

## 3<sup>rd</sup> Colloquium on Fundamental Research in Pediatric Oncology

**Programm** 

### **Organizing comittee**

U1035 & U1218 INSERM / Bordeaux university



**Christophe Grosset** 

Véronique Trézéguet





**Aksam Merched** 

**Philippe Veschambre** 





**Patrick Auguste** 

Sarah Lesjean





**Stéphane Ducassou** 



**Béatrice Turcq** 

#### Scientific comittee



Valérie Castellani Marie Castets Christophe Grosset Isabelle Janoueix Véronique Maguer-Satta Eddy Pasquier Celio Pouponnot Françoise Redini Annie Schmid-Alliana Abdoulazize Souleymane

#### Welcome

W e are pleased to welcome you to the 3rd edition of the colloquium of Fundamental Research in Pediatric Oncology organized in partnership with the REACT-4KIDS network (https://react4kids.apps-dev.io/).

This meeting, which brings together different fundamental research French teams working in Pediatric Oncology, is an excellent opportunity for all of us to share our latest discoveries and create new collaborations or strengthen existing ones.

This year again, we are pleased to welcome two internationally renowned scientists, Carolina Armengol from Barcelona in Spain and Giuseppe Gasparre from Bologna in Italia.

On Thursday evening, we offer a special opening to the civil society such as patients and concerned families as well as to governmental representatives and medical and research professionals. A scientific session for the general public will be dedicated to all these partners to present some scientific topics in a popularized language.

Christophe Grosset and the organizing committee

### Thanks for our sponsors and partners





























#### **Thursday 12 December**

14:00 Welcoming and Introduction. Marie Castets - Christophe Grosset

#### Session 1

Chairmen: Marie Castets - Christophe Grosset

- 14:10 Carolina Armengol (IGTP, Barcelona) Invited Speaker Hepatoblastoma and the PHITT project.
- 14:55 Cédric Maurange (UMR7288, Marseille) Selected Speacker Coopted temporal patterning governs cellular hierarchy, heterogeneity and metabolism in Drosophila neuro-developmental tumors.
- 15:15 Marina Pierrevelcin (UMR7021, Strasbourg) Selected Speacker Hypoxia involvement in pediatrics osteosarcomas and its recreation in 2D and 3D preclinical models.
- 15:35 Sponsor presentation: NEB

15:50-16:10 **Coffe break** 

#### Session 2

Chairmen: Béatrice Turcq - Célio Pouponnot

- 16:10 Valérie Castellani (Institut NeuroMyoGène, Lyon) Invited Speaker Generating human tumors and derived metastasis in targeted tissues of the avian embryo: a paradigm to investigate the interplay between tumoral cells and the embryonic microenvironment for cancers with prenatal origin.
- 16:40 Olivier Ayrault (Institut Curie, Paris) Invited Speaker
- Using quantitative proteomics to decipher the biology of medulloblastoma. 17:10
  - Cecile Thirant (U830, Paris) Selected Speacker
- Neuroblastoma tumor heterogeneity and cell plasticity. 17:30
- Quentin Bailleul (U908, Lille) Selected Speacker Impact of H3.3K27M mutation on Diffuse Intrinsic Pontine Glioma's resistance to treatment.

17:50-18:10 Break

Session in french for all Cancers de l'enfant : Quoi d'neuf Doc?

#### 18:10-18:40

#### La parole aux doctorants

La parole à l'expert

Stéphane Ducassou

Repositionnement de droques.

Jeremy Ariey-Bonnet, (UMR7258, Marseille)

Hétérogénéité tumorale et approche single cell. Mélanie Lavaud, (UMR1238, Nantes)

Identité des tumeurs par séquençage et médecine personnalisée. Paul Huchedé, (U1052, Lyon)

19:00-19:30

18:40-19:00

Débat général

Échanges associations/chercheurs/public

Spécificités des cancers pédiatriques.

(CHU/ Inserm U1218, Bordeaux)

19:30 Cocktail apéritif

#### Friday 13 December

#### Session 3

Chairmen: Françoise Rédini - Patrick Auguste

08:30 Gilles Pagès (IRCAN, Nice) Invited Speaker

Towards the development of new therapeutic strategies for medulloblastoma.

09:00 Chloé Dhunputh (U1218, Bordeaux) Selected Speacker

Combination of the CDK4/CDK6 inhibitor palbociclib and the mTOR inhibitor everolimus in childhood T-lineage acute lymphoblastic leukemia with CDKN2A/2B deletion and/or PTEN loss.

09:20 Quentin Fuchs (UMR7213, Strasbourg) Selected Speacker

HIF2 in pediatric high grade glioma and its targeting.

09:40 Maïlys Rossi (UMR7258, Marseille) Selected Speacker

Repositioning of beta-blockers as anticancer agents for the treatment of medulloblastoma: new hope for children with high-risk brain tumors?

10:00 Sponsor presentation: Active Motif

#### Session 4

Chairmen: Isabelle Janoueix - Stéphane Ducassou

10:35 Françoise Pflumio (U1274, Fontenay-aux-Roses) Invited Speaker

T-ALL chemoresistance: also a question of bone marrow microenvironment and oxygen levels?

11:05 Franck Bourdeaut (Institut Curie, Paris) Invited Speaker

Mouse models to unveil the mysteries of rhabdoid tumors.

11:35 Sandrine Valsesia-Wittmann (UA8, Lyon) Selected Speacker

Repurposing Rotavirus Vaccines for Intratumoral Immunotherapy can overcome Resistance to Immune Checkpoint Blockade through RIG-I activation.

11:55 Kathrin Weber (CRCL, Lyon) Selected Speacker

Induction of TLR3-dependent cell death in caspase-8 deficient neuroblastoma cells.

12:15-14:30 Lunch + Poster session

#### Session 5

Chairmen: Véronique Trézéguet - Aksam Merched

14:30 Giuseppe Gasparre (CRBA, Bologna) Invited Speaker

How twisting cancer cell metabolism may affect macrophages.

15:15 Clémence Deligne (EA2465, Lens) Selected Speacker

Understanding DIPG chemoresistance using a human in vitro model of the blood-tumor barrier.

15:35 Raj Sewduth (VIB, Ghent) Selected Speacker

Role of Lztr1 inactivation in schwannomatosis versus Noonan syndrome.

15:55 Nicola Mosca (U1035, Bordeaux) Selected Speacker

Role of LHX2 in hepatoblastoma.

16:15-16:30 Award winners, concluding remarks and conference ending.

