PoxSTG-EEV: a Novel Enveloped Virion–Based Formulation of a Chimeric Oncolytic PoxVirus Adapted for Systemic Delivery

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Johann Foloppe, Juliette Kempf, Michèle Klein, Julie Hortelano, Baptiste Moreau, Laurine Gault, Isabelle Farine, Philippe Erbs

Transgene S.A., Illkirch-Graffenstaden, France

BACKGROUND

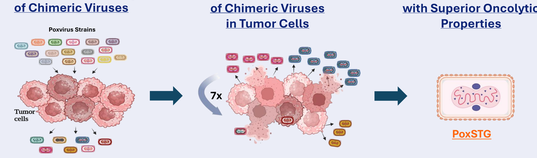
- Systemic delivery remains a major challenge for oncolytic virotherapy due to rapid neutralization by complement and antiviral antibodies.
- PoxSTG is a tumor-selective chimeric poxvirus with enhanced intratumoral dissemination, high EEV production capacity, and superior oncolytic potency relative to vaccinia virus (VACV)*.
- We developed **PoxSTG-EEV**, an EEV-enriched formulation optimized for systemic delivery.
- Here, we report the development, characterization, immune evasion properties, and systemic antitumor efficacy of **PoxSTG-EEV.**

METHODS

Generation of a Library of Chimeric Viruses

Specific Enrichment of Chimeric Viruses in Tumor Cells

Selection of Chimeric Variant with Superior Oncolytic Properties



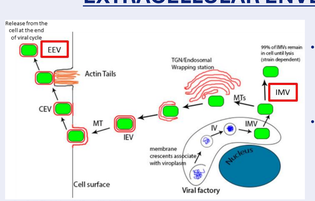
PoxSTG Produces Drastically Higher Levels of EEV Particles

Development of a Novel Manufacturing Process for the Production of an EEV-Based Oncolytic Virus Candidate



PoxSTG-EEV

EXTRACELLULAR ENVELOPED VIRION



From Smith GL et al. Journal of General Virology. 2002

- **IMV**: The **I**ntracellular **M**ature **V**irion is released following cell lysis and constitutes the most abundant viral form
- **EEV**: **E**xtracellular **E**nveloped **V**irion, a poxvirus-specific viral form released at the end of the viral replication cycle and coated with an outer membrane derived from the host cell.

EEVs possess a host-derived membrane that promotes immune evasion and facilitates systemic dissemination.

** Foloppe et al. A chimeric oncolytic poxvirus overcomes barriers to systemic cancer virotherapy. Research Square. 2026. doi:10.21203/rs.3.rs-10348774/v1*

DEVELOPMENT OF A NOVEL METHOD FOR EEV PARTICLE ISOLATION, PURIFICATION, AND QUANTIFICATION

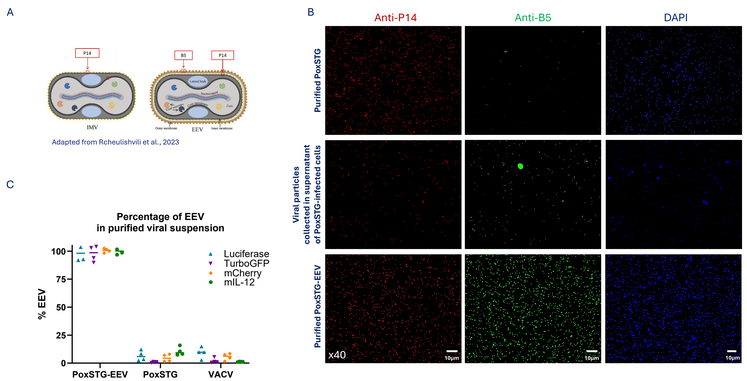


Figure 1: Production, purification and quantification of EEV particles. (A) Schematic representation of IMV and EEV, highlighting the localization of the viral proteins P14 and B5 used as immunostaining markers. (B) Representative immunofluorescence images of purified PoxSTG, viral particles present in the supernatant of PoxSTG-infected cells collected 24 h post-infection (MOI = 0.1), or purified PoxSTG-EEV. Viral particles were stained with anti-P14 and anti-B5 antibodies and counterstained with DAPI. (C) Percentage of EEVs in the different viral preparations, determined by quantifying B5-positive (EEV) particles relative to the total number of DAPI-positive viral particles using ImageJ software.

