Childhood cancers represent the second cause of death in children in developed countries. Most of these tumors develop from embryonal tissues and constitute, in contrast to adult tumors, accidents of development rather than of tissue renewal or ageing. Different characteristics distinguish childhood from adult cancers and may appear as elements of relative simplicity to study their mechanisms of initiation and progression: the low number of genetic alterations which probably account for a limited role of genetic instability, the low exposure of children to environmental mutagenic factors and the very rapid development of these tumors which suggest a limited number of oncogenic steps.

Our objectives are to define the molecular lesions that characterize pediatric tumors and to investigate the functional consequences of these alterations in the appropriate cell background. The three main groups of tumors of interest are sarcomas with a specific focus on Ewing sarcoma, rhabdoid tumors and neuroblastomas. In past years we could contribute to define their primary genetic alterations, i.e. EWSR1-FLI1 fusion, SMARCB1 inactivation, BCOR fusions, and ALK activation mutations (Delattre et al, Nature 1992; Versteege et al, Nature, 1998; Janoueix-Lerosey et al, Nature, 2008; Pierron et al, Nat Genet, 2012, Watson et al, J Pathol, 2018). These molecular alterations constitute the starting point to achieve a better understanding of the specific processes that underlie the development of these tumors as well as to elaborate new tools for diagnosis and prognosis and to propose new therapeutic options. These projects are developed in tight collaborations with our colleagues from the clinical department of the SIREDO pediatric oncology center and of the Curie hospitals.

Our strategy develops along three major axes:

1- **Functional analyses** of the altered genes through the set-up of appropriate biochemical, cellular and animal models.

2- Study of the **inter- and intra-tumor heterogeneity** with particular attention to genetic and non-genetic plasticity. This theme takes advantage of single cell analyses, epigenetic profiling and live-imaging approaches. We have also a strong interest in the elucidation of the **cells-of-origin** of these cancers to understand the normal biology of these cells and its reprogramming in the cancer cells.

3- Investigation of the **interplay between germline and tumor genomes**, to decipher how germline polymorphisms may synergize with acquired mutations to promote oncogenesis.
Ewing Sarcoma

Cell Heterogeneity and plasticity

Ewing’s sarcoma is characterized in most cases by the expression of a fusion protein associating the prion-like domain of EWSR1 and the FLI1 DNA binding. We have recently shown that the level of expression of this oncogene is heterogeneous within tumors and impacts on cell behavior. EWSR1-FLI1<sub>low</sub> cells have a mesenchymal phenotype with migratory and invasive capacities, while EWSR1-FLI1<sub>high</sub> cells proliferate with cellular plasticity between these 2 states (Franzetti GA et al., Oncogene, 2017). A more precise evaluation of Ewing cells at the single cell level further indicate that cells exhibit a broad range of EWS-FLI1 activity and that proliferation is associated with a window of EWS-FLI1 activity (IC-EWS<sup>OPT</sup> on Fig.1)(Aynaud et al, Cell reports, 2020). We are now investigating cell- and non-cell-autonomous factors controlling EWS-FLI1 activity.

Fig1. Left) Scheme of Ewing cell heterogeneity. Cells with a low EWS-FLI1 activity (light blue) have mesenchymal-like features with cell matrix adhesion and high migration potential. Right) single cell RNA-seq analysis showing that proliferation (Y-axis) is associated with a window of EWS-FLI1 activity (X-axis)
Study of STAG2 mutations

STAG2 is one of the most recurrently mutated genes in human cancer, and the secondary most frequent mutation in Ewing sarcoma. They are associated with poor outcome for these patients (Tirode, Surdez et al., Cancer Discovery, 2014). STAG2 encodes an integral member of the cohesin complex, a ring-shaped multi-protein structure, which is important for proper sister chromatid cohesion and release during mitosis. Cohesin, together with CTCF, is also essential to shape the architecture of the genome through its ring structure that allows for chromatin loops formation. We investigated STAG2 function using isogenic proficient and deficient Ewing sarcoma models, as well as new NGS technologies to assess 3D genome interactions (HiChIP). We show that STAG2 Loss-of-Function strongly alters the anchored dynamic loop extrusion process and dramatically decreases promoter-enhancer interactions, particularly at EWSR1-FLI1-bound GGAA microsatellites. (Surdez et al, Cancer Cell, 2021). Therefore, STAG2-LOF reduces the activity of the EWSR1-FLI1 oncogene and hence promotes and EWSR1-FLI1\textsuperscript{low} phenotype with increased migration and invasion ability of Ewing cells. Accordingly, a lower expression of genes down-regulated by STAG2 and
displaying EWSR1-FLI1-bound microsatellites is of adverse prognostic significance in patients with Ewing sarcoma. We are currently investigating the role of EWSR1-FLI1 in the genome architecture of Ewing cells.

