



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



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Background

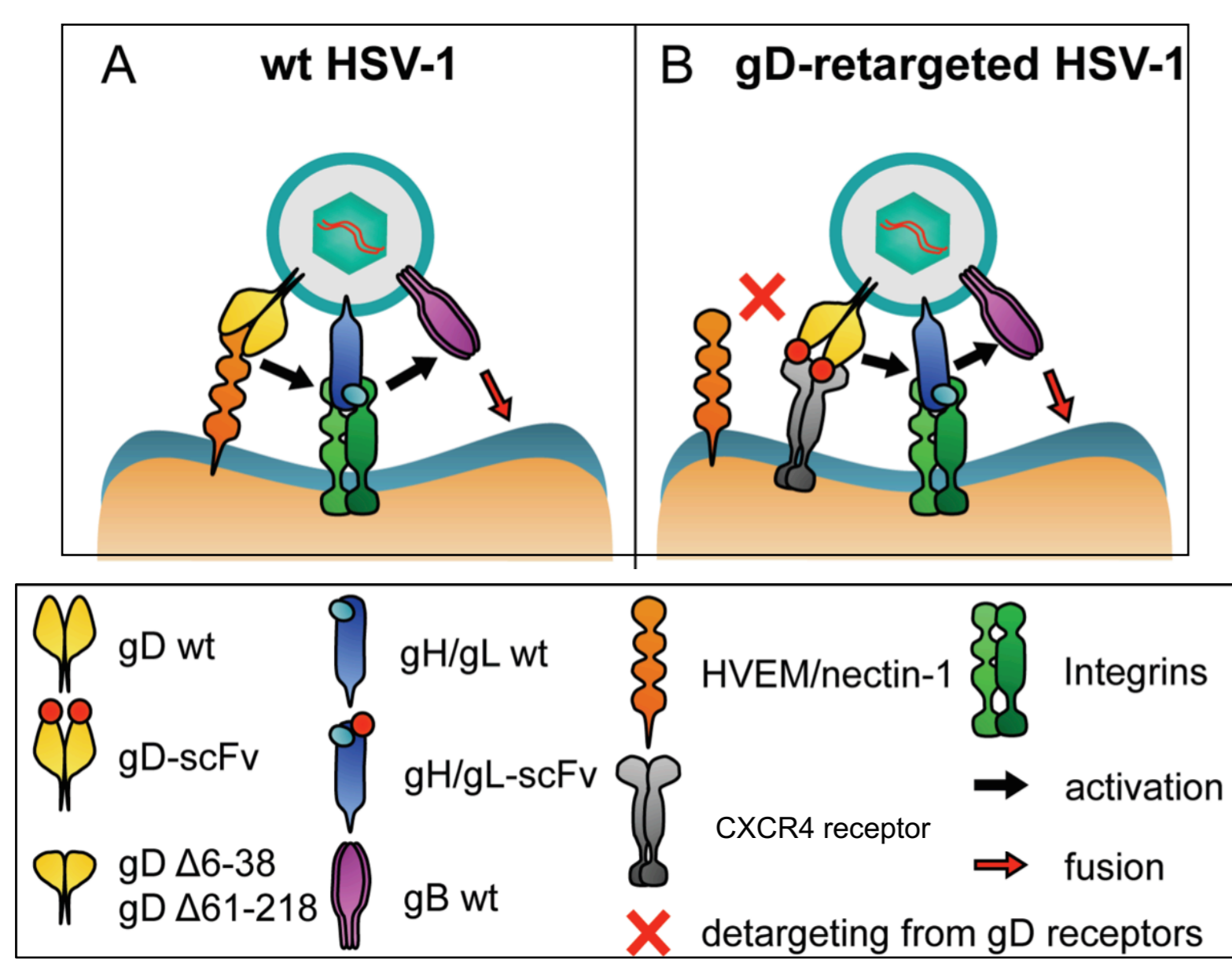
Glioblastoma is the most common and aggressive primary brain tumor in adults. Surgical resection followed by radiotherapy and/or chemotherapy is the standard treatment. However, despite improvement of these treatments, glioblastoma patients have a poor prognosis, mainly due to recurrence of the tumor. 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. These cells have been shown to be CXCR4+ and to be attracted by CXCL12 secreted by the SVZ cells. Finally, they 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 oHSV able to target specifically the GSCs and to induce their apoptosis, constituting thereby a proof-of-concept of a "targeted" and "armed" oHSV.