Marie Castets - Christophe Grosset

## **Invited Speakers**

## **Thursday 12 December**

	Hepatoblastoma and the PHITT project.
16:10	Valérie Castellani (Institut NeuroMyoGène, Lyon) Generating human tumors and derived metastasis in targeted tissue of the avian embryo: a paradigm to investigate the interplay betwee tumoral cells and the embryonic microenvironment for cancers wit prenatal origin.
16:40	Olivier Ayrault (Institut Curie, Paris)  Using quantitative proteomics to decipher the biology of

Carolina Armengol (IGTP, Barcelona)

medulloblastoma.

14:10

## **Friday 13 December**

08:30	Gilles Pagès (IRCAN, Nice)  Towards the development of new therapeutic strategies for medulloblastoma.
10:35	Françoise Pflumio (U1274, Fontenay-aux-Roses)  T-ALL chemoresistance: also a question of bone marrow microenvironment and oxygen levels?
11:05	Franck Bourdeaut (Institut Curie, Paris)  Mouse models to unveil the mysteries of rhabdoid tumors.
14:30	Giuseppe Gasparre (CRBA, Bologna)  How twisting cancer cell metabolism may affect macrophages.



#### **Carolina Armengol**

Barcelona, Spain

Germans Trias i Pujol Research Institute (IGTP)

Program of Predictive and Personalized Medicine of Cancer

(PMPPC)

Childhood Liver Oncology Group

ead of the Childhood Liver Oncology Group (@cLOG\_Lab) at Germans Trias i Pujol Research Institute (IGTP, Barcelona) and head of the G0033 and 2017-SGR-490 research groups of the CIBERehd (Liver and Digestive Diseases Networking Biomedical Research Centre) and AGAUR (Agency for Management of University and Research) with more than 20 years committed to translational research in the liver cancer field. During her training, she worked under the direction of two internationally recognized liver cancer experts: Drs. Jordi Bruix (PhD director) and Marie Annick Buendia (post-doct). In 2009, she boosted the creation of the Spanish Group for the Study of Pediatric Liver Tumors (GEETHI), a multidisciplinary group created to improve the clinical management of pediatric patients with liver cancer by integrating clinical, pathological and molecular knowledge. The research lines that she directs are focused on identifying diagnostic and prognostic biomarkers as well as molecular drivers as potential therapeutic targets for patients with liver cancer by using next-generation sequencing and omic technologies. At present, she is also working in the establishment of a European collection of biological samples from childhood patients with liver cancer and the translational research of these samples in the context of the "Children's Liver Tumour European Research Network" project (H2020) and the first Pediatric Hepatic International Tumour Trial (PHITT).



#### Valérie Castellani

Lyon, France
Institut Neuromyogène (INMG)
Université Claude Bernard Lyon1

CNRS5310/U1217 Inserm

Neuro-development, pediatric cancer and signaling team

Valérie Castellani is a CNRS research director specialized in Neuroscience and developmental biology at the Institut NeuroMyoGène in Lyon. The Castellani lab studies the prenatal development of the nervous system, focusing on the molecular signaling providing topographic landmarks to orient cell polarity and migration. This fundamental research led to the development of various experimental paradigms that the lab transferred to study pediatric cancers under the angle of the developmental biology. The lab conceived an innovative model allowing human cancer cells to form tumors into targeted tissues of the avian embryo. Initially designed for neuroblastoma, the paradigm successfully recapitulated key aspects of the disease progression leading to the identification, through integrated transcriptomic approaches of novel pro-metastatic gene programs. The lab extended its scope of interest to additional pediatric cancers and exploits its model to investigate the mechanisms by which the embryonic microenvironment impacts on the metastatic dissemination.



### **Olivier Ayrault**

Orsay, France
Institut Curie / CNRS UMR 3347 / INSERM U1021
Signaling in development and brain tumors team

r. Olivier Ayrault is a group leader at Institut Curie (France). He performed his post-doctoral training in the laboratory of Pr. Martine Roussel (Saint-Jude Children's Research Hospital, Memphis, TN, USA) in which he dissected the role of several signaling pathways for cancer development. Now, his general team goal is to decipher fundamental mechanisms related to the complex biology of medulloblastoma, the tumor that originates from the cerebellum. Because signaling pathways that are dysregulated in brain cancer are also at the basis of normal cerebellar development, they perform their studies back and forth in the normal development and cancer contexts. Recently, his group integrated high-resolution genomic, transcriptomic, epigenetic, proteomic and phosphoproteomic data to specifically profile distinct medulloblastoma subgroup.



### Gilles Pagès

Nice, France

Université Cote d'Azur, Institut Cancer et vieillissement de Nice UMR CNRS 7284/INSERM U 1081 Normal and Pathological Angiogenesis team Centre Scientifique de Monaco Mechanism of resistance to targeted therapies team

Gilles Pagès is director of research first class at INSERM and in charge of mission for the Centre Scientifique de Monaco. He is an expert of cell signaling and tumor angiogenesis for 20 years. He is a member of the National Committee of INSERM (CSS2) (https://eva3accueil.inserm.fr/). He belongs to the council of the European Vascular Biology Organization(https://evbo.org) and he is the honorific president of the French Society of Angiogenesis (http://www.angiogenese.fr/). GP is an expert for the French, National Institute of Cancer (INCA), and Agency for Research (ANR), and several Agencies Fighting against cancer in France and Europe. GP received several awards (LNCC, Roche, the charity ARTUR, the Estée Lauder group (Pink Ribbon award) and the National Academy of Medicine). GP published 145 papers (Science, Nat Cell Biol, EMBO J, PNAS,..). His H factor is 54 (> 10000 citations WOS). He deposited 10 patents (last six years). He coordinated ancillary studies for three clinical trials.



### Françoise Pflumio

Fontenay-aux-Roses, France

Laboratoire des cellules Souches Hématopoïétiques et leucémiques

Equipe Niche et Cancer dans l'Hématopoïèse

UMR « Stabilité Génétique, Cellules Souches et Radiations »

CEA - Inserm U1274- Universités de Paris et Paris-Sud

Françoise Pflumio, PhD, is a Research Director at INSERM. She works in the field of Hematology. She is heading the team « Niche and Cancer in Hematopoiesis » in UMR E008/U1274 « Genetic Stability Génétique, Stem Cells and Radiations », that is supported by INSERM, CEA and the Universities of Paris-Sud and of Paris, located in the Fontenay-aux-Roses site of CEA. The research directed by F Pflumio is focused on two main aspects. Her lab studies the mechanisms that regulate the hematopoietic potential of normal stem and progenitor cells, in particular in humans. The lab is also interested in understanding the interactions between the bone marrow niche cells and the leukemic cells from acute leukemia, with a particular interest in pediatric T cell acute lymphoblastic leukemia T (-ALL). The team has wellknown expertise in setting up xenograft models of human normal and pathologic hematopoiesis.





