Therapeutic Application of Targeted Synthetic Vaccine Particles (*t*SVP) in HPV Cancer Model: Durable CTL Activity and Adjuvant Sparing

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ABSTRACT

We have earlier reported that targeted Synthetic Vaccine Particle (*t*SVP™) technology, which enables nanoparticle co-encapsulation of antigens and adjuvants leads to induction of strong humoral and cellular immune responses with minimal systemic production of inflammatory cytokines. Such cellular responses provided for significant activity against OVA-expressing tumor cells *in vivo* and generated long-term central and effector CTL memory. Here we extend these findings using tSVP immunization for treatment of TC-1 epithelial tumor, which expresses HPV-16 oncogene E7. Therapeutic treatment with *t*SVP[E7.I.49] containing the dominant MHC class I peptide from E7 protein in combination with TLR7/8 or 9 agonists led to profound suppression of tumor growth after subcutaneous or metastatic seeding and thus to a 3-5 week delay in animal mortality. A single injection of tSVP[E7.I.49] carrying TLR9 agonist CpG resulted in strong and consistent levels of CTL activity as early as 4 days and as late as 31 days after particle inoculation. tSVP-encapsulated CpG, *t*SVP[CpG], provided superior CTL induction and therapeutic benefit when compared to equal or higher amounts of free phosphorothioate-modified CpG. Moreover, *t*SVP-entrapment enabled use of native CpG containing a nuclease-labile phosphodiester backbone (PO-CpG). Notably, repeated multiple injections of therapeutically active *t*SVP[PO-CpG] as needed for maintenance of anti-tumor activity in vivo did not result in local tissue inflammation, which was observed when PS[CpG] was utilized.

Therapeutic Treatment of Modeled Lung Metastases with SVP

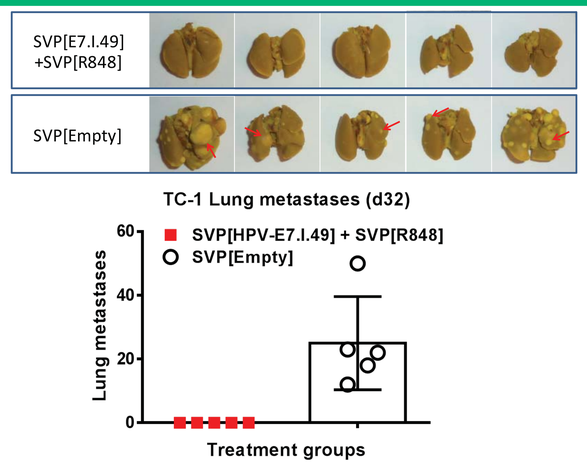

Figure 1. 5 × 10^4 TC-1 cells (transformed lung epithelium expressing HPV-16 oncogenes E6 and E7) were injected i.v. into C57BL/6 mice (5/group). Therapeutic treatment with SVP-entrapped E7.I.49 peptide and TLR7/8 agonist R848 was administered at days 3, 7, 14 & 21. On day 32 lungs were harvested and metastases counted.

SVP Entrapment of Both Antigen and Adjuvant is Essential for CTL Activity and Tumor Therapy

