INVITED REVIEW

Emerging biomarkers for programmed death-1 pathway cancer therapy

Joline SJ Lim[1,2], Raghav Sundar[1,2], Maxime Chénard-Poirier[1], Juanita Lopez[1] and Timothy A Yap[1,3]

[1]Royal Marsden Hospital, London, UK; [2]National University Cancer Institute of Singapore, Singapore, [3]The Institute of Cancer Research, London, UK

***Corresponding author:**

Dr Timothy A Yap MBBS PhD MRCP BSc PgDip

Clinician Scientist and Consultant Medical Oncologist

Drug Development Unit and Lung Cancer Unit

The Institute of Cancer Research and Royal Marsden Hospital,

Downs Road,

London SM2 5PT,

United Kingdom.

Tel: 44-20-8722-3539

Fax: 44-20-8642-7979

E-mail: timothy.yap@icr.ac.uk

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Abstract

The field of immuno-oncology has witnessed unprecedented success in recent years, with several programmed cell death 1 (PD-1) and programmed cell death-ligand 1 (PD-L1) inhibitors obtaining FDA registration and breakthrough drug therapy designation in multiple tumor types. Despite its clear efficacy in certain cancers, treatment with these agents carries a risk of immune-related toxicities and substantial financial burden. It is therefore critical to identify patients likely to benefit from such immunotherapies and develop strategies to differentiate responders from non-responders early during treatment. Here we discuss the development of predictive and treatment response biomarkers for immune checkpoint inhibitors. We first examine the role of PD-L1 expression, the most extensively studied predictive biomarker of response, and further discuss emerging putative predictive biomarkers. We also detail challenges faced in the development of response assessments for immunotherapeutics and propose other biomarkers that may be useful as surrogate intermediate endpoints of response.

Introduction

While immuno-oncology has been explored for many years with varied success, the Food and Drug Administration (FDA) approval of ipilimumab (Yervoy, Bristol-Meyers Squibb) in metastatic melanoma in 2011 finally heralded the advent of well tolerated and effective immune checkpoint inhibitors. A succession of novel immunotherapeutics have now achieved FDA Breakthrough Therapy Designation and/or obtained accelerated regulatory approval for use in multiple different cancers, including the programmed cell death 1 (PD-1) inhibitor nivolumab (Opdivo, Bristol-Meyers Squibb) in melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC) and Hodgkin's lymphoma, PD-1 inhibitor pembrolizumab (Keytruda, Merck Sharp & Dohme) in melanoma and NSCLC, and most recently, the programmed cell death-ligand 1 (PD-L1) atezolizumab (Tecentriq, Roche) for bladder cancer and NSCLC. Many other PD-1 and PD-L1 inhibitors are currently at different stages of drug development and it is likely that most will also achieve similar regulatory success. As an increasing number of immune checkpoint inhibitors approach regulatory approval, newer compounds from the same class of drugs will face the challenge of identifying niche indications that are distinct from more established agents in this increasingly competitive clinical arena, rather than designing more "me too" studies. Many investigators are also now pursuing rational combination strategies using a PD-1/PD-L1 inhibitor backbone, so as to overcome drug resistance to improve patient outcomes.

Immune checkpoint blockade has been show to activate cellular-mediated immune response against tumor cells. Preclinical studies have shown that blockade of either PD-1 or its ligands improve T-cell effector function, and blockade of PD-L1 increased

infiltration of tumor-reactive CD8+ T cells in mouse models [1, 2]. Besides the PD-1/PD-L1 pathway, another immunomodulatory mechanism that has been exploited in the clinical setting is cytotoxic T-lymphocyte antigen-4 (CTLA-4), a surface receptor on effector T cells that interacts with antigen-presenting cells (APC), leading to T-cell arrest [3]. While an in-depth discussion of all other immunoregulatory molecules is beyond the scope of this review, other new targets of immunomodulation that are under active investigation include TIM3, an immune checkpoint molecule frequently co-expressed with PD-1, and LAG3, a co-stimulatory receptor that decreases cytotoxic T cell function [4, 5].

