PoxSTG, a novel chimeric poxvirus with improved oncolytic potency

Philippe Erbs, Annie Findeli, Johann Foloppe, Juliette Kempf, Isabelle Farine, Baptiste Moreau, Julie Hortelano, Christelle Pichon, Jules Deforges, Eric Quemeneur

Transgene SA, Illkirch-Graffenstaden, France

AACR 2023
Abstract # 6796

Download the poster here

ABSTRACT

Oncolytic virus (OV) therapy has emerged as a promising approach for cancer treatment with the potential to be less toxic and more efficient than classic cancer therapies. Various types of OVs in clinical development, including Vaccinia virus (VACV)-derived OVs, have shown good safety profiles, but limited therapeutic efficacy as monotherapy in some cancer models. We used a directed evolution process, pooling multiple species of poxviruses to generate chimeric poxviruses with increased oncolytic properties. Through selective pressure by successive passages on human tumor cells, a new chimeric oncolytic poxvirus, named PoxSTG, was derived. The chimeric viral genome contains, in addition to sequences from several strains of Vaccinia virus, sequences of Rabbitpox virus and Cowpox virus. Compared with its parental viruses, PoxSTG has demonstrated superior oncolytic characteristics, and was notably attenuated in normal primary cells. PoxSTG has superior secretion of extracellular-enveloped virus (EEV) compared to all parental strains inducing higher dissemination of the virus into the tumors and more resistance to neutralization. PoxSTG was saved and showed potent antitumor effects in several syngeneic and xenograft mouse models of cancers. Furthermore, PoxSTG exerted low-dose antitumor effects in virus-injected and non-virus-injected distant tumors in a CRC xenograft model, demonstrating strong virus spread to distant tumors.

Directed evolution for selection of chimeric Poxvirus

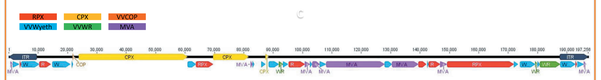
The directed evolution strategy employed to generate chimeric VACV comprised two steps:

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 one of the clone, designated **PoxSTG**, we have obtained a superior oncolytic activity.

PoxSTG is a chimeric Poxvirus

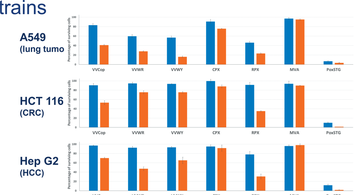
Sequence analysis of POXSTG revealed that the virus genome is derived from Cowpox virus (CPX), Rabbitpox virus (RPX) and four strains of Vaccinia virus (Copenhagen, Western Reserve, Wyeth, MVA)



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

PoxSTG shows superior oncolytic activity

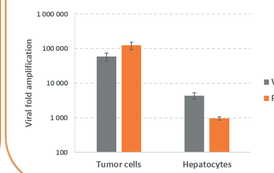
PoxSTG showed a higher oncolytic activity than parental strains



Oncolytic effect of PoxSTG on human tumor cells. Tumor cells were infected at 2 MOIs (10^{-5} blue and 10^{-4} orange) and cell viability was determined 4 days later. The 6 parental orthopoxviral strains were used as reference.

PoxSTG shows increased attenuation in normal cells

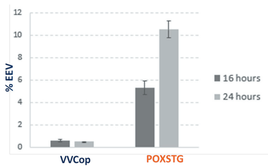
Compared to Vaccinia Virus (VVcop), PoxSTG produced more viral particles in tumor cells (HCC) but demonstrated a reduced replication on primary cells (hepatocytes).

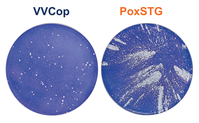


Replication in tumor cells and in primary human cells. HepG2 tumor cells were infected at MOI 10^{-5} and harvested 3 days post infection. Human primary hepatocytes were infected at MOI 10^{-4} and harvested 3 days post infection. Results are expressed as viral fold amplification (corresponding to output/input ratio).

PoxSTG produces more EEV and form comets

PoxSTG produced large amounts of EEV (extracellular-enveloped virus) and displayed enhanced *in vitro* spreading (comets)





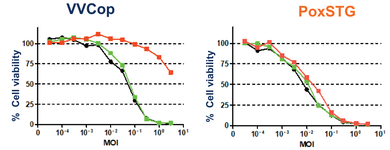
Comet assays. The indicated viruses were plated on monolayers of human tumor cell. After 2 days, cells were stained with crystal violet

Production of EEV form at early times after infection of a A549 monolayer. A549 cells were infected with the indicated viruses. Supernatant or cell fraction were collected at 16- and 24-hours post-infection. Virus titers from the supernatants alone (EEV) and from both supernatants and cells (IMV + EEV, total progeny virus) at 16 hours and 24 hours were determined. Ratio of EEV at 16- and 24-hours post-infection are presented

