

Multiplex Cocktails for Immunotherapy Targets: PD-L1 with Tumor Specific Transcription Factors

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Introduction

Blocking interaction between the programmed cell death (PD-1) protein and one of its ligands, PD-L1, has been reported to generate impressive antitumor responses.¹ Therapeutics targeting PD-L1 in several cancers are currently in clinical trials, and the FDA has granted accelerated approval for pembrolizumab to treat patients with (a) advanced, unresectable melanoma that has progressed after ipilimumab treatment, and (b) advanced (metastatic) non-small cell lung cancer (NSCLC).¹⁻³ Various scoring and interpretation methods have been put forth to assay PD-L1 expression. Non-PD-L1 staining targets across tumors may also have important prognostic aspects.^{4,5} PD-L1 expression on tumor cells can be upregulated via activation of CD8⁽⁺⁾ cytotoxic T lymphocytes. PD-L1 expression is also associated with certain subtypes of tumor infiltrating lymphocytes (TILs), macrophages and dendritic cells.⁴⁻⁶ PD-L1 expression concomitant with expression of both CD8⁽⁺⁾ and FOXP3⁽⁺⁾ TIL regulatory factors in NSCLC may also help inform adjuvant chemotherapy decisions.⁷ We have observed in certain cancers, PD-L1 expression with macrophages and other immune effectors can make interpretation and scoring difficult. High Ki-67 and pHH3 proliferation can define active growth in melanoma; and high CD8 T-cell infiltrates in melanomas are more likely to be associated with PD-L1 expression in tumor cells.^{8,9} Thus, a strategy leveraging IHC multiplex stains could aid in resolving challenging cases of PD-L1 cases by cocktailing nuclear transcription factor antibodies or with other potential markers.

Design

Formalin-fixed, paraffin-embedded tissues and tissue microarrays including lung cancer, bladder cancer and melanoma were processed in the usual way and tissue sections cut at 4-5 microns. All reagents, antibodies and chromogens were provided by Biocare Medical. PD-L1 rabbit monoclonal antibody [CAL10] was cocktailled with the following antibodies: TTF-1 [SPT24] and p40 [BC28] (lung cancer); CD163 [10D6] (lung alveolar macrophages); p40 and GATA-3 [L50-823] (bladder cancer); and SOX10 [BC34] (melanoma). A triple stain including SOX10 + PD-L1 and CD8 [C8/144B] were tested on melanoma samples to help identify high proliferation zones in melanoma. Antibody cocktails were detected with double and triple stain detection systems utilizing DAB (brown), fast red (red) and Ferangi blue (blue) chromogens for visualization. Sections can be counterstained with standard hematoxylin, or other nuclear stains can be used such as Weigert's iron hematoxylin (black) and nuclear fast red.

A cell-line control slide for PD-L1 with high, medium, low and negative spots was used to ensure proper titers.

Results

The control slide with cell lines for PD-L1 staining intensity was staining properly according to the vendor's guidelines.

Double stains consisting of TTF-1 + PD-L1, CD163 + PD-L1 and p40 + PD-L1 were well adapted in cases of lung cancer. The nuclear staining of TTF-1 in lung adenocarcinoma (blue) helped define PD-L1⁽⁺⁾ stained tumor cells (brown, Figure 1). CD163 (red) + PD-L1⁽⁺⁾ (brown) helped separate PD-L1⁽⁺⁾ and CD163⁽⁺⁾ macrophages from PD-L1⁽⁺⁾ stained tumor cells (Figure 2). Tumor cells (nuclear) in lung squamous cell carcinoma were stained discretely with p40 (blue) and PD-L1 membrane staining (brown, Figure 3A, B).

PD-L1 expression in the urothelial carcinoma cells that also expressed either p40 or GATA-3 was co-expressed and provided robust nuclear staining in bladder tumor cells (Figure 4).

