Development and Characterization of Two OVA-Expressing Immunogenic Syngeneic Models: CT26-OVA and B16-OVA

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INTRODUCTION

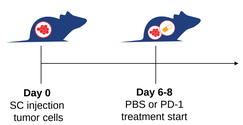
Syngeneic mouse tumor models have been the workhorse for investigating immuno-oncology (I/O) therapeutics. However, there are a limited number of such models available, and among them, only a few respond to immune checkpoint inhibitors (ICI). One potential reason for this ICI-insensitivity may be their intrinsic low-/non-immunogenicity. Chicken ovalbumin (OVA) is a protein known to be highly immunogenic, and engineered syngeneic cell lines expressing OVA could potentially render the cell more immunogenic, therefore providing further tools for I/O research. To this end, we have developed immunogenic variants of both colon cancer CT26.WT and melanoma B16-F10 syngeneic tumors by introducing the OVA transgene.

METHODS

Cell line transduction: CT26.WT and B16-F10 cells were transduced by lentiviral vector (pLVX-EF1a-IRES-PURO) with a chicken ovalbumin coding cDNA, and selected in culture in the presence of the antibiotic puromycin. OVA expression was confirmed by western blotting analysis and the cells denoted as CT26-OVA and B16-OVA.

***In vivo* experiments:** The engineered cells were evaluated for tumorigenicity following subcutaneous (SC) implantation in immune competent mice and for *in vivo* response to anti-PD-1 treatment. Mice bearing CT26-OVA and B16-OVA tumors were treated with anti-PD-1 antibodies (10mg/kg) or vehicle (intraperitoneally (i.p.) twice weekly for up to 3 weeks). Tumors were collected at termination and subjected to flow analysis for the frequency of total tumor-infiltrating CD8+ T cells and H-2Kb-restricted OVA tetramer (SIINFEKL)-positive cells (B16-OVA model only). The dosing regimen has been previously described, and the sampling plan is depicted below.

Fig 1. CT26-OVA and B16-OVA sampling timeline



B16-OVA	CT26-OVA
Model terminated ~**Day 17**	Model terminated ~**Day 40**
Tumor: OVA-specific antibodies	Spleen: FACS analysis
Tumor and spleen: FACS analysis	Spleen and lymph nodes: CD3+ T cells obtained for adoptive cell transfer (ACT)

RESULTS

OVA-expressing models showed slower SC tumor growth compared to the parental lines, which was probably due to immune-mediated rejection. Both B16-OVA and CT26-OVA models produced a higher sensitivity to anti-PD-1 treatment, superior to corresponding untransduced parental models. In addition, adoptive T cell transfer was conducted by isolating CD3+ T cells from the spleens of cured mice, and subsequently inoculating into naive mice. Tumor growth in ACT mice was prohibited, indicating the presence of tumor-specific memory T cells.

RESULTS

Fig 2. Evaluation of OVA expression in B16-OVA and CT26-OVA stable clones using western blot

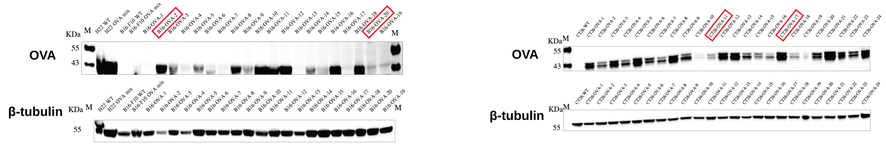
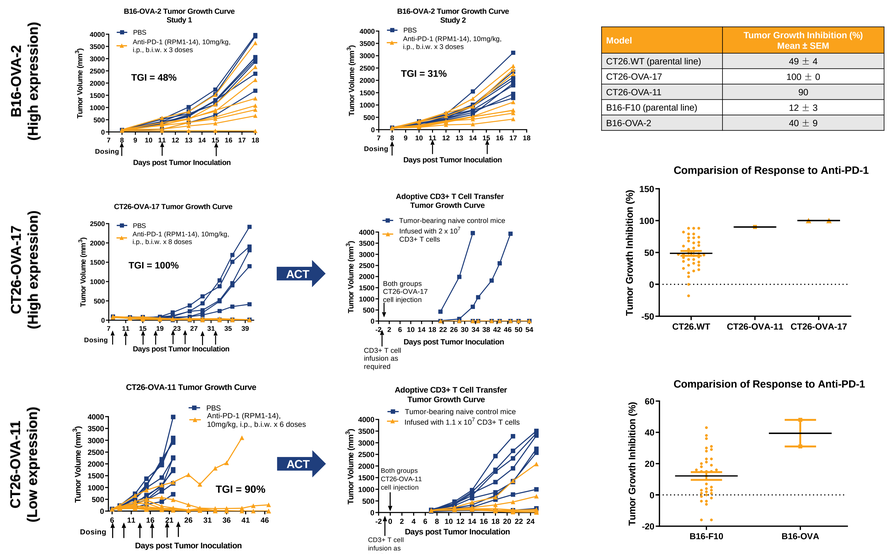


