TG7010: a Novel Chimeric Oncolytic Poxvirus Encoding IL-12 with Improved Properties for Systemic Delivery

***J. Foloppe*, J. Kempf, L. Gault, I. Farine, J. Hortelano, B. Moreau, M. Klein, S. Cochin, E. Quéméneur and P. Erbs**

Oncolytic Virus Department, Research & Innovation, Transgene SA, Illkirch-Graffenstaden, France

international oncolytic virus conference
rotterdam | the netherlands 2024

BACKGROUND

Oncolytic virotherapy has emerged as a novel cancer therapeutic approach with the potential to be more effective and less toxic than current therapies due to the selective growth and amplification of the virus in tumor cells. Various types of oncolytic viruses (OVs) in clinical development, including Vaccinia virus-derived OVs, have shown good safety profiles, but have generally failed to achieve the expected therapeutic value as monotherapies. Consequently, new approaches to generate powerful oncolytic viruses are needed. We used a new directed evolution process, pooling several orthopoxvirus species to create a highly potent oncolytic chimeric poxvirus, named PoxSTG.

Compared to classical oncolytic vaccinia virus (VACV), PoxSTG demonstrates superior tumor lytic capacity, higher dissemination in tumors and greater resistance to humoral immunity. Armed with IL-12, a pleiotropic and potent cytokine involved in the activation of natural killer and T cells, PoxSTG-IL12, named TG7010, showed potent antitumor effects in several syngeneic and xenograft mouse models. Furthermore, IV injection of TG7010, in combination with anti-PD-1 treatment, exerted significant antitumor effects leading to tumor rejection. All these data demonstrate the potential of TG7010 as a novel therapeutic agent for cancer treatment.

TG7010 WAS GENERATED BY DIRECTED EVOLUTION

The directed evolution strategy employed to generate chimeric VACV comprised two steps:

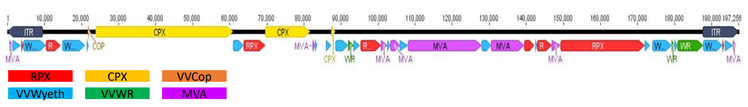
1. a library of viruses was generated by co-infecting human cancer cell line with 16 Poxviruses.
2. amplification of viral progeny under stringent conditions towards clonal isolation of virus candidates was performed by seven successive passages on human tumor cells.

From the last passage, 48 individual plaque-purified viruses were isolated and screened for their oncolytic potential on tumor cell lines. We selected one of the clone, designated PoxSTG, having superior oncolytic activity.

Erbs P et al. "PoxSTG, a novel chimeric poxvirus with improved oncolytic potency." *Cancer Research* 83.7_Supplement (2023): 6796-6796

TG7010 IS A CHIMERIC POXVIRUS

Sequence analysis of POXSTG revealed that the virus genome is derived from Cowpox virus (CPX), Rabbitpox virus (RPX) and four strains of Vaccinia virus Copenhagen, Western Reserve, Wyeth, MVA)

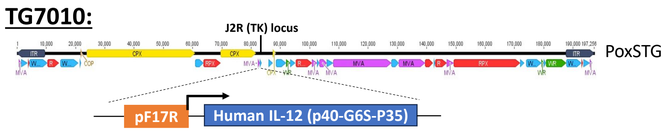


Homology maps between PoxSTG and each parental genome
PoxSTG genome annotated with results from global pairwise alignments.

Erbs P et al. "PoxSTG, a novel chimeric poxvirus with improved oncolytic potency." *Cancer Research* 83.7_Supplement (2023): 6796-6796

TG7010 IS ARMED WITH INTERLEUKIN-12

TG7010:

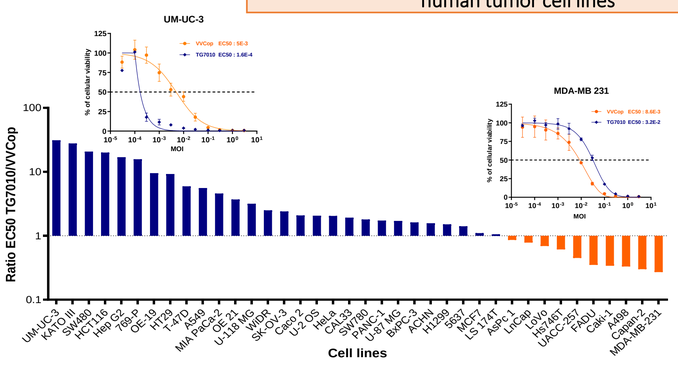


IL-12 exhibits antitumor effect through pleiotropic activities:

