11TH INTERNATIONAL PEDIATRIC RENAL TUMOUR BIOLOGY CONFERENCE

MARSEILLE / FRANCE
SEPTEMBER 7th-9th, 2022

# ABSTRACT'S BOOKLET

CHILDREN'S ONCOLOGY GROUP

canceropôle Provence-Alpes-Côte d'Azur

SIOP

RENAL TUMOUR STUDY GROUP | RTSG

# Selected talks

## N° 1 - **The role of structural variation: both 1q gain and CTNNB1/WT1-mutated Wilms tumors show Wnt pathway activation**

Authors:
**Jayne Hehir-Kwa, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS***
Ianthe Van Belzen, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Marc Van Tuil, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Shashi Badloe, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Eric Strengman, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Alex Janse, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Eugene Verwiel, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Kerstens Hindrik, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Douwe Van Der Leest, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Alissa Groenendijk, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Ronald De Krijger, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Jarno Drost, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Marry Heuvel-Eibrink, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Bastiaan Tops, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Frank Holstege, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*
Patrick Kemmeren, *Princess maxima center for pediatric oncology, Utrecht, NETHERLANDS*

Structural variants (SVs) and large chromosomal alterations are of special interest in pediatric cancers with a low mutation burden. For example, Wilms tumors (WT) display high genetic heterogeneity but a low mutation burden which limits the identification of driver mutations. Recurrent chromosomal alterations and SVs are potential biomarkers, e.g. loss of heterozygosity of 1p/16q is associated with poor prognosis and the prognostic value of 1q gain is actively studied. However, for most SVs the functional effects and clinical relevance remain unknown.

In a cohort of 30 WT patients, we identified SVs, copy number (CN) alterations, SNVs and indels using paired tumor-normal WGS, and quantified gene expression with RNA-seq. Combined genome-wide CN and SV profiles showed that tumors profoundly differ in both their types of 1q gain and genomic stability, and can be divided broadly into three subsets: CN neutral, those carrying 1p-/1q+ and genomically unstable WTs. Analysis of SNVs, SVs and CNAs demonstrated that diverse events can have similar disruptive effects on tumor suppressor genes, eg. WT1, AMER1 and TP53. Moreover, we identified expression clusters with enrichment for distinct biological processes e.g. proliferation, muscle differentiation and early renal development. Integration with WGS data showed that these expression clusters group tumors with diverse genetic alterations that disrupt or activate these biological processes. For example, the expression cluster characterised by increased expression of muscle differentiation genes and Wnt pathway activation consists of tumors with CTNNB1/WT1 mutations and a subset of 1q+ tumors. By integrating CNs and SVs, we resolved distinct mechanisms underlying 1q gain which are reflected in differences in overexpressed genes among 1q+ tumors. In conclusion, we show that joint analysis of diverse mutation types provides insights into the contribution of SVs and helps to elucidate the heterogeneity of WT.

# N°2 - DNA methylation patterns, but not genetic sequence alterations, are concordant across multiply sampled sites within individuals with unilateral and bilateral Wilms tumors

Authors :
**Jack Brzezinski, *Hospital for sick children, Paediatric haematology and oncology, Toronto, CANADA***
Sanaa Choufani, *Hospital for sick children, Genetics and genome biology, Toronto, CANADA*
Cheryl Shuman, *University of Toronto, Molecular genetics, Toronto, CANADA*
Rodrigo Romao, *Iwk hospital, Pediatric surgery, Halifax, CANADA*
Armando Lorenzo, *Hospital for sick children, Pediatric urology, Toronto, CANADA*

Wilms tumor, the most common renal malignancy of childhood, is a disease marked by heterogeneity. One tumor can harbor multiple histologic subtypes and multifocal or bilateral disease is common occurring in up to 10% of patients. Several molecular features have been associated with Wilms tumors and some are used for risk stratification. However, it is unclear which, if any, features are consistent throughout a single tumor or shared between tumors in cases of bilateral disease.

To answer this question, we generated genomic and epigenomic data from multiply sampled Wilms tumors from 10 patients - 3 with unilateral disease and 7 with bilateral disease. Multiple samples from each tumor as well as matched normal tissues were included when available. All tumors and matched normal tissues had DNA methylation array data available. Segmental chromosomal aberrations (SCAs) were inferred from methylation array data. Sequencing data were available for all bilateral patients but not for unilateral patients.

We found that SCAs were discordant both between pairs of bilateral tumors and within multiply sampled unilateral tumors. This discordance included SCAs used for clinical risk stratification (chromosome 1q gain, 1p loss). Sequence variants were also rarely shared between pairs of bilateral tumors. On the other hand, DNA methylation patterns were concordant across all samples for each individual. We previously described 3 distinct DNA methylation patterns in Wilms tumors and found that all samples from each patient classified into the same subgroup. Furthermore, DNA methylation at Wilms tumor-specific and subgroup-specific differentially methylated regions had high (>0.8) correlation in all samples from all cases.

Our data demonstrate that, in contrast to genomic changes such as SCAs and sequence alterations, DNA methylation patterns in Wilms tumors are frequently shared between paired tumors in bilateral disease and in multiple regions within individual tumors. This is consistent with earlier studies demonstrating that gain of methylation at the H19 imprinted region is often the earliest event in Wilms tumors and in some cases likely occurs in the embryonic intermediate mesoderm prior to the development of individual kidneys. Our findings demonstrate the utility of Wilms tumor DNA methylation patterns in overcoming tumor heterogeneity for risk stratification in cases of bilateral or multifocal Wilms tumors.

## N°3 - **Genotype-phenotype correlations in patients with germline WT1-variants in a national cohort**

Authors:
**Sophie Van Peer, *Princess máxima center, Utrecht, NETHERLANDS***
Janna Hol, *Princess máxima center, Utrecht, NETHERLANDS*
Sanne Egging, *Princess máxima center, Utrecht, NETHERLANDS*
Maria Paola Lombardi, *Amsterdam university medical center, Clinical genetics, Amsterdam, NETHERLANDS*
Marry Van Den Heuvel-Eibrink, *Princess máxima center, Utrecht, NETHERLANDS*
Roland Kuiper, *Princess máxima center, Utrecht, NETHERLANDS*
Marjolijn Jongmans, *Princess máxima center, Utrecht, NETHERLANDS*

WT1 disorder is characterized by combinations of glomerulopathy, urogenital anomalies and Wilms tumor. There is bias towards description of patients with variants in the DNA-binding/Zinc-finger domain of WT1 (exon 8/9). We observed an enrichment of variants outside this region in children with Wilms tumor and found it challenging to predict their glomerulopathy risk. This prompted us to study phenotype-genotype correlations in a national cohort of individuals with WT1-variants.

We approached all Dutch genetic laboratories and requested pseudoanonymized data of all patients with germline WT1-variants.
We identified 36 patients with (likely) pathogenic WT1-variants (median age at inclusion 13.3 years, range 2.8-58). Seventeen patients had truncating variants, 12 of which located outside the DNA-binding/Zinc-finger domain. All 17 patients had Wilms tumors (median age 12 months, range: 6-35), two developed glomerulopathy leading to kidney failure (age 26 and 40 years). Nine patients had missense variants, all in the DNA-binding/Zinc-finger domain. Of those, two developed Wilms tumors (age 12 and 18 months), 9/9 glomerulopathy (median age 1 month, range: 0 months-54 years) and 8/9 kidney failure (median age 1 month, range: 0-23). Six patients had splice variants, of which 3 in exon 9 and the others in introns 1, 6 and 7. Of this last group, two developed Wilms tumor (intron 6-7) and 3/3 kidney failure (median age 33 months, range: 10-333). Of the 3 patients with intron 9 splice variants, none developed Wilms tumor, all had kidney failure (median age 173 months, range: 24-240). Four patients had a (partial) WT1 deletion, all had Wilms tumor and none glomerulopathy. Four patients experienced WT1-related glomerulopathy onset after Wilms tumor treatment (median age 26.5 years, range: 2-40). Urogenital malformations were present in 20 patients, of those, karyotype was XY in 10, XX in 3 and unknown in 7.

We observed remarkable few patients with infantile/childhood glomerulopathy onset when the WT1-variant is outside the DNA-binding/Zinc-finger domain. These patients can however be at risk for glomerulopathy later in life. Therefore, we suggest life-long surveillance for glomerulopathy for all patients with a germline WT1 mutation and kidney-preserving treatment when affected by Wilms tumor. Alertness of a WT1-variant is especially recommended for young girls with Wilms tumor who often present without additional phenotype.

References:
Falcone MP, Pritchard-Jones K, Brok J, Mifsud W, Williams RD, Nakata K, Tugnait S, Al-Saadi R, Side L, Anderson J, Duncan C, Marks SD, Bockenhauer D, Chowdhury T. Long-term kidney function in children with Wilms tumour and constitutional WT1 pathogenic va, Lipska BS, Ranchin B, Iatropoulos P, Gellermann J, Melk A, Ozaltin F, Caridi G, Seeman T, Tory K, Jankauskiene A, Zurowska A, Szczepanska M, Wasilewska A, Harambat J, Trautmann A, Peco-Antic A, Borzecka H, Moczulska A, Saeed B, Bogdanovic R, Kalyoncu M, S, Lehnhardt A, Karnatz C, Ahlenstiel-Grunow T, Benz K, Benz MR, Budde K, Büscher AK, Fehr T, Feldkötter M, Graf N, Höcker B, Jungraithmayr T, Klaus G, Koehler B, Konrad M, Kranz B, Montoya CR, Müller D, Neuhaus TJ, Oh J, Pape L, Pohl M, Royer-Pokora B, Quer, Segers H, Kersseboom R, Alders M, Pieters R, Wagner A, van den Heuvel-Eibrink MM. Frequency of WT1 and 11p15 constitutional aberrations and phenotypic correlation in childhood Wilms tumour patients. Eur J Cancer. 2012 Nov;48(17):3249-56.

