Development of an Inhaled TLR9 Agonist for the Immunotherapy of Lung Cancer

Gallotta M, Assi H, Janatpour M, Coffman RL and Guiducci C

Dynavax Technologies Corporation, Berkeley, California

Background

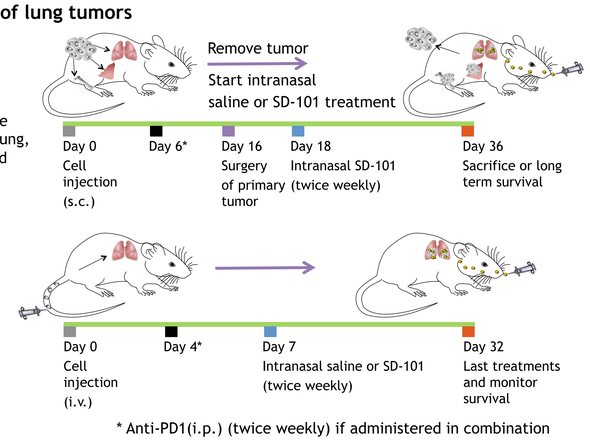
- Lung cancer is the most common cause of cancer-related death in the world.
- Anti-PD-1 antibodies have been approved as first line therapy (Pembrolizumab) in patients with metastatic NSCLC having high PD-L1 expression and as second-line therapy for all metastatic NSCLC patients. However response rates are low, with only a 40% ORR in the PD-L1 high patient group and a 20% ORR among all patients. This suggests that combination therapies will be needed to fully unlock the potential of this line of therapy.
- The elimination of cancer cells by PD-1 blockade relies on unlocking the cytolytic capacity of tumor-specific CTL. Thus, the success of anti-PD-1 therapy correlates with the potential immunogenicity of the tumor and the presence of pre-existing infiltrates of T cells specific for tumor antigens.
- The current challenge for increasing the rate of response to PD-1 blockade therapies is to increase the level of CTL infiltrating the tumor by priming de-novo responses and by rendering the tumor more permissive to T cell infiltration.
- We have demonstrated that resistance to PD-1 blockade in solid tumors can be reversed by intratumoral injection of a CpG oligonucleotide, SD-101. SD-101 is a TLR9 agonist that induces high levels of Type I IFN, induces maturation of antigen presenting cells, and stimulates and expands tumor-specific T cells (*Wang S, Campos J et al. PNAS 2016*).
- Lung tumors are not suited for repeated intratumoral injections; however, delivery focused on tumor sites in the lung can be accomplished by inhalation of a TLR9 agonist.

Rationale and Objective of the Study

- The purpose for pulmonary administration of SD-101 is to achieve immunostimulatory concentrations in or near the tumor and tumor-draining lymph nodes. This localized administration will achieve active doses at the optimum site for enhancing anti-tumor immune responses while minimizing unnecessary systemic stimulation.
- The objective of this study was to test the ability of SD-101, delivered intranasally, to induce an effective antitumor response against lung tumors and to assess synergy with anti-PD1 treatment.

Experimental Model

Figure 1: Mouse models of lung tumors



* Anti-PD1(i.p.) (twice weekly) if administered in combination

Results

Intranasal SD-101 induces a Type I IFN gene signature in tumor bearing lungs and controls lung tumors and distant site metastasis

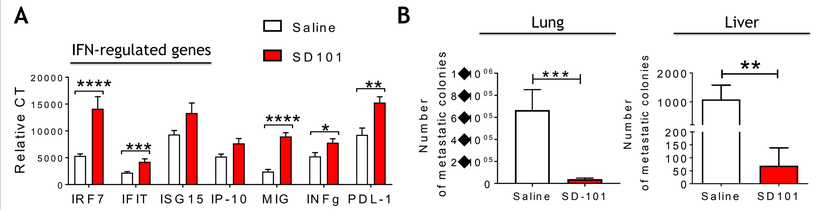


Figure 2: (A) Mice were injected s.c. with 4T1 cells and the schedule of treatment was as in Figure 1. Gene expression level was analyzed by TAQMAN performed on lung single cell suspension. Data are presented as relative threshold cycle (CT) of the gene of interest relative to Ubiquitin. (B) Tumor burden in lung and liver was assessed using a clonogenic assay. Differences were examined using the Mann-Whitney U test.

Intranasal SD-101 induces CD8+ T cell recruitment in "cold" lung tumors and the formation of tertiary lymphoid structures (TLS) adjacent to the tumor

