CONSTRUCTION OF AN ONCOLYTIC HERPESVIRUS (oHSV) FOR INDUCING APOPTOSIS IN GLIOBLASTOMA: PROOF OF CONCEPT

Sánchez Gil Judit[1], Lebrun Marielle[1], Alice Collignon[1], Rogister Bernard[2], Chevigné Andy[3], Sadzot Catherine[1].
1: Laboratory of Virology and Immunology,*GIGA-I3, University of Liège*.
2: Laboratory of Nervous System diseases and Therapy, GIGA-Neurosciences, University of Liège
3: Immuno-Pharmacology and Interactomics, Department of Infection and Immunity, Luxembourg Institute of Health

Background

Glioblastoma is the most common and aggressive primary brain tumor in adults. Surgical resection followed by radiotherapy and/or chemotherapy is the standard treatment. However, despite improvement of these treatments, glioblastoma patients have a poor prognosis, mainly due to recurrence of the tumor. In addition, it has been shown in an orthotopic xenograft model that GBM cells can escape the tumor mass and specifically invade the subventricular zones (SVZ) of the adult brain (1). These cells have been shown to be CXCR4+ and to be attracted by CXCL12 secreted by the SVZ cells. Finally, these cells have been characterized as glioblastoma stem cells (GSC), the only tumor cells population able to initiate a tumor growth. Despite many efforts, molecular targeted agents have not really improved the patients survival, justifying the search for new approaches among which the use of oncolytic viruses. Due to its large genome, its rapid infectious cycle and the fact that, if warranted, it can be controlled by acyclovir, Herpes simplex virus (HSV) has been engineered to replicate exclusively in tumor cells and to be used as an oncolytic virus (oHSV).

Aim

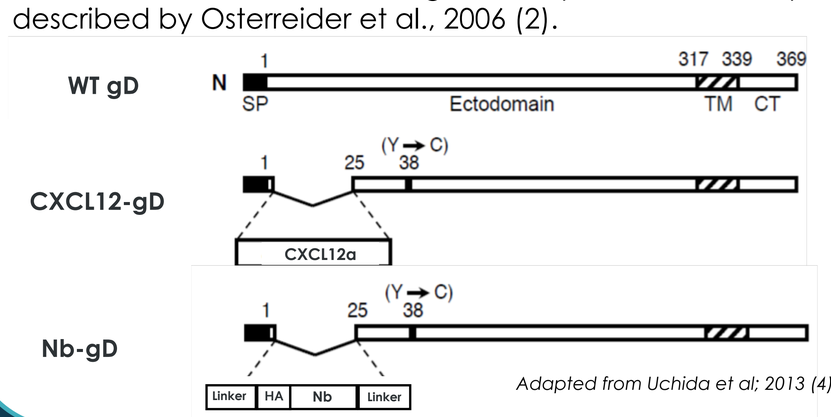
The aim of this project is to build an oncolytic Herpesvirus (oHSV) able to target specifically the GSCs and to induce their apoptosis, constituting thereby a proof-of-concept of a "targeted" and "armed" oHSV.

Results

1. Targeting: modification of gD to target to CXCR4+ cells

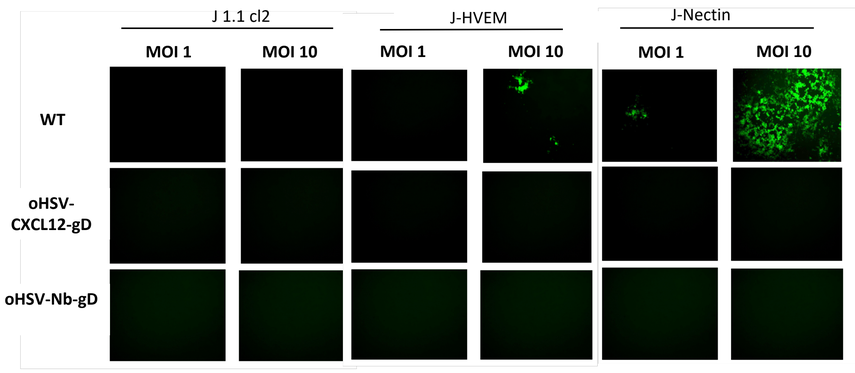
Glycoprotein D retargeting constructions

An oHSV (ICP6-/ICP34.5-) with a modified glycoprotein targeting CXCR4 has been created using the "en passant" technique described by Osterreider et al., 2006 (2).



Adapted from Uchida et al; 2013 (4)

