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A Potent STING Agonist Induces Endothelial PD-L1 and Enhances Antitumor Efficacy of a Novel PD-L1/PD-L2 Antibody

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Background

Antibody therapies targeting the T cell co-inhibitory receptor programmed cell death-1 (PD-1) and its ligand programmed death-1 ligand 1 (PD-L1) have become a pillar of modern oncology, though their effectiveness is often limited to tumors with pre-existing immune infiltration. To address the challenges posed by the immunosuppressive tumor microenvironment (TME), we developed 8803 (IMGS-203), an activator of stimulator of interferon genes (STING), evaluated its efficacy in combination with a novel anti-PD-L1/PD-L2 antibody (27907),[1] and reported that the combination therapy resulted in curative responses in check-point refractory tumor models.[2,3] Here, we analyzed the TME from tumors treated locally with 8803 and systemically with the dual specific antibody 27907 to elucidate the mechanism of action via immunohistochemistry (IHC) and flow cytometry analysis.

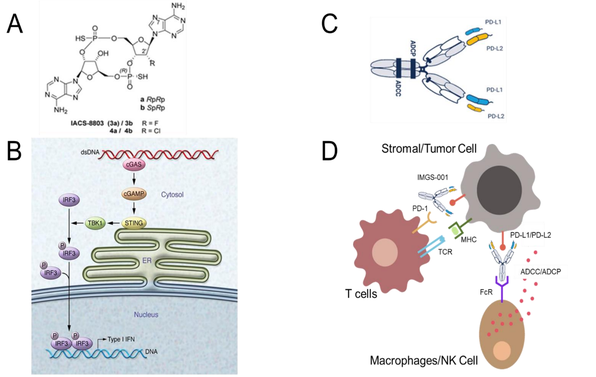

Figure 1. STING agonist 8803, dual functioning 27907 antibody and their mechanisms of action. (**A**) Structure of the 2',3'-linked phosphorothioate diadenosine monophosphate (2',3'-S2-CDA) STING agonists 8803.[4] (**B**) Overview of the STING pathway.[5] STING activation leads to activation of TBK1 and IRF3, leading to production of type I IFNs. (**C**) Graphical representation of 27907 monoclonal antibody. Each antigen binding site is capable of binding either PD-L1 or PD-L2 and blocking their interactions with PD-1. Point mutations within the Fc region enhance the cell-mediated effector functions of the antibody, including antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC). (**D**) Mechanism of Action of 27907.

Methods

8803 was tested in vivo in combination with 27907 using murine melanoma B16F10-PDL2, expressing both PD-L1 and PD-L2 and mammary adenocarcinoma TS/A, expressing only PD-L1. Mice with established tumors received intratumoral 8803 (10 μg/injection (twice for B16F10-PDL2 and 3 times for TS/A)) and systemic 27907 (10 mg/kg, twice weekly for 1.5–2 weeks). Tumors were harvested 18–20 days post-implantation and analyzed via hematoxylin and eosin (H&E) staining, IHC, and fluorescence microscopy to assess necrosis, the TME, and vascularization. For histopathological assessment, formalin-fixed, paraffin-embedded tumor sections from B16F10-PDL2 and TS/A models were stained with H&E. Whole-slide images were analyzed using NPDview software to quantify necrotic areas as a percentage of total tumor area.

For flow cytometry, tumors were freshly excised, minced, and enzymatically digested with collagenases and DNase to generate single-cell suspensions. Cells were washed, stained, and analyzed using a NovoCyte cytometer.

In vitro, human (HUVEC) and mouse (bEnd.3) endothelial cells were treated with 8803 was administered at the indicated concentrations for 24 hours. Cytokine in supernatants was evaluated via reporter assay or ELISA, and PD-L1 surface expression was evaluated by flow cytometry. For cytotoxicity assays, mouse bone marrow (BM) or human peripheral blood mononuclear cells (PBMCs) were pre-treated with GM-CSF, IL-2, and IL-6 and co-cultured with endothelial cells with constitutive luciferase expression. Treatments included 8803, 27907, or the combination. Endothelial cell viability was measured to assess immune-mediated cytotoxicity (expressed as relative luminescence activity).

Results

8803 and 27907 Combination Treatment Inhibits Tumor Growth and Improves Survival in Cold Tumor Models

The anti-tumor efficacy of the STING agonist 8803 and the anti-PD-L1/PD-L2 antibody 27907 was evaluated in the syngeneic B16F10-PDL2 model as previously described.[3] Treatment with 8803 and 27907 significantly inhibited tumor growth and enhanced survival, with 60% of animals alive by the end of the study (Fig.2A). The combination was also tested in the TS/A syngeneic tumor model (Fig. 2B). Treatment commenced when tumors reached a mean diameter of 6-8 mm, with 8803 administered intratumorally (10 μg/ per injection, 3 times at 3–4-day intervals) and 27907 (10 mg/kg) intraperitoneally twice a week for 2 weeks. The combination treatment resulted in the best tumor growth inhibition.

