

# Shedding light on the molecular determinants of response to anti-PD-1 therapy

Ross A. Soo<sup>1,2,3</sup>

<sup>1</sup>Department of Haematology-Oncology, National University Cancer Institute, National University Health System, Singapore; <sup>2</sup>Cancer Science Institute of Singapore, National University of Singapore, Singapore; <sup>3</sup>Department of Surgery, University of Western Australia, Western Australia, Australia

Correspondence to: Ross A. Soo. Department of Haematology-Oncology, National University Cancer Institute, National University Health System, 1E Kent Ridge Road, NUHS Tower Block Level 7, Singapore. Email: ross\_soo@nuhs.edu.sg.

**Abstract:** Immune checkpoint inhibition targeting the programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) axis has emerged as a very promising therapeutic avenue in the treatment of patients with advanced stage non-small cell lung cancer but only a subset of patients derives clinical benefit from PD-1/PD-L1 inhibitors. Studies to date have reported patients with PDL-1 expressing tumors have a better outcome than those with PDL-1 negative tumors but assays used to identify PD-L1 positive patients has been challenging. Through whole exome sequencing of tumors, investigators have recently described mutational burden in non-small cell lung cancer (NSCLC) was associated with response to pembrolizumab. In two independent patient cohorts, it was reported a high somatic nonsynonymous mutation burden was associated with greater durable clinical benefit, higher objective response rates (ORRs) and a longer progression free survival. In addition clinical efficacy was associated with a molecular smoking signature, certain DNA repair mutations and the burden of neoantigens.

**Keywords:** Programmed death-1 (PD-1); programmed death-ligand 1 (PD-L1); immunotherapy; whole exome sequencing; non-small cell lung cancer (NSCLC)

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Progress in the understanding of the role of the immune system in tumor immunosurveillance has led to the recognition that tumors can evade immune destruction via the dysregulation of co-inhibitory or checkpoint signals. Lung tumorigenesis is not only dependent on genetic aberrants within cancer cells but also on interactions with the immune system (1). In the normal physiological state, programmed death-1 (PD-1), an immune checkpoint or co-inhibitory molecule found on activated T cells, acts to prevent autoimmunity (2). The binding of PD-1 with one of its ligands, programmed death-ligand 1 (PD-L1) (or CD274, B7-H1) or PD-L2 (CD 273, B7-DC), leads to the down-regulation of cytotoxic T cell function (2). However tumors can co-opt the PD-1/PD-L1 pathway to evade T-cell-induced antitumor response (2,3). The inhibition of the PD-1/PD-L1 pathway with immune checkpoint inhibitors can interrupt the engagement of PD-1 with its

ligands and block inhibitory signals in T cells, resulting in tumor recognition by cytotoxic T cells.

The development of immune checkpoint inhibitors such as nivolumab, pembrolizumab (PD-1 inhibitors) and MPDL3280A (PD-L1 inhibitors) represent an important breakthrough in the treatment of cancer. Early phase studies of PD-1/PD-L1 inhibitors have reported impressive clinical activity and durable responses in patients with refractory tumors including melanoma, renal cell cancer, Hodgkin's lymphoma, bladder cancer, and non-small cell lung cancer (NSCLC) (4-10) and recent phase III studies have reported pembrolizumab and nivolumab conferred OS benefit in melanoma versus ipilimumab (11) and dacarbazine (12) respectively and improvement in OS in patients with advanced stage NSCLC treated with second line nivolumab versus docetaxel (13,14).

As clinical efficacy is seen only in a subset of patients

**Table 1** ORR of PD-1/PD-L1 inhibitors in patients with advanced stage NSCLC according to smoking status and histologic subtype

Immune checkpoint inhibitor	ORR (95% CI)				Reference
	Smoking status		Histologic subtype		
	Never	Former/current	Squamous	Non squamous	
Nivolumab	0 [0-25]	27 [17-38]	16.7 [7.9-29.3]	17.6 [9.7-28.2]	(15)
Pembrolizumab	10.3 [5.6-17]	22.5 [18.3-27.1]	23.5 [15-34]	18.7 [15-22.9]	(10)
MPDL3280A	10	42	27 [8-61]	21 [11-36]	(8)

ORR, objective response rate; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; NSCLC, non-small cell lung cancer; CI, confidence interval.

**Table 2** ORR of PD-1/PD-L1 inhibitors in patients with NSCLC according to PD-L1 IHC assay

Drug	Nivolumab		Pembrolizumab		MPDL3280A		MEDI4736					
Pharmaceutical	BMS		Merck		Roche/Genentech		Astra-Zeneca/MedImmune					
Ab clone (source)	28-8 (Dako)		22C3 (Dako)		SP142 (Ventana)		SP263 (Ventana)					
Tissue	Archived		Recent		Archived/recent		Archived/recent					
Cell scored	Tumor cell membrane		Tumor cell or stroma		Infiltrating immune cells		Tumor cell membrane					
Study (reference)	CheckMate (9,15,21)		KEYNOTE-001 (10)		NCT01375842 (8)		NCT01693562 (22)					
	012	063										
Treatment line	1 <sup>st</sup>	≥2 <sup>nd</sup> line	≥2 <sup>nd</sup> line	1 <sup>st</sup>	≥2 <sup>nd</sup> line	≥2 <sup>nd</sup> line	≥2 <sup>nd</sup> line					
Cutoff	5%	1%	5%	5%	1-49 ≥50	1-49 ≥50	1-<5 ≥5-<10 ≥10	NR				
ORR (%) in PD-L1 +ve	50	13	15	24	19.2	50	15.6	43.9	15	14	83	39
ORR (%) in PD-L1 -ve	0	17	14	14	17	9.1	20	5				

