. The liver metastases appeared decreased in number and size (largest being 7 mm from 11 mm).

On follow up at 11 months of age, he remained asymptomatic with decreasing thoracic mass (3 cm cephalo-caudal axis), no diffusion restriction, still with contrast enhancement, cord compression at the T2-T3 level without contrast enhancement and heterogeneous hepatic parenchyma without definitive masses. He is due for re-evaluation at 15 months of age (September 2022).

Due to the significant residual intra-thoracic and intra-spinal residual disease the question for the need of surgical debulking needs to be discussed.

CP_31
A CASE OF PRIMARY PROGRESSIVE NEUROBLASTOMA: IS CURE STILL POSSIBLE?

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2. Department of Paediatric Radiology, Great North Children's Hospital, U.K
3. Department of Medical Physics, Royal Victoria Infirmary, Newcastle, U.K.

CASE

A 9-year old girl was diagnosed with metastatic high-risk neuroblastoma with a left suprarenal primary, bone marrow and widespread bone metastases with a SIOPEN score of 16. There was widespread spinal involvement at multiple sites with spinal canal extension. Histology of a bone metastasis showed poorly differentiated NB, MYCN not amplified, ALK negative, and presence of atypical segmental chromosomal abnormalities (SCAs) only. LDH was 730 U/L and urinary catecholamines raised. The patient was treated according to the SIOPEN HRNBL-1 protocol but, the mIBG scan showed progression at the end of COJEC. MRI scan showed stable primary disease and continued 95–100% involvement of bone marrow.

Due to primary progressive disease the patient was ineligible for VERITAS and received four cycles of cyclophosphamide and topotecan after which the mIBG scan and bone marrow revealed static disease. A stem cell harvest was performed.
A further bone biopsy was obtained (due to inaccessibility of the primary tumour) and sent for whole genome sequencing and SMPaeds but the tumour cell content was too low to detect the previous SCA and no actionable somatic mutations were found.
She then had 8 x Bevacizumab, irinotecan, and temozolomide (BIT) after which she had static mIBG and MRI results and a partial bone marrow response. Interestingly, an FDG- PET scan showed mild FDG activity in the primary tumour and only minimal activity in the skeleton.
MIBG therapy through the MiNivAN trial was offered but declined for practical reasons. She has recently received mIBG & topotecan treatment with stem cell reinfusion. She will be reassessed soon with a view to proceed to surgery followed by busulfan/melphalan high dose chemotherapy.

EDUCATIONAL POINT

Primary progressive neuroblastoma is very rare (< 5% of all neuroblastoma). Is this linked to the unusual genetics ? Should we use PET rather mIBG scan to guide treatment response? Should we include baseline FDG PET in some mIBG positive neuroblastoma patients?

QUESTION

Although the chances of cure are significantly lower in primary progressive followed by refractory neuroblastoma could we still achieve cure with the rest of standard high risk treatment if there is some response to mIBG therapy?

CP_32
A CASE OF A 3,5 YEAR OLD GIRL WITH PARAVERTEBRAL NEUROBLASTOMA WITH MANY THERAPEUTIC CHALLENGES

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CASE

A 3,5 year old girl after suffering abdominal pain for 15 days was diagnosed with neuroblastoma. A large pelvic mass was found with intravertebral extension and penetration in the sciatic foramen. Because of the presence of surgical risk factors the mass was inoperable at the beginning. Biopsy was taken and histopathology report was positive for NBL poorly differentiated without NMYC amplification or segmental mutations. The patient was staged at L2 – Group 8 NBL Intermediate risk. After receiving chemotherapy according to LINES SIOPEN protocol Group 8 (6 cycles), she was operated on but the mass was not removed because it was very hemorrhagic and the patient kept on having surgical risk factors. After 1st surgery the patient was found to suffer sciatic nerve paresis. Embolization of the mass was performed by interventional radiologist in order to minimize the risk of hemorrhage. New surgical approach was performed and the mass was partially excised. The residual mass was (5,3×5,2×7,6)cm with intravertebral extension. We communicated with foreign operating centers but the mass was characterized as inoperable. She continued with radiotherapy with neurological improvement. After having received 6 cycles of 13-cis retinoic acid the patient had the same residual mass with positive 123-MIBG and that's why we proceeded to transdermal cryopexy. The sciatic paresis was deteriorated but was improved later on. The patient has stable residual disease for 11 months.