- Activates B and T lymphocytes and NK cells
- Increases IFN-γ production
- Increase PD-L-1 expression in tumor cells
- Restore antitumor immunity → shift cold to hot tumors
- Reduce tumor immunosuppressive actvity

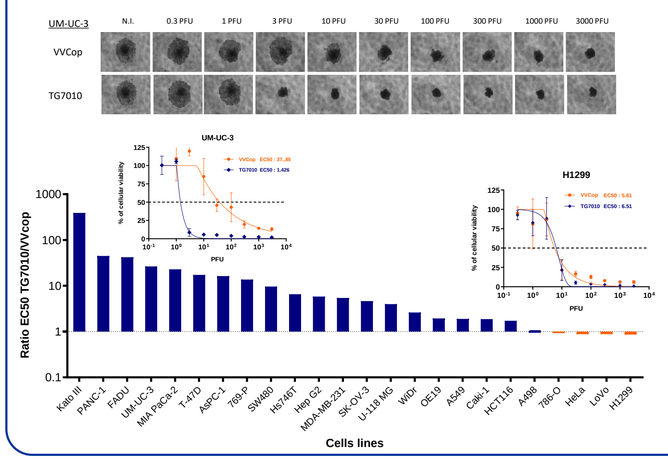
TG7010 DEMONSTRATES IMPROVED ONCOLYTIC PROPERTIES

TG7010 demonstrates better oncolytic activity in a panel of human tumor cell lines



Oncolytic activity assay in human tumor cell lines. Tumor cells were infected at 10 different MOIs (from 10-5 to 1) and cell viability was determined 4 days later using cell titer blue cell viability assay. The EC50 was calculated for each virus using Graphpad prism software and the ratio between the EC50 obtained with TG7010 and VVcop was represented.

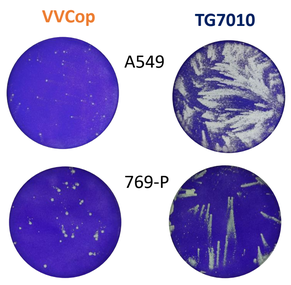
TG7010 displays better antitumor activity in 3D human tumor spheroid model



Antitumor activity assay in spheroid model of human tumor cell lines. Tumor cells lines were incubated at 2000 cells/well in an ultra low attachment 96 wells plate. Four days after cell inoculation, the 3D tumor cells were infected at 9 different virus quantities (0.3 to 3000 PFU). Seven days later, pictures of each well were taken, and cell viability was determined using cell titer blue cell viability assay. The EC50 was calculated for each virus using Graphpad prism software and the ratio between the EC50 obtained with TG7010 and VVcop was represented.

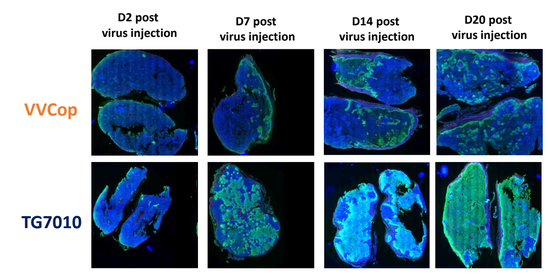
TG7010 DISPLAYS SIGNIFICANT ENHANCED SPREADING IN TUMOR DUE TO HIGH LEVEL OF EEV PRODUCTION AND SYNCITIA FORMATION

TG7010 displays a significantly improved *in vitro* spreading



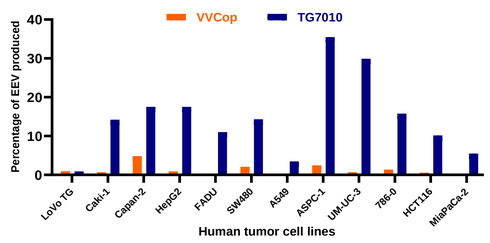
***In vitro* spreading (comet assay)**. Monolayers of A549 and 769-P human tumor cell were infected by the indicated viruses. After 2 days, cells were stained with crystal violet.

