PROJECT 1

DoS: Gaetano Finocchiaro, M.D.

Title: An in-depth appraisal of cytokine-induced killer (CIK) cells in glioblastomas with unmethylated MGMT promoter.

Curriculum: See the attachment

Link to OSR/UniSR personal page: Not applicable

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

An in-depth appraisal of cytokine-induced killer (CIK) cells in glioblastomas with unmethylated MGMT promoter.

With a 5-year survival rate of 4% with standard treatment (surgery, radiotherapy with concurrent temozolomide and adjuvant temozolomide) glioblastoma (GBM) is one of the most aggressive cancers. The methylation of the methyl-guanine methyltransferase (MGMT) gene, present in one-third of the patients, increases significantly the response to temozolomide and the overall survival.

Several randomized immunotherapy trials in GBM have been completed in recent years with limited clinical benefit: the highly suppressive tumor microenvironment (TME) is one major obstacle to the development of clinically meaningful immune responses.

Adoptive immunotherapy might have the potential to increase such responses, *per se*, and also in synergy with other approaches including immune checkpoint inhibitors or dendritic cell immunotherapy.

Cytokine-induced killer (CYK) cells are one approach for adoptive immunotherapy (Zhang and Schmidt-Wolf, 2020). In the last decade, CYK cells have been used in clinical studies targeting different solid tumors. CYK include heterogeneous cells comprising CD3+CD56- T cells, CD3-CD56 + NK cells and CD3+CD56+ NKT cells. Their ratio (and possibly efficacy) may depend on the cytokine cocktails used for the expansion of Peripheral Blood Mononuclear Cells (PBMC) that usually includes IFN-gamma, antiCD3 mAb, and IL-2 (Introna, 2017).

Two studies in GBM showed that CYK treatment was associated with prolonged survival but the evidence for associated immune responses and the molecular characterization of the tumors were insufficient to draw firm conclusions.

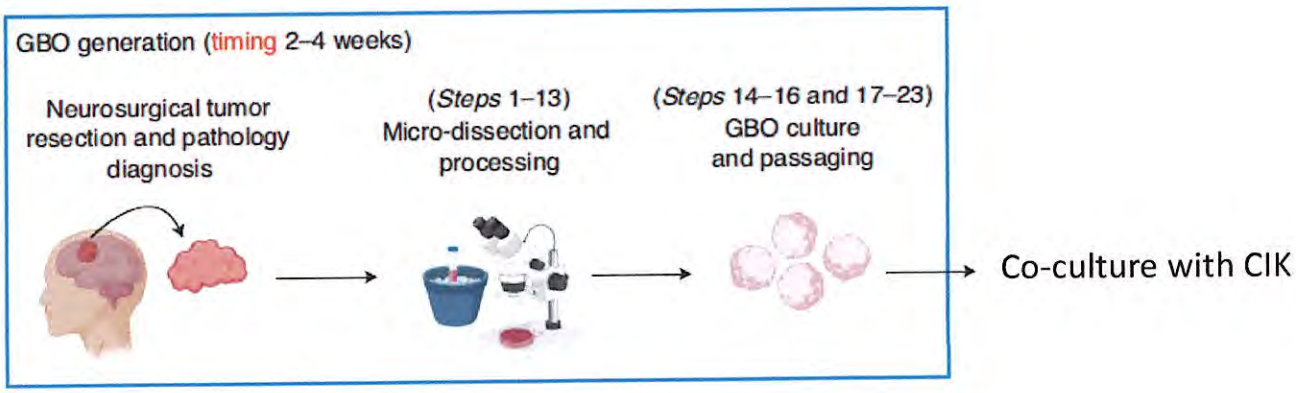
In this 3 year program, we plan a first preclinical part to establish GBM organoids and to match them with autologous CYK cells (Jacob *et al.*, 2020). Their characterization will include the expression of the recently identified inhibitor of tumor-infiltrating lymphocytes in GBM CD161. In parallel, studies with different cytokine cocktails will be performed to test their effectiveness in vitro and the timing necessary to reach cell numbers in the range of 10e9 for patients infusions. After obtaining EC approval, in the second part of the program, we propose to start a pilot study in GBM patients at first diagnosis with no MGMT methylation and

in the absence of temozolomide, which in this setting has low efficacy and because previous data suggest that it may inhibit the cytotoxic activity of T cells. CYC cell infusions could be repeated until disease progression or evidence of grade 3-4 toxicity.

The study may give important information for the targeted use of CYK cells, identifying tumors with mutational and TME profiles associated with clinical responses. If previous results on survival prolongation in the absence of major toxicity will be confirmed, CYK cells have the potential for wider diffusion, due to the relative simplicity and low cost of their production.

Skills to be acquired by the student:

Preparation of glioblastoma organoids (GBOs) (Jacob *et al.*, 2020)



Pros.

- By preserving cell-cell interactions and minimizing clonal selection, GBOs maintain the cellular heterogeneity of parent tumors.
- We are confident that any motivated individual can reproduce this protocol with access to quality resected patient tumor tissue, the proper resources, and careful attention to detail.

Cons.

- The large size of GBOs limits the ability to uniformly load fluorescent dyes or indicators. This makes it difficult to quantify cell killing using the equivalent assays to those used in single-cell-suspension killing assays.

Preparation of cytokine-induced killer cells (CIK)

Peripheral blood mononuclear cells (PBMCs)are initially with interferon-γ , followed one day later by the addition of the monoclonal anti-CD3 antibody OKT-3 and human recombinant interleukin-2 (rhIL-2). The addition of these reagents, all available as clinical products, makes possible a GMP (Good Manufacturing Practice) compliant procedure for the preparation of CIK cells (Introna, 2017).

References (max. 3)

Introna M. CIK as therapeutic agents against tumors. J Autoimmun 2017; 85: 32–44.

Jacob F, Ming G-L, Song H. Generation and biobanking of patient-derived glioblastoma organoids and their application in CAR T cell testing. Nat Protoc 2020; 15: 4000–33.

Zhang Y, Schmidt-Wolf IGH. Ten-year update of the international registry on cytokine-induced killer cells in cancer immunotherapy. J Cell Physiol 2020; 235: 9291–303.