Intratumoral administration of a TLR9-adjuvanted nanoparticle cancer vaccine stimulates more effective immunity in both injected and un-injected tumor sites compared to conventional route of administration

Naik E, Ying C, Milley R, Calacsan C, Chipman S, Tüting T, Coffman RL, and Guiducci C

Dynavax Technologies Corporation, Berkeley, California

Background and Objective of the Study

- Therapeutic success of a cancer vaccine requires substantial expansion of vaccine-primed CTL and efficient differentiation to polyfunctional CD8+ T cells capable of migrating from the site of vaccination to metastatic sites. Lack of migration from vaccine draining LN to tumor sites is likely a major limiting factor for the efficacy of cancer vaccines.
- We and others have shown that intratumoral injection of a CpG oligonucleotide TLR9 agonist, alone or with anti-PD-1, stimulates CD8-mediated anti-tumor responses leading to effective clearance of both injected and uninjected tumors. This has been shown in multiple mouse tumor models and is now being explored in clinical studies.
- This potent immunostimulatory activity suggests that addition of an optimally immunogenic form of one or more tumor antigens to an intratumoral CpG treatment regimen would lead to further enhancement of the anti-tumor T cell response.
- Dynavax has developed a highly efficient nanoparticle platform incorporating multiple copies of a CpG oligonucleotide and long tumor antigen peptides, both covalently linked to the sucrose polymer Ficoll.
- In this study we have evaluated the tumor itself as a potential site of T cell priming in response to intratumoral vaccination with these CpG-antigen nanoparticle conjugates.

Experimental Model

Figure 1. A) Components of the nanoparticulate vaccine containing the CpG oligonucleotide, DV230, and an ovalbumin (OVA) or melanocyte (Mel) antigen long peptide. The peptides and the CpG are covalently linked to Ficoll polysaccharide.

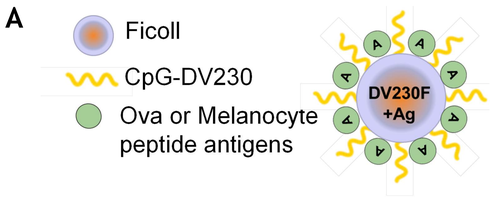
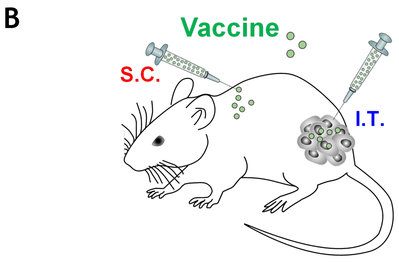


Figure 1: B) The vaccine is injected either in established tumors (I.T.) or at a subcutaneous site distant from the tumor (S.C.).



Results

Figure 2. Intratumoral vaccination induces superior anti-tumor activity, compared to distant site injection or to intratumoral DV230F (CpG-Ficoll without antigen)

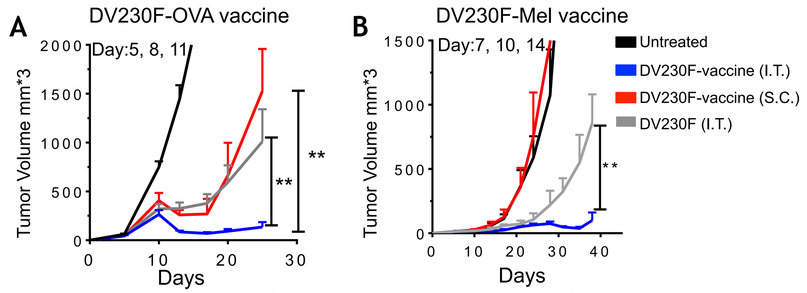


Fig. 2: (A-B) Change in tumor volume following intratumoral (IT) or distant site (SC) vaccination. (A) DV230F-OVA vaccine in EG7-OVA lymphoma model (B) DV230F-Mel vaccine in HCMEL-12 melanoma model. DV230F given IT was used to compared the antitumor activity of adjuvant alone versus adjuvant conjugated with antigen (DV230F-Vaccine).

Figure 3. Maximal antitumor activity requires co-delivery of antigen and CpG on the same particle

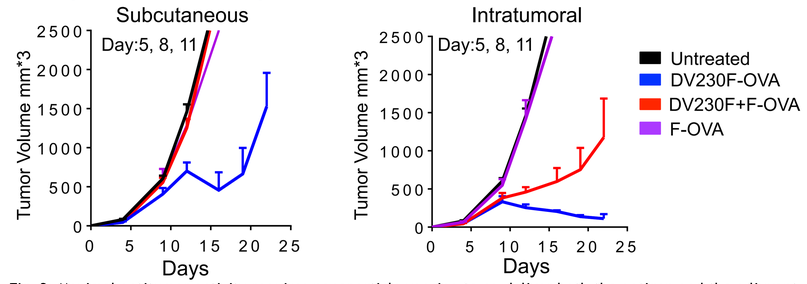


Fig. 3: Maximal antitumor activity requires nanoparticle vaccine to co-deliver both the antigen and the adjuvant. EG7OVA-bearing mice were evaluated for their response to DV230F and OVA antigen conjugated to the same nanoparticle (DV230F-OVA; blue) or DV230F and OVA antigen conjugated to different nanoparticles but co-administered in one injection (DV230-F+Ficoll-OVA; purple) or Ficoll-OVA administered alone (purple). Vaccine were administered either SC (left graph) or IT (right graph). Days of vaccination are indicated for each graph.

