Unleashing the immune system: PD-1 and PD-Ls in the pre-treatment tumor microenvironment and correlation with response to PD-1/PD-L1 blockade

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Abbreviations: CRPC, castration-resistant prostate cancer; FFPE, formalin-fixed paraffin-embedded; NSCLC, non-small cell lung carcinoma; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; RCC, renal cell carcinoma; TIL, tumor-infiltrating lymphocytes.

Focal tumor cell PD-L1 expression adjacent to TIL can be used as a surrogate marker of an ongoing antitumor host response, which may be unleashed by PD-1 blockade. Tumor cell PD-L1 expression is superior to TIL PD-1 expression and the presence of TIL alone, when predicting response to anti-PD-1 therapy.

In addition to exciting durable tumor regressions, one of the more provocative findings associated with PD-L1/PD-1 pathway blockade involves the potential predictive value of pre-treatment specimen PD-L1 expression. We first reported a small series of nine patients from the MDX-1106/BMS-936558 trial, suggesting that tumor cell surface (membranous) PD-L1 expression may be associated with responsiveness to PD-1 blockade.1 These findings were supported in a larger series of 42 patients in the follow-up trial.2 Specifically, of the 25 patients who had a formalin-fixed paraffin-embedded (FFPE) pre-treatment specimen that was PD-L1 (+), 36% had an objective response to anti-PD-1. In contrast, no patients whose tumors were PD-L1(−) demonstrated a clinical response (p = 0.006).

More recently, our group published the results of an expanded analysis conducted on 68 FFPE pre-treatment specimens from 41 patients with advanced cancers who were treated with anti-PD-1. The cohort included 16 patients with melanoma, 12 with non-small cell lung carcinoma (NSCLC), 6 with kidney cancer, 5 with colorectal carcinoma (CRC), and 2 with castration-resistant prostate cancer (CRPC).3 Fifty-three of these 68 specimens had previously been assessed for PD-L1 expression.2 The extended analysis included additional histologic and immune features in the pre-treatment tumor microenvironment, and how they related to each other and to patient outcomes. This involved a focus on infiltrating immune cell subsets, PD-1, PD-L1 and PD-L2 expression.

We found that tumor PD-L1 expression varied significantly by tumor type. Approximately 60% of the melanoma, NSCLC, and kidney cancer specimens tested demonstrated PD-L1 expression, in contrast to only one of 12 (8%) colorectal and CRPC specimens (p = 0.005). When tumor cell PD-L1 expression was observed, it was focal and seen in immediate geographic association with tumor infiltrating lymphocytes (TIL) in all but one case (33/34). Such constancy supports our hypothesis that PD-L1 expression by tumor is a mechanism of adaptive immune resistance.4 We also observed PD-L1 expression on infiltrating immune cells in the absence of tumor cell expression. For example, even though only 1 of 8 CRC cases demonstrated PD-L1+ tumor cells, 4 of the CRC cases (50%) had PD-L1 displayed on TIL and associated macrophages.

The finding that TIL PD-1 was displayed adjacent to PD-L1 (and sometimes with PD-L2) suggests an immunosuppressive microenvironment that may be altered by the administration of anti-PD-1 therapy. Accordingly,
we examined how these factors in pre-
treatment tumor specimens predicted 
response to anti-PD-1. We found that 
PD-L1 expression by tumor was the 
strongest single factor predicting objective
response (Fig. 1), when compared 
to TIL PD-1 expression, or the presence of 
TIL alone. This is likely because 
focal tumor cell PD-L1 expression 
adjacent to TIL reflects an ongoing antitu-
more immune response, which may 
be protected by anti-PD-1. While PD-1 is 
the direct target of anti-PD-1, it only 
demonstrated a borderline association 
with response in our series. Similarly, 
the presence of TIL alone is not a sig-
ificant factor predicting response to 
anti-PD-1. This latter finding suggests 
various functional states of TIL. Future 
 studies will undoubtedly focus on fur-
ther characterizing lymphocyte subsets, 
including regulatory T-cells, as well as 
other immunoreactive cell types, such as 
myeloid-derived suppressor cells, and 
how these populations relate to response 
to anti-PD-1.

A proportion of the patients in our 
cohort had multiple pre-treatment speci-
mens available for testing. PD-L1 expres-
sion was also heterogeneous across 
different pathologic specimens from a sin-
gle patient. For the purpose of the afore-
mentioned analysis where PD-L1 expression 
was correlated with response to 
anti-PD-1, a patient was considered PD-
L1(+) if any of their specimens demon-
strated tumor cell PD-L1 expression. 
For example, one melanoma patient who 
demonstrated a complete response had 
three different pre-treatment specimens 
available for study. The primary mela-
noma was PD-L1(+), and the lymph
node and subsequent subcutaneous metas-
tases were both PD-L1(−). By our meth-
ology, the patient was considered PD-
L1(+), due to PD-L1 expression of the 
primary tumor. Notably, if only one of 
the patient’s latter specimens had been 
tested, and PD-L1 status was used as a 
selection criteria for PD-1/PD-L1 block-
de, the patient would have been consid-
ered ‘PD-L1(−)’ and may have missed the 
opportunity to receive anti-PD-1.

Identifying and validating markers that 
could enrich for clinical response would 
have great significance for optimal thera-
peutic development. We, and now others 
5-8 have demonstrated that PD-L1 expres-
sion in the tumor microenvironment 
 enriches for response to anti-PD-1, 
though the association is not absolute. 
Uncertainty remains as to whether PD-L1 
expression in a single pathologic specimen 
will routinely be used to pre-select indi-
vidual patients for anti-PD1 therapy. Fea-
tures such as the temporal and geographic 
heterogeneity of PD-L1 expression across 
specimens from a single patient call this 
approach into question. Our findings sup-
port the proposed mechanism of action of 
anti-PD-1 and suggest that study of the 
pre-treatment pathologic specimens may 
be used to help identify tumor types likely 
to respond to this therapy. Pre-treatment 
pathologic specimens will also likely be 
useful in identifying additional dominant 
or co-dominant pathways that may be tar-
geted in combination with anti-PD-1 to 
further increase the proportion of patients 
who benefit from these exciting agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were 
disclosed.

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Figure 1 Tumor PD-L1 is the strongest single predictor of response to anti-PD-1. When analyzing 
either the highest scoring sample among multiple biopsies from individual patients or the speci-
men obtained closest to therapy, tumor cell PD-L1 expression correlated with objective response to 
anti-PD-1 therapy. This association was stronger than the borderline association with PD-1 expres-
sion. Simply the presence of intratumoral immune cell infiltrates did not correlate with response. 
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