# Engineering Oncolytic Vaccinia Virus with Improved Cancer Killing Abilities

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## ABSTRACT

Although recent progress has been made in the field of virotherapy with the development of second generation armed oncolytic vectors, there is still a need to design new improved viruses that could overcome cancer cells less permissive to cytolysis.

To this aim a new generation of vaccinia virus able to inhibit host cell factor involved in cellular resistance was developed. As the replication of vaccinia virus (VACV) occurs in the cell cytoplasm, a small hairpin RNA-based knockdown strategy is not possible. Thus, the strategy was to design VACV expressing a soluble ScFv directed against host cellular proteins implicated in resistance to viral cytolysis. The ScFv was engineered to block the targeted cell factor by altering its cellular localization hence mimicking siRNA knockdown.

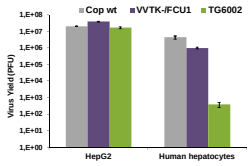
The identification of pathways and host cell genes which, when silenced with siRNAs, potentiate the cytopathic effect of a VACV specifically in a tumor cell line was performed by screening a siRNA library targeting the whole human genome in combination with VACV infection. The selection of the soluble ScFv is done by phage display, the feasibility of the relocalization strategy is evaluated and the oncolytic VACVs expressing ScFv will be generated.

In conclusion, we identified targetable key cellular components implicated in the mechanisms of resistance towards VACV cytopathic activity in tumor cells. Furthermore, we will generate new improved oncolytic VACV expressing soluble ScFv against these cellular factors.

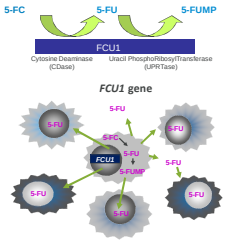
## ABOUT TG6002

### TG6002 = VVTK-RR-/FCU1

- VV: Vaccinia virus strain Copenhagen
- Deletion of *TK* and *RR* genes: attenuated replication in healthy Cells
- Express *FCU1* gene: combined therapy of oncolytic activity and targeted chemotherapy

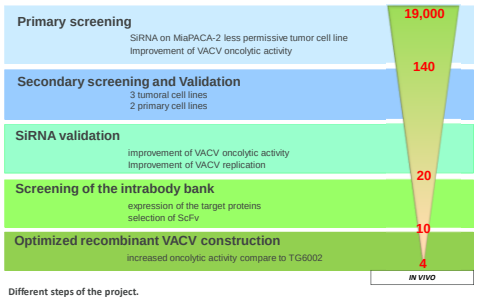



**Virus production of the different VV in tumor cells and primary normal cells.** Human hepatocarcinoma HepG2 cells and human hepatocytes were infected by VV wild type (Copwt), VVTK-/FCU1 (single deleted) and TG6002 (double deleted) at 100 pfu. Virus produced after 48 h was titrated by plaque assay.



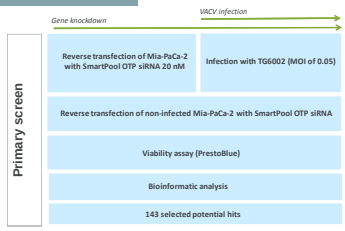

**Bystander effect in tumor**

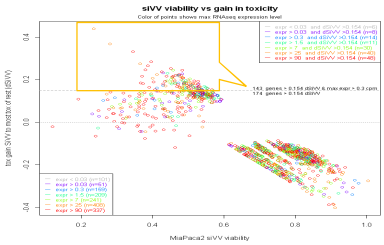
## Project summary




**Different steps of the project.**

## Primary screen

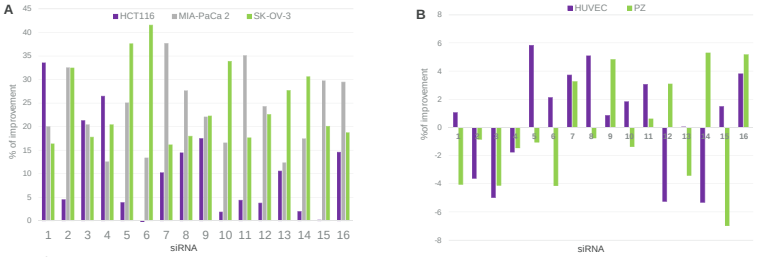



**Protocol and Results of the primary RNAi screen and selection of the hits.**
Results from RNAi screen were initially corrected for systematic zonal effects, normalized and filtered based on a statistical test of each combiantion TG6002 + siRNA against TG6002 alone, 1670 siRNAs were retained. Gain in toxicity (dSiVV) was determined by evaluating the distance of the combination siRNA + TG6002 measures to the most toxic of TG6002 or siRNA alone. In the graphical representation all points (siRNA) above dSiVV =0 have thus greater toxicity than TG6002 or siRNA alone. Finally, combination TG6002 + siRNA with low viability and high gain in toxicity (dSiVV) were selected in the upper left quadrant of the graphical representation using dSiVV > 0.154 as threshold. Points were colored according to transript abundance by RNA-Seq and transcripts with low expression levels (<0.3 cpm) finally discarded to yield 143 siRNA target genes.

