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Mini Review

Response Predictors for Pembrolizumab in Advanced NSCLC beyond PD-L1 Expression

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Abstract:

Pembrolizumab has significantly improved outcome of advanced NSCLC. PD-L1 expression has limited utility as a prognostic and predictive biomarker. To improve this several other biomarkers have been evaluated. Useful amongst them are 1. Tumor specific biomarkers include tumor mutation burden, immune cell infiltration (phenotype, genotype, site, type), 2. Changes in cellular, cytokine in peripheral blood. The article provides review of the current status.

Keywords: pembrolizumab; NSCLC; tumor mutation burden; PD-L1 expression; hyper progression; response predictors; tumor immune infiltrate; CD8+ T cells; CD4+ T cells; macrophages

Introduction:

Majority of patients diagnosed to have Non-small cell lung cancer (NSCLC) have advanced disease at diagnosis. This makes it difficult to provide curative treatment options. Prior to introduction of pembrolizumab five year survival rate used to be around 5%. This has improved to more than 25% following introduction of pembrolizumab in treatment of NSCLC. This dramatic improvement is seen in patients treated with pembrolizumab having programmed-death ligand 1 (PD-L1) expression of ≥ 50% [1]. Currently pembrolizumab is approved in combination with chemotherapy as a first line treatment of squamous (paclitaxel or nab-paclitaxel and platinum) and non-squamous (pemetrexed and platinum; with no EGFR or ALK genomic tumor aberrations) metastatic NSCLC [2, 3, 4]. It is also approved as a monotherapy in first-line management of patients with PD-L1 expression ≥ 50% having metastatic NSCLC with no Epidermal growth factor receptor (EGFR) or Anaplastic lymphoma kinase (ALK) genomic tumor aberrations [3, 5] and as a second line for the patients whose tumors express PD-L1 (TPS ≥1%) [6]. Currently, PD-L1 expression level measurement by immunohistochemistry is approved as a companion diagnostic for use with pembrolizumab. PD-L1 expression more than 1% [7, 8, 9, 10, 11, 12] in advanced NSCLC is seen approximately 50% [7, 8] with around 30% have it more than 50% [8, 9]. In spite of approval by FDA, it does not have a very high specificity and sensitivity as predictive biomarker. Response to therapy is also seen in absence of PD-L1 expression and failure to respond is also known in those having >50% PD-L1 expression. The response to pembrolizumab is proportional to the amount of PD-L1 expression in non-squamous NSCLC (61.4% vs 47.6% for > 50% and 1-49% respectively) as well as non-selected NSCLC but no significant difference is seen in Squamous NSCLC (60.3% vs 57.9% for > 50% and 1-49% respectively). The survival benefit is also not uniform in spite of use of PD-L1 as a biomarker (Table-1). In spite of all this, more than 10% of patients progress rapidly on therapy. This is not seen with other therapy including chemotherapy [13, 14, 15, 16].

To further improve outcome, efforts are being made to understand parameters contributing to response/no response to therapy with a purpose of better selection of patients as well as identifying novel co-therapies. Most of the information generated so far are based on small studies, and or retrospective analysis of clinical trial samples and so needs to be confirmed in a well-designed study. In this article, current information about various parameters affecting the outcome of pembrolizumab in advanced NSCLC are reviewed.

Table 1. Overall survival (OS) and PD-L1 expression

PD-L1 expression	OS Keynote 189 - non squamous With chemotherapy [2]	OS Keynote 407 – Squamous With chemotherapy [4]	OS Keynote 042- unselected Monotherapy [17]
>50%	0.42	0.64	0.69
1-49%	0.55	0.57	0.81
< 1%	0.59	0.61	-

1. Tumor characteristics:

Pembrolizumab decreases tumor associated immunosuppression by its action on intratumoral PD-1/PD-L1 interaction and proliferation of intratumoral immune cells. This has led to evaluation of tumor characteristics like Tumor mutation burden (TMB), immune infiltrates, expression of other inhibitory molecules etc. for their role in response to therapy.

