Development of a Prototype Immunohistochemistry Assay to Measure Programmed Death Ligand-1 Expression in Tumor Tissue

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INTRODUCTION

Merck and QualTek, now part of Discovery Life Sciences (DLS), jointly developed the prototype companion diagnostic immunohistochemistry (IHC) assay for measuring PD-L1 expression among patients in clinical trials to predict those who have a higher likelihood of experiencing a response with pembrolizumab.

The prototype IHC assay was utilized prospectively in early MK3475 (pembrolizumab) NSCLC and melanoma patient enrolled trials.

Following early approvals for pembrolizumab, DLS continues to serve as the preferred specialty lab for PD-L1 CDx testing in Merck Investigator Study Protocol (MISP) studies, Merck External Collaborator Studies (MECS) as well as Merck sponsored clinical studies.

In this presentation, data presented is adapted to only IHC experiments from the publication in Archives of Pathology Laboratory Medicine (2016; 140:1259-1266; doi: 10.5858/arpa.2015-0544-OA).

METHODS & MATERIALS

The anti-human PD-L1 IgG1/κ antibody (clone 22C3) was generated at Merck Research Laboratories (Kenilworth, NJ) by immunizing mice with a proprietary fusion protein containing the human PD-L1 extracellular domain.

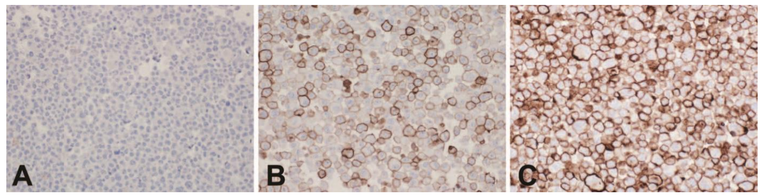
NSCLC cell lines with varying PD-L1 expression were used to initially test sensitivity and specificity of the 22C3 antibody, followed by testing in normal tonsil.

In multi-tumor tissue blocks, which include larger ‘sausage’ shaped pieces of tissue compared to tissue microarrays, assay optimization was carried out, inclusive of testing retrieval methods, assay range and linearity, and assay accuracy. Assay sensitivity and precision was also carried out.

The optimized assay was tested in larger sets of NSCLC (n=142) and melanoma (n=79) FFPE tissues with a modified H-score (MHS) utilized to semi-quantitatively assess tumor PD-L1 (considering whole or partial membrane expression), where the modification to a typical H-score was to include reactive mononuclear inflammatory cells (MICs) within tumor. Positivity was defined as an MHS of 1 or more or the presence of distinctive PD-L1 interface expression (i.e., PD-L1 staining of MICs present at the leading edge or margin of the tumor mass or nodules/nests or closely associated with tumor cells in the stromal environment). Interface was assessed as *present* or *absent* and did not contribute to the MHS.

