Improving PD-L1 quality control using a dynamic range cell line and Qualitopix analysis

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Introduction

Immunohistochemistry (IHC) with PD-L1 is in use to predict ICI response in NSCLC patients. Inter-observer, inter- and intra-laboratory variability is, however, a known issue. In predictive IHC and to a lesser extent in panel based prognostic IHC, quality assurance principles and methods are becoming more relevant for optimal scoring. To improve scoring of PD-L1, artificial intelligence (AI) might be the way forward. Before AI can be used for daily practice, however, laboratories need to make sure that their IHC is a consistent assay instead of a routine stain.

Methods

To define variability of PD-L1 (1:50 22C3 laboratory developed test (LDT)) a dynamic range cell line (HistoCyte, NewCastle, UK) is repeatedly stained. Staining results were quantified using intensity scores (Qualitopix, Visiopharm, Hørsholm, DK). To illustrate relevance of a dynamic range control, dilutions of the primary antibody (1:80; 1:100 and 1:150) and staining with or without amplification were introduced to stain a NSCLC-TMA.

Figure 1, Qualitopix results showing approved individual stains (grean spots), unexplained faulse stains (yellow), explained faulse stain (red) and dotted white cut-off lines (SD)

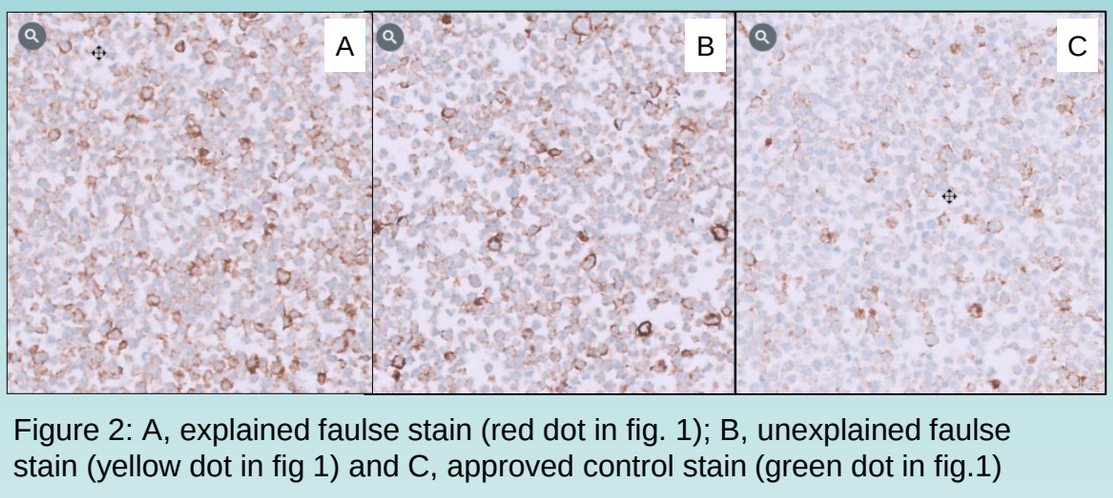

Figure 2: A, explained faulse stain (red dot in fig. 1); B, unexplained faulse stain (yellow dot in fig 1) and C, approved control stain (green dot in fig.1)

Results

Quantification of PD-L1 expression in a cell line (n=100) showed: negative 0.0 ± 0.1% (mean ± SD), weak 11.5 ± 13.7%, intermediate 94.2 ± 1.7% and high 97.3 ± 11.4% cores (fig 1). Furthermore, repeated measurements of the dynamic range cell line, plotted in a Levey-Jennings graph, revealed a few cases with inconsistent stain quality that required a re-stain. These cases were not identified when routinely reviewed (fig 2). Despite that cell line controls are within one SDs of the mean, the NSCLC-TMA (n=40) illustrated a significant ($p<0.05$) decrease in positive cases at 1:80 (20/25) and 1:100 (17/25). A dilution of 1:150 or 1:50 without amplification, compared to standard 22C3 LDT at 1:50 with amplification did show a low reading in the cell line (fig 3).

