Elucidate ACKR3 expression and function in glioblastoma-associated microenvironment

Kuppens, A.*; Leurs, R.**; Chevigné, A.***; Rogister, B.*; Neirinckx, V*.

*GIGA Neurosciences - Laboratory of Nervous System Disorders and Therapy, Université de Liège
** Division of Medicinal Chemistry, Faculty of Science, Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam
***Immuno-Pharmacology and Interactomics, Department of Infection and Immunity, Luxembourg Institute of Health

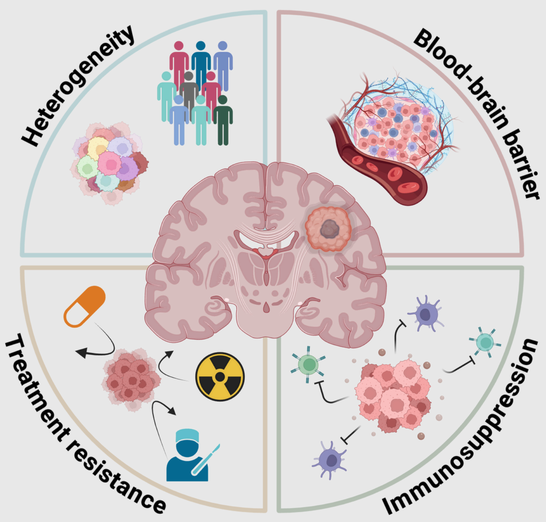

Figure 1. Representation of the aspects limiting the development of efficient GB treatments.

Glioblastoma (GB) is the most common and aggressive primary brain tumor. The current treatment based on a combination of surgery, radio- and chemotherapy is unsuffisiant and patient survival usually does not exceed one year. GB treatment is limited by several factors, including the immunosuppressive tumor microenvironment (TME) that drastically reduces the efficiency of immunotherapies.

The CXCL12/CXCR4 chemokine axis is responsible for the migration of glioblastoma cells towards the subventricular zone and their radioprotection. Accumulating results indicate that ACKR3, the other receptor for CXCL12, might also participate in GB severity as its expression correlates with a poorer prognosis. However, its function in cancer remains to be determined.

The aim of this study is to characterize the expression and function of ACKR3 in GB-associated TME and evaluate its potential relevance as therapeutic target.

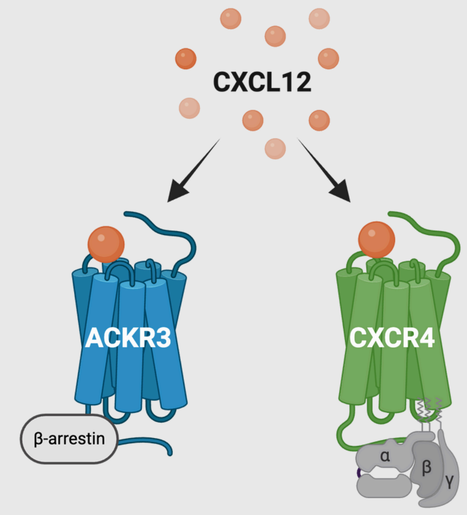

Figure 2. Representation of the CXCL12/CXCR4/ACKR3 signaling. CXCL12 binding to CXCR4 induces the recruitment of G-protein, while ACKR3 recruits β-arrestin.

The pharmacological modulation of the receptors was tested in a syngeneic mouse model.
ACKR3 was modulated with either a specific agonist **VUF11207** (5mg/kg/day) or an inverse agonist **VUF25550** (100mg/kg/day), **AMD3100** was administered as CXCR4 antagonist (5mg/kg/day).
On day 18 post engrafment, brains were collected, dissociated and immune cells were fluorescently labelled for spectral flow cytometry analysis.