## N°4 - **Resolving the pathogenesis of treatment-resistant anaplastic Wilms tumors through spatial mapping of cancer cell evolution**

Authors:
**Bahar Rastegar, *Lund university, Division of clinical genetics, department of laboratory medicine, lund university, and division of pediatrics, skåne university hospital, Lund, SWEDEN***
Natalie Andersson, *Lund university, Division of clinical genetics, department of laboratory medicine, lund university, Lund, SWEDEN*
Alexandra Petersson, *Lund university, Division of oncology and therapeutic pathology, department of clinical sciences, lund university, Lund, SWEDEN*
Jenny Karlsson, *Lund university, Division of clinical genetics, department of laboratory medicine, Lund, SWEDEN*
Subhayan Chattopadhyay, *Lund university, Division of clinical genetics, department of laboratory medicine, Lund, SWEDEN*
Anders Valind, *Lund university, Division of clinical genetics, department of laboratory medicine, and division of pediatrics, skåne university hospital, Lund, SWEDEN*
Caroline Jansson, *Lund university, Division of clinical genetics, department of laboratory medicine, Lund, SWEDEN*
Geoffroy Durand, *Lund university, Division of clinical genetics, department of laboratory medicine, Lund, SWEDEN*
Patrik Romerius, *Lund university, Department of pediatrics, skåne university hospital, Lund, SWEDEN*
Karin Jirström, *Lund university, Division of oncology and therapeutic pathology, department of clinical sciences, Lund, SWEDEN*
Linda Holquist Mengelbier, *Lund university, Division of clinical genetics, department of laboratory medicine, Lund, SWEDEN*
David Gisselsson Nord, *Lund university, Division of clinical genetics, department of laboratory medicine, and division of oncology and therapeutic pathology, department of clinical sciences, Lund, SWEDEN*

While intermediate-risk Wilms tumors (WT) now has an overall survival rate of almost 90%, high-stage tumors with diffuse anaplasia (DA) has overall survival of only around 50%. We here identify key events in the pathogenesis of DA by mapping cancer cell evolution over anatomic space in WTs.

We mapped subclonal landscapes across tumor space in a retrospective cohort of 20 WTs using high-resolution copy number profiling and targeted mutation analysis followed by clonal deconvolution and phylogenetic analyses. Tumor whole mount sections (WMS) were utilized to characterize the anatomic distribution of subclones across anatomic tumor compartments.

Compartments demarcated by fibrous septae or necrosis/regression were frequently (73%) associated with the emergence of a new clonal copy number aberration (CNA), while clonal sweeps were rare within compartments. Our data indicate that DA WTs are signified by rich compartmentalization of mutations across anatomic space as well as high levels of phylogenetic species richness, divergence and irregularity compared to non-DA WTs. TP53 mutations are frequently followed by saltatory evolution and convergent loss of the remaining wild-type allele across tumor space. While DA tumors often had a high CNA burden along with necrosis, CNAs appeared to protect against necrosis and other signs of regression in non-anaplastic tumors.

WTs with DA have significantly more complex phylogenies compared with intermediate risk and blastemal WTs including features of saltatory and parallel evolution. The subclonal landscape of individual tumors were constrained by anatomic compartments, which should be taken into account at clinical sampling for precision diagnostics.

# N°5 - **Anaplastic Wilms tumour cells proliferate despite double stranded DNA breaks and a high burden of copy number aberrations**

Authors:
**Kaname Uno,** ***Department of laboratory medicine, lund university, Division of clinical genetics, Lund, SWEDEN***
David Gisselsson, *Department of laboratory medicine, lund university, Division of clinical genetics, Lund, SWEDEN*
Bahar Rastegar, *Department of laboratory medicine, lund university, Division of clinical genetics, Lund, SWEDEN*
Caroline Jansson, *Department of laboratory medicine, lund university, Division of clinical genetics, Lund, SWEDEN*
Geoffroy Durand, *Department of laboratory medicine, lund university, Division of clinical genetics, Lund, SWEDEN*
Anders Valind, *Department of laboratory medicine, lund university, Division of clinical genetics, Lund, SWEDEN*
Alessia Bertolotti, *Fondazione irccs instituto nazionale dei tumouri, Department of diagnostic pathology and laboratory medicine, Milan, ITALY*
Sara Ciceri, *Fondazione irccs istituto nazionale dei tumori, Molecular bases of genetic risk and genetic testing unit department of research, Milan, ITALY*
Filippo Spreafico, *Fondazione irccs istituto nazionale dei tumori, Pediatric oncology unit, department of medical oncology and hematology, Milan, ITALY*
Paola Collini, *Fondazione irccs istituto nazionale dei tumori, Soft tissue and bone pathology and pediatric pathology unit, Milan, ITALY*
Daniela Perotti, *Fondazione irccs istituto nazionale dei tumori, Molecular bases of genetic risk and genetic testing unit department of research, Milan, ITALY*
Linda Holmquist Mengelbier, *Department of laboratory medicine, lund university, Division of clinical genetics, Lund, SWEDEN*

The prognosis of patients with Wilms tumour (WT) is largely favourable due to multimodal treatment strategies. However, patients diagnosed with advanced-stage WT with diffuse anaplasia have a poor prognosis despite intensive chemotherapy treatment. We hypothesized that anaplastic cells have the ability to continue to grow during chemotherapy because they can tolerate ongoing DNA breakage and a high burden of copy number aberrations (CNAs).

Tumours from 27 children diagnosed with WT in either Lund or Milan were analysed. All patients had been treated with neoadjuvant chemotherapy followed by tumour resection. Seventeen had been classified as SIOP high risk (12 diffuse anaplastic, 5 blastemal type) and 10 as intermediate risk tumours. Multiple samples were collected from each tumour and patient specific tissue microarrays (TMAs) were created, which enabled spatial analyses. To strictly compare the different morphological features of anaplasia with other factors, each tumour region was scored for aspects of cellular pleomorphism, grading 0-3 for: hyperchromasia, multipolar mitosis, and enlarged nuclei. Grade 2 and 3 were defined as high-grade pleomorphism. Immunohistochemistry was used to detect p53, ki67 (proliferation) and γH2AX (double strand DNA breaks). The expression of γH2AX was divided into strong staining for detection of apoptosis, and dot staining corresponding to double stranded DNA breaks in absence of apoptosis. The amount of CNAs and the TP53 mutation status for most of the tumour regions, were obtained by SNP array and next generation sequencing, respectively.

A total of 787 tumour regions were analysed: 65 areas (8.3%), were defined with high-grade pleomorphism. Most of the high-grade pleomorphism areas (61/65) were from diffuse anaplasia cases, whereas 4/65 areas were from blastemal-type cases (3 was grade2 and 1 was grade3 in deeper sections). Almost all areas in the intermediate risk cases had grade 0. The extent of apoptosis, assessed by strong γH2AX staining, was not significantly different between anaplastic and blastemal-type cases ($p = 0.373$). In contrast, the amount of double stranded DNA breaks i.e. γH2AX dot staining, was significantly higher in anaplastic compared to blastemal-type WTs ($p < 0.001$). When comparing scoring of pleomorphism, γH2AX dot, p53 and ki67 expression were significantly higher when the pleomorphism grade was 2 or 3 ($p<0.001$, $p<0.001$, $p<0.001$, respectively). There was a significantly higher number of CNAs in areas with grade 2 or 3, as well as in areas with TP53 mutations ($p<0.001$ and $p<0.001$, respectively). Nearly 80% of high-grade pleomorphism areas had TP53 mutation.

Our data suggest that diffuse anaplastic WTs, especially in areas with high-grade pleomorphism, are highly proliferative even though the cells in these areas have a high burden of double stranded DNA damage and CNAs. The phenomenon was related to high p53 expression and the presence of TP53 mutations.

References:
Jenny et al. Four evolutionary trajectories underlie genetic intratumoral variation in childhood cancer. Nat Genet 2018;50(7):944-950., Natalie et al. Extensive Clonal Branching Shapes the Evolutionary History of High-Risk Pediatric Cancers. Cancer Res 2020;80(7):1512-1523., Ariadne et al. Significance of TP53 Mutation in Wilms Tumors with Diffuse Anaplasia: A Report from the Children's Oncology Group. Clin Cancer Res 2016;22(22):5582-5591, Jenny et al. TP53 alterations in Wilms tumour represent progression events with strong intratumour heterogeneity that are closely linked but not limited to anaplasia. J Pathol Clin Res 2017 ;3(4):234-248.

## N°6 - **Features of Wilms Tumors at the Single Cell Transcriptomic Level**

Authors:
**Alireza Paikari, *University of wuerzburg, Germany, Developmental biochemistry, Wuerzburg, GERMANY***
Emmanuel Saliba, *University of Wuerzburg, Helmholtz institute for rna-based infection research, Wuerzburg, GERMANY*
Taryn Treger, *Wellcome sanger institute, Cambridge, UNITED KINGDOM*
Sam Behjati, *Wellcome sanger institute Cambridge, UNITED KINGDOM*
Jenny Wegert, *University of Wuerzburg, Developmental biochemistry, Wuerzburg, GERMANY*
Manfred Gessler, *University of Wuerzburg, Developmental biochemistry, Wuerzburg, GERMANY*

Despite decades of clinical and basic research on Wilms tumor, the most common renal malignancy of childhood, the outcome of patients with unfavorable histologic and molecular features remains unsatisfactory. Advances in the understanding of Wilms tumor biology, known as the intersection between disrupted organogenesis and tumorigenesis, may translate into a better classification, improve risk stratification, the introduction of new molecular biomarkers, identification of potential new therapeutic targets, and better outcome in the end. To reach these goals, we performed single nucleus RNA sequencing on 42 pediatric Wilms tumors (all under 12 years old) composed of different subtypes and relapse cases, before and after chemotherapy. We also included six samples of patients' normal kidney tissues, which came from the unaffected parts of nephrectomy specimens. We compared our results to the publicly available fetal kidney (9w, 11w, 13w, 16w, and 18 weeks of pregnancy) single cell RNA seq data.

Frozen tissues were used to generate single nuclei suspensions for processing on the Chromium 10X controller (V3/3 3′ chemistry). Libraries were produced according to the manufacturer's instructions and sequenced on an Illumina HiSeq4000 device. Single cell RNA-seq data were quantified using the 10X software package, Cellranger, to map sequencing data to the GRCh38 reference genome supplied by 10X. Using the Seurat version 4, data were normalized, and log scaled. After the determination of high variable genes by the FindVariableGenes function, clusters were identified using the community identification algorithm as implemented in the Seurat "FindClusters" algorithm. Cells were then annotated by mapping them to accessible fetal kidney references. Differential expression of genes identified by FindMarkers and FindConservedMarkers commands.

Wilms tumor components are highly comparable to normal developing fetal kidneys but, several genes are upregulated or downregulated in each component. The Wilms tumor's epithelial subtype includes almost all ranges of nephron progenitor cell-derived components, while the blastemal subtype includes more of the early development stages. While the ratio of nephron progenitor cell count is highest in blastemal tumors and lowest in stromal tumors, the ratio of interstitial progenitor cell count is the opposite. Chemotherapy results in a decreased number of proliferating cells and changes in the gene expression profile of the cells.

Wilms tumors are comparable to fetal developing kidneys and exhibit fetal cellular signals, but there are several gene expression differences in each cellular compartment. While various subtypes of Wilms tumor correspond to different developmental stages, common compartments reveal differences in gene expression and various gene set enrichment changes. There are several genes related to the tumor state that are candidates for prognostic approaches or as therapeutic targets which need to be validated.

