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Supplemental information

TheraVision: Engineering platform technology

for the development of oncolytic viruses

based on herpes simplex virus type 1

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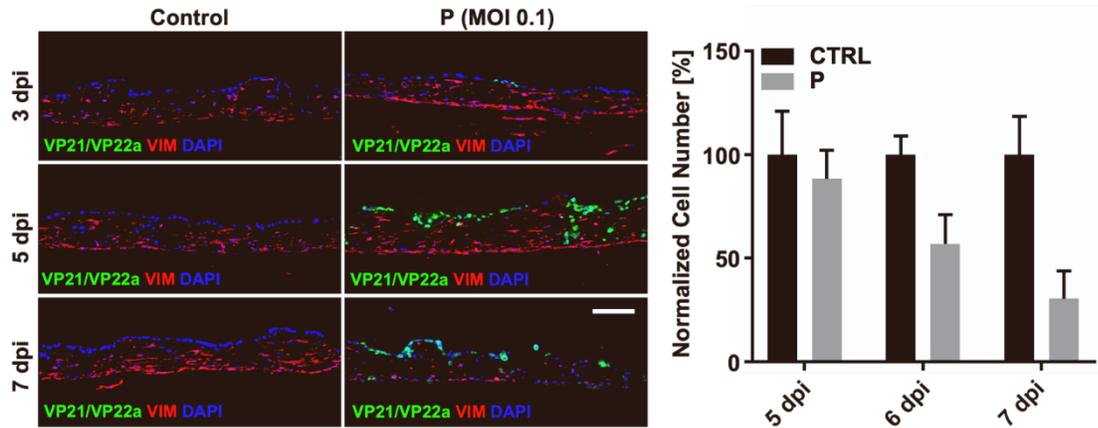


Figure S1 – Safety and efficacy evaluation of P virus in the 3D tissue tumor model

P virus does reduce cell number of tumor cells cultured on SIS muc for 14 days cells evaluated 5, 6 and 7 dpi with MOI 0.1 (mean +/-SD of 5 pictures). Immunofluorescence staining of paraffin sections illustrate that viral infection starts at the time point of 3 dpi (HSV1 proteins, VP21/VP22a, green) and also infects fibroblasts (vimentin, red) over time (5 and 7 dpi). Nuclei were visualized by DAPI staining. The scale bar corresponds to 100 μ m.

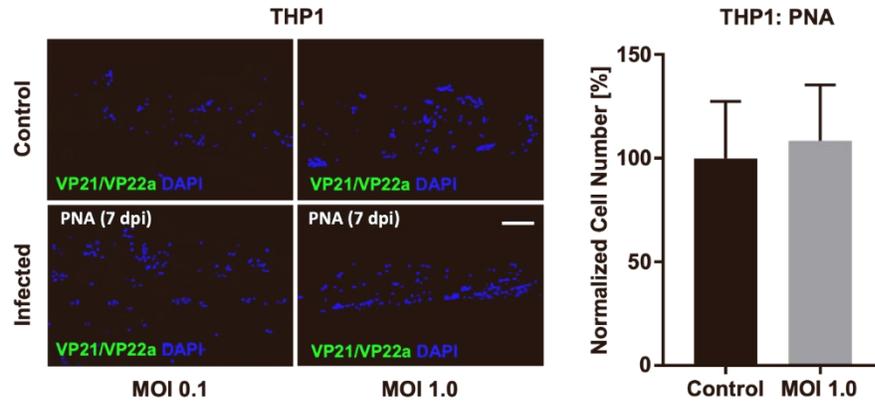


Figure S2 – Safety evaluation of PNA in the 3D tissue tumor model

Quantification of THP1 immune cells in PNA infected 3D tissue model (MOI 1.0, 7 dpi) is shown. Five pictures were analyzed for each time point. The error bars represent SD. The scale bar corresponds to 100 μm .

