Oncolytic Vaccinia Virus expressing Cytidine Deaminase induces DNA damage and shows potent anti-tumor effects

J. Foloppe[1], J. Kempf[1], C. Pichon[1], A. Findeli[1], I. Farine[1], J. Hortelano[1], B. Moreau[1], M. Klein[1], S. Cochin[1], R. Brandely[1], V. Koerper[1], N. Silvestre[1], C. Machon[2], J. Guitton[2], E. Quémeneur[1] and P. Erbs[1]

[1] Transgene S.A., 400 boulevard Gonthier d'Andernach, Illkirch-Graffenstaden, 67405, France

[2] Hospices Civils de Lyon, Centre Hospitalier Lyon-Sud, Laboratoire de biochimie-toxicologie, Pierre Bénite, 69495, France

contact: erbs@transgene.fr

Abstract 4387
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Abstract

The recombinant Vaccinia virus Copenhagen strain deleted in Thymidine Kinase and Ribonucleotide Reductase (VVTK-RR-) is a potent and versatile oncolytic platform that has demonstrated strong activity in various preclinical models. The deletion of such VV genes inhibits viral replication in normal cells, while retaining its therapeutic replication in tumor cells. TG6002, a VVTK-RR- expressing the suicide gene *FCU1**, is under investigation in Phase I trials in patients with advanced gastrointestinal tumors (NCT03724071).

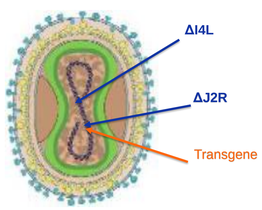
We have developed a new product based on the VVTK-RR- vector, named TG6010, expressing the human cytidine deaminase (hCD) that efficiently catalyzes the deamination of cytidine and deoxycytidine to uridine and deoxyuridine, respectively.

The tumor specific expression of hCD by the VV leads to a depletion of cytidine and deoxycytidine. This cytidine/deoxycytidine depletion resulting from hCD overexpression, activated a DNA damage response highlighted by an induction of γH2AX phosphorylation. Next, to validate the potential therapeutic use of TG6010, we analyzed the effects of the virus on human xenograft tumors implanted in mice. We observed, after systemic injection of TG6010, high expression of hCD in the tumors with a significant increase in DNA damage as revealed by the γH2AX foci assay. In addition, we observed that TG6010 significantly reduced tumor growth compared to control groups.

* Foloppe et al. The Enhanced Tumor Specificity of TG6002, an Armed Oncolytic Vaccinia Virus Deleted in Two Genes Involved in Nucleotide Metabolism. *Mol Ther Oncolytics*. 2019;14:1-14.

Our oncolytic virus platform

VVTK-RR-



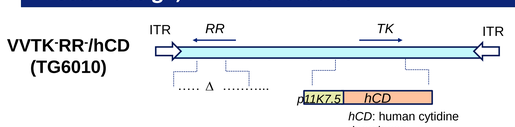
Characteristics of our oncolytic vaccinia virus

- VV: Vaccinia virus strain Copenhagen
- Deletion of *Thymidine kinase (J2R/TK)* and ribonucleotide reductase (*I4L/RR)* genes: attenuated replication in healthy cells

Advantages of oncolytic vaccinia virus

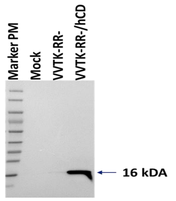
- Large spectrum of tumor types
- Good safety profile and high therapeutic index
- Pure cytoplasmic replication (no risk for genome integration or mutagenesis)
- Good immunological balance (Th1 vs Th2, anti-tumor vs anti-viral responses)
- Large genome capacity (up to 25 kb), accommodating multiple transgenes at different loci
- Well established processes for GMP manufacturing