**Interactions between germline and somatic alterations**

Ewing sarcoma is mostly observed in populations of European ancestry. This has long suggested genetic susceptibility to this tumor. In collaboration with the Chanock and Machiela groups at NCI we performed a GWAS of Ewing patients which demonstrated a highly statistical association of Ewing sarcoma with six genomic loci (Postel-Vinay et al, *Nature Genetics*, 2012; Machiela et al, *Nature Communication*, 2018). Further investigation of the chromosome 10 locus showed that the risk allele presents a polymorphism at a GGAA sequence that is bound by EWS-FLI1 to regulate the EGR2 gene, which is essential in Ewing sarcoma (Grunewald et al, *Nature Genetics*, 2015). We are presently investigating other Ewing-susceptibility loci.

**New therapeutic approaches**

Currently, we are studying the mechanisms involved in the transition between EWSR1-FLI1\textsuperscript{low} and EWSR1-FLI1\textsuperscript{high} cells to better understand treatment resistance mechanisms. In addition, we are developing cellular models expressing an endogenous EWSR1-FLI1 protein fused to a fluorescent protein to study the impact of pharmaceutical compounds on protein stability in collaboration.
Neuroblastoma

Heterogeneity and plasticity of cell identity

Tumor cell plasticity has now been identified as a source of intra-tumor heterogeneity that may contribute to treatment failure in several types of cancer. Neuroblastoma, a tumor derived from multipotent neural crest cells (NCC), accounts for around 15% of children cancer-related deaths. Through the analysis of the super-enhancer landscape, we recently revealed two types of cell identity: a sympathetic noradrenergic identity (NOR) driven by the PHOX2B, HAND2 and GATA3 transcription factors (TFs) and a NCC-like identity (MES) driven by a module containing AP-1 TF (Boeva et al, Nature Genetics, 2017). MES cells are less sensitive to chemotherapy. Recent evidence indicates that some neuroblastoma cells exhibit plasticity and can shift between an NCC-like/mesenchymal (MES) and a noradrenergic (NOR) identity and vice versa. A main objective is to understand the mechanisms that control reprogramming of neuroblastoma cell identity and define the signaling pathways involved in the noradrenergic and mesenchymal cells and in the NOR-MES transition. Recent results show that both external...
environmental signals and intrinsic factors control plasticity and cell identity in neuroblastoma. In particular, a cell line derived from a PDX model (established from a stage 4 patient at relapse) composed exclusively by NOR cells in vivo can grow in vitro as a bi-phenotypic population, with adherent cells and floating neurospheres, single-cell RNA-seq documenting MES and NOR identities, respectively (Figure 4). We are investigating in depth through scRNA-seq and genetic engineering the mechanisms of the NOR to MES transition which may be crucial to account for treatment failure.

**Deciphering the tumor microenvironment of neuroblastoma**

We have recently explored biopsies from patients by scRNA-seq (10X Genomics technology available at the NGS platform of Institut Curie) to investigate both the heterogeneity of tumor cells and the tumor microenvironment of neuroblastoma. In parallel, we characterize mouse tumors generated in the TH-MYCN transgenic model, which constitutes a preclinical model of neuroblastoma in an immunocompetent background. In these analyses, cell populations are annotated through the expression of canonical cell type gene markers and/or known signatures. We are exploring similarities in population structure between both organisms with a focus on myeloid populations and Cancer-Associated Fibroblasts. Interactions between different populations of the microenvironment and/or with tumor cells are analyzed.

*Fig 5. Single-cell transcriptomic analysis of the TH-MYCN mouse neuroblastoma model. UMAP of 5650 cells after integration with Seurat of three tumors analysed by scRNA-seq.*
using bio-informatic tools and functional assays, in order to further define therapies targeting both the microenvironment and the tumor cells.

**Role of MYCN overexpression and Alk mutations in tumorigenesis**

In 2008, the identification of activating mutations of the ALK gene in a subset of sporadic and familial neuroblastomas (Mossé et al, Chen et al, George et al, Janoueix-Lerosey et al, Nature, 2008) constituted a major advance in the understanding of the disease and opened the way to potential targeted treatments. The ALK gene encodes a tyrosine kinase receptor (RTK) that is preferentially expressed in the central and peripheral nervous systems. To better understand the early stages of tumorigenesis, we are exploring how overexpression of the MYCN gene (that is amplified in 20% of primary tumors) and/or Alk mutations modify the development of the adrenal gland and the sympathetic ganglia ecosystems. This work is carried out using mouse models, taking advantage of scRNA-seq approaches combined to multiplex imaging.

