Poster ID: LB-287
Abstract control N° 10504

Pseudocowpox (PCPV): A next generation viral vector for cancer immunotherapy

A poxviral vector selected for its remarkable ability to induce IFN-alpha

Karola Rittner, Marine Ricordel, Caroline Tosch, Christine Thioudellet, Christelle Remy-Ziller, Marie-Christine Claudepierre, Virginie Nourtier, Isabelle Farine, Chantal Hoffmann, Doris Schmitt, Benoit Sansas, Johann Foloppe, Philippe Erbs, Nathalie Silvestre, Kaïdre Bendjama, Eric Quéméneur.

***Transgene** SA, Illkirch-Graffenstaden, France*

AACR 2018 CHICAGO April 14-18, 2018

OBJECTIVE → NEW TOOLS FOR THERAPEUTIC VACCINATION

Recombinant viruses constitutes a promising modality of immunotherapy. An increasing amount of evidence supports the use of oncolytic viruses in various clinical indications. Also, better understanding of immune mechanisms and progress in immunomonitoring protocols had led to the conclusion that viral induced immune response plays a major role in the anti-tumoral response of this class of viral-based therapeutics. Historically, oncolytic viruses were selected on their ability to lyse tumor cells.

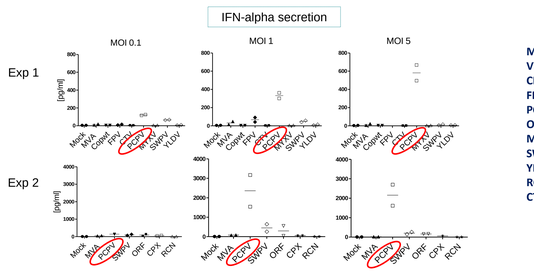
Herein, we describe a next generation anti-cancer virus selected on its capacity to prime and stimulate the immune response.

Anti-tumoral efficacy and effects on the immune system were shown in tumor models, by the vector on its own as well as by recombinant virus modified to express relevant tumor antigens.

SCREENING OF *POXVIRIDAE* IN HUMAN PBMCs

We screened a variety of *wt* Poxviridae to identify variants more likely to stimulate the immune response. To this, we exposed human PBMCs to these viruses and measured their capacity to trigger the release of IFN-alpha as a surrogate marker of their pro-immune properties. In parallel, we have screened the impact of virus on viability of PBMC in order to exclude agents with high toxicity for non tumoral cells.

PCPV showed remarkable ability to induce IFN-alpha in human PBMCs.



MVA MVAN33.1
VV Cop *wt* Vaccinia Virus Copenhague
CPX Cowpox
FPV Fowlpox
PCPV Pseudocowpox
ORF Parapoxvirus ovis
MYXV Myxomavirus
SWPV Swinepox
YLDV Yaba-like disease virus
RCN Raccoonpox
CTV Cotiavirus

Figure 1: Human Peripheral Blood Mononuclear cells (PBMCs) from two healthy donors per experiment were infected with *wt Poxviridae* at MOI 0.1, 1 and 5. After o/n incubation, supernatants were isolated and a variety of cytokines and chemokines were quantified (Luminex analysis). The results for IFN-alpha are shown.
→ Among all tested *Poxviridae*, the Parapoxvirus PCPV turned out to be best inducer of IFN-alpha.

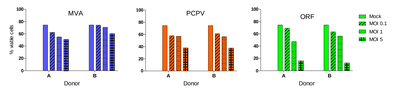


Figure 2: Human Peripheral Blood Mononuclear cells (PBMCs) from healthy donors (A, B) were infected with MVA, and the parapoxviruses PCPV and ORF at the MOIs 0.1, 1 and 5. After o/n incubation, cells were stained with LiveDead, the percentage of viable cells is shown. → Compared to ORF virus, PCPV showed lower toxicity to PBMCs.

ABOUT PCPV AND THE GENERATION OF ITS RECOMBINANTS

wt Pseudocowpox PCPV

- PCPV strain TJS (ATCC, VR-634) was isolated from a human case of "Milker's nodules", described in Friedman-Kien *et al*, 1963 Science.
- Patients with Milker's nodules did not develop immunity to vaccinia et *vice versa*.
- PCPV induces the generation of inclusion and elementary bodies, as it is characteristic for poxviruses.