# N°7 - **Inter-Ethnic Variations in Methylation and Spatial Transcriptomics Affecting Local Immune Cell Regulation in Wilms Tumor**

Authors:
**Amos Loh Hp, *Kk women's and children's hospital, Viva-kkh paediatric brain and solid tumour programme, children's blood and cancer centre, SINGAPORE***
Weng Khong Lim, *Duke nus medical school, Singhealth duke nus genomic medicine centre, SINGAPORE*
Wong Meng Kang, *Kk women's and children's hospital, Viva-kkh paediatric brain and solid tumour programme, children's blood and cancer centre, SINGAPORE*
Willie Shun Shing Yu, *Duke nus medical school, Centre for computational biology, SINGAPORE*
Cedric Cy Ng, *National cancer centre Singapore, Laboratory of cancer epigenome, SINGAPORE*
Tan Sheng Hui, *Kk women's and children's hospital, Viva-kkh paediatric brain and solid tumour programme, children's blood and cancer centre, SINGAPORE*
Khaing Cho Thet, *Kk women's and children's hospital, Department of pathology and laboratory medicine, SINGAPORE*
Kuick Chik Hong, *Kk women's and children's hospital, Department of pathology and laboratory medicine, Singapore*
Kenneth Tou En Chang, *Kk women's and children's hospital, Department of pathology and laboratory medicine*
Victor Kwan Min Lee, *National university health system, Division of pathology, SINGAPORE*
Thuan Chong Quah, *Division of paediatrics SINGAPORE*
Steven George Rozen *Duke nus medical school, Cancer and stem cell biology programme SINGAPORE*
Bin Tean The, *Duke nus medical school, Cancer and stem cell biology programme, SINGAPORE*

The clinical and pathological profile of Wilms tumor differs significantly between ethnic groups, with disproportionately low incidence among Asians. Since nephrogenic development is largely epigenetically-controlled, and map to parallel ontogenic subgroups of Wilms tumors, we hypothesized that differences in gene expression in the tumor and microenvironment may be associated with these inter-ethnic differences. We compared favorable histology Wilms tumors of Asian and non-Asian (Caucasian) children using a multi-omic approach, in particular profiling their DNA methylation landscape using EPIC850K Infinium array and regional distribution of differential gene expression using NanoString GeoMX® Digital Spatial Profiling.

In a discovery set of 14 Asian (Chinese) and 15 non-Asian (White Caucasian) tumor-normal pairs, whole genome sequencing showed a higher mutational burden among non-Asians, with WT1, CTNNB1, WTX variants the most frequent. DNA methylation identified a subset (n=7) of Asian patients' normal kidney specimens that clustered distinctly from other Asians, non-Asians, and normal fetal controls. They were characterized by a signature of 152 significantly-hypomethylated genes involved in CD17/CD28/NFKB myeloid cell regulation, CD101-mediated immunoglobulin regulation, and Rho-GTPase-mediated innate immune response. Tumor DNA methylation did not differ significantly by ethnicity, but instead clustered patients by metastatic status. Bulk RNAseq however did not identify significantly differentially expressed genes among tumor and normal specimens, so spatial transcriptomics was employed to identify potential regions of differential gene expression.
In a validation set of 15 Asian and 8 non-Asian patients, we defined regions of interest (ROIs) of normal and tumor, near or away from the tumor-normal interface (TNI), at cortex and renal sinus.

First, comparing gene expression by site, genes encoding for histone H3 family members H3C2, H3C10, H3C8 were the most overexpressed in tumor compared to normal ROIs, with WT1 and CTNNB1 also among the most upregulated genes; genes regulating mesenchymal connective tissue were the most downregulated. Correspondingly, in tumor ROIs, WNT and senescence-associated signalling pathways were most significantly enriched, while complement cascade and interferon/IL10 signalling and were the most significantly downregulated pathways.

Next, comparing gene expression at these regions by ethnic group, Asians had significant upregulation of HLA-DMB and anti-inflammatory factor BM7 in TNI-adjacent tumor, and significant upregulation of H3C8 in TNI-adjacent normal kidney. At regions away from the TNI, Asians were significantly enriched for senescence-associated signalling and p53-dependent DNA damage-response pathways. Non-Asians were enriched for extracellular matrix organization and interferon signalling-associated pathways. Correspondingly, immune cell deconvolution with safeTME showed greater immune cell infiltration in non-Asian samples, with enrichment of monocyte-macrophage lineage cells in normal ROIs, and neutrophils and other myeloid cells in tumor ROIs.

In summary, this multi-omic profiling of favourable histology Wilms tumor revealed inter-ethnic differences in immune regulatory mechanisms in the microenvironment of the tumor-adjacent kidney, with interferon-mediated signalling predominating in non-Asians, and differential upregulation of histone H3 family genes in Asians. These differences may regulate recruitment and gene expression of tissue-resident immune cell populations and could modulate individual treatment responses in children of different ethnic groups and requires further study and international collaboration.

N°8 - **In search of mutational drivers leading to Wilms tumor recurrence**

Authors:
***Daniela Perotti, Fondazione irccs istituto nazionale dei tumori Department, Milan, ITALY***
*Sara Ciceri, Fondazione irccs istituto nazionale dei tumori, Dep research, Milan, ITALY*
*Paola Collini*, *Fondazione irccs istituto nazionale dei tumori, Pathol dept, Milan, ITALY*
*Paolo Radice, Fondazione irccs istituto nazionale dei tumori, Dep research, Milan, ITALY*
*Filippo Spreafico, Fondazione irccs istituto nazionale dei tumori, Pediatric dept, Milan, ITALY*

Whereas 90% of patients with Wilms tumor (WT) reach cure, approximately half of patients developing a recurrent tumor die of the disease. Therefore, to disclose events leading to recurrence represents a clinical need. To study paired primary/recurrent tumor samples, being aware of the intra-tumoral heterogeneity, might help finding these answers.

Preliminary data suggested that mutations in SIX1 and DROSHA underlie WT recurrence. Centers of the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) that had registered relapsing WTs in the AIEOP-2003 and SIOP-2001 protocols were asked to participate in this study. Overall, we recruited 27 paired primary/recurrent tumor samples, 10 primary tumor samples from patients who relapsed, and 50 tumor samples from 50 non-relapsing patients which were investigated for mutations in the SIX1/2 and micro RNA processing (miRNAPG) genes. We disclosed miRNAPG mutations in 7, and co-occurring SIX1 and miRNAPG mutations in 2 out of the 27 primary WTs, and miRNAPG mutations in 5 and co-occurring SIX1 and miRNAPG mutations in 4 out of the 27 paired recurrences.

Noteworthy, we could observe that whereas in primary tumors the mutations could be heterogeneously present, in all cases these mutations were present across all the analyzed recurrent tumor samples. One SIX1 mutation and 2 miRNAPG mutations were identified in the 10 primary WTs from relapsing patients. Within the 50 WTs from non- relapsing patients, one SIX2 and 7 miRNAPG mutations were identified. A borderline statistically significant association was observed between miRNAPG mutations and the occurrence of relapse (p value: 0.05). These data suggest that SIX1 and miRNAPGs mutations may provide mechanistic roles during tumor progression to recurrence and represent oncogenic drivers in WT development. However, in approximately 2/3 of the relapsing cases, the genetic event driving to recurrence is presently unknown.

N°9 - **Differential expression of miRNAs in blood of Wilms Tumor patients at diagnosis**

Authors:
***Nicole Ludwig, Saarland university, Human genetics, Homburg, GERMANY***
*Viktoria Wagner, Saarland university Chair for clinical bioinformatics, Saarbrücken, GERMANY*
*Norbert Graf, Saarland university, Department of pediatric oncology and hematology, Homburg, GERMANY*
*Rhoikos Furtwängler*, *Saarland university, Department of pediatric oncology and hematology, Homburg, GERMANY*
*Andreas Keller, Saarland university, Human genetics, Homburg, GERMANY*
*Eckart Meese*, S*aarland university, Chair for clinical bioinformatics, Saarbrücken, GERMANY*

Previous research showed a role of miRNAs in WT tumorigenesis and suggested circulating miRNAs in blood or serum as potential minimal-invasive biomarkers in renal tumours. Especially at diagnosis, biomarkers able to differentiate WT from non-WT, to identify metastases or predict outcome are urgently needed. To identify such circulating miRNAs, we profiled miRNAs in 273 blood samples from patients before start of SIOP treatment using microarrays.

We further analysed these profiles to identify miRNAs potentially indicating histological subtype, presence of primary metastasis, or correlating with tumor stage, level of regression upon treatment, initial tumor volume or blastemal volume. Amongst others, we found miR-10b-5p significantly elevated in the blood of WT patients compared to patients with non-WT renal tumors and gradually increasing from stage I to stage III WTs. Furthermore, blood levels of miR-10b-5p were increased in patients with metastases and positively correlated with initial tumor volume, suggesting the tumor as cellular source of elevated blood miR-10b-5p levels. Additional miRNAs with significant associations included miR-483-3p (tumor stage and initial tumor volume), miR-378d (metastasis), miR-186-5p (blastemal volume) and mir-193b-3p (regression).

A validation study assessing the expression of 30 selected miRNAs based on our microarray results in an independent patient cohort using microfluidic Fluidigm RT-qPCR is currently ongoing.

N°10 - **Cell-lineage Crosstalk in Kidney Development and Wilms Tumor**

Authors:
**Keri Drake, *Ut southwestern medical center, Pediatrics, Dallas, UNITED STATES***

Wilms tumors arise from embryonic renal precursor cells, demonstrating a link between the dysregulation of normal development and tumorigenesis. While it is well known that reciprocal signaling interactions are critical in coordinating normal kidney development, much remains to be understood about the role(s) of cell-lineage crosstalk in the pathogenesis of Wilms tumor.

Genetically engineered mouse models were used to target Wilms tumor gene mutations, including activation of beta-catenin (Ctnnb1) and loss of Wilms tumor 1 (Wt1), to renal progenitor cells. Embryonic kidneys were then examined for cell-autonomous and non cell-autonomous effects disrupting normal cell-lineage crosstalk. Results: We and others have shown that activation of Ctnnb1 in the nephrogenic lineage paradoxically results in loss of nephron progenitor cell (NPC) renewal, a phenotype opposite to Wilms tumor. However, activation of Ctnnb1 specifically targeting the stromal lineage showed non cell-autonomous effects on adjacent NPCs, with a block in their differentiation and altered gene expression. Additionally, stromal-specific mutants showed transcriptomic changes similar to human Wilms tumors, suggesting that signals from the stroma regulate the balance of NPC maintenance/differentiation, and when disrupted, may result in abnormally maintained progenitor cells reminiscent of nephrogenic rests/blastema. Regarding the role Wt1 in Wilms tumor, its loss has been shown to cell-autonomously block NPC differentiation; however, human tumors with Wt1 mutations interestingly show stromal-predominant histology. This led us to re-examine mouse models with Wt1 mutations, and somewhat surprisingly, we found that loss of Wt1 specifically targeting the NPC lineage results in abnormal expansion of the nephrogenic stroma. This model suggests that signals from the NPCs and/or early nephron structures non cell-autonomously regulate stromal development. Ongoing studies are aimed at examining how these developmental defects identified in the above mutant mouse models may be recapitulated in human Wilms tumors.