TG7010 shows enhanced spreading in human tumors



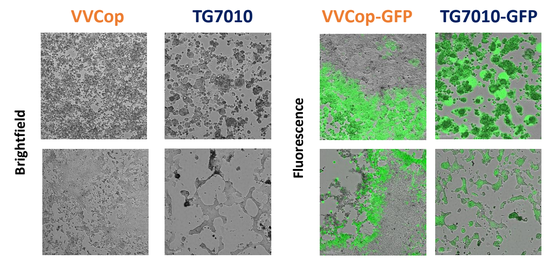
Virus detection in tumor xenografts. Immunofluorescence in HCT116 xenograft tumors implanted subcutaneously. Immunofluorescence of the tumors was performed 2, 7, 14 and 20 days after a single intravenous injection of the indicated virus at 1 X 1E+05 PFU. Cellular DNA was stained in blue with DAPI and viruses were stained in green.

TG7010 produces a large amount of EEV in human tumor cell lines allowing enhanced tumor spreading



EEV production in human tumor cell lines. Production of EEV form at early times after infection of tumor cell line monolayer. Cells were infected with the indicated viruses. Supernatant or cell fraction were collected at 24-hours post-infection. Virus titers from the supernatants alone (EEV) and from both supernatants and cells (IMV + EEV, total progeny virus) at 24 hours were determined and represented.

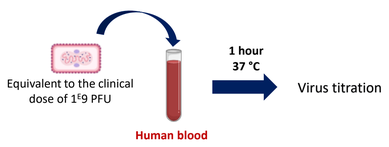
TG7010 induces syncytia formation in tumor cell enabling increasing virus spreading within tumor

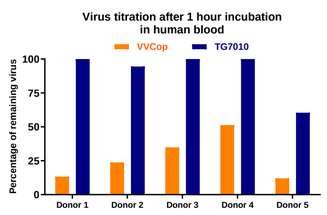


Syncitia formation. Monolayers of A549 and HCT116 human tumor cell were infected by the indicated viruses at MOI 1E-02. After 2 days, infected cells were visualized under a fluorescence microscope. Photographs representing the morphologies of cells infected with either VVCop or TG7010 were taken in Brightfield and under fluorescent conditions.

TG7010 EXHIBITS ENHANCED FEATURES FOR IV DELIVERY

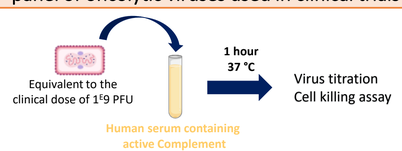
TG7010 is more persistent in human blood

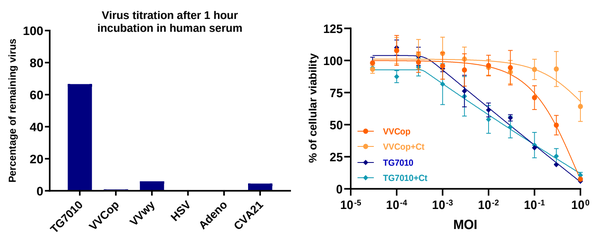




Impact of human blood on virus stability. Viruses were incubated for 1 hour at 37 °C in PBS or in blood from different donors at 2E+05 PFU/mL, corresponding to the clinical dose of 1E+09 PFU. The quantity of remaining infectious virus was determined using titration assay by plaque assay method. Results are represented for each virus as the percentage of infectious virus after incubation in blood versus PBS.