SOX10 nuclear staining (blue) was observed in most melanoma cells including conventional melanoma, desmoplastic / spindle cell melanoma and metastatic melanomas. PD-L1⁽⁺⁾ membranous staining (brown) was co-expressed with SOX10 and was morphologically distinct (Figure 5A, B).

A triple stain consisting of SOX10 (blue) + PD-L1 (brown) and CD8 (red) helped define high proliferation zones in melanoma. High CD8 cytotoxic T-cell staining was associated with strong PD-L1 expression in tumor cells (Figure 5C, D).

Figure 1

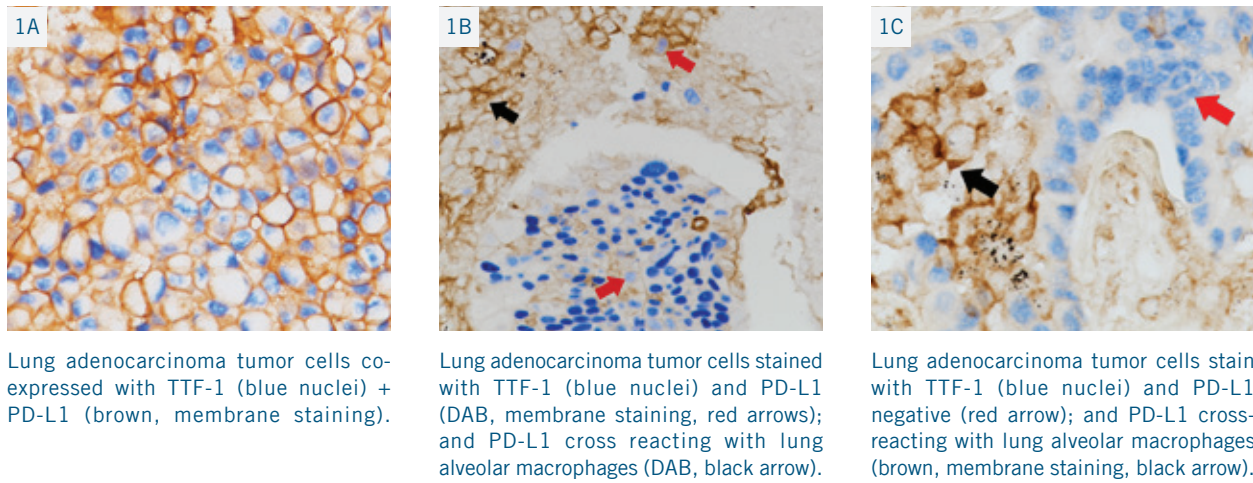


Figure 2

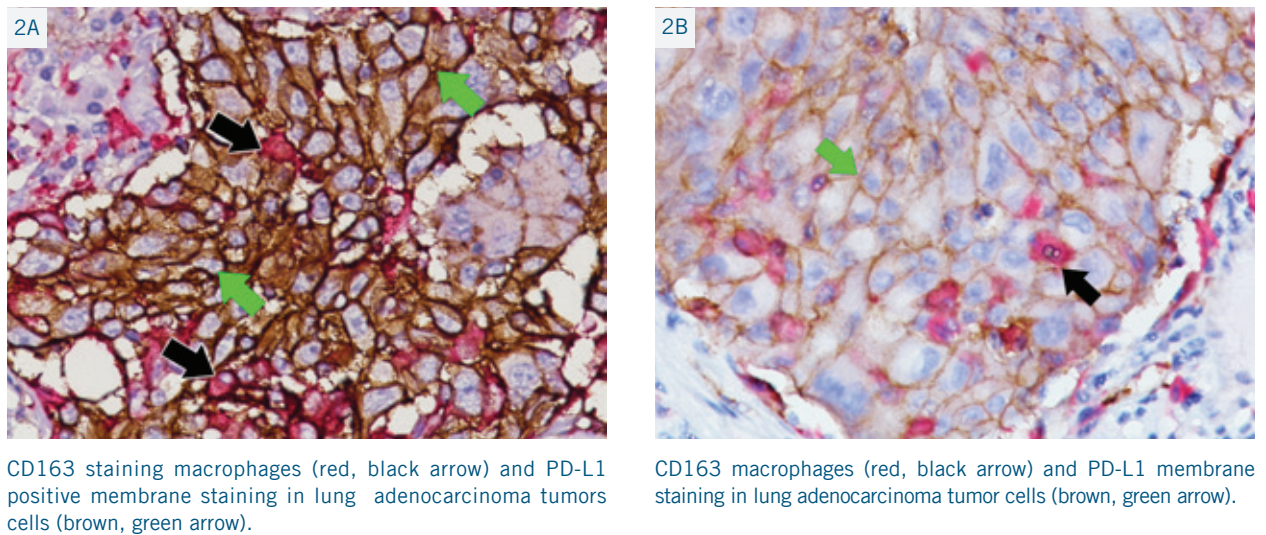


Figure 3

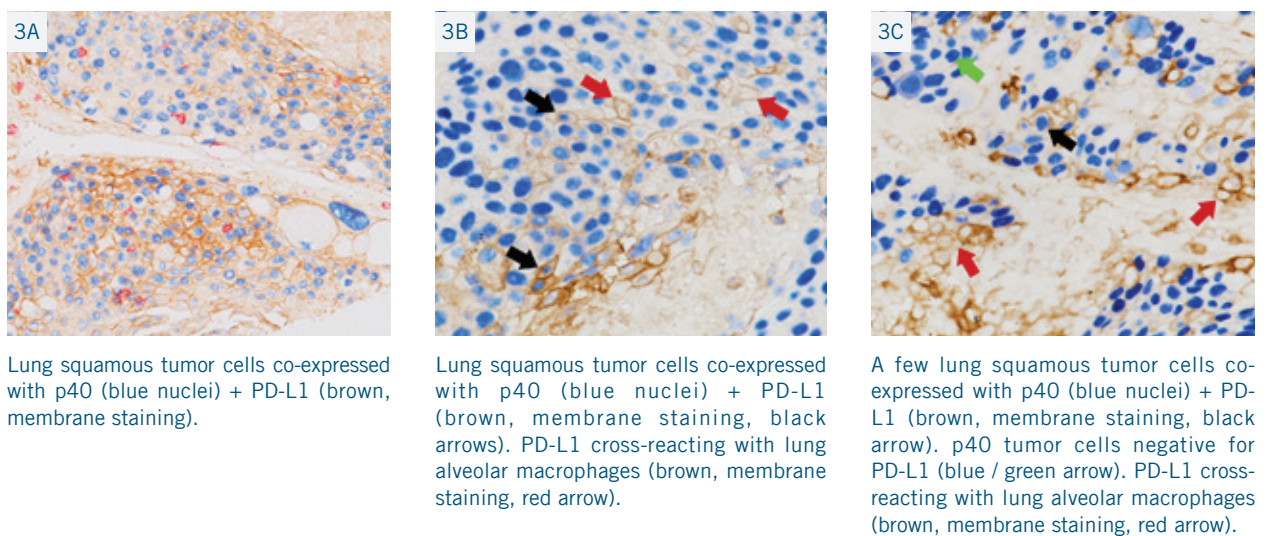
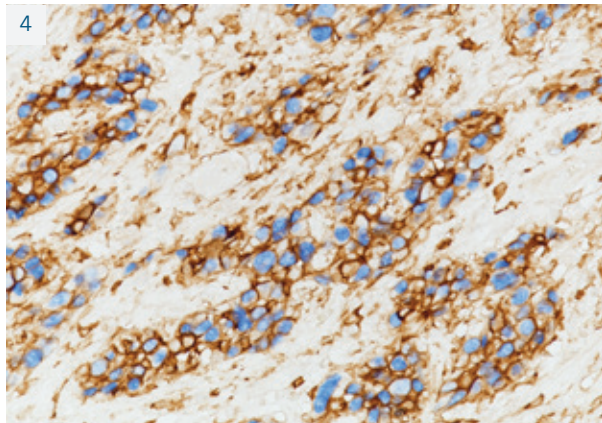
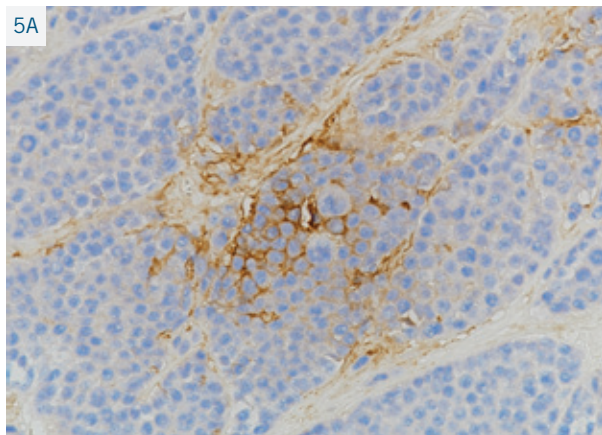


