The use of proteomics to analyze whole tumors and identify unique immuno-oncology targets for antibody-based therapeutics

Jim Ackroyd[1], Arnima Bisht[2], Jason Allen[1], Lindsey Hudson[1], Martin Barnes[1], Christian Rohlff[1], Keith Wilson[2], Robert Boyd[1], Dee Aud[2].

[1]Oxford BioTherapeutics Ltd, Abingdon, United Kingdom; [2]Oxford BioTherapeutics Inc, San Jose, CA

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Introduction

The recent clinical success of the mAb therapeutics targeting immune checkpoint inhibitor proteins (PD-1/PD-L1, CTLA-4) has led to an increased appreciation of the potential of utilizing the immune system in oncology. There are two major strategies to elicit either a novel immune anti-tumor response or to reactivate a pre-existing anti-tumor response: by releasing a checkpoint inhibitory pathway via cell surface receptors (such as PD-1/PD-L1, CTLA-4) or by activation of co-stimulatory receptors (such as CD40, OX40, or GITR). Both of these strategies of immune modulation utilize cell surface receptors, and the targeting of antibody therapeutics with the appropriate functional activity to those receptors, to modify immune cell responses and allow for anti-tumor activity. The identification of novel immune-modulatory receptors with the potential to be immuno-oncology therapeutic targets could be of high value to this anti-tumor approach.

OGAP is a unique proteomic database that integrates information at the tissue, disease and protein isoform level across diseases, indications, and normal tissues to clarify membrane protein expression levels and profiles. Specifically, it currently holds information on ~16,000 human proteins sequenced, ~7,000 membrane proteins, ~40 tissues/organs, and ~19 cancers. OGAP has been used to identify novel oncology therapeutic targets for both ADC and BiTE-like approaches. We are creating a new knowledge base incorporating immune proteomic, structural and functional data to assist in the discovery of novel immuno-oncology targets.

Sample Preparation: Activated T Cells

Buffy coats were obtained from normal human donors (Stanford Blood Bank) and untouched Pan Human T Cells were isolated with the Miltenyi kit according to manufacturer's directions following PBMC enrichment by Ficoll gradient. T cells from 3 donors were pooled to generate a cell pellet for untouched, resting T cells. Cells were cultured for 20 hours in the presence of anti-CD3/anti-CD28 activation beads (Invitrogen/Dynal) according to manufacturer's directions. A small aliquot for each donor was cultured without stimulation to provide a baseline for FACS analysis of activation by CD69 upregulation. T cells from 3 activated donors were also pooled to form the pellet for proteomic analysis.

Bioinformatic Analysis and Identification of Immuno-Oncology Proteins

5987 human proteins were identified by the above proteomic analysis, we then applied two approaches to the identification of immuno-oncology proteins. Firstly sequence analysis of the human proteome was performed to find proteins with a similar structure to known immune checkpoint proteins (PD-L1, VISTA, PD-1, CTLA4). Consensus sequences were generated from both the extracellular domain and cytoplasmic domain of these proteins. The analysis revealed that 407 proteins had conserved residues to known immune checkpoint proteins. Using spectral analysis[1] to measure protein abundance, 756 membrane proteins were identified in resting T-cells and CD3 / CD28 activated T-cells (see Figure 2). The detection of known T-cell activation antigens (CD3, CD4 CD8, CD69, HLA-DRA, CD40L see Figures 1 and 3) further confirmed the successful activation of the T-cells. Proteins displaying differential expression in resting vs. activated T-cells were then investigated further. Combining the bioinformatics and experimental expression based approaches produced a list of 55 potential immuno-oncology targets (see Figures 4 and 5). Analysis of the proteins using OGAP showed the list included T-cell specific and cancer specific proteins. Figure 6 depicts two novel immuno-oncology targets which exhibit differential expression in T-cells and high cancer expression in indications with unmet clinical needs.

