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Pseudocowpox virus (PCPV), a potent viral vector for both antigen-dependent and independent cancer immunotherapy

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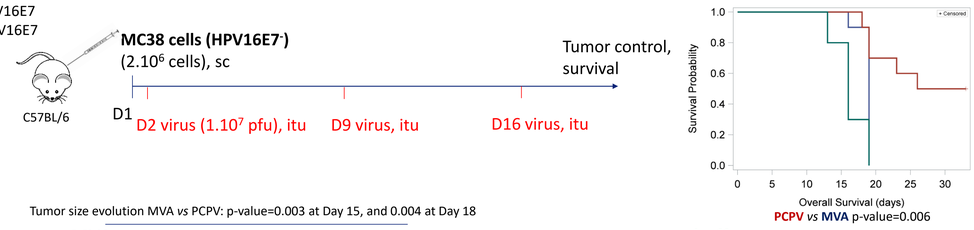
BACKGROUND

Viral vectors expressing tumor antigens and/or cytokines have proven to be clinically effective approaches to stimulate anti-tumor immunity and to change the tumor microenvironment. However, novel viral strains with improved immunogenic properties would be beneficial to expand the scope of virotherapeutic approaches. We identified Pseudocowpox virus (PCPV) from a Poxviridae screening program. Compared to well established poxvirus strains like MVA or oncolytic Vaccinia virus (VACV), PCPV induced the secretion of **1,000-fold more IFN-alpha in human PBMCs**. PCPV treatment induced **efficient activation of antigen-presenting cells**, and a **less suppressive phenotype in *in vitro* derived M2-like macrophages and MDSCs** [see AACR 2018, poster LB-287]. A recombinant PCPV encoding the tumor associated antigen (TAA) HPV16E7 was generated to assess the anti-tumor activity in the syngeneic murine tumor models MC38 (HPV16E7$^-$) and TC1 (HPV16E7$^+$).

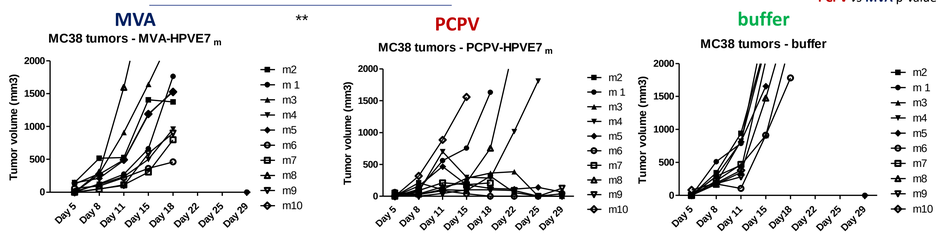
TAA-INDEPENDENT EFFECTS IN MC38 TUMOR MODEL

PCPV injected into MC38 tumors reduced tumor size and increased survival

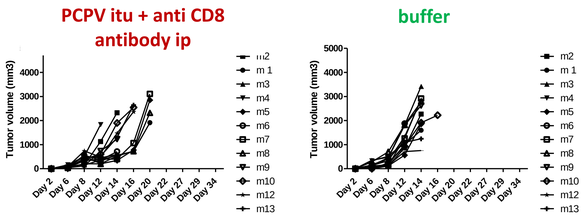
- MVA-HPV16E7
- PCPV-HPV16E7
- buffer



Tumor size evolution MVA *vs* PCPV: p-value=0.003 at Day 15, and 0.004 at Day 18

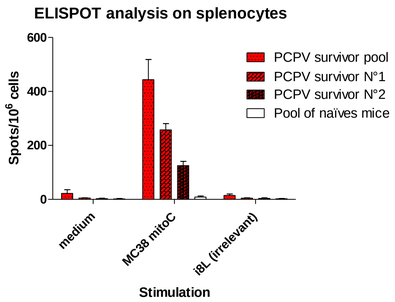


Depletion of CD8+ T cells reverts effects of PCPV



13 mice per group were injected with buffer of PCPV as indicated above.
Left: mice were treated D-2, D-1, D6 and D13 with anti CD8 antibody (clone 53-6-7, 200 µg/injection, ip). N=1.
Depletion of CD8+ T cells was confirmed in blood and spleen Day 14.

