HAWK Biosystems

Immune Checkpoint Interaction Quantification by QF-Pro® Whitepaper

HAWK Biosystems have published a case study in the Journal of Clinical Oncology titled "*Functional engagement of the PD-1/PD-L1 complex but not PD-L1 expression is highly predictive of patient response to immunotherapy in NSCLC*". This is a ground-breaking result which could increase patient responses to immune checkpoint inhibition (ICI) therapies in non-small cell lung carcinoma (NSCLC) by up to 280%.

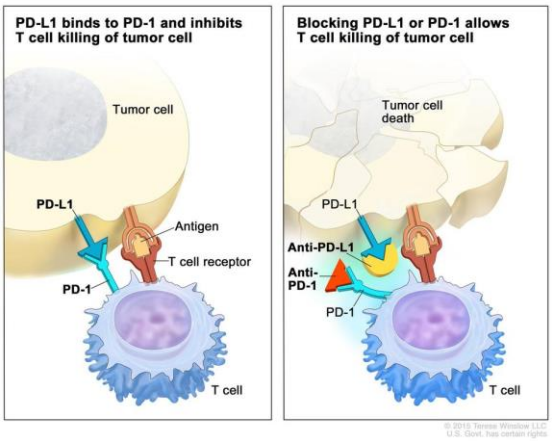

Figure 1: PD-1/PD-L1 is an immune checkpoint designed to switch off the immune system to prevent auto-immune disease. However, some cancers upregulate the ligand to evade the immune system, which is inherently programmed to destroy neoplastic cells. Drugs blocking these interactions between the immune system and cancer cells can allow the immune system to find and clear cancer cells from the body.

What are Immune Checkpoints:

Immune checkpoints are ligand-receptor pairs which have evolved within the immune system to prevent dysregulated over activation of the immune system. Well known immune checkpoints include:

- Programmed death receptor-1/programme death ligand-1 **(PD-1/PD-L1)**
- Cytotoxic T-lymphocyte associated protein-4/ (CD)-80 - **(CTLA-4/CD80)**
- T-cell immunoreceptor with Ig and ITIM domains/CD155 – **(TIGIT/CD155)**

Immune checkpoint receptors, found on immune cells such as lymphocytes, may interact with their cognate ligands on antigen presenting cells or normal body cells. Upon interaction, these checkpoints downregulate the immune system, preventing it from attacking healthy body cells. However, many cancers, including NSCLC, highjack this pathway by upregulating the cognate ligands of these immune receptors, thus preventing the immune system from attacking and clearing the cancer.

This interaction can be blocked using therapeutic monoclonal antibodies which disrupt the engagement of these checkpoints, thus re-enabling the immune system to attack neoplastic cells from the

body. Despite showing strong promise to reduce tumour volume and progression, many patients develop primary or acquired resistance and do not respond fully to these therapies. It is though that this is, in part, due to these drugs being prescribed to an ill-defined patient subset.

How are patients selected for immune checkpoint inhibition therapy:

Currently, NSCLC patients are selected for ICI therapy based on the expression levels of the checkpoint ligand. Within our case study, we analysed arguably the most well-known immune checkpoint, PD-1/PD-L1. The expression of the ligand, PD-L1 is used to select patients for ICI therapies aimed at blocking PD-1/PD-L1 interactions. However, this approach has several limitations which may in fact prevent patients from receiving the correct treatment.

Firstly, detecting ligand expression utilises immunohistochemistry (IHC) approaches which label the ligand (PD-L1 here) with an antibody. However, antibodies are rarely 100% specific and may recognise epitopes other than PD-L1, thus leading an operator to believe there is more PD-L1 within a sample. Secondly, these readouts are subjective and are often visually assessed by operators and graded with a scoring system which contains a low dynamic range. This leads to discrepancies in results between laboratory personnel and different laboratories. Thirdly, and more crucially, ligand expression does not correlate with receptor-ligand engagement. A patient may present with high levels of PD-L1, but this does not ensure that the ligand is in fact interacting with the receptor, PD-1.