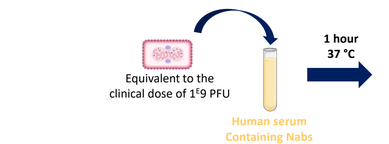
TG7010 is more resistant to complement inhibition than a panel of oncolytic viruses used in clinical trials

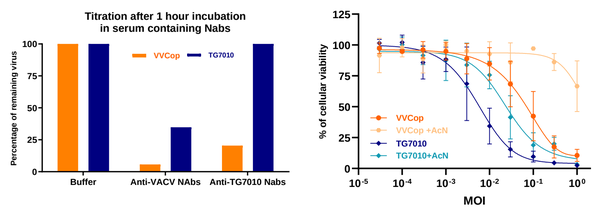




Impact of human complement on virus inhibition. Viruses were incubated for 1 hour at 37 °C in PBS or in human serum containing active complement at 2E+05 virus/mL, corresponding to the clinical dose of 1E+09 PFU. A. The quantity of remaining infectious virus was determined using titration assay. Results are represented for each virus as the percentage of infectious virus after incubation in blood versus PBS. B. After incubation, A549 human tumor cell line were infected in suspension with 10 different MOI of each virus in different conditions. Four days later, cell viability was determined using cell titer blue cell viability assay. The results were represented as the percentage of cellular viability.

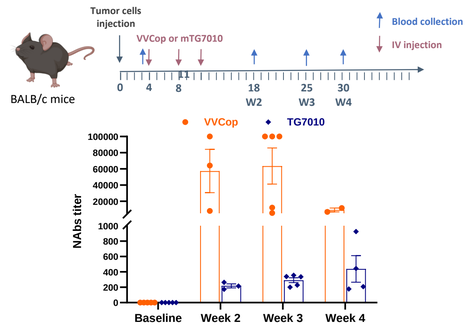
TG7010 is less inhibited by Nabs compared to VVCop





Impact of Nabs on virus inhibition. Viruses were incubated for 1 hour at 37 °C in PBS or in serum containing NAbs at 2E+05 PFU/mL, corresponding to the clinical dose of 1E+09 PFU. A. The quantity of remaining infectious virus was determined using titration assay. Results are represented for each virus as the percentage of infectious virus after incubation in blood versus PBS. B. After incubation, A549 human tumor cell line were infected in suspension with 10 different MOI of each virus in different conditions. Four days later, cell viability was determined using cell titer blue cell viability assay. The results were represented as the percentage of cellular viability.

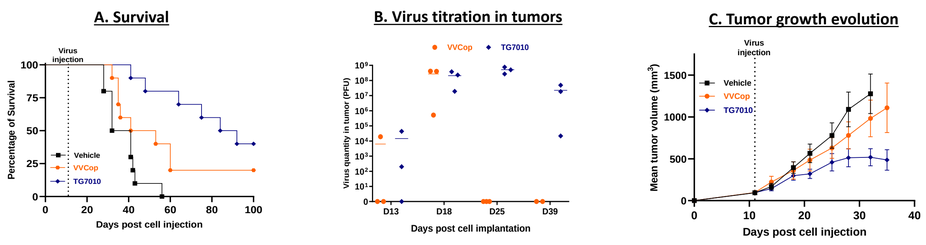
TG7010 is less prone to induce Nabs production in immunocompetent mice



Titer quantification of NAbs produced in immunocompetent mice. Viruses (3E+06 PFU) were IV injected in BALB/c mice at 4, 8 and 11 days after subcutaneous implantation of CT26 tumor cells. Blood was collected at baseline, one day before first virus injection, and 2, 3 and 4 weeks after first virus injection by tail vein sampling using capillaries. Blood samples were stored at -80 °C until NAbs titer determination. NAbs titers were determined by neutralization assay using 2E+03 Pfu of virus. The NAbs titer was defined as the dilution factor of the highest blood dilution resulting in a 50% reduction in the number of plaques. Nabs titers were calculated using Graphpad Prism software.

TG7010 IS EFFECTIVE AND PERSISTENT IN HUMAN TUMOR

TG7010 shows enhanced antitumor activity and persistence in human tumor inducing tumor growth inhibition



Therapeutic activity in human colorectal HCT116 xenograft model. Viruses were injected I.V. at 1x1E+05 PFU in nude mice bearing HCT116 human tumors implanted subcutaneously. (n = 10 per group). (A) Kaplan-Meier survival analysis. (B) Virus titer in tumors at days 13, 18, 26 and 38 post-cell injection (C) Tumor growth dynamics. (D). Graph represents the mean tumor volume ± sem from 10 mice/group.

