Supplemental Information

Enhanced Therapeutic Efficacy of a Novel

Oncolytic Herpes Simplex Virus Type 2 Encoding

an Antibody Against Programmed Cell Death 1

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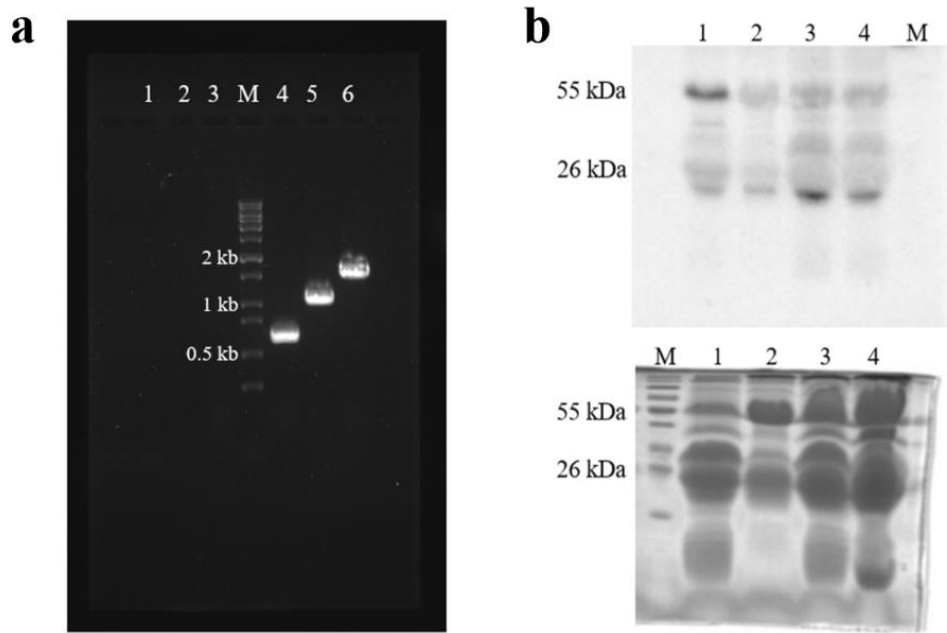
Figure Supplemental 1. The anti-hPD1mAb encoding sequence was inserted and translated correctly

(a) DNA ladder analysis of the modified oHSV2-aPD1 sequences. Lane 1: water light chain (blank control), lane 2: water heavy chain (blank control), lane 3: oHSV2 light chain (negative control), lane 4: oHSV2 heavy chain (negative control), lane 5: oHSV2-aPD1 light chain, lane 6: oHSV2-aPD1 heavy chain. M: molecular marker. **(b)** Culture supernatants from oHSV2-aPD1-infected Vero (lanes 1), A549 (lane 2), CT26 (lane 3) or B16R (lane 4) cells were collected, purified using protein G agarose, and then loaded on SDS-PAGE gels under denaturing conditions. Upper panel: picture following transfer onto PVDF membrane. Lower panel: matched picture of the gel stained with Coomassie brilliant blue. M: molecular marker.
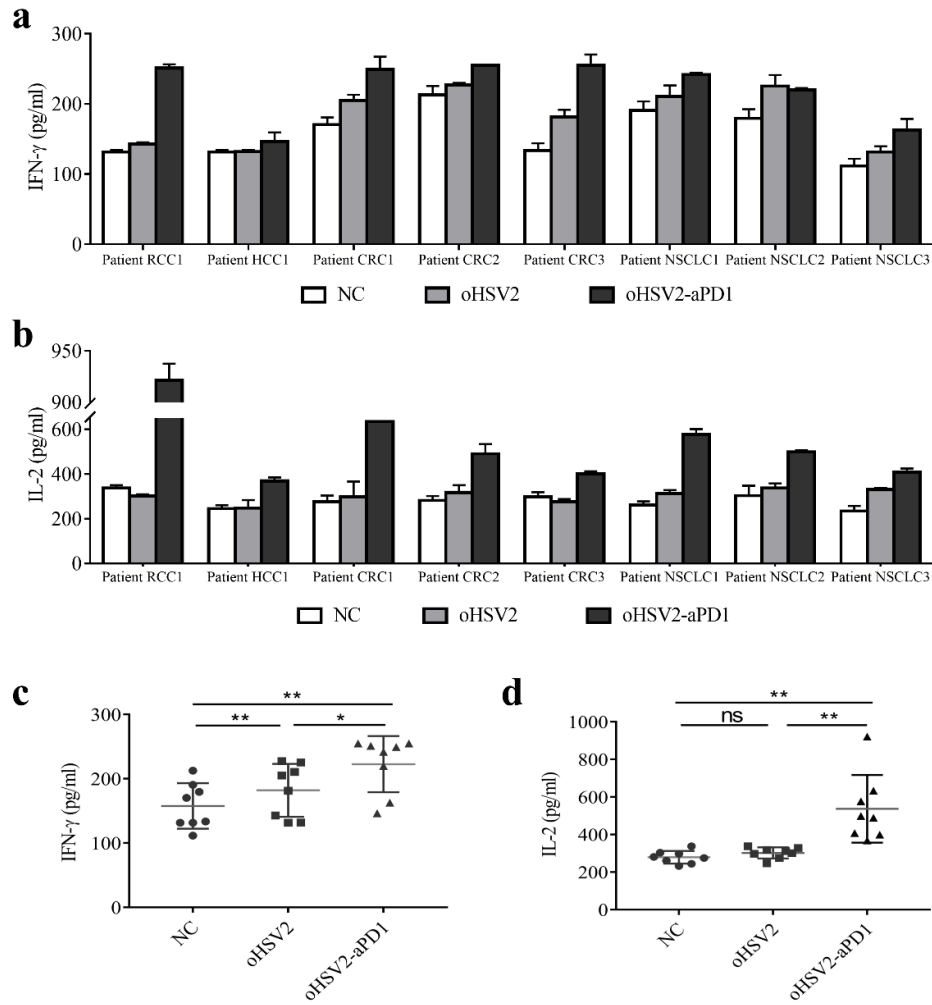
Figure Supplemental 2. Production of IFN-γ and IL-2 in anti-hPD1mAb-stimulated PBMCs from cancer patients

