Synthetic Vaccine Particles Induce Durable CTL Responses and Demonstrate Synergy with Checkpoint Inhibitors for Anti-Tumor Therapy

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ABSTRACT

We have reported that synthetic vaccine particle (SVP) technology enables nanoparticle encapsulation of antigens and TLR agonists resulting in augmentation of immune responses with minimal systemic production of inflammatory cytokines. Here we evaluated several antigen formulations for their ability to induce CTL activity in combination with SVP-entrapped adjuvants. One of these formulations led to efficient cross-presentation of antigen resulting in rapid and durable CTL activity and providing for expansion of $CD8^+$ T cell effector memory cells locally and centrally after a single immunization. SVP treatment resulted in superior survival following therapeutic dosing in several mouse cancer models. Immunological memory in SVP-immunized animals persisted for 1-2 years. Treatment of tumors induced by cervical cancer model cell line TC-1 using a SVP-entrapped peptide led to suppression of tumor growth and a considerable delay in mortality. SVP-encapsulated phosphodiester CpG (PO-CpG) provided superior therapeutic benefit when compared to equal or higher amounts of free phosphorothioate backbone-modified CpG. Mutated HPV-16 oncogenic proteins encapsulated into SVP induced broad CTL activity and strong tumor suppression with the majority of mice surviving the challenge even when treatment was delayed until 14 days after tumor inoculation when nearly all animals presented with sizeable tumors. Similarly, treatment with SVP-encapsulated peptide derived from Trp2 protein generated cytotoxicity in vivo leading to prolonged survival in mice inoculated with B16 melanoma cells. Combining SVP treatment with anti-PD-L1 antibodies showed strong synergistic activity.

Two SVP formulations (F1 and F2) Induce Different Levels of Antigen-specific Cytotoxicity, Anti-tumor Activity and Immune Memory

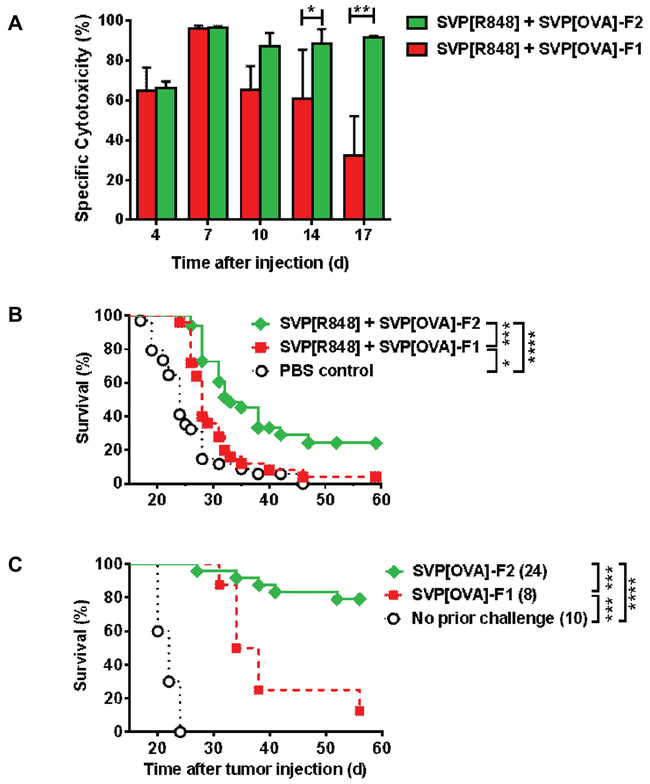
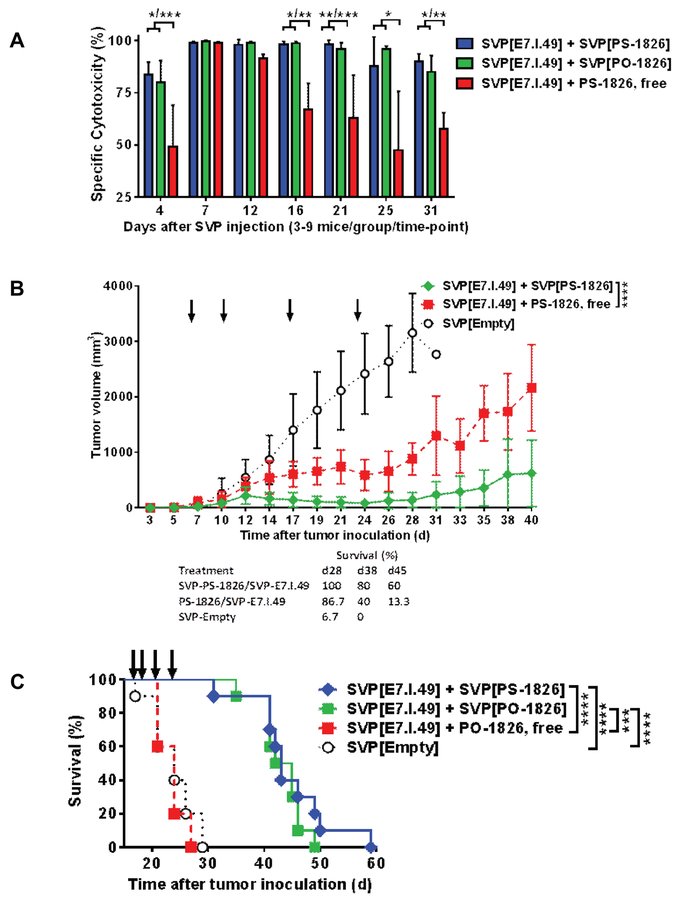