ORR, objective response rate; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; NSCLC, non-small cell lung cancer; IHC, immunohistochemistry.

with advanced stage NSCLC treated with PD-1/PD-L1 inhibitors, biomarkers are needed to improve patient selection for immune checkpoint inhibition. Whilst histologic subtype was not associated with response, current or former smoking status has been reported to be associated with an increased response to treatment in several studies (Table 1). A possible explanation for this finding is smoking-associated lung cancers have a higher mutational load, resulting in the creation of more tumor neoantigens and increased immunogenicity (16,17).

Since the PD-1/PD-L1 pathway is implicated in immune escape in NSCLC, tumor PD-L1 expression with immunohistochemistry (IHC) has been used as a predictive biomarker. In one study, no association between tumor PD-L1 expression and response to nivolumab was found (15) whereas in KEYNOTE 001, tumor PD-L1 expression was associated with response to pembrolizumab (10). A possible confounder was PD-L1 expression was determined using

archival tumor samples in the nivolumab study whereas a fresh tumor biopsy was obtained in the latter study.

The development of anti-PD-L1 IHC companion diagnostics by several pharmaceutical companies has created some challenges as these assays are using different IHC antibody clones, different staining protocols and platforms, different scoring systems, and different cutoffs defining positivity. Other issues influencing PD-L1 assessment include tumor heterogeneity, the dynamic nature of PD-L1 expression (18-20) and differences in the assessment of PD-L1 in the tumor microenvironment (tumor cell, stroma, or both) (Table 2). Regarding the latter factor, PD-L1 expression in the tumor microenvironment may be an important determinant of response. Herbst and colleagues found PD-L1 expression in tumor infiltrating immune cells, instead of tumor cells, was associated with response in patients with advanced stage NSCLC treated with MPDL3280A (8) whereas immune cells at the invasive

margin expressing PD-1 and PD-L1 was associated with response in patients with advanced stage melanoma treated with pembrolizumab (19). Furthermore, whilst high PD-L1 expression is associated with a higher response rate, responses of 5-20% are also seen in PD-L1 negative patients, adding further complexity to using PD-L1 as a biomarker (Table 2).

As immune checkpoint inhibitors are active in bladder cancer (6), NSCLC (8,10,15), and melanoma (11,12), tumors typically associated with high somatic mutation rates (16), it has been hypothesized that the mutational landscape is a determinant of response to PD-1/PD-L1 inhibitors. Based on this premise, investigators have recently reported mutational burden in NSCLC was associated with response to PD-1 inhibition. In this seminal Science paper, the subject of this editorial, Rizvi and colleagues performed whole-exome sequencing of tumors from a phase I study of patients with advanced stage NSCLC treated with pembrolizumab (23). In two independent patient cohorts they found a high somatic nonsynonymous mutation burden was associated with greater durable clinical benefit (defined as a partial or stable response of at least 6 months), higher objective response rates and a longer progression free survival. In addition, clinical efficacy was associated with a molecular smoking signature, certain DNA repair mutations and the burden of neoantigens. Interestingly the molecular smoking signature, rather than self-reported smoking status, correlated with efficacy.

Furthermore, a high nonsynonymous mutational burden correlated with a greater number of putative neoantigens with high binding affinity to patient-specific HLA alleles, and patients who had a durable clinical benefit had a higher neoantigen burden than those who did not. These findings are significant as they support the hypothesis that the recognition of neoantigens, formed as a result of somatic mutations, is important for anti PD-1 activity and provides further proof of principle that tumor genomics can dictate responses to immunotherapy. The same investigators have recently reported mutational load and a neoantigen landscape that is specifically present in melanomas was associated with response to CTLA-4 inhibition (17). Another notable finding is T-cell response against a mutation-associated neoantigen was detected in peripheral blood lymphocytes from one patient who responded to pembrolizumab. This important finding creates an opportunity to develop blood-based assays to monitor response to PD-1 inhibition.

What is unknown from this study is the correlation

between mutational load and PD-L1 density and more studies are needed to determine the relationship between mutational burden and PD-L1 expression. Further studies are also required to confirm these findings with other PD-1/PD-L1 inhibitors and in other malignancies. Research in identifying mechanisms of resistance to immune checkpoint inhibitors will also aid in the development of rational therapeutic strategies and ultimately improve patient outcomes. As PD-L1 IHC assays may be introduced into clinical use in the near future, the International Association for the Study of Lung Cancer is planning an international characterization study of PD-L1 companion diagnostics to gain a better understanding of the assays, their performance on different sample types (large specimens, small biopsies, cytology) and different on platforms.

In summary, through a better understanding of the molecular determinants of response to immunotherapy, Rizvi *et al.* has provided insight on how tumor mutational load in NSCLC influence the efficacy of anti-PD-1 therapy and T-cell responses to neoantigens created by somatic mutations might underpin pembrolizumab activity in NSCLC. The results also underscore the importance of incorporating biomarkers in studies to characterize molecular mechanisms of sensitivity.

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*Comment on:* Rizvi NA, Hellmann MD, Snyder A, *et al.* Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124-8.

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