Biomarkers that predict response to immunotherapy – no magic bullet

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Abstract

Immunotherapeutic agents have shown impressive clinical efficacy in a broad range of tumour types, particularly in non-small cell lung cancer and melanoma. An effective predictive biomarker is needed to provide patients with the most effective available treatments, avoid unnecessary toxicity and improve cost effectiveness. While it has been an area of very active research in recent years, the ideal biomarker for predicting response to immune check point inhibitor therapy has not yet been universally agreed upon. Approaches to date have focussed on assessment of tumour related factors such as immunohistochemical expression of programmed death ligand-1 (PD-L1), mutational load and DNA mismatch repair gene or protein status. Alternatively, assessment of the immune microenvironment by techniques such as gene expression profiling or measurement of tumour infiltrating lymphocytes can also be informative. Identifying and validating effective biomarkers is particularly challenging for immunotherapy because the dynamic and multifactorial nature of the interaction between tumours and host immunity. In this review, we discuss the relative advantages and disadvantages of different biomarker approaches in the quest to identify a clinically effective predictive biomarker that can improve the overall utility for immune checkpoint inhibitors.

Recent successful clinical development of immunotherapeutic agents that inhibit immune checkpoints has revolutionised therapeutic approaches in a wide range of human malignancies and improved clinical outcomes. Clinical responses to immune checkpoint inhibitors have been demonstrated in a variety of solid tumours including melanoma, non-small cell lung cancer (NSCLC), bladder urothelial carcinoma, head and neck squamous cell carcinoma (HNSCC), gastrointestinal carcinoma, renal cell carcinoma (RCC), triple negative breast carcinoma and refractory Hodgkin lymphoma, with a significant proportion of patients having durable responses.[1] While these agents show good responses in some patients, overall response rates in unselected populations are relatively low, highlighting the need for an effective predictive biomarker.

Malignant tumours use a variety of mechanisms to evade or disarm host immunity, which has recently been recognised as a hallmark of cancer.[2] This involves creation of an immunosuppressive tumour

micro-environment (TME) with functional analogy to physiological immune-privileged sites such as the eye or testis.[3] One means of evading tumour-reactive effector T-cells is up-regulation of inhibitory molecules such as PD-L1 on cancer cells and infiltrating monocytes. Chronic antigen exposure, such as in cancer, also drives effector T-cell expression of inhibitory CTLA-4; which binds with greater avidity and affinity with tumour/TME B7 proteins than its stimulatory homologue, CD28. Such inhibitory receptors are an essential means of tolerance since it is impossible to expose developing T-cells to *all* self-antigens in the thymus; peripheral signals are needed to indicate self versus pathogen and avoid auto-immunity. On binding of an inhibitory molecule with its ligand, activated T-cells abort clonal expansion and either apoptose or become anergic. By blocking this interaction, therapeutic monoclonal antibodies, such as those targeting PD-1, PD-L1 and CTLA-4, can invigorate the immune response to cancer.

To date, biomarker strategies in clinical trials of immune checkpoint inhibitors have focussed on immunohistochemical (IHC) expression of PD-L1. While increased PD-L1 expression generally enriches for patient response to treatment, this biomarker is imperfect in many tumour types and may have limited clinical application. Development of a biomarker strategy that could more accurately predict patient response would be of great clinical and economic assistance. Other biomarker approaches that have been investigated can be broadly divided into those that assess tumour-related factors (such as mutational load, neoantigens and PD-L1) and those that focus on host immunity (such as tumour infiltrating lymphocytes (TILs) or a T-cell inflamed phenotype). The role of host germline predisposition to auto-immunity is also being investigated as a source of variation in both response and toxicity.

PD-L1 expression in tumours

Checkpoint inhibitors that obstruct the immunosuppressive interaction of PD-L1 on tumour cells with the receptor PD-1 on immune cells, unblock anti-tumour activity, making PD-L1 expression a logical biomarker of interest. PD-L1 expression on tumour cells is dynamic and induced by inflammatory cytokines, especially IFN-γ produced by activated T-cells and NK-cells (adaptive immune resistance).[4] In contrast, PD-L1 upregulation may be constitutive due to activation of oncogenic pathways including phosphoinositide 3-kinase, mitogen-activated protein kinase, JAK2/STAT, PTEN or EGFR.[4,5] In many solid tumours, adaptive PD-L1 expression commonly occurs in association with TILs and IFN-γ in the TME.[4,6,7] Indeed, expression of PD-L1 occurs in a wide variety of solid tumours including NSCLC, melanoma, HNSCC, gastrointestinal carcinomas and some breast carcinomas,[8-12] potentially making these tumours ideal candidates for checkpoint inhibition (figure 1).