Our findings suggest that disruptions in progenitor cell crosstalk and normal reciprocal signaling interactions may alter the embryonic microenvironment to perturb development and drive tumorigenesis, potentially offering new insights into mechanisms of Wilms tumor biology.

References:
Drake KA, Chaney CP, Das A, Roy P, Kwartler CS, Rakheja D, Carroll TJ. (2020). Stromal activation of beta-catenin in the developing kidney impacts nephron progenitor differentiation and may contribute to Wilms tumor. Development. 147(21): dev189597

## N°11 - **Establishment and functional characterization of Wilms tumor organoid cultures**

Authors:
**Lisa Götz**, ***University of würzburg, Developmental biochemistry, Würzburg, GERMANY***
Manfred Gessler, *University of würzburg, Developmental biochemistry, Würzburg, GERMANY*
Jarno Drost, *Princess máxima center for pediatric oncology, Utrecht, NETHERLANDS*
Sabrina Bausenwein, *University of würzburg, Developmental biochemistry, Würzburg, GERMANY*
Jenny Wegert, *University of würzburg, Developmental biochemistry Würzburg, GERMANY*

Wilms tumor (WT) arises from multipotent embryonic renal precursor cells and these tumors often present with a classic triphasic histology composed of blastemal, epithelial and stromal elements. Due to diverse genetic drivers, different histological appearances may prevail in WTs.

Functional analysis of these drivers and testing of novel drugs have been hampered by the lack of suitable in vitro model systems. Established adherent cell cultures mostly resemble the stromal WT subtype. We have been able to generate 3D WT spheroid cultures predominated by blastemal elements, but stromal and especially epithelial cells are rare under these conditions and efficiency was low. Thus, optimization of in vitro models, preferably in 3D was highly needed. Organoid technology represents a promising additional tool for cultivation of kidney tissue and childhood kidney cancers to capture disease heterogeneity.

Using organoid culture conditions we observed the growth of organoids from single cell suspensions of both, viable WT material (60% efficiency, 57 WTs) and healthy adjacent kidney tissue (100% efficiency, 25 kidneys). This method allows for long-term expansion (up to eight months as yet) of WT and kidney organoids that maintain high proliferative capacity. For the majority of WT organoids, long-term cultivation selects for compact, ball-like morphology with sparse cystic elements, whereas kidney organoids exclusively display cystic morphology. All of these cultures could be further characterized as epithelial differentiated cells by cytokeratin staining. These findings were supported by high expression of epithelial markers and downregulation of kidney stem cell markers.

Comparison of adherent, spheroid and organoid cultures suggested that they differentially favour growth of either the stromal, blastemal or epithelial components of tumors and they could clearly be distinguished based on their RNA-seq expression profile. Subclustering of organoid cultures strongly correlated with both MYCN status and organoid morphology, the latter also being associated with differential expression of genes involved in WNT-signaling. We could also demonstrate that these organoids are a promising tool for patient specific drug screening. Testing of inhibitors that are highly effective in other solid tumors revealed sensitivity of organoids as well as spheroids and adherent cultures in a MYCN-dependent manner.

Wilms tumor organoids are promising in vitro models especially for epithelial WT and they will facilitate further characterization of candidate genes and testing of novel treatment concepts.

N°12 - **In vitro compound efficacy and toxicity testing by Organotypic Tissue Slicing in paediatric renal tumours, a novel rapid approach, with potential clinical relevance**

Authors :
**Daphne Komninos*, Prinses máxima center, Utrecht, NETHERLANDS***
Chris Oudmaijer*, Prinses máxima center, Utrecht, NETHERLANDS*
Winnie Van Den Boogaard*, Prinses máxima center, Utrecht, NETHERLANDS*
Jan Hoeijmakers*, Prinses máxima center, Utrecht, NETHERLANDS*
Marry Van Den Heuvel-Eibrink*, Prinses máxima center, Utrecht, NETHERLANDS*
Wilbert Vermeij*, Prinses máxima center, Utrecht, NETHERLANDS*

Renal tumours represent 6% of all childhood cancers. In children, malignant rhabdoid tumours of the kidneys, clear cell sarcomas, congenital mesoblastomas and renal carcinomas are the most common types, with Wilms tumours (WT; nephroblastoma) being most prevalent. Over the last decades survival of paediatric renal tumour patients has further improved[1].This is the result of better stratification of specified treatment per tumour type, and a higher efficacy of cancer therapy. During and after treatment renal kidney cancer patients are frequently confronted with severe side-effects. Chemo- and radiotherapy, both meant to target tumour cell DNA, also induce DNA damage and consequent toxicity in (surrounding) healthy tissue. This leads to for example acute kidney injury (AKI) and increased risk of secondary malignancies[2]. Recently we started a clinical study to investigate a possibility to reduce these toxicities, by a Fasting Intervention for Unilateral Renal Tumours to reduce Toxicity (FIURTT)[3]. In this study, children with a localised renal tumour are invited to follow a pre-operative short-term fasting (STF) regimen, by which we aim to decrease surgery-induced organ damage and to prevent AKI. To further examine the possible protective effects of STF in vitro, we developed a culturing method using organotypic tissue slices (OTS), where 0,5 cm punch biopsies from both tumour and healthy kidney tissue are sliced in 300 µm thick sections. OTS can be cultured, treated and assessed for several days ex vivo post-surgery. During that time, they maintain tissue morphology and microenvironment, thereby closely mimicking the in vivo situation. During the first year of this study, we found that it is feasible to use this method for healthy kidney tissue as well as WT material. An LDH cytotoxicity assay proved to be effective to measure cell death in cultured OTS, thereby enabling the analysis of chemotherapy-induced damage over time. With histological and immunofluorescent (IF) stainings we were able to assess viability of the tissue. We have found that after a week the tissue is still viable, showing cell proliferation and RNA synthesis. This OTS method allows for rapid drug sensitivity as well as -efficacy testing in tumour and normal kidney tissue of one and the same patient with a targeted compound approach. This can be of value for future kidney cancer patients, for preclinical drug screening, in adverse prognostic patients, but also to evaluate interventions that aim to decrease toxicity.

References:
[1]Brok, J., Treger, T. D., Gooskens, S. L., van den Heuvel-Eibrink, M. M., & Pritchard-Jones, K. (2016). Biology and treatment of renal tumours in childhood. European Journal of Cancer, 68, 179-195.,
[2] van den Boogaard, W., Komninos, D. S., & Vermeij, W. P. (2022). Chemotherapy Side-Effects: Not All DNA Damage Is Equal. Cancers, 14(3), 627.,
[3]Oudmaijer, C. A., van den Boogaard, W., Komninos, D.S.J., ... Heuvel-Eibrink, M. M. and Vermeij, W. P. (2022). Fasting Intervention for Children With Unilateral Renal Tumors to Reduce Toxicity. Frontiers in pediatrics, 17.,
[4]van den Boogaard, W. M., van den Heuvel-Eibrink, M. M., Hoeijmakers, J. H., & Vermeij, W. P. (2021). Nutritional preconditioning in cancer treatment in relation to DNA damage and aging. Annual Review of Cancer Biology, 5, 161-179.

# N°13 - **Identification of Candidate Resistance Mechanisms in Favorable-Histology Wilms Tumor**

Authors:
**Andrew Fleming, St. jude children's research hospital, Surgery, Memphis, UNITED STATES**
Karissa Dieseldorff Jones, St. jude children's research hospital, Computational biology, Memphis
Christopher Morton, St. jude children's research hospital, Surgery, Memphis, UNITED STATES
Allison Woolard, St. jude children's research hospital, Surgery, Memphis, UNITED STATES
Carolyn Jablonowski, St. jude children's research hospital, Surgery, Memphis, UNITED STATES
Prahalathan Pichavaram, St. jude children's research hospital, Surgery, Memphis, UNITED STATES
Xiang Chen, St. jude children's research hospital, Computational biology, Memphis, UNITED STATES
Sivaraman Natarajan, St. jude children's research hospital, Computational biology, Memphis, U.S.
John Easton, St. jude children's research hospital, Computational biology, Memphis, U.S.
Emilia Pinto, St. jude children's research hospital, Pathology, Memphis, UNITED STATES
Jerold Rehg, St. jude children's research hospital, Pathology, Memphis, UNITED STATES
Teresa Santiago, St. jude children's research hospital, Pathology, Memphis, UNITED STATES
Gerard Zambetti, St. jude children's research hospital, Pathology, Memphis, UNITED STATES
Andrew Davidoff, St. jude children's research hospital, Surgery, Memphis, UNITED STATES
Andrew Murphy St. jude children's research hospital, Surgery, Memphis, UNITED STATES

To identify candidate mechanisms of therapeutic resistance in favorable-histology Wilms tumor (WT), we treated KT-47 xenografts with repeated cycles of actinomycin-D, vincristine, and doxorubicin (VAD) until complete chemotherapy resistance developed. Histology, gene expression, protein expression, and methylation status were analyzed in pretreated, preresistant and fully resistant KT-47 clones and compared to primary WT patient samples.

VAD-chemoresistance was selected using our patient-derived favorable-histology WT KT-47 xenograft model in CB17 scid−/− mice. (Figure 1A). mRNA-Seq fragments per kilobase of exon per million mapped fragments (log2FPKM) values were used to identify differential expression of genes (DEGs). Log ratio and mean average (MA) plots were generated. RNA-seq Log2FPKM values for genes of interest were compared among 38 WT patient samples and correlated with neoadjuvant chemotherapy (nCT) exposure. DEG heatmaps were generated utilizing z-scores clustered by kidney development and WT gene sets. Gene set enrichment analysis (GSEA) was performed utilizing normalized enrichment scores with false-discovery rate (FDR) thresholds of <0.25. Immunohistochemistry (IHC) was performed to evaluate protein-level expression of relevant DEGs. 850K methylation bead chip analysis was performed, and global methylation status was compared between resistance phenotypes utilizing unsupervised hierarchical clustering (UHC) and principal component analysis (PCA).