PBMCs from eight advanced tumor patients were treated with filtered culture supernatants from mock-infected (NC, negative control) or OV-infected cells and stimulated with ionomycin and phorbol myristate acetat (PMA). After 36 hours, supernatants were harvested to measure the production of IFN-γ and IL-2 by ELISA. IFN-γ levels **(a)** and IL-2 **(b)** levels in cancer patient PBMCs, with each specimen presented individually. IFN-γ levels **(c)** and IL-2 levels **(d)** in cancer patient PBMCs are shown as statistical results. RCC: renal cell carcinoma, HCC: hepatocellular carcinoma, CRC: colorectal cancer, NSCLC: nonsmall cell lung cancer. Data are presented as the mean ± SD. (*$p < 0.05$; **$p < 0.01$; ns, not significant)
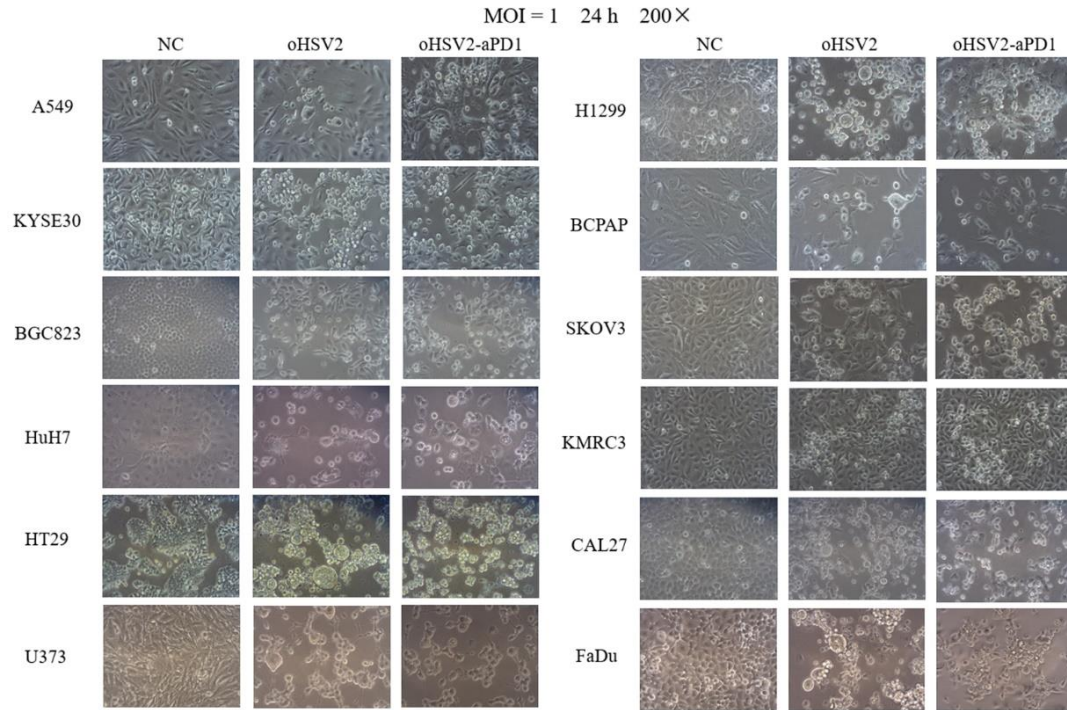
Figure Supplemental 3. Comparison of infectious activities of oHSV2 and oHSV2-aPD1 viruses in human tumor cells