PD-L1 IHC as a biomarker in clinical trials

While clinical effects of the agents most advanced in development (anti-PD-1 antibodies nivolumab and pembrolizumab; and anti-PD-L1 antibodies atezolizumab, avelumab and durvalumab)[13] are promising, the overall response rate is relatively low in unselected populations. In the initial phase 1 study of nivolumab in melanoma, NSCLC, RCC, colorectal and prostate carcinoma, PD-L1 positive patients (≥5% tumour cells positive) had a 36% overall response rate (ORR) compared to no responses in PD-L1 negative cases.[14] While PD-L1 expression enriches for patients more likely to respond to PD-1/PD-L1 inhibition, many studies have also demonstrated clinical responses in PD-L1 negative patients, including approximately 10-15% of NSCLC[15] and 33% of melanomas treated with nivolumab.[16] The poor negative predictive value of PD-L1 IHC in some studies precludes its use as an exclusive biomarker across all tumour types.

Treatment response association with PD-L1 expression in NSCLC

Response rates and survival benefits have generally correlated with tumour PD-L1 expression in clinical trials of immune checkpoint monotherapy in NSCLC. In the phase 1 KEYNOTE-01 study of second line pembrolizumab treatment in NSCLC, there was a response rate (RR) of 45.2% in patients showing high expression of PD-L1 (≥50% of tumour cells) versus 10.7% in patients with low PD-L1 (<1%).[17] In the phase 3 KEYNOTE-024 trial of first line pembrolizumab in advanced NSCLC, patients with high PD-L1 (≥50%, present in 30.2% of cases) showed improved overall response rate (ORR) and overall survival (OS) compared to standard chemotherapy, providing the strongest clinical rationale for use of PD-L1 IHC as a predictive marker in the clinical setting.[18] The analogous first line

trial of nivolumab, however, CheckMate-026, used a lower cut-off for PD-L1 positivity of ≥5% and was a negative trial.[19] There was no improvement in the ORR or OS, furthermore the pre-planned subgroup analysis of patients with tumour PD-L1 expression ≥50% also failed to show benefit, although high rates of crossover and differences in baseline treatment group characteristics were cited as potential confounding factors.

In clinical trials of atezolizumab in advanced NSCLC in the second line, PD-L1 expression has been assessed in both tumour cells (TC) and infiltrating immune cells (IC) using the SP142 clone antibody.[13] Subgroup analysis of the phase 2 POPLAR study showed improved OS amongst patients with TC or IC PD-L1 in at least 1% of cells compared to docetexal but not in those with lower PD-L1.[20] Hazard ratios for improved survival were higher in those with greater PD-L1 expression.[20] Similarly durvalumab showed an ORR of 16.4% in advanced NSCLC patients with high PD-L1 (≥25%) versus 7.5% amongst low PD-L1 patients in a phase 2 study.[21]

Treatment response association with PD-L1 expression in melanoma

Unlike in NSCLC, clinical benefits with PD-1/PD-L1 blockade in melanoma are not as closely linked to PD-L1 expression and the proportion of PD-L1 IHC negative patients that respond to PD-1 inhibitors has precluded its use as a clinically useful biomarker. In the Checkmate-037 study of nivolumab in melanoma patients previously treated with ipilimumab, 20.3% of PD-L1 negative patients (<5% TC) responded to treatment, as opposed to 43.6% of PD-L1 positive patients.[22] Similarly, in a study of nivolumab versus dacarbazine (Checkmate-066), PD-L1 status did not correlate with survival advantage and 33.1% of PD-L1 IHC negative patients responded to immunotherapy, compared to 52.7% of PD-L1 positive patients.[16] In addition, in the KEYNOTE-006 study of pembrolizumab versus ipilimumab, improved PFS was observed in both PD-L1 negative and positive patients using a cut-point of 1%.[23] Recently, clinical trials have been reported comparing the efficacy of combined immune checkpoint inhibitor therapy of nivolumab with ipilimumab versus nivolumab alone. Early data suggests that melanoma patients whose tumours express PD-L1 derive no benefit from combination immunotherapy over monotherapy, indicating that such patients can be spared the risk of potentially severe toxicity from combination therapy. In contrast, patients with low PD-L1 expression (<5%) had improved ORR and survival from combination immunotherapy, suggesting PD-L1 may be useful in identifying melanoma patients in whom anti-PD1/anti-CTLA-4 combination therapy should be considered.

