Background  PD-1/PD-L1 checkpoint blockade has led to improvements in clinical outcomes in various advanced cancers. However, response rates remain low and most patients eventually present with intrinsic resistance to PD-1/PD-L1 inhibitors. There is a great need to elucidate tumor-intrinsic mechanisms involved in immune-evasion to improve patient survival. Elevated levels of soluble PD-L1 (sPD-L1) can be detected in peripheral blood and are associated with poor survival in many cancer indications. sPD-L1 secreted by tumors retains its immunosuppressive function by interacting with PD-1 expressing cells. Furthermore, sPD-L1 is not as effectively neutralized by therapeutic anti-PD-L1 antibodies as PD-L1 expressed on the cell surface. Therefore, targeting sPD-L1 represents a promising therapeutic approach to improve the efficacy of PD-1/PD-L1 inhibitors and restore antitumor immunity. Herein, we evaluated the efficacy of engineered NaNot® nanoparticles in depleting sPD-L1 and restoring antitumor immunity in humanized murine tumor models.

Methods  Proprietary NaNot® nanoparticles were conjugated with a monoclonal antibody against human PD-L1 (H1A clone). The capture efficiency of NaNot® against sPD-L1 was validated using recombinant PD-L1 protein, sPD-L1-containing cell culture media and patient plasma. To evaluate the antitumor activity of NaNot®, humanized PD-L1/PD-1 mice were inoculated with human PD-L1 secreting E0771 (triple negative breast cancer) or human PD-L1 non-secreting B16F10 (melanoma) tumor cells. When tumors reached ~125 mm³, mice were treated with intravenous PBS or NaNot® every other day for 5 total injections. Cytometry by time-of-flight (CyTOF) and flow cytometry were used to determine immune cell phenotypes within tumors and spleens in response to NaNot®.

Results  NaNot® captured 100% of sPD-L1 (concentration range: 0.16–3.21 ng/ml) in patient plasma. Similar to in vitro results, a significant depletion of sPD-L1 was observed in vivo after a single tail vein injection of NaNot® (p=0.025). Following 5 injections with NaNot®, median tumor size was 307 mm³ compared to 877 mm³ for the PBS-treated arm (p=0.001). Treatment with NaNot® was associated with higher CD8 T cell tumor infiltration compared to PBS. Immunophenotyping of tumors and spleens revealed a reduction in exhausted CD8 T cells and regulatory T cells in NaNot® treated mice. In PD-L1 non-secreting B16F10 tumor-bearing mice, no difference in tumor growth was observed between NaNot® and PBS groups supporting the selectivity of NaNot® for sPD-L1.

Conclusions  Our work demonstrates that selective capture of tumor-derived sPD-L1 with a novel nanotherapeutic platform can elicit local and systemic antitumor immunity.

Ethics Approval  All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC). All patient samples were collected with approved Mayo Clinic Institutional Review Board (IRB #20–003367) and in accordance with the World Medical Association’s Declaration of Helsinki.