

increased intratumoral granzyme B secreting CD8+ T cells. Additionally, targeting CD47 within organoids increased IFN γ 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 γ 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 α (SIRP α) on macrophages leading to decreased phagocytic activity. Blockade of CD47/SIRP α 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.¹ Several Sec61-targeting compounds have been identified to suppress translocation in a signal sequence-specific manner.² We previously described Sec61 inhibitors capable of selectively targeting immune checkpoint proteins and enhancing T cell function.³ 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.

REFERENCES

1. Park E, Rapoport TA. Mechanisms of Sec61/SecY-mediated protein translocation across membranes. *Annu Rev Biophys* 2012; **41**:1–20.
2. Van Puyenbroeck V, Vermeire K. Inhibitors of protein translocation across membranes of the secretory pathway: novel antimicrobial and anticancer agents. *Cell Mol Life Sci* 2018; **75**:1541–1558.
3. Whang J, Anderl J, Fan A, Kirk C, Lowe E, McMinn D, et al. Targeting multiple immune checkpoint proteins with novel small molecule inhibitors of Sec61-dependent cotranslational translocation. 34th Annual Meeting & Pre-Conference Programs of the Society for Immunotherapy of Cancer (SITC 2019): part 2. *J Immunother Cancer* 2019; **7**: 283. Abstract 815.

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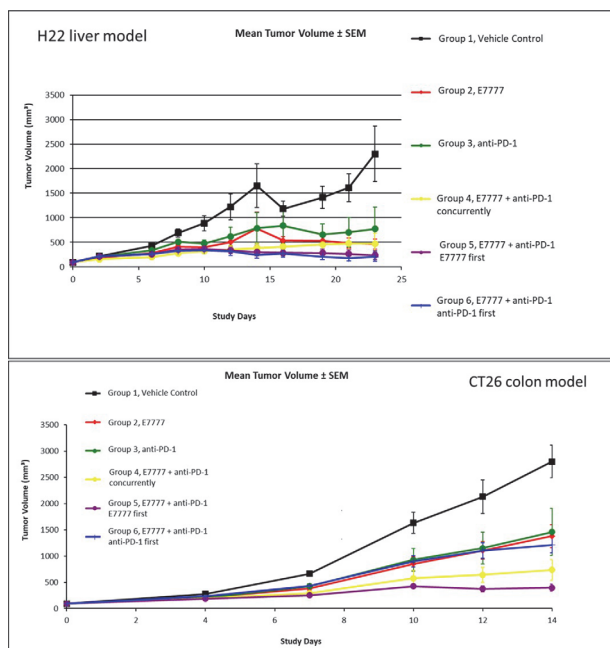
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E7777 (DENILEUKIN DIFTITOX) ENHANCES ANTI-TUMOR ACTIVITY AND SIGNIFICANTLY EXTENDS SURVIVAL BENEFIT OF ANTI-PD-1 IN SYNGENEIC SOLID TUMOR MODELS

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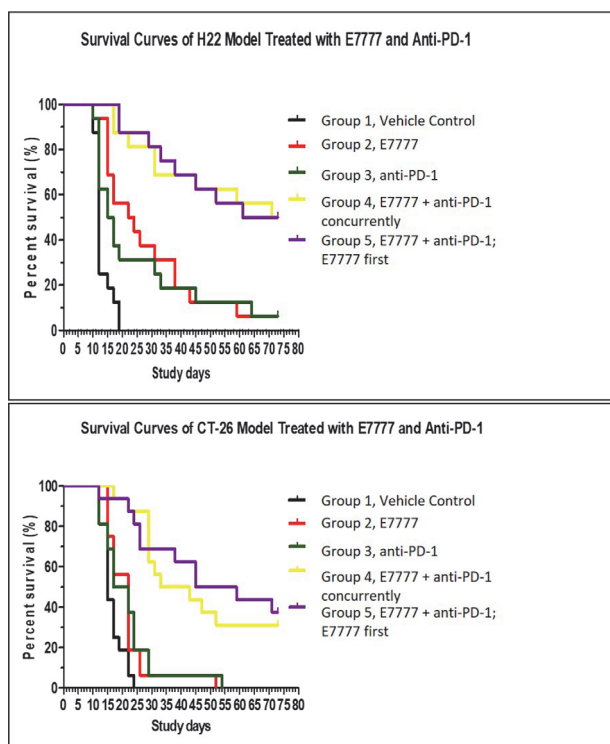
Background Regulatory T cell (Tregs) inhibit activity of anti-tumor T cells, and have been shown to limit checkpoint inhibitor effectiveness. Depletion of Tregs seems desirable during immunotherapy, but chronic Treg depletion with antibody therapies can lead to serious autoimmune adverse events. Compared 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.