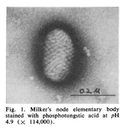


Fig. 1. Milker's node elementary body stained with phosphotungstic acid at pH 4.9 (× 114,000).

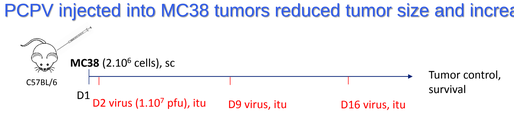
From Friedman–Kien *et al.*

→ Recombinant PCPVs

- Recombinant PCPV were generated by insertion of genes into the non-essential VEGF loci by homologous recombination in Bovine Turbinate cells (BT, ATCC CRL-1390).
- Preclinical batches were generated in HeLa cells, crude harvest of infected cells was disrupted using high shear homogenizer, clarification by filtration, and purified by tangential flow filtration.
  - **PCPV *wt***
  - **PCPV-mCherry**
  - **PCPV-GFP**
  - **PCPV-MUC1**
  - **PCPV-HPV16E7$_m$**

REDUCTION OF MC38 TUMOR GROWTH

PCPV injected into MC38 tumors reduced tumor size and increased survival



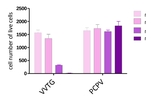


Figure 8: Viability of MC38 cells infected at MOI 1, 10^{-1}, 10^{-2} and 10^{-3} with PCPV or a oncolytic VV (VVTG). After 5 days, living cells were determined.
→ No lytic activity of PCPV in MC38 cells.

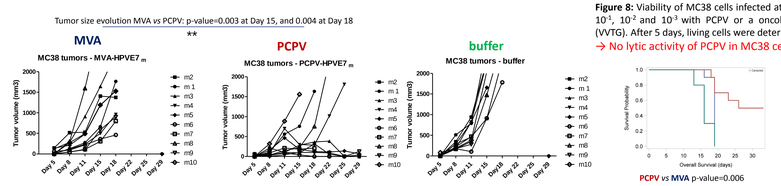


Figure 7: The capacity of PCPV to control tumor growth and to increase survival rates was probed in the colon carcinoma cell line MC38 (kindly provided by J.W. Hodge) injected subcutaneously in syngeneic C57BL/6 mice (10 mice per group). Day 2, 1x10^7 pfu of a PCPV or a MVA virus, or buffer were injected at the cell line injection site (D2) and later in the emerging tumor (D9 and D16). Tumor size evolution and survival probability are shown.
→ Compared to buffer or MVA, PCPV induced statistically significant reduction of tumor size over time and increased survival .

EFFECTS IN HUMAN IMMUNE CELLS

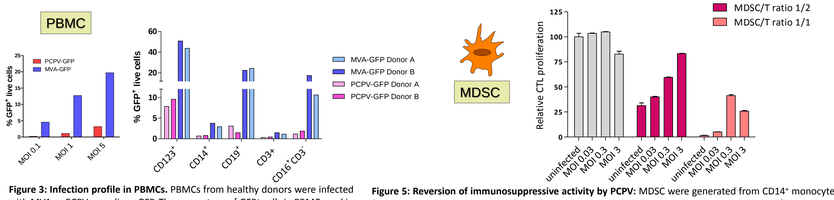


Figure 3: Infection profile in PBMCs. PBMCs from healthy donors were infected with MVA or PCPV encoding eGFP. The percentage of GFP$^+$ cells in PBMCs and in subpopulations was measured (MOI 1).
→ PCPV was less efficient in infecting PBMCs than MVA. Among tested subpopulations, PCPV infected preferentially CD123$^+$ cell types.