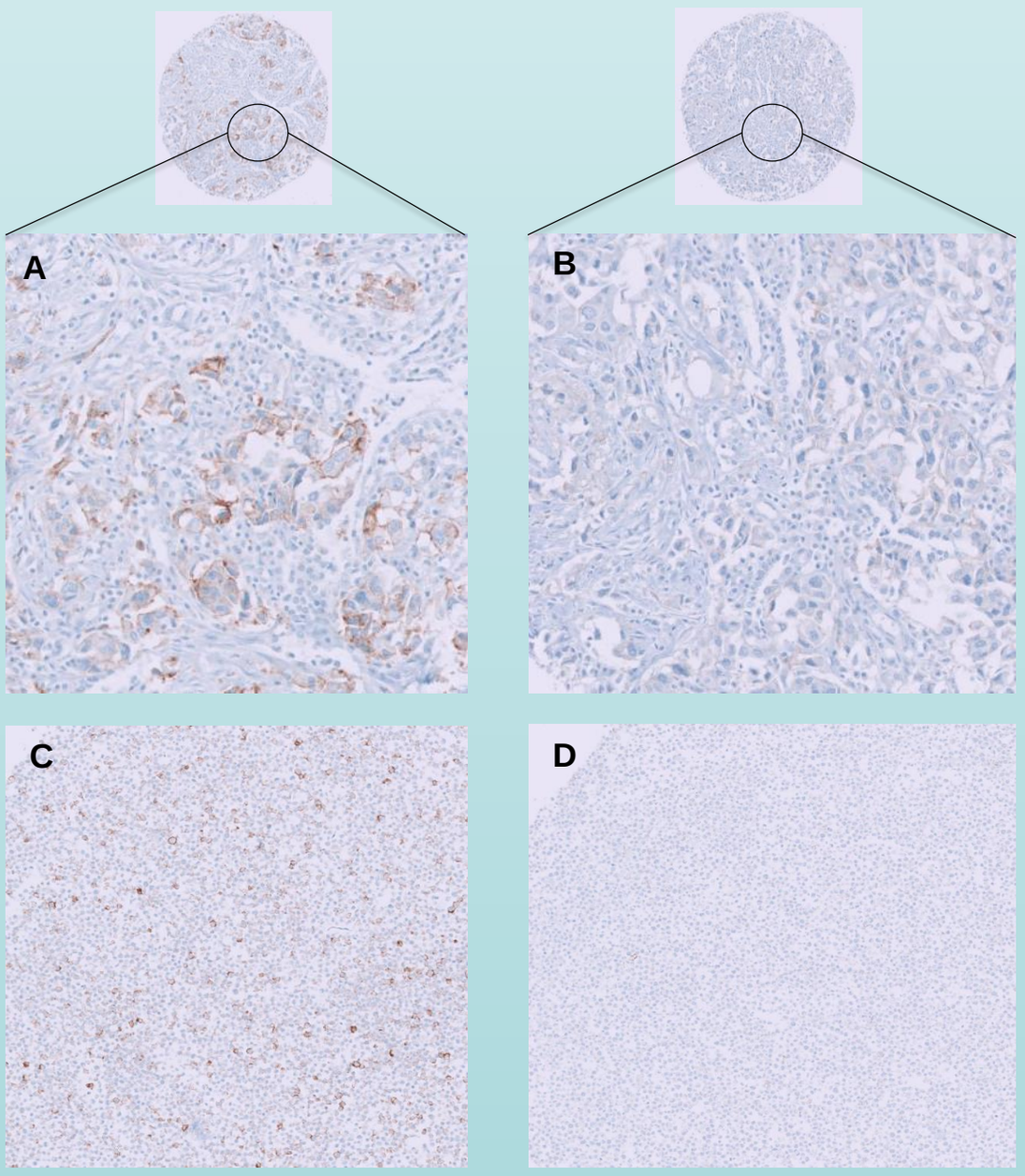

Figure 3: A, 1:50 PD-L1 stained tumor tissue. B, 1:150 PD-L1 stained tumor tissue. C and D, corresponding control cell line ‘weak spot’ for A and B, respectively.

Discussion

Magnani and Taylor stated: ‘*IHC employed as a tissue stain differs significantly from IHC used as an assay’* [1]. IHC quality control of PD-L1 using a dynamic range cell line with results plotted in a Levey-Jennings graph shows variability in stain intensity. Daily measuring stain variability allows early detection of technical issues as well.

Pathologists can be swayed by the issues of the day and miss subtle changes in IHC performance. To reduce intra-laboratory variability and ensure reliable consistent immunohistochemical assays for PD-L1, a dynamic range cell line shows to be a good control. In conclusion, a dynamic range cell line combined with daily analysis of stain intensity shows an improvement in quality control. We propose to improve predictive immunohistochemistry analysis using Levey-Jennings plots, especially when stain intensity is near or at its analytical cut-off.

[1], Magnani & Taylor, Arch Pathol Lab Med 2023