- **EEV is important for the efficient dissemination of the virus**
- **EEV is resistant to neutralization by antibody. It is also resistant against complement activity**

PoxSTG escapes neutralization by immunized serum

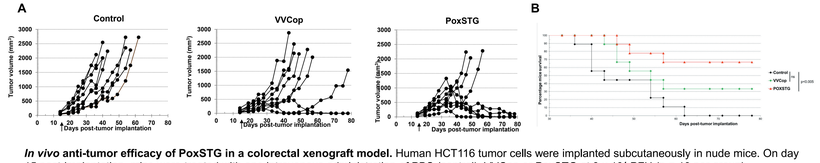
In vitro activity of PoxSTG despite neutralizing anti-VACV antibodies



Neutralization assay. Viability of human tumor cells infected by VV or PoxSTG in presence of patient-derived serum collected after IV administration of VVCop (immunized serum). Condition without serum (control) and condition using serum collected from the same patient before administration of VVCop (pre-immunized serum) were used as negative controls.

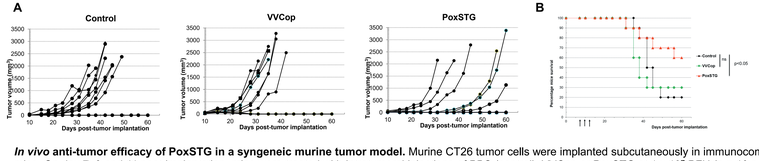
PoxSTG shows enhanced oncolytic potency in various tumor mouse models

PoxSTG showed potent antitumor effects in xenograft and syngeneic mouse models of cancers



***In vivo* anti-tumor efficacy of PoxSTG in a colorectal xenograft model.** Human HCT116 tumor cells were implanted subcutaneously in nude mice. On day 15 post-implantation, mice were treated with one intravenous administration of PBS (control), VVCop or PoxSTG at 3 x 10^4 PFU (n = 10 per group).

(A) Tumor growth dynamics of individual mice. (B) Kaplan-Meier survival analysis.

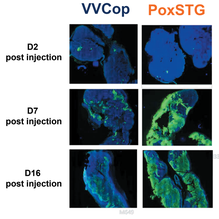


***In vivo* anti-tumor efficacy of PoxSTG in a syngeneic murine tumor model.** Murine CT26 tumor cells were implanted subcutaneously in immunocompetent mice. On day 7, 9 and 11 post-implantation, mice were treated with intratumoral injections of PBS (control), VVCop or PoxSTG at 1 x 10^7 PFU (n = 10 per group).

(A) Tumor growth dynamics of individual mice. (B) Kaplan-Meier survival analysis.

PoxSTG shows enhanced capacity for tumoral spread

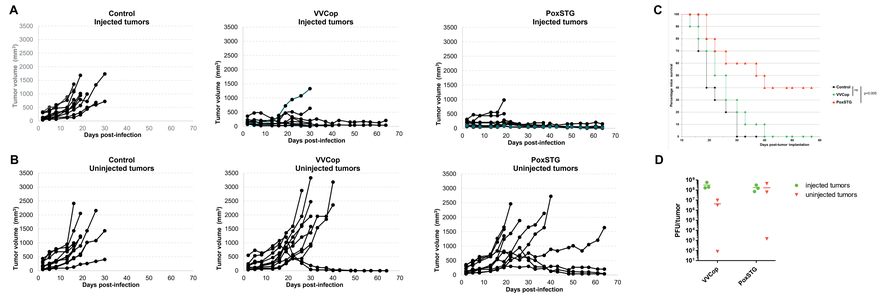
PoxSTG displayed enhanced tumor spreading



Viral immunostaining in HCT116 xenograft tumors implanted subcutaneously. Immunostaining of the tumors performed 2, 7 and 16 days after a single intravenous injection of the indicated virus at 1 x 10^5 PFU. Cellular DNA was stained in blue with DAPI and virus was stained in green .

PoxSTG efficiently spreads to distant tumors in a bilateral flank xenograft mouse model

PoxSTG was able to disseminate to distant unijected tumors



***In vivo* anti-tumor efficacy of PoxSTG in a bilateral flank xenograft mouse model.** Subcutaneous HCT116 xenografts were injected intratumorally with 1 x 10^6 PFU of VVCop, PoxSTG or PBS (control) only in the right-sided tumors (n = 10 per group). (A) Tumor growth dynamics of injected tumors. (B) Tumor growth dynamics of uninjected tumors. (C) Kaplan-Meier survival analysis. (D) Virus titer in injected and uninjected tumors at days 13 post-virus injection.

KEY FINDINGS

PoxSTG is a chimeric oncolytic poxvirus with improved anticancer activity

- ✓ PoxSTG shows superior oncolytic potency accompanied by increased attenuation in normal cells
- ✓ PoxSTG shows superior viral spread and viral load in infected tumors
- ✓ PoxSTG is less sensitive to neutralizing antibodies