Paris, France Institut Curie SIREDO Pediatric Oncology Center

Laboratory of Translational Research in Pediatric Oncology - INSERMU830

r. Franck Bourdeaut (MD, PhD) is a pediatric oncologist and Researcher at Institut Curie where he supervises the Rhabdoid Team Research, Laboratory of Translationnal Research in Pediatric Oncology (Institut Curie, the French National Center of Health and Medical Research Inserm). After a MD degree in pediatrics obtained in 2004 and a PhD on the fundamental basis of oncology in 2008, he did a post-doctoral sabbatical in the Charles Robert's laboratory at the Dana Farber Cancer Institute, Boston, in 2015. His lab is developping mouse models of rhabdoid tumors, and aims to identify cells of origin, mechanisms of immune escape and role of epigenetic actors in the oncogenesis of this very agressive malignancy. Dr Franck Bourdeaut is also the current chair of the ATRT working group of the SIOP-Europe Brain Tumour Society.



#### **Giuseppe Gasparre**

Bologna, Italy
Center for Applied Biomedical Research (CRBA)
Dept. of Medical and Surgical Sciences - DIMEC
Medical Genetics Unit

.Gasparre is professor of Medical Genetics and Director of the Center for Applied Biomedical Research at the University of Bologna, Italy. He coordinated the MEET - Mitochondrial European Educational Training, a European consortium on mitochondrial medicine, funded within the FP7 ITN Program by the EU, and is currently the WP leader and co-coordinator of the H2020 Marie Curie ITN TRANSMIT - Translating Mitochondria in Tumorigenesis. He graduated at the University of Bologna in 2003 in Pharmaceutical Biotechnologies and in 2008 he obtained a PhD in Human Genetics at the University of Turin, Italy, where he served as mentor for the PhD program. His research focuses on dissecting the details of the metabolic status of oncocytic tumors presenting mitochondrial hyperplasia as a distinctive hallmark. As a consequence, he turned on investigating the role of mtDNA mutations in the metabolic rewiring of cancer cells, touching aspects such as resistance to therapy, induction of cell senescence, and the recognition of diagnostic/prognostic markers. He receives funding from the EU, The Worldwide Cancer Research, the Italian Ministry of Health and of University, the Italian Association for Cancer Research and the Fondazione Umberto Veronesi. He authored 90 among papers and book chapters, mainly on cancer and mitochondria.

## **Session 1**

Chairmen : Marie Castets - Christophe Grosset

> Thursday 12.12 14:10 to 15:50

## Coopted temporal patterning governs cellular hierarchy, heterogeneity and metabolism in Drosophila neuro-developmental tumors.

Sara Genovese<sup>1</sup>, Raphaël Clément<sup>1</sup>, Cassandra Gaultier<sup>1</sup>, Florence Besse<sup>2</sup>, Luis Nuno Miguel<sup>1</sup>, Fabrice Daian<sup>1</sup>, Karine Narbonne-Reveau<sup>1</sup>, Sophie Foppolo<sup>1</sup>, <u>Cédric Maurange</u><sup>1,\*</sup>

- 1 : Institut de Biologie du Développement de Marseille. CNRS : UMR7288
- <sup>2</sup>: Institut de Biologie Valrose CNRS INSERM, Université de Nice Sophia-Antipolis

Cancers of the central nervous system (CNS) represent less than 2% of all cancers in adults, but more than 25% in children. This suggests that the developing CNS is particularly prone to malignant transformation. Yet, the underlying mechanisms and gene networks involved are unclear. We use Drosophila and its simple nervous system amenable to powerful genetic manipulations to investigate why the developing brain is susceptible to tumorigenesis. During Drosophila larval development, asymmetrically-dividing neural stem cells, called neuroblasts, progress through an intrinsic temporal patterning program that ensures cessation of divisions before adulthood. We previously showed that temporal patterning also delineates an early developmental window during which neuroblasts are susceptible to tumor initiation (Narbonne-Reveau et al., 2016). Using single-cell transcriptomics, clonal analysis and numerical modeling, we now identify a network of twenty larval temporal patterning genes that are redeployed within neuroblast tumors to trigger a robust hierarchical division scheme that perpetuates growth while inducing predictable cell heterogeneity. The RNA-binding proteins Imp/Igf2bp and Syncrip exhibit antagonistic roles along the hierarchy to control the population of cancer stem cell-like cells. Along the hierarchy, Imp, Syncrip and other temporal patterning genes define a differentiation trajectory that regulates glucose metabolism genes to determine the proliferative properties of tumor cells. We propose that partial redeployment of the temporal patterning program encoded in the cell of origin may govern the hierarchy, metabolic heterogeneity and growth properties of cells in neural tumors with a developmental origin.

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## Hypoxia involvement in pediatrics osteosarcomas and its recreation in 2D and 3D preclinical models.

<u>Marina Pierrevelcin</u><sup>1</sup>, Benoît Lhermitte, Isabelle Lelong-Rebel<sup>1</sup>, Monique Dontenwill<sup>1</sup>, Natacha Entz-Werlé<sup>1,\*</sup>

- $^{1}$  : Laboratoire de Bioimagerie et Pathologie, université de Strasbourg : UMR7021, CNRS. Strasbourg
- \*: Corresponding author: Natacha.Entz-Werle@chru-strasbourg.fr

Osteosarcoma (OTS) is the first bone cancer diagnosed in adolescents and young adults. Multiple studies showed the involvement of hypoxia in cancers. Indeed, this bone malignancy is already known as a highly necrotic cancer even at diagnosis. The necrosis might be a consequence of the initial excessive growth of cancer cells with a potential induction of hypoxic-realted signaling pathways. The rapid proliferation of OTS cells is also creating an abnormal neoangiogenesis, probable consequence of different hypoxia levels. That's why it is important to recreate the hypoxia microenvironment using 5 to 1% oxygen in incubators where are cultured 2D and 3D OTS models. Our objectives in this study were to determine, first, the presence of hypoxia deregulation in a cohort of pediatric OTS at diagnosis, in patient-derived cell lines (PDCLs) and PDX tumors in mice, and, secondly, produce different models mimicking closely the OS progression and micro-environnement. Indeed, we explored by immunohistochemistry and immunocytochemistry all tumor specimens and models by looking at protein expressions of key hypoxia-related biomarkers (HIF1a, phosphomTor, pS6 and Carbonic anhydrase IX) and did drug screening experiences to target mTor and HIF1a genes. On the patient tumors, half of them were hyperexpressing HIF1a and 68% pS6 with a significant link for both with worst survivals (OS and EFS). The 5 PDCL from diagnostic samples and the 2 PDCL from tumor relapses were cultured and characterized in normoxic and hypoxic conditions showing a more realistic and closer patient reality at 5% of oxygen. We observe the presence of HIF1a and pS6 expressions in the nucleus by immunofluorescence in all PDCLs and PDX obtained from those cells and a higher proliferation rate at 5% O2. By Western Blot and RTqPCR, we confirmed those hypoxia induced variations of HIF-1a, pS6, mTor and HIF2a overtime. We also create 3D OTS models composed of bone matrix environment associated to immunity cells and/or vessels. Those 3D models had also the same hypoxic protein expressions and might be a new way to study targeted therapies. The OTS models were sensitive to HIF1a inhibitor (irinotecan) at 21 and 5% of O2 and completely resistant to mTor inhibition in hypoxia. Adding both compounds was resulting in a synergism especially in hypoxic environment. In conclusion, these experiments showed the importance of hypoxia environment in 2D and 3D OTS models to study drug targeting and show the validity of new therapeutic strategies.

