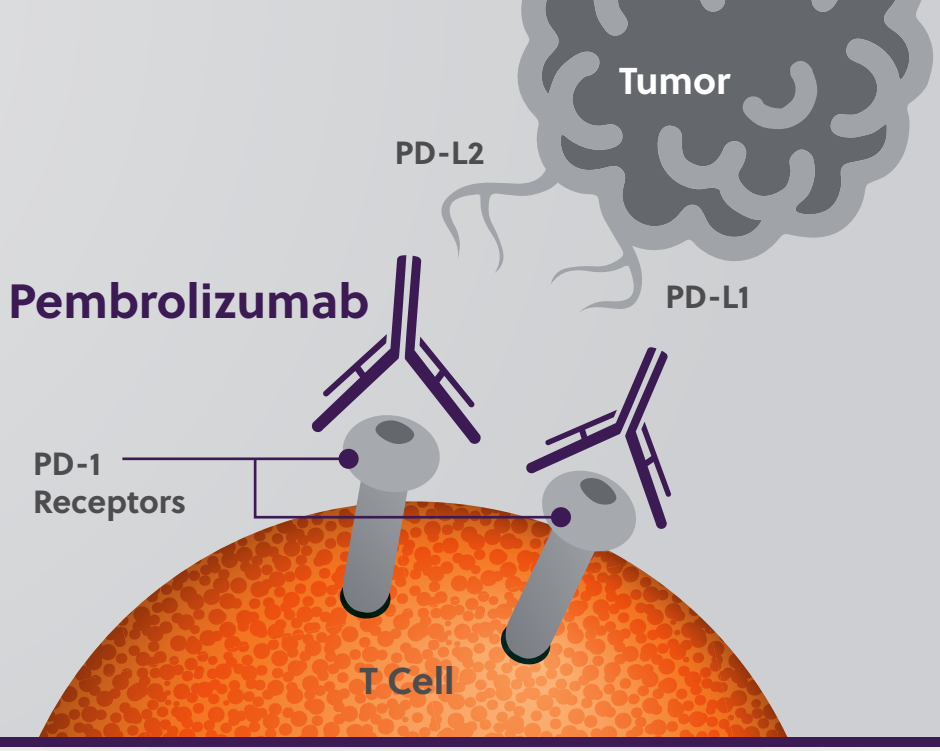

CASE STUDY:

Development of the Prototype Immunohistochemistry Assay that Advanced Development of Pembrolizumab

Our prototype assay's expert development resulted in an assay with wide dynamic range, high precision and reproducible results, laying the foundation for the successful 22C3 PD-L1 companion diagnostic assay widely utilized today to select patients eligible for treatment with the anti-PD-1 immunotherapy pembrolizumab.

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BACKGROUND:

The programmed death receptor-1 (PD-1) pathway is one of the major immune-checkpoints that engages with tumor cells to avoid T-cell–mediated immune surveillance.[1] The PD-1 receptor-1 ligand (PD-L1), which is often expressed by tumor cells, binds with PD-1 on T cells and delivers an inhibitor signal to down-regulate T cell proliferation and activation – enabling tumor expansion and growth.[2, 3]

Given its role in tumor progression, the PD-1 pathway is a common target for immunotherapies. The first clinical trial of a PD-L1-blocking drug began in 2006 in patients with a variety of tumors including melanoma, kidney, and lung. Today, six PD-1/L1 checkpoint inhibitor immunotherapies have been approved by the U.S. Food and Drug Administration for 14 different types of cancer, and PD-1/L1 inhibitors are being evaluated in more than 2,250 clinical trials.[4,5]

SCOPE OF PROJECT:

