Identification of Novel Cancer-Specific Splice Variants Using Oxford Biotherapeutics' Ultra-High Sensitivity Tissue Membrane Proteomics Database, OGAP®-Verify, for First-in-class ADCs and Other Therapeutic Antibodies.

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Abstract

Oxford BioTherapeutics (OBT) is a clinical stage oncology company with a pipeline of antibody-based therapies, including Antibody-Drug Conjugates (ADCs), T-Cell Engagers (TCEs) and Immuno-Oncology (IO) antibodies. OBT utilizes its proprietary, proteomic discovery platform, OGAP®, to identify novel cancer therapeutic targets to develop therapies in different unmet indications. Leveraging OGAP®, OBT is focussing on developing first-in-class therapies (ADC, IO) with partnerships including, Boehringer Ingelheim (TCE), ImmunoGen (ADC) and Genmab (IO).

OGAP® is one of the world's largest quantitative membrane protein expression libraries, generated using tissue proteomics. Alongside canonical peptide sequences, OGAP® contains a large dataset of peptides which do not map to SwissProt canonical sequences. OBT is analysing this data to identify novel cancer-specific splice variants. Directly measuring membrane protein abundance circumvents the problem of poor correlation of mRNA abundance with protein expression. This has allowed OBT to identify isoform targets containing peptide sequences which do not map to the canonical proteins. Two partnered programs have been yielded so far from this analysis, but the vast majority of the space remains unexplored. Targets identified from this space represent first-in-class opportunities and are available for partnering.

Furthermore, the cancer-specific splice variant target pipeline has been greatly enhanced through OGAP®-Verify which now has higher sensitivity than IHC, down to 50 copies-per-cell in patient tissues, enabling OBT to measure protein abundance (copies-per-cell) in normal and cancer patient tissues as opposed to cell lines, removing ambiguity of IHC antibody specificity and the difficulty to make splice variant specific antibodies.

Introduction

- OBT leverages its proprietary, proteomic target discovery platform - OGAP®, to identify first-in-class antibody-based therapeutic targets by directly measuring membrane protein abundance in patient tissues using quantitative mass spectrometry.
- Along with canonical peptide sequences, OGAP® contains a ***large dataset of >75,000 peptides*** which do not map to SwissProt canonical proteins.

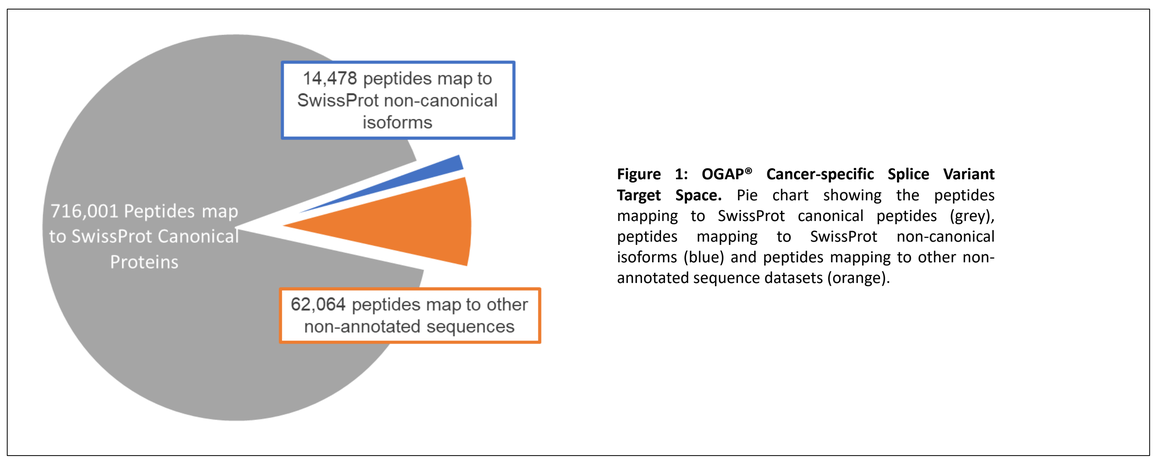

Figure 1: OGAP® Cancer-specific Splice Variant Target Space. Pie chart showing the peptides mapping to SwissProt canonical peptides (grey), peptides mapping to SwissProt non-canonical isoforms (blue) and peptides mapping to other non-annotated sequence datasets (orange).

