Novel Immuno-oncology Strategy for Targeted Cytotoxic Lymphocyte Activation

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Background

- IL2 is the original immunotherapy with the promise for a curative tumor immune response. Significant off target side effects, however, limit wide spread application[1].
- The trimeric IL2 receptor, consisting of the high affinity α chain and signaling βγ chains, is expressed on multiple cell types (Figure 1a). Cytotoxic lymphocytes, such as NK cells and CD8+ T cells, thus have to compete for IL2 with multiple hematopoietic and non-hematopoietic cells
- Higher levels of α chain expression of CD4+Foxp3+ regulatory T cells and endothelial cells also decreases efficacy and contributes to complications of IL2 therapy
- NKG2D is an activating receptor that can be expressed on both human and murine NK and CD8$^+$ T cells but is virtually absent from other lymphocytes or non-hematopoietic cells such as vascular endothelium[2]
- We recently described the construction and function of an IL2 fusion protein that is targeted to both NK cells and CD8+ T cells using a high affinity viral NKG2D ligand called orthopoxvirus major histocompatibility complex class I-like protein, or OMCP [3] (Figure 1b). The NKG2D ligand does not co-stimulate through this activating receptor but rather acts as a surface delivery mechanism for IL-2.

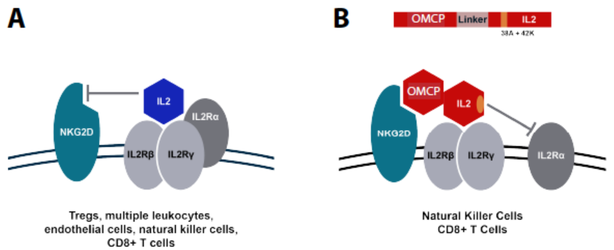

Figure1. (A) Wild-type IL2 is captured at the cell surface by the α chain of the IL2 receptor which is widely expressed on many cell types. (B) Our fusion protein, called OMCP-mutIL2, is captured at the cell surface by NKG2D and is thus targeted to NK cells and CD8+ T cells.

Hypothesis

We hypothesize that OMCP-mutIL-2 will be advantageous for immunotherapy with limited side effects associated with traditional high-dose IL-2.

Results

OMCP-mutIL-2 Ameliorates Tumor Growth, Facilitates Tumor Penetration, and Decreases Exhaustion of Tumor-Resident Lymphocytes

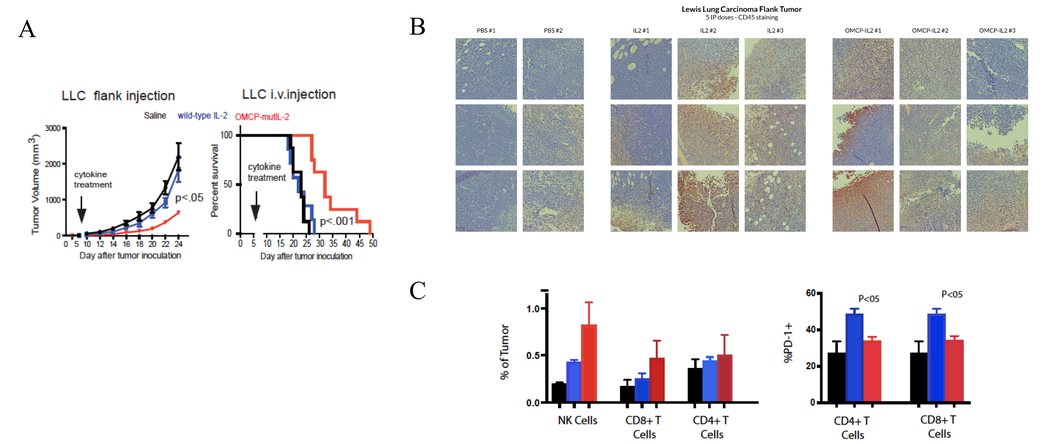

Figure 2: (A) As previously described[3] OMCP-mutIL-2 treatment decreases growth and improves survival of lung cancer. (B) Immunohistochemistry of flank tumors demonstrates an increase in tumor-resident lymphocytes in both IL-2 and OMCP-mutIL-2 treated mice. (C) Flow cytometry of tumor digests demonstrates a trend for increase in NK cells (NK1.1+DX5+) and CD8+ T cells after OMCP-mutIL-2 treatment. A lower percentage of PD-1+ tumor-resident CD4+ and CD8+ T cells is evident in OMCP-mutIL-2 treated mice.