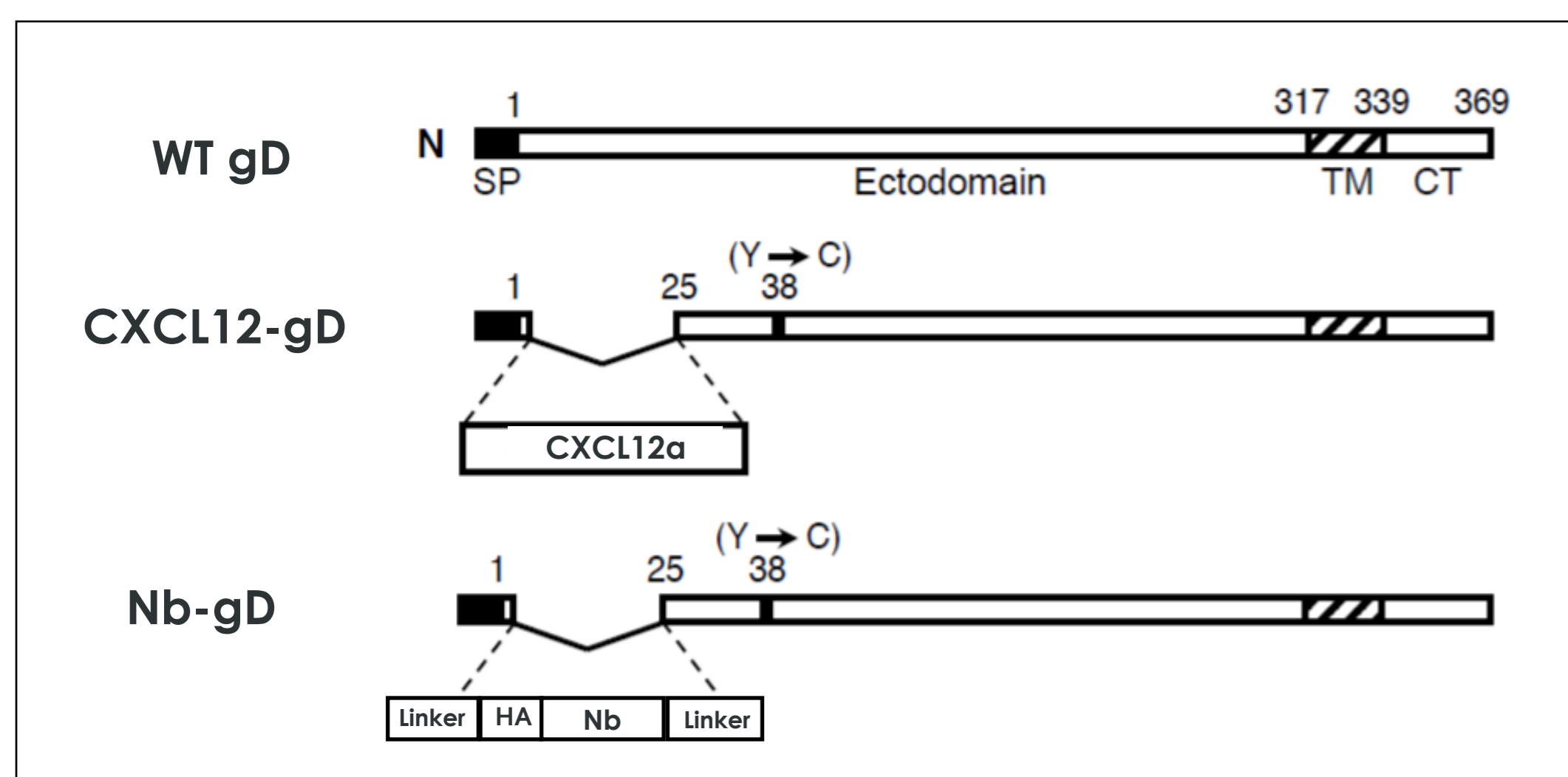
Results

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

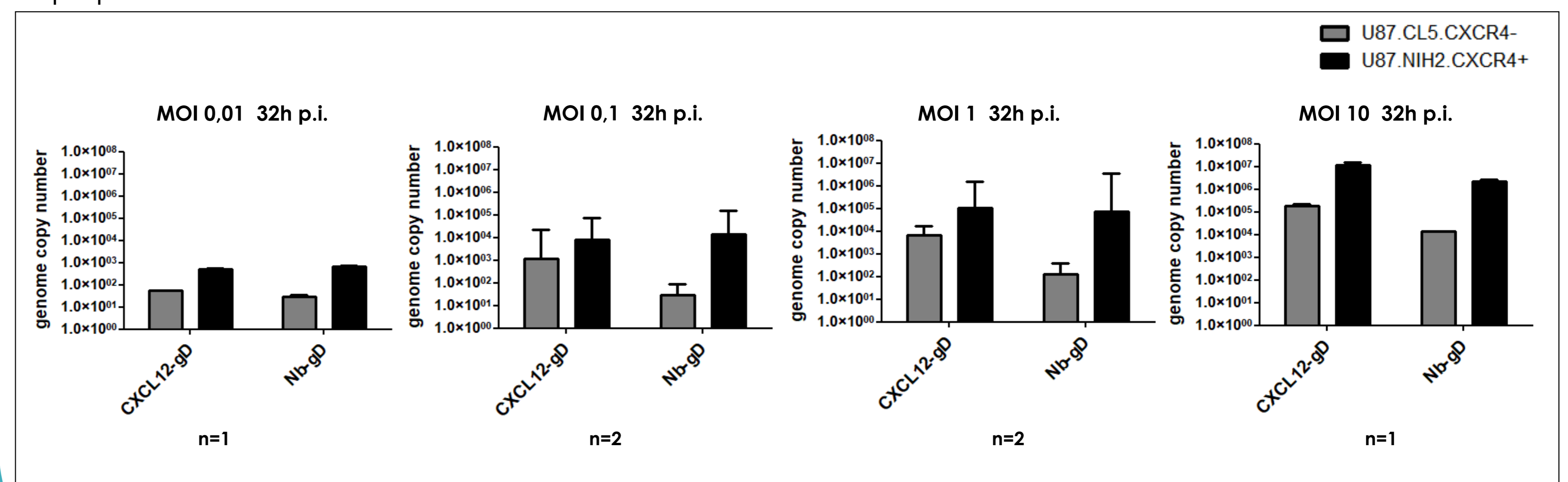


Adapted from Petrovic, B., et al., 2017

Glycoprotein D retargeting constructions

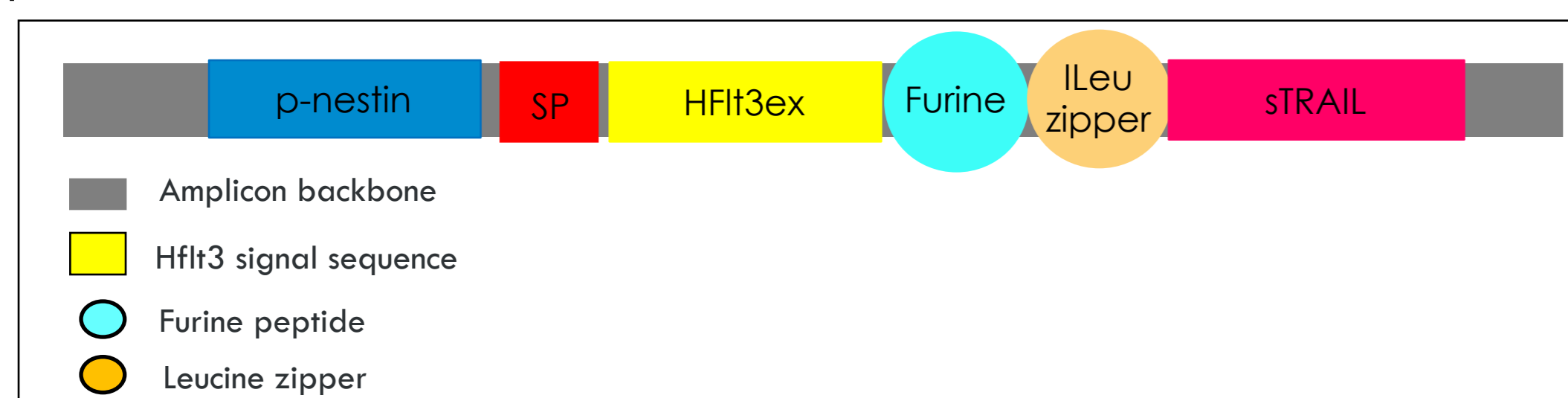


An oHSV virus with a modified glycoprotein targeting CXCR4 has been created using the "en passant" technique described by Osterreider et al., 2006 (2): the gD domain required for the interaction with the natural receptors has been deleted and replaced by either CXCL12 or a anti CXCR4-nanobody. Its capacity to infect only cells expressing CXCR4 has been analysed 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 showed 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.