- These peptides potentially map to sequences from SwissProt non-canonical splice variant, Trembl, Ensembl and other sequence datasets.
- OBT has established a pipeline to identify novel, cancer-specific splice variants. To date, it has ***analysed just <5% percent of these 'un-mapped' peptides*** and has already identified a number of cancer-specific splice variants which can be uniquely targeted by an antibody. Two programs are in development from this initial pilot study.
- ***>95%*** of the target search space is still unexplored.
- Based on OBT's extensive experience, there are three types of targetable splice variant neo-epitopes -
  - (A) Sequence deletion, creating a neo-junction (Figure 2A).
  - (B) Exon insertion, alternate start/stop codon creating a neo-epitope (Figure 2B).
  - (C) Novel splice variant of a cytosolic protein which due to creation of a transmembrane span, becomes membrane bound (Figure 2C).
- ***OBT*** has the ***capability to identify*** such ***unique, targetable epitopes***.
- Exact strategy to identify and validate such cancer-specific splice variants will be tailored for each target.

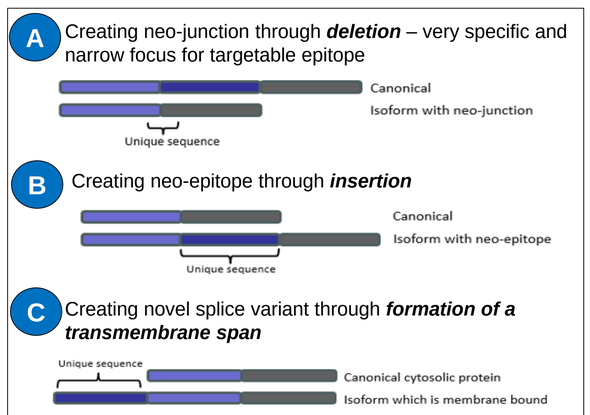

Figure 2: Types of Targetable Splice Variant Neo-epitopes. (A) Formation of neo-junction through deletion. (B) Formation of neo-epitope through neo-e epitope via alternate start/stop codon. (C) Formation of a novel membrane-bound splice variant of cytosolic canonical protein due to creation of transmembrane span.

OGAP® Cancer-specific Isoform Identification Workflow

Manual data curation of protein sequences into protein sequence families (canonical and isoforms)

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Manual data mining to generate list of putative cancer-specific isoforms

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Manual validation of mass spectrometry data generates a refined putative cancer-specific isoform shortlist

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Validation of mass spectrometry data with supporting lab experimentations

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Full cancer-specific isoform target validation and selection of cancer-specific isoform

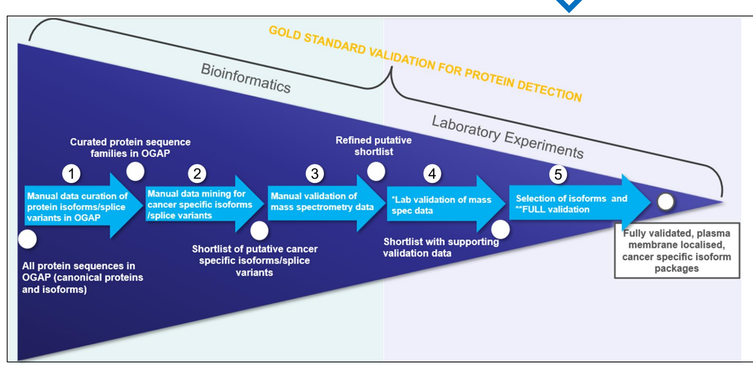

Figure 3: OGAP® Cancer-specific Isoform Identification Workflow Using Bioinformatics and Laboratory Experiments. (1) Manual data curation of protein sequences to group them into protein sequence families for both canonical and isoforms. (2) Manual data mining to generate a putative list of cancer-specific isoforms. (3) Laboratory validation of mass spectrometry data is confirmed by comparing experimental observations to synthetic peptide standards. (4) Full validation of cancer-specific isoform targets include, confirmation of plasma membrane expression, generation of validated tool antibodies and cell lines against the isoform, confirmation of normal tissue expression profile and cancer prevalence by immunohistochemistry for selection of the isoform target.

