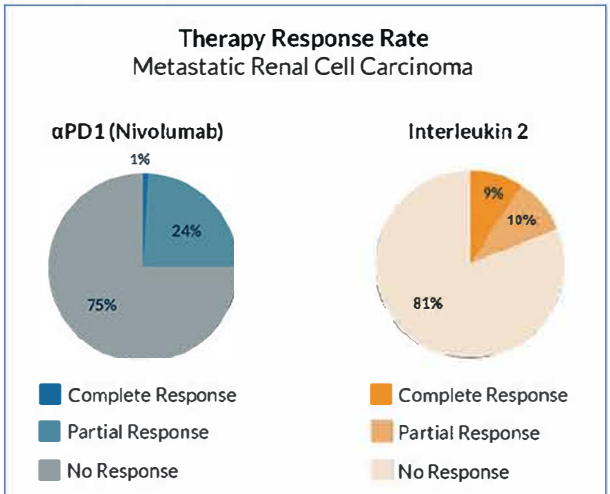
SPECIFIC AIMS

The cytokine interleukin-2 (IL-2) is considered a master regulator of the immune system(1). Its anti-tumor effects are utilized for expansion of tumor reactive lymphocytes ex vivo(2) and its in vivo administration was the first immunotherapy approved by the FDA(3-7). In renal cell carcinoma high dose IL-2 (HD IL-2) induces long-term cure of 5-9% of patients, while the anti-PD-1 therapy Nivolumab, which recently received FDA fast-track approval, has a <1% complete remission rate(8) (**Summary Figure**). Despite these promising results HD IL-2 has fallen out of favor due to adverse side effects including fever, malaise, and life-threatening systemic capillary leak(9). These side effects result in a 50% rate of therapy discontinuation and a mortality of 2-5% (10). If a safe and efficacious form of IL-2 were available it would have wide-spread utility for multiple malignancies.



Summary Figure of Complete Response (Cure) Achieved with IL-2 vs. PD-1 Blockade

The structure of the IL-2 receptor (IL-2R) provides opportunities to modify IL-2 to reduce toxicity. The signaling portion of IL-2R consists of the β and γ chains, while the non-signaling high affinity α-chain functions to efficiently capture IL-2 at the cell surface. Together these chains form the high affinity trimeric IL-2αβγ receptor (IL-2Rαβγ) which is broadly expressed across many hematopoietic and non-hematopoietic cells. For these reasons HD IL-2 broadly activates multiple cells and tissues resulting in many "off target" side effects. For example HD IL-2 promotes the proliferation of cytotoxic lymphocytes (NK cells and CD8+ T cells) but it also promotes the expansion of regulatory T cells (T_{regs}) that inhibit the immune responses. Activation of vascular endothelium, which also expresses IL-2Rαβγ, induces devastating and life-threatening complications(11). An improved form of IL-2, that solely activates cytotoxic lymphocytes (CTLs) without activating T_{regs} or vascular endothelium, may offer the opportunity to expand IL-2 use in the clinic.

NKG2D is an activating receptor that is expressed solely on CTLs, such as NK cells and activated/memory $CD8^+$ T cells. It is not present on T_{regs}, vascular endothelium or other lymphocytes(12). We have recently described the construction of a fusion protein that delivers IL-2 directly to NKG2D-expressing cells through a ligand known as orthopoxvirus major histocompatibility complex class I-like protein (OMCP). Thus NKG2D substitutes for the high affinity α-chain of the IL-2 receptor, resulting in specific and precise activation of cytotoxic lymphocytes. Such a strategy avoids activation of most other cells, including vascular endothelium and T_{regs} (13). Our fusion protein, called OMCP-mutIL-2, offers a dramatic reduction in adverse side effects and superior tumor control over wild-type IL-2(13). In this application we seek to further develop OMCP-mutIL-2 into a viable drug candidate and broaden its application for stimulation of CTLs both in vitro and in vivo.

Specific Aim #1: To utilize computational modeling to define immunogenicity of OMCP-mutIL-2. Since the A0 application we have eliminated the murine humoral immune response to OMCP-mutIL-2 by modifying the peptide linker region. We now plan to utilize in silico analysis to test human immunogenicity to these constructs in direct comparison to proteins in clinical use already. Such data will allow us to refine our final lead product for clinical work.

***Specific Aim 2:* To evaluate OMCP-mutIL-2 as an adjuvant for checkpoint blockade immunotherapy.** We hypothesize that the complementary and non-overlapping mechanisms of action of OMCP-mutIL-2 will substantially decrease the growth of lung cancer without an increase in adverse events when combined with checkpoint blockade.

***Specific Aim 3:* To evaluate the utility of OMCP-mutIL-2 for expansion of human tumor-reactive lymphocytes in vitro.** Preliminary data generated in murine models demonstrates that OMCP-mutIL-2 offers a quantitative and qualitative advantage for T cell expansion. Here we plan to compare OMCP-mutIL-2 to conventional cytokine stimulation for expansion of tumor-reactive lymphocytes from patients vaccinated against malignant melanoma.