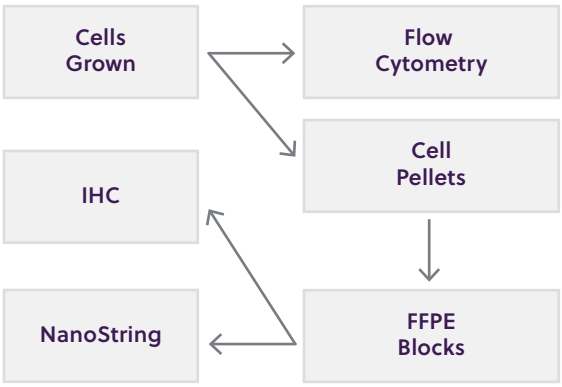
The ability to reliably and simply measure PD-L1 expression in tumor tissue may identify subsets of patients who might achieve better outcomes with anti–PD-1 or anti–PD-L1 therapeutics. QualTek experts (now part of Discovery Life Sciences) collaborated with experts from Merck Research Laboratories to develop a prototype immunohistochemistry (IHC) assay for measuring PD-L1 expression among patients enrolled in Merck-sponsored clinical trials to predict those who have a higher likelihood of responding to Merck's immune checkpoint inhibitor, pembrolizumab.[6]

SOLUTION

Develop and optimize a highly precise, specific and reproducible prototype IHC assay that accurately and robustly measures PD-L1 in non-small cell lung cancer (NSCLC) samples and can be expanded for large-scale use as a companion diagnostic assay.

ENSURING SPECIFICITY

Antibody generation and specificity for the prototype assay were determined using an orthogonal approach that included messenger RNA, flow cytometry and IHC analyses (Figure 1).[6] FFPE blocks of NSCLC cell lines that had a broad spectrum of known PD-L1 expressions at the RNA level were expanded into cell lines analyzed using flow cytometry. Cell pellets from the same lines were used for PD-L1 IHC analysis at QualTek (now Discovery Life Sciences) and mRNA analysis using the NanoString platform. The prevalence and intensity of PD-L1 staining detected for each cell line using the 22C3 antibody (mouse anti-human PD-L1 IgG1/κ antibody generated at Merck Research Laboratories, Kenilworth, NJ) was compared with expression of PD-L1 mRNA detected via the NanoString platform (Figure 2)[6] and measured by anti–PD- L1 clone 29E.2A3 using flow cytometry (Figure 3).[6]



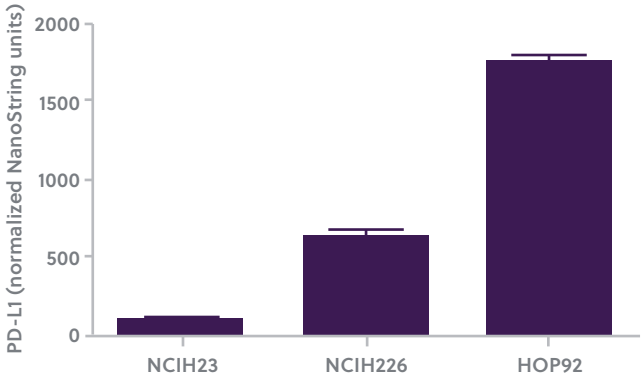


Figure 1. *Overview of process for orthogonal validation of anti-programmed death ligand-1 (anti-PD-L1) clone 22C3 for formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer. Abbreviation: IHC, immunohistochemistry.*[6]

Figure 2. *NanoString analysis of anti-programmed death ligand-1 (anti-PD-L1) clone 22C3 on formalin-fixed, paraffin-embedded non-small cell lung cancer cell pellets. Expression of PD-L1 messenger RNA using the NanoString platform for NCIH23, NCIH226, and HOP92 cell lines.*[6]