Figure 1. A. In vivo cytotoxicity. Animals (3-6 per time-point) were injected with SVP[OVA]-F1 or SVP[OVA]-F2 in combination with SVP[R848] and CTL activity measured (summary of 3 independent experiments). * p <0.05, ** p <0.01. **B.** Animals were treated (s.c., hind limb) with SVP[OVA]-F1 or SVP[OVA]-F2 in combination with SVP[R848] at days 3, 7, 14, and 21 after inoculation of EG.7-OVA tumor cells (summary of five independent experiments). **C.** SVP-treated animals surviving EG.7-OVA challenge were re-challenged with the same cells without further treatment. Summary of two independent experiments is shown. *** p<0.001, **** p<0.0001

SVP-induced CTL Activity Against Dominant CD8 Epitope of HPV-16 E7 Protein Corresponds to Therapeutic Benefit Against TC-1 Tumors



Treatment	Survival (%) d28	d38	d45
SVP-PS-1826/SVP-E7.I.49	100	80	60
PS-1826/SVP-E7.I.49	86.7	40	13.3
SVP-Empty	6.7	0	

Figure 2. A. Mice were immunized with SVP-entrapped E7.I.49 peptide (formulation 2) and SVP-entrapped or free forms of CpG ODN and in vivo CTL activity measured at different time-points. **B.** Treatment of TC-1 tumors by SVP[E7.I.49] combined with SVP-entrapped or free CpG PS-1826 (summary of three independent experiments). **C.** SVP-entrapped PS-1826 or PO-1826 vs. free PO-1826 (summary of two independent experiments). Treatments in **B** and **C** were administered on days 6, 10, 17 and 24 after tumor inoculation (shown by arrows). *** p < 0.001, **** p < 0.0001.

Treatment of TC-1 tumors by SVP-entrapped HPV Antigens and Adjuvants

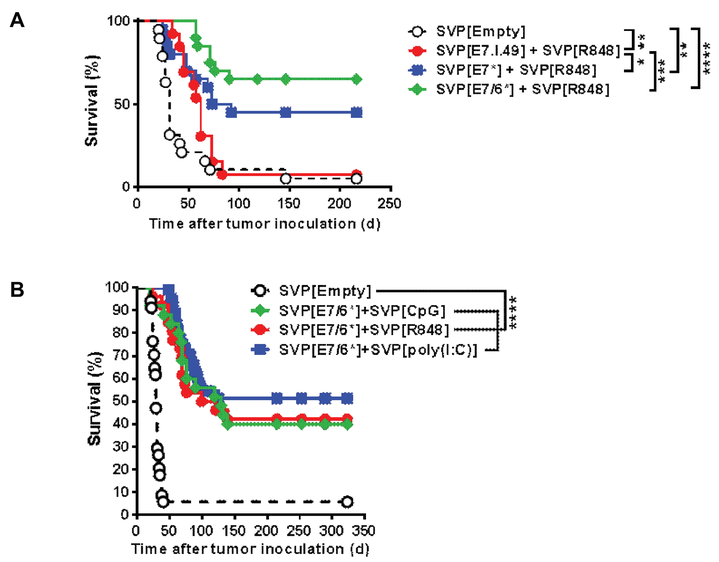


Figure 3. A, B: SVP-entrapped E7.I.49 or modified E7* and E7/E6* proteins combined with SVP[R848] (**A**) or (**B**) E7/E6* combined with SVP-entrapped R848, CpG or poly(I:C); treatments administered on days 10, 14, 21 and 28 (**A**) or 13/14, 17, 24 and 31 (**B**) after tumor inoculation. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001