I. Tumor mutation burden (TMB) /neoantigen [7, 13, 18, 19, 20, 21, 22]: Nonsynonymous mutations seen in NSCLC appears to be an independent marker of response to anti PD-1 therapy [19]. Hyper mutated tumours are known to be amenable to therapy in absence of PD-L1 expression [19, 22] and higher TMB is considered as an independent marker of response to therapy as well as control of disease [18, 23, 24]. Higher TMB in squamous NSCLC may be responsible for discrepancy in relationship between PD-L1 expression level and outcome. Tumor burden more than 10 is seen in 37% in non-squamous NSCLC and 42% in squamous NSCLC [19]. TMB more than 20 is found to be associated with better outcome (OS 16.8 months vs 8.5 months) compared to TMB<20 [25]. Neoantigens are generated following tumor mutation and TMB correlates with neoantigen load.

TMB can also be measured in circulating tumor cells and is found to correlate with TMB [26].

II. Number of Metastatic lesions: Hyper progression is more frequent in patients with more than 2 metastatic lesions [14].

III. Pre-treatment tumor growth rate: Response to treatment is more frequent in patients with higher pre-treatment growth rate [16].

IV. Immune cell infiltration [13, 20, 27]: Expansion of infiltrating immune cells are known to contribute to response to therapy. Response to anti PD-1 therapy depends on amount, type and site of immune cell infiltration. Based on the site of immune cell infiltrate tumor is designated to one of three immune phenotypes. Inflamed phenotype has abundance of infiltrating immune cells within and surrounding the tumor. This phenotype is associated with response to the therapy. Excluded phenotype has immune cells restricted to margin of the tumor. Desert phenotype has absence of immune cells within tumor (stroma as well as surrounding). Absence of immune cells is associated with lack of the response. However, this is too simplistic as response is dependent on amount and type (phenotype and functional characteristic) of immune infiltrate.

a. Phenotype of immune cells: Tumor regression following therapy is dependent on decreased immune suppression and increased immunostimulation. Higher CD3 and/or CD8 cells (considered immunostimulant type) are associated with better prognosis and higher amount of FOXP3 and PD-1 represent immunosuppressive microenvironment and is associated with poor outcome.

Both of above (immunostimulant and immunosuppressive) when used as a ratio rather than in isolation e.g. FOXP3/CD8 and PD-1/CD8 ratio provide better prognostication [28]. Lower ratio is associated with increased response rate. Based on above, it is suggested that on immunohistochemistry CD3 T cells ≥ 120 per HPF with n CD8+ T cells and FOXP3+ T cells in a ratio of ≥ 4:1 can be used to prognosticate better outcome [27].

Intratumoral M2-like CD163+ CD33+ PD-L1+ clustered epithelioid macrophages are associated with hyper progression [15].

b. Genotype (gene expression profile) :

i. Gene profiling: It is possible to identify desired genes in a pretreatment samples and gene profiling is used to identify a biomarker for response to therapy. Interferon gamma is essential for immune response. Cytolytic activity of immune cells is dependent on secretion of cytolytic enzymes like granzymes and perforins. Based on this Interferon gene expression signature profiles are identified to predict response to therapy [29].

ii. Epigenetic profiling [30]: Epigenetics plays a role in manifestation of gene function. Based on demethylation status of selected CPG loci, epigenetic signature was identified. Positive signature was associated with improved progression free survival (PFS) and OS but not with PD-L1 expression, TMB or CD8+ cells. Negative signature was associated with increased tumor associated macrophages, neutrophils and fibroblasts. Unmethylated T-cell differentiation factor FOXP1 was associated with improved survival. These findings are specific to immunotherapy only.

c. Proliferative potential: Based on expression of BUB1, CCNB2, CDK1, CDKN3, FOXM1, KIAA0101, MAD2L1, MELK, MKI67 (better known as Ki-67), and TOP2A tumors can be divided into tumors with high proliferative potential, moderate proliferative potential and low proliferative potential. Tumors with a low or high proliferative potential fail to respond (primary resistance). Amongst patients with moderately proliferation potential, PD-L1 expression predicts survival. Higher expression > 50% is associated with better survival compared to PD-L1 expression <50%. [31]

d. Co-expression of inhibitory molecules: Pembrolizumab inhibits action of PD-1 expressing immune cells. PD-1 is one of the inhibitory molecules expressed by T cells. There are other inhibitory molecules like LAG-3 and TIM3, which are also expressed by them. Co-expression of inhibitory molecules like LAG3 with PD-1 is indicative of primary resistance (no response) to therapy. [32, 33]

