LB-110 AACR ANNUAL MEETING | APRIL 14-18, 2018 | CHICAGO, IL, USA

Activating OX40 receptor promotes the expansion of CD8+ TIL with enhanced T-cell effector function

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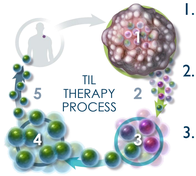
BACKGROUND

- Adoptive cell therapy (ACT) using tumor infiltrating lymphocytes (TIL) 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 TIL.[2]
- OX40 (CD134) belongs to the tumor necrosis factor receptor super family and is mainly expressed by activated T lymphocytes.
- Activation of OX40 signaling promotes proliferation and survival of T cells via the NF-κB pathway.[3]
- Agonistic anti-OX40 antibodies (Ab) have been developed as potential immunotherapies for the treatment of cancer. One such Ab was recently shown to increase reactivity of tumor antigen-specific CD8+ T cells in patient's peripheral blood.[4]

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.

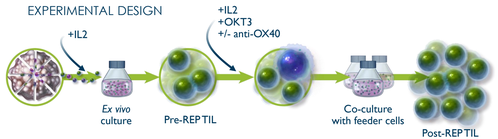
OVERVIEW OF TIL THERAPY PROCESS



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-Rapid Expansion Protocol (REP).
3. pre-REP TIL are further propagated in a REP protocol in the presence of irradiated PBMCs, anti-CD3 antibody, and IL-2 (3000 IU/mL).
4. TIL products are assessed 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 2) and fludarabine (25 mg/m^2, day 3 to 7). Following infusion of TIL, patients receive a short duration (up to 6 doses) of high-dose IL-2 (600,000 IU/kg) to support growth and engraftment of transferred TIL.

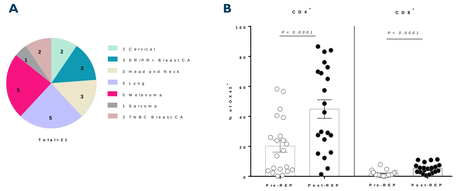
EXPERIMENTAL DESIGN

- Twenty-one human tumor samples derived from melanoma, head and neck, sarcoma, cervical, 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.
- Extensive phenotypic and functional characterization was done on the final products.



RESULTS

Figure 1. OX40 is enriched in the CD4+ TIL subset, and up-regulated following T-cell activation.



Evaluation of OX40 expression in CD4+ and CD8+TIL. (A) Twenty-one tumor samples from 7 different histologies were assessed. **(B)** Expression of OX40 at the surface of pre- (white dots) and post- (black dots) REP TIL was analyzed by flow cytometry. Shown are the percentages of positive CD4+ (left panel) and CD8+ (right panel) T-cells. Means ± SEM are indicated by horizontal and vertical bars, respectively. P value was calculated by paired student's t-test and considered statistically significant when <0.05.

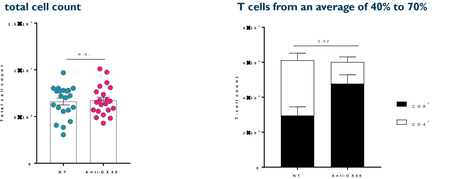
The percentage of OX40-positive cells is significantly higher in CD4+ than CD8+ subset at both stages of TIL expansion. Overall OX40 expression was markedly higher in post-REP TIL relative to pre-REP TIL, demonstrating OX40-receptor up-regulation upon TIL activation.

References

1. Dudley, M. E. et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 23, 2346-2357, doi:10.1200/JCO.2005.00.240 (2005).
2. Radvanyi, L. G. et al. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. *Clin Cancer Res.* 18, 6758-6770, doi:10.1158/1078-0432.CCR-12-1177 (2012).
3. Song, J., So, T. & Croft, M. Activation of NF-kappaB1 by OX40 contributes to antigen-driven T cell expansion and survival. *J Immunol.* 180, 7240-7248 (2008).
4. Curti, B. D. et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. *Cancer Res.* 73, 7189-7198, doi:10.1158/0008-5472.CAN-12-4174 (2013).

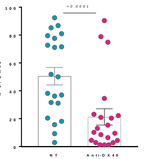
RESULTS

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



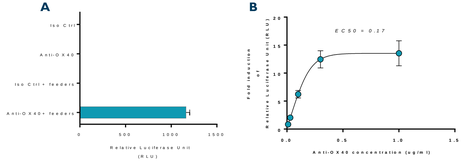
Cell count and T-cell subsets characterization of post-REP TIL expanded with anti-OX40 agonist. Pre-REP TILs from different tumor histologies were expanded with or without anti-OX40 agonist (n=21). **(A)** Total cells were counted and results plotted for each individual sample expanded in the presence (pink dots) and absence (blue dots) of anti-OX40. **(B)** Post-REP TILs were stained for T-cell lineage markers including CD3, CD4, and CD8 and analyzed by flow cytometry. Average total T-cell (whole bar), CD8+ (black), and CD4+ (white) counts and respective mean ± SEM are shown for control (NT) and treatment (anti-OX40) groups. A significant increase in the proportion of CD8+TIL was demonstrated when treated with anti-OX40 ($p < 0.02$). P value was calculated by paired student's t-test and considered statistically significant when <0.05.

Figure 3. Anti-OX40 antibody decreased the levels of OX40 receptor on CD4+ T cells.