## **Session 2**

Chairmen : Béatrice Turcq - Célio Pouponnot

> Thursday 12.12 16:10 to 17:50

#### Neuroblastoma tumor heterogeneity and cell plasticity.

<u>Cecile Thirant<sup>1,\*</sup></u>, Simon Durand<sup>1</sup>, Cecile Pierre-Eugène<sup>1</sup>, Caroline Louis-Brennetot<sup>1</sup>, Nadege Gruel<sup>1</sup>, Melissa Saichi<sup>2</sup>, Valentina Boeva<sup>2</sup>, Ana Costa<sup>1</sup>, Agathe Peltier<sup>1</sup>, Didier Surdez<sup>1</sup>, Justine Zulini<sup>1</sup>, Sakina Zaidi<sup>1</sup>, Angel M. Carcaboso<sup>3</sup>, Gudrun Schleiermacher<sup>1</sup>, Olivier Delattre<sup>1</sup>, Isabelle Janoueix-Lerosey<sup>1</sup>

- 1: U830, INSERM, Institut Curie -PSL Research University. Paris
- <sup>2</sup>: Institut Cochin. Département Développement, Reproduction, Cancer
- 3 : Institut de Recerca Sant Joan de Deu, Barcelona
- \*: Corresponding author: cecile.thirant@curie.fr

Neuroblastoma is a pediatric malignancy of the peripheral sympathetic nervous system affecting mainly young children. Around half of patients are diagnosed with a high risk disease, which is associated to less than 50% of survival, despite current intensive therapies. Development of new treatment options is urging.

Neuroblastoma is complex in terms of clinical presentation and evolution, but also in terms of intra-tumor molecular and cellular identities. The lab and others have recently demonstrated non-genetic heterogeneity based on the analyses of superenhancer profiles highlighting the existence of two main tumor identities: noradrenergic and mesenchymal. Both cell identities are governed by distinct core regulatory circuitries. However, their co-existence in patients, their interactions and dependencies between each other, and their roles in resistance to treatment need now to be deeply addressed.

We are dissecting patient's tumor heterogeneity using single cell transcriptomic approach on neuroblastoma samples. We already sequenced 10 different cases (4 biopsies and 6 patient-derived xenografts). Using bioinformatics, we demonstrated the co-existence of noradrenergic and mesenchymal tumor cells in some patients, and propose specific gene signatures in good accordance to super-enhancer profiles. Thanks to a couple of tumors obtained from the same patient at diagnosis and relapse, we could also follow mechanisms of disease evolution. In parallel, we have recently established a new human patient-derived cell line that allows us to decipher neuroblastoma cell plasticity. Using a surrogate mesenchymal cell surface marker, we could sort the noradrenergic and mesenchymal tumor cells and proved their trans-differentiation potential in vitro and in vivo. Finally, we demonstrated mesenchymal tumor component supports the chemotherapies observed in patients. We are now using single cell sequencing and trajectories reconstruction within this model to dissect the molecular mechanisms supporting the "Noradrenergic to Mesenchymal Transition" and inversely during spontaneous trans-differentiation or in response to chemotherapies, in order to identify new potential therapeutic targets for this poor prognosis disease.

## Impact of H3.3K27M mutation on Diffuse Intrinsic Pontine Glioma's resistance to treatment.

<u>Quentin Bailleul<sup>1,\*</sup></u>, Mélanie Arcicasa<sup>1</sup>, Audrey Hochart<sup>2</sup>, Pierre-Olivier Angrand<sup>1</sup>, Eric Adriaenssens<sup>1</sup>, Chann Lagadec<sup>1</sup>, Xuefen Le Bourhis<sup>1</sup>, Pierre Leblond<sup>4</sup>, Samuel Meignan<sup>1</sup>

- $^{\rm 1}$  : Centre Oscar Lambret, Centre Oscar Lambret, Inserm U908, Université de Lille Sciences et Technologies. Lille
- <sup>2</sup> : Centre Hospitalier Régional Universitaire de Lille
- <sup>4</sup> : Institut d'Hématologie et d'Oncologie Pédiatrique. Lyon
- \* : Corresponding author : qbailleul@laposte.net

Diffuse Intrinsic Pontine Glioma is one of the worst pediatric brain tumors regarding prognosis due notably to intrinsic cell resistance to radio and chemotherapy. One of the main characteristics of DIPG cells is the presence of a mono-allelic mutation on the lysine 27 of histone H3 (H3K27M). This mutation inhibits the trimethylation of this lysine that leads to strong modifications of gene expression. Until now, even though this mutation seems to be a driver event in tumorigenesis, its role in cell resistance to treatment has not been deciphered, due to a lack of relevant cellular models.

This way, in order to evaluate the role of the mutation on resistance to treatment, we first induced the mutation in three H3K27-unmutated pediatric glioma cell lines. In parallel, using the CRISPR/Cas9 technology, we are establishing DIPG cellular models in which the mutation will be reversed. By gene trapping approach, we aim to restore an H3F3Awt/wt genotype. After validation, these models would result in original tools to study the impact of H3K27M mutation in DIPG cells resistance to treatment.

For the model of induction, the transfected cell lines exhibit the mutation accompanied by a loss of H3K27me3 mark and H3.3 overexpression. For now, we showed an increased cell growth due to the mutation in two cell lines, under normoxia as well as under hypoxia. On contrary there was no impact on resistance to chemotherapy or ionizing radiation. In the third cell line, we didn't observe any impact on cell growth, but an increase of cell radioresistance. Concerning the mutation reversion, our preliminary results show homologous recombination at the right locus in the genome, and some clones present a loss of the mutation confirmed by sequencing. After the removal of resistance cassette by action of a recombinase protein, we will be able to evaluate the biological effects of mutation reversion.