oHSV2 or oHSV2-aPD1 were used to mock-infect or infect human tumor cells, including A549, KYSE30, BGC823, HuH7, HT29, U373, H1299, BCPAP, SKOV3, KMRC3, CAL27 and FaDu cells, at MOI = 1 for 24 hours. Images were collected at 20× objective magnification with an inverted phase contrast microscope.
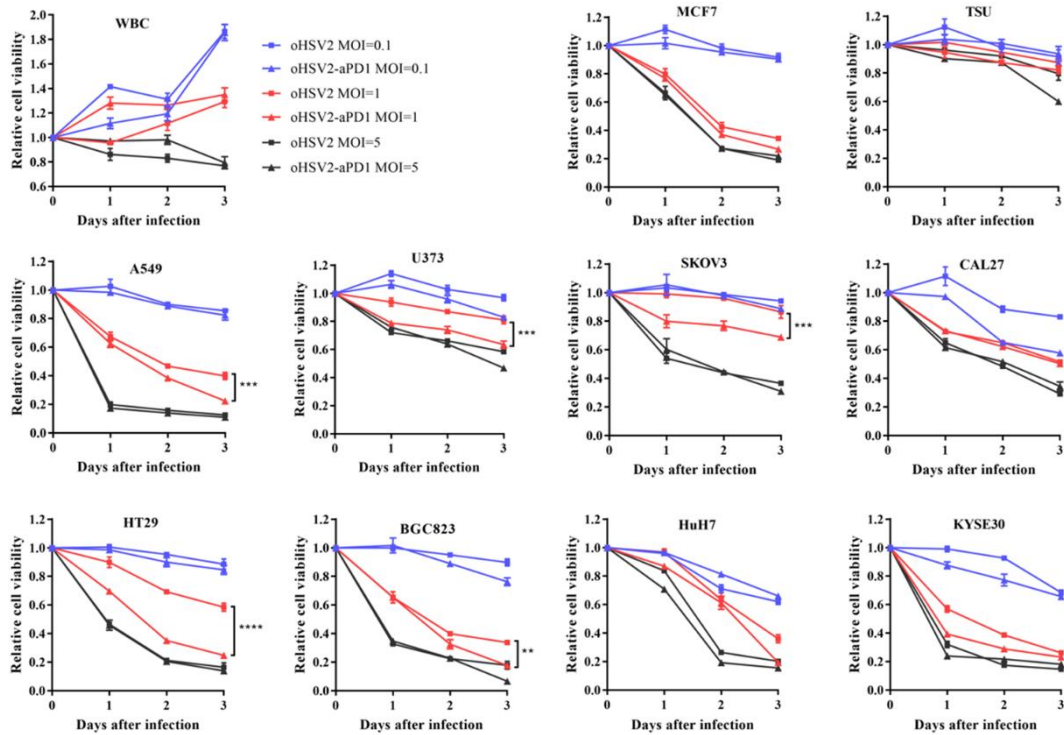
Figure Supplemental 4. Comparison of oncolytic activities of oHSV2 and oHSV2-aPD1 viruses in human cells