Histologic analysis demonstrated development of blastemal predominance in the resistant clones (Fig. 1B). MA plots revealed enrichment of a LIN28B, a known oncogene, and doxorubicin resistance-associated ABCB1 in the resistance clones (FDR<0.01). The tumor suppressor WIF1 and the WT1 target IGFBP5 were downregulated (FDR<0.01) (Figure 1C). Borderline enrichment of LIN28B was observed in the preresistance group (FDR<0.1). The heatmap display of DEGs revealed upregulation of genes associated with blastemal cell types in WT and kidney development and downregulation of differentiated cell types including epithelia and stroma (Fig. 1D). LIN28B was not enriched among 11/38 patient tumors that received nCT (P=0.2684). GSEA revealed significant downregulation of genes associated with WT compared to fetal kidney (FDR<0.0001) and global downregulation of gene sets associated with differentiated skeletal muscle (FDR<0.0001). IHC revealed strong LIN28B expression in the blastemal component of both resistance clones and weak cytoplasmic LIN28B expression in the blastemal component of the preresistance group (Figure 1E). Neither the pretreatment group nor the original KT-47 patient tumor expressed LIN28B. A dendrogram generated by UHC of samples by global methylation status clustered each resistance phenotype (Figure 1F). A three-component PCA of global methylation status similarly demonstrated significant clustering by resistance phenotype (Figure 1G).

Chemotherapy resistance was associated with blastemal enrichment, oncologically-relevant DEGs, and changes in global methylation status in KT-47. Upregulation of LIN28B appears to be the earliest event associated with chemotherapy resistance in this model of favorable histology WT. We aim to determine the mechanisms of LIN28B upregulation and clonal evolution associated with chemotherapy resistance in favorable histology WT.

## N°14 - **Functional analysis of Trim28 knockout mouse kidneys**

Authors:
**Balázs Pálházi, *University of würzburg, Department of developmental biochemistry, Würzburg, GERMANY***
Silke Appenzeller, *University of würzburg, Comprehensive cancer center, core unit bioinformatics, Würzburg, GERMANY*
Manfred Gessler, *University of würzburg, Department of developmental biochemistry, Würzburg, GERMANY*

Wilms tumors (WT) are thought to result from interruption of normal differentiation, which highlights the importance of deeper insight into early renal development. Epithelial predominant WTs are often characterized by inactivation of TRIM28. 1,2 The Trim28 protein is part of transcriptional regulatory complexes and plays a crucial role e.g. in transcriptional silencing together with KRAB-ZNF transcription factors. Silencing of transposable elements (TEs) by such complexes is essential for proper embryonal development. 3 Therefore, loss of Trim28 in kidney precursor cells may lead to abnormal development or function of the kidney.

Our aim was to employ conditional Trim28 knockout mice to follow kidney differentiation and to determine the relevance of this protein during developmental processes.

To generate kidney-specific Trim28 knockout (KO) mice, we intercrossed Six2-Cre and Trim28-flox mice. We examined mouse kidneys at different developmental stages to find differences between conditional Trim28ΔSix2/ΔSix2 KO and Trim28flox/flox or Trim28flox/wt control mice. We performed RNA-seq and qPCR to detect transcriptional differences in kidney transcriptomes. To visualize expression changes and morphological abnormalities, we used H&E, immunofluorescence and immunohistochemistry staining as well as RNA in situ hybridization.

Most Trim28 KO (Trim28ΔSix2/ΔSix2) offspring died within the first few days of life with signs of kidney failure. Their phenotype is characterized by reduced kidney size but normal body weight in comparison to control littermates. RNA-seq results from bulk kidney RNA showed that especially TE expression (e.g., MMERVK10C, RLTR10B) is significantly upregulated in KO kidneys, while several markers of Six2+ derived cell types are deregulated - preferentially those expressed in proximal tubules. Derepression of TEs due to the lack of Trim28 protein appears to influence expression of neighboring coding and non-coding genes.

Trim28 is essential for normal kidney development and function, but early lethality precludes possible tumor formation. Impairment of the ability to silence TEs leads to abnormal kidney development and transcription patterns. Further analysis of cell type composition and gene/protein expression patterns are under way to elucidate the molecular pathways affected by loss of Trim28.

N°15 - **Towards rational epigenetic combination therapy of rhabdoid tumors based on the mechanisms of clinical resistance to EZH2 inhibition in SMARCB1-deficient sarcomas**

Authors:
**Alex Kentsis,** ***Tow Center for Developmental Oncology, Sloan Kettering Institute, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY, USA***
Yaniv Kazansky, *Tow Center for Developmental Oncology, Sloan Kettering Institute, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Daniel Cameron, *Tow Center for Developmental Oncology, Sloan Kettering Institute, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Neil Farrow, *Epizyme, Inc, Cambridge, MA, USA*
Mrinal Gounder, *Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Rhabdoid tumors (RT) and epithelioid sarcomas (ES) that cannot be cured with surgery are resistant to all known forms of therapy and are almost uniformly lethal. Recent work has revealed essential functions of dysregulated gene expression in sarcoma development, including deletions of SMARCB1, a key component of the BAF chromatin remodeling complex. Based on the functional antagonism between BAF and EZH2, the histone methyltransferase subunit of the PRC2 complex, we recently completed the clinical trial of the EZH2 inhibitor tazemetostat (TAZ), leading to its FDA approval.

We have now defined genetic mechanisms of resistance to TAZ using analysis of patient tumors, including matched specimens from clinical trial patients whose tumors initially regressed and subsequently progressed. This included acquired mutation of *EZH2*, which confers resistance to TAZ in *EZH2*-mutant RT cells and can be overcome with the allosteric EED inhibitor MAK683, but not with the EZH1/2 inhibitor valemetostat.

We also observed inactivating mutations of *RB1*, which cause EZH2-independent resistance, as evidenced by effective suppression of H3K27me3 in isogenic pairs of *RB1*-deficient and proficient RT cells. Using transcriptomics and cell cycle analysis, we found that RB1 loss allows cells to escape G1/S arrest caused by TAZ-induced upregulation of the cell cycle inhibitor CDKN2A. Thus, an intact RB1/E2F axis is required for therapeutic response to TAZ, suggesting a general mechanism for effective epigenetic therapy of this disease and nominating prognostic biomarkers for stratifying future therapy for patients. Translational studies of a panel of SMARCB1-deficient cell lines, including renal rhabdoid tumors and patient-derived mouse xenografts revealed effective combinations with TAZ, including epigenetic, synthetic lethal, and immune mechanisms.nIn particular, combination with the recently developed ATR inhibitor elimusertib led to differentiation and apoptosis of RT cells due to the epigenetic induction of the rhabdoid tumor mutator PGBD5, causing unrepaired dsDNA breaks in the absence of DNA replication stress.

In all, this work defines essential requirements for effective epigenetic therapy of rhabdoid tumors, which should lead immediately to new clinical trials to improve the cure rates of children with these cancers.

References :
https://pubmed.ncbi.nlm.nih.gov/28504702/
https://pubmed.ncbi.nlm.nih.gov/33035459/

N°16 - **Establishing a pediatric kidney tumor progression model to study SFPQ-TFE3 translocated Renal Cell Carcinoma**

Authors:
**Maroussia Ganpat, *Princess maxima center, Pediatric oncology, Utrecht, NETHERLANDS***
Francisco Morales-Rodriquez, *Princess maxima center, Pediatric oncology, Utrecht, NETHERLANDS*
Sepide Derakshan, Princess maxima center, Pediatric oncology, Utrecht, NETHERLANDS
Arianna Fumagalli, *Princess maxima center, Pediatric oncology, Utrecht, NETHERLANDS*
Shanzheng Wang, *German cancer research center (dkfz) and german cancer consortium (dktk), Pediatric neurooncology, Heidelberg, GERMANY*
Peter Zeller, *Hubrecht institute, Utrecht, NETHERLANDS*
Dilara Ayyildiz, *Princess maxima center, Pediatric oncology, Utrecht, NETHERLANDS*
Marcel Kool, *German cancer research center (dkfz) and german cancer consortium (dktk) Department : Pediatric neurooncology, Heidelberg, GERMANY*
Marry Van Den Heivel-Eibrink, *Princess maxima center, Pediatric oncology, Utrecht, NETHERLANDS*
Alexander Van Oudenaarden*, Hubrecht institute, Utrecht, NETHERLANDS*
Susana Chuva De Sousa Lopes, *Leiden university medical center, Anatomy and embryology, Leiden, NETHERLANDS*
Jarno Drost, *Princess maxima center, Pediatric oncology, Utrecht, NETHERLANDS*

Pediatric renal cell carcinomas (RCC) account for approximately 3% of all renal malignancies in children. Their genetic driver landscape is fundamentally different from their adult counterparts. Translocations involving the MITF transcription factor family members TFE3 and – less frequently – TFEB represent one of the main oncogenic driver events, but the exact cellular origin and underlying biology remain largely unclear.

To identify essential driver events and to study their contribution to RCC tumorigenesis, we utilized healthy human kidney organoid (i.e., tubuloids) and expressed one of the most commonly found translocations in RCC, the SFPQ-TFE3 fusion. We found that expression of the fusion in tubuloids induces histological features similar to clear cell RCC, with cells having distinctive clear cytoplasms. RNA-sequencing revealed that tubuloids expressing SFPQ-TFE3 adopt an RCC-like gene expression signature. In addition, mapping genome-wide binding of the fusion revealed distinctive binding patterns of the fusion compared to wildtype TFE3, possible explaining its oncogenic properties. Finally, SFPQ-TFE3 expressing tubuloids grow as RCC upon orthotopic transplantation in mice.

In conclusion, our findings imply that expression of the SFPQ-TFE3 gene fusion in renal tubular cells is sufficient for RCC development. Studies are ongoing to further elucidate the biology underlying this rare pediatric tumor.

## N°17 - Investigating structural associations of the lymphatic vasculature in Wilms tumour

Authors:
**Daniyal Jafree, *Ucl great ormond street institute of child health, Developmental biology and cancer programme, London, UNITED KINGDOM***
Kevin Cao, *Ucl great ormond street institute of child health, Developmental biology and cancer programme, London, UNITED KINGDOM*
Reem Al-Saadi, *Great ormond street hospital, Histopathology department, London, UNITED KINGDOM*
Guillaume Morcrette, *Ucl great ormond street institute of child health, Developmental biology and cancer programme, London, UNITED KINGDOM*
Tanzina Chowdhury, *Great ormond street hospital, Department of haematology and oncology, London, U.K.*
Aleksandra Letunovksa, *Great ormond street hospital, Histopathology department London, U.K.*
Man Cheuk Yan*, Ucl great ormond street institute of child health, Developmental biology and cancer programme, London, UNITED KINGDOM*
Paul Winyard, *Ucl great ormond street institute of child health, Developmental biology and cancer programme, London, UNITED KINGDOM*
Peter Scambler, *Ucl great ormond street institute of child health, Developmental biology and cancer programme, London, UNITED KINGDOM*
Kathy Pritchard-Jones, *Ucl great ormond street institute of child health, Developmental biology and cancer programme, London, UNITED KINGDOM*
David Long, *Ucl great ormond street institute of child health, Developmental biology and cancer programme, London, UNITED KINGDOM*

The lymphatic vasculature maintains tissue fluid balance and mediates the resolution of inflammation in health and disease. Lymphatic infiltration in some solid tumours is associated with metastatic spread, whilst in other cases, lymphatics may modulate protective anti-tumour immune surveillance. Comparatively, there is very little information on the role of lymphatic vessels in childhood kidney cancer; one of the most common solid tumours in children. Here, we use emerging technologies to examine lymphatic vessels in Wilms tumour, the most prevalent childhood kidney cancer.