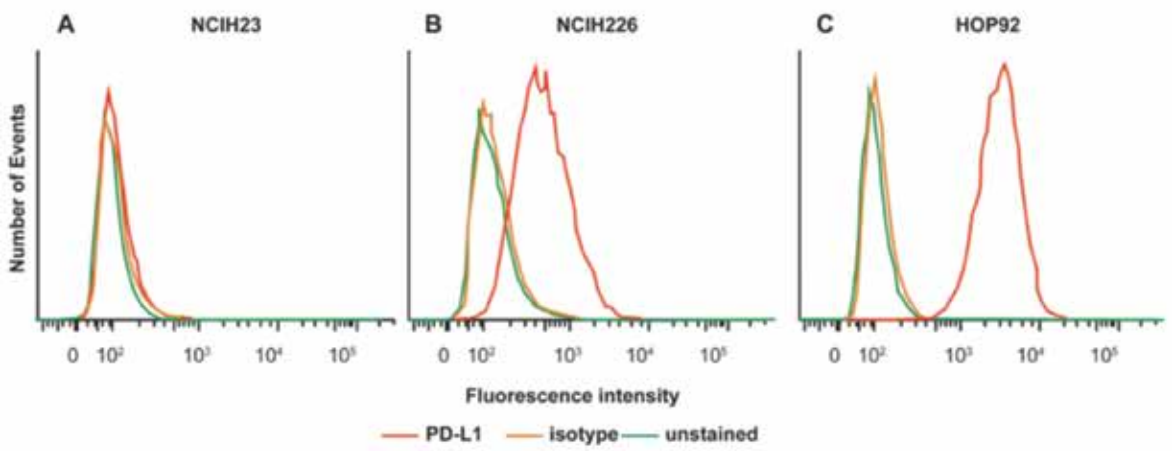


Figure 3. *Flow cytometry analysis of anti-programmed death ligand-1 (anti-PD-L1) clone 22C3 on formalin-fixed, paraffin-embedded non-small cell lung cancer (NSCLC) cell pellets. Identification of PD-L1⁻ and PD-L1⁺ NSCLC cell lines by flow cytometry with BioLegend (San Diego, California) clone 29E.2A3 for NCIH23 (A), NCIH226, (B), and HOP92 (C).*[6]

Flow cytometry and mRNA results were compared against IHC analyses (Figure 4) conducted at QualTek (now Discovery Life Sciences). Results confirmed specificity via correlating patterns of PD-L1 expression across all methodologies.[6]

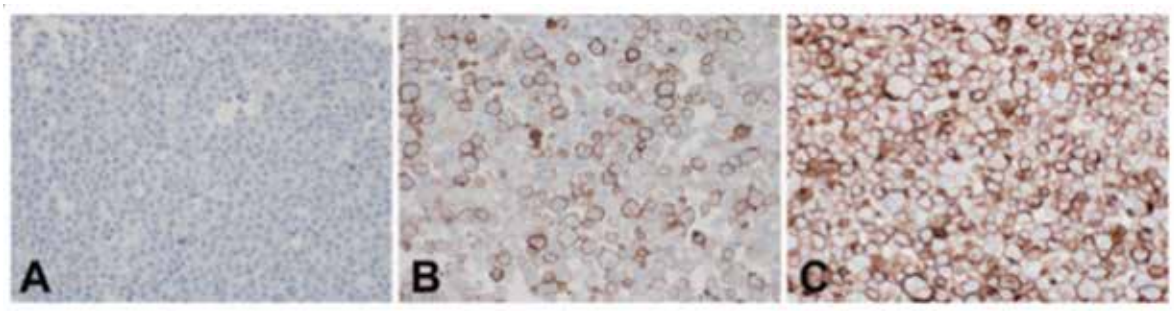


Figure 4. *Immunohistochemistry analysis of anti-programmed death ligand-1 (anti-PD-L1) clone 22C3 on formalin-fixed, paraffin-embedded non-small cell lung cancer cell pellets. PD-L1 immunohistochemistry expression analysis with anti-PD-L1 clone 22C3 is shown on NCIH23 (A), NCIH226 (B), and HOP92 (C) cell lines (original magnification x20 [A through C]).*[6]

ASSAY AND PROTOCOL OPTIMIZATION:

