FINAL REPORT – Summary

OCEANCHArCoT
OCEAN CHemodiversity Against Cell cycle Targets

Aim: Research of new marine inhibitors targeted against disease-relevant protein kinases from (i) the Great Barrier Reef, and (ii) the phytoplankton biodiversity.

Protein kinases (PKs) are involved in various human pathologies such as cancer, neurodegenerative diseases or can be targeted to alter the life cycle of various parasites such as those responsible neglected parasitic diseases including leishmaniasis and malaria. Protein phosphorylations are key events involved in the vast majority of cellular pathways. Therefore, PKs constitute currently the first class of targets used by pharmaceutical companies for the characterization of novel bioactive compounds. In a project intended to exploit the ocean biodiversity, Jean-Baptiste Charcot (1867-1936), a pioneer in oceanography established large collections of marine organisms. Taking this one step further, OCEANCHArCoT project aimed to source marine crude extracts from the Nature Bank collection (QLD, Australia), and from the world unique Roscoff Culture Collection of microalgae (France) to identify new PK inhibitors.
At the early stage of the project at Griffith Institute for Discovery (Australia), we chose to focus notably on necroptosis pathway. Necroptosis is one of the most recent discovered programmed cell death that has been shown to be of central pathophysiological relevance in multiple disorders (hepatitis, brain and cardiac ischemia, pancreatitis, viral infection and inflammatory diseases). Currently, there is no therapy for necroptosis prevention and cure. This pathway is driven by two kinases, RIPK1 (Receptor Interacting Protein Kinase 1) and RIPK3, and a pseudo-kinase MLKL (Mixed Lineage Kinase domain-Like). Due to the intrinsic characteristics of these targets, it was extremely difficult to produce a significant and stable amount of them during the first year. However, after three years of hard work with two protein production facilities, we succeeded and we obtained more than 3 mg of both MLKL isoforms that will be useful to continue the project. Perseverance is a key word for OCEANCHArCoT project. Simultaneously, our major goal became the identification of new MLKL inhibitors. The original workflow had to be redefined to allow a rational and efficient exploration of marine chemodiversity from Great Barrier Reef biodiversity housed in Nature Bank (Eskitis Institute, Griffith University) despite the lack of target availability. With a cell-based assay that we developed for a robotic platform, we screened 35,585 Lead-Like Enhanced fractions corresponding to 7,117 marine biota. We obtained 102 good hits from this screening. Then, the strategy was to implement NMR fingerprint based dereplication to focus our workflow on the best potential leads. This step needed to implement a bioinformatic solution dedicated to metabolomics: Workflow4metabolomics. Developments of this solution are still ongoing, and there is still a lot to do in order to make annotation/identification step efficient for marine natural products, the final goal being the full dereplication of raw extracts.

During the return phase at Station Biologique de Roscoff (CNRS, France), the aim was to develop a strategy to explore microalgae chemodiversity from the knowledge acquired at Griffith Institute for Drug Discovery (outgoing phase, Australia). Since microalgae metabolome access is not trivial, extraction procedure is very important to take into account (Serive *et al*, 2012). We applied first a protocol developed during my PhD in order to focus straight away on the production of lead-like enhanced fractions (drug-like fractions) and the rest of the metabolomics workflow allowing to dereplicate them. Although the development of some parts is still ongoing and consistent, the overall strategy is the future in blue biotechnologies projects to identify as early as possible molecules of interest. With the advent of OMICs techniques and bioinformatic tools, this will be possible. Despite dereplication methodology, it is still important to prioritize the purification work on the best fractions with biological activities determination. Thus, simultaneously, we developed a combined « bottom-up » and « top-down » approach: (i) a bottom-up strategy through *in vitro* screening with various bioassays (ii) a top-down strategy through *in silico* screening of marine metabolites. *In vitro* screening was related to PKs, which are involved notably in the control of the cellular division (*e.g.* mitotic kinases including Haspin and Auroras). Cell-based assays have been performed as well, including assays on fibroblasts, acute T lymphocyte leukemia, malignant melanoma skin cells, and a particular cell line dedicated to study necroptosis. Amongst all the interesting results that we obtained, we can highlight one related to the chemodiversity of dinoflagellates. A toxin, which is non-