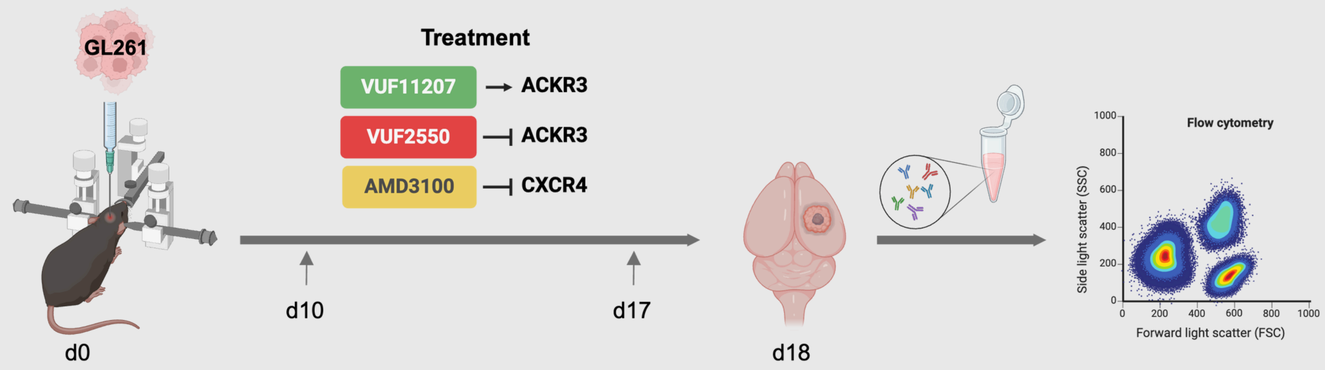

Figure 3. Experiment workflow for ACKR3 and CXCR4 modulation *in vivo*.

Overview of ACKR3 and CXCR4 expression in tumor and CD45+ cells (syngeneic murine GB model)

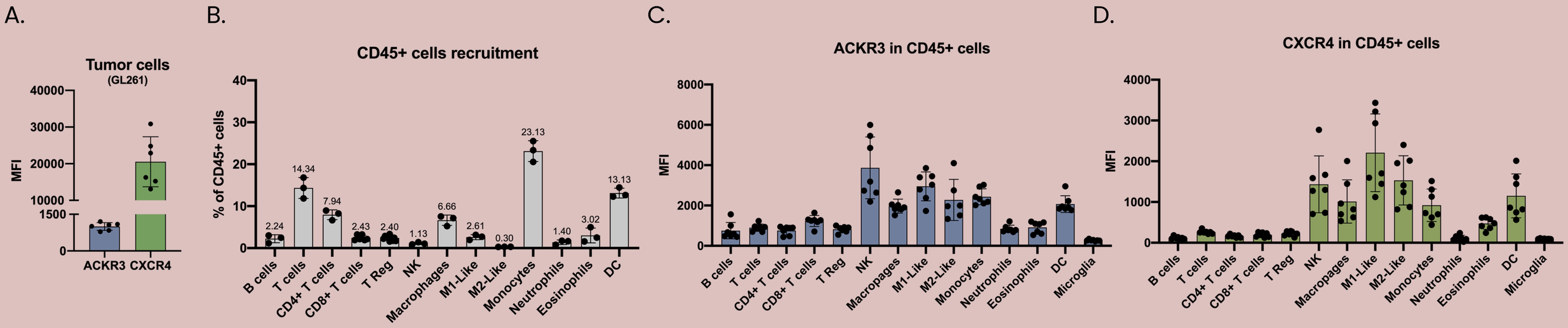

Figure 4. ACKR3 and CXCR4 expression in tumor and immune cells from GB-bearing mice (untreated). A. Receptor expression in GL261 murine GB cells *in vivo*. B. Overview of leukocyte infiltration in the brain. C. and D. Representation of ACKR3 and CXCR4 expression (MFI) in all CD45+ cell populations, respectively.

CXCL12 plasma concentration

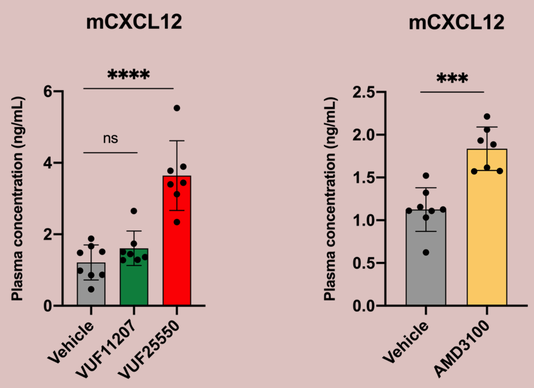

Figure 5. Quantification of CXCL12 plasma concentration. Plasma was collected at experiment endpoint and CXCL12 concentration was evaluated by ELISA.