OMCP-mutIL-2 Does not Lead to Cytokine Storm

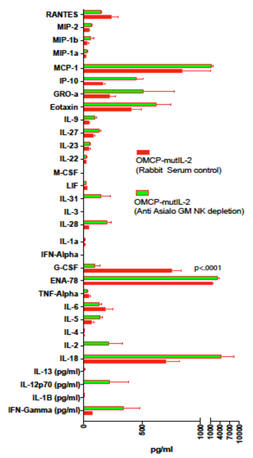

Figure 3: Some forms of immunotherapy, such as CAR T cells, result deleterious side effects from elaboration of proinflammatory cytokines. As admiaitration of OMCP-mutIL-2 results in massive expansion of systemic NK cells[3] we administered supratherapeutic doses of OMCP-mutIL-2 to mice depleted of NK cells by Anti-Asialo GM or control mice treated with rabbit serum. Only levels of G-CSF were higher in NK sufficient mice.

OMCP-mutIL-2 Combined with TCR Stimulation Results in Superior Expansion of T Cells

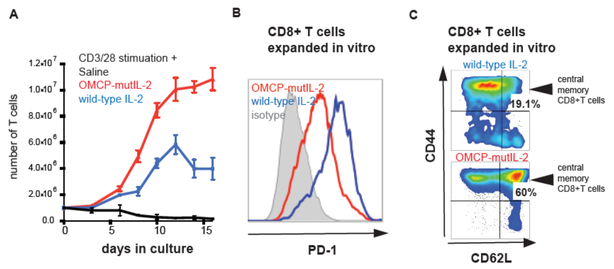

Figure 4: (A) After a 72 hour period of in vitro stimulation with anti-CD3/CD28 antibodies more robust T cell expansion is evident after culture in OMCP-mutIL-2 over IL-2. (B) Lover levels of PD-1 are evident in OMCP-mutIL-2 expanded cells. (C) A higher proportion of central memory CD44+CD62L+ CD8+ T cells are evident in OMCP-mutIL-2 expanded cultures.

Combined with PD-1 checkpoint Blockade OMCP-mutIL-2 results in Near Complete Control of Lung Cancer Growth

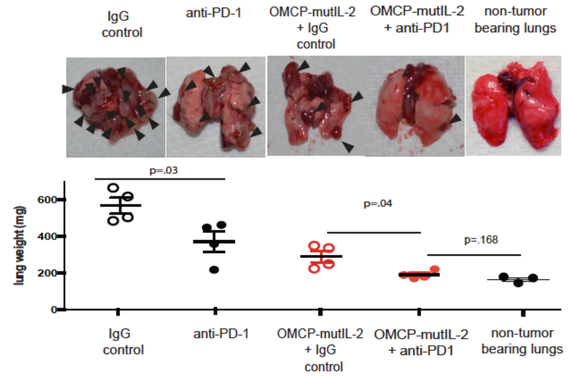

Figure 6: In order to study combination immunotherapy we injected Lewis Lung Carcinoma bearing the model tumor antigen ovalbumin into C57BL/6 mice. Five days after tumor injection the mice were treated with PD-1 blocking antibody (clone RMP1-14) every three days and/or OMCP-mutIL2 at 750,000IU in 10 doses over 5 days. Some mice received control IgG. Using lung weight as a surrogate marker of tumor growth significant augmentation anti-tumor immunotherapy was evident with combination OMCP-mutIL-2 and PD-1 blockade.

Conclusions

- Targeted delivery of a no α chain binding IL-2 mutant offers superior immunotherapy potential over traditional high dose IL-2
- OMCP-mutIL-2 facilitates tumor infiltration by cytotoxic lymphocytes with lower levels of exhaustion as defined by surface PD-1 expression
- OMCP-mutIL-2 may offer an advantage for in vitro expansion of T cells due to generation of CD44hiCD62Lhi central memory cells lower levels of exhaustion as defined by surface PD-1 expression
- Despite massive systemic activation of NK cells minimal difference exist in serum cytokine production are evident between NK sufficient and deficient mice suggesting lack of NK-mediated cytokine storm
- Combination of checkpoint blockade and OMCP-mutIL-2 may have significant immunologic advantages over monotherapy for lung cancer

References

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- 3) Ghasemi, R., *et al.* Selective targeting of IL-2 to NKG2D bearing cells for improved immunotherapy. *Nature Communications* **7**(2016)