**Sarcoma**

Sarcomas consist in a group of rare malignant tumors of mesenchymal origin and are characterized by clinical, pathological and molecular heterogeneities. From a pathological point of view, more than 150 sarcoma subtypes have been described, and this classification has even been enlarged during the last decades due to the discovery of multiple molecular abnormalities specific to certain groups of tumors. Our research projects focus on three main axes.

**Genomic and transcriptomic characterization of sarcoma**

We use Next Generation Sequencing approaches (WES, RNA-seq) to study the genetic, and transcriptomic features of sarcomas, to identify new tumor entities and characterize their tumor and immune characteristics, and to develop bioinformatics tools for standard diagnosis.

**Intratumor heterogeneity of high-grade soft tissue sarcoma**

We also study the intratumor heterogeneity of specific high-grade sarcoma such as dedifferentiated liposarcoma and undifferentiated pleomorphic sarcoma by single-cell sequencing. The objective is to study the biological mechanisms leading to the existence of distinct tumor cell populations within these tumors, as well as their interactions with the tumor microenvironment in order to identify new therapeutic targets.
Deciphering the cell of origin of specific sarcoma subtypes

Most sarcoma subtypes are thought to derive from the transformation of mesenchymal stem cells (MSC) at various stages of differentiation. We perform an extensive epigenetic and transcriptomic profiling of a cohort of various sarcoma subtypes to identify specificities related to their putative cell-of-origin. For this purpose, tumor profiles are being integrated and combined with epigenetic and transcriptomic profiles of non-malignant mesenchymal cells and tissues. The objective is to determine the cellular context in which specific genomic alterations can lead to the development of these tumors.

All these projects are to develop new therapeutic approaches and improve patient outcome.

Key publications

Year of publication 2021

Olivier Saulnier, Katia Guedri-Idjouadiene, Marie-Ming Aynaud, Alina Chakraborty, Jonathan Bruyr, Joséphine Pineau, Tina O’Grady, Olivier Mirabeau, Sandrine Grossetête, Bartimée Galvan, Margaux Claes, Zahra Al Oula Hassoun, Benjamin Sadacca, Karine Laud, Sakina Zaïdi, Didier Surdez, Sylvain Baulande, Xavier Rambout, Franck Tirode, Martin Dutertre, Olivier Delattre, Franck Dequiedt (2021 May 19)

ERG transcription factors have a splicing regulatory function involving RBFOX2 that is altered in the EWS-FLI1 oncogenic fusion.

Nucleic acids research: DOI: 10.1093/nar/gkab305

Didier Surdez, Sakina Zaïdi, Sandrine Grossetête, Karine Laud-Duval, Anna Sole Ferre, Lieke Mous, Thomas Vourc'h, Franck Tirode, Gaelle Pierron, Virginie Raynal, Sylvain Baulande, Erika Brunet, Véronique Hill, Olivier Delattre (2021 Apr 30)

STAG2 mutations alter CTCF-anchored loop extrusion, reduce cis-regulatory interactions and EWSR1-FLI1 activity in Ewing sarcoma.

Cancer cell: DOI: 10.1016/j.ccell.2021.04.001

Year of publication 2020

Transcriptional Programs Define Intratumoral Heterogeneity of Ewing Sarcoma at Single-Cell Resolution.
*Cell reports* : 30 : 1767-1779.e6 : [DOI: 10.1016/j.celrep.2020.01.049]

Year of publication 2019


Study of chromatin remodeling genes implicates SMARCA4 as a putative player in oncogenesis in neuroblastoma.

Year of publication 2018


Transcriptomic definition of molecular subgroups of small round cell sarcomas

Year of publication 2017

Céline Chauvin, Amaury Leruste, Arnault Tauziede-Espariat, Mamy Andrianteranagna, Didier Surdez, Aurianne Lescure, Zhi-Yan Han, Elodie Anthony, Wilfrid Richer, Sylvain Baulande, Mylène Bohec, Sakina Zaidi, Marie-Ming Aynaud, Laetitia Maillot, Julien Masliah-Planchon, Stefano Cairo, Sergio Roman-Roman, Olivier Delattre, Elaine Del Nery, Franck Bourdeaut (2017 Nov 16)

High-Throughput Drug Screening Identifies Pazopanib and Clofilium Tosylate as Promising Treatments for Malignant Rhabdoid Tumors.
*Cell reports* : 1737-1745 : [DOI: S2211-1247(17)31539-5]