Treatment response association with PD-L1 expression in other tumours

In HNSCC and bladder urothelial carcinoma, there is some evidence for a role of PD-L1 expression in helping to predict response to PD-1/PD-L1 inhibitors, while in several other tumour types such as clear cell RCC, PD-L1 expression does not appear to have a role.[24] Hodgkin lymphomas show a response rate of 87% to nivolumab for all-comers and unlike in most solid tumours, these tumours typically display constitutive PD-L1 expression resulting from copy number gain or amplification of PD-L1 and PD-L2 genes on chromosome 9p24.1.[25] In triple-negative breast and basal-like breast carcinomas, gastric cancer, and a subgroup of lung cancers, PD-L1 amplification has also been described, although the clinicopathological relevance is currently uncertain.[26]

Challenges of PD-L1 IHC as a Biomarker

Different assays and different criteria for PD-L1 positivity

Comparison of the performance of PD-L1 IHC as a predictive biomarker across different clinical trials and different tumour types is challenging due to the use of different PD-L1 IHC clones and assays and different criteria to determine high PDL-1 expression. Most PD-L1 IHC biomarkers used in clinical trials have examined membranous expression in tumour cells only, however, the proportion of positive tumour cells used to determine a “positive” result has ranged from 1% to 50% (including 5%, 10% and 25% cut points)[13,15] with higher cut-points correlating with higher RRs. PD-L1 is not a binomial marker so there is no clear biological cut-point, in marked distinction to the binomial distribution of other molecular predictive biomarkers such as *EGFR* status in lung adenocarcinomas. ROC characteristics were used in the phase 1 study of pembrolizumab to identify a threshold of 50% as the most

appropriate cut-point for the PD-L1 22C3 clone in NSCLC.[17] Threshold development for PD-L1 in other clinical trials is less clear and may have less of a biological basis. These differing criteria for positivity, along with the use of different antibody clones, has until recently made it impossible to compare the efficacy of PD-L1 as a biomarker across different clinical trials.

More recently, an increasing number of correlation studies between the various PD-L1 IHC assays have been undertaken in NSCLC and have consistently shown fairly equivalent performance characteristics of 3 of the clones (22C3, 28-8 and SP263 but not SP142) using the commercial assays but not laboratory developed tests (LDTs),[27-31] enabling comparisons between relevant clinical trials. The ultimate testing paradigm in NSCLC will depend on results of these studies and the ability of LDTs to perform to the standard of commercially developed assays.

Heterogeneity in PD-L1 staining from different sites and from different time points

PD-L1 expression is not uncommonly heterogenous within a single tumour specimen with areas of positive and negative staining,[32] so small biopsy samples can potentially give misleading results. In a study of PD-L1 status (SP142 clone) in 160 NSCLC resection specimens and matched preoperative biopsies, the concordance of PD-L1 status was 81% in tumour cells but only 52% when both tumour and immune cells were assessed.[33] Tumour samples from different sites can also differ and matched samples of primary NSCLC and nodal metastases have shown PD-L1 status concordance of 70.3%-89%.[34,35] Similarly, a discordance rate of 34% has been reported between primary and metastatic HNSCC.[12] In melanoma specimens, PD-L1 IHC status was discordant in matched longitudinal specimens in 50% of 46 patients.[11] Prior chemotherapy or radiotherapy may increase PD-L1 expression by eliciting a T-cell immune reaction.[36] This would suggest that contemporary, rather than archival pre-treatment biopsies are more informative, however, data is lacking to support this conclusively.