Cancer treatment strategies have evolved from an all-comer approach with cytotoxic chemotherapies to biomarker-driven strategies with molecular targeted agents where patients with potentially actionable mutations are matched with rational antitumor therapies [6]. However, to date, the search for robust analytically validated and clinically qualified biomarkers that can accurately predict response to immune checkpoint inhibitors has been more complex. In addition, conventional treatment response criteria to immunotherapies using standard imaging techniques are not optimal assessment strategies since they may sometimes be misleading due to the tumor flare phenomenon associated with immunotherapy, whereby initial scans may show pseudoprogression of tumor due to inflammatory cell infiltration or necrosis of tumor due to treatment response. This is further compounded by the poor correlation between response rates and overall survival (OS) as evidenced by the subgroup of patients who achieve durable long-term responses despite not developing an objective radiological response. As such, in addition to predictive biomarkers, we

also need to seek biomarkers that can reflect treatment response more accurately and to serve as surrogate intermediate endpoints markers.

In this review, we focus on the development of predictive and treatment response biomarkers for immune checkpoint inhibitors. We first examine the role of PD-L1 expression, the most extensively studied predictive biomarker of response, and further discuss emerging putative predictive biomarkers including mutational load, neoantigens and non-genomic signatures. We also detail the challenges faced in the development of response assessments for immunotherapeutics, and propose other biomarkers that may be useful as surrogate intermediate endpoints for response.

Predictive biomarkers of response for immunotherapy

The development of robust predictive markers of response for immunotherapy has been challenging due in part to the underlying complexities of the immune system. For example, immunoediting leads to constant changes in the tumor microenvironment, posing challenges in obtaining a true reflection of the dynamic state of tumor immunogenicity. In addition, factoring in the substantial fiscal burden and increased (albeit uncommon) risk of significant immune-related toxicities involved with the administration of immunotherapy, there is a pressing need to develop analytically validated biomarker assays that can identify patients who are most likely to respond to such treatments.

PD-L1 expression

PD-1, PD-L1 and programmed cell death-ligand 2 (PD-L2) have all been assessed as predictive markers of response to PD-1/PD-L1 inhibitors. While PD-1 expression

may be the most intuitive choice as a predictive biomarker of response to PD-1 inhibitors, early studies indicated that PD-L1 expression showed better correlation with antitumor response compared to PD-1 [7]. PD-L2, another ligand for PD-1, has also been examined in early studies, and was found to be expressed in similar regions as PD-L1 [8, 9]. Nonetheless, correlation with clinical outcomes has not been consistent, and thus PD-L1 remains the frontrunner as a predictive marker, albeit with its own limitations.

PD-L1 can be expressed both constitutively due to oncogene activation and the dysregulation of signaling pathways [10], or induced in an adaptive fashion by proinflammatory factors such as interferon gamma (IFNγ) [7, 11]. Tumors with constitutive PD-L1 expression have also been linked to specific oncogenes, such as PTEN loss for colorectal cancer and glioblastoma multiforme (GBM) [12], epidermal growth factor receptor (EGFR) activation in NSCLC [13] and the janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway in T-cell lymphoma [14]. However, the majority of other tumors, which express PD-L1 appear to have dynamic levels of PD-L1 expression, which are influenced by the tumor microenvironment and a host of inflammatory factors. The upregulation of PD-L1 leads to increased binding to T-cells through PD-1, creating an overall dampening effect on immune feedback, permitting tumor cells to escape immune surveillance [11].

The first evidence that PD-L1 expression was predictive of patient outcomes was in a phase I trial of nivolumab in patients with advanced melanoma, NSCLC, CRPC, RCC and colorectal cancers. Pre-treatment tumor biopsy specimens were tested