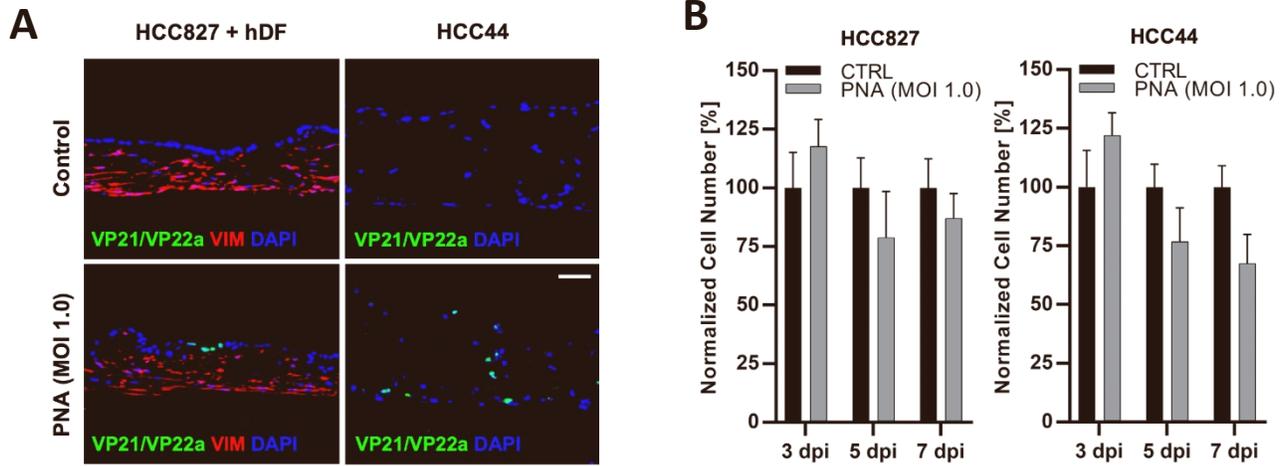


Figure S3 –Efficacy of OV in invasive tumor cells

A: 3D tissue model with either HCC827 or HCC44 cells were infected with PNA (MOI 1.0) and evaluated 7 dpi. Infection of tumor cells is illustrated in green by immunofluorescence staining (HSV1 proteins, VP21/VP22a). HDFs were visualized by staining of vimentin (red), cell nuclei by staining with DAPI. The scale bar corresponds to 100 μ m. B: Quantification of HCC827 and HCC44 carcinoma cells in PNA infected 3D tissue model (MOI 1.0) is displayed over time. Five pictures were analyzed for each time point. The error bars represent SD. White arrows indicate VP21/22a-positive tumor cells.

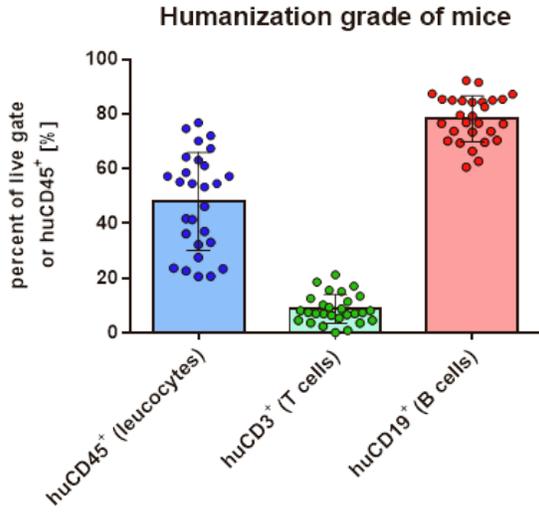
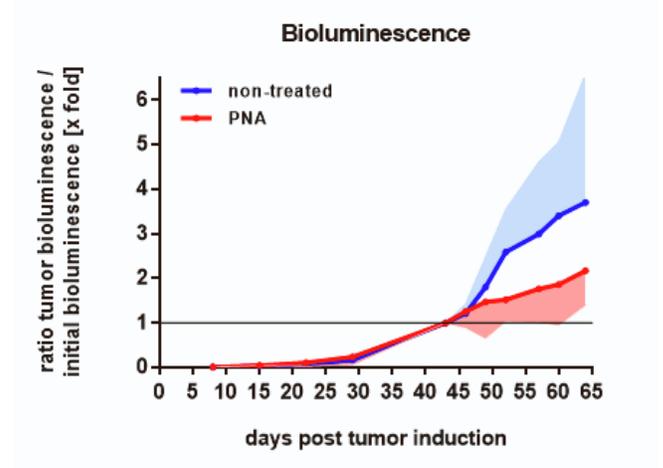
A**B**