Challenges in PD-L1 IHC interpretation

Reliable and reproducible quantification of PD-L1 IHC staining can be challenging for pathologists but is essential for PD-L1 to be useful as a clinical biomarker. In a study of 120 NSCLC stained with PD-L1 (22C3 clone), 10 Australian pathologists showed substantial interobserver agreement with overall agreement of 81.9% when assessing the 50% cut-point and 84.2% when assessing the 1% cut-point (kappa 0.64 and 0.68).[37] The intra-observer agreement was 91.3% and 89.7%, respectively. In another study, nine pathologists also showed substantial agreement scoring PD-L1 in tumour cells from 15 NSCLCs (kappa scores 0.6-0.8 with cut-points of 1, 5, 10 and 50%), however, concordance was only slight to fair when scoring PD-L1 in immune cells (kappa 0.12-0.25).[31]

Advantages/disadvantages of IHC as a biomarker

As a biomarker for routine clinical use, the technique of IHC has many strengths including being rapid, relatively low cost compared to molecular techniques and widely available in all pathology laboratories. However, IHC is not without technical challenges especially with regards to reproducibility of LDTs versus commercial assays. PD-L1 IHC expression can also be altered based on pre-analytical factors including ischaemic time, formalin fixation time, use of different IHC clones that recognise different epitopes on PD-L1, different IHC amplification or detection methods.[38] Use of external quality controls (such as tonsil or placenta showing constitutive PD-L1 expression) and ongoing internal and external quality assurance measures are essential for clinical use of PD-L1 IHC as a predictive biomarker.

Mutational burden

Somatic (tumour) mutations generate foreign proteins not found in normal tissue, forming neo-epitopes recognised by effector T-cells.[39] Somatic gene mutation frequency is highest in carcinogen-associated tumours led by melanomas, followed by lung squamous cell and adenocarcinoma and bladder carcinoma,[40] and immune checkpoint inhibitors have exhibited most clinical effect in these tumours. Greater clinical benefit with pembrolizumab has been demonstrated in NSCLCs bearing higher non-synonymous mutational load as measured by whole exome sequencing, and these cases

were associated with smoking and higher PD-L1 expression.[41] The survival benefits in NSCLC treated with immune checkpoint inhibitors are not seen in NSCLCs with simpler genotypes such as those harbouring *EGFR* driver mutations.[42]

Similar studies in melanoma have shown higher mutational load correlates with clinical response to ipilumumab[43] although not all these tumours responded to treatment. Treatment of melanomas with PD-1 inhibitors also show a correlation between high mutational burden and improved RR and OS.[44] Furthermore, high mutational load has been associated with increased PDL-1 expression in melanoma.[45] Higher mutational load has also been associated with patient response to atezolizumab in locally advanced or metastatic urothelial cancer.[46]

While these studies support the biological rationale that the genomic landscape of cancer determines treatment response, there are significant limitations to using this approach clinically, including lack of a clear definition of how to measure mutational load, the cost of whole exome sequencing and ability to obtain sufficient DNA from tumour samples. It is inevitable that costs of sequencing will continue to reduce, however, surrogate biomarkers of mutational burden may be more practical in clinical practice.

Mismatch repair deficiency protein deficiency

Mutations in genes controlling DNA repair and replication such as MSH2, BRCA2, POLD1 and POLE correlate with higher somatic mutational burden, in keeping with impaired DNA repair mechanisms.[41] While the median mutation frequency in colorectal carcinomas (CRC) is relatively low, those that harbour microsatellite instability (MSI) have an average mutational frequency 10-50 times greater than that seen in microsatellite stable CRC.[47] In a phase 2 study of pembrolizumab the ORR was 40% in 11 mismatch repair deficient (MMRD) CRC (as determined by microsatellite instability analysis) and 0% in 18 MMR proficient patients.[10] PD-L1 expression was only seen in MMRD tumours and CD8+ T cell density was higher in the invasive front of these tumours.[10]

This study provided important data supporting MMRD as a predictive biomarker to select patients for PD-1 inhibition, regardless of the histological tumour type. However, MMRD tumours represent only a small subset of solid tumours, and is most commonly seen in colorectal carcinomas (~15%), endometrioid adenocarcinomas (~25%) and a lower proportion of gastric, pancreatic and other carcinomas.[47,48] Multiple ongoing clinical trials are investigating immune checkpoint inhibitors in MSI high CRC and other solid tumours.[47,48]

MSI-H tumours usually arise sporadically through hypermethylation of the MLH1 gene promoter or biallelic mutations, however, a significant proportion result from germline mutations providing a screening opportunity for Lynch syndrome. MMRD can be identified through IHC staining for MMR proteins (typically MLH1, MSH2, MSH6 and PMS2)[49] or demonstration of MSI using PCR or NGS.[47,48] As evidence for efficacy of checkpoint inhibition in MMRD tumours develops, results of these assays are likely to play an important role in treatment decisions.

