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14. ABSTRACT

The goal of this project is to engineer T-cells for adoptive cell therapy of prostate cancer, by conjugating drug-loaded nanoparticles to therapeutic cells to enable targeted drug delivery to tumor sites and lymph nodes. Nanoparticles with cytokine proteins bound to their surfaces will be attached to the surface of T-cells– the equivalent of delivering a drug-loaded pill to each individual anti-tumor T-cell. These drug-carrying 'T-Pharmacytes' will be continuously stimulated by the protein and carry these cytokine-loaded particles wherever they traffic in the body. We hypothesize that this strategy will provide a safe and effective means to augment adoptive cell therapy and allow the great promise of this immunotherapy to be realized for treatment of prostate cancer. The proposed studies will provide a preclinical test of this concept. If successful, this approach to enhancing an immunotherapy strategy already in clinical trials might be translated to patient treatment in a relatively short timespan.

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T-Pharmacytes for Prostate Cancer Immunotherapy

Progress Report

Dane Wittrup

May 2012

In this Synergistic Idea Development Award, the Wittrup group has been contributing to Specific Aim #1 "Synthesis of biodegradable nanoparticles as cell surface drug carriers, and engineering of cytokines for enhanced T-cell stimulation", engineering novel cytokines for delivery via intratumoral nanoparticle injection. We constructed monovalent Fc fusions with wild-type murine IL-2 and two different mutants, one with enhanced affinity for CD25 and one with ablated binding to CD25. To determine the effect of CD25 binding affinity on IL-2 immunostimulatory effects and toxicity more directly, we evaluated the effects of an affinity series of IL-2, consisting of high-affinity CD25-binding QQ 6.2-10, wild-type IL-2, and a rationally designed non-CD25 binding IL-2 mutant named E76G. Importantly, evaluating these cytokines *in vivo* in mice, we employed only cytokines of mouse origin, unlike the human IL-2 often employed in such studies.

Monovalent heterodimeric Fc/IL-2 fusion constructs were expressed as two separate chains, the Fc domain of murine IgG2a isotype with a His6 tag, and the same Fc fused to the murine IL-2 of interest (wt, QQ6.2-10, or E76G) and a FLAG tag (Figure 1). Sequential purification on cobalt resin and anti-FLAG resin results in pure heterodimer, presenting IL-2 monovalently. Each of the Fc sequences was mutated with the D265A mutation that significantly decreases effector function, so as not to invoke ADCC or CDC against the targeted NK and T cells.

Each of the three Fc/IL-2 fusions was injected into mice and the effect on T cell and NK cell levels was measured (Figure 2). Paradoxically, the non-CD25-binding E76G mutant led to considerable increases in CD3+CD8+, CD3+CD4+, CD4+CD25+Foxp3+, and NK cell levels. Subsequently however, the E76G Fc/IL-2 was found to exert a lesser therapeutic effect in tumor models, and so the wild-type Fc/IL-2 has been used for all subsequent work.

Figure 1. Monovalent Fc/IL-2 construct topology.

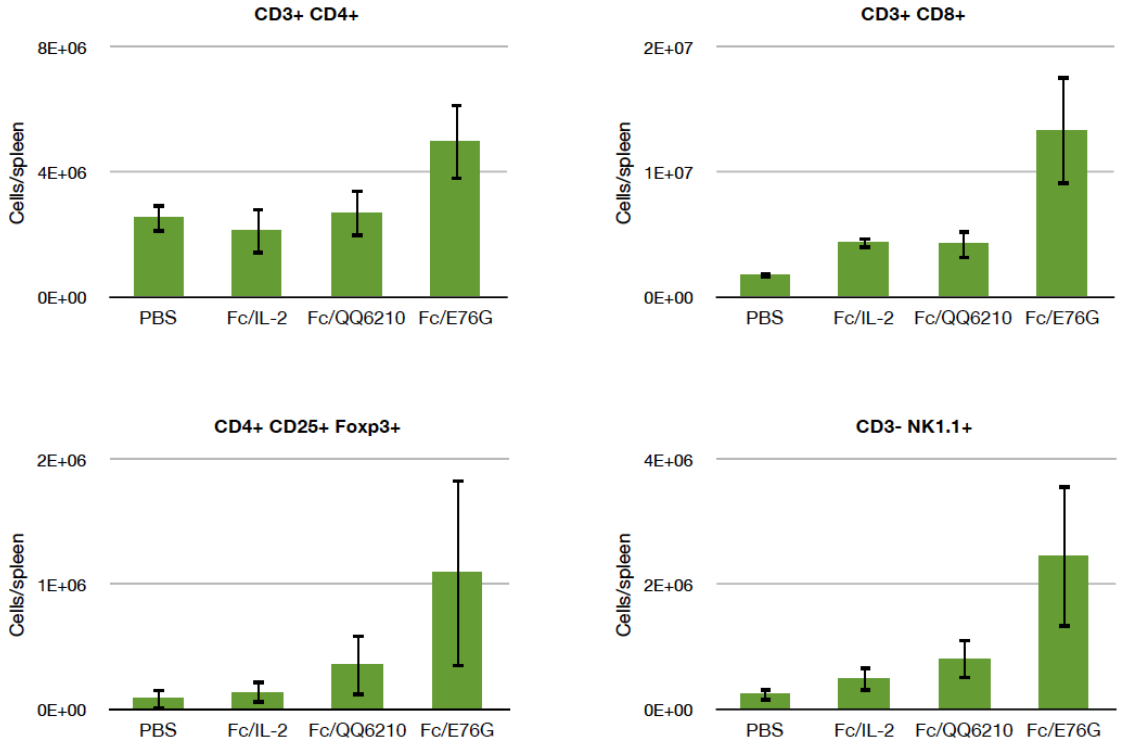

Figure 1. C57BL/6 mice (*n* = 3 mice per group) were injected intravenously with PBS, or 25 µg of Fc/IL-2, Fc/QQ6210, Fc/E76G. Four days after treatment, spleens were analyzed for T and NK cell composition.