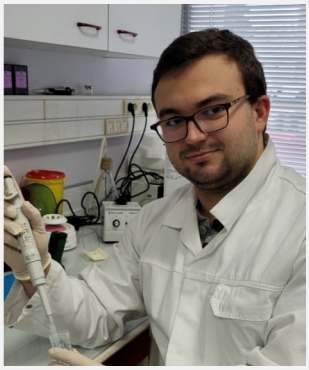
ImmunoTools *special* Award 2021



Jérémy Raguin, PhD-student

Supervisor: Dr. Marc-André MOUTHON

UMRE008, University of Paris, U1274, INSERM, LRP/SDRR/iRCM/JACOB/DRF, CEA, 18, route du Panorama, BPn°6, F-92265 Fontenay-aux-Roses, France

Modeling the glioblastoma tumor niche on human brain organoids

Glioblastoma (GBM) is the most severe primary brain tumor with a median survival of 15 months despite aggressive multimodal treatments combining surgery, radiotherapy and chemotherapy (Wen and Kesari, 2008). Recurrence is due to the presence of glioblastoma stem cells (GSCs) that are resistant to treatment (Bao et al., 2006). In addition, invasiveness of GSCs that is enhanced by radiation therapy (Gauthier et al., 2020) represents a major obstacle for curative therapy (Vollmann-Zwerenz et al., 2020). Moreover, GSCs are located in a tumor microenvironment, called a tumor niche, that supports their aggressive characteristics (Broekman et al., 2018). In particular, GSCs are associated with blood vessels that regulate their proliferation and survival (Jhaveri et al., 2016). Macrophages and microglial cells also represent the majority of non-tumor cells in GBMs. The presence of tumor-associated macrophages (TAMs) is correlated with GBM severity and poor survival (Müller et al., 2017; Yeini et al., 2021). TAMs represent a heterogeneous population and originate primarily from the recruitment of circulating monocytes/macrophages (Pombo Antunes et al., 2021; Van Hove et al., 2019). In one hand, TAMs create immunosuppressive conditions with the synthesis of cytokines such as TGFβ; and, on the other hand, synthesize factors promoting the proliferation and survival of GSCs (Gutmann and Kettenmann, 2019).

Recently, a model of GBM on cerebral organoids (GLICO) was developed and allowed to obtain invasion signatures reminiscent to those observed in surgical specimens from GBM patients (Krieger et al., 2020; Linkous et al., 2019). My project aims at optimizing a culture protocol of GSCs on brain organoids with a complex tumor microenvironment containing TAMs.

For this project, cerebral organoids will be derived from human pluripotent stem cells (iPS) and vascularization will be initiated by VEGF treatment during organoid formation (Ham et al., 2020). Then, GSCs (genetically modified to express Enhanced Green Fluorescent Protein) will be added together with normal human blood monocytes. The CSGs, TAMs and vascularization quantified characterized by microscopy (migration…). In addition, GLICO will be analyzed in more details by flow cytometry for the modification of the polarization of TAMs (CD11b/c, CD163...) and the synthesis of cytokines (IL-6, IFNγ…). The different cell populations (organoid, GSCs, TAMs,

endothelial cells...) will be also characterized by RNA sequencing after sorting by flow cytometry.

ImmunoTools allows me to characterize the phenotype of TAMs by flow cytometry with anti-human antibodies: CD11b-PE, CD11c-PE, CD14-PerCP, CD31-PE, IL-6-PE, IFN gamma-PE, IgG2b-PE, Annexin V-APC, and with the recombinant human cytokine rh IL-4 and rh VEGF-A/VEGF-165.

ImmunoTools *special* AWARD for **Jérémy Raguin** includes 10 reagents

PE - conjugated anti-human CD11b, CD11c, CD31, IFN gamma, IL-6, IgG2b

PerCP - conjugated anti-human CD14

APC - conjugated Annexin-V

recombinant human rh IL-4, rh VEGF-A/VEGF-165

DETAILS more AWARDS