Collagen Anchoring Agonist Antibodies for Cancer Immunotherapy

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Cancer immunotherapy represents a paradigm shift in how we think about and treat cancer. While traditional cancer interventions such as surgery, radiation, and chemotherapy are aimed at killing or removing the tumor cells themselves, immunotherapies instead seek to establish long-lasting, robust antitumor immune responses. A wide range of modalities exist in this space, including cytokines, cell therapies, antibodies, oncolytic viruses, and cancer vaccines. One approach that has shown promising results in preclinical mouse models is the use of agonist antibodies targeting costimulatory, or activating, receptors on effector immune cells, particularly $CD8^+$ T cells. However, translation of these antibody therapeutics into the clinic has been hampered by severe, sometimes fatal, on-target, off-tumor toxicities. Thus, the field at large has shifted focus to developing agonist antibodies with tumor restricted activity. To that end, we sought to develop collagen anchored agonist antibodies, an approach we have previously validated with collagen anchored cytokines. When injected directly into the tumor these collagen anchoring therapies are preferentially retained in the tumor microenvironment (TME), enhancing efficacy while limiting systemic toxicities.

We first attempted to engineer a generalizable antibody anchoring platform by constructing fusions of immunoglobulin G (IgG) binding domains to collagen binding domains. However, due to the low to moderate affinity of existing IgG binding domains and rapid *in vivo* exchange with endogenous IgG, this platform underperformed at retaining agonist antibodies in the TME. We then pivoted to constructing and expressing direct agonist antibody fusions to collagen binding domains, demonstrating that this is a strategy generalizable to a range of antibody therapeutics. We primarily focused on evaluating agonist antibodies targeting 4-1BB and CD28, two well studied costimulatory receptors, fused to the collagen binding domain LAIR (α4-1BB-LAIR and αCD28-LAIR, respectively) in a range of both monotherapies and combination therapies.

We observed that while combination treatment of α4-1BB-LAIR with an antitumor antibody (TA99) displayed only modest efficacy in the B16F10 murine melanoma model, simultaneous depletion of $CD4^+$ T cells during treatment boosted cure rates to over 90% of mice. We elucidated two mechanisms of action for this synergy: αCD4 eliminated tumor draining lymph node Tregs, enhancing priming and activation of $CD8^+$ T cells, and TA99 + α4-1BB-LAIR supported the cytotoxic program of these newly primed $CD8^+$ T cells within TME. Replacement of αCD4 with αCTLA-4, a clinically approved checkpoint inhibitor that enhances T cell priming, produced equivalent cure rates while additionally generating robust immunological memory against secondary tumor rechallenge. Together, my thesis work demonstrates that collagen anchoring is an effective strategy to improve the therapeutic index of agonist antibody therapies and uncovers a fundamental two-step approach to designing effective cancer immunotherapy combinations.

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