OGAP® Cancer-specific Isoform Example Case Study: Claudin 18.2

- ***OGAP® data*** for Claudin 18.2 splice variant ***correlates*** with ***existing publicly available data***.
- Clinical data from several phase I, phase II and phase III clinical trials reported to express Claudin 18.2 in 95% of gastric signet ring carcinomas, 60% of pancreatic adenocarcinoma tumors and healthy stomach mucosa by IHC.
- OGAP® reports expression of Claudin 18.2 in 100% of gastric tumors and 50% of pancreatic tumors analysed.
- OGAP® detects Claudin 18.2 expression in all normal gastric samples analysed.
- Claudin 18.2 is not reported in public databases, Peptide Atlas and Proteomics DB, but OGAP® has the capability to identify such splice variants with data close to clinical scenario.

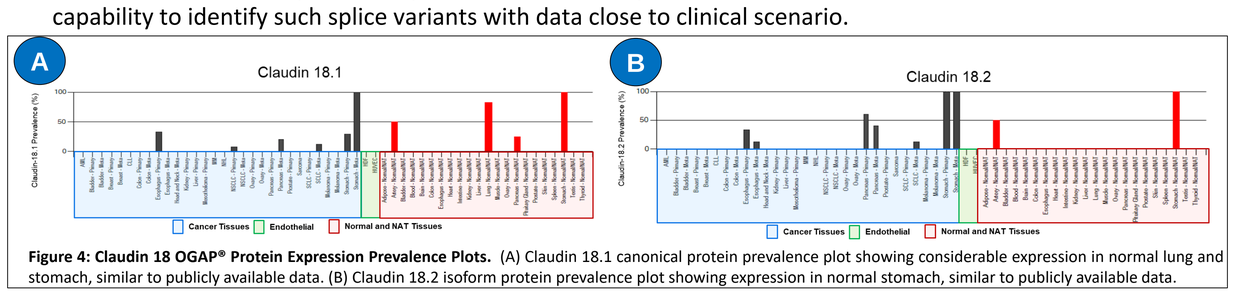

Figure 4: Claudin 18 OGAP® Protein Expression Prevalence Plots. (A) Claudin 18.1 canonical protein prevalence plot showing considerable expression in normal lung and stomach, similar to publicly available data. (B) Claudin 18.2 isoform protein prevalence plot showing expression in normal stomach, similar to publicly available data.

- Sequence alignment of Claudin 18.2 isoform and Claudin 18.1 canonical proteins demonstrates 22 amino acid substitutions in the N-terminal region of Claudin 18.2 (Figure 6), making it uniquely targetable by antibodies.
- ***OBT*** has ***identified eight Claudin 18.2 isoform unique peptides*** containing substitutions, signifies unique targetability of the isoform with an antibody.
- ***Public mRNA data alone fails to predict Claudin 18.2 as a target*** in stomach, pancreatic and esophageal cancers, as being found to be overexpressed in clinical trials (Figure 5).
- ***OGAP® enables extensive identification of such targets***, that are ***unable to be identified by mRNA predictions*** and with little to no normal/normal adjacent tissue expression.

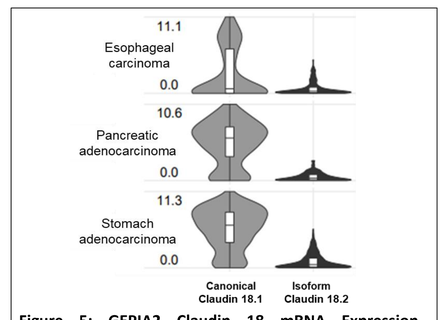

Figure 5: GEPIA2 Claudin 18 mRNA Expression Distributions in Canonical Versus Isoform. In all the clinical indications including, stomach, pancreatic and esophageal cancers, the Claudin 18.2 isoform could not be predicted using mRNA expression (http://gepia2.cancer-pku.cn/#index).