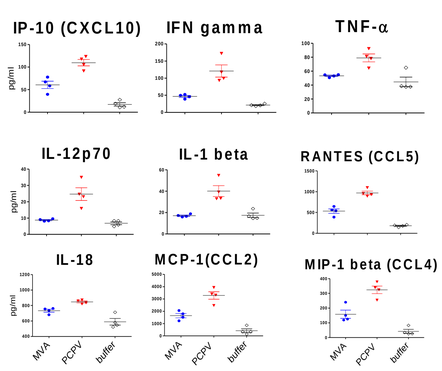
PCPV treatment induced MC38-specific responses in survivors (Day 34)



Detection of MC38-specific T cells in splenocytes from pooled or individual survivors (D34), after stimulation with MC38 cells treated with Mitomycin C or irrelevant peptide IRL. Control: splenocytes from naïve mice.

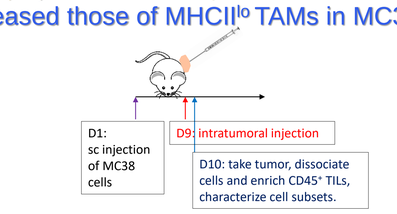
PARTICULARITIES OF PCPV vs MVA

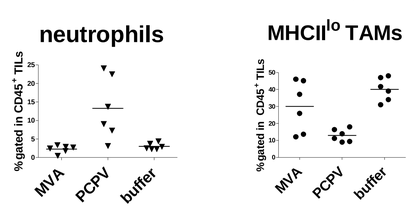
PCPV increased local of Th1 Cytokines and Chemokines release



4 mice/group were injected in both flanks applying 5.10^5 pfu/flank. After 16h, skin samples were taken and mechanically dissociated. Cleared supernatant was analyzed (Luminex).

PCPV (itu) increased frequencies of neutrophils and decreased those of MHCII^{lo} TAMs in MC38 tumors



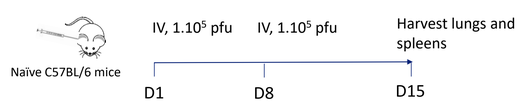


The day after virus injection, tumors were enzymatically dissociated and TILs were enriched using CD45+ magnetic beads. Subpopulations were identified by flow cytometry according to Brauner *et al.* (AACR 2017 poster N°1672).

TAA-DEPENDENT RESPONSES IN NAÏVE AND TC1-BEARING MICE

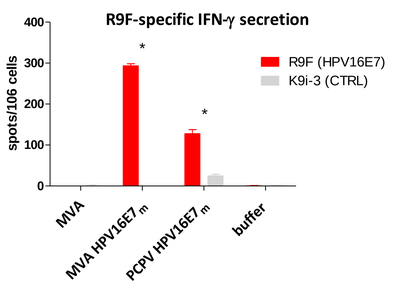
PCPV-HPV16E7 induced strong cellular response against HPV16E7 detectable in spleen and lung

- MVA
- MVA-HPV16E7
- PCPV-HPV16E7
- buffer



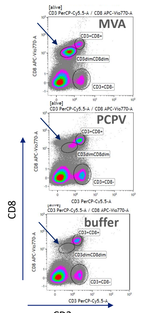
A

ELISPOT analysis in splenocytes



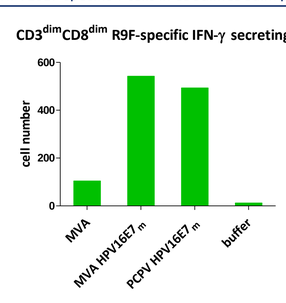
B

Lung CD3^{dim}CD8^{dim} T cells



C

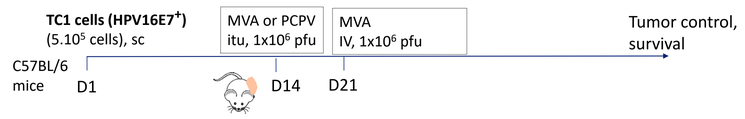
Intracellular cytokine staining (ICS) in lung cells: HPV16E7-specific T cells in CD3dimCD8dim population