A full assessment of 22C3 antibody assay conditions were tested as part of assay optimization, including antibody concentration, antibody incubation time, antigen-retrieval reagents and method, and the antibody detection system. Progressive iterations were employed to identify conditions that exhibited accurate cellular localization of PD-L1, broad dynamic range of PD-L1 expression, an appropriate signal-to-noise ratio, and acceptable performance in positive and negative tissue controls.[6]

Based on this robust serial process, the optimal protocol for using 22C3 as an IHC reagent for detecting PD-L1 expression was determined and further tested in downstream studies.[6]

UTILIZATION AND SCORING OF THE OPTIMIZED PROTOTYPE ASSAY:

Using the prototype assay in 142 non–small cell lung cancer (NSCLC) and 79 melanoma archival tumor-bank tissue samples, PD-L1 staining was observed at the plasma membrane of nucleated tumor and nontumor cells and, in some cases, as a distinct lichenoid pattern at the tumor-stroma border (Figure 5).[6]

Using a preliminary scoring method, 56% (80 of 142) of non–small cell lung cancer and 53% (42 of 79) of melanoma samples were defined as PD-L1+ based on a modified H-score of 1 or more or the presence of a distinctive staining pattern at the tumor-stroma interface.[6]

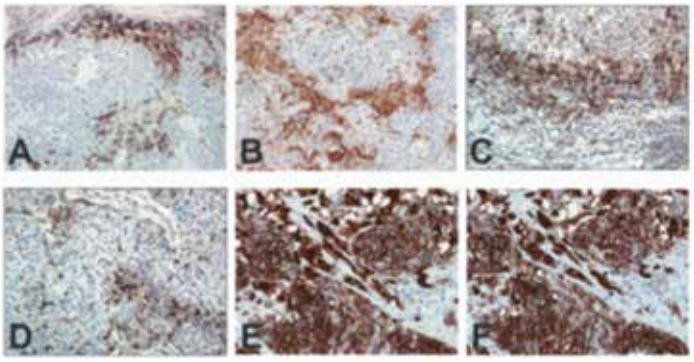


Figure 5. *Programmed death ligand-1 (PD-L1) immunohistochemistry (22C3 antibody) staining patterns. Interface pattern in non-small cell lung cancer (A and B) and melanoma (C). D, Dendritic pattern, melanoma. E and F, Melanin interference in melanoma, where PD-L1 staining (E) is not distinguishable from the isotype-negative control (F) (original magnification x20).*[6]

Pathology		Samples, No.	Tumor MHS Positive (≥1%), No. (%)	Stroma Positive, No. (%)	Dendritic Positive (Melanoma Only), No. (%)	Overall PD-L1+ (MHS and/or Stroma), No. (%)[a]
NSCLC	Squamous cell	53	22 (42)	32 (60)	—	33 (62)
	Adenocarcinoma	78	28 (36)	35 (45)	—	39 (50)
	Large cell	11	6 (55)	8 (73)	—	8 (73)
	Total	**142**	**56** (39)	**75** (53)	—	**80** (56)
Melanoma	Total	79	23 (29)	30 (38)	8 (10)	42 (53)

Abbreviation: *MHS, modified H-score.*

[a] *Samples may be positive if either or both MHS and stroma are positive. Most MHS-positive samples are also stroma-positive.*[6]

OUTCOME

A prototype assay suitable for analyzing PD-L1 expression in Merck-sponsored clinical trials and epidemiologic studies to assess the utility of PD-L1 as a potential prognostic biomarker, predictive biomarker, or both, was successfully developed quickly and cost-effectively. This assay is now being used to determine eligibility in select clinical studies of pembrolizumab with the intent of enriching the population of patients with NSCLC who may be more responsive to pembrolizumab and for defining the prognostic role of PD-L1 in melanoma, NSCLC, head and neck cancer, gastric cancer, and Hodgkin lymphoma.

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