Tissue volumes were subsampled from nephrectomies for post-chemotherapy Wilms tumours and adjacent non-tumorous renal tissue, as part of the international UMBRELLA study. All tumours had been subject to a course of pre-operative chemotherapy. Tissues were processed using wholemount immunofluorescence and optical clearing to enable 3D imaging of lymphatic vasculature. We compared 3D images for the presence of lymphatic vessels as well as their diameter and vessel density, defined as the number of lymphatic vessel branches per unit volume of tissue.

3D imaging revealed lymphatic infiltration to be a feature of post-chemotherapy Wilms tumour. Lymphatic density was greater within the tumour microenvironment than adjacent non-tumorous kidney tissue or kidneys without malignancy. Distinct lymphatic patterns were observed dependent on tumour subtype, with greater tumour lymphatic density and smaller vessel diameter observed in epithelial-predominant Wilms tumours compared to stromal-predominant regions. In ongoing work, we are employing computational interrogation of transcriptomics datasets to complement our imaging findings and assess how the lymphatic transcriptome varies with clinical outcome.

These results suggest that lymphatic infiltration is a feature of the Wilms tumour microenvironment after chemotherapy. Comparative evaluation of lymphatic structure in pre-chemotherapy tumours will help to elaborate if lymphatic infiltration is a cellular hallmark of the response to chemotherapy. Further, the observed variation in lymphatic density by histological risk group could provide a rationale to investigate lymphatic-derived molecules as prognostic biomarkers for Wilms tumour. Ultimately, our ongoing work may yield insights to harness the lymphatic vasculature to enhance targeted treatments, such as immunotherapy, for Wilms tumour and other renal cancers.

N°18 - **Predicting the Percentage of Stromal Tissue in Wilms Tumours using Advanced Diffusion Imaging**

*Authors:*
***Harriet Rogers, Guy's and st thomas' trust, Mri physics, London, UNITED KINGDOM***
*Susan Shelmerdine, Great ormond street hospital for children nhs foundation trust, Department of radiology, London, UNITED KINGDOM*
*Patrick Hales, Perspectum, Mri physics, Oxford, UNITED KINGDOM*
*Martijn Verhagen, University medical center groningen, university of Groningen, Department of radiology, Groningen, NETHERLANDS*
*Reem Al-Saadi, Ucl great ormond street institute of child health, Developmental biology & cancer research & teaching department, London, UNITED KINGDOM*
*Tanzina Chowdhury, Great ormond street hospital for children nhs foundation trust, Department of haematology and oncology, London, UNITED KINGDOM*
*Kathy Pritchard-Jones, Ucl great ormond street institute of child health, Developmental biology & cancer research & teaching department, London, UNITED KINGDOM*
*Chris Clark, Ucl great ormond street institute of child health Developmental imaging and biophysics section, London, UNITED KINGDOM*

Classifying Wilms tumour histological subtypes is crucial in staging and treatment planning. Knowing a tumour is intermediate risk and a stromal subtype could avoid intensification of therapy in cases where pre-operative chemotherapy has not resulted in tumour shrinkage and could aid in earlier decisions regarding treatment and surgical planning. Currently, this relies on histological analysis of the excised tumour, however MRI may provide a non-invasive biomarker. The Apparent Diffusion Coefficient (ADC) derived from diffusion-weighted MRI (DWI) is related to cellular density and morphology[1]. Stromal subtypes have increased ADC values compared to other subtypes, suggesting a decreased cellularity in stromal tissue[2]. It has also been demonstrated that non-Gaussian models such as IVIM (Intravoxel Incoherent Motion) better describe DWI data in Wilms tumours compared to ADC[3]. The derived IVIM parameters D and f represent the slow diffusion coefficient (a measure of cellularity) and the perfusion fraction, respectively[4]. It is hypothesised that these parameters may be able to predict the percentage of stromal tissue within a given Wilms tumour.

**Data:** MRI data from 24 Wilms tumour lesions were retrospectively (March 2009–March 2017) collected from Great Ormond Street Hospital (London, UK), following chemotherapy prior to surgery. Cases were included if they had a known %stromal from histopathology from subsequent resection of the tumour.

**MRI:** A 1.5T Siemens Magnetom Avanto scanner equipped with 40 mT/m gradients. DWI was acquired with 7 or 8 b values (0, 50, 100, 250, 500, 750, 1000 or 0, 50, 100, 150, 200, 250, 500, 1000 s/mm2), in 3 orthogonal directions. Patients also received axial T1w MRI without contrast.

**Analysis:** Tumour ROIs were generated and verified by two radiologists. These were refined by removing the necrotic tissue (defined by image-analysis, based on a previous published method[5]) to ensure only viable tumour tissue remains within the ROIs (Figure. 1). A voxel-wise IVIM fit was applied to the DWI data to produce the quantitative diffusion parameter maps D and f.

The 25th percentile from the D map and 75th percentile from the f map were extracted from the ROIs to further ensure no necrotic tissue was included. A multiple linear regression was performed to identify whether these two parameters could predict the %stromal tissue. Additionally, a regression using ADC values was used as a comparison model.

Multiple linear regression demonstrated that D and f significantly predicted %Stromal: $F(2, 21)=39.6$, $p <0.001$, , $R2 = 0.79$ and Adjusted $R2 = 0.77$. Figure 2 demonstrates this significant positive regression. Linear regression from ADC was significant, however it was not as strong as the IVIM model: $F(2, 22)=33$, $p <0.001$, ,$R2 = 0.6$ and Adjusted $R2 = 0.58$. Utilising D and f from the IVIM model strongly predicted %stromal tissue in Wilms tumour lesions. This method used existing standard clinical MRI sequences with no extra scan time

or change to the current patient clinical pathway.
It is possible to non-invasively identify the percentage of stromal tissue in Wilms tumours by using DWI.

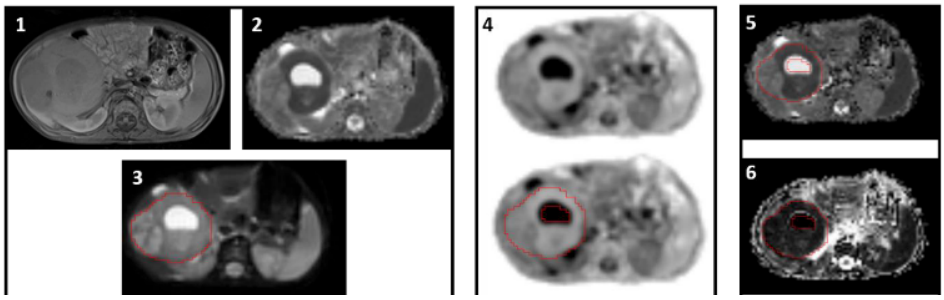


Figure 1. An example of Wilms tumour imaging. Non-contrast imaging is acquired including; (1) T1wand (2) ADC. Whole tumour ROIs (shown in red) are drawn and verified by two radiologists on the (3)b0 image (non-diffusion weighted image). These images are used to generate (4) enhancement maps[4] which allows automatic segmentation and subsequent removal of the necrotic tissue from within the whole tumour ROIs. IVIM Diffusion parameter maps (5) D and (6) f are generated and data is extracted from the ROIs which now have necrotic tissue removed.

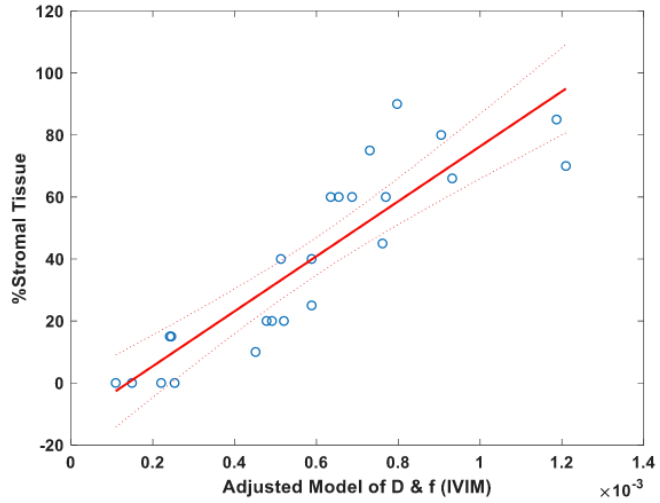


Figure 2. A multiple linear regression of D and f predicting %stromal tissue in 24 Wilms tumour lesions ($F(2, 21) = 39.6$, $p < 0.001$, $R2 = 0.79$). Blue Circles: Individual tumours, Red Line: Model fit, Red Dotted Line: 95% Confidence Interval.

References:
[1] P. W. Hales, Ø. E. Olsen, N. J. Sebire, K. Pritchard-Jones, and C. A. Clark, 'A multi Gaussian model for apparent diffusion coefficient histogram analysis of Wilms' tumour subtype and response to chemotherapy', NMR Biomed., vol. 28, no. 8, pp. 948–957, 2015, doi: 10.1002/nbm.3337.
[2] H. J. Rogers, M. V. Verhagen, C. A. Clark, and P. W. Hales, 'Comparison of models of diffusion in Wilms' tumours and normal contralateral renal tissue', Magma N. Y. N, vol. 34, no. 2, pp. 261– 271, Apr. 2021, doi: 10.1007/s10334-020-00862-4.
[3] D. Le Bihan, E. Breton, D. Lallemand, M. L. Aubin, J. Vignaud, and M. Laval-Jeantet, 'Separation of diffusion and perfusion in intravoxel incoherent motion MR imaging.', Radiology, vol. 168, no. 2, pp. 497–505, Aug. 1988, doi: 10.1148/radiology.168.2.3393671.
[4] H. J. Rogers, M. V. Verhagen, S. C. Shelmerdine, C. A. Clark, and P. W. Hales, 'An alternative approach to contrast-enhanced imaging: diffusion-weighted imaging and T1-weighted imaging identifies and quantifies necrosis in Wilms tumour', Eur. Radiol., Dec. 2018, doi: 10.1007/s00330-018-5907-z.