identified yet, is extremely active *in vitro* against acute leukemia model (IC_{50}= 24,3 ng.mL^{-1}) and against malignant melanoma skin model (IC_{50}= 18,9 ng.mL^{-1}). Work is still ongoing to identify the molecule and understand the mechanism of action. *In silico* screening was performed to predict 25 major therapeutic activities (allergy, Alzheimer, angina, arthritis, asthma, bacterial infections, cancer, depression, diabetes, HIV, heart failure, hyperlipidemia, hypertension, inflammation, migraine, mycosis, obesity, osteoporosis, pain, Parkinson, psoriasis, schizophrenia, skin diseases, thrombosis, and viral infections). Predictions of adverse toxic effects have been performed as well. A total of 76 marine metabolites has been studied with this top-down approach highlighting a potential of interest with some molecules for further studies.

Return phase was also the opportunity to develop and continue various collaborations about the valorization of marine bioresources. We have worked with LEMAR laboratory (Brest, France) on the characterization of biological effects and to the exploration of the mechanism of action of exotoxins from *Alexandrium minutum*. We continued to work with Griffith Institute for Drug Discovery (Australia) about the identification of lead-like molecules from Nature Bank collection. We explored the signaling pathways inhibited by some metabolites from ancient dinoflagellates (collaboration with IFREMER). Within the axis 7 of OCEANOMICs program (Innovation platform for plankton screening for active compounds and metabolites), we have worked to the research of applications in cosmeceutical, nutraceutical and feed/aquaculture fields in collaboration with Veolia Environnement. In the same work package, we have worked on the research of bioactive metabolites from cultivatable microalgae (industry) (collaboration with Université Nice Sophia Antipolis, Greensea, and Soliance SMEs). Finally, I have been implied as a consultant for the valorization of starfish in the framework of bloomings in oyster and mussel farms (Région Bretagne).

IMPACT

OCEANCHArCoT fellowship was a great opportunity to transform my ideas into actions and unleash the potential of marine natural products for human health. The impact of such a project is multiple. It constitutes a strong expertise for my career (various set of skills in high throughput screening, therapeutic targets, marine molecule management, bioassays, *in silico* screening) and helps to become an independent researcher. This is a real valuable research experience in the emerging field of marine molecules for cancer treatment and other human pathologies, so-called blue biotechnologies. During this project, I had the opportunity to forge important scholarly contacts within the community of my European and international peers (academic and private sectors). Moreover, my understanding of scientific priorities and strengths of Queensland government are an asset to consider further projects between Europe and Queensland. OCEANCHArCoT project is multidisciplinary and is a fertile ground for long-term collaborations as a central hub in blue biotechnologies.

The EU has the world's largest maritime territory including its outlying regions. Europe has a major role to play in the therapeutic valorization of the marine chemodiversity. In this context, OCEANCHArCoT contributes to the strengthening of the Europe's research Excellence and its competitiveness in focusing on highly innovative discoveries about new inhibitors targeted against disease-relevant protein kinases. Some of these projects presented above are applied. An economic valorization in the next years can be expected. It is particularly true for Veolia Environment, Greensea, Soliance companies, and also Région Bretagne (all from France). In term of academic research, we plan to valorize our results with LEMAR (France), Griffith University (Australia), IFREMER (France), University Nice Sophia Antipolis (France), and University of Dundee (Scotland) through scientific publications. Finally, OCEANCHArCoT has a social impact through various outreach activities. We engaged the general public in various events like EKKA Royal Queensland Show, National Science week, Facebook and Twitter pages, public conferences and hosting of High School students.

Thus, overall, OCEANCHArCoT contributed and will continue to contribute, from Australia to France, to the research of marine bioactives to discover new therapeutic avenues to fight growing medical and social burdens.

Contact

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