

Analysis of tumor infiltrating lymphocytes and in vitro exhausted T cell models to identify unique Immuno-Oncology targets

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Study rationale

T cells in tumors are exposed to persistent antigen stimulation and/or inflammatory signals. Such prolonged stimulation is often associated with deterioration of T cell effector functions resulting in a functional state termed 'exhaustion'. Identification of cell surface proteins involved in induction and maintenance of the exhausted state could inform novel immunomodulatory cancer therapies. We conducted in-depth expression profiling of membrane proteins from intact tumors (Colorectal, Pancreatic, Lung, Breast and Prostate cancer), different *in vitro* models of T cell exhaustion and primary tumor-derived lymphocytes (Colorectal, Breast and Lung cancer) using proteomics to identify novel Immuno-Oncology (IO) targets. Analysis of proteomic expression data revealed potential novel IO targets in primary tumor-derived lymphocytes (TILs) with significant cancer expression.

To rapidly validate IO targets emerging from the above approach we analyzed fresh tumors using an integrated evaluation methodology. Tumors were subject to multiple parallel analysis steps: (i) Unbiased proteomic analysis of the tumor and TILs; (ii) Flow cytometry analysis of TILs; (iii) Immuno-histochemical analysis of tumor sections; (iv) *in vitro* functional modulation of PBMCs; (v) *ex vivo* functional modulation of TILs and normal resident lymphocytes (NRLs). We show the data for one specific I-O target, OXBT185. Antibody modulation of this target results in potent T cell activation.

i. Exhausted T cell and TIL proteomics

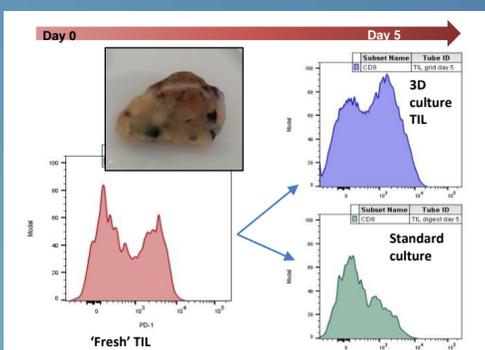


Figure 1. FACS Analysis comparing PD-1 expression in fresh isolated TILs and 3D cultured TILs after 5 days.

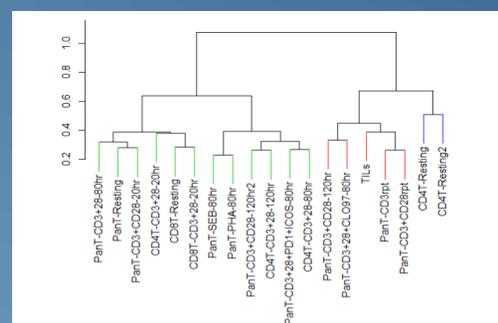


Figure 2. Hierarchical clustering of TILs and exhausted T cell models. Generated using a binary distance matrix and Ward scoring methods available in R (Version 3.2.0). Exhausted T cell models exhibit close expression to TILs isolated from CRC.

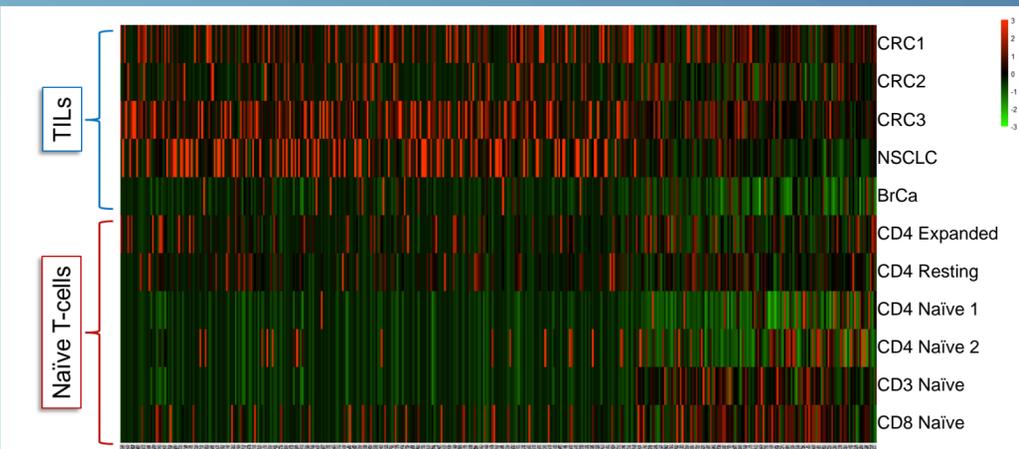


Figure 3. Proteomic expression in TILs and naive T cells isolated from buffy coat. Generated with the pheatmap package available in R (Version 3.2.0) using a binary distance matrix and Ward scoring methods. TILs display unique proteomic signature as compared to naive T cells allowing for discovery of unique immuno-oncology targets and biomarkers (Kast. J et al. (2013) ASMS 61st meeting Poster session MP25: 497).