## N°19 - **Clinical translation of a non-oncogene encoded vulnerability to Exportin 1 inhibition in pediatric renal tumors**

Authors:
***Michael V. Ortiz, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY.***
*Julia L. Glade Bender, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY.*
*Diego F. Coutinho, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY.*
*Prabhjot Mundi, Department of Medicine, Columbia University Medical Center, New York, NY.*
*Lianna J. Marks, Department of Medicine, Columbia University Medical Center, New York, NY.*
*Chelsey Burke, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY.*
*Kelly L. Vallance, Cook Children's Hematology and Oncology, Fort Worth, TX.*
*Audrey Mauguen, Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY.*
*Andrea Califano, Department of Systems Biology, Columbia University Medical Center, New York, NY.*
*Andrew L. Hong, Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, GA.*
*Andrew L. Kung, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY.*
*Filemon S. Dela Cruz*, *Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY.*

Malignant rhabdoid tumors (MRT) and Wilms tumors (WT) are rare and aggressive renal tumors most often seen in infants and young children. Outcomes remain poor for patients with MRT and a subset of WT patients with high-risk biologic features. Both MRT and WT generally lack therapeutically targetable genetic mutations so we analyzed gene expression profiles of both tumor types in search of non-genetically encoded vulnerabilities.

An integrated systems biology approach based on the VIPER algorithm [Alvarez et al. Nat Genet 2016] was used to computationally infer protein activity from transcriptomic data of MRT and WT patients, as available in the TARGET database and to compare it to that of all tumors in the TCGA and TARGET datasets. This unbiased analysis identified common inferred dependencies for MRT and WT, particularly markedly aberrant activation of the nuclear export protein Exportin-1 (XPO1) in MRTs and WTs compared to all other tumor types. Assessment of a large *in vitro* cohort of MRT and WT cell lines demonstrated baseline activation of XPO1 in nearly all cases. Treatment with the selective XPO1 inhibitor selinexor demonstrated exquisite sensitivity, resulting in cell cycle arrest and induction of apoptosis in both MRT and WT cell lines. *In vivo* anti-tumor activity was assessed in 2 MRT and 5 WT patient-derived xenograft models which revealed that XPO1 inhibitors induce significant tumor growth inhibition and effective disease control with sustained treatment. We also present a case report of multiply relapsed WT in a child who has experienced prolonged disease control on Selinexor.

As a result of these findings, we are advancing a single agent, multi-center, open-label, phase II clinical trial of Selinexor monotherapy in children and adults. The study will allow for intra-patient dose adjustments along with enhanced supportive care, as well as on-therapy surgery and/or radiation for patients who respond to Selinexor or have durable stable disease. The study will consist of separate cohorts including those for relapsed or refractory WT, MRT, as well as additional childhood tumors where XPO1 inhibition appears a promising therapeutic modality.

The primary objective of this trial is to define the antitumor activity of Selinexor in relapsed and refractory WT. Secondary objectives include (1) evaluating the antitumor activity of Selinexor in MRT and selected additional childhood solid tumors, (2) characterizing the pharmacokinetic and safety profile of the liquid formulation in young children, and (3) determining whether intra-patient dose adjustments with enhanced supportive care allows for a safely augmented dosing of Selinexor. Exploratory objectives include evaluating the use of a clinically validated integrated systems biology approach, Darwin OncoTarget/OncoTreat, as well as immunohistochemical assessment of potential response biomarkers of XPO1, TP53, and TRIP13 based on promising orthogonal findings from the laboratory of Dr. Andrew Hong [Mittal K, Lee BP, Cooper GW, et al. *bioRxiv*, 2022].

# Posters

## N°1 - Familial renal tumor associated with a novel loss-of-function germline variant in DICER1

Authors:
Milda Mikalonyte, *Vilnius university, Student, Vilnius, LITHUANIA*
Egle Preiksaitiene,*Vilnius university hospital santaros klinikos,Hematology,oncology and transfusion center,Vilnius, LITHUANIA*
Rolanda Nemaniene, *Vilnius university hospital santaros klinikos, Center for pediatric oncology and hematology, Vilnius, LITHUANIA*
Rutkauskaite Vilma, *Vilnius university hospital santaros klinikos, Center for pediatric oncology and hematology, Vilnius, LITHUANIA*
Goda Vaitkeviciene, *Vilnius university hospital santaros klinikos, Center for pediatric oncology and hematology, Vilnius, LITHUANIA*
Irina Adomaitiene, *Vilnius university hospital santaros klinikos, Radiology center, Vilnius, LITHUANIA*
Karin Wadt, *Rigshospitalet, copenhagen university hospital, Department of clinical genetics, Copenhagen, DK*
Thomas Van Overeem Hansen, Rigshospitalet, copenhagen university hospital, Department of clinical genetics, Copenhagen, DENMARK
Kjeld Schmiegelow, *Rigshospitalet university hospital, Department of pediatrics and adolescent medicine, Copenhagen, DENMARK*
Jelena Rascon, *Vilnius university hospital santaros klinikos, Center for pediatric oncology and hematology, Vilnius, LITHUANIA*

We present a case of a 2-year-old girl with a clinically diagnosed Wilms tumor (WT), which after surgical removal appeared to be a pediatric cystic nephroma (CN). A heterozygous germline variant in the DICER1 gene, that has not been previously reported in literature, was detected in the patient and her relatives. Heterozygous germline pathogenic variants in DICER1 are proved to promote tumorigenesis and are associated with an increased risk of developing a variety of different tumors. The purpose of this case report is to illustrate the connection between a germline DICER1 variant and a familial renal tumor.

DICER1 encodes a protein that acts as a ribonuclease and is a haploinsufficient tumor suppressor gene. Although DICER1 syndrome is characterized by a variety of tumors, cystic nephroma is the most common of renal manifestations.
We analyzed a case of a 2 years and 9 months old girl, diagnosed with Wilms tumor, which after surgical removal and histological examination appeared to be a pediatric cystic nephroma. Due to a history of renal tumors in the family next-generation sequencing was performed on the genomic DNA of a proband and a heterozygous variant NM_177438.2:c.4173_4174del in exon 22 of DICER1 was detected. Our case revealed that germline DICER1 frameshift variant c.4173_4174del, that has not been previously reported in literature, can cause the development of a familial renal tumor.
Cancer predisposing syndromes (CPS) are of increasing importance in trying to understand the development of both benign and malignant tumors. DICER1 syndrome is one of the most recently discovered CPS in children. In our family, renal tumor manifested in two of four family members with germline pathogenic variant in DICER1 gene. Although penetrance is not complete, genetic testing and counseling are highly recommended for individuals with a CN diagnosis and their family.

In conclusion, this case demonstrates that germline DICER1 frameshift variant c.4173_4174del can cause the development of a familial renal tumor. It is important to note that pediatric CN can be mistaken with WT and a thorough examination is necessary to make a correct diagnosis. Our case also confirms the suggestion that patients with a pediatric CN diagnosis and their families should be tested for DICER1 variants.

References:
Kumar MS, Pester RE, Chen CY, et al. Dicer1 functions as a haploinsufficient tumor suppressor. Genes Dev. 2009;23(23):2700-2704. doi:10.1101/gad.1848209, Schultz KAP, Williams GM, Kamihara J, et al. DICER1 and Associated Conditions: Identification of At-risk Individuals and Recommended Surveillance Strategies. Clin Cancer Res. 2018;24(10):2251-2261. doi:10.1158/1078-0432.CCR-17-3089, Lambertz I, Nittner D, Mestdagh P, et al. Monoallelic but not biallelic loss of Dicer1 promotes tumorigenesis in vivo. Cell Death Differ. 2010;17(4):633-641. doi:10.1038/cdd.2009.202, Robertson J, Jorcyk C, Oxford J. DICER1 Syndrome: DICER1 Mutations in Rare Cancers. Cancers. 2018;10(5):143. doi:10.3390/cancers10050143

# N°2 - Unique Prognostic Associations of Copy Number Changes of Genomic Loci in Asian Wilms Tumors

Authors:
Amos Loh, *Kk women's and children's hospital, Department of paediatric surgery, Singapore, SINGAPORE*
Kia Teng Lim, *National university of Singapore, Yong loo lin school of medicine, Singapore, SINGAPORE*
Wong Meng Kang, *Kk women's and children's hospital, Singapore, SINGAPORE*
Do-Thi Ngoc Thuy, *Children's hospital 1, Department of pathology, Ho chi minh city, VIETNAM*
Tan Sheng Hui, *Kk women's and children's hospital, Viva-kkh paediatric brain and solid tumour programme, Singapore, SINGAPORE*
Isshani Devaraj, *National university of Singapore, Depart of biological sciences, Singapore SINGAPORE*
Kuick Chik Hong, *Kk women's and children's hospital, Department of pathology and laboratory medicine, Singapore, SINGAPORE*
Tawng D Khawn, *Kk women's and children's hospital, Paediatric haematology oncology service, Singapore,*
Prasad Iyer, *Kk women's and children's hospital, Paediatric haematology oncology service, Singapore,*
Soh Shui Yen, *Kk women's and children's hospital, Paediatric haematology oncology service, Singapore,*
Lee York Tien, *Kk women's and children's hospital, Department of paediatric surgery, Singapore, SINGAPORE*
Yeo Eric, *National university hospital, Division of pathology, Singapore, SINGAPORE*
Victor Lee, *National university hospital, Division of pathology, Singapore, SINGAPORE*
Quah Thuan Chong, *National university hospital, Division of paediatric haematology oncology, Singapore,*
Bui Chi Bao, *Vietnam university hospital, School of medicine, Ho chi minh city, VIETNAM*
Phan-Dang Anh Thu, *University of medicine and pharmacy, Department of pathology, Ho chi minh city, VIETNAM*
Kenneth Chang, K*k women's and children's hospital, Department of pathology and laboratory medicine, Singapore, SINGAPORE*

Low incidence rate and the presentation of favorable prognosis is a unique feature of Wilms tumors among Asians. Our previous study showed that 1p/16q co-loss of heterozygosity (LOH) was not prognostic in Asians. Here, we profiled the copy number (CN) changes within genomic loci of interests to determine an association with relapse in a multiracial pan-Asian population-based cohort. In addition, we profiled a subset of relapsed cases for the presence of Wilms driver genes mutations that identified additional molecular signatures for disease relapse.