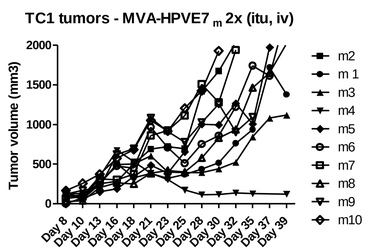
A) Detection of HPV16E7-specific T cells in pooled splenocyes from 6 mice (ELISPOT), after stimulation with R9F peptide, specific for HPV16E7. B) Detection of T lymphocytes in dissociated lung tissue from 6 mice / group, according to Remy-Ziller et al, 2017. Appearance of CD3^{dim}CD8^{dim} population after repeated virus treatment. C) Detection of HPV16E7-specific IFN-γ secreting T cells within the CD3dimCD8dim population in the lung of iv treated mice.

Heterologous prime boost PCPV (*itu*) / MVA (*iv*) superior to homologous MVA (*itu*) / MVA (*iv*) vaccination

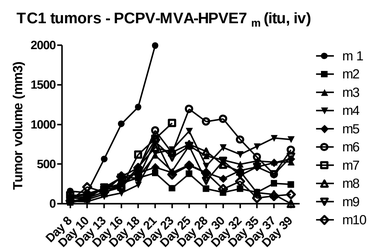
- MVA-HPV16E7
- PCPV-HPV16E7
- buffer



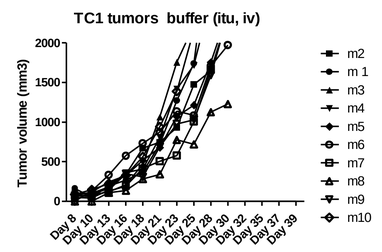
MVA *itu* – MVA *iv*



PCPV *itu* – MVA *iv*



Buffer *itu* – buffer *iv*



TC1 cells were sc injected into C57BL/6 mice (10 mice /group). After 14 days of tumor growth, mice were randomized and first intratumorally injected with MVA or PVPV encoding HPV16E7, and one week later intravenously with MVA HPV16E7. Tumor growth and survival were followed.

ABOUT PCPV AND THE GENERATION OF ITS RECOMBINANTS

Pseudocowpox PCPV (Parapoxvirus)

- PCPV strain TJS (ATCC, VR-634) was isolated from a human case of “Milker’s nodules”.
- Patients with Milker’s nodules did not develop immunity to vaccinia et *vice versa*.
- Recombinant PCPV were generated by insertion of genes into the non-essential VEGF loci.
- PCPV has detectable but low oncolytic potential in human and murine cells (Ricordel *et al.*, OncoTarget, paper *under revision*). Lower oncolytic activity *in vitro* observed than for the prototype Parapoxvirus ORF.
- Less than 80% pairwise identity with ORFvirus.

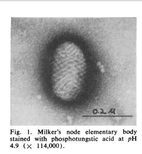


Fig. 1. Milker's node elementary body stained with phosphotungstic acid at pH 4.9 (× 114,000).

From Friedman and Kien, 1963

CONCLUSIONS AND PERSPECTIVES

Our recent data confirm the potential of PCPV as a new vector for anti-tumor vaccination, in particular for its intrinsic ability to control tumor growth in a tumor antigen-independent manner. Heterologous prime boost regimen with our individualized vaccine approach myVac or combination with oncolytic virotherapy can be envisaged. Detailed studies in the human immune cells from patients are ongoing to dissect effects of PCPV on immunosuppressive and immunostimulating cell populations. Patent application filed.

myvac

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