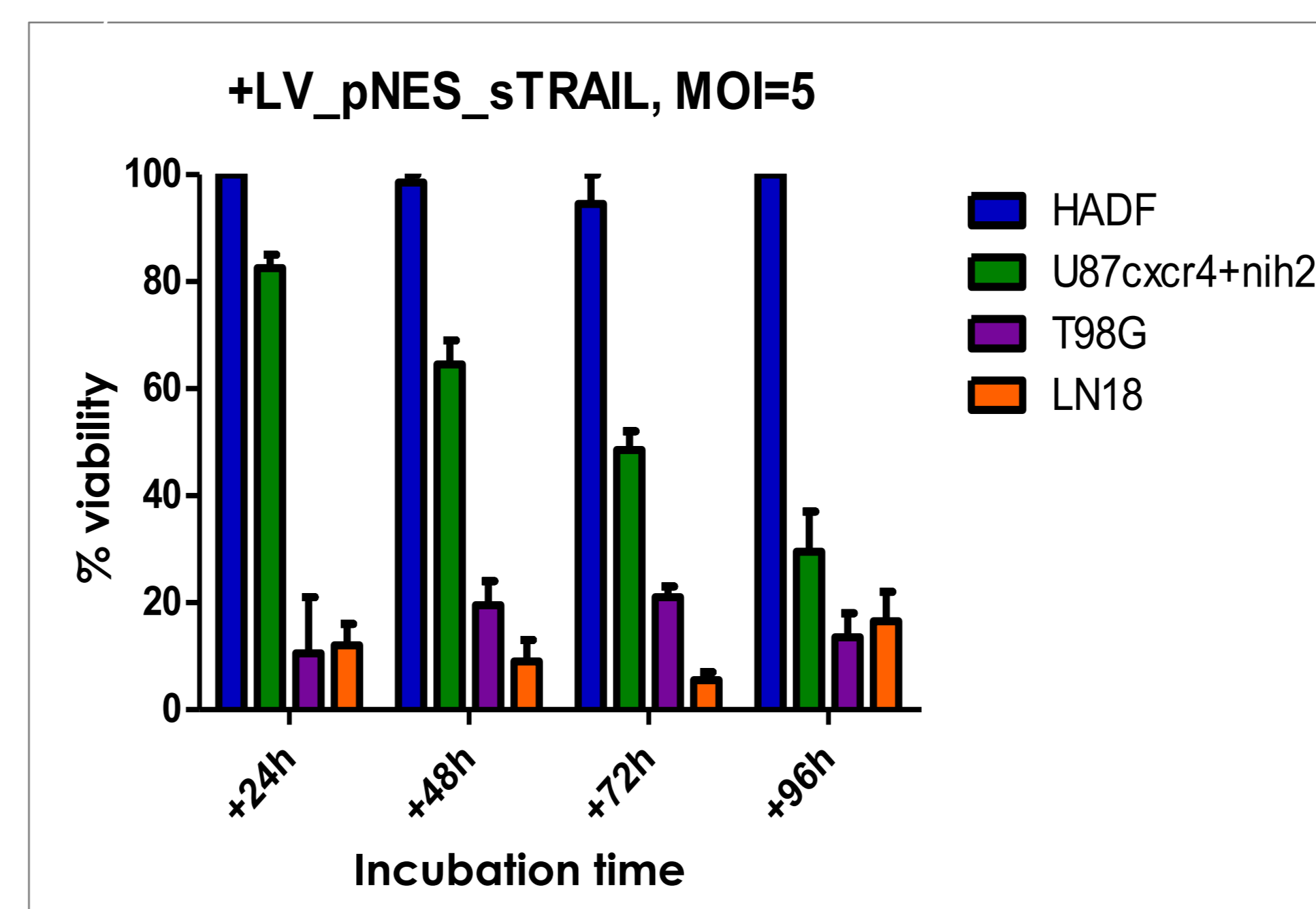
2. Arming: introduction of a transgene coding for a soluble form of TRAIL

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

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 with and MOI of 5. Fibroblasts (HADF) do not express nestin and are used as negative control. U87, T98G and LN18 are GBM nestin⁺ 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 oHSV works correctly, being able to infect specifically CXCR4⁺ GBM cells. This virus will be further modified to increase its efficiency and will be armed with a gene coding for the soluble form of TRAIL driven by the nestin promoter, to trigger apoptosis. After its *in vitro* characterization, the safety and efficacy of this oHSV will be evaluated *in vivo* in an orthotopic xenograft model.

- Goffart, N., Kroonen, J., Di Valentin, E., Dedobbeleer, M., Denne, A., Martinive, P., & Rogister, B. (2014). Adult mouse subventricular zones stimulate glioblastoma stem cells specific invasion through CXCL12/CXCR4 signalling. *Neuro-oncology*, 17(1), 81-94.
- Karstenischer, B., Einem, J. V., Kaufer, B., & Osterrieder, N. (2006). Two-step Red-mediated recombination for versatile high-efficiency markerless DNA manipulation in Escherichia coli. *BioTechniques*, 40(2), 191-197.