This results in two groups of mistreated patients. One group of patients present with high ligand expression but do not have significant checkpoint interaction, therefore are receiving treatments that will not benefit them (and are missing out on treatments that would). The second group of patients present with lower PD-L1 levels, but, despite this, the PD-L1 present in their samples is interacting significantly with the receptor PD-1. These patients are excluded from ICI therapies that would greatly benefit them.

Therefore, a better solution is required to select patients for ICI therapies.

The use of QF-Pro® to stratify patients for ICI therapies:

QF-Pro® assays are based on a smart proprietary adaptation of Förster Resonance Energy Transfer (FRET) that for the first time enables a reliable and robust use of this technique in pathology samples, due to its unprecedented signal to noise ratio.

The goal of QF-Pro® is to detect protein post-translational modifications (PTMs) and protein-protein interactions (PPIs) within cell samples or fixed tissue samples. The labelling of QF-Pro® is as follows. To detect PTMs, a biomarker is labelled on two distinct sites with species-specific primary antibodies. These are in turn labelled with proprietary secondary reagents conjugated to either a donor or acceptor chromophore. To detect PPIs, the two proposed interacting biomarkers are labelled with the species-specific antibodies, one antibody for each biomarker, and these are labelled as above with secondary reagents. In this case study, we labelled PD-1 (donor) and PD-L1

(acceptor) to allow for the quantification of this receptor-ligand interaction.

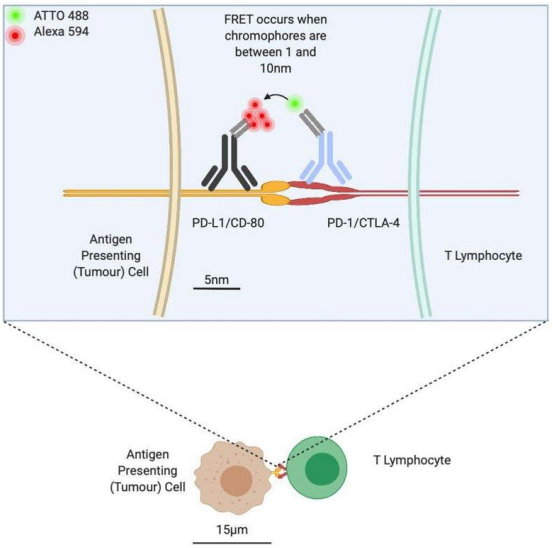

Figure 2: QF-Pro® detects FRET between the receptor (PD-1) and ligand (PD-L1) when they are interacting within 1-10nm.

The detection and quantification of FRET between the donor and acceptor chromophores within this assay allows for the precise calculation of 1-10nm distances, acting as a “chemical ruler” which reports on the above phenomenon with a high sensitivity and dynamic range. The quantification of this interaction allows for the discovery and measurement of a functional biomarker in NSCLC, PD-1/PD-L1 **interaction state.**

This is crucial as it is this checkpoint engagement, rather than PD-L1 expression, which drives immune evasion and disease progression in NSCLC. QF-Pro® therefore provides a tool whereby researchers and clinicians can stratify NSCLC patients for ICI therapies.

Our case study consisted of a multi-site blinded analysis across a cohort of 188 ICI-treated NSCLC patients.

QF-Pro® initially demonstrated the ability to detect and quantify intra- and intertumoural heterogeneity of the PD-1/PD-L1 immune checkpoint engagement, a metric which is not obtainable using other techniques within patient tissue samples.