Figure 4. Intratumoral vaccination induces a more effective systemic CD8+T cell response

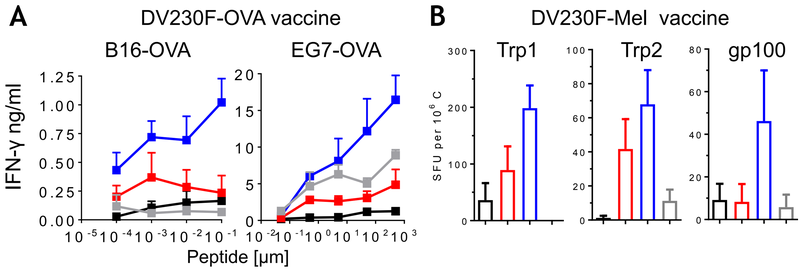


Fig. 4: B16-OVA or EG7OVA tumor bearing mice were immunized three times with DV230F-OVA (A) or DV230F-Mel (B) vaccine given I.T. or at distant site S.C. DV230-F alone was used as control (grey). Four days after last immunization, splenocytes were collected and stimulated with various concentrations of OVA peptide and the production of IFN-γ in the tissue culture supernatant assessed at 72 h by ELISA (A). In (B) IFN-γ positive T cells were detected by ELISPOT after stimulation with the indicated peptides.

Results

Figure 5: Intratumoral vaccination elicits more multifunctional antigen specific CD8⁺ T cells than distant site vaccination

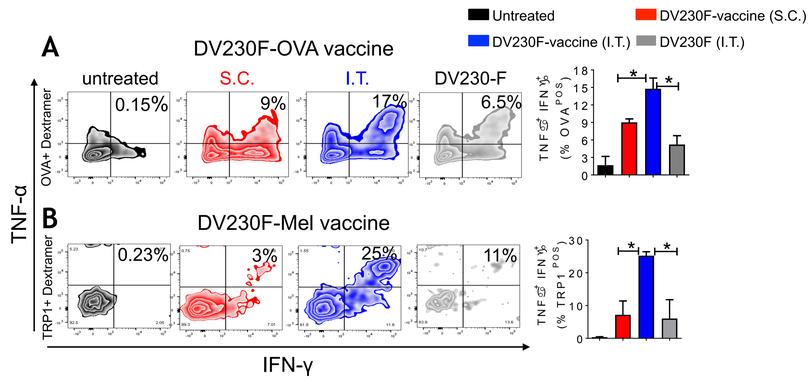


Fig. 5: Mice bearing established EG7OVA (A) or B16F10 (B) tumors were untreated or vaccinated three times with DV230F-OVA (A) or DV230F-Mel (B) vaccine given I.T. or at distant site (S.C.) on days 0, 3, and 7. DV230-F given I.T. was the control. TNF-α and IFN-γ production by $CD8^+OVA^+$ or $CD8^+Trp\text{-}1^+$ tumor infiltrating CD8 T cells was assessed by FACS 4 days after the last vaccination. Representative dot plots are shown on the left, and cumulative data of three independent experiments is shown on the right.

Figure 6: Intratumoral vaccination induces superior antitumor activity on distant site metastases

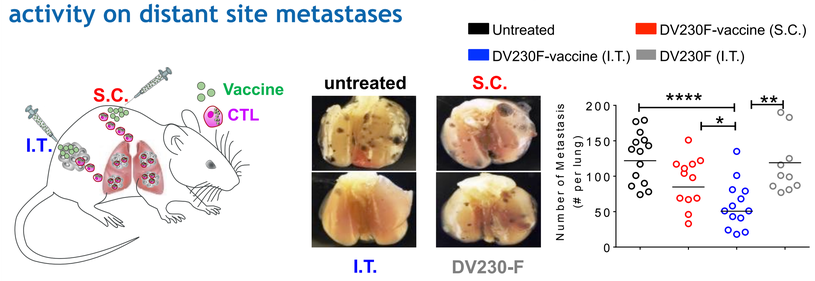


Fig. 6: Mice bearing both B16-OVA subcutaneous tumors and lung metastasis were vaccinated I.T. or S.C. at day 0, 4, 7, and 10. Seven days after the last immunization lungs were collected, and the number of macroscopic lung tumors was counted. The right graph shows cumulative data from two independent experiments.

Conclusions

- Intratumoral vaccination with a CpG-adjuvanted nanoparticle vaccine significantly increases the magnitude and quality of vaccine-primed CTL and enhances control of metastatic disease.
- Intratumoral vaccination substantially increases the magnitude of the systemic CTL response measured in the spleen and enhances CTL homing capacity to distant site tumors.
- A higher proportion of tumor-antigen specific CD8+ T cells generated by I.T. injection were polyfunctional, expressing both IFN-γ and TNF-α. They were also less exhausted as measured by decreased overall PD-1 levels, increased T bet, and the increased number of PD-1+ CD8 T cells that retained cytotoxicity and proliferative capacity (not shown here).