## Secondary screen

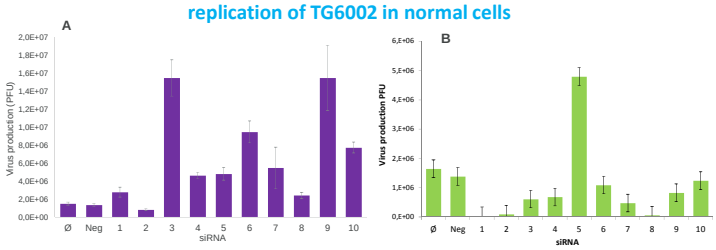
### The combination TG6002 infection and siRNA transfection displayed specific improvement of cytotoxicity in tumor cells




**Results of the secondary screen.**
Percentage of improvement in cytotoxicity of the combination of TG6002 and siRNA in comparison to the cytotoxicity of TG6002 or siRNA alone in tumor (A) or normal (B) cell lines. Cells were transfected by each siRNA (1 to 16) and then infected by TG6002. 72h after virus infection, cellular viability was evaluated by presto blue method. The results are represented as a percentage of improvement of cytotoxicity of the combination SiRNA and TG6002 versus siRNA or TG6002 alone.

## siRNA Validation

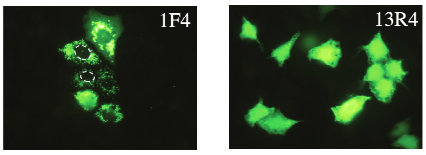
### The combination of TG6002 with target siRNA highly improved replication of TG6002 in tumor cells. The combination with the same SiRNA reduced replication of TG6002 in normal cells



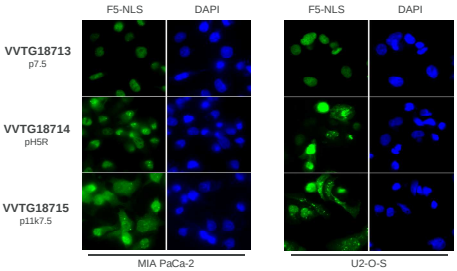

**Replication rate of the TG6002 alone or in combination with 10 siRNA selected in secondary screen in tumor and primary normal cell lines.**
Human tumor HCT116 (A) and human normal MRC5 (B) cell lines were first transfected by siRNA. 48h post-transfection, the cells were infected byTG6002 at a MOI of 0,0001. At 72h post infection, the plaques were frozen and virus titration was performed by plaque assay on CEF.

## Intrabodies expression by TG6002

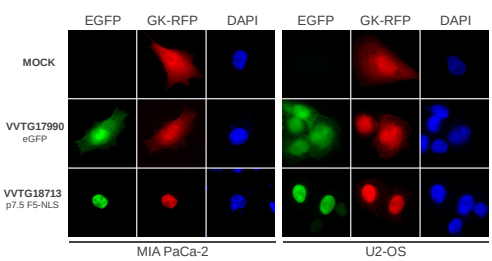
### TG6002 is able to express a soluble intrabody in tumor cells. The expressed ScFc is functional to bind specific protein and promotes the transport of the whole cytosolic fraction of gankyrin into the nucleus, suggesting that they are adequate for intrabody-mediated relocalization of target proteins.




**Intracellular expression of single-chain Fv molecules after transfection.**
HeLa cells were transfected with constructs that express the coding sequence of either scFv 1F4, cloned from a murine hybridoma, or scFv 13R4, a human scFv that was engineered to fold soluby in the absence of disulfide bridge formation. 48 post-transfection, the cells were fixed and the expressed scFv polypeptides were revealed with the anti-myc tag 9E10 antibody, followed by incubation with Alexa 488-labbelled anti-mouse rabbit globulins. FITC filter (green). Magnification: x 630




**Intracellular expression of the NLS-tagged scFv after infection with the scFv F5/NLS armed VACV.**
MIA PaCa-2 and U2OS cells were infected with recombinant virus at a moi of 0.01. After 24 hr, the cells were fixed and the expressed scFv molecules were revealed. Representative images of cells infected with the recombinant viruses VVTG18713, VVTG18714 and VVTG18715 are shown. These viruses harbor the scFv F5/NLS coding region under the control of the promoters p7.5, pH5R and p11k7.5, respectively. Nuclei were counterstained with DAPI (blue). Magnification: x 400




**Relocalization of ectopically-expressed gankyrin protein by scFv F5/NLS expressed by the VACV.**
MIA PaCa-2 and U2OS cells were transfected with a construct encoding the gankyrin protein tagged with the red fluorescent protein at the C-terminus (GK-RFP). 24 hr post-transfection, the cells were infected with the recombinant viruses VVTG17990 (encoding eGFP) and VVTG18713 as indicated. The subcellular distribution of GK -RFP and the expressed eGFP or scFv F5/NLS polypeptides were visualized using a fluorescent microscope, 24 hr after infection. Nuclei were stained with DAPI (blue). Magnification: x 630.

## CONCLUSION AND NEXT STEPS

**This project enables the identification of pathways and host cell genes which, when silenced with siRNAs, potentiate the cytopathic effect of TG6002 in tumor cell lines but not in primary cells. The selection of recombinant target relocalizing intrabodies mimicking siRNA knockdowns is ongoing. These intrabodies will contain a cellular localization signal that will relocate the target protein to cell compartments in which it will not be able to exert its function. A new generation of improved TG6002-derived VACVs expressing recombinant target relocalizing intra-solubodies will be generated. The therapeutic activity of these new viruses will be evaluated *in vitro* on cancer cell lines and *in vivo* in xenografted cancer mouse models.**