To sum up, these different models would allow us to decipher cellular and molecular mechanism induced by the H3.3K27M mutation in DIPG cells including resistance to treatment, and thus, to possibly identify putative therapeutic targets.

## **Session 3**

Chairmen : Françoise Rédini - Patrick Auguste

> Friday 13.12 08:30 to 10:15

# Combination of the CDK4/CDK6 inhibitor palbociclib and the mTOR inhibitor everolimus in childhood T-lineage acute lymphoblastic leukemia with CDKN2A/2B deletion and/or PTEN loss.

<u>Chloé Dhunputh</u> $^{1,*}$ , Valérie Lagarde $^{1}$ , Valérie Prouzet-Mauleon $^{1}$ , Catherine Sawai $^{1}$ , Béatrice Turcq $^{1}$ , Stéphane Ducassou $^{1}$ 

1: Univ. Bordeaux, INSERM U1218 ACTION, Institut Bergonié. Bordeaux

\*: Corresponding author: chloe.dhunputh@orange.fr

Acute lymphoblastic leukemia (ALL) is the most frequently observed cancer in children, representing 30% of all pediatric tumors. Although important advances have been made in the treatment of pediatric T-cell ALL (T-ALL), 20% of patients relapse or have refractory disease. CDKN2A/2B deletions are present at diagnosis in 70% of cases of ALL, but the impact of these abnormalities on the overall prognosis is not yet well understood. CDKN2A/2B encode p16 and p15 respectively and are selective inhibitors of CDK4/CDK6 that regulate cell division and apoptosis. CDK4/CDK6 pharmacological inhibitors exist, and while they have been tested in clinicals trials, their efficacy in treating pediatric T-ALL is unknown. PTEN expression and function is also altered in T-ALL. PTEN is a tumour suppressor gene and a main negative regulator of the PI3K pathway. The PTEN/ PI3K/AKT pathway is widely involved in the regulation of cell proliferation, cell cycle and apoptosis. Everolimus is a mTOR inhibitor and it has been shown that inhibiting mTORC1 induces apoptosis in human T-ALL cell lines with PTEN loss. The aim of this study is to explore combination therapy of palbociclib and everolimus in order to determine whether there is a synergistic or additive effect on cell death and self-renewal of ALL cells.

We performed preclinical drug testing of palbociclib and everolimus using pediatric T-ALL cell lines: DND-41, P12-ICHIKAWA, MOLT-16. We tested the effect of each drug alone or in combination on a number of cellular functions including cell cycle status, proliferation and apoptosis. Proliferation was evaluated by MTS assay. Cell cycle status was measured by 5'-ethynyl-2'deoxyuridine incorporation. Apoptosis was determined by AnnexinV staining. Protein expression and phosphorylation were determined by western blot analysis.

We found that palbociclib treatment reduced cell viability and proliferation in a concentration dependent manner. In addition, cell apoptosis was significantly higher after 72 hours of exposure to palbociclib in all cell lines. Moreover, palbociclib induced cell cycle arrest at the G0/G1 phase by dephosphorylation of Rb. Indeed, incubation of T-ALL cell lines resulted in a dose- and time-dependent loss of phosphorylated Rb, which is a major substrate of CDK4/CDK6 and regulates cell cycle progression. We also found that everolimus induces cell death and decreases cell proliferation in T-ALL cells. However, everolimus had no major effect on cell cycle status in these cells. Finally, the combination of palbociclib and everolimus, synergistic effects were exhibited on apoptosis, cell proliferation and cell cycle arrest.



#### HIF2 in pediatric high grade glioma and its targeting.

<u>Quentin Fuchs</u>¹, Anne-Florence Blandin¹, Isabelle Lelong-Rebel², Marina Pierrevelcin², Monique Dontenwill², Natacha Entz-Werlé²,\*

- <sup>1</sup>: Faculté de Pharmacie (LBP) CNRS: UMR7213. Illkirch
- <sup>2</sup>: Laboratoire de Bioimagerie et Pathologie. Université de Strasbourg: UMR7021, CNRS
- \* : Corresponding author : Natacha.Entz-Werle@chru-strasbourg.fr

Pediatric high-grade gliomas (pHGGs) represent a very dismal disease that is needing new innovative compound for treatment. Despite the past discovery of histone H3 driver mutations, we are not able for instance to stop this induced epigenetic process and remodulate it. Therefore, there is since a decade proactive works aimed to discover new targetable proteins in these devastating tumors. In our recent previous works in pHGGs, we highlighted HIF2alpha as a biomarker of worse prognosis and outcome and a key in treatment resistance. Therefore, this new project was designed to determine in several patient-derived cell lines (5 PDCLs) the presence of HIF2alpha, its role and its induction in normoxic and hypoxic microenvironment concomitantly by immunofluorescence, western blot assessments and RNAsequencing analyses. Complementary ChipSeq were also performed using HIF2 antibodies to determine its role as a transcription factor. After the confirmation of its frequent presence in multiple PDCLs initiated from thalamic pHGGs and DIPGs, we were using allosteric inhibitors to target HIF2alpha. Surprisingly, this protein was expressed constantly in hypoxic and normoxic conditions and specifically in PDCLs bearing stem cell features. Specific protein expressions were established between stemness markers and HIF2alpha. To go further, we tested specific HIF2alpha inhibitors, which were having an impact on cell proliferation and on the decrease of the target itself. In conclusion, HIF2 seem to be a major biomarker in pHGGs that might be targeted and it is a useful new opportunity for pHGGs treatments.

#### Repositioning of beta-blockers as anticancer agents for the treatment of medulloblastoma: new hope for children with high-risk brain tumors?

<u>Maïlys Rossi</u><sup>1</sup>, Julie Talbot<sup>2</sup>, Marie-Pierre Montero<sup>1</sup>, Patricia Piris<sup>1</sup>, Duje Buric<sup>1</sup>, Eddy Pasquier<sup>1</sup>, Laetitia Padovani<sup>1,3</sup>, Olivier Ayrault<sup>2</sup>, Manon Carre<sup>1,\*</sup>, Nicolas Andre<sup>1,4,\*</sup>

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Medulloblastoma (MB) is the most frequent brain malignancy of the childhood. Their current multimodal treatment – surgery, radiotherapy and chemotherapy – allows 60% of children to survive up to 10 years. However, this comes at the expense of serious and often long-lasting side effects. Moreover, alternative treatment options are still urgently needed for MB with a very poor prognosis, i.e. for TP53-mutated SHH and c-myc-amplified group 3 patients. Drug repositioning, which consist of testing already approved drugs for new medical indications, represents an increasingly popular strategy to fast-track anti-cancer therapy with very little toxicity in the clinic.