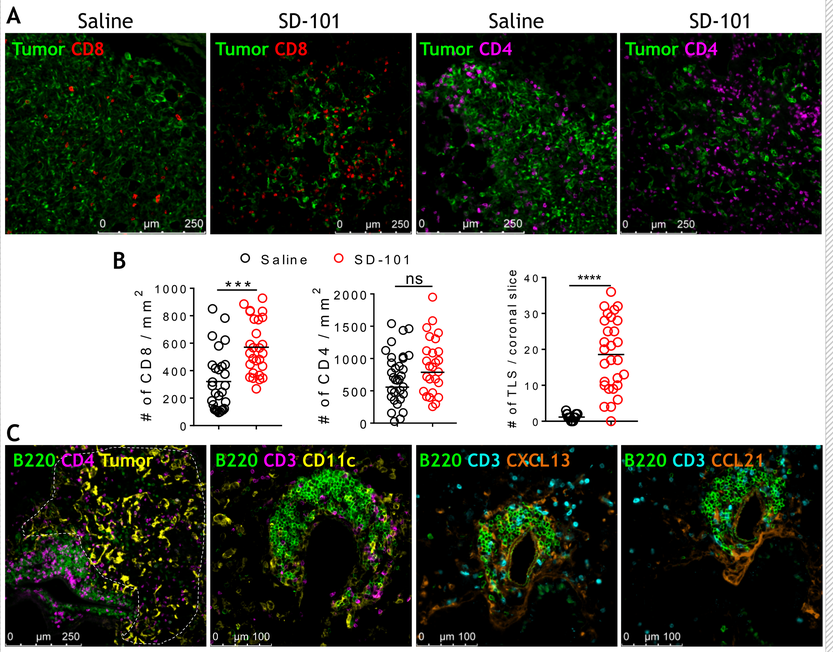


Figure 3: (A) Representative lung images showing 4T1 tumor cells (green) infiltrated with CD8+ (red) or CD4+ (purple) T cells. Tumors from mice treated with SD-101 are significantly more infiltrated with T cells if compared to saline. (B) The number of CD8+ or CD4+ T cells infiltrating 4T1 lung tumors and the number of tertiary lymphoid structures (TLS) adjacent to tumors, was determined by immunofluorescence. (C) *Left*: Representative images of lymphoid aggregates characterized by B cells, T cells and DCs aggregates. *Right*: Representative images showing co-localization of chemokines CXCL13 and CCL21 in the lymphoid structures.

Treatment with SD-101 in combination with PD-1 blockade reduces lung tumors & results in long term survival of mice

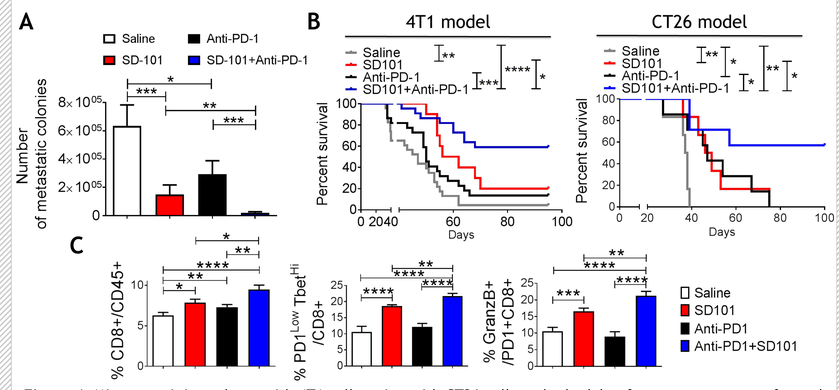


Figure 4: Mice were injected s.c. with 4T1 cells or i.v. with CT26 cells and schedule of treatments was performed as in Figure 1. (A) For the 4T1 model, lung tumor burden was assessed using a clonogenic assay. (B) Long term survival of treated mice demonstrates that mice treated with the combination of SD-101 plus anti-PD-1 rejected lung tumors and disseminated metastasis (in the case of the 4T1 model). Differences in survival were examined using Log-rank (Mantel-Cox) test. (C) Lungs were collected 2 days after the last treatment and processed to obtain a single cell suspension and analyzed by flow cytometry. SD-101 plus Anti-PD1 treatment results in higher infiltration of CD8 T cells associated with increased effector ability compared to controls.

Both CD8+ and CD4+ T cells are required for the efficacy of SD-101 plus Anti-PD-1 treatment of lung tumors

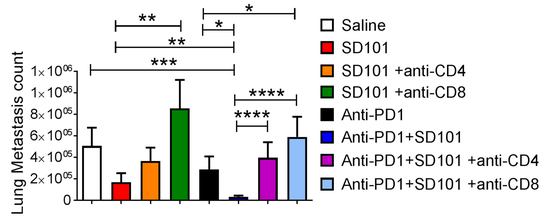


Figure 5: Mice were injected s.c. with 4T1 cells and schedule of treatments was performed as in Figure 1. Depletion of CD4 and CD8 T cells were started the day before the start of SD-101 treatment and given every 3 days until the end of SD-101 treatment. Lung tumor burden was assessed using a clonogenic assay. Differences were examined using Mann-Whitney U test (*P < 0.05, **P < 0.01, ***P < 0.001; ****P < 0.0001).

Conclusions

- Efficacy studies in lung tumor models showed significant decreases in lung tumor burden in animals treated with intranasal SD-101 alone or in combination with PD-1 blockade. The combination of SD-101 plus anti-PD-1 led to a significant increase in the long term survival of treated animals.
- SD-101, alone or in combination with anti-PD-1, demonstrated a strong immunomodulatory effect on the lung microenvironment including an increase in the number of effector T cells infiltrating the tumors and the formation of lymphoid structures adjacent to the tumors.
- This study suggests that combination treatment with an inhaled TLR9 agonist could significantly enhance the efficacy of PD-1 blockade, which is rapidly becoming standard of care for the immunotherapy of lung cancer.
- Dynavax has developed a novel CpG oligonucleotide - DV281 - that is optimized for aerosol delivery and plans to initiate clinical studies in NSCLC patients in combination with anti-PD-1 therapies this year.