Figure S4 – Safety of OV in mouse models

A: Examination of humanization grade in 10-12 week old NSG mice after postnatal injection of human stem cells (CD34+) by flow cytometric analysis of blood. The percentages of human leukocytes (huCD45+) of all cells after cell aggregate exclusion and distribution of human T cells (huCD3+) and B cells (CD4+) within human leukocyte fraction are shown (n=28). B: NSCLC tumor was induced in immunodeficient NSG mice using 2×10^5 HCC827Luc cells on day 0 by subcutaneous injection into the flank and intratumorally treated with $3,3 \times 10^6$ PFU of PNA on day 44. Tumor progression of non-treated (blue) or PNA treated (red) mice (n=10) compared to initial tumor volume on day 44 is shown.

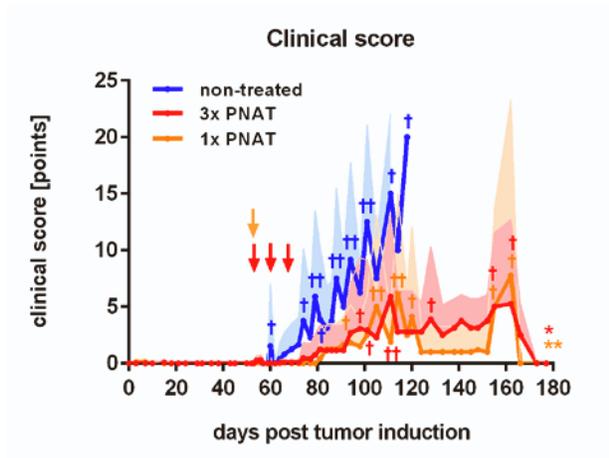


Figure S5 – Efficacy of OV in mouse models

NSCLC tumor was induced in immunodeficient NSG mice using 2×10^5 HCC827Luc cells on day 0 by subcutaneous injection into the flank and intratumorally treated once with $3,3 \times 10^6$ PFU of PNAT on day 53 (n=11, orange). Another group received PNAT intratumorally three times at 7-day intervals (n = 13, red). Additionally, a non-treated tumor group was included as control (n = 13, blue). Clinical score was determined for all animals and mean values and corresponding standard deviations of each group are shown. Statistical analysis was performed by Mann-Whitney U test in comparison to non-treated control.

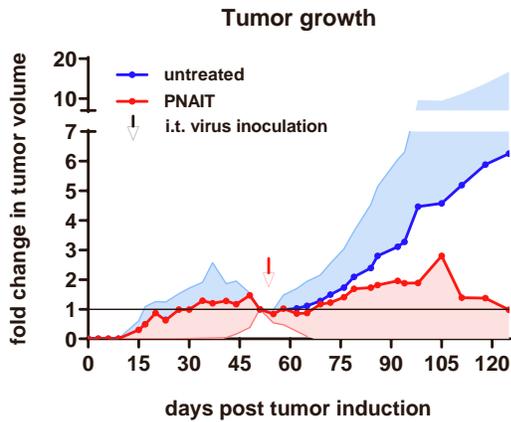


Figure S6 - Efficacy evaluation of the functionalized PNAIT virus in presence of immune cells

Immunodeficient NSG mice were humanized by intrahepatically injection of $2-4 \times 10^5$ human CD34+ hematopoietic stem cells 24-48 hours after birth (humanization status after 12 weeks: $\geq 20\%$ huCD45+ events of total CD45+ events). NSCLC tumor was induced using 2×10^5 HCC827Luc cells on day 0 by subcutaneous injection into the flank and intratumorally treated once with 1.6×10^5 PFU of PNAIT on day 55 (n = 13, red). Additionally, a non-treated tumor group was included as control (n = 11, blue). Figure 6F shows tumor progression compared to initial tumor volume on day 55.