V. Classification based on tumor immune microenvironment (TIMIT): Pembrolizumab induces changes in PD-1/PD-L1 axis and infiltrating immune cells. PD-1 is expressed by immune cells and PD-L1 is expressed by tumor cells. Combining the two (PD-1/PD-L1 and CD8+ TIL) and grading their expression as high or low provides a novel way of classifying TIMIT. High CD8+ and low PD-1/PD-L1 predicts better outcome whereas low CD8+ and high PD-1/PD-L1 predicts the worst outcome. TMB is not found to differ in these subtypes.[34]

1.Gut microbiome:

Gut microbiome is found to play important role in response to anti PD-1 therapy. In animal studies, they are known to alter T cell and dendritic cell repertoire.

I. Amount: Patients having higher amount of gut microbiome as revealed by higher bene count respond favourably to treatment. Those who receive antibiotic prior to or during treatment do not respond to anti-PD1 therapy. [13]

II. Type: Patients having dysbiosis (can be antibiotic induced) fail to respond to therapy [13]. Decreased level of specific microbiome (Ruminococcus bromii, Dialister and Sutterella) and increased level of specific microbiome (Akkermansia muciniphila, Bifidobacterium

longum, Faecalibacterium prausnitzi, Propionibacterium acnes, Veillonella parvula,) are seen in patients responding to anti-PD-1 therapy.

2. Changes in Peripheral blood:

There is a constant exchange between tumors and peripheral blood and some of the changes taking place within tumor gets reflected in the peripheral blood. Peripheral blood evaluation offers advantage of having sample at multiple time points before and after therapy. This is not practical for biopsy of tumor tissues and thus provides very useful information. The changes in pre-treatment samples and during treatment are found to be associated with outcome.

I. Pre-treatment biomarkers: Lymphocytes are key immune cells for response to therapy. Based on markers expressed by them they can be divided into various subtypes with specific function assigned to them. Their relative frequency is also indicative of magnitude of response.

a) Pre-treatment lymphocyte subtype: Circulating lymphocytes are responsible for immune surveillance and they reflect intratumoral immune surveillance.

i. Expression of PD1 and FOXP3 are indicative of immunosuppressive phenotype. Their preponderance is associated with poor outcome [35, 36, 37].

ii. Highly differentiated (CD27 negative CD28 low / negative) CD4 cells are indicative of better immunosurveillance and higher count is associated with objective response and better PFS [32]. Base line low percentages of highly differentiated memory CD4 T cells is associated with primary resistance (Lack of response to therapy) [38]. The response rate was 50% in a group with high percentage of highly differentiated memory CD4 cells and improves to > 70% on combining with PD-L1 positivity.

b) Ratio of peripheral blood cells.

i. Neutrophil-to-lymphocyte ratio [39, 40, 41]: Lymphocytes play a key role in immune response while neutrophils do not play a significant role. Decrease in lymphocyte as determined by higher neutrophil to lymphocyte ratio is associated with shorter OS and PFS. Ratio of 5.9 or higher prognosticates progression of disease [40].

ii. Monocyte-to-lymphocyte ratio: Similar to neutrophil to monocyte ratio, monocyte to lymphocyte ratio of 11.3 or higher is associated with disease progression [40].

c) Proliferative response of pretreatment CD4 cells on exposure to anti-PD1 antibody: Response to pembrolizumab is dependent on proliferation of lymphocyte. Attempts has been made to use this phenomenon as a marker for response to therapy. CD4 cell proliferation on exposure to anti-PD1 antibody ex vivo is seen in responders. It is absent in nonresponders. This absence of proliferation is a unique feature and found to persist even after three cycles of pembrolizumab [32].

d) Red blood cell distribution width (RDW) [42]: Red blood cell distribution width while indicating erythropoiesis is also useful as inflammatory parameters. Increased RDW is found to be associated with poor prognosis. This seems to be an independent marker.

e) Lung Immune Prognostic Index (LIPI) [13]: LIPI is a combination of two parameters; Neutrophil to lymphocyte ratio (NLR) and lactic dehydrogenase (LDH) level. Based on these two parameters a patient can be assigned to one of the three groups 1. Low NLR and normal LDH 2. High NLR and normal LDH and 3. High NLR and high LDH. Patients with high NLR and LDH (group 3) has the worst prognosis and patients with low NLR (group 1) carry the best prognosis.