Fig 3. Characterization of *in vivo* growth kinetics, response to anti-PD-1 treatment, and TGI comparison between parental and OVA-expressing lines



Model	Tumor Growth Inhibition (%) Mean ± SEM
CT26.WT (parental line)	49 ± 4
CT26-OVA-17	100 ± 0
CT26-OVA-11	90
B16-F10 (parental line)	12 ± 3
B16-OVA-2	40 ± 9

Fig 4. Tumor concentration of OVA-specific antibodies, measured by ELISA, at the end of the B16-OVA model efficacy study

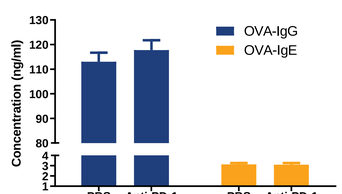


Fig 5. OVA-specific CD8+ T cell detection by tetramer assay at the end of the B16-OVA model efficacy study

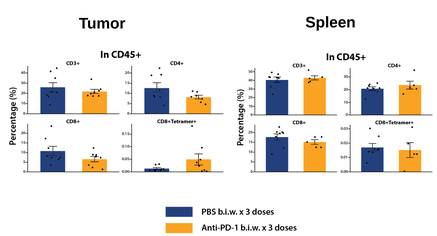
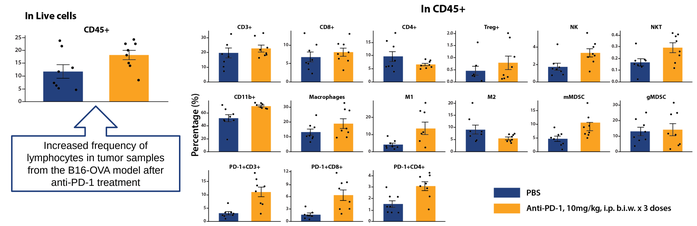
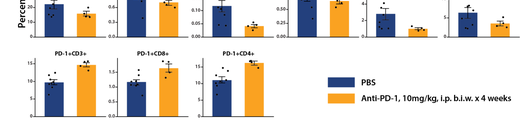


Fig 6. Phenotype analysis of tumor-infiltrating lymphocytes in the B16-OVA model at the end of the efficacy study



Increased frequency of lymphocytes in tumor samples from the B16-OVA model after anti-PD-1 treatment

Fig 7. Phenotype analysis of spleen in the CT26-OVA model (Clone 17) at the end of the efficacy study



SUMMARY

- OVA expression was confirmed by western blot assay on cells and the presence of OVA specific antibodies in tumors, which also confirmed that IgG1 is the predominant anti-OVA immunoglobulin isotype in tumors
- Both CT26-OVA and B16-OVA tumor cell lines are more immunogenic than their parental cell lines, reflected by the therapeutic effect of anti-PD-1 being enhanced in the OVA-expressing compared to parental models. Anti-PD-1 completely cured CT26-OVA tumor bearing mice and the CD3+ T cells from these tumor free mice were able to prohibit growth of CT26-OVA tumors in an ACT study, confirming the existence of long-lived memory CD8+ T cells
- Poor tumor take rate and big intra-tumor variance was observed in the OVA high-expression clone (#17), while the OVA low expression clone exhibits very good tumor take rate and relatively small variance, therefore the low expression clone (#11) is recommended for use in efficacy studies
- OVA-specific CD8+ T cells were barely detectable within tumor and spleen in the B16-OVA model by tetramer assay
- Upon anti-PD-1 treatment, an increased frequency of CD45+ cells in tumor samples from B16-OVA and an increased percentage of CD3+ T cells, CD8+ T cells, while decreased immunosuppressive myeloid cells (macrophage, MDSCs) in spleen samples from CT26-OVA were observed by flow cytometry analysis. Frequency of PD-1+CD3+ cells increased upon PD-1 treatment, which was observed in tumor and spleen samples from both models

REFERENCES

- Lelliott *et al*. A novel immunogenic mouse model of melanoma for the preclinical assessment of combination targeted and immune-based therapy. *Sci Rep* 2019;9(1):1225.