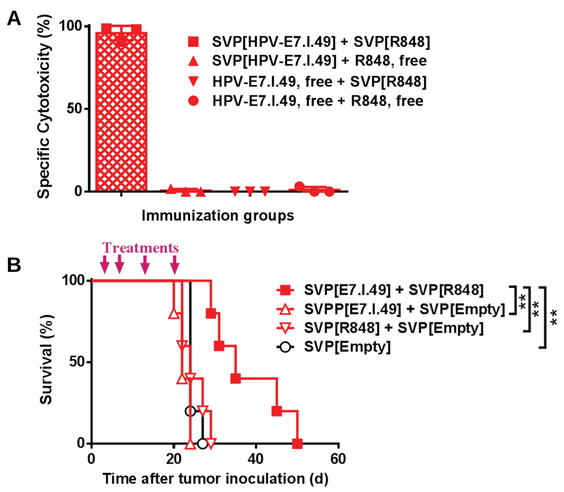

Figure 2. Combinations of SVP-entrapped and free E7.I.49 peptide (2 μg) and R848 (4.7 μg) were used in vivo for (A) CTL assay on day 7 after a single injection of naïve mice and (**B**) for therapeutic treatment on days 3, 7, 14 and 21 after s.c. inoculation of 5 × 10^4 TC-1 cells which result in development of solid tumors.

SVP[CpG] is More Potent than SVP[R848] in TC-1 Tumor Therapy

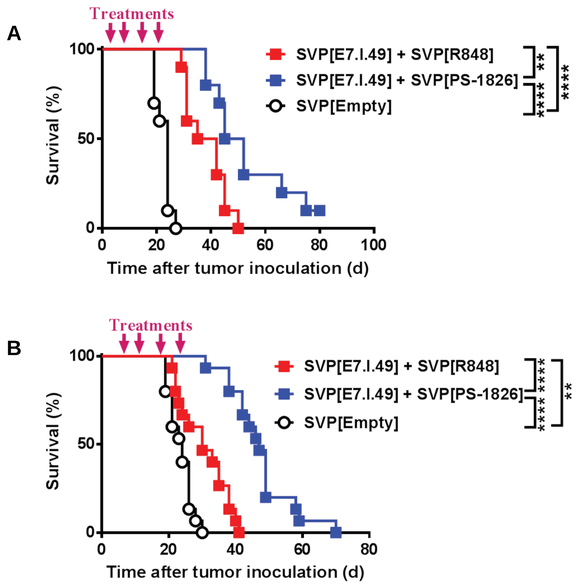

Figure 3. SVP-entrapped R848 or TLR9 agonist CpG were used for therapeutic treatment of solid TC-1 tumors starting at day 3 (**A**) or 6 (**B**) after tumor inoculation. Summary of three (**A**) or four (**B**) independent experiments is shown.

SVP-entrapped PS-CpG is More Therapeutically Potent than Free PS-CpG

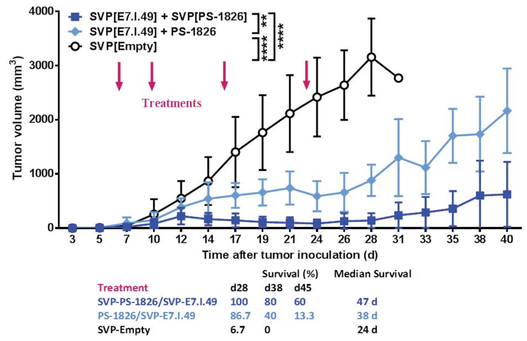

Figure 4. SVP-entrapped or free PS-CpG was used for treatment of solid TC-1 tumors starting at day 6 post tumor inoculation.

SVP Delivery Enables use of Native Phosphodiester (PO) CpG

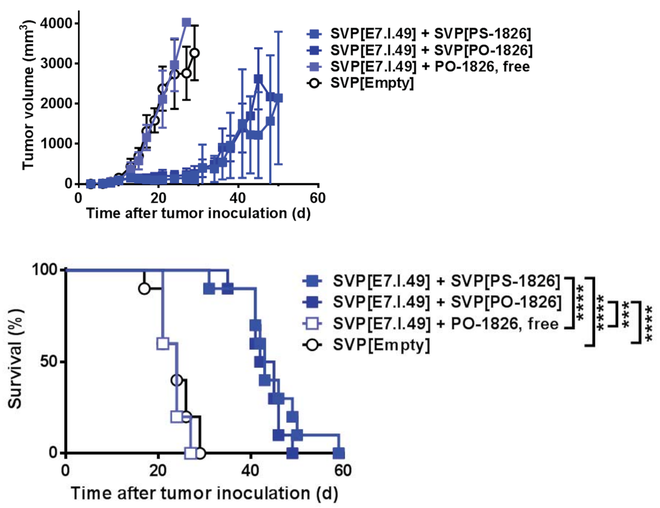

Figure 5. SVP-entrapped or free PO-CpG (as well as SVP-entrapped PS-CpG) was used for treatment of solid TC-1 tumors starting at day 6 post tumor inoculation. Summary of two independent experiments is shown.