Figure 5: Reversion of immunosuppressive activity by PCPV: MDSC were generated from CD14$^+$ monocytes from healthy donors in the presence of IL-6 and GM-CSF (CD1a$^-$, CD14$^-$, CD33$^+$, HLA-DR$^+$, CD80^{lo}, CD86$^+$, PD-L1$^+$, CD68$^+$), and co-cultivated with CFSE-labeled autologous CD8$^+$ T cells in the presence of TransAct/IL-2 over 4 days. T cells or co-cultures were infected with PCPV at indicated MOI.
→ The suppressive activity provided by MDSCs on CTL proliferation could be reversed by PCPV treatment (ratio 1/2).

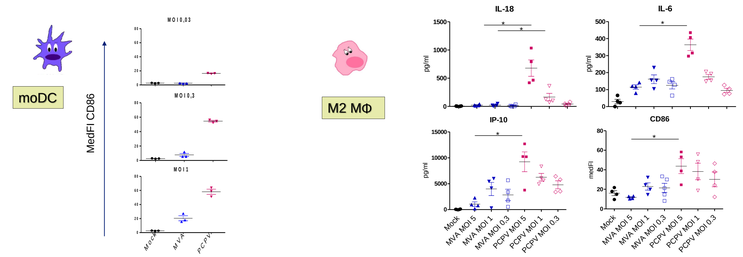


Figure 4: Activation of moDC (APCs) by PCPV: Monocyte-derived dendritic cells (moDCs) generated in the presence of GM-CSF, IL-4, were infected with MVA or PCPV. Median fluorescent intensity of the activation marker CD86 was measured after o/n incubation by flow cytometry.
→ Activation of moDCs was superior after treatment with PCPV.

Figure 6: Reversion of immunosuppressive phenotype - M2-like Macrophages: Generation of M2-like human macrophages from monocytes (4 donors) cultured first in M-CSF, and then in IL-4, IL-10 and TGF-beta (Mia *et al*. 2014). Resulting CD163$^+$CD206$^+$ cells were infected with MVA or PCPV. Cytokine profile in the supernatant and cell activation (CD86) were probed after o/n incubation.
→ Compared to MVA (MOI 5), PCPV induced higher secretion of IL-18, IL-6, IP-10 and expression of CD86.

EFFECTS ON LOCAL CYTOKINE /CHEMOKINE AND TIL PROFILES

PCPV induced ample local release of cytokine/chemokine in mouse skin. Within MC38 tumors, PCPV increased the percentage of neutrophils while reducing those of MHCII^{lo} TAMs.

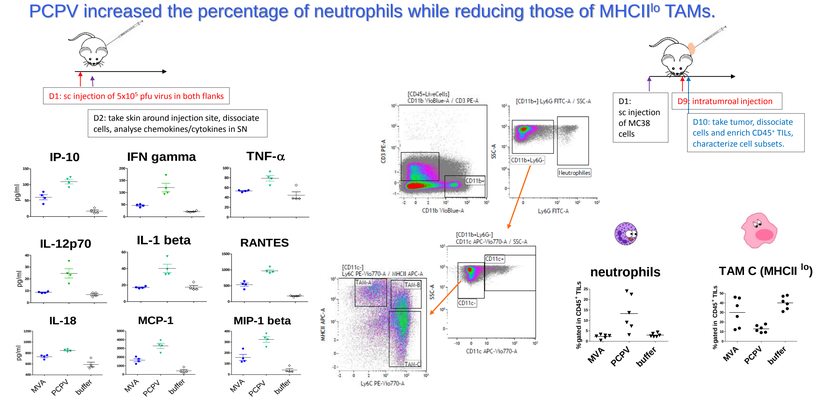


Figure 9: For local cytokine and chemokine detection, 4 mice/group were injected in both flanks applying 5.10^5 pfu / flank. Skin samples were mechanically dissociated. Cleared supernatant was analyzed (Luminex).
→ Compared to MVA, PCPV induced significantly higher local levels of IP-10, IFN-gamma, TNF-alpha, IL-12, IL-1 beta, RANTES, IL-18, MCP-1 and MIP1-beta.

