Application to be sent to Claire Mendoza and Clemence Grosnit:
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Deadline: March 1st, 2024

EUR CARe PhD program pre-proposal

(2 pages maximum)

PhD Director: Bruno Ségui; bruno.segui@inserm.fr
(Name and email)

PhD Director affiliation: Cancer Research Center of Toulouse, MELASPHINX team, France.

PhD co-Director: Yusuf Hannun; Yusuf.Hannun@stonybrookmedicine.edu
(Name and email)

PhD co-Director affiliation: Medical School, Stony Brook University, New York, USA.

Research project title: Targeting glycosphingolipid metabolism to improve efficacy of immune checkpoint inhibitors in melanoma.

Research program abstract (max 500 words):

Cutaneous melanoma is a skin cancer with a poor prognosis that can be treated with immune checkpoint inhibitors (ICI) such as anti-PD-1 and anti-CTLA-4. However, only fifty percent of patients benefit from this therapy due to primary or acquired resistance mechanisms. Sphingolipids are a class of lipids containing a sphingoid base, which play a key role not only in membrane structures, but also in cell signalling pathways. The central molecule in sphingolipid metabolism is ceramide, which can be metabolized to sphingomyelin or glycosphingolipids, including gangliosides. Ceramide can also be degraded to sphingosine, which can be phosphorylated to generate sphingosine 1-phosphate (S1P). While ceramide is considered as anti-oncometabolite, S1P and some gangliosides behave like oncometabolites. Our laboratory has recently shown that dysregulation of sphingolipid metabolism in melanoma probably contributes to immune escape and resistance to ICI. Conversely, reprograming ceramide metabolism, by promoting ceramide accumulation or limiting S1P, can overcome melanoma's resistance to ICI.

We and others have shown that melanoma cells exhibit a strong metabolism of ceramide to glycosphingolipids. Our unpublished data indicate that certain glycosphingolipid concentrations increased during treatment in the plasma of patients who did not respond to ICI. Among glycosphingolipids, glucosylceramide is the precursor of lactosylceramide, which can be further metabolized in 3 main branches of complex glycosphingolipids, including lactosides, globosides and gangliosides. We hypothesize that, among the glycosphingolipids, some are required for the response to ICI while others may exert an opposite function. Furthermore, depending on the mutational status of melanoma cells (*BRAF*, *NRAS*, *CDKN2A*), we expect a different impact on sphingolipid metabolism as well as on the reprogramming of this metabolism on melanoma growth, immune responses and response to ICI.

This **transdisciplinary project** involves the generation of knock out melanoma cells for genes encoding key enzymes involved in the synthesis of the different branches of glucosylceramide-derived glycosphingolipids by **CRISPR/Cas9** (year 1: in B. Ségui's laboratory); (ii) monitoring the consequences on intracellular sphingolipid composition by **mass spectrometry** and

assessing the impact of *BRAF*/*NRAS*/*CDKN2A* mutations on sphingolipid metabolism. Unique **metabolic flux experiments** utilizing a d17 dihydrosphingosine probe, developed by this laboratory, will be applied for this purpose (year 2 in Y. Hannun's laboratory); (iii) monitoring the consequences on **tumor growth, immune responses and response to ICI** in immunocompetent mice (year 3 in B. Ségui's laboratory). The latter part will be carried out using **spectral flow cytometry** as well as by **transcriptomic and bioinformatic approaches** to identify the mechanisms by which glycosphingolipid reprogramming overcomes resistance to ICI.
The ultimate goal of this proposal is to identify some glycosphingolipid-metabolising enzymes to target in order to overcome ICI resistance in melanoma. This proposal could open up new avenues for cancer therapies combining glycosphingolipid-metabolizing enzyme inhibitors and ICI.

Describe in 50 words max for each how this project fits the 3 defining criteria of the CARe graduate programme:

1) **Relation to CARe topics of Cancer, Ageing and/or Rejuvenation**
   This project is focused on cancer, from basic findings to identify resistance mechanisms to ICI, to lipid metabolism reprogramming to overcome resistance to ICI.
2) **Multidisciplinary aspect**
   Molecular and cellular biology, Immunology, lipid biochemistry including mass spectrometry and metabolic flux experiments, transcriptomics and bioinformatics.
3) **International and/or industrial aspect(s)**
   Thesis in cotutelle between Paul Sabatier and Stony Brook Universities.

5 keywords in line with EUR CARe

Cancer, Resistance mechanisms, Therapy, Mass Spectrometry, Bioinformatics

5 references of the teams, highlighting the co-signatory students:
(Last 5 years)

Montfort A#, Bertrand F#, Rochotte J#, Gilhodes J, Filleron T, Milhès J, Dufau C, Imbert C, Riond J, Tosolini M, Clarke CJ, Dufour F, Constantinescu AA, Junior NF, Garcia V, Record M, Cordelier P, Brousset P, Rochaix P, Silvente-Poirot S, Therville N, Andrieu-Abadie N, Levade T, **Hannun YA**, Benoist H, Meyer N, Micheau O, Colacios C, **Ségui B**.
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Cancer Immunol Res. 2021 May;9(5):568-582. doi: 10.1158/2326-6066.CIR-20-0342. #co-first authors.

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Clin Cancer Res. 2021 Feb 15;27(4):1037-1047. doi: 10.1158/1078-0432.CCR-20-3449.
#co-last and co-corresponding authors.

Imbert C, Montfort A, Fraisse M, Marcheteau E, Gilhodes J, Martin E, Bertrand F, Marcellin M, Burlet-Schiltz O, Peredo AG, Garcia V, Carpentier S, Tartare-Deckert S, Brousset P, Rochaix P, Puisset F, Filleron T, Meyer N, Lamant L, Levade T, **Ségui B**, Andrieu-Abadie N, Colacios C.
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