ii. FACS Expression of OXBT185 in TILs

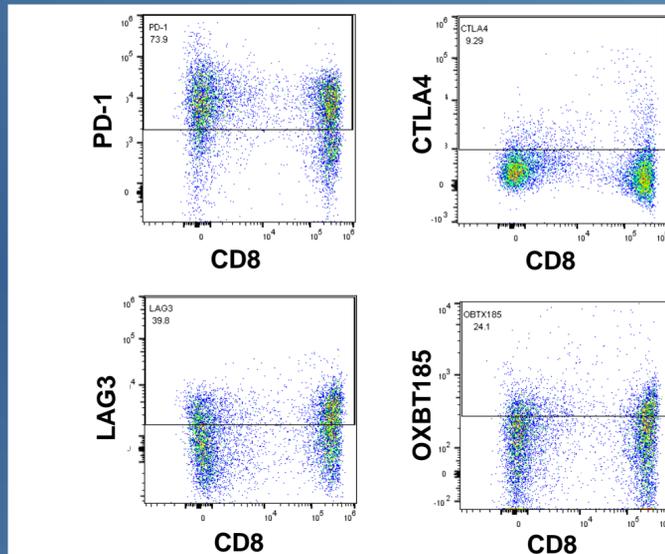


Figure 4. Protein expression of known IO targets and OXBT185 on TILs from NSCLC. Using a mouse anti-OXBT185 for FACS analysis, expression of OXBT185 was seen on TILs isolated from NSCLC.

iii. IHC Expression of OXBT185 in TILs

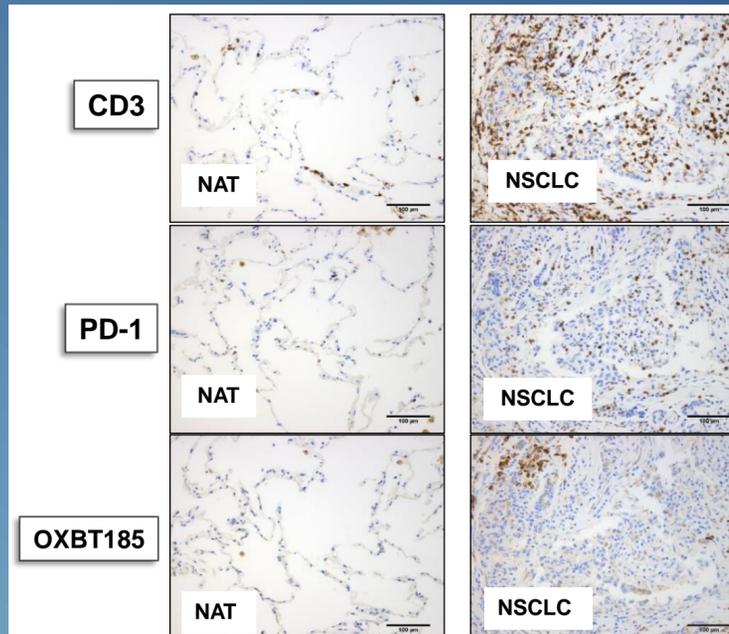


Figure 5. IHC analysis of matched tumor and normal adjacent tissue. Expression of OXBT185 was confirmed by positive membrane staining in the immune infiltrate of NSCLC. While staining was observed in the NAT it was of weaker intensity.

iv. Functional anti-OXBT185 activates T cells

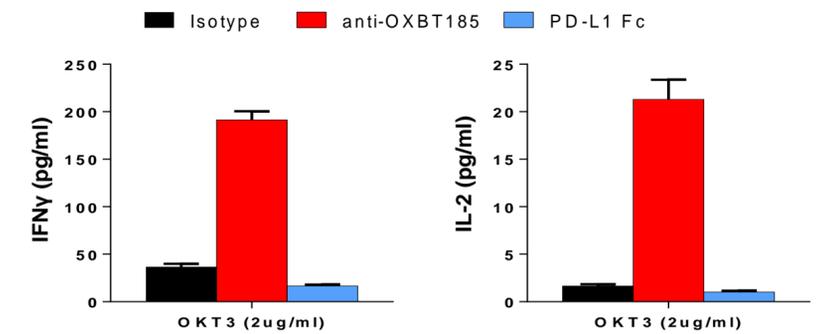


Figure 6. *In vitro* cytokine release using PBMCs primed with OKT3. IFN γ and IL-2 were measured using ELISA kits available from ebioscience. As expected PD-L1-Fc (R&D systems) inhibited cytokine release, while anti-OXBT185 stimulated production of IFN γ and IL-2.

v. Functional anti-OXBT185 activates TILs in tumor explant

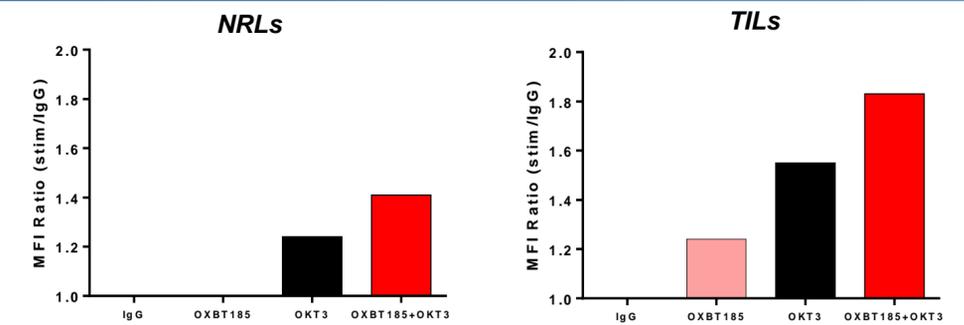


Figure 7. *Ex vivo* cytokine release using NRLs (A) and TILs (B) primed with OKT3. Ratio against IgG isotype control indicated more IFN γ producing cells from TILs when stimulated with anti-OXBT185. Anti-OXBT185 stimulation induced IFN γ production in TILs but not in NRLs. Moreover, anti-OXBT185 synergised with low-level CD3 stimulation to boost IFN γ production.

Conclusions

- 3D culture of TILs maintains their tumor phenotype.
- Proteomic analysis of these TILs reveals a unique protein signature compared to naive T cells allowing identification of IO targets and biomarkers.
- Agonist anti-OXBT185 antibody activates peripheral T cells and TILs from 3D tumor explant culture.

OBT's unique discovery path allows rapid identification and validation of IO targets.