II. Post treatment changes as a prognostic parameters: It is easy to have blood withdrawn at multiple time points during therapy. The attempts are made to analyse various parameters to identify changes in peripheral blood associated with response or its absence. Some of the changes can be detected as early as two-three weeks of initiation of therapy.

a) Changes in subtypes of immune cells:

i. Increase in CD8+ve cell in responders.

a. CD8+ve CD4-ve CD45RO+ve phenotype representing effector memory cells is seen in responders (p=0.002)[43].

b. CD8+ve cells is seen in responders, particularly expressing CD28 [32].

ii. Increase PD-1 expressing CD4 cells: PD-1 expression on CD4 cells is indicative of immune suppression. Increased expression during therapy is indicative of increased immunosuppression and is associated with progression [35].

iii. Change in Ki67 expressing cells at 3 weeks: Ki67 is a marker of proliferation.

a. Four fold increase in Ki67 expressing immune cells predict response while no change is seen in non-responders [33]

b. This increase in Ki67 PD1+ CD8+ T cell subset is twofold in responders[44]

iv. Change in Granzyme-B expressing immune cells: Granzyme- B expression is associated with killing of cancer cells. Responders also have increased Granzyme -B in Ki67 CD8 cells at 3 weeks [33].

b) **Speed of immune response** / PD1+ CD8+ T cell response: Early proliferation (4 week) of PD1+CD8+ T cells predicts response to therapy (seen in 57% with ORR) while late or absence of proliferation is associated with absence of response. (84.6% of non-responders) [33].

c) Cytokine levels:

i. Decrease in CXCL2 and increase in MMP2 at six weeks there is associated with improved survival [45].

ii. Increase in Serum IL-8 levels suggests increase in immunosuppression and is associated with progression while decrease is associated with response to therapy [46].

d) Change in serum tumor markers :

Carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), cytokeratin-19 fragments (CYFRA 21-1) and neuron specific enolase (NSE) are serum tumor markers (STM). Decrease in STM is associated with better outcome while increase in STM is associated with poor outcome. (Median PFS 11M vs 6 months with increase < 2 fold (p=0.001). The outcome is also proportional to the amount of decrease (6 months for < 2-fold increase and 2 months for ≥2-fold increase). OS also follows same trend (not reached for decrease in STM, 14 months for increase <2 and 4 months for STM >20 [47].

e) Change in circulating tumor cells :

i. Change in PD-L1 expression in circulating tumor cells [48]: PD-L1 expression in circulating cells do not correlate with PD-L1 expression on tumors. However, decrease in PD-L1 expression in circulating

tumor cells is associated with response to therapy and increase expression is seen in non responders.

ii. Change in ctDNA: Response to therapy is associated with decrease/non detectable ctDNA at eight weeks in responders. Increase in ctDNA is seen in nonresponders [49].

iii. Circulating tumor cells are also found to provide information about TMB [26].

3. Others

I. Treatment related adverse events [50]: Generally adverse events seen with pembrolizumab are due to hyper activation of immune system. Their manifestation is associated with response to therapy. Responses seen are durable and persists even when therapy is discontinued for managing adverse events.

II. Sex [51]: Better efficacy is seen in males compared to females.

III. Age [52]: Use of pembrolizumab in advanced NSCLC is associated with better outcome in younger than 65 years of age compared to those more than 65 years of age. The difference in HR is > 0.2 for first line therapy. It reduces to 0.13 when used as a subsequent therapy. Age more than 65 years is also associated with hyperprogression of disease [14, 16].

Summary:

Ongoing research has identified various tumor characteristics, gut microbiome profile, changes reflected in peripheral blood associated with outcome. Changes in peripheral blood can be monitored during therapy also. Currently they are outcome of small studies and/ or retrospective analysis of clinical trials. Their validation will pave way for better selection of patients for monotherapy as well as combination therapy. This will also help in identifying novel co-therapies.

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