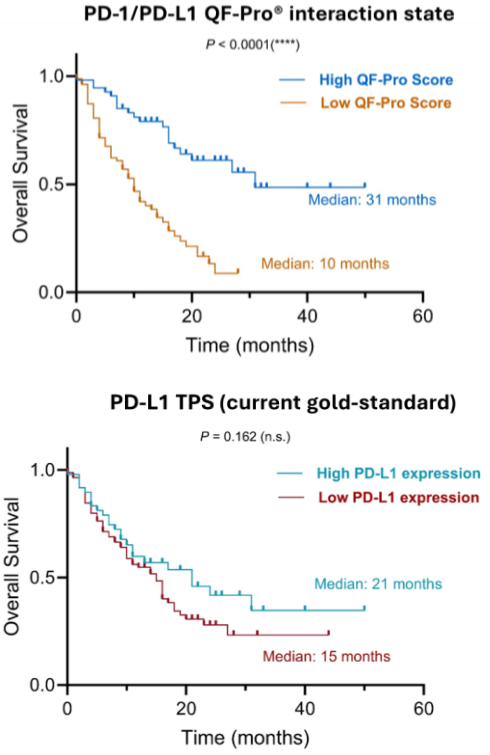

Figure 3: High PD-1/PD-L1 interaction state, quantify with QF-Pro® better correlates with enhanced OS in response to immunotherapy than PD-L1 expression. Patients were stratified into two groups: those with high interaction states and those with low interaction states.

Notable, we showed no correlation between the extent of PD-1/PD-L1 interaction and PD-L1 expression (the current gold-standard biomarker for patient stratification in NSCLC. Importantly, PD-L1 expression scores used clinically to stratify the patients, correlated poorly with overall survival, by contrast patients showing a high PD-1/PD-L1 interaction had significantly better responses to anti-PD-1/PD-L1 treatments, as evidenced by increased OS (Figure 3). This relationship was particularly strong in the setting of first line treatments.

Therefore, the functional read out of this PD-1/PD-L1 interaction as a predictive biomarker for the stratification of NSCLC patients, can significantly improve the response rates to immunotherapy by up to 280%. This would both capture patients excluded from checkpoint immunotherapy (high PD-1/PD-L1 interaction but low PD-L1 expression, 24% of patients), and additionally avoid treating patients which despite their high PD-L1 expression do not respond and suffer from side effects.

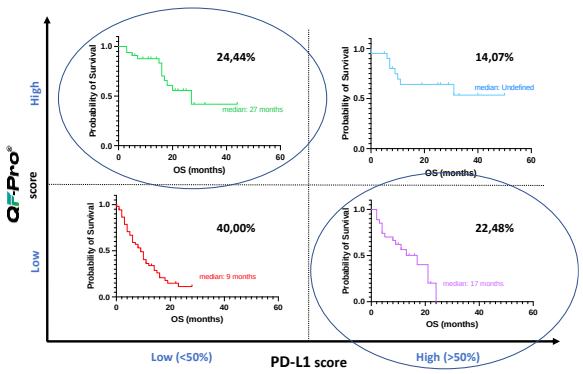

Figure 4: QF-Pro® identifies that 24.44% of NSCLC patients have significant PD-1/PD-L1 interaction state despite showing low PD-L1 scores. These NSCLC patients are currently missed from correct treatment groups.

This has proven to be a huge leap forward in world personalised medicines agenda, as there is now a platform which can accurately stratify patients for ICI therapies and can increase NSCLC ICI response rates by 280%.

Context and Future Perspectives.

Given the significance of the results above and their potential to change the landscape of precision medicine in NSCLC we are seeking to extend the reach of our technology to benefit the greatest number of patients. If you are researching in the field of NSCLC ICI therapies, planning to run, or running clinical trials in this field, please contact us as we would relish the opportunity to deploy our technique in a larger cohort prospective study to help increase response rates of NSCLC patients in a clinical setting.

Whilst QF-Pro® has advanced our progress in personalised medicine and advanced patient stratification, the benefits of QF-Pro® are not limited solely to immune oncology. If you are also working in fundamental research or clinical trials in other pathologies or disciplines, contact us to find out how we can tailor a QF-Pro® solution to your needs.

Contact Us.

Find out more information about our QF-Pro® services at: www.hawkbiosystems.com.

Alternatively, please write to us at: contact@hawkbiosystems.com.