for cell surface PD-L1 protein expression using immunohistochemistry (IHC), with a expression threshold cut off of 5% designated to be PD-L1 positive. Using these criteria, the authors found that in their limited series of patients, PD-L1 negative tumors had a response rate of 0% (0/17 patient response), whereas PD-L1 positive tumors had a response rate of 36% (9/25 patient response) [15]. Multiple biomarker studies have since been undertaken to investigate PD-L1 expression as a predictive biomarker of response to anti-PD-1 and anti-PD-L1 therapies, with varying degrees of success **(Supplementary Table 1)**. In general, while increased PD-L1 expression correlates with improved response rates to PD-1/PD-L1 inhibitor treatment, it has also become clear that there is a proportion of patients with PD-L1 negative tumors who still respond to therapy, highlighting a clear limitation with this biomarker assay [16]. Currently, the only companion diagnostic for PD-L1 expression approved by the FDA is the pharmDx test of PD-L1 IHC 22C3 to assess PD-L1 expression prior to pembrolizumab use. In KEYNOTE-001, a substudy of patients with advanced NSCLC showed that patients harboring tumors with at least 50% PD-L1 expression had improved response rates (RR) and median overall survival (mOS) compared to those with tumors expressing less than 50% PD-L1 expression (RR 45% vs 11-17%, p=0.01; mOS not reached vs 10.4 months) [17].

Nivolumab, which has now been approved for use in melanoma, NSCLC and RCC, has been FDA-approved with a complementary but not mandatory diagnostic PD-L1 IHC 28-8 PharmDx diagnostic [18-21]. More importantly, while PD-L1 positivity may potentially correlate with improved response rates and survival, multiple studies have repeatedly shown that there is a small but definite proportion of patients with PD-L1 negative tumors who continue to benefit from treatment. Excluding such patients

from potentially life-prolonging treatments on the basis of IHC expression thus remains a clinical and ethical dilemma. Overall, the biological and technical complexities of PD-L1 expression add a challenging dimension to its utility as a predictive biomarker of response.

Multiple tumor factors contribute to the challenge of assessing PD-L1 expression of an isolated tumor specimen at a single timepoint as a critical indicator in determining if a patient may benefit from anti-PD-1 or anti-PD-L1 therapy. Due to the adaptive nature of PD-L1 and its upregulation by pro-inflammatory conditions [7], PD-L1 expression levels tend to be dynamic rather than stagnant, raising questions as to whether a single tumor sample that may have been taken at the time of a patient's original diagnosis is truly reflective of the current state of disease, especially after multiple lines of antitumor therapy. PD-L1 expression also tends to be focal, mainly bordering areas of tumor cells and lymphocytes, leading to concerns of sampling error by missing the tumor-immune interface and thereby rendering false negative results [11]. In a study that assessed matched patient samples from primary and secondary sites of disease from patients with RCC, there was discordant tumor cell PD-L1 staining in 20.8% of patients, raising concerns that intertumor immunologic heterogeneity may confound the accuracy of overall tumor PD-L1 expression status.

Apart from such tumor factors, technical issues also play an important role in determining PD-L1 expression by IHC. The development of multiple anti-PD-1/PD-L1 inhibitors by different pharmaceutical companies with separate proprietary companion diagnostics to explore the utility of PD-L1 expression as a biomarker has led to an array of antibodies currently in use for PD-L1 IHC testing [22]. Confounded

by the fact that different studies have used various cut-off criteria to define PD-L1 positivity, the lack of unification in testing has led to much confusion, and significant challenges in collating a large dataset to determine the benefit of PD-L1 expression as a predictive biomarker or to make cross-study comparisons of patient outcomes [23, 24]. Additionally, PD-L1 is expressed not only on tumor cells but other components in the tumor microenvironment, such as macrophages and lymphocytes. Determining if positive PD-L1 expression is actually meaningful on such cells is currently an area of active research [25].

Nonetheless, efforts are currently underway in an attempt to address some of these technical challenges. Over the past year, the Blueprint Proposal has been put in place as an initiative between pharmaceutical and companion diagnostic companies to develop consensus in the use of PD-L1 as a predictive biomarker for anti-PD-1/PD-L1 therapies. The aim of the proposal is to work via cross industry collaboration to generate information upon which analytic comparison of various diagnostic assays may be conducted, potentially paving the way for post-market standardization or practice guideline development. Initial findings were presented this year, where a comparison of three PD-L1 diagnostic assays (Ventana SP263, Dako 28-8 and Dako 22C3) was undertaken on 81 tumor biopsy samples from patients with NSCLC treated with pembrolizumab or nivolumab, which showed good concordance of 96% [26]. While these results are promising, further confirmatory data are awaited in other tumor types. It is unlikely that a single PD-L1 diagnostic will ever be completely predictive, and multiple groups have now presented new evidence that automated, digital analysis of multiplex IHC is more predictive than any one marker [27, 28]. A

multifactorial approach to immunotherapeutic biomarker assay development is likely to be essential, although the optimal strategy has yet to be determined.