Other potential biomarkers

Other potential biomarkers have been investigated including IHC expression of PD-L2,[50,51] IFN-γ levels,[52,53] tumour infiltrating lymphocytes,[7,54] immune gene signatures[55,56,57] or various combinations of markers, with varying degrees of success. Like other immunotherapy biomarkers, standardised methods of assessment and the cut-point for determining “positive” from “negative” is unclear.

Conclusions

Immune checkpoint inhibitors are currently being evaluated in numerous clinical trials across most cancer types, with evidence of efficacy established or emerging in several major groups such as NSCLC and melanoma. As the range of indications increases, so too does the number of patients exposed to the drugs, who either gain significant benefit or risk unwarranted toxicity. There is a great need to identify and evaluate effective predictive biomarkers in a prospective fashion. Of all potential predictive biomarkers, PD-L1 IHC has been the most studied, particularly in NSCLC, but its exact

clinical applications are not well established. Other biomarker strategies include MMR status, mutational load, tumour infiltrating lymphocytes and immune gene signatures have also been evaluated. To date, however, development of a single successful predictive biomarker has been largely unsuccessful despite overwhelming efforts from the scientific and pharmaceutical communities. The solution may ultimately be a combination of biomarkers taking into account host and tumour, and may not be a “one size fits all” across all tumour types. Regardless, developing a strategy to personalise immuno-therapeutics is essential to achieve optimal patient outcomes in this realm.

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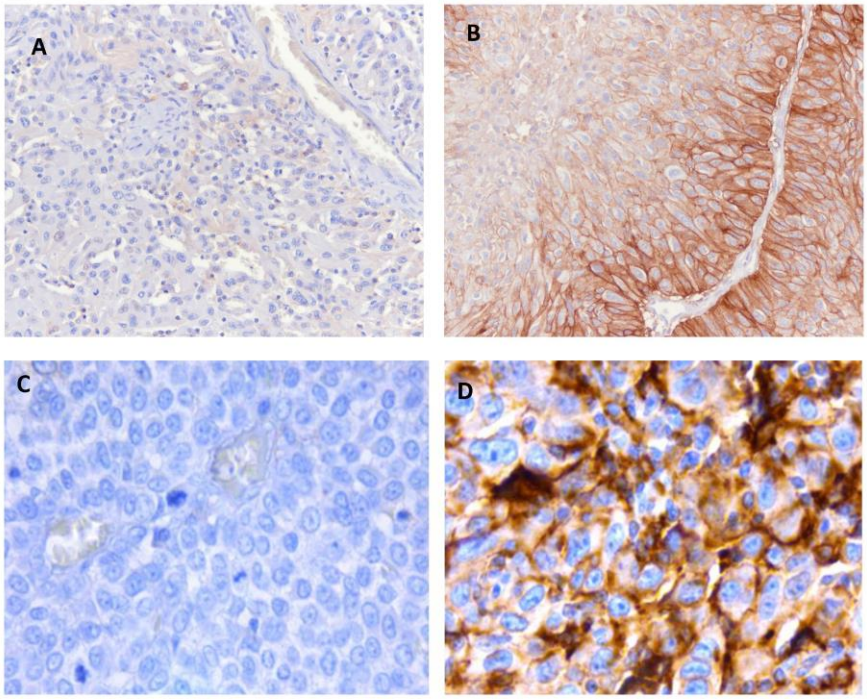
Figure 1: *PD-L1 IHC expression (22C3 clone) in NSCLC and melanoma. (A) NSCLC with membranous staining in 1% of tumour cells. (B) NSCLC with membranous staining in >50% of tumour cells. (C) and (D) In melanoma samples PD-L1 IHC status is not always concordant. In this example, the primary tumour was negative (C), while the locoregional metastasis had membranous staining in >50% of tumour cells.*