oHSV2 or oHSV2-aPD1 were used to mock-infect or infect WBC, TSU, MCF7, A549, U373, SKOV3, CAL27, HT29, BGC823, HuH7 and KYSE30 cells at the indicated three MOIs for the indicated lengths of time. Each value is presented as the mean ± SD of three measurements. Statistical significance is indicated by **($p < 0.01$), ***($p < 0.001$) and ****($p < 0.0001$).
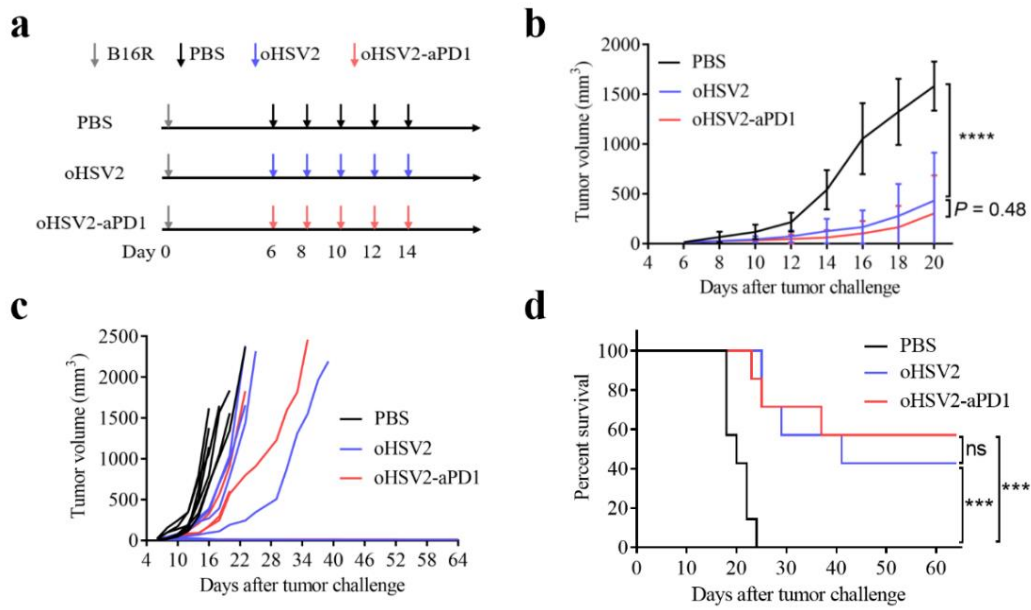
Figure Supplemental 5. Comparison of therapeutic effects of oHSV2 and oHSV2-aPD1 viruses in B16R melanoma model in normal C57BL/6J mice

Mice were subcutaneously inoculated with B16R cancer cells and treated via intratumoral injection as described in the Methods section (n = 7 per group). **(a)** Schematic representation of the experiment. **(b)** Tumor volumes within different groups were measured every other day and data are presented as the mean ± SD. **(c)** Tumor volumes from each specimen are shown individually. **(d)** Overall survival is shown using Kaplan-Meier curves and was analyzed using the Log rank test. (***$p < 0.001$, ****$p < 0.0001$, ns, not significant)
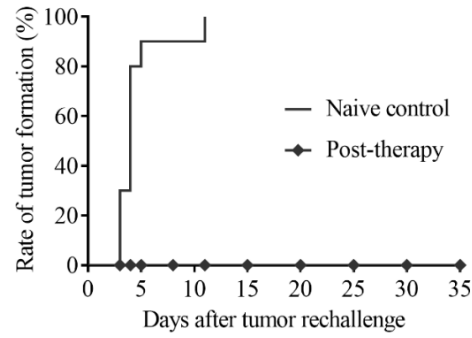
Figure Supplemental 6. Administration of oHSV2 and PD-1 antibody alone or their union led to an antitumor immune memory

Ten tumor-free mice from the four kinds of immunotherapies (anti-PD1, n = 1; oHSV2, n = 2; oHSV2-aPD1, n = 4; oHSV2 + anti-PD1, n = 3) were rechallenged at day 64 after the first tumor inoculation, with 2×10^5 B16R cells per mouse in the contralateral flank. And ten naive C57BL/6J-Pdcd1 mice received same dose tumor cells as a control.

Table Supplemental 1. Patient characteristics.

Patient ID	Age (year)	Gender	Type	TNM stage	Treatment
Patient RCC1	50	Male	[a]RCC	T3NxM1	None
Patient HCC1	59	Male	[b]HCC	TxN0M1	None
Patient CRC1	55	Male	[c]CRC	T3N2M0	Radiotherapy
Patient CRC2	55	Female	CRC	T3N1M0	None
Patient CRC3	65	Female	CRC	T3N2M1	Chemotherapy
Patient NSCLC1	84	Female	[d]NSCLC	T2N3Mx	None
Patient NSCLC2	61	Male	NSCLC	T2N2Mx	None
Patient NSCLC3	79	Male	NSCLC	T2N2M1	None

[a]RCC: renal cell carcinoma, [b]HCC: hepatocellular carcinoma, [c]CRC: colorectal cancer, [d]NSCLC: nonsmall cell lung cancer.