

increased intratumoral granzyme B secreting CD8+ T cells. Additionally, targeting CD47 within organoids increased IFN $\gamma$  and granzyme B released, indicating enhanced CD8+ T cell cytolytic capacity. Differential cellular bioenergetics was observed between cancer and T cells suggesting a shift in metabolism in the tumor microenvironment. CD47 targeted T cells had an increased glycolytic rate compared to WT T cells. Conversely CD47 targeted TNBC cells had a decreased glycolytic rate, which may be correlated with decreased PD-L1 expression.

**Conclusions** Targeting CD47 enhanced granzyme B and IFN $\gamma$  expression suggesting potential mechanisms to increase tumor immunogenicity. CD47 targeted monotherapy or combination with anti-PD-L1 preserves T cell bioenergetics and antitumor function, resulting in decreased TNBC tumor burden. Alternatively, CD47 targeted TNBC had a decreased glycolytic rate and decreased PD-L1 expression, which is reported to regulate glycolysis through Akt/mTOR signaling. Targeting CD47 on T cells enhances their bioenergetics and antitumor function while decreasing TNBC cell bioenergetics, making them more susceptible to immune cell killing. Our data indicates that CD47 targeted monotherapy or combination with anti-PD-L1 may enhance TNBC patient response and improve overall survival.

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**Ethics Approval** Animal studies were approved by the Institutional Care and Use Committee, Wake Forest Health Sciences.

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#### SMALL MOLECULE INHIBITORS OF SEC61 COTRANSLATIONAL TRANSLOCATION REGULATE THE PHAGOCYTOSIS CHECKPOINT MOLECULE CD47

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**Background** Many tumor cells escape immune cell clearance by overexpressing CD47, a multi-pass transmembrane protein, which binds signal regulatory protein  $\alpha$  (SIRP $\alpha$ ) on macrophages leading to decreased phagocytic activity. Blockade of CD47/SIRP $\alpha$  interactions enhances macrophage phagocytosis and is being targeted with antibody-based drugs, some of which are used in combination therapies in clinical trials. A novel method to target CD47 is through the inhibition of cotranslational translocation of transmembrane proteins. Immediately after exiting the ribosome, signal sequences that are unique to each protein are directed through the Sec61 channel into the ER for extracellular expression.<sup>1</sup> Several Sec61-targeting compounds have been identified to suppress translocation in a signal sequence-specific manner.<sup>2</sup> We previously described Sec61 inhibitors capable of selectively targeting immune checkpoint proteins and enhancing T cell function.<sup>3</sup> Here, we demonstrate the blockade of CD47 expression on tumor cells and enhancement of macrophage phagocytosis with small molecule inhibitors of Sec61.

**Methods** Sec61-dependent expression of target proteins was assayed using HEK293 cells overexpressing constructs comprised of signal sequences fused to a luciferase reporter. Stimulated PBMCs or tumor cells were incubated with Sec61 inhibitors, and surface expression of checkpoint molecules

were examined by flow cytometry. Necrotic and apoptotic cells were assessed by Annexin V and 7AAD labeling. Human CD14+ monocytes were differentiated to M1- or M2-type macrophages. Jurkat or SKBR3 cells were incubated with Sec61 inhibitors, labeled with a pH sensitive dye and co-cultured with macrophages to assess phagocytosis.

**Results** We identified Sec61 inhibitors that block select immune checkpoint proteins. Compounds demonstrated either selective or multi-target profiles in transient transfection screens, which was supported by decreased protein expression on activated T cells. KZR-9275 targeted multiple checkpoint molecules, including PD-1, LAG-3 and CD73, along with a potent inhibition of the CD47 signal sequence reporter. CD47 surface expression was decreased on Jurkat and SKBR3 cells following 72 hours of compound treatment. KZR-9275 treatment of SKBR3 cells induced a minor increase in apoptotic cells, which was not detected in Jurkat cells. Increased macrophage phagocytosis, especially with M2-type macrophages, was observed when Jurkat or SKBR3 cells were pre-treated with KZR-9275.

**Conclusions** Our findings demonstrate that Sec61 inhibitors can block the expression of CD47, a phagocytosis checkpoint protein, on tumor cells and subsequently modulate macrophage phagocytic activity. Small molecule inhibitors of Sec61 provide an opportunity to target multiple checkpoint proteins on various cell populations. Future in vivo tumor models will assess the efficacy of Sec61 inhibitors to provide combination-like therapy.

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#### E7777 (DENILEUKIN DIFTITOX) ENHANCES ANTI-TUMOR ACTIVITY AND SIGNIFICANTLY EXTENDS SURVIVAL BENEFIT OF ANTI-PD-1 IN SYNGENEIC SOLID TUMOR MODELS

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**Background** Regulatory T cell (Tregs) inhibit activity of anti-tumor T cells, and have been shown to limit checkpoint inhibitor effectiveness. Depletion of Tregs seems desirable during immunotherapy, but chronic Treg depletion with antibody therapies can lead to serious autoimmune adverse events. Compared to antibodies, the fusion protein E7777 (IL-2/diphtheria toxin) has a relatively short half-life in circulation, which allows for transient and selective Treg depletion. The potential therapeutic benefit of combining E7777 with anti-PD-1 was tested in syngeneic solid tumor models.

**Methods** CT26 colon and H22 liver cancer tumors were implanted subcutaneously in immunocompetent BALB/c mice. E7777 (2.5 mcg/mouse, i.v.) was given on a Q7Dx3 schedule. Anti-murine PD-1 was given (100 mcg/mouse, i.v.) Q4Dx5.