Vector design, *in vitro* evaluation



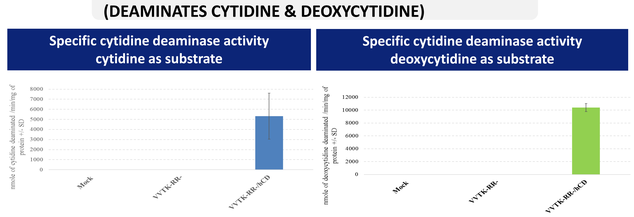
- Virus backbone similar to TG6002
- hCD inserted in the TK locus under the control of the vaccinia synthetic p11K7.5 promoter (strong promoter driving high transcription rate)
- VVTK-RR-/hCD express the expected 16-kDa hCD protein
- hCD expressed by VV is functional (deaminates cytidine and deoxycytidine)
- No negative effect on the in vitro oncolytic activity of VV

VVTK-RR-/HCD EXPRESS THE EXPECTED 16-kDa hCD PROTEIN



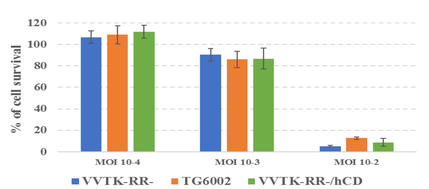
Western Blot shows specific expression of hCD in tumor cells infected by VVTK-RR-/hCD at MOI 10^{-1}

hCD EXPRESSED BY VV IS FUNCTIONNAL (DEAMINATES CYTIDINE & DEOXYCYTIDINE)



Results show high cytidine deaminase activity in tumor cells infected by VVTK-RR-/hCD at MOI 10-2

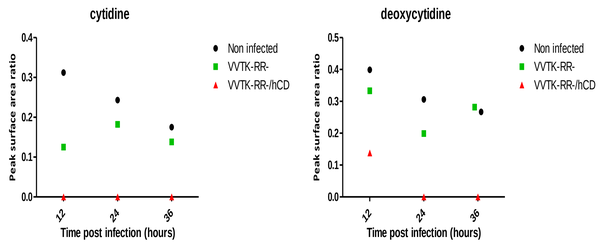
EXPRESSION OF hCD HAS NO NEGATIVE EFFECT ON THE *IN VITRO* ONCOLYTIC ACTIVITY OF VV



Results show equivalent viability of tumor cells infected by VVTK-RR- and VVTK-RR/hCD indicating that hCD expression does not affect the oncolytic activity of VV

In vitro measurements of endogenous pools of nucleosides

OVEREXPRESSION of hCD LEADS TO DEPLETION OF ENDOGENOUS CYTIDINE AND DEOXYCYTIDINE IN TUMOR CELLS

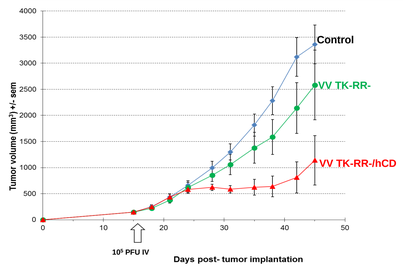


Results show a depletion of cytidine and deoxycytidine in tumor cells infected by VVTK-RR-/hCD at MOI 10^{-2}.

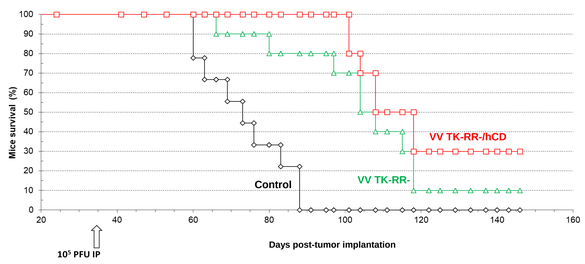
The determination of nuclosides was performed with a method based on an online extraction coupled with LC-MS/MS