Figure 6: Sequence Alignment of Claudin 18.1 (Canonical) and Claudin 18.2 (Isoform). Sequence alignment of Claudin 18.1 canonical and Claudin 18.2 isoform showing a stretch of 22 amino acid substitution in the N-terminal region of Claudin 18.2 isoform.

OGAP® Identified Novel Cancer-specific Isoform Example

- Example of a novel cancer-specific isoform (Figure 7) identified using OBT's proprietary proteomic database, OGAP®, which has a C-terminal deletion compared to the canonical sequence
- OBT's proteomics data shows expression in nine cancer indications and no expression detected in normal tissues as compared to the canonical protein which shows extensive expression across most normal tissues (Figure 8).
- Cancer-specific isoform is predicted to contain six transmembrane domains.
- Cancer-specific isoform potentially targetable through a C-terminal neo-junction/large deletion causing a change in tertiary structure compared to the canonical protein.

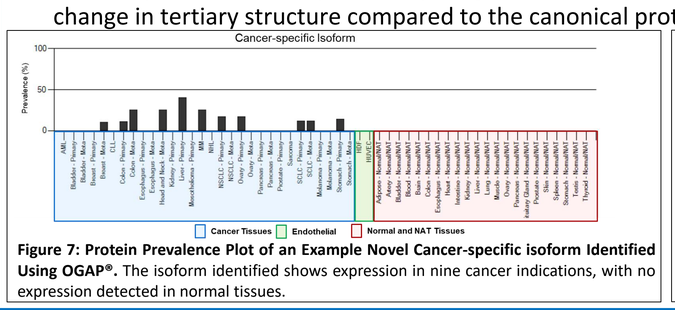

Figure 7: Protein Prevalence Plot of an Example Novel Cancer-specific isoform Identified Using OGAP®. The isoform identified shows expression in nine cancer indications, with no expression detected in normal tissues.

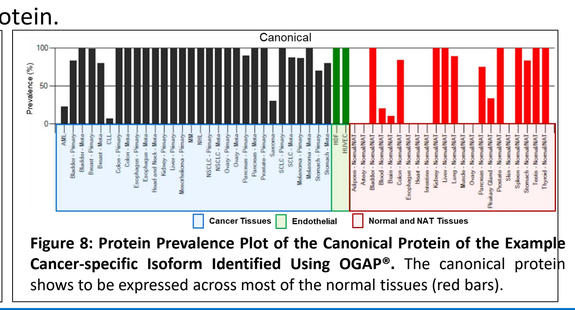

Figure 8: Protein Prevalence Plot of the Canonical Protein of the Example Cancer-specific Isoform Identified Using OGAP®. The canonical protein shows to be expressed across most of the normal tissues (red bars).

OBT's Novel Approach Identifies Unique Cancer-specific Isoform Targets

- Built upon a ***solid foundation of technical expertise***, ***addressing the shortcomings of existing discovery*** and ***validation platforms***, ***OGAP®*** provides access to a ***superior toolkit and proteomic database*** for novel cancer-specific target identification.
- In addition to OGAP® existing capabilities, the ability of cancer-specific splice variant target pipeline has been greatly enhanced through ***OGAP®-Verify*** which now has ***higher sensitivity than IHC***, which can measure protein abundance down to ***50 copies-per-cell*** in normal and cancer patient tissues as opposed to cell lines, removing ambiguity of IHC antibody specificity.
- ***OGAP® contains peptide sequences which do not map to SwissProt canonical sequences***, analysis of such sequences have yielded two partnered programs, and a ***vast majority of the space remains unexplored***.
- ***Isoform targets identified*** from this space are ***first-in-class opportunities and available for partnering***.