Here, we investigated the preclinical efficacy of beta-blockers and of their original combination with radiotherapy for the treatment of MB. We first showed that propranolol, carvedilol and nebivolol –lipophilic  $\beta$ -blockers that cross the bloodbrain barrier – decreased both MB cell survival and migration in 2D and 3D spheroid models. These beta-blockers are able to disrupt MB bioenergetics, i.e. both glycolysis and mitochondrial respiration, in agreement with our previous results in infantile neuroblastoma.

We then showed that low doses of propranolol, carvedilol or nebivolol potentiate clinically-relevant radiation protocols (1.8 Gy/day, 5 days a week) in 2D cultures and 3D micromasses of human MB cell lines. All these results were confirmed in MB cells from group 3 patient-derived xegrografts (PDX) cells. Lastly, to evaluate the most promising combinations in more in vivo-like situations, we have developed a tumor-grafted organotypic cultures of cerebellar tissue: stably DsRed-expressing MB spheroids are grafted in mice healthy cerebellum slices. Progression of the MB cells exposed to radiotherapy +  $\beta$ -blockers is being analyzed over time in these innovative co-culture models, using a live cell analysis system and a sensitive microplate reader with an advanced matrix scanning feature.

Our results provide a rationale for further study of  $\beta$ -blockers in combination with conventional radiotherapy in medulloblastoma, with the ultimate goal of improving the quality of life of children with high-risk brain tumors.



## **Session 4**

Chairmen : Isabelle Janoueix - Stéphane Ducassou

> Friday 13.12 10:35 to 12:15

## Repurposing Rotavirus Vaccines for Intratumoral Immunotherapy can overcome Resistance to Immune Checkpoint Blockade through RIG-I activation.

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Immune checkpoint targeted therapies (ICT) against PD-1, PD-L1 and CTLA-4 are currently revolutionizing cancer care. However, only a minority of patients generate objective tumor responses with these treatments. Therefore, new therapeutic interventions are needed to increase the immunogenicity of tumors in order to overcome the resistance to immune checkpoint blockade therapy. Pattern recognition receptors (PRR) such as toll-like receptor agonists have been shown to overcome resistance to immune checkpoint targeted therapy in pre-clinical models. Because the pediatric development of PRR agonists will likely start many years after the development for adults, we sought to use anti-infectious disease vaccines as a potential source of clinical-grade PRR agonists to prime the immunity in pediatric cancers. Almong all the vaccines we checked, we found that rotavirus vaccines, non GMO, have both immunostimulatory and oncolytic properties. These attenuated viruses can directly kill cancer cells with features of immunogenic cell death. Moreover, they have pro-inflammatory properties and can activate NFKb pathways in a Toll independent manner. These in vitro biological properties translate into in vivo anti-tumor activity. Intra-tumoral (IT) rotavirus therapy has anti-tumor effects which are mainly immune mediated as demonstrated by their weaken activity in NSG immunodeficient mice. Interestingly, in immunocompetent syngeneic murine tumor models of neuroblastoma and lymphoma, IT rotavirus therapy can overcome resistance to ICT and in particular synergies with anti-CTLA4 and PDL1, leading to complete tumors regression and abscopal effect. This therapeutic effect relied on specific modifications of tumor immune infiltrates and immune activation pathways. IT rotavirus vaccines was associated to a switch in the immunosuppressive myeloid infiltrating cells that were reactivated by expressing CD86 and upregulation of activation markers such as OX40/CD137 on CD8 T cells, leading to adaptive immune memory response that protect mice upon rechallenge. Most importantly, heat and UV inactivated rotavirus lost their oncolytic activity but kept their synergy with ICT through the up regulation of RIG-I as shown by nanostring analysis.

Significance & Impact: Rotavirus vaccines are clinical grade products, including for children. Therefore, in situ immunization strategies with intra-tumoral inactivated rotavirus can therefore be implemented quickly in the clinic including in pediatric cancers. Intra-tumoral priming of the anti-tumor immunity with oncolytic and immunostimulatory rotavirus vaccines could be a feasible strategy to overcome resistance to anti-PD-1/anti-CTLA-4 therapy in patients with cancer.

Shekarian T et al., Science Translational Med. In press.



## Induction of TLR3-dependent cell death in caspase-8 deficient neuroblastoma cells.

<u>Kathrin Weber</u><sup>1,\*</sup>, Joseph Bisaccia<sup>1</sup>, Juliette Geoffray<sup>1</sup>, Severine Fagault<sup>1</sup>, Constance Desombre<sup>1</sup>, Serge Lebecque<sup>1</sup>, Marie Castets<sup>1,\*</sup>

Neuroblastoma (NB) is the most frequent extracranial cancer in children, with approximately 150 new cases diagnosed in France each year. It is responsible for 15% of childhood cancer-related mortality.

Targeted cell-death based treatments are currently considered as new putative therapeutic paths, but still lack sufficient efficiency due to apoptosis-resistance. In NB, caspase-8 expression is notably frequently suppressed by genetic and epigenetic mechanisms or functionally silenced by increase of its inhibitors, both situations leading to intrinsic apoptosis blockade. Thus, successful anti-cancer therapy requires the induction of alternative programmed cell death pathways. We studied the possibility to restore cell death functionality, using Toll-like receptor 3 (TLR3). We showed that activation of this receptor is sufficient to restore death of caspase-8 deficient cells and could open new therapeutic opportunities.

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## **Session 5**

Chairmen : Véronique Trézéguet - Aksam Merched

> Friday 13.12 14:30 to 16:15

## Understanding DIPG chemoresistance using a human in vitro model of the blood-tumor barrier.

<u>Clémence Deligne<sup>1,\*</sup></u>, Johan Hachani<sup>1</sup>, Sophie Duban-Deweer<sup>1</sup>, Fabien Gosselet<sup>1</sup>, Samuel Meignan<sup>2</sup>, Pierre Leblond<sup>3</sup>, Marie-Pierre Dehouck<sup>1</sup>, Caroline Mysiorek<sup>1,\*</sup>

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#### Introduction:

Pediatric Diffuse Intrinsic Pontine Glioma (DIPG) are particularly aggressive and represent one of the most devastating and lethal brain tumors in children, with a median survival of 12 months. Therapeutic failure can be explained by the inability for surgeons to perform a resection, due to the diffuse growth pattern and midline localization of the tumor. Alternative strategies, unfortunately palliative, consist of a chemotherapeutic approach, either alone or in combination with radiotherapy. However, the Blood-Brain Barrier (BBB), located at the brain capillary endothelial cells, tightly controls and restricts the access of chemicals, like chemotherapeutic drugs, to the brain parenchyma. Moreover, the BBB specific properties are modified in most pathological conditions, hence impacting the access of drugs to the brain. To understand how the BBB is impacted by the presence of DIPG tumors, an in vitro approach is necessary to decipher the cellular and molecular mechanisms responsible of the chemoresistance.

