OMTM, Volume *29*

Supplemental information

Capsid-modified adeno-associated virus vectors as novel vaccine platform for cancer immunotherapy

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Table S1: Characterization of eGFP-encoding AAV preparations.

Capsids engineered to display Ova epitopes or full-length Ova were packaged with vector genomes encoding for eGFP in a self-complementary (sc) vector genome conformation. Following iodixanol density gradient ultracentrifugation, the genomic titer, the capsid titer, and the infectious titers of the indicated preparations were determined by qPCR, ELISA, and flow cytometry, respectively. Based on these values, the packaging efficiency as capsids (Cap)/vector genome (vg) and the infectivity as Cap/infectious units (IU) were calculated.

	Vector genomes/µL	**Capsids/µL**	**Cap/vg**	**Infectious units/µL**	**Cap/IU**
AAV2::eGFP (scGFP)	$5.68x10^8$	$5.63x10^9$	9.9	$3.47x10^8$	16.2
AAV-Vac_Ova8^{587} (scGFP)	$4.26x10^8$	$4.34x10^9$	10.2	$2.32x10^6$	1886.9
AAV-Vac_Ova4^{587} (scGFP)	$4.71x10^8$	$3.40x10^9$	7.2	$4.27x10^5$	7962.5
AAV-Vac_Ova4+8^{VP2} (scGFP)	$3.06x10^8$	$4.15x10^9$	13.6	$6.97x10^7$	59.5
AAV-Vac_cOva^{VP2} (scGFP)	$3.23x10^8$	$3.51x10^9$	10.9	$7.12x10^7$	49.4

AAV2 = wild-type AAV 2 capsid; AAV-Vac_Ova8^{587} = capsid with MHC-I-restricted Ova epitope inserted at I-587; AAV-Vac_Ova4^{587} = capsid with MHC-II-restricted Ova epitope inserted at I-587; AAV-Vac_Ova4+8^{VP2} = capsid with MHC-I- and MHC-II-restricted Ova epitope inserted into the capsid as fusion to VP2; AAV-Vac_cOva^{VP2} = capsid with full-length cOva inserted into the capsid as fusion to VP2; scGFP = vector genome encoding for eGFP; self-complementary vector genome conformation

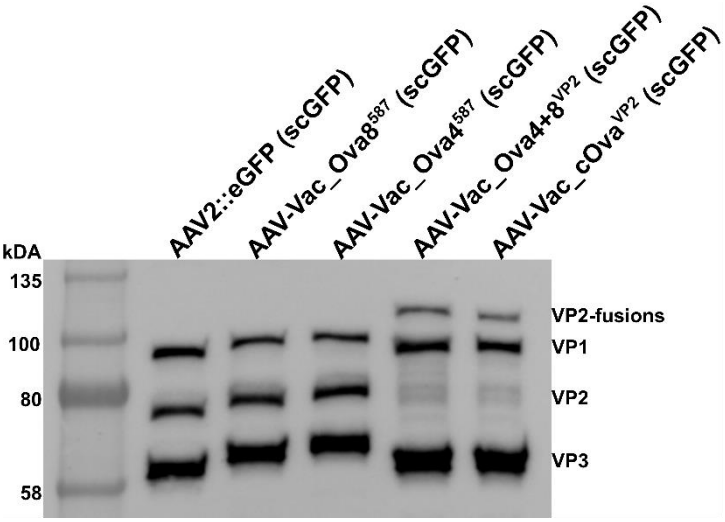

Figure S1: Western blot analysis of antigen-decorated capsids packaged with vector genomes encoding for eGFP.

$5x10^9$ vector genomes of indicated preparations, encoding for eGFP (as self-complementary genome configuration (scGFP)), were separated on a 10% SDS PAGE. The capsid protein-specific antibody B1 was used for visualizing the three different capsid proteins VP1, VP2, and VP3 in their wild-type as well as modified form (VP1: 87 kDa, VP2: 72 kDa, VP3: 62 kDa, Ova8+4-eGFP-VP2: 98 kDa, cOva-VP2: 96 kDa).

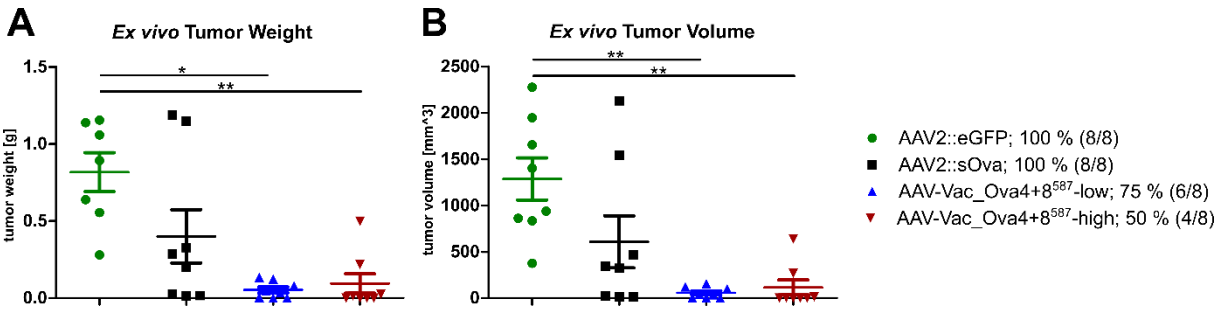

Figure S2: Animals of the antigen-decorated AAV vector-based vaccine cohorts demonstrated reduced B16/F10-Ova tumor growth, also according to post-mortem examinations.