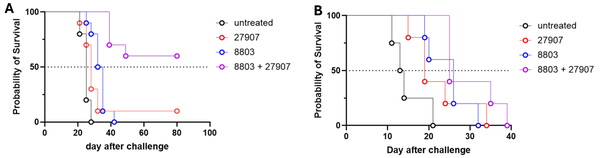

Figure 2. Kaplan Meier plots showing overall survival of B16F10-PDL2 (A) and TS/A (B) tumor-bearing mice treated with the combination of 8803 and 27907. The combination treatment resulted in better overall survival compared to the individual treatments.

Combination Therapy With 8803 and 27907 Increases Tumor-Infiltrating Immune Cell Activation and Upregulates PD-L1 Expression

B16F10-PDL2 tumor-bearing mice were treated with the STING agonist 8803 and anti–PD-L1/PD-L2 antibody 27907, as previously described. Tumors were analyzed ex vivo by flow cytometry (Fig. 3). The combination therapy led to a significant rise in CD45+ cells within the TME, and elevated levels of CD3+ T cells and CD44+PD-1+ CD8+ T cells, indicative of robust T-cell activation. A decrease in CD206+ tumor-associated macrophages (TAMS) was also observed, suggesting a shift towards a less immunosuppressive phenotype. Analysis of PD-L1 expression showed that 27907 mitigates PD-L1 upregulation coinciding with inflammatory response induced by 8803.

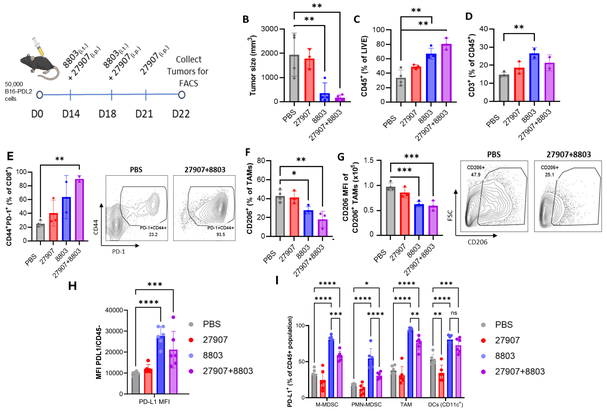

Figure 3. Characterization of the TME in B16F10-PDL2 tumors following treatment with 8803 in combination with 27907. (**A**) Schematic representation of the treatment and dosing schedule. (**B**) Tumor volume at the time of tissue collection. (**C–F**) Analysis of immune cell subsets within the tumor: CD45+ leukocytes (C), CD3+ T cells (D), CD44+PD-1+ CD8+ T cells (E), and CD206+ tumor-associated macrophages (TAMs) (F). (**G**) Mean fluorescence intensity (MFI) of CD206 expression on TAMs. (**H-I**) PD-L1 expression on CD45- (H) and CD45+ (I) cells within the TME.

Tumor Necrobiotic Effect of 8803 In Vivo Is Potentiated By 27907

To assess treatment-induced tumor necrobiosis, B16F10-PDL2 and TS/A tumor-bearing mice were treated with 8803 alone or in combination with 27907, as outlined in Figure 3A. H&E staining of excised tumors revealed enhanced necrobiotic changes in the combination-treated groups. Representative sections (×40 magnification) showed. The expansion of necrotic regions observed in both groups, 8803 and the combination 8803/27907, induced tumor-destructive effects compared to the control

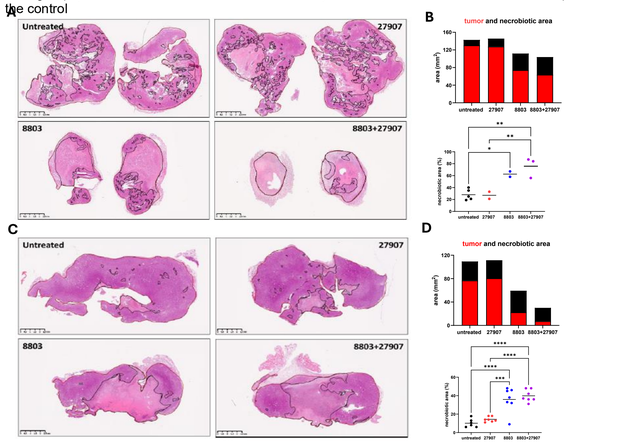

Fig.4. Histopathological Analysis of B16F10-PDL2 and TS/A tumors treated with 8803 and 27907. Representative H&E-stained sections of B16F10-PDL2 (A–B, top) and TS/A tumors (C–D, bottom) following treatment as described in Figure 3A. Treated tumors exhibited increased necrosis. (B,D) Quantification of necrotic areas was performed using NPDview software on whole-slide scanned images. Data represent necrotic area as a percentage of total tumor area.