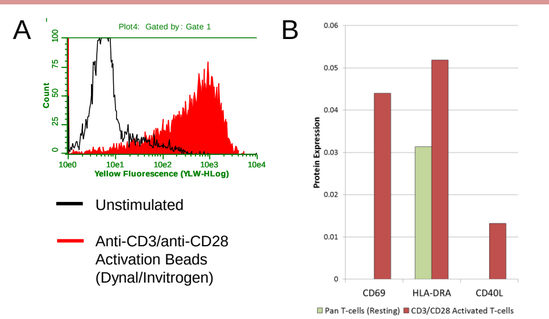

Figure 1. ***A*** Anti-CD69 PE (BD) FACS staining of anti-CD3/anti-CD28 activated Pan T Cells shows an increase in CD69 brightness and proportion of positive cells (red filled histogram) vs. unstimulated Pan T cells (black open histogram). Up-regulation of CD69 indicates successful activation of pan T-cells. ***B*** Up-regulation of CD69 and other activation markers also detected by proteomics.

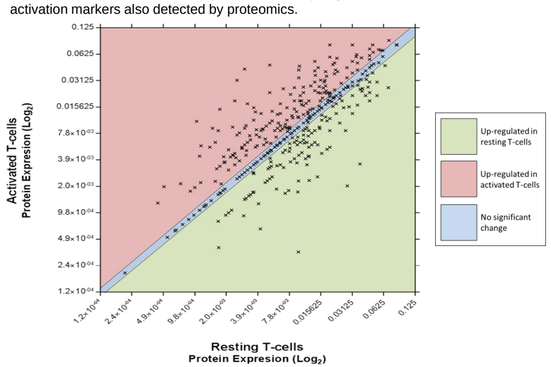

Figure 2. Proteomic analysis of resting and activated T cells identified 756 membrane proteins. The above graph plots proteins with no expression difference between resting and activated T cells (blue), proteins up regulated in resting T cells (green) and proteins up regulated in activated T cells (pink). Relative protein expression was calculated according to reference 1.

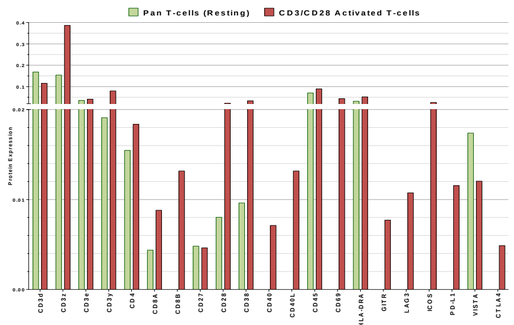

Figure 3. Known T-cell antigen protein expression in resting and activated T-cells. Key T cell markers such as CD3, CD4, CD8, PD-L1, VISTA, GITR and CTLA4 have been selected. Protein expression in resting T cells (green) and activated T cells (red) is shown. Relative protein expression was calculated according to reference 1.

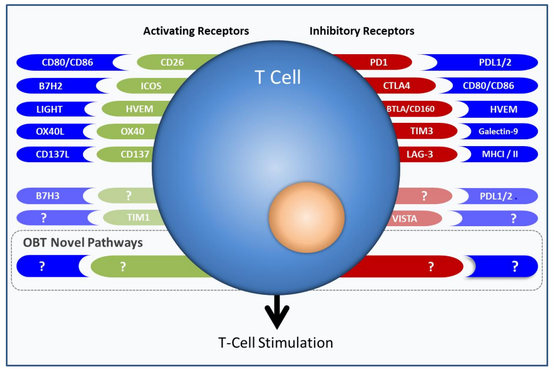

Figure 4. Known ligand-receptor interactions.
OBT proteomic analysis of immune cells facilitates the discovery of novel immuno-oncology targets for immune checkpoint blockade

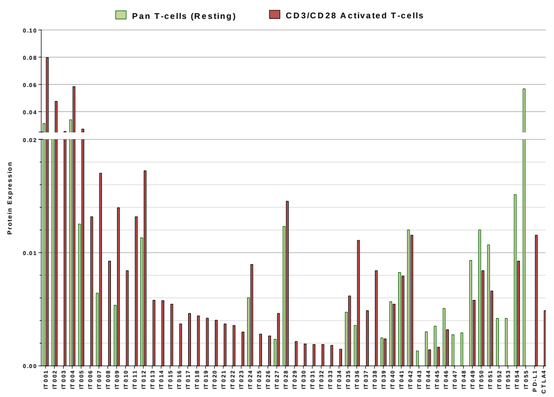

Figure 5. Potential immuno-oncology targets expression in pan T-cells and CD3/CD28 activated T-cells
The above targets displayed differential expression in resting vs. activated T-cells and/or conserved amino acid sequence homologous to known checkpoint proteins. Analysis of targets in OGAP revealed that some targets had high cancer specificity while others were highly specific to T-cells.

