# Targeting of CXCR4+ Glioblastoma Initiating Cells with a retargeted oncolytic herpesvirus

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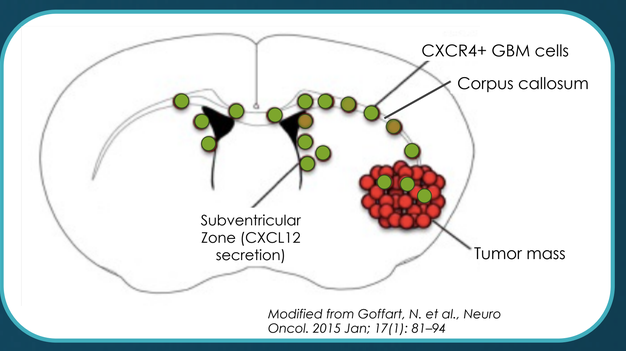
## Background

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. 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 (Goffart, N. et al., *Neuro-oncology*, 2014, *17*(1), 81-94). These cells are the only tumor cells able to initiate a tumor and thus show characteristics that identified them as Glioblastoma Initiating Cells (GIC). In addition, these cells, which are not removed with the surgical resection are more resistant to radiotherapy and are probably responsible for the recurrence.



*Modified from Goffart, N. et al., Neuro Oncol. 2015 Jan; 17(1): 81–94*

## Aim

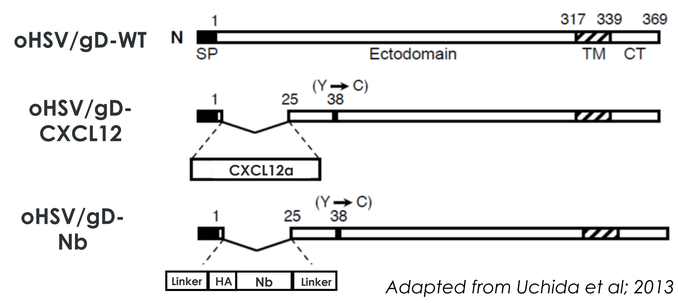
The aim of this project is to build an oncolytic HSV (oHSV) able to (1) target specifically the CXCR4+ GICs and to (2) induce their apoptosis.

## Results

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

An oHSV virus with a modified glycoprotein targeting CXCR4 has been created using the "en passant" technique described by Osterreider et al., 2006 (Karstentischer, Bet al., 2006, BioTechniques, 40(2),191-197):

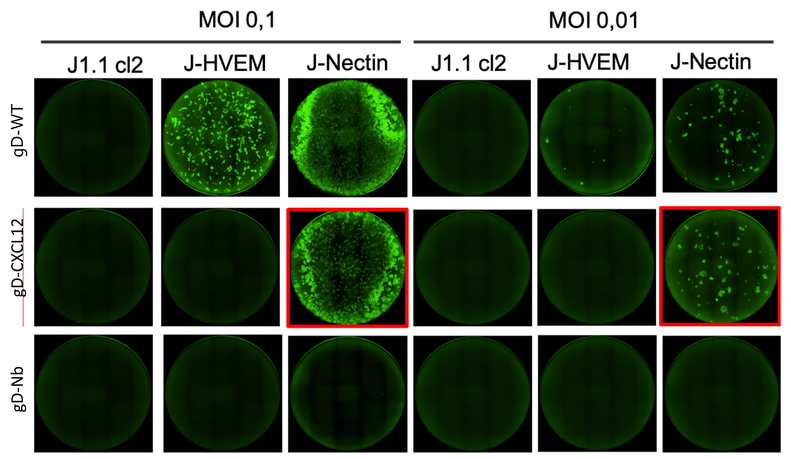
- The gD domain required for the interaction with the natural receptors HVEM and Nectin) has been deleted or mutated respectively and replaced by either CXCL12 or a anti CXCR4-nanobody.
- Tyr 38 important for the interaction with Nectin has been mutated into Cyst.



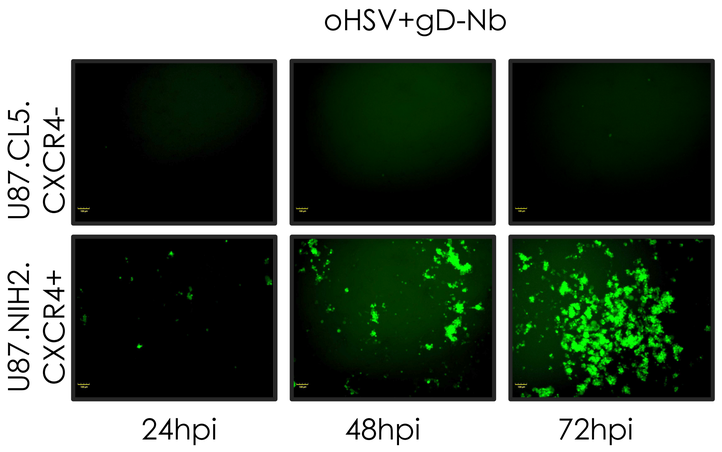
*Adapted from Uchida et al; 2013*

The correct de-targeting of the modified oHSVs have been evaluated by infecting J1.1 cells (HSV resistant) J-HVEM+ and J-Nectin+ cells (kind gift of G. Campadelli-Fiume) with a MOI of 1, 0,1 or 0,01 (72 hpi).