Depiction and description of the experimental setup are provided in figure 5. After dissection of the animals at D30 post-vaccination, B16/F10-Ova tumors were isolated and weighted (A). Tumor volumes were measured using a digital caliper (B). Each animal is represented by one data point. All visible tumors were included in the analyses, even when very tiny. The percentage and number of tumor-bearing mice in each cohort are indicated in the figure legend.

Statistical analysis: Kruskal-Wallis test with Dunns post-test; (*) $p \leq 0.05$, (**) $p \leq 0.01$. Data are represented as Mean with SEM.

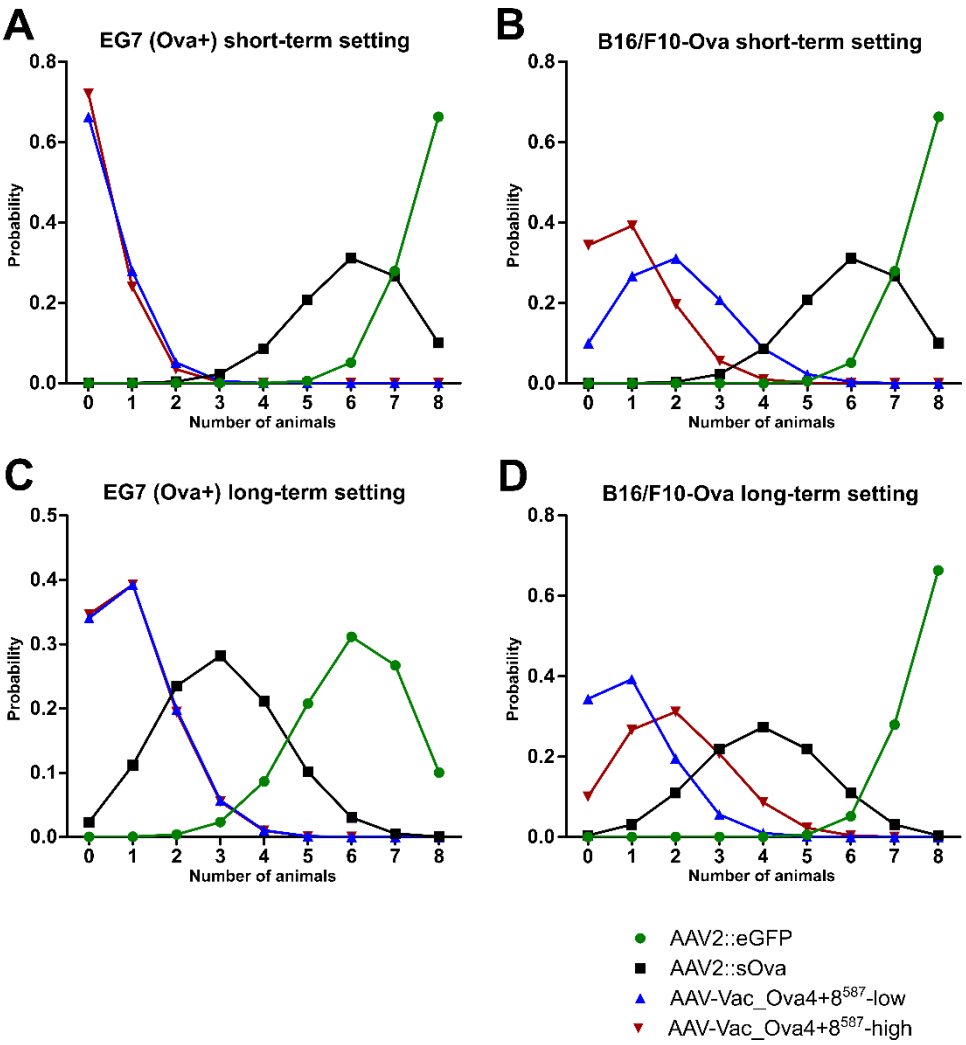

Figure S3: The probability of developing tumors is lower for mice that are vaccinated with the antigen-decorated AAV vector based vaccine.

Graphical depiction of the probability of developing tumors in the indicated treatment groups using the experimentally observed tumor frequency as the basis and projecting a possible outcome for repetition using a group size of 8 mice. Depiction and description of the experimental setup are provided in figures 4 (EG7 model in short-term setup; A), 5 (B16/F10-Ova model in short-term setup; B), 6 (EG7 model in long-term setup; C), and 7 (B16/F10-Ova model in long-term setup; D).

Vaccination with an unrelated vector (AAV2::eGFP) is the least effective, as indicated by probabilities in the range of 0.31 - 0.66 for finding tumors in 6 - 8 animals of the cohort (A-D). Similar to AAV2::eGFP, vaccination with AAV2::sOva is predicted to result in a probability of 0.31 for finding tumors in 6 of 8 mice in the short-term setting (A, B). For the long-term setting,

due to the effect of the sOva-expression in AAV2::sOva-transduced cells, the highest probabilities for finding tumors shift to fewer animal numbers (between 0.27 - 0.28 for finding tumors in 3 - 4 animals (C, D)). In contrast, for the antigen-decorated AAV vector-based vaccine, probability values range between 0.28 - 0.72 for finding tumors in 0 - 2 animals (short-term setting), or 0.31 - 0.39 for finding tumors in 1 - 2 animals (long-term setting).

Curves were generated by utilizing the function "dbinom()" in R:Base 4.3, with the experimentally observed frequency as probability.