#### Material and methods:

Our human syngenic in vitro BBB model consists of a triple culture of human endothelial cells (differentiated from CD34+ stem cells), pericytes and astrocytes. Once validated in terms of BBB phenotype, the model was adapted to develop a blood-tumor barrier (BTB) specific to pediatric DIPG (with the use of HSJD-DIPG-007, -013 and -014 cell lines). The physical and metabolic properties of the BTB were analyzed.

#### Results:

The results showed that the integrity of the BTB remained intact until 7 days of incubation with DIPG cells, which is consistent with clinical observations. The transcriptional expression of several efflux transporters at the BTB was evaluated, as well as the functionality of efflux transporters. Both transcriptional expression and activity did not seem to be modified by the presence of DIPG. Evaluation of the transport of chemotherapeutic drugs is currently conducted.

#### Conclusion:

This original human BTB model allows a better understanding of the influence of DIPG on the chemotherapeutic transport through the BBB, which could be targeted to increase tumor exposure to chemotherapeutic agents and consequently improve treatment efficiency.



## Role of Lztr1 inactivation in schwannomatosis versus Noonan syndrome.

Raj Sewduth<sup>1,2,\*</sup>, Zhao Peihua, Mikhail Steklov, Benoit Lechat, Maria Francesca Baietti, Anna Sablina,

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Loss of lztr1 in mice partially recapitulates Noonan Syndrome pathogenesis with phenotypes such as cardiological, skeletal dysfunction (Steklov et al., Science 2018) as well as hydrops fetalis, hemmorhages and coagulation defects (under revision). Germline mutations of the Lztr1 gene in patients also predispose to distinct tumor syndromes: schwannoma related tumors (vestibular, sciatic nerve, schwannomatosis) and astrocytoma. The mechanisms by which Lztr1 germline mutations predispose to schwannomas versus Noonan syndrome are still unknown. Here, to understand the origin of these different Lztr1 related phenotypes, we generated tissue- and developmental stage specific conditional knockout mice carrying Lztr1 using an inducible Nestin creERT2 promoter. Lztr1 loss in adults did not promote any tumor related phenotypes as assessed by MRI/ PET-scan, while Lztr1 deletion in utero lead to early embryo lethality. As secondary malignant neoplasms such as schwannoma are commonly occur in young patients treated with irradiation, we are now developing a novel procedure for administering fractionated cranial irradiation and investigating the incidence and genetic profile in Lztr1 mutant mice irradiated to a moderate (15 Gy) or high dose (30 Gy). We hope that Lztr1 inactivation cooperates with irradiation to induce more solid tumors and myeloid malignancies, with tumors presenting a different genetic profile. As Lztr1 functions as a E3 ubiquitin ligase and have different proteins associated to DNA repair/ damage, we expect for example a shift from p53 to Ras mutations.

#### Role of LHX2 in hepatoblastoma.

<u>Nicola Mosca<sup>1,\*</sup></u>, Antonin Marchais<sup>2</sup>, Charlotte Mussini<sup>3,4</sup>, Sandrine Fedou<sup>1</sup>, Pierre Thiebaud<sup>1</sup>, Philippe Veschambre<sup>1</sup>, Christophe F. Grosset<sup>1,4</sup>

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Hepatoblastoma (HB) is the most common form of liver cancer in children. It is characterized by an abnormal activation of the Wnt pathway mainly due to activating mutations in  $\beta$ -catenin/CTNNB1 gene. LHX2, a member of the LIM homeobox gene family, has been reported to play important roles in embryogenesis and oncogenesis. Besides its roles in physiological conditions, LHX2 is highly expressed in a variety of human cancers, but its role in liver cancer is completely unknown. The aim of the present study was to clarify the role of LHX2 in hepatoblastoma and to explore the molecular mechanisms under its influence. We studied LHX2 expression in different datasets of HB samples and found a strong and recurrent downregulation of this gene in tumors compared to normal liver. Using different hepatoma cell lines with mutated (MUT) or wild-type (WT) β-catenin, we found that an ectopic expression of LHX2 reduces the proliferation, migration and survival of hepatoma cell in a p53-dependent fashion. The anti-proliferative effects of LHX2 was further demonstrated in vivo using the chick chorioallantoic membrane (CAM) assay. Next, we performed RNA-sequencing analysis to identify the signalling pathways, disease states and functions associated with an overexpression of LHX2 in hepatoma cells. Finally, we found that LHX2 strongly impedes Wnt signaling activity and Wnt-associated genes expression in cells with WT  $\beta$ -catenin, while mutations in  $\beta$ -catenin exon 3 abrogate this inactivation. Collectively, our findings suggest that LHX2 is a strong tumor suppressor gene in hepatoblastoma.

## **Posters**

Friday 13.12 12:15 to 14:30

## MT2A is an early predictive biomarker of response to chemotherapy and a potential therapeutic target in osteosarcoma.

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**Context**: Osteosarcoma is the most prevalent primary bone malignancy in children, adolescents and young adults. The current protocols combine surgery to neoadjuvant and adjuvant chemotherapy regimen, leading to a five-year overall survival rate levelled off around 60-70% over the past fifty years. The major obstacles for a more favourable outcome are the frequent occurrence of drug resistance, and metastatic relapses.

Metallothioneins (MTs) are a group of cysteine-rich metal-binding proteins exhibiting significant chelating properties. Hence, MTs play a key role in trace elements (zinc, copper...) homeostasis, protection against oxidative stress, and toxic heavy metals. We previously reported that high MT2A mRNA level in tumour tissue at diagnosis correlates with poor response to chemotherapy and poorer outcome in a small cohort of osteosarcoma patients, suggesting a prognostic significance.

The aim of the present study was to investigate the preventive role of metallothionein-2A (MT2A) in response to cytotoxic effects of chemotherapy.

**Methods**: A panel of human and murine osteosarcoma cell lines, modified for MT2A were evaluated for cell viability, and motility (wound healing assay). Cell-derived xenograft models were established in mice. FFPE tumour samples were assessed by IHC.

**Results**: In vitro experiments indicated a positive correlation between half-maximal inhibitory concentration (IC50) for drugs in clinical practice, and MT2A mRNA level. This reinforced our previously reported correlation between MT2A mRNA level in tumour samples at diagnosis and overall survival in patients with osteosarcoma. In addition, MT2A/MT2 silencing using shRNA strategy led to a marked reduction of IC50 values in vitro and to enhanced cytotoxic effect of chemotherapy drugs on primary tumour in murine xenograft models.

