A NOVEL IMMUNOSTIMULATORY TLR7/8 AGONIST IS CURATIVE AS A MONOTHERAPY IN LEWIS LUNG CARCINOMA AND SYNERGIZES WITH ANTI-PD-1 IN B16F10 AND MC38 TUMOR MODELS

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Background Over the past 130 years, the field of immunotherapy has progressed to the use of cytokines and of immune checkpoint inhibitors, namely anti-PD-1 and anti-CTLA4. Despite these advances, response rates remain stubbornly low. New immunotherapies that stimulate the immune system in different ways and can synergize with and expand the population of patients who respond to existing immunotherapies are urgently needed. Inimmune has developed and evaluated a novel TLR7/8 agonist as an immunotherapy for cancer.

Methods To test the activity of our novel TLR7/8 agonist, fresh human peripheral blood mononuclear cells (hPBMCs) were collected from healthy adult donors and stimulated with TLR7/8 agonist for 24 hours. Supernatants were then analyzed for cytokine production by ELISA. For pre-clinical murine studies to determine the efficacy of our novel TLR7/8 agonist as a cancer immunotherapy, LLC, B16F10, or MC38 tumor cells were implanted in the flank of C57BL/6 mice. Our novel TLR7/8 agonist was injected IV, SC, IP, or IT on days 5 and 12 post-implantation. For experiments where anti-PD-1 was used, anti-PD-1 was administered via IP injection on days 3, 6, and 9 post-implantation. To investigate changes in immune cell populations in the TME post-treatment, B16F10 tumors were treated with TLR7/8 agonist on days 5 and 12 post-implantation, or anti-PD-1 on days 3, 6, and 9 post-implantation, or both, and were harvested on day 12 post-implantation. Untreated tumors were harvested on day 12 post-implantation as controls. Tumors were disaggregated to single cell suspensions, stained with phenotyping antibodies, and analyzed by flow cytometry.

Results The lead formulation of our novel TLR7/8 agonist was able to eliminate Lewis Lung Carcinoma (LLC) flank tumors in 80% of mice after just two treatments. Further, as a monotherapy, our novel TLR7/8 agonist slowed growth of MC38 and B16F10 flank tumors and synergized with anti-PD-1 therapy, leading to a 100% rejection rate in MC38 flank tumors and a 75-100% rejection rate in B16F10 tumors when both treatments were used in combination depending on the route of administration and dose schedule. Mechanistically, the combination of our TLR7/8 agonist plus anti-PD-1 lead to increases in monocytes, B cells, and CD8 T cell populations in the TME of B16F10 flank tumors when compared to treatment with TLR7/8 agonist or anti-PD-1 alone.

Conclusions As we advance our novel synthetic TLR7/8 agonist to Phase 1 clinical trials, these data suggest potential efficacy as a monotherapy or in combination with checkpoint inhibitors in patients with solid tumors.