PS- and PO-forms of SVP-entrapped CpG are Equally Potent in CTL Induction and Exceed the Activity of Free PS-CpG

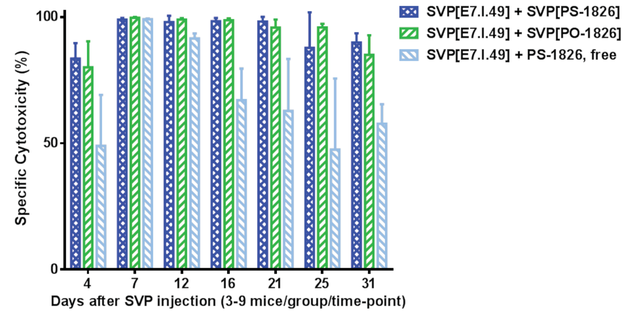

Figure 6. *In vivo* CTL activity against E7.I.49 after a single inoculation of SVP-entrapped PS- or PO-CpG or free PS-CpG. Summary of seven independent experiments.

Therapeutic Synergy of SVP and Antibodies Blocking Immune Exhaustion Signaling

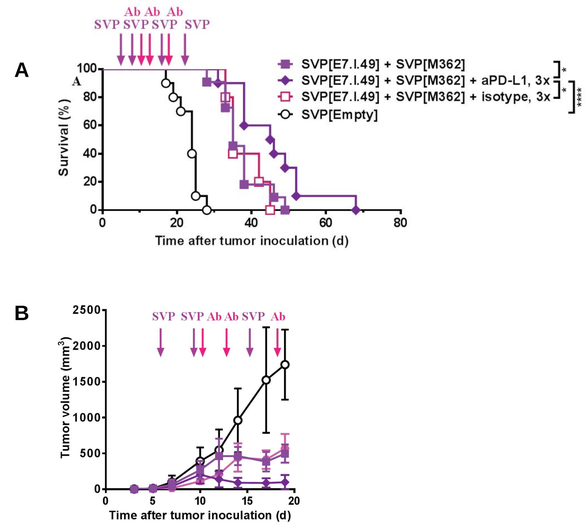

Figure 7. SVP-entrapped type C CpG M362 was used for treatment of solid TC-1 tumors with or w/o co-administration of monoclonal antibodies against PD-L1. **A** – survival; **B** – tumor volumes. No effect was observed when aPD-L1 alone was used (not presented). Summary of two independent experiments is shown.

CONCLUSIONS

1. *t*SVP™ nanoparticle was successfully used to encapsulate an adjuvant and a therapeutically-relevant peptide antigen derived from E7 oncogene of human papilloma virus (HPV-16)
2. Therapeutic treatment with *t*SVP[E7.I.49] in combination with TLR7/8 or 9 agonists led to profound suppression of E7-expressing TC-1 tumor growth after subcutaneous or metastatic seeding
3. A single injection of *t*SVP[E7.I.49] carrying TLR9 agonist CpG resulted in strong and consistent levels of CTL activity as early as 4 days and as late as 31 days after particle inoculation
4. *t*SVP-encapsulated CpG, *t*SVP[CpG], provided superior CTL induction and therapeutic benefit when compared to equal or higher amounts of free phosphorothioate-modified CpG
5. *t*SVP encapsulation enabled effective CTL induction by native CpG containing a nuclease-labile phosphodiester backbone (PO-CpG) without local tissue inflammation

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