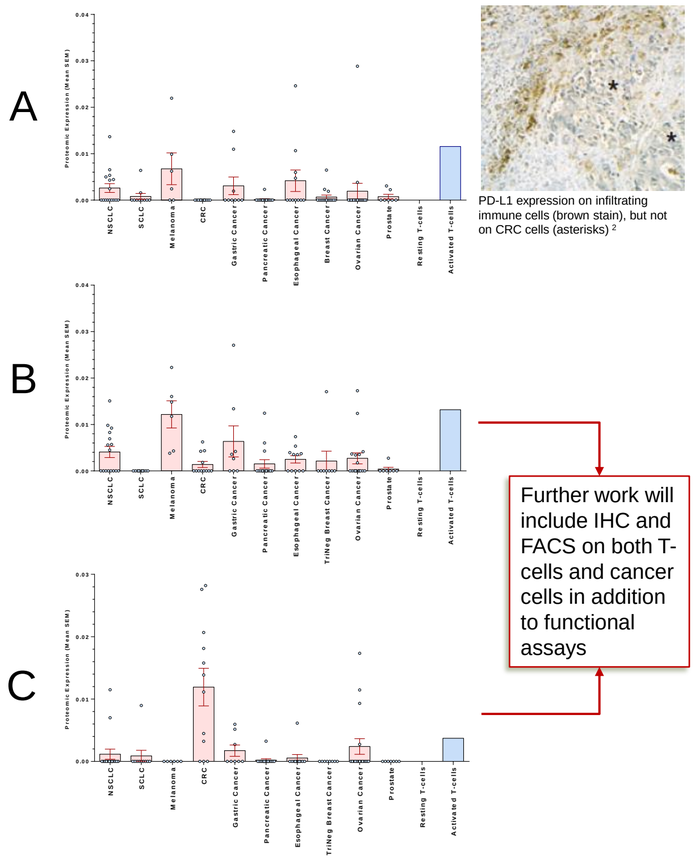

Figure 6. ***A*** PD-L1 shows proteomic expression across a range of cancers including NSCLC and melanoma, but was not detected in CRC and Pancreatic cancer. ***B*** IT011 has similar domains to CTLA4 and is up-regulated in activated T-cells, cancer tissues and cancer cell lines. ***C*** IT016 shows expression in CRC, ovarian cancer tissue and cancer cell lines. IT016 is also upregulated in activated T-cells.

Conclusion

Proteomic analysis of resting pan T cells and anti-CD3 / anti-CD28 activated pan T-cells has provided a unique approach to the discovery of novel immuno-oncology targets. The analysis resulted in the identification of a number of potential immuno-oncology targets exclusive to T cells and/or shared expression on cancer cells in indications with a high unmet clinical need. Using this approach also allowed the assessment of differences between activated T cell vs. resting T cell protein levels. This approach identified expected T cell protein activation markers (CD69, HLA-DR, CD40L, CTLA-4, ICOS and CD38) and may lead to the identification of novel T cell markers. Analysis of this data set with additional data mining strategies is likely to reveal more potential targets and T cell markers which will strengthen OBT's immuno-oncology pipeline and lead to novel therapies for the treatment of cancer.

Further Work

The analysis of T cell subsets, B cells, monocytes and other immune cell subsets with and without activation is underway. OBT is generating affinity reagents to potential immuno-oncology targets and will also generate more extensive expression data. The ability of these potential immuno-oncology targets to modulate immune cell activity will also be investigated.

1. Kast J. *et al.* Improved proteomic quantitation for the validation of therapeutic targets (2013) ASMS 61st meeting Poster session MP25: 497
2. Taube JM *et al*. Clin Cancer Res. 2014 Oct 1;20(19):5064-74