EDUCATIONAL POINT

Intraspinal residual mass is permitted at the end of treatment, provided no functional problem exists.
Interventional radiology can be very helpful when a tumor in inoperable.

QUESTION

Since there is still residual mass what do you suggest to do with it? Provide additional therapy?

CP_33

IS NF1 MUTATION ASSOCIATED WITH VERY AGGRESSIVE DISEASE AND POOR OUTCOME?

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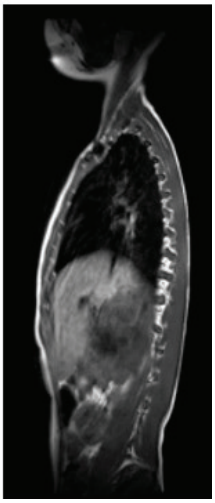
Princess Maxima Center, Utrecht, The Netherlands

Patient

- 11 year old boy
- Presented with fatigue and pain in the left hip
- Phsyical examination: mass in the right abdomen

MRI

10 cm mass - right adrenal gland

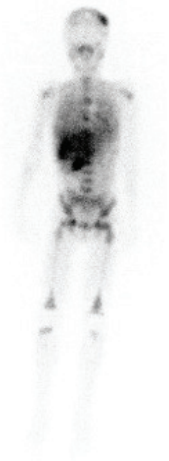


Urinary catecholamines: all extremely high!

HVA:	47 (7)
VMA:	45 (5)
Dopamine:	**105872 (431)**
3MT:	**10880 (57)**
Norepinephrine:	641 (64)
Normetanephrine:	6447 (31)
Epinephrine:	146 (18)
Metanephrine:	383 (37)

MIBG

SIOPEN: 46



Bone marrow

Biopsy's left and right:

90% infiltration of undifferentiated neuroblastoma

PA

Poorly differentiated neuroblastoma

RNA sequencing: no clinical relevant mutations

WES:

- **Somatic mutation NF1** → skip in exon 5
- Loss chromosome 17p (inclusief NF1)
- No MYCN amplification
- No ALK amplification
- Partial loss chromosome 1p
- Gain 17q

Therapy

Neuroblastoom stage M
- Right adrenal gland
- Multiple bonemarrow and bone metastasis

HR NBL2, no randomisation

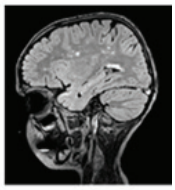
15-09-2021 start treatment GPOH

1e N6-kuur (start 11-10) -> did not finish the course

Complains of double vision & headache

MRI: sinustrombosis

Blood clot in the venous sinuses, which drain blood from the brain



Therapy: anticoagulants (medications that suppress blood clotting)

3 weeks later: tumor bleed

Progression of disease

Progression: liver, bone marrow, lungs, primary tumor

Poor physical condition because of toxicity (sinus trombose and high intracranial pressure)

No qualification for VERITAS because of progression

MIBG 5-11-2021

SIOPEN: 59

→ No treatment options anymore

Discussion

- Very fast progression of disease (after 1x N5/N6 chemo)
- Extremely high SIOPEN score, especially after progression (59)
- No MYCN (elevated 3MT in urine, myc activity?)
- Somatic mutation NF1 and loss chromosome 17p (homozygote loss of NF1) → this leads to activitation of the MAPK pathway
  - Treatment with MEK inhibitors?
  - See literature on the next slide (NF1 deficient neuroblastoma's have extremely poor outcome, that fits our case report)
- Fase 1 trial: selumetinib to patients with NF1 deficient tumors (neurofibroma's); Activity of Selumetinib in Neurofibromatosis Type 1-Related Plexiform Neurofibromas - PubMed (nih.gov)

NF1 Is a Tumor Suppressor in Neuroblastoma that Determines Retinoic Acid Response and Disease Outcome

Michael Hölzel 6 Sidong Huang 6, Jan Koster, Ludwine Messiaen
Rogier Versteeg, René Bernards
Cell, july 2010

Loss of *NF1* activates **RAS-MEK signaling**, which in turn represses ZNF423, a critical transcriptional coactivator retinoic acid receptors. Neuroblastomas with low levels of both *NF1* and *ZNF423* have extremely poor outcome. We find *NF1* mutations in neuroblastoma cell lines and in primary tumors. Inhibition of MEK signaling downstream of NF1 restores responsiveness to RA, suggesting a therapeutic strategy to overcome RA resistance in *NF1*-deficie neuroblastomas.

