Extracellular vesicles (EV) - mediators of therapeutic vaccination?

In vivo and in vitro characterization of EVs generated after infection of human and murine cells with therapeutic poxviruses

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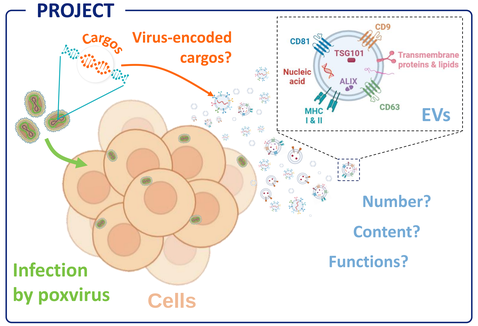
INTRODUCTION

Poxviral vectors are among the most promising therapeutic vaccines, demonstrated in ongoing clinical trials: TG4001, a MVA encoding the HPV oncoviral antigens E6 and E7 applied in HPV⁺ anogenital cancers (AACR poster CT045) and TG4050, a personalized MVA vaccine against ovarian and head and neck cancer (AACR poster LB205).

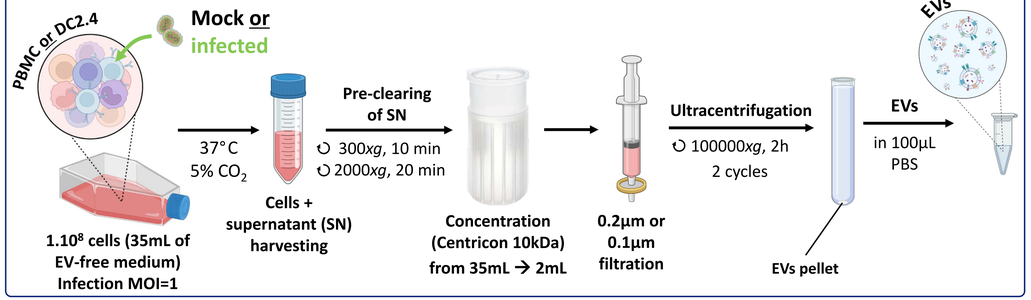
All cells, but especially virus-infected cells, communicate with their environment by secreting extracellular vesicles (EVs). In this work, we inquired whether EVs played a role in the therapeutic vaccination process with MVA vaccines:

- **How can we separate EVs from poxviruses after infection** (PBMCs or murine DC2.4 cells) **?**
- **How does poxvirus infection modulate the secretion and content of EVs ?**
- **Are EVs immunogenic ?**
- **Do they mediate therapeutic effects ?**

PROJECT

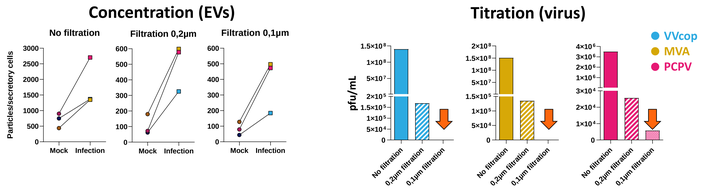


MATERIAL & METHODS



RESULTS

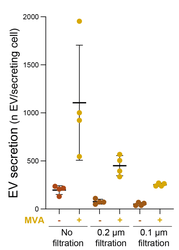
❶ Isolation and separation of PBMC EVs from infectious poxviral particles



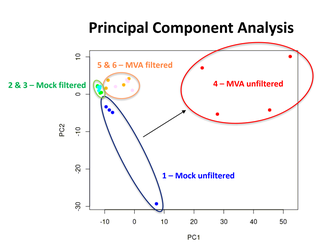
- The amount of EVs secreted by PBMC is increased after infection with all three viral strains tested
- Small EVs are separated from infectious viral particles by 0,1µm filtration (except for PCPV)

❷ Proteomic analysis of PBMC EVs by mass spectrometry

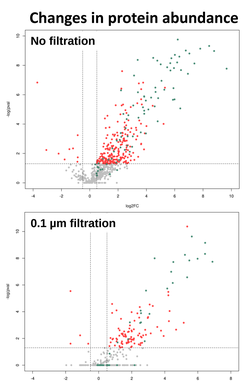
EVs isolated from PBMC mock or infected by MVA-GFP :
MOI = 1, +/- filtration, n = 4



5x more EVs (+/- viral particles) in MVA-infected conditions

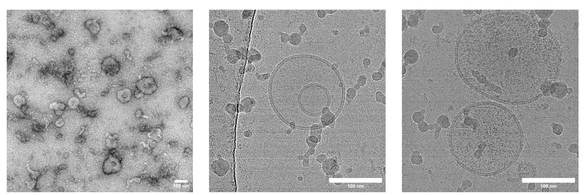


Effect of the infection on EVs protein abundance



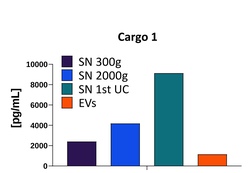
Differentially expressed **viral** and **cellular** proteins in EV fractions