IHC Staining of TME in B16F10-PDL2 and TS/A Tumors Treated with 8803 and 27907

Combined 8803 and 27907 treatment reshaped the TME in both B16F10-PDL2 and TS/A models. In B16F10-PDL2 tumors, the treatment increased $CD3^+$ T-cell infiltration, reduced $Foxp3^+$ regulatory T cells, and decreased $CD206^+$ M2-like macrophages, suggesting a shift toward an immunostimulatory state. TS/A tumors showed similar immune changes, though with less impact on $CD206^+$ cells.

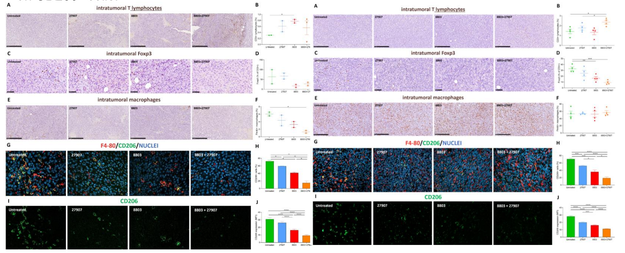

Figure 5. IHC analysis of tumor immune cell infiltration after 8803 and 27907 treatment. In B16F10-PDL2 (left panels), combination treatment increased CD3+ T cell infiltration, reduced Foxp3 expression, and decreased CD206 expression, with similar trends in TS/A (right panels) tumors.

Combination Therapy Increases CD62p Expression on Endothelium Cells and Platelets

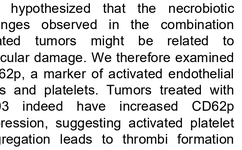

Figure 6. CD62p Expression in B16F10-PDL2 (A) and TS/A (B) Tumors. CD62p was detected on endothelial cells and platelets following 8803 and combination treatment, indicating vascular damage.

We hypothesized that the necrobiotic changes observed in the combination treated tumors might be related to vascular damage. We therefore examined CD62p, a marker of activated endothelial cells and platelets. Tumors treated with 8803 indeed have increased CD62p expression, suggesting activated platelet aggregation leads to thrombi formation and necrosis.

8803 Induces PD-L1 Upregulation And, In Combination With 27907, Enhances Immune-Mediated Cytotoxicity In Mouse Endothelial Cells

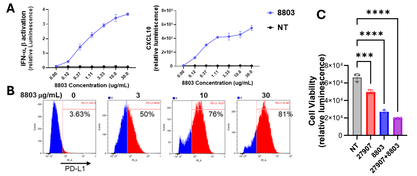

Figure 7. 8803 activates mouse endothelial cells. (A) STING pathway activation in bEnd.3 cells, demonstrated by cytokine induction. (B) PD-L1 upregulation in bEnd.3 cells following 8803 treatment. (C) Increased killing of bEnd.3 cells when co-cultured with activated PBMC cells and treated with 8803, 27907, or both compared to control.

Treatment of bEnd.3 cells with 8803 induced IFNs and CXCL10 secretion and upregulated PD-L1 expression (Fig. A-B). Co-culture with pre-activated PBMC cells enhanced immune-mediated killing, further potentiated by 27907 (Fig. C) Importantly, 8803 treatment did not impair bEnd.3 cell viability, supporting its role as a selective immune activator in the TME (data not shown).

8803 Induces PD-L1 Upregulation And, In Combination With 27907, Enhances Immune-Mediated Cytotoxicity In Human Endothelial Cells

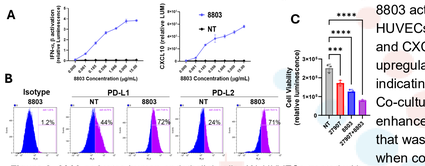

Figure 8. 8803 activates human endothelial cells. (**A**) HUVECs treated with 8803 secrete IFNs and CXCL10. (**B**) Flow cytometric analysis of PD-L1 surface expression on HUVECs. (**C**) Immune-mediated cytotoxicity assay showing increased killing of HUVECs co-cultured with human PBMCs in the presence of 8803, 27907, or their combination.

8803 activated the STING pathway in HUVECs, leading to the secretion of IFNs and CXCL10 (Fig. A). Additionally, 8803 upregulated PD-L1 expression (Fig. B), indicating an immunomodulatory effect. Co-culturing HUVECs with human PBMCs enhanced immune cytotoxicity, an effect that was further potentiated by 27907 when compared to control (Fig. C). Importantly, direct exposure to 8803 did not compromise endothelial cell viability, further supporting its function as an immune modulator (data not shown).

Conclusions

We have shown that the combination of 8803, given IT, with 27907, given systemically, induces profound tumor responses in immune-excluded melanoma (B16F10-PDL2) and mammary adenocarcinoma (TS/A) tumor models resulting in significant necrobiotic areas within the tumors, increased infiltration of immune cells (CD45+), reduction of M2 macrophages (CD206+), and influx of T cell populations (CD3+). Furthermore, intratumoral (IT) administration of 8803 or 8803/27907 significantly upregulated PD-L1 expression in tumor microenvironment (TME) cells, as demonstrated by increased CD62p expression on endothelial cells within the treated tumors. These findings underscore the potent therapeutic synergy of 8803 with 27907 and support their further development for clinical applications.

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