Abstract 208 Figure 1 Tumor growth in s.c. syngeneic solid tumors. N=16/group

Groups of 16 mice received each agent as monotherapy or in combinations. Sequencing of combination administration was also varied: Group 4 started treatment on the same day; Group 5 received E7777 2 days prior to start of anti-PD-1; Group 6 received anti-PD-1 first. Tumor growth was compared across all groups. In survival studies, mice were treated for 3 weeks and observed with twice weekly tumor measurements. In other experiments, tumors, tumor-draining lymph



Abstract 208 Figure 2 Overall survival in s.c. syngeneic models. N=16/group

nodes, and spleens were examined by IHC and by flow cytometry of immune cells from dissociated tissues at defined points, for immune biomarkers.

Results Figure 1 shows additive benefit from the E7777 + anti-PD-1 combinations over either monotherapy. Most importantly, figure 2 and table 1 show significantly enhanced overall survival from a 3 week course of combinations compared to either agent alone ($p < 0.005$) or to vehicle controls ($p < 0.000001$). There was no clear distinction among different sequencing regimens. Benefit correlated with enhanced CD8: Treg ratios in tumors.

Abstract 208 Table 1 Calculated median survival

	Group 1	Group 2	Group 3	Group 4	Group 5
Treatment	Vehicle Control	E7777 Q7Dx3	α -PD-1 Q4Dx5	E7777 + α -PD-1	E7777 + α -PD-1
H22 Liver	12	23	16	72	67
CT26 Colon	15	22	19.5	38	52

Conclusions Depletion of Tregs by E7777 significantly increased anti-tumor activity and durably extended overall survival compared to treatment with anti-PD-1 alone in syngeneic solid tumor models. Clinical studies of a combination of the two agents are planned.

Ethics Approval All studies were conducted at Crown Bio, and were approved by the Crown Bio IACUC.

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PRECLINICAL MECHANISTIC AND CLINICAL EVALUATION OF THE CORTICOSTEROID DEXAMETHASONE'S DETRIMENTAL EFFECTS ON IMMUNE CHECKPOINT BLOCKADE IN GLIOBLASTOMA CANCER

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Background Increasing data indicate that corticosteroids can exert a detrimental effect on immunotherapy for oncology patients. Dexamethasone, a uniquely potent corticosteroid, is frequently administered to brain tumor patients to decrease tumor-associated edema, but limited data exist describing how dexamethasone affects the immune system systemically and intratumorally in glioblastoma patients – particularly in the context of immunotherapy.

Methods We evaluated the dose-dependent effects of dexamethasone when administered with PD-1 blockade and/or radiotherapy on survival and tumor response in immunocompetent C57BL/6 mice with syngeneic GL261 and CT-2A glioblastoma tumors. The immune microenvironment was comprehensively profiled using flow cytometry analysis. Clinically, the effect of dexamethasone on survival was evaluated in 181 IDH-wildtype glioblastoma patients treated with PD-(L)1 blockade, with adjustment for relevant prognostic factors using multivariable Cox regression.

Results Despite the inherent responsiveness of GL261 to immune checkpoint blockade, concurrent dexamethasone administration with anti-PD-1 therapy reduced survival in a dose-dependent manner (figure 1). Concurrent dexamethasone