CASEPOSTERS SIOPEN 2022

CPP_34

CATECHOLAMINE-NEGATIVE NEUROBLASTOMA PATIENTS: THE ROLE OF ADRENERGIC-TO-MESENCHYMAL TRANSITION?

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Amsterdam UMC, Amsterdam & Princess Maxima Center, Utrecht (The Netherlands)

Timeline

November 2019

- 11 year old boy
- **Since 9 months:** back pain with tingling sensation in fingers
  - Symptoms were explained with the diagnosis of syringomyelia (cyst in spinal cord)
  - Physiotherapy
- **2 weeks ago:** fever, back pain, vomiting, diarrhoea
  - Submitted with viral gastro enteritis and discharged few days later
  - Did not recover while at home
- **November 2019:** again progressive abdominal pain, back pain and vomiting
  - Again submitted
  - Imaging → patient was referred to the Princess Máxima Center.

November 2019: physical examination

Pale, clinically not well

Respiratory:
left side thorax: normal respiratory sounds
right side thorax: reduced to no air entry middle and lower part of the thorax

Circulatory: normal heart sounds

Abdomen: palpable mass right flank

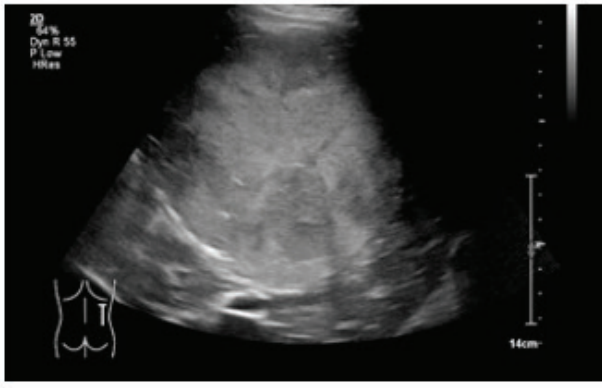
Lymph nodes: no palpable lymph nodes.

Neurological examination: double images when looking to the right

November 2019:

Ultra sound

Tumour originating from the right adrenal gland measuring 11.5 x 10.8 x 15 cm Signs of retroperitoneal lymph node involvement.



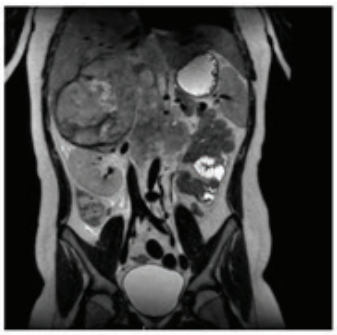
Urine catecholamines

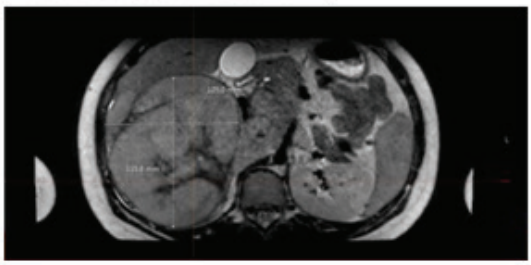
HVA:	3.8	(upper limit 5.1)
VMA:	4.6	(upper limit 7.5)
Dopamine:	*420*	*(upper limit 374)*
3MT:	44	(upper limit 58)
Normetanephrine:	*45*	*(upper limit 42)*
Norepinephrine:	*100*	*(upper limit 65)*

November 2019

MRI scan:

Large retroperitoneal mass in the right adrenal gland, multiple lesions retroperitoneal para-aortal most likely to be lymph node metastases. Lesion suspect of intra-thoracic lymph node metastases, close to the diaphragm crux.
Suspicion of a bone lesion S1





DD: malignant rhaboïd, neuroblastoma, sarcoma

December 2019

Clinically:

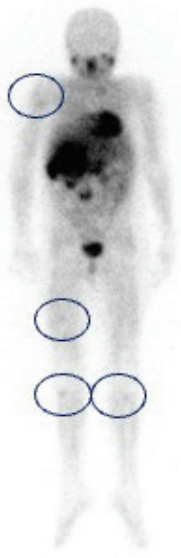
Pain and nausea increasing, distended abdomen and fever

MIBG scan: SIOPEN 4

Lesion suspicious for a neuroblastoma originating from the right adrenal gland with retroperitoneal lymph node metastases.