❸ Isolation and characterization of 0.1µm-filtered EVs from murine dendritic DC2.4 cell line infected with MVA_pH5R-eGFP-OVA-fusion_p11k7.5_Cargo1_pH5R_Cargo2

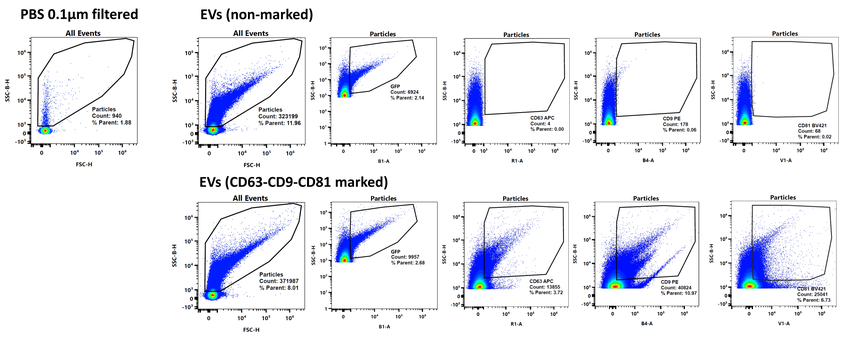


Classical cup-shaped vesicles in negative staining (TEM)

Bilayered vesicles in EV fraction were visualized (Cryo-EM)



The soluble Cargo1 was detected in the EV fraction, suggesting it was associated to sedimentable structures



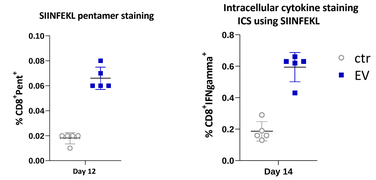
First attempts of EV characterization by flow cytometry: EV preparations contain GFP-positive events. The tetraspanins CD63, CD9 and CD81 are detectable after staining with specific antibodies.

❹ Immunogenicity of isolated DC2.4 EVs (MVA-GFP-OVA-Cargo1-Cargo2) n=1

Vaccination ip Day 1 (2.8 E+09 particles) and Day 7 (3.0 E+09 particles)

5 naïve C57BL/6 mice

Day **12**: harvest blood,
Day **14**: harvest splenocytes
Detection of CD8⁺ T cells, specific for the OVA CD8 epitope SIINFEKL



Virus-free EVs from MVA-GFP-OVA-Cargo1-Cargo2 infected DC2.4 cells induce SIINFEKL-specific CD8⁺ T cells.

❺ Therapeutic efficacy of DC2.4 EVs (MVA-GFP-OVA-Cargo1-Cargo2) n=1

6 C57BL/6 mice

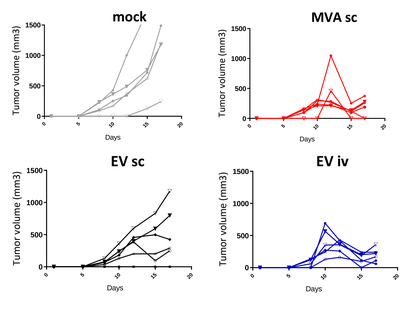
Day 1 — Day 5 — Day 11 → Tumor growth, TILs, specific response

Day 1: E.G7-OVA (ATCC CRL-2113, a murine T lymphoblast cell line) cell grafting sc

Day 5 and **Day 11**: treatment with MVA (sc / sc) or EVs (sc/sc) or EVs (iv/iv).

MVA: MVA-GFP-OVA-Cargo1-Cargo2: 1x10⁷ pfu.

EVs: virus-free EVs from DC2.4 infected with MVA-GFP-OVA-Cargo1-Cargo2.
Day 5: 4.0 E+09 particles. Day 11: 1.2 E+09 particles.



Virus-free EVs from MVA-GFP-OVA-Cargo 1-Cargo 2 infected DC2.4 showed therapeutic efficacy visible by a decrease in tumor burden after intravenous injection.

CONCLUSIONS

- **A protocol was established to separate small EVs from poxviruses.**
- **Poxviral infection increased EVs secretion from infected cells (PBMCs & DC2.4 cell line) and modified their protein content.**
- **Murine EVs isolated from DC2.4 cells, infected with an armed MVA-GFP-OVA virus, contain therapeutic cargo and GFP-OVA fusion protein.**
- **In vivo, such EVs generate OVA-specific CD8⁺ T cells in naïve mice and reduce tumor growth in EG.7-OVA tumor-bearing upon iv injection (n=1).**

PERSPECTIVES

- **Avenue 1: Ex vivo generation of loaded EVs in allogenic stem cells (off the shelf approach).**
- **Avenue 2: Design poxviral vectors which further enhance loaded EV secretion in vivo.**

DISCLOSURES

All authors affiliated to Transgene SA are or used to be employees of Transgene SA. Other authors do not have competing interest.