EEV CONFERS RESISTANCE TO COMPLEMENT AND NEUTRALIZING ANTIBODIES

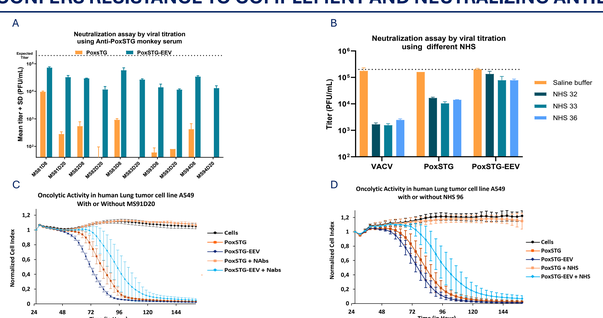


Figure 2: Impact of complement and neutralizing antibodies on PoxSTG viral activity. Viral preparations (2 × 10^5 PFU/mL) were incubated for 1 h at 37°C in PBS or in immune monkey sera (MS) containing various titers of anti-PoxSTG neutralizing antibodies (Nabs) (A and C) or in 50 % human normal serum (NHS) containing active complement (B and D). A and B: Residual infectious virus was quantified by plaque titration assay. Results are expressed as the titer of non-neutralized virus (PFU/mL). C and D: Viral neutralization mediated by anti-PoxSTG Nabs (C) or NHS (D) or was further evaluated using the xCELLigence real-time cell analysis (RTCA) system. Cell proliferation and virus-induced cytotoxicity were continuously monitored for 6 days. Results are expressed as the normalized Cell Index over time.

PoxSTG-EEV ACHIEVES ANTITUMOR EFFICACY FOLLOWING INTRAVENOUS ADMINISTRATION

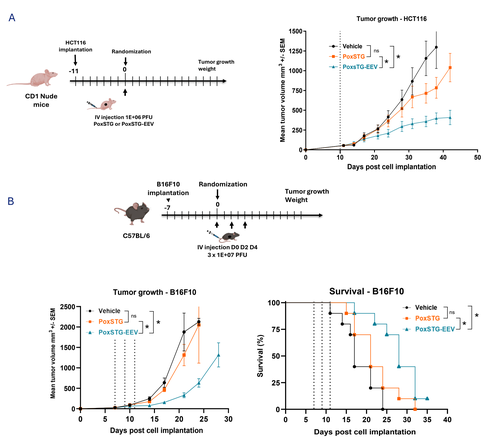
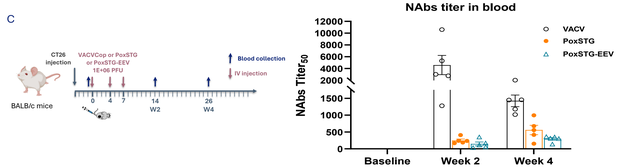
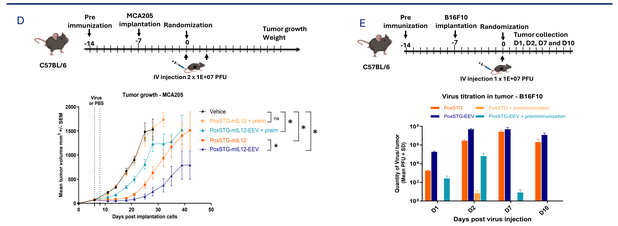


Figure 3: Therapeutic Activity and Tumor Targeting Following Systemic Administration. (A, B, C, D and E) Schematic representation of the experimental design for each study, including the mouse strain, tumor cell line, treatment dose, and administration schedule. For tumor growth curves, statistical significance was assessed at each time point using pairwise two-sided Mann-Whitney U tests. Asterisks indicate statistical significance based on unadjusted p-values for panel A and on p-values adjusted for multiple comparisons for panels B and D. The statistical results were further supported by a quadratic mixed-effects model including day, day^2 and their interactions with treatment group. Kaplan-Meier survival curves showing overall survival; statistical significance was evaluated using the log-rank test. (C) NAb titers were determined by a plaque reduction neutralization assay using 2 × 10^3 PFU of virus. Titers were defined as NT50 values correspond to the serum dilution achieving 50% neutralization, determined by 5-parameter logistic regression (E) Viral titers in B16F10 tumors were quantified at different time points following intravenous administration of PoxSTG or PoxSTG-EEV in either naive or pre-immunized mice. Data are presented as mean ± SEM (n = 3 tumors per time point). ns: not significant.

PoxSTG-EEV: ENHANCED IMMUNE EVASION THROUGH REDUCED IMMUNOGENICITY AND HUMORAL ANTIVIRAL RESPONSES



PoxSTG-EEV RETAINS ANTITUMOR EFFICACY DESPITE ANTIVIRAL NEUTRALIZING ANTIBODIES AFTER IV ADMINISTRATION



PoxSTG-EEV

PoxSTG

Efficient tumor-selective chimeric backbone

- Improved cytotoxicity versus conventional OV
- Efficient amplification and spread throughout the tumor mass
- Optimized dissemination from one tumor site to another
- Enhanced capacity to produce EEV particles
- Payload-flexible platform

&

EEV

Enriched enveloped formulation

- Resistance to neutralization by NAbs & the complement system
- A “stealth” virus that limits the action & generation of neutralizing antibodies
- Improved IV antitumor activity
- An innovative process for producing systemically deliverable EEV-based viral products

PoxSTG-EEV COMBINES POTENT TUMOR-SELECTIVE ONCOLYSIS WITH EEV-MEDIATED IMMUNE EVASION, ENABLING EFFECTIVE INTRAVENOUS DELIVERY AND SUSTAINED ANTITUMOR ACTIVITY DESPITE ANTIVIRAL HUMORAL IMMUNITY