Activating OX40 receptor promotes the expansion of CD8+ T-cell effecter function
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BACKGROUND
• Adoptive cell therapy (ACT) using tumor infiltrating lymphocytes (TILs) has demonstrated efficacy in metastatic melanoma patients, with ~50% objective responses.1
• A high proportion of CD8+ T cells in the infusion product is recognized as possibly important for the efficacy of ACT with TILs.2
• OX40 (CD134) belongs to the tumor necrosis factor receptor super family and is mainly expressed by activated T-lymphocytes.3
• Activation of OX40 in antigen-pulsed stimulation promotes proliferation and survival of T lymphocytes via the NF-κB and JNK pathways.4
• Anergic anti-OX40 agonistic antibodies (Ab) have been developed as potential immunomodulators for the treatment of cancer. One such Ab was recently shown to increase reactivity of tumor antigen-specific CD8+ T lymphocytes in vivo.5

STUDY OBJECTIVE
• To fully examine the expression of the OX40 receptor on TIL and investigate the impact of an anti-OX40 agonistic antibody on the ex vivo expansion and effector function of TIL derived from different histologies.

OVERVIEW OF TIL THERAPY PROCESS
1. The tumor is excised from the patient and transported to the GMP Manufacturing facility.
2. Upon arrival, the tumor is fragmented and placed in flasks with IL-2 for a pre-RP Expansion Protocol (REP).
3. pre-FE-TIL are further propagated in a REP in the presence of irradiated PRMC, anti-CD8 antibody and IL-2 (5000 IU/ml).
4. TIL products are tested for phenotype and effector function.
5. Prior to infusion of expanded TIL, patients receive a non-myeloablative lymphodepletion regimen consisting of cyclophosphamide (60 mg/kg, day 1) and fludarabine (25 mg/m2, day 3) to 7). Following infusion of TIL, patients receive a short duration (up to 6 days) infusion of high-dose IL-2 (600,000 IU/kg) to support growth and engraftment of transferred TIL.

EXPERIMENTAL DESIGN
• Twenty-one human tumor samples derived from melanoma, head and neck, sarcoma, ovarian, and breast cancers were subjected to research-scale pre-REP. Pre-REP-TIL were subsequently expanded in the presence or absence of anti-OX40 agonistic antibody. Pre- and post-REP-TIL were analyzed for OX40 expression.
• Exposure of effector phenotypic and functional characterization was done on the final products.

RESULTS
Figure 1. OX40 is enriched in the CD8+ T-cell subset, and upregulated following T-cell activation.

Figure 2. Anti-OX40 agonist specifically enhances the expansion of CD8+ T cells.

Figure 3. Anti-OX40 antibody decreased the levels of OX40 receptor on CD8+ T cells.

Figure 4. Anti-OX40 antibody induced NF-κB signaling in a dose- and clustering-dependent manner.

Figure 5. Diversity of the TCR-Vβ repertoire was conserved in both CD8+ and CD4+ T-cell subsets in TIL expanded with anti-OX40 L4050.

Figure 6. TIL expanded with anti-OX40 exhibited a more differentiated phenotype.

Figure 7. TIL expanded with anti-OX40 exhibited decreased expression of the exhaustion markers PD-1 and TIM-3.

Figure 8. TIL expanded in the presence of anti-OX40 increased production of IFN-γ in the CD4+ T-cell population.

REFERENCES

CONCLUSIONS
Summary of the Study
• OX40 was mainly expressed by the CD4+ TIL subset, and highly up-regulated following TIL activation.
• Anti-OX40 promoted CD8+ TIL expansion at the expense of CD4+ T cells, while maintaining the TCR-Vβ repertoire in both CD8+ and CD4+ T-cell subsets.
• The OX40 receptor was specifically down-regulated in CD8+ T cells in response to anti-OX40-expanded TIL, likely due to antibody internalization, and suggesting that the CD4+ T cells are primary antibody targets.

NuP-β-CXCF was induced by anti-OX40 in REP-like culture conditions, demonstrating that the activity was dose-dependent and required clustering.

A decrease in CD28 and increase in CD69 expression in post-REP-TIL expanded with anti-OX40 indicated a more differentiated phenotype.

TIL expanded with anti-OX40 decreased PD-1 and TIM-3 expression, suggesting a less exhausted phenotype.

TIL expanded in the presence of anti-OX40 demonstrated heightened IFN-γ production upon re-stimulation, indicating enhanced T-cell effecter function.

These data illustrate the impact of OX40 activation on TIL, using an agonistic antibody, and suggest that therapeutic products with enhanced activity can be designed for a number of tumor histologies by supplementing REP cultures with the anti-OX40 antibody.

Disclaimers and Statement
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