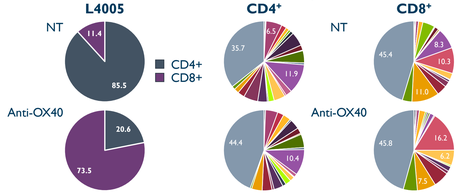
OX40 expression on CD4+TIL following anti-OX0 treatment TIL expanded with (pink dots) and without (blue dots) anti-OX40 were analyzed for cell surface expression of OX40 by flow cytometry. Shown are the percentages of positive CD4+T cells. Means ± SEM are indicated by horizontal and vertical bars, respectively. P value was calculated by student's t-test and considered statistically significant when <0.05. A marked decrease in OX40 expression was observed within the CD4+ subset of T cells, consistent with antibody binding-induced internalization of the receptor.

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



Mechanism of action studies. The impact of anti-OX40 agonist on NF-κB signaling was studied in an HEK-293 cell-based luciferase reporter assay. Cells were cultured in the presence of feeder cells (irradiated PBMCs) to mimic REP conditions and stimulated with anti-OX40. **(A)** No induction was detected in the absence of feeder cells or by the human IgG isotype control antibody, suggesting that anti-OX40 activity is specific and requires clustering (n=2). **(B)** Reporter activity was induced in a dose-dependent manner in the luciferase reporter cells stimulated with anti-OX40 at various concentrations ranging from 1 to 0.01 ug/ml. An EC_{50} of 0.17 was calculated (n=2). Error bars are shown as mean ± SEM.

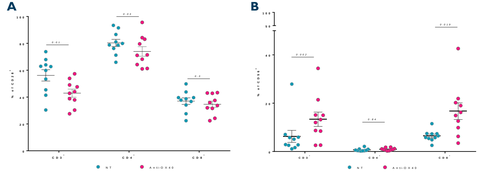
Figure 5. Diversity of the TCR-V(β) repertoire is conserved in both CD4+ & CD8+ T cells subsets in TIL expanded with anti-OX40.



Analysis of T cell receptor (TCR)-V(β) repertoire. TIL were derived from a lung tumor sample (L4005) in the presence (Anti-OX40) or absence (NT) of agonistic antibody. TIL subsets and TCR-V(β) repertoire were quantified by flow cytometry using IO Test Beta Mark TCR V beta Repertoire Kit. TIL expanded with or without anti-OX40 presented with comparable repertoires of TCR Vβ in both CD4+ and CD8+ T-cell subsets despite dramatically different representation of each T-cell subset in the two conditions.

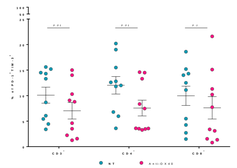
Results indicate that anti-OX40-induced skewing of the CD8+TIL subsets occurs without significant clonal selection.

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



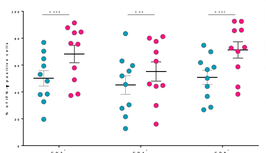
Phenotypic characterization of post-REP TIL expanded with anti-OX40 agonist. Pre-REP TIL were rapidly expanded in the presence of OX40 agonistic antibody (Anti-OX40) or in the absence of any treatment (NT) (n=10). Post-REP TIL were phenotypically characterized for T-cell lineage, differentiation, and exhaustion markers including CD3, CD4, CD8, CD28, CD56, PD-1, and TIM-3. A significant decrease in CD28 expression **(A)** and increase in CD56 expression **(B)** was observed in total CD3+ post-REP TIL expanded with anti-OX40 agonist relative to control, indicating a more differentiated phenotype. Bars are shown as mean ± SEM. $p<0.05$ is considered statistically significant.

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



Analysis of T-cell exhaustion markers in post-REP TIL expanded with anti-OX40. Total CD3+ TIL expanded with anti-OX40 agonist had lower PD-1 and TIM-3 expression (n=10). **(A)**The reduction was particularly significant in the CD4+ subset. Error bars are shown as mean ± SEM. $p<0.05$ is considered statistically significant.

Figure 8. TIL expanded in the presence of anti-OX40 increased production of IFN-γ in the CD8+ TIL subset.



Error bars are shown as mean ± SEM. $p<0.05$ is considered statistically significant.

Assessment of effector cytokine production following T cell re-stimulation. Pre-REP TIL were rapidly expanded in the presence of the OX40 agonistic antibody (Anti-OX40), no treatment (NT), and re-stimulated with PMA and Ionomycin (n=10). IFN-γ was measured in the CD3+, CD4+, and CD8+ TIL subsets by intracellular flow cytometry. Total CD3+TIL expanded with anti-OX40 produced significantly higher level of IFN-γ than those expanded in control condition. The increase was specific for the CD8+ subset.

Higher levels of IFN-γ production are indicative of enhanced effector functions, which may translate in enhanced cytotoxic activity of the CD8+ T cells.

SUMMARY

- 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 diverse TCR-V(β) repertoire in both CD8+ and CD4+ cell subsets.
- OX40 receptor was specifically down-regulated in CD4+ subset in response to anti-OX40-treated TIL, likely due to antibody internalization, and suggesting that the CD4+ T cells are the primary antibody targets.
- NF-κB signaling 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 CD56 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 agonist demonstrated heightened IFN-γ production upon re-stimulation, indicating enhanced T cell effector 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 obtained for a number of tumor histologies by supplementing REP cultures with the anti-OX40 antibody.

Disclosure & Funding Statement

- This study and poster are sponsored by Iovance Biotherapeutics, Inc.
- All authors are employees of Iovance Biotherapeutics, Inc. and may have stock options.