Mutational load

With multiple issues associated with the use of PD-L1 expression as a predictive biomarker of response to immune checkpoint inhibitors, it is unlikely that a PD-L1 IHC assay will ever be completely predictive. Much effort is thus now focused on the discovery and development of alternative strategies **(Table 1)**. While correlation of response to immune checkpoint inhibitors with driver mutations has been largely unsuccessful [29], there is mounting evidence that mutational load may correlate with response to immune checkpoint inhibitors [30, 31]. In a study examining the mutational load of a range of cancers through the interrogation of available databases, it was noted that different tumor types express somatic mutations to varying degrees. Melanoma, lung and bladder cancers – malignancies in which PD-1 and PD-L1 inhibitors are now approved in - ranked as the tumors with the highest median mutational load [32].

The use of mutational load as a predictive biomarker of response has already been assessed in different immunotherapy trials. In a cohort of patients with advanced melanoma treated with ipilimumab, whole-exome sequencing of DNA from matched tumor and normal tissue (blood) specimens form patients was undertaken for correlation between mutational burden and clinical outcomes. These data showed that patients with a high mutational load had a better OS in the discovery set, with a trend towards improved OS in the validation set [33]. A retrospective analysis of tumor mutational load assessed in 2 cohorts of patients with advanced NSCLC

treated with pembrolizumab showed that patients with durable clinical benefit, defined as a partial or stable response of more than 6 months, expressed a higher nonsynonymous mutational burden (median 299 vs 127, p=0.0008), with a higher degree of non-synonymous mutations correlating with an improved median progression-free survival (HR 0.19, p=0.0004) [30]. Most recently, a phase II study of atezolizumab in patients with advanced bladder cancer evaluated the mutational load of enrolled patients, and showed that the median mutational load was significantly higher in responders compared to non-responders (12.4 vs 6.4 per megabase, p<0.0001), independent of The Cancer Genome Atlas (TCGA) subtype or immune cell subgroup [34].

While costs of genomic sequencing has been steadily decreasing over the past decade, whole exome and whole genome sequencing and their respective data analyses remain challenging in terms of the associated relative financial costs and time implications, as well as bioinformatic challenges, limiting their large scale application in the routine clinical setting. Although results from PD-1 inhibitor trials in colorectal cancer have been disappointing, further analysis has shown that patients with mismatch-repair (MMR) deficient tumors - determined by microsatellite instability (MSI) analysis - had an improved ORR (62% vs 0%) and disease control rate (DCR 92% vs 16%) [31]. A separate study looking at mutational load using a targeted next-generation sequencing (NGS) panel showed that a mutational load of ≥20 mutations correlated with MMR deficiency [35]. While further studies are needed, these data suggest that targeted NGS panels or MSI analyses may potentially be a more time and cost-effective surrogate of the mutational load of tumors.

Neoantigens

The link between mutational load and response to immune checkpoint inhibitors is likely to be due to the production of neoantigens by tumor cells compared to somatic cells. Single nucleotide mutations may lead to changes in peptide sequences producing T-cell neoantigenic peptides that are prone to immune attack. A greater mutational load may thus translate to a higher number of neoantigens, which may potentially lead to greater T-cell dependent cytokine production and tumor cell kill when immune checkpoint blockade is lifted [36].

In the study by Snyder and colleagues investigating the relationship between mutational burden and treatment outcomes in patients with advanced melanoma treated with ipilimumab, the authors examined neoantigen load by translating all nonsynonymous missense mutations into mutant and nonmutant peptides. A neoepitope signature that correlated with survival was established in a discovery series of patients and subsequently confirmed in a validation set [33]. Similar results were observed in a study undertaken in a separate cohort of patients with melanoma treated with ipilimumab, where a higher neoantigen load was associated with antitumor response to ipilimumab ($p=0.027$) [37].