Primary tumour and lymph node metastases partially MIBG avid

Limited uptake in skeletal lesions. Recommendation to perform FDG PET CT.



December 2019

FDG PET CT
Suspicious for metastatic neuroblastoma,
more bone lesions compared to MIBG scan.

Bone marrow biopsy
Representative biopsy, cellular tumour
Fast dividing undiff tumor, Ki67 70%
Markers: keratine -, S100 -, PHOX2B-
No rhabdomyosarcoma markers
Morphologically not suspicious for adrenal carcinoma
Loss of SMARCA4, TH neg

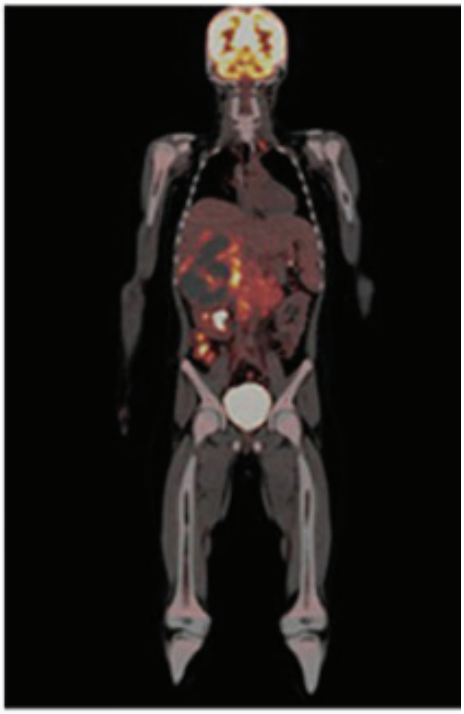
Molecular: SMARCA4 c.4574T>C Leu1525Pro (VAF 88%).
No other mutations.

Normal chr1 and 11q, gain 17q, no ampl ALK/MYCN,
LOH 19p (other allele of SMARCA4)

DNA methylation→ rhaboïd tumor

Clinic:

Ill, further clinical deterioration, high GI obstruction,
pain → start chemotherapy treatment (N6 course).



January 2020

Conclusion biopsy right adrenal gland:

Malignant rhabdoid diagnosis with bone metastasis

No MYCN

SMARC A4 positive

→ Start VDCy IE courses for rhabdoid tumour (2x VDCy, IE)

March 2020

Resection primary tumour

- Same but now also focally ganglioncells, which are intratumorally → indication of maturation of tumor under chemotherapy.
- Methylation still classifies as rhabdoid tumor however calibration score is far too low to draw solid conclusions.
- Genetically same as diagnosis

Based on these now the conclusion is that this is a **NBL with mutation/ loss of SMARCA4**

→ radiotherapy with continuation of rhaboïd chemotherapy schedule.
After completion of radiotherapy: continuation neuroblastoma protocol

June 2020: HD BuMel

August 2020: RTH prox humerus right

September 2020

September 2020: start immunotherapy

December 2020: At evaluation tumor progression after 3 courses of immunotherapy (biopsy) → TOTEM

February 2021: 2x TOTEM and further progression of disease → no therapy options

March 2021: progression brain, thorax, abdomen

April 2021: deceased

Addition analysis (research): Bone marrow qPCR

At diagnosis: high expression of adrenergic mRNA markers (CHRNA3,GAP43))

After 2 courses of TOTEM: very high expression of adrenergic mRNA markers (PHOX2B, CHRNA3, GAP43)

Discussion

- Based on catecholamines → NB?
- Somatic SMARCA4 variants have been associated with NBL
- Loss of SMARCA4 to be associated poor prognosis (Bellini et al., 2019)
- Regular screening for NBL? The lifetime penetrance of SMARCA4 is possibly very low
- Currently, case series under submission on SMARCA4 neuroblastoma cases (paper from research group in Canada)

Case Reports > J Pediatr Hematol Oncol. 2003 Jul;25(7):572-4.
doi: 10.1097/00043426-200307000-00016.

Neuroblastoma mimicking rhabdoid tumor of the kidney

Peter H Shaw [1], Paul S Dickman

Affiliations + expand

PMID: 12847328 DOI: 10.1097/00043426-200307000-00016

Abstract

Rhabdoid tumor of the kidney (RTK) has mimicked other renal tumors histologically, but there has been only one previous report of neuroblastoma mimicking RTK. The authors present the case of a 17-month-old boy who presented with a large left renal mass that was diagnosed as RTK. At the completion of therapy he was found to have residual masses. They were biopsied and found to be viable neuroblastoma.