**Conclusions**: Our results identified the key role of high MT2A expression level in the resistance of osteosarcoma cells to chemotherapeutic drugs. We revealed a negative correlation between MT2A level and drug efficacy (IC50 values), and antitumour effects for various drugs when cells were silenced for MT2A. Our results thus support a model whereby MT2A contributed to lowering the drug cytotoxic effects and could be a valuable therapeutic target to improve osteosarcoma sensitivity to chemotherapy. In conclusion, we demonstrated that MT2A could be considered as a predictive biomarker of the efficacy of chemotherapy for patients with osteosarcoma, and as a potential therapeutic target to develop novel treatment strategy to prevent or de-escalate chemo-resistance in osteosarcoma.

## EZH2 oncogene in pediatric brainstem glioma: A new therapeutic target?

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Diffuse Midline Glioma (DMG) is a rare and highly aggressive pediatric tumor affecting children's brainstem. The average survival time after diagnosis is less than one year. The only available treatment options are chemotherapy and radiotherapy. Therefore, novel effective treatment modalities are urgently needed.

DMG is characterized by a mutation in histone H3 leading to a substitution of Lysine 27 to Methionine which deregulates Polycomb Repressive Complex 2 (PRC2), including enzymatic activity of EZH2. Previous studies have shown that inhibition of EZH2 by chemical agents decreases DMG cell proliferation and inhibits tumor growth in vivo. Our project aims to further validate EZH2 as therapeutic target using chemical EZH2 inhibitors, small interfering RNAs and a CRISPR/Cas9 approach in a series of DMG tumor cell lines and to determine underlying molecular mechanisms of action.

Efficacy of EZH2 inhibitors and protein down-regulation were evaluated in DMG cell lines by Western blot, proliferation and cell death assay. A knockout (KO) of the EZH2 gene was also realized using the CRISPR/Cas9 system. GSK126 is an efficient inhibitor with consistent anti-proliferative and pro-apoptotic effects in several DMG cell lines. When tumor cells where genetically depleted of EZH2, no growth inhibition was observed, neither by siRNA-mediated reduction or knock-out using the CRPSR/Cas9 system. Treatment with GSK126 in EZH2 depleted cells preserves comparable cytotoxic activity as in wild-type cell lines.

Proteomic analysis is underway to identify additional novel targets of GSK126. Preliminary results show that GSK126 induces a strong and selective over expression of 34 proteins are highly and selectively enriched in biological processes implicating cholesterol metabolism, perhaps pointing to a compensatory resistance mechanism. Inhibitory effects will be further confirmed using murine DMG tumor models. To minimize the toxic effects of GSK126 treatment, we envision vectorization of EZH2 inhibitors using a prodrug delivery system, which is a promising new concept that improves the effectiveness of anti-tumor therapy and significantly reduce its toxicity.

## GRAFTING HUMAN TUMORAL CELLS IN AN AVIAN EMBRYO TO MODEL PEDIATRIC MALIGNANCIES IN AN IMMATURE ORGANISM.

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#### BACKGROUND, OBJECTIVES

Pediatric malignancies share the unique particularity to occur in developing organisms. Half of them even arise at embryonic stages, thus concomitant to morphogenetic processes characterized by intense cell proliferation and migration. To which extent cancer cells exploit and hijack the embryonic programs to widely spread and colonize particular developing tissues is a fundamental question. It has long remained inscrutable, mainly due to the lack of models recapitulating prenatal tumoral events and allowing experimental manipulations. Based on our recent achievements on neuroblastoma, we aimed to develop additional models recapitulating the embryonic environment and adapted to patient-driven analyses.

#### **MFTHODS**

We focused on three pediatric malignancies with migration specificities: neuroblastoma (NB), medulloblastoma (MB) and germ cell tumors (GCT). Graft of cell lines and patient samples in avian embryos were performed in selected tissue sites and developmental stages to reproduce and study the etiological steps of primary tumor formation and/or secondary dissemination modalities.

#### **RESULTS**

We analyzed by 3D imaging using light sheet confocal microscopy the sequence of events and physical pathways leading to NB secondary metastasis to distant organs such as the bone marrow. In the same line, grafting MB cells in selected brain territories revealed stereotypic migratory patterns. At last, placing GCTs cells in the migratory path of their cells of origin successfully led to the formation of tumors in gonadal sites, opening the possibility to challenge the hypotheses underlying their etiology.

#### **CONCLUSION**

Thus, our in vivo paradigm consisting in confronting tumoral cells to microenvironments specific to the developing organism and selected according to the territories of tumor occurrence, appears as a powerful in vivo paradigm to model pediatric malignancies. The data obtained on three major pediatric indications pave the way to a range of embryonal malignancies with yet no available or suitable in vivo models.

## Novel Lipid-Modulating Role of Meprin a in Liver Cancer.

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Hepatoblastoma (HB) is the most common pediatric liver cancer that develops on a normal liver of babies. The incidence rate of this disease has increased over the last decade to score 0.5-1.5 cases per million children. Meprin a is a metalloprotease known to be involved in many biological processes associated with cancer, metastases and inflammation. This protein is overexpressed in many cancers including colorectal, colon, breast cancer and most interesting is hepatocellular carcinoma (HCC). In HCC, it induces migration and invasion and is associated with very poor prognosis. Therefore, in this project, we aim to study the potential oncogenic role played by meprin a in the progression of HB.

First, RNAseq analysis was performed in HB tumor samples (T) from patients and compared to their corresponding non-tumoral (NT) parts, which showed overexpression of MEP1A transcript in T vs. NT tissues. To create hepatic cancer cell lines overexpressing MEP1A, we used lentiviral transduction system in HepG2 and Huh7 cells. We assessed the effect of meprin overexpression in the secretome of HepG2 by proteomics. Using this approach, we showed that high expression of meprin affects the amount of several proteins involved in modulating lipid metabolism and the uptake of lipoproteins such as low density lipoproteins (LDL). This finding led us to explore the effect of stating, such as simvastatin, which are known to modify the expression of LDL receptor (LDLR). The effects of simvastatin treatment on tumor cell lines were assessed using western blot, proliferation assay, and other functional assays. Simvastatin treatment was capable of reversing the lipid metabolism profile of Huh7 and HepG2 cells by increasing the expression of LDLR, stimulating the uptake of LDL and reducing the endogenous biosynthesis of cholesterol. Moreover, simvastatin treatment led to the inhibition of the cellular proliferation in a dose-dependent manner. In conclusion, we report that meprin a is overexpressed in HB tumors and show for the first time that this overexpression may affect the metabolism of cancer cells. This metabolic signature can be potentially overridden by available drugs such as statins, which opens up new avenues for the treatment of HB. More work is needed to further explore and understand these different aspects.