Figure 10: Mice were split into 3 groups of 6 animals. Palpable MC38 tumors were injected intratumorally 9 days after tumor cell implantation with 1x10^7 pfu of recombinant PCPV, MVA or buffer. The day after virus injection, mice were sacrificed, tumors were isolated, enzymatically dissociated and TILs were enriched using CD45$^+$ magnetic beads technology Subpopulations within the CD45$^+$ enriched cells were identified by flow cytometry according to Brauner *et al*. (AACR 2017 poster abstract N° 1672).
→ PCPV led to more neutrophils and less MHC II ^{lo} TAM-Cs within the TME.

IMMUNOGENICITY STUDIES

PCPV-HPV16E7 induced a strong cellular response against HPV16E7$_m$ detectable in spleen and lung and reduced growth of TC1 tumors

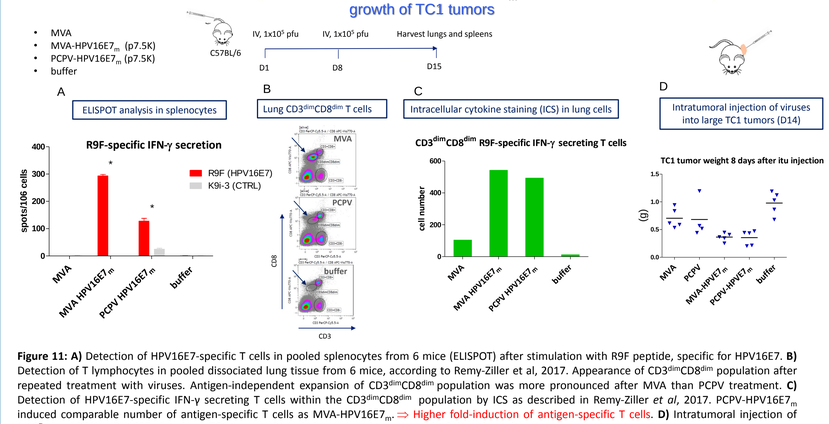


Figure 11: A) Detection of HPV16E7-specific T cells in pooled splenocytes from 6 mice (ELISPOT) after stimulation with R9F peptide, specific for HPV16E7. **B)** Detection of T lymphocytes in pooled dissociated lung tissue from 6 mice, according to Remy-Ziller et al, 2017. Appearance of CD3^{dim}CD8^{dim} population after repeated treatment with viruses. Antigen-independent expansion of CD3^{dim}CD8^{dim} population was more pronounced after MVA than PCPV treatment. **C)** Detection of HPV16E7-specific IFN-γ secreting T cells within the CD3^{dim}CD8^{dim} population by ICS as described in Remy-Ziller *et al*, 2017. PCPV-HPV16E7$_m$ induced comparable number of antigen-specific T cells as MVA-HPV16E7$_m$. ⇒ Higher fold-induction of antigen-specific T cells. **D)** Intratumoral injection of 1.10^7 pfu of PCPV-HPV16E7$_m$ into large TC1 tumors (D14), led to reduced tumor weight 8 days thereafter.

CONCLUSIONS AND PERSPECTIVES

- ❑ PCPV was selected among a panel of attenuated and oncolytic *Poxviridae* for its capacity to induce the secretion of high levels of IFN-alpha in human peripheral blood mononuclear cells.
- ❑ In human immune cells, PCPV induced efficient activation of antigen-presenting cells and a less suppressive phenotype in *in vitro* derived M2-like macrophages and MDSCs.
- ❑ Recombinant PCPV vectors could be generated by insertion of transgenes under the control of the pH5R promoter in the VEGF loci.
- ❑ PCPV-HPVE7$_m$ useful as cancer vaccine: generation of T cell response in spleen and lung of iv injected animals.
- ❑ Control of fast growing MC-38 tumors were observed after itu injection of PCPV vectors. This observation was associated with increased local release of IP-10, IFN-gamma, IL-12p70, RANTES *etc,*. and reduction of percentage of intratumoral suppressive macrophages (TAM MHCII^{lo}).
- ➢ **PCPV is proposed as a future virotherapeutic with local responses to reshape the TME and prime specific responses against vector-encoded or tumor-presented antigens (*in situ* vaccination).**

DISCLOSURES

All authors affiliated to Transgene SA are or were employees of Transgene