Modulation of leukocyte recruitment upon CXCR4 or ACKR3 modulation

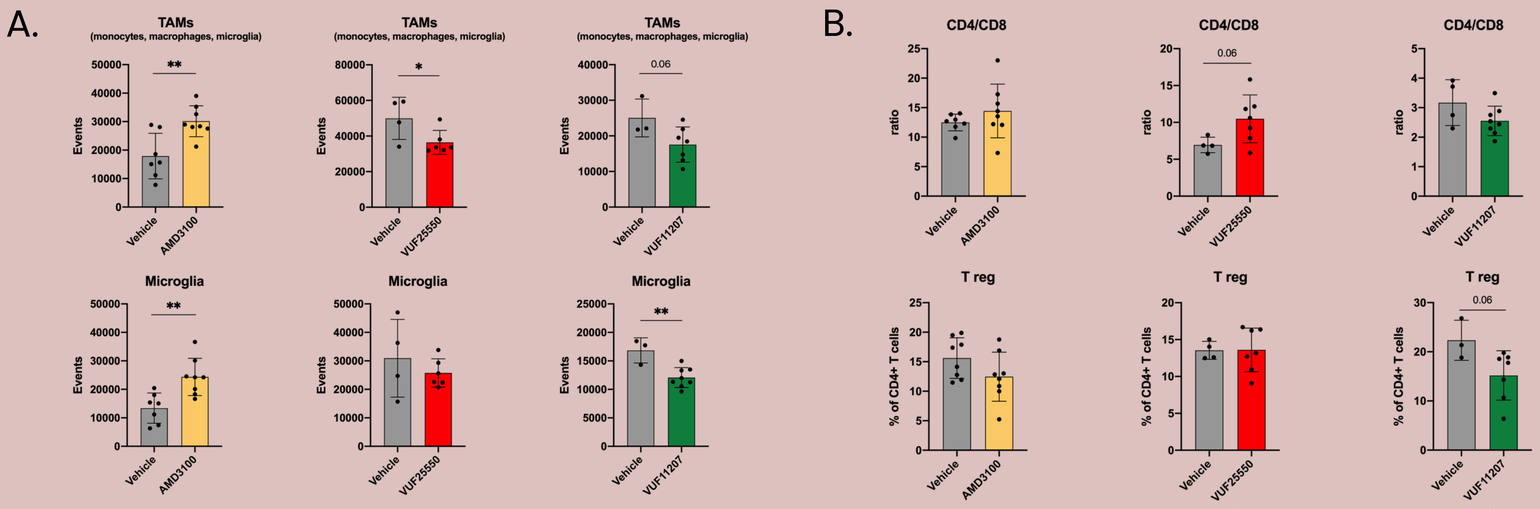

Figure 6. Modulation of leukocyte recruitment from the myeloid and lymphoid compartments (A and B, respectively).

Altogether, results obtained by spectral flow cytometry indicate that ACKR3 and CXCR4 are expressed in different immune cell types from GB-associated TME. Both receptors show similar expression profiles in CD45+ cells; they are highly expressed in myeloid and NK cells and at very low level in microglia (CD45int). Their expression in murine GB cells is however different; ACKR3 is almost absent in tumor cells.
As ACKR3 is found at the surface of various immune cells, we addressed its function using two pharmacological modulators. VUF11207, as agonist, induces the recruitment of β-arrestin and subsequent receptor's internalization; VUF25550, as inverse agonist, reduces this interaction. AMD3100 (Plerixafor) is used as CXCR4 antagonist, blocking the recruitment of G protein. Both VUF25550 and AMD3100 administration resulted in an upregulated CXCL12 plasma concentration, while this effect was limited with ACKR3 agonist. Focusing on the impact on leukocytes recruitment, ACKR3 and CXCR4 modulation had distinct consequences. CXCR4 modulation induces the increase of TAMs, mostly due changes in microglial cells, while a decrease is observed upon ACKR3 modulation. ACKR3 agonism or antagonism appear to have opposite effects on CD4/CD8 T cells ratio and influence regulatory T cells proportions.
These data support the hypothesis that ACKR3, as well as CXCR4, participate to the immunosuppressive TME of glioblastoma.

contact: amandine.kuppens@uliege.be