Case Report

Primary Adrenal Malignant Rhabdoid Tumor in a 14-Year-Old Female: A Case Report and Literature Review

International Journal of Surgical Pathology
1-5

DOI: 10.1177/10668969211024331
journals.sagepub.com/home/ijs
SAGE

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CPP_35
A CASE OF A NEONATE WITH CONGENITAL NEUROBLASTOMA AND THROMBASTHENIA

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2. Haemophilia center / Haemostasis and Thrombosis Unit, Agia Sofia Children's Hospital, Athens, Greece

CASE
A newborn boy was diagnosed with abdominal mass on the 1st day of life. Biopsy was taken and histopathology report was positive for congenital NBL poorly differentiated without NMYC amplification or segmental aberations. The primary mass was bilateral on adrenal glands. The liver and the bone marrow were infiltrated. The patient was staged as MS. Chemotherapy was started immediately on 8th day of life in the Neonatal Unit according to LINES SIOPEN protocol Group 5 because of the presence of life threatening symptoms, liver dysfunction with coagulation disorders and respiratory distress. He received 2 cycles of chemotherapy (Carboplatina, Etoposide). Two months after the last therapy the boy was admitted to the department with seizures and status epilepticus resistant to anticonvulsant therapy. The brain MRI revealed extended subarachnoid hemorrhage. Neurosurgical decompression was performed. Despite the intervention, the focal seizures could not be controlled, so thiopental therapeutic coma was used. The patient improved gradually within 7 days. The fact that the subarachnoid hemorrhage occurred while there were no detectable coagulation disorders, neither low platelets, made us perform a hematological investigation for rare coagulation disorders. The investigation for qualitative disorders of platelets was positive with pathologic value of PFA -100 (Coll/Epi, Coll/ADP). The aggregation tests, that are more specific for detection of qualitative disorders of platelets, were indicative for Throbasthenia Glanzmann. Both parents were tested, and mother's sample had the same but milder disfunction. The whole screening is about to be completed with flow cytometry for glycoproteins GPIIb, GPIIIa .

EDUCATIONAL POINT
It is a very rare case of a child patient to have a congenital neuroblastoma and thrombasthenia.

QUESTION
The question is whether thrombasthenia is primary or secondary to neuroblastoma or chemotherapy. Have you seen any other children with neuroblastoma and thrombasthenia ?

CPP_36
EXCEPTIONAL REPONSE TO TOTEM CHEMOTHERAPY RECHALLANGE OF A PATIENT WITH HIGH-RISK NEUROBLASTOMA: WHAT DO WE DO NEXT?

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CASE
A 21-month girl was diagnosed in April 2017 with metastatic high-risk neuroblastoma with a left suprarenal primary tumor and bone/bone marrow metastases. Histology confirmed diagnosis of neuroblastoma, MYCN amplified, ALK negative. She was included in the HRNBL1 trial, randomized to N7 modified chemotherapy, with complete metastatic response after the end of induction chemotherapy . Treatment was then followed by primary tumor surgery, high-dose chemotherapy busulfan-melphalan, local radiotherapy of the primary tumour and maintenance therapy (retinoic acid and dinituximab beta). Disease evaluation at the end of treatment showed a persistent complete response (August 2018).

In February 2019 she was hospitalised due to a pathological fracture. Disease evaluation showed a metastatic relapse exclusively to the bone (score SIOPEN 8). She started treatment with TOTEM with a complete response after 3 cycles and continued with a total of 18 months of TOTEM followed of 18 months of temozolomide (TMZ). After 3 years of persistent complete reponse under TOTEM/TMZ chemotherapy, treatment was stopped (February 2022).

In Juin 2022 she was hospitalized again due to a pathological fracture. As in the first relapse, disease evaluation confirmed the metastatic relapse, limited to the bone (score SIOPEN 17). One of the metastatic lesions was biopsied to perform a full molecular profiling. Based on her previously good response to TOTEM and disease relapse after TMZ was stopped , she received again TOTEM chemotherapy. Disease evaluation after 2 months showed again complete remission of her disease (September 2022).

EDUCATIONAL POINT

Patients with relapse/refractory high-risk neuroblastoma with prolonged disease response to TOTEM can be challenged in case of a new disease relapse.

QUESTION

What would you propose for this patient at this moment?