References

1. Hamanishi J, Mandai M, Matsumura N, Abiko K, Baba T, Konishi I. PD-1/PD-L1 blockade in cancer treatment: perspectives and issues. International Journal of Clinical Oncology. 2016;21(3):462-473.
2. Hanahan D, Weinberg R. Hallmarks of Cancer: The Next Generation. Cell. 2011;144(5):646-674.
3. Joyce J, Fearon D. T cell exclusion, immune privilege, and the tumor microenvironment. Science. 2015;348:74-80.
4. Teng MWL, Ngiow SF, Ribas A, Smyth MJ. Classifying Cancers Based on T-cell Infiltration and PD-L1. Cancer Research. 2015;75(11):2139.
5. Akbay EA, Koyama S, Carretero J, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discovery. 2013.
6. Taube JM, Anders RA, Young GD, et al. Colocalization of Inflammatory Response with B7-H1 Expression in Human Melanocytic Lesions Supports an Adaptive Resistance Mechanism of Immune Escape. Science Translational Medicine. 2012;4(127):127ra137-127ra137.
7. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568-571.
8. Beckers RK, Selinger CI, Vilain R, et al. Programmed death ligand 1 expression in triple-negative breast cancer is associated with tumour-infiltrating lymphocytes and improved outcome. Histopathology. 2016;69(1):25-34.
9. Cooper WA, Tran T, Vilain RE, et al. PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. Lung Cancer. 2015;89(2):181-188.
10. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. New England Journal of Medicine. 2015;372(26):2509-2520.
11. Madore J, Vilain RE, Menzies AM, et al. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. Pigment Cell & Melanoma Research. 2015;28(3):245-253.
12. Roper E, Lum T, Palme CE, et al. PD-L1 expression predicts longer disease free survival in high risk head and neck cutaneous squamous cell carcinoma. Pathology. 2017;49(5):499-505.
13. Tsao MS, Kerr KM, Dacic S, Yatabe Y, Hirsch FR, eds. IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer. Colorado: Editorial Rx Press; 2017.
14. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. New England Journal of Medicine. 2012;366(26):2443-2454.
15. Chae YK, Pan A, Davis AA, et al. Biomarkers for PD-1/PD-L1 blockade therapy in non-small-cell lung cancer: Is PD-L1 expression a good marker for patient selection? Clinical Lung Cancer. 2016;17(5):350-361.
16. Robert C, Long GV, Brady B, et al. Nivolumab in Previously Untreated Melanoma without BRAF Mutation. New England Journal of Medicine. 2015;372(4):320-330.
17. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015;372(21):2018-2028.
18. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2016;375(19):1823-1833.
19. Carbone DP, Reck M, Paz-Ares L, et al. First-Line Nivolumab in Stage IV or Recurrent Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2017;376(25):2415-2426.
20. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. The Lancet. 2016;387(10030):1837-1846.
21. Garassino M, Vansteenkiste J, Kim J-H, et al. PL04a.03: Durvalumab in 3rd-Line LocallyAdvanced or Metastatic, EGFR/ALK Wild-Type NSCLC: Results from the Phase 2 ATLANTIC Study. Journal of Thoracic Oncology.12(1):S10-S11.
22. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. The Lancet Oncology. 2015;16(4):375-384.
23. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. New England Journal of Medicine. 2015;372(26):2521-2532.
24. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. New England Journal of Medicine. 2015;373(19):1803-1813.

25. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 Blockade with Nivolumab in Relapsed or Refractory Hodgkin's Lymphoma. New England Journal of Medicine. 2015;372(4):311-319.
26. Budczies J, Bockmayr M, Denkert C, et al. Pan-cancer analysis of copy number changes in programmed death-ligand 1 (PD-L1, CD274) – associations with gene expression, mutational load, and survival. Genes, Chromosomes and Cancer. 2016;55(8):626-639.
27. Adam J, Rouquette I, Damotte D, et al. PL04a.04: Multicentric French Harmonization Study for PD-L1 IHC Testing in NSCLC. Journal of Thoracic Oncology. 2017;12(1):S11-S12.
28. Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. Journal of Thoracic Oncology. 2017;12(2):208-222.
29. Ratcliffe MJ, Sharpe A, Midha A, et al. Agreement between Programmed Cell Death Ligand-1 Diagnostic Assays across Multiple Protein Expression Cutoffs in Non–Small Cell Lung Cancer. Clinical Cancer Research. 2017;23(14):3585.
30. Rimm DL, Han G, Taube JM, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for pd-l1 expression in non–small cell lung cancer. JAMA Oncology. 2017;3(8):1051-1058.
31. Scheel AH, Dietel M, Heukamp LC, et al. Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. Modern Pathology. 2016;29(10):1165-1172.
32. McLaughlin J, Han G, Schalper KA, et al. Quantitative assessment of the heterogeneity of PD-L11 expression in non–small-cell lung cancer. JAMA Oncology. 2016;2(1):46-54.
33. Ilie M, Long-Mira E, Bence C, et al. Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies. Annals of Oncology. 2016;27(1):147-153.
34. Ameratunga M, Asadi K, Lin X, et al. PD-L1 and Tumor Infiltrating Lymphocytes as Prognostic Markers in Resected NSCLC. PLOS ONE. 2016;11(4):e0153954.
35. Kim M-Y, Koh J, Kim S, Go H, Jeon YK, Chung DH. Clinicopathological analysis of PD-L1 and PD-L2 expression in pulmonary squamous cell carcinoma: Comparison with tumor-infiltrating T cells and the status of oncogenic drivers. Lung Cancer. 2015;88(1):24-33.
36. Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature. 2015;520(7547):373-377.
37. Cooper WA, Russell PA, Cherian M, et al. Intra- and Interobserver Reproducibility Assessment of PD-L1 Biomarker in Non–Small Cell Lung Cancer. Clinical Cancer Research. 2017;23:4569-4577.
38. Ilie M, Hofman V, Dietel M, Soria J-C, Hofman P. Assessment of the PD-L1 status by immunohistochemistry: challenges and perspectives for therapeutic strategies in lung cancer patients. Virchows Archiv. 2016;468(5):511-525.
39. Desrichard A, Snyder A, Chan TA. Cancer Neoantigens and Applications for Immunotherapy. Clinical Cancer Research. 2016;22(4):807.
40. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415-421.
41. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science (New York, N.Y.). 2015;348(6230):124-128.
42. Lee CK, Man J, Lord S, et al. Checkpoint Inhibitors in Metastatic EGFR-Mutated Non-Small Cell Lung Cancer: A Meta-Analysis. Journal of Thoracic Oncology. 2016;12(2):403-407.
43. Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science (New York, N.Y.). 2015;350(6257):207-211.
44. Johnson DB, Frampton GM, Rioth MJ, et al. Hybrid capture-based next-generation sequencing (HC NGS) in melanoma to identify markers of response to anti-PD-1/PD-L1. Journal of Clinical Oncology. 2016;34(15_suppl):105-105.
45. Madore J, Strbenac D, Vilain R, et al. PD-L1 Negative Status is Associated with Lower Mutation Burden, Differential Expression of Immune-Related Genes, and Worse Survival in Stage III Melanoma. Clinical Cancer Research. 2016;22:3915-3923.
46. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. The Lancet. 2016;387(10031):1909-1920.
47. Link J, Overman M. Immunotherapy progress in mismatch repair-deficient colorectal cancer and future therapeutic challenges. Cancer J. 2016;22:190-195.

48. Colle R, Cohen R, Cochereau D, et al. Immunotherapy and patients treated for cancer with microsatellite instability. Bulletin du Cancer. 2016;104.
49. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer). New England Journal of Medicine. 2005;352(18):1851-1860.
50. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment with Response to Anti–PD-1 Therapy. Clinical Cancer Research. 2014;20(19):5064-5074.
51. Yearley JH, Gibson C, Yu N, et al. PD-L2 Expression in Human Tumors: Relevance to Anti-PD-1 Therapy in Cancer. Clinical Cancer Research. 2017;23(12):3158.
52. Higgs B, Morehouse C, Streicher K, et al. Relationship of baseline tumoral IFNγ mRNA and PD-L1 protein expression to overall survival in durvalumab-treated NSCLC patients. Journal of Clinical Oncology. 2016;34(15_suppl):3036-3036.
53. Herbst RS, Soria J-C, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563-567.
54. Bremnes RM, Busund L-T, Kilvær TL, et al. The Role of Tumor-Infiltrating Lymphocytes in Development, Progression, and Prognosis of Non-Small Cell Lung Cancer. Journal of Thoracic Oncology. 2016;11(6):789-800.
55. Ji R-R, Chasalow SD, Wang L, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunology, Immunotherapy. 2012;61(7):1019-1031.
56. Ribas A, Robert C, Hodi F, et al. Association of response to programmed death receptor 1 (PD-1) blockade with pembrolizumab (MK-3475) with an interferon-inflammatory immune gene signature. Journal of Clinical Oncology. 2015;33(15_suppl):3001-3001.
57. Hugo W, Zaretsky JM, Sun L, et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell. 2016;165(1):35-44.