Antitumor activity of VVTK-RR-/hCD (TG6010) in xenograft tumor models

HIGH EFFICIENCY OF VVTK-RR-/hCD IN XENOGRAFT TUMOR MODELS



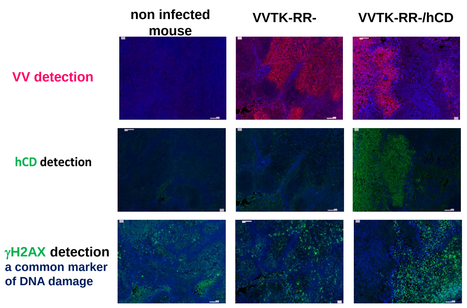
Antitumor activity of VVTK-RR-/hCD in a CRC subcutaneous xenograft model
VVTK-RR-/hCD was injected I.V. at 1.10^6 PFU (arrow) in nude mice bearing HCT116 human tumors implanted subcutaneously. Graph represents the mean tumor volume ± sem from 12 mice/group.
I.V. injection of VVTK-RR-/hCD significantly improves the antitumoral effect compared to the VVTK-RR- treated mice.



Antitumor activity of VVTK-RR-/hCD in a peritoneal carcinomatis xenograft model
VVTK-RR-/hCD was injected I.P. at 1.10^5 PFU (arrow) in nude mice bearing SK-OV-3 human tumors implanted intra peritoneally. Graph represents average survival of 10 mice/group..
I.P. injection of VVTK-RR-/hCD significantly improves the survival of animals compared to the VVTK-RR- treated mice.

Evaluation of DNA damage in xenograft tumors

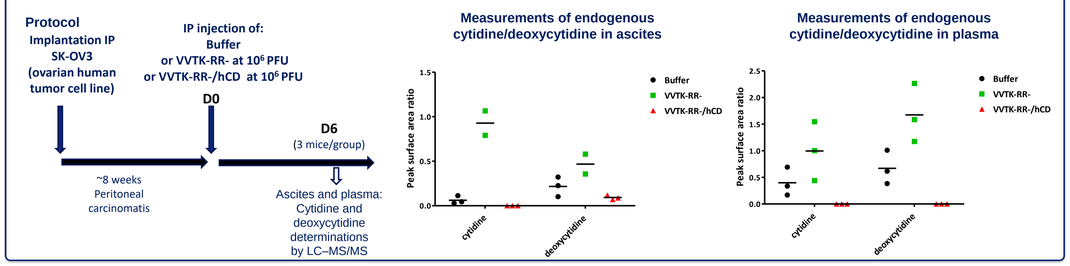
VVTK-RR-/hCD INDUCES DNA DAMAGE IN TUMOR CELLS (INCREASE OF γH2AX POSITIVE CELLS AFTER VVTK-RR-/hCD INFECTION)



Single IV injection of VV at 10^7 PFU in subcuteaous xenograft model (HCT116). Immunohistochemistry on tumors 4 days post infection.

Measurements of endogenous pools of nucleosides in peritoneal carcinomatis xenograft model

IP INJECTION OF VVTK-RR-/hCD INDUCES A DEPLETION OF CYTIDINE/DEOXYCYTIDINE IN ASCITES AND IN PLASMA



Conclusion

VVTK-RR- (Copenhagen strain) is a potent and versatile oncolytic platform that has demonstrated strong specificity and antitumoral activity in various preclinical models after systemic injection. TG6010 (VVTK-RR-/hCD) showed a specific expression of human cytidine deaminase (hCD) in the tumor.

Overexpression of hCD leads to a depletion of cytidine/deoxycytidine and an increase of γH2AX positive tumor cells was observed after TG6010 infection compared to non injected and VVTK-RR- (empty) infected mice. TG6010 displays enhanced tumor growth control in xenograft models.

In conclusion, an oncolytic vaccinia virus expressing the cytidine deaminase has shown potent anti-tumor effects both *in vitro* and *in vivo*. Mechanistically, due to the cytidine deaminase overexpression, we observed induction of a DNA damage response.