Multiplex ligation-dependent probe amplification (MLPA) assay was optimized for formalin-fixed paraffin embedded (FFPE) specimens and validated against matched frozen tissues. MLPA screening was then applied to 2 sets of patient specimens: a discovery set of 61 tumors from 55 Asian and non-Asian patients treated in Singapore with National Wilms Tumor Study regimens between 2001 – 2022, and a validation set of 14 tumors from the Vietnamese population diagnosed between 2015 – 2021. Log2 CN ratios and LOH (gain/loss of ≥2 consecutive loci) were correlated with clinical variables. Whole-exome sequencing (WES) was also performed on FFPE specimens from 6 patients within the discovery set who developed relapse.
Clustering analysis identified a subgroup of predominantly non-Asian and relapsed patients with 1p, 16q loss, and 1q gain, while a subgroup (21.0%) with predominantly Asian ethnicity, low stage and few relapses was characterized by gain of MYCN exon 2 with/ without PAX3 gain, and minimal 1p/1q/16q aberrations. Overall, 23.0% of the discovery set and 37.5% of the validation set had MYCN exon 2 gain. Univariate t-test verified that PAX3 and MYCN gain were significantly associated within Asians ($P=0.02$, $P=0.001$, respectively) and PAX3 loss was associated with relapse ($P=0.02$). WT1 LOH was associated with older age and metastases ($P=0.02$, $P=0.03$, respectively), and TP53 LOH with older age, metastases and anaplasia ($P=0.04$, $P=0.004$, $P<0.001$, respectively). Notably, 1q gain was associated with relapse ($P=0.001$), but not 1p/16q co-LOH. Relapse was associated with older age ($P<0.05$), higher stage and unfavorable histology ($P<0.01$). WES showed 5/6 of relapse cases (4 Asians, 1 non-Asian) to carry the polymorphic P72R SNP allele in TP53, which has been associated with poorer survival outcome in other cancers. Of these 5 relapsed cases, 1 Asian case acquired PAX3 loss post-relapse while the non-Asian case carried a T257I SNP allele in beta-catenin, though the clinical significance of this mutation remains unclear.

Among Asians, 1q gain, but not 1p/16q co-LOH is prognostic for Wilms tumor relapse. In addition, the frequent gain of MYCN and its downstream target PAX3 may represent a unique signature for favorable outcome. While the association between PAX3 loss and relapse is strong, WES identified 2 further subsets within Asian relapse cases that carry either TP53 P72R mutant allele only or the ‘double-positives’ profile of both TP53 P72R mutant and PAX3 loss.Taken together, our results identified distinct molecular signatures for favorable prognosis and relapse within the Asian population.

## N°3 **- Familial Wilms tumor associated with germline heterozygous DIS3L2 variants**

Authors:
Sophie Van Peer, Princess máxima center, Utrecht, NETHERLANDS
Esmé Waanders, University medical center Utrecht, Department of genetics, Utrecht, NETHERLANDS
Roland Kuiper, Princess máxima center, Utrecht, NETHERLANDS
Marjolijn Jongmans, Princess máxima center, Utrecht, NETHERLANDS
Janna Hol, Princess máxima center, Utrecht, NETHERLANDS
Marry Van Den Heuvel-Eibrink, Princess máxima center, Utrecht, NETHERLANDS

Background In recent years, many new genes have been associated with Wilms tumor predisposition. Approximately 2% of children with Wilms tumor have a positive family history for Wilms tumor. Many of those families are explained by mutations in these recently discovered genes, but in some the cause remains unknown. We recently identified that heterozygous germline DIS3L2 variants are a significant contributor to Wilms tumor predisposition. We integrated this newly identified predisposition in our familial Wilms tumor screening program.

Methods We identified a Dutch family including four relatives with Wilms tumor and re-analyzed whole exome sequencing data of the proband for newly identified genes.
Results A heterozygous germline exon 9 deletion in DIS3L2 was identified in the index patient. Using a long-range polymerase chain reaction flanking the deleted exon, we confirmed the variant in a distantly related family member who deceased due to Wilms tumor. Of the other two relatives affected by Wilms tumor, no germline DNA was available. The index patient passed the variant on to three of her four children, all below seven years of age, who did not develop Wilms tumor till date. Single nucleotide polymorphism array analysis on blood DNA of five relatives in this family with the exon 9 deletion showed a shared haplotype surrounding the region of the DIS3L2 gene. Array data was also compared to three unrelated previously identified patients with DIS3L2 exon 9 deletion to investigate a founder effect, which showed no shared haplotype in the DIS3L2 region between these patients.

Conclusion We present the first case of familial Wilms tumor based on heterozygous DIS3L2 variants. As we did not identify a founder effect in unrelated patients, we conclude that exon 9 deletions in DIS3L2 are a recurrent genomic event, probably caused by repeat elements flanking this exon.

References:
Hol JA, Kuiper RP, van Dijk F, Waanders E, van Peer SE, Koudijs MJ, Bladergroen R, van Reijmersdal SV, Morgado LM, Bliek J, Lombardi MP, Hopman S, Drost J, de Krijger RR, van den Heuvel-Eibrink MM, Jongmans MCJ. Prevalence of (Epi)genetic Predisposing Fac, Astuti D, Morris MR, Cooper WN, et al: Germline mutations in DIS3L2 cause the Perlman syndrome of overgrowth andWilms tumor susceptibility. Nat Genet 44: 277-284, 2012, Perlman M. Perlman syndrome: familial renal dysplasia with Wilms tumor, fetal gigantism, and multiple congenital anomalies. Am J Med Genet. 1986 Dec;25(4):793-5.,

## N°4 **- Investigating whether chemotherapy-induced senescence affects the behaviour of cancer stem cells in wilms' tumour**

Authors:
Masoumeh Mousavinejad, University of Liverpool, Institute of systems, molecular and integrative biology, Liverpool, UNITED KINGDOM

Wilms' tumour (nephroblastoma) is the most common renal tumour occurs in children that mainly arises from renal progenitor tissue in the kidneys during embryonic development. The treatment regimens may vary by the stage of cancer and usually involves pre-operative chemotherapy with nephrectomy and tailored post-operative treatment involving chemotherapy and radiotherapy. Although the treatment of WT is approximately 90% successful, in about 10% of children, disease relapse occurs commonly with blastemal-type tumours. In this study we hypothesis that cancer stem cells (CSCs) in the blastema component can undergo senescence in response to chemotherapy thereby escaping the cytotoxic effects of therapies. Upon cessation of treatment, CSCs can become released from senescence, allowing tumours to relapse and potentially metastasise.

To determine the incidence of stem cells and senescent cells 10 tumour archived nephrectomy specimens from Alder Hey Children's Hospital Liverpool (AHCH) and Royal Manchester Children's Hospital (RMCH) were evaluated using immunohistochemistry. Results: Immunohistochemistry analysis revealed the expression of renal progenitor markers, PAX2 and SIX2, along with cancer stem cell associated marker, NCAM, in WT cases. Co-Expression of PAX2 and NCAM principally labels undifferentiated and proliferating WT Blastema. Expression of P53, the cell cycle arrest marker, was confirmed the presence of the senescence cells in the Blastema component.

Senescence in the blastema component in WT allows the CSCs to survive, supports their stemness, and subsequently affects the aggressiveness of CSCs.

## N°5 **- Development of a DNA methylation assay in cell free DNA as a surveillance tool in Wilms tumor predisposition syndromes**

Authors:
Jack Brzezinski, Hospital for sick children, Paediatric haematology and oncology, Toronto, CANADA
Sanaa Choufani, Hospital for sick children, Genetics and genome biology, Toronto, CANADA
Nicole Martin, Hospital for sick children, Clinical metabolics and genetics, Toronto, CANADA
Cheryl Cytrynbaum, Hospital for sick children, Clinical metabolics and genetics, Toronto, CANADA
Armando Lorenzo, Hospital for sick children, Urology, Toronto, CANADA
Celine Aziz, Hospital for sick children, Clinical metabolics and genetics, Toronto, CANADA
Trevor Pugh, University of Toronto, Medical biophysics, Toronto, CANADA
Rosanna Weksberg, Hospital for sick children, Clinical metabolics and genetics, Toronto, CANADA

Non-invasive tumor diagnostics from cell-free DNA in plasma represents an exciting advance in oncology. As it is often a sensitive tool, it has the potential to be used not just for diagnosis but for surveillance for patients at high risk of developing these tumors. Wilms tumors (WT) are an apt model as a disease that could be detected early in development since at least 10% of these tumors are associated with congenital anomalies and recent evidence has demonstrated that an even larger number are associated with non-syndromic inheritable predisposition syndromes.

Although several studies have been published showing that Wilms tumor circulating tumor DNA can be identified through detection of copy number variants, this approach has several limitations. Up to 20% of Wilms tumors will not have measurable copy number variants at diagnosis. Furthermore, investigations into tumor evolution suggest that copy number variants are heterogeneous and are infrequently the early events that would be targetd when monitoring cell free DNA as a surveillance tool. However, it may be possible to overcome these limitations by measuring DNA methylation along with copy number variants. We have recently shown that all Wilms tumors have one of several consistent DNA methylation patterns and that these are likely to be present even in the early development of disease.

In this early stage feasibility study we demonstrated the potential of using combined methods for detecting Wilms tumor circulating tumor DNA in plasma – cell-free MeDIP-Seq for DNA methylation detection and ultra-low pass whole genome sequencing for copy number detection. We initially demonstrated that Wilms tumor ctDNA can be detected by our combined assay in three plasma samples from children with WT compared to controls (Figure). We now show data in 40 plasma samples from patients with Wilms tumors demonstrating that a refined classifier using multiple differentially methylated regions identified in tumor samples robustly identifies the diagnosis with high accuracy.

These results have laid the groundwork to pilot this assay as a surveillance tool for children with Wilms tumor predisposition syndromes. We have established a consortium of 20 centers that look after children with predisposition syndromes in order to collect plasma samples obtained before a tumor diagnosis. We expect to enroll >200 such patients within the next two years. For these WT cases, banked plasma samples obtained before and at diagnosis will be tested using our cell-free DNAm classifier to determine how early the WT signal is detectable before the imaging diagnosis.

References : Mahamdallie et al, Identification of new Wilms tumor predisposition genes, Lancet Child Adol Health, 2019, Madunat-Harjuoja et al; Circulating Tumor DNA as a Biomarker in Patients With Stage III and IV Wilms Tumor: Analysis From a Children's Oncology Group Trial, AREN0533; J Clin Oncol; 2022, Brzezinski et al; Clinically and biologically relevant subgroups of Wilms tumour defined by genomic and epigenomic analyses; Br J Cancer; 2021, Shen et al; Sensitive tumour detection and classification using plasma cell-free DNA methylomes; Nature; 2018

# Acknowledgment

SFCE Société Française de lutte contre les Cancers et les leucémies de l'Enfant et de l'adolescent

RESOP

LA LIGUE CONTRE LE CANCER

Roche

arc Fondation pour la recherche sur le cancer

SFC Société Française du Cancer

Hôpitaux Universitaires de Marseille | ap hm

RÉGION SUD PROVENCE ALPES CÔTE D'AZUR

Aix*Marseille université Socialement engagée

enfants cancers santé

11TH INTERNATIONAL PEDIATRIC RENAL TUMOUR BIOLOGY CONFERENCE

MARSEILLE / FRANCE

SEPTEMBER 7th-9th, 2022