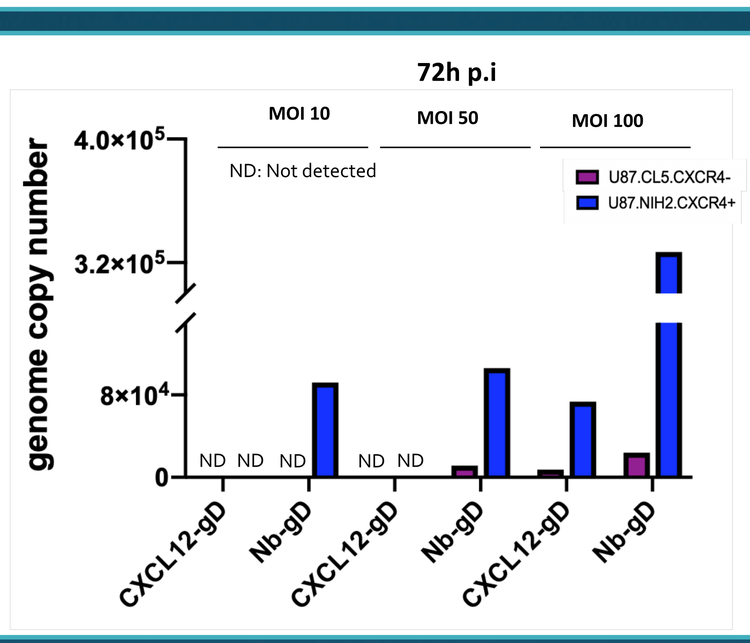
The gD domain required for the interaction with the natural receptor HVEM has been deleted and replaced by either CXCL12 or a anti CXCR4-nanobody and the AA38, important for the interaction with nectin has been mutated (Y>C) (according to Uchida et al; 2009(3)). To verify the de-targeting of these viruses, J1.1 cells (HSV resistant) J-HVEM and J-Nectin cells (kind gift of Dr. G. Campadelli Fiume) were infected with the WT or the mutated virus (MOI 1 or 10). J-CXCR4 cells are being generated and will be infected to confirm the re-targeting.



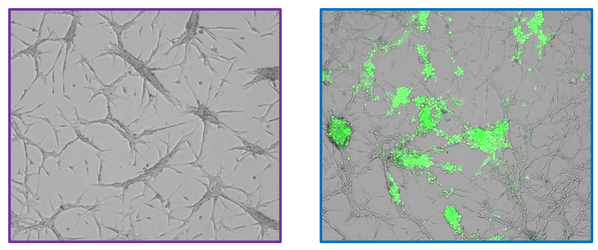
72h p.i

The capacity of these targeted oHSV to specifically infect CXCR4+ cells has been analyzed by infection of glioblastoma cells lines that are negative or positive for CXCR4 (U87.CL5.CXCR4- or U87.NIH2.CXCR4+ respectively) and further quantification by qPCR of the viral genome copy number after various time of infection.

Preliminary results show a higher viral genome copy number in the cells over-expressing CXCR4 compared to the CXCR4$^-$ cells, indicating that the retargeting of oHSV allows to specifically infect a cell sub-population (n=1).

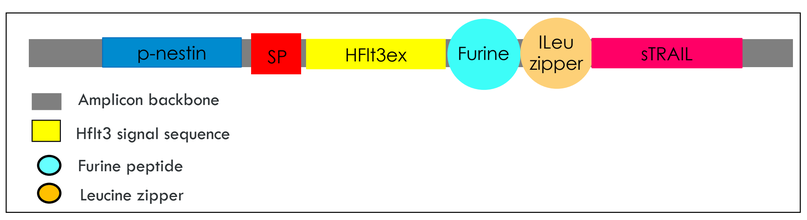


oHSV-Nb-gD
MOI 10 72h p.i.



2. Arming: introduction of a transgene coding for a soluble form of TRAIL

A gene coding for a soluble form of TRAIL driven by the nestin promoter will be introduced in the oHSV. However, to test more easily the efficacy and specificity of this transgene, cells have been first transduced with a Lentivirus expressing this transgene, these latest being more easy to produce.



Adapted from Khalid et al; 2004 (5)

Analyse of cell viability by trypan-blue technique:

Alice Collignon, Master thesis

Cell lines were transduced with the LV during 24, 48, 72 and 96h (MOI of 5). Fibroblasts (HADF) do not express nestin and are used as negative control. U87, T98G and LN18 are nestin$^+$ GBM cell lines.

Results show that the cell viability decreases only in cells expressing nestin, suggesting that the apoptotic soluble protein TRAIL is expressed only in nestin$^+$ cells.

This transgene will be introduced in the retargeted oHSV backbone.

Conclusions

In conclusion, our preliminary results suggest that the retargeted oHSVs work correctly, being able to infect specifically CXCR4$^+$ GBM cells. These viruses will be further modified to increase their efficiency and will be armed with a gene coding for the soluble form of TRAIL driven by the nestin promoter, to trigger apoptosis. After their *in vitro* characterization, the safety and efficacy of these oHSVs will be evaluated *in vivo* in an orthotopic xenograft model.

1. Goffart, N., Kroonen, J., et al; 2014. Adult mouse subventricular zones stimulate glioblastoma stem cells specific invasion through CXCL12/CXCR4 signaling. *Neuro-oncology*, *17*(1), 81-94.
2. Karstentischer, B., Einem, J. V.,et al; 2006. Two-step Red-mediated recombination for versatile high-efficiency markerless DNA manipulation in Escherichia coli. BioTechniques, 40(2),191-197.
3. Uchida H., Shah WA., et al; 2009. Generation of herpesvirus entry mediator (HVEM)-restricted herpes simplex virus type 1 mutant viruses: resistance of HVEM-expressing cells and identification of mutations that rescue nectin-1 recognition. J Virol. 2009 Apr;83(7):2951-61.
4. Uchida H., Marzulli M., et al; 20013. Effective treatment of an orthotopic xenograft model of human glioblastoma using an EGFR-retargeted oncolytic herpes simplex virus. Mol Ther. 2013 Mar;21(3):561-9. doi: 10.1038/mt.2012.211.
5. Khalid S., Ching-Hsuan T., et al; 2004. Inducible release of TRAIL fusion proteins from a proapoptotic form for tumor therapy. Cancer research, Vol 64-9, 3236-42.