KEY MESSAGES

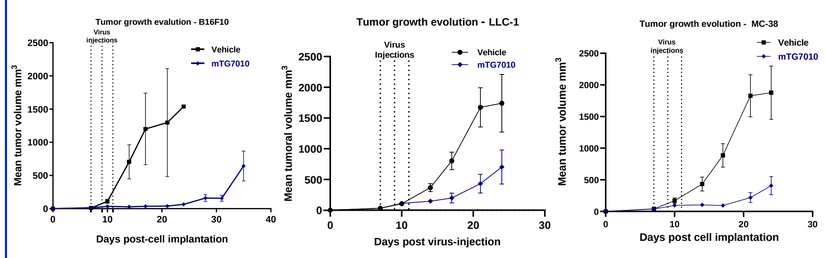
TG7010 is a chimeric oncolytic poxvirus encoding IL-12 with improved anticancer properties

- TG7010 shows superior spreading within tumors and high oncolytic potency
- TG7010 is adapted for IV delivery with reduced neutralization by complement and Nabs and persistence in human blood
- TG7010, in combination with anti-PD-1 treatment, demonstrated synergistic effect leading to complete tumor shrinkage after IV injection

TG7010 DISPLAYS SIGNIFICANT ANTITUMOR ACTIVITY ENHANCED BY ANTI-PD-1 TREATMENT

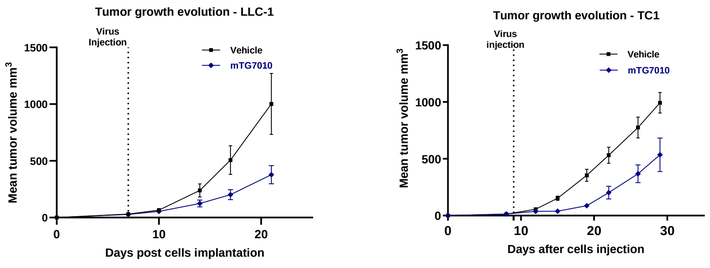
TG7010 displays significant reduction of tumor growth after IT injection in several syngeneic models

A



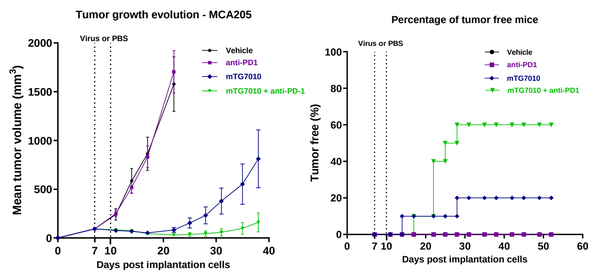
TG7010 is effective in reducing tumor growth after one single IV injection

B



C

TG7010 demonstrates high efficacy after IV injection in MCA205 syngeneic model, enhanced by anti-PD-1 treatment, bringing the percentage of tumor-free mice to 60%.



Therapeutic activity of TG7010 in syngeneic model. Viruses were injected in immunocompetent mice bearing murine tumors implanted subcutaneously (n = 10 per group).

(A) mTG7010 (TG7010 surrogate encoding murine IL-12) was injected I.T. at 1E+07 PFU at days 7, 9 and 11 after cell implantation. Graph represents the mean tumor volume ± sem from 10 mice/group.

(B) mTG7010 was injected once IV at 1E+07 PFU at day 7 or 9 post-cell injection for LLC-1 or TC1 model respectively. Graph represents the mean tumor volume ± sem from 10 mice/group.

(C) mTG7010 was injected I.V at 1E+07 PFU at days 7 and 10 after cell implantation. Treatment by anti-PD-1 antibody was injected I.P. at 250 µg/mice at days 4, 8 ,11, 14, 18 and 22 post first virus injection. Graph on the left represents the mean tumor volume ± sem from 10 mice/group. The graph on the right represents the percentage of tumor free mice from 10 mice/group.

international oncolytic virus conference
rotterdam | the netherlands 2024

Presented at the 16th International Oncolytic Virotherapy Conference 2024
Rotterdam, 27-30 October 2024