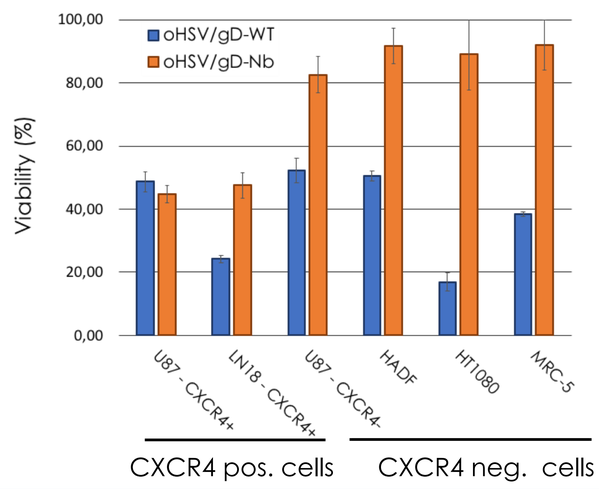
The oHSV/gD-Nb is efficiently detargeted while the gD-CXCL12 has reverted and is still able to infected Nectin+ cells




The capacity of oHSV/gD-Nb to infect only cells expressing CXCR4 has been analysed by infecting U87.CL5.CXCR4- or U87.NIH2.CXCR4+ and confirmed by qRT PCR (data not shown).

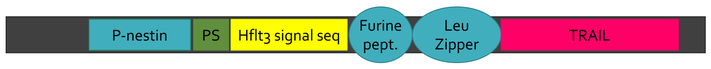



When compared with the gD-WT that can induce mortality in all cell lines, the retargeted virus induces mortality only in CXCR4+ cells indicating that the retargeting of oHSV allows to specifically infect CXCR4+ cells.



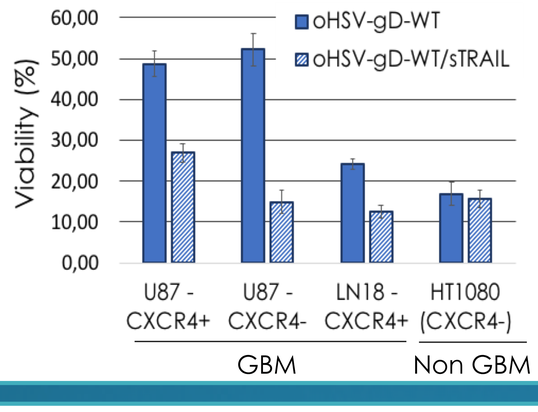

### 2. Arming: introduction of a transgene coding for a soluble form of TRAIL

A gene coding for a soluble form of TRAIL driven by the nestin promoter has been further introduced in oHSV/gD-Nb.

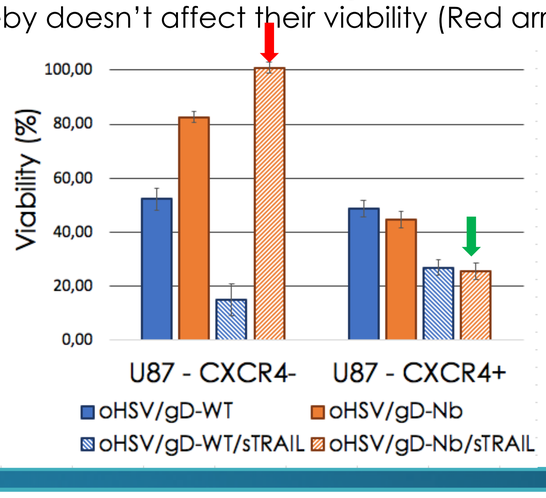


*Adapted from Khalid et al., 2004, 64: 3236-3242*

The specificity and efficacy of the armed oHSV was evaluated by quantification of the viability of cells 72hpi (MOI:1) (MTT assay). The viability of HT1080 in which the nestin promoter is not active is not improved by sTRAIL demonstrating the specificity of the promotor.




GBM cells viability is affected in GBM cells only if they are CXCR4 positive (Green arrow) while the virus could not infect GBM negative cells and thereby doesn't affect their viability (Red arrow)




## Conclusions

These preliminary results suggest that the retargeted oHSV expressing gD fused to a nanobody specific to CXCR4 works correctly, being able to specifically infect CXCR4+ GBM cells. When armed with pnestin-sTRAIL, It is able to induce apoptosis in GBM cells in which the nestin promoter is active. This virus is currently under characterisation in neurospheres and its safety and efficacy will be soon evaluated *in vivo* in an orthotopic xenograft model.