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

James Ackroyd1, Joanne Berry1, Yaoyao Fu2, Jason Allen1, Martin Barnes1, Alexander Patterson1, Angelo Kaplan2, Nickolas Attanasio2, Rachel Dusek2, Christian Rohllf2, Haining Huang3, Livija Deban1, Robert Boyd1. 1Oxford BioTherapeutics Ltd, Abingdon, United Kingdom; 2Oxford BioTherapeutics Inc, San Jose, CA

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 (CancerL, Pancreatic, Lung, Breast and Prostate cancer), different in vitro models of T cell exhaustion and primary tumor-derived lymphocytes (CancerL, 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 analysed 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) Immunohistochemical 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 IO 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 3.2.2). Exhausted T cell models exhibit close expression to TILs isolated from CRC.

Figure 3. Protein expression in NSCLC naïve T cells and naïve TILs was assessed using the pheatmap package available in R (Version 3.2.2) using a binary distance matrix and Ward scoring methods. TILs display unique proteomic signature as compared to naïve T cells allowing for discovery of unique immunomodulatory targets and biomarkers (Rast, J et al. (2013) ASMS 61st meeting Poster session MIPS: 497).

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.

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

Figure 6. In vitro cytokine release using PBMCs primed with OKT3. IFNγ 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 IFNγ and IL-2.

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

Conclusions
• 3D culture of TILs maintains their tumor phenotype.
• Proteomic analysis of these TILs reveals an unique protein signature compared to naïve 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.