SVP-induced CTL Activity Against Trp2(180-188) Peptide Corresponds to Therapeutic Benefit Against B16-F10

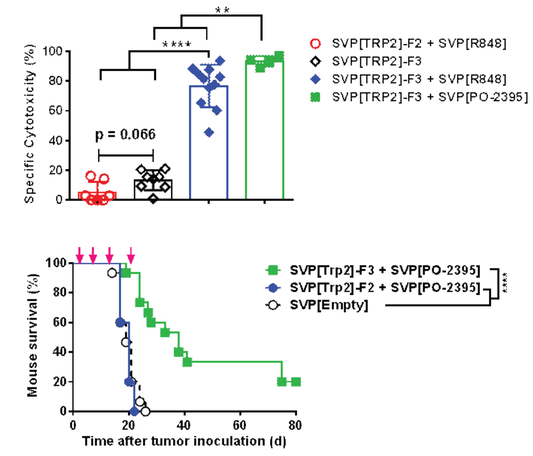


Figure 4. A. Mice were immunized with SVP-entrapped Trp2 peptide (formulations 2 or 3) and TLR agonists and in vivo CTL activity measured at day 7. **B.** Treatment of B16 tumors by SVP[Trp2]-F2 or F3 combined with SVP-entrapped CpG PO-2395 (summary of three independent experiments). SVP administered on days 3, 7, 14 and 21 after tumor injection (shown by arrows); **** p < 0.0001.

Synergy of SVP-induced Anti-tumor Activity and Immune Therapy Against Checkpoint Molecules

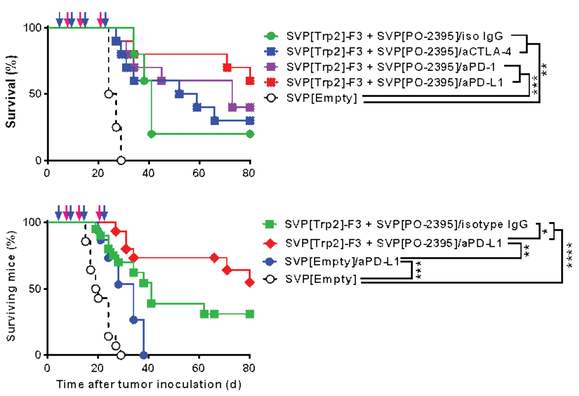


Figure 5. A. Mice were injected with B16 cells and treated with SVP[Trp2]-F3/SVP[PO-2395] or SVP[Empty] alone or combined with isotype IgG or antibodies to CTLA-4, PD-1 and PD-L1. **B.** The summary of three independent experiments with aPD-L1. SVP administered on days 3, 7, 14 and 21 (⬇), and therapeutic antibodies on days 6, 13 and 20 after tumor injection (⬇); * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

CONCLUSIONS

- Synthetic vaccine particle (SVP) technology enables nanoparticle encapsulation of antigens and TLR agonists resulting in augmentation of immune responses
- We identified several SVP formulations capable of producing potent CTL activity against model and disease-associated peptides and proteins
- These formulations directed efficient cross-presentation of antigen, induced rapid and durable CTL activity after a single immunization
- SVP therapeutic treatment resulted in superior survival in several mouse cancer models, including cervical cancer and melanoma cell lines TC-1 and B16-F10
- Mutated HPV-16 oncogenic proteins encapsulated into SVP were therapeutically active in combination with different adjuvants with majority of mice surviving TC-1 challenge even if treatment was initiated two weeks after tumor inoculation in mice presenting with sizeable tumors
- Treatment with SVP-encapsulated Trp2 peptide lead to prolonged survival in mice inoculated with B16-F10, especially in combination with anti-PD-L1 antibodies, with majority of animals surviving over 80 days after challenge

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