DOCTORAT BIOLOGIE BRETAGNE SANTE LOIRE

Year 2022/2023

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☒ **Requested** ☐ **Acquired**	**Funding origin:**
Thesis title: **Characterization and RNAi targeting of oncogenic gene fusions in glioblastoma**		3 keywords: Glioblastoma; gene fusion targeting; RNAi
Unit / team: **CRCI2NA, INSERM U1307, CNRS U6075 / Team GLIAD**		
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Socio-economic and scientific context (approximately 10 lines):

Glioblastomas (GB) are the most common and most aggressive primary tumors of the central nervous system (CNS). Their incidence has increased over the past decades, reaching 7 new cases per year per 100,000 inhabitants, and the standard therapeutic approach has not significantly improved their prognosis. Conventional treatments consist of maximum safe surgical resection followed by external radiotherapy combined with temozolomide (concomitant radiochemotherapy), followed by 6 cycles of temozolomide. Despite improvements in neurosurgical techniques and molecular characterization of CNS tumors, the median overall survival of GB patients is 15 months. Most GB (> 90%) are classified as *IDH*-wild type in contrast to *IDH*-mutant diffuse gliomas, which develop in younger adults and are associated longer survival. *IDH*-wild type GB is characterized by genomic instability leading to genome duplication and chromosome rearrangements such as gene fusions, which may be oncogenic and drive tumor cell proliferation. Such rearrangements may contribute to treatment resistance and fuel tumor recurrence. The identification of oncogenic gene fusions and the validation of the gene products (mRNA, chimeric protein) as targets (intrinsic or as a proxy for the tumor ecosystem) open up new areas of research and therapeutic innovation. In this context, the use of RNA interference (RNAi) associated with the development of new locoregional approaches to control the disease represents a technological challenge. If successful, such a strategy would circumvent the blood-brain barrier and reduce systemic toxicity. Moreover, it offers opportunities for precision medicine strategies with potential socio-economic impact (longer survival and reduced side effects, treatment costs and hospital stay).

Working hypothesis and aims (approximately 8 lines):

While some chromosome rearrangements in GB lead to the loss of tumor suppressor genes or amplification of oncogenes, others lead to the juxtaposition of coding sequences of two genes resulting in potentially oncogenic chimeric genes. The identification of the *FGFR3-TACC3* fusion in GB allowed the development of drugs targeting the constitutively active tyrosine kinase domain of the chimeric protein (European phase II clinical trial TARGET, AstraZeneca, Jansen). With regard to targeted therapies the clinical trials in GB but also in other cancers such as lung adenocarcinomas have yielded promising results, stressing the need to thoroughly dissect the genetic underpinnings of cancers. We recently identified new candidate fusion genes using first pangenomic SNP arrays in 205 adult *IDH*-wild type GB. We selected GB cases displaying clustered chromosome rearrangements suggestive of chromothripsis, which is known to give rise to gene fusions. Those rearranged GB cases were then RNA-sequenced and the detected gene fusions were validated by RT-PCR followed by Sanger sequencing. Novel potentially oncogenic gene fusions were further selected to develop cell models suitable for assessing the functional roles of the chimeric proteins in GB. The main goal is to understand the pathogenesis of gene fusion-driven GB and to develop new approaches to target the activated pathway(s). We are implementing an innovative protein vectorization of RNAi for locoregional delivery. Even though a given oncogenic gene fusion is hardly detected in over 5% of GB, all the oncogenic gene fusions combined may represent 20% of GB patients. Targeting the fusion gene products (mRNA, protein) may slow disease progression and allow for survival times of GB patients more akin to those observed in *IDH*-mutant diffuse gliomas.

Main milestones of the thesis (approximately 12 lines):

1) Assessing the functional role and potential gain of function of the constitutively active chimeric protein within tumor cells and healthy cells. GB cells and normal human astrocytes have been modified to express the fusion gene. The properties of the modified cell lines (proliferation, adhesion, migration, radiation resistance) will be studied by standard biochemistry, cell biology and molecular techniques. The cellular bio-distribution of the chimeric protein and the downstream activated signaling pathways (phosphoproteins) will be investigated.
2) Assessing the impact of exogenous modulation of the expression of the chimeric protein *in vitro*. The modulation of the gene fusion by specifically designed siRNAs will be tested through commercially available systems (RNAiMax, NTER) and siRNA/Argonaute-2 (AGO2) nanoparticles currently being developed in the laboratory. Coupling the system with existing drugs is also a possibility.
3) Assessing the *in vivo* impact through a locoregional implant with prolonged release of RNAi targeting the gene fusion. One or two models will be developed *in vivo* (orthotopic xenograft in nude mice) for therapeutic interventions at the preclinical level through targeting of the gene fusion by sustained release of RNAi.
4) Assessing the tumorigenic potential resulting from expression of the fusion gene in a modified non-tumoral astrocytic cell line *in vivo* (orthotopic xenograft in nude mice) (link with 1)).

Scientific and technical skills required by the candidate (2 lines):

Molecular and cell biology, Bio-engineering, *in vitro* and *in vivo* experimental models.
Team spirit, motivation, autonomy, good oral and writing skills.

3 publications de l'équipe d'accueil relatives au domaine (5 dernières années) :

1. Ah-Pine F, Casas D, Menei P, Boisselier B, Garcion E, Rousseau A. RNA-sequencing of IDH-wild-type glioblastoma with chromothripsis identifies novel gene fusions with potential oncogenic properties. Translational Oncology, 4 (2021) 100884. (IF: 3.558)
2. Anthiya S, Griveau A, Loussouarn C, Baril P. Garnett M, Issartel JP, Garcion E. MicroRNA-based drugs for brain tumors. *Trends in Cancer*, 4 (2) (2018) 37-53. (IF: 8.884)
3. Séhédic D, Chourpa I, Tétaud C, Griveau A, Loussouarn C, Avril S, Legendre C, Lepareur N, Wion D, Hindré F, Davodeau F, Garcion E. Locoregional confinement and major clinical benefit of ^{188}Re-loaded CXCR4-targeted nanocarriers in an orthotopic human to mouse model of

glioblastoma. *Theranostics*, 7 (18) (2017) 74517-4536. (IF: 8.537)

Collaborations nationales et internationales : Patrick Baril, CBM, Orléans ; Moreno GALLENI, CIP, Liège ; Benjamin Lemasson, GIN, Grenoble ; Consortium GLIOSILK Euronanomed III ; LABEX IRON ; CGO.