Figure 4



Bladder cancer tumor cells co-expressed with GATA-3 + p40 (blue nuclei) + PD-L1 (brown, membrane staining).

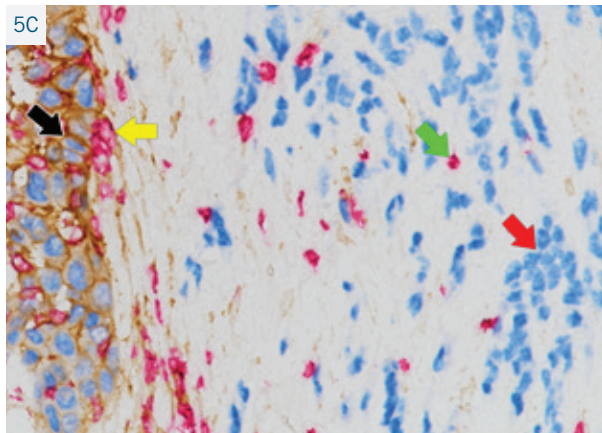
Figure 5



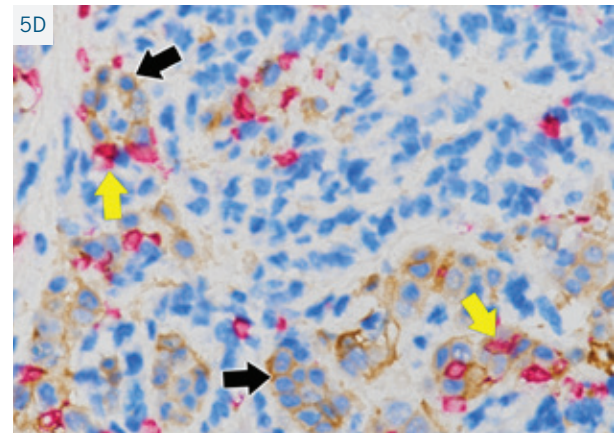
Melanoma stained with SOX10 (nuclei, blue) and PD-L1 (negative).



Melanoma stained with SOX10 (nuclei, blue) and PD-L1 (negative).



Melanoma stained with SOX10 (nuclei, blue) and PD-L1 (brown, membrane) and CD8 (red, cytotoxic T-cells). Notice high CD8 staining adjacent to strong PD-L1 staining (black, yellow arrows). SOX10 staining (blue nuclei), non-detectable PD-L1 membrane staining (red arrow) and low CD8 staining (green arrow).



Melanoma stained with SOX10 (nuclei, blue) and PD-L1 (brown, membrane, black arrows) and CD8 (red, cytotoxic T-cells, yellow arrows). Notice CD8 cytotoxic T-cells are highly associated with PD-L1 stained tumor cells.

Conclusion

PD-L1 cocktails with nuclear transcription factor antibodies or PD-L1 cocktailed with cytoplasmic antibodies can help discriminate tumor cells from non-tumor cells and may facilitate quantifying or immunoscore for accurate assessment.

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