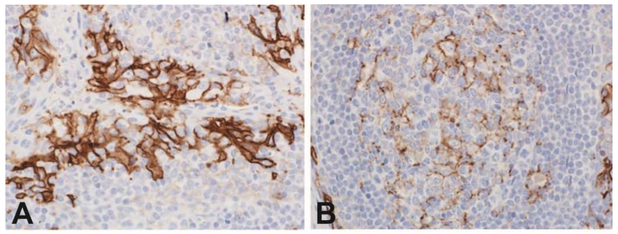
RESULTS

Figure 1



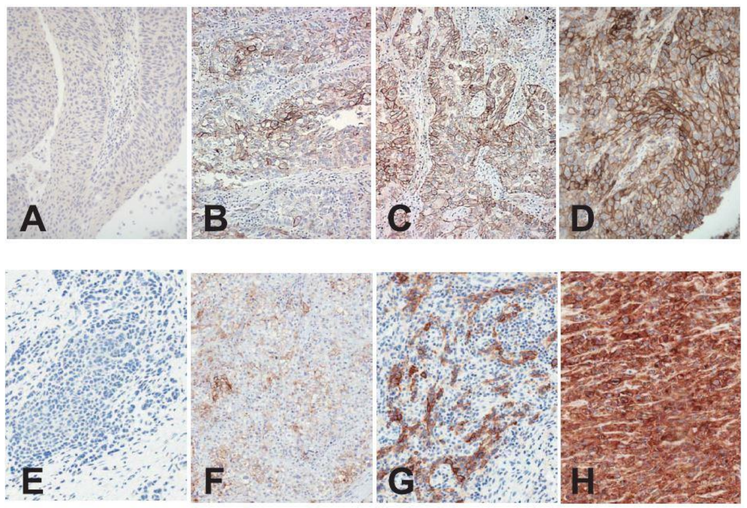
IHC analysis of anti-programmed death ligand-1 (anti-PD-L1) clone 22C3 on FFPE NSCLC cell pellets. PD-L1 IHC expression analysis with anti-PD-L1 clone 22C3 is shown on NCIH23 (A), NCIH226 (B) and HOP92 (C) cell lines (20x objective).

Figure 2



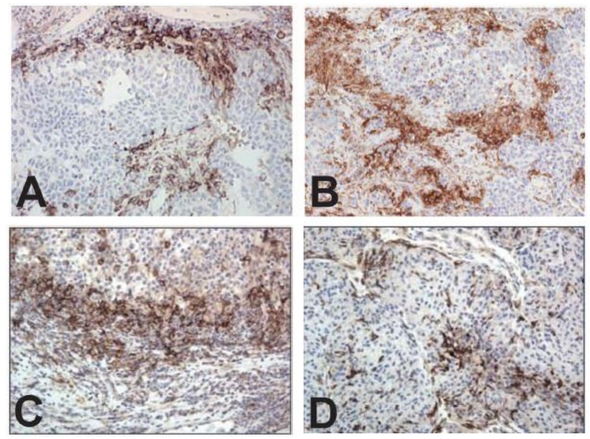
PD-L1 expression in healthy human tonsil crypt epithelium (A) and follicular macrophages (B) (60x objective).

Figure 3



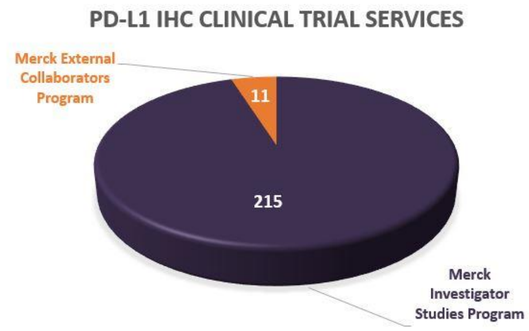
Representative PD-L1 tumor staining with 22C3 antibody. A, NSCLC; MHS 0. B, NSCLC; MHS 40. C, NSCLC; MHS 150. D, NSCLC; MHS 250. E, Melanoma; MHS 0. F, Melanoma; MHS 90. G, Melanoma; MHS 130. H, Melanoma; MHS, 300. (20x objective).

Figure 4



PD-L1 IHC staining patterns. Interface pattern in NSCLC (A and B) and melanoma (C). D, dendritic pattern, melanoma. (20x objective).

Figure 5



In addition to Merck sponsored studies, and in partnership with Merck, DLS has contracted PD-L1 IHC testing in over 200 external clinical studies from 2015 to 2022.

CONCLUSIONS

- Following development of the prototype IHC assay, DLS supported Merck in clinical trials contributing toward commercial approval of pembrolizumab.
- Further IHC testing with the prototype assay was later carried out in multiple tumor indications for both research and clinical trials.
- Currently, DLS is providing PD-L1 IHC CDx testing as well as other service offerings for Merck.

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The methods and protocols mentioned in this poster is not for diagnostic procedures.