There are inherent challenges in the study of neoantigens, as not all may elicit a T-cell response. While there may be shared antigens present in the majority of tumors, it may be the unique antigens present within each patient that are necessary to elicit immunogenicity. Preclinical studies have suggested that T-cell reactivity may rely on a small proportion of neoantigens that are particularly immunogenic rather than an overall quantitative neoantigen load [38], while a more recent study suggests that

certain unique neoepitopes may even confer a protective effect by the negative stimulation of T-cell activity [39].

Secondly, the immune system interacts with the tumor and its microenvironment extensively through the process of cancer immunoediting, whereby tumors go through the three phases of elimination, equilibrium, and finally escape, to avoid innate immunosurveillance [40]. T-cells are central to this process of immunoediting, eliminating tumor cells that express highly immunogenic neoantigens [41], or engaging epigenetic mechanisms to silence neoantigen expression [42]. This continuous evolution of neoantigen production is dependent on ongoing DNA alterations that may accumulate with tumor growth, as well as silencing of the immunogenicity of these neoantigens through T-cell modulation. This may lead to heterogeneity in the neoantigen landscape, and serial monitoring may be required in order to accurately reflect the effects of immunoediting.

More recently, further studies on clonal neoantigens have confirmed that a high neoantigen repertoire was associated with improved patient outcomes in terms of PFS and OS. Interestingly, interrogation of neoantigens from the analysis of individual specimens taken from multiple regions of the same tumor showed evidence of neoantigen intratumor heterogeneity and the presence of clonal and subclonal neoantigens. From the series of lung adenocarcinomas evaluated, decreased neoantigen intratumoral heterogeneity was associated with improved OS ($p=0.025$), while patients with durable clinical benefit had lower neoantigen intratumor heterogeneity compared to patients without clinical benefit ($p=0.006$) [43].

While the correlation of mutational load and neoantigens may be scientifically promising as a tool for the prediction of response to immune checkpoint inhibitors, further investigation is still required to confirm its clinical utility. Current bioinformatic studies harbor limitations in the identification of neoantigens derived from passenger mutations that may not actually induce a T-cell immune response. Several studies have already shown that while there may be a high number of neoantigenic epitopes shortlisted through current computational algorithms, only a small proportion may actually bind to the MHC I groove and elicit a T-cell response [44, 45]. One of the current challenges lies in the filtering of data obtained from whole exome sequencing to identify meaningful neoantigens that are responsible for T-cell responses. This requires the formulation of algorithms to identify neoantigens with high MHC binding affinities that are recognized by the T-cell receptor repertoire to elicit meaningful T-cell responses.

Non-genomic signatures as predictive markers of response and resistance

Non-genomic signatures are also being studied as possible predictive biomarkers for treatment response. Transcriptional signatures have been studied among patients with melanoma treated with anti-PD-1 therapies, and a distinct signature was found to be related to innate anti-PD-1 drug resistance [46]. Twenty-eight tumors from patients with melanoma treated with anti-PD-1 therapy were characterized with RNA-seq and gene ontology (GO) enrichment analysis, leading to the identification of a group of 26 transcriptome signatures, collectively referred to as the innate anti-PD-1 resistance (IPRES) signature. Patients whose tumors were IPRES enriched had a poorer survival ($p=0.04$) compared to patients with non-IPRES enriched tumors. Similar transcriptomic subsets were also found in other tumor types upon analysis of

RNA-seq datasets found in TCGA, suggesting that the IPRES signature may also potentially be applicable to other tumor histologies, although this will require confirmation from further studies.

In patients with melanoma treated with anti-CTLA-4 and anti-PD-1 inhibitors, immune signatures of tumor specimens assessed at multiple timepoints during treatment were shown to be predictive of response to such therapies [47]. Patients in this study underwent serial tumor biopsies at multiple timepoints, including sampling pre-treatment, early and late on-treatment, as well as at disease progression. Targeted gene expression profiling using NanoString panels composed of immune-related genes and genes involved in common cancer signaling pathways showed distinct immune signatures of 411 differentially expressed genes (FDR-adjusted $p<0.05$) between responders and non-responders in early on-treatment biopsies. The investigators suggest that early on-treatment biopsies may be more predictive of response than pre-treatment biopsies, although this could be confounded by the fact that such signatures were a result of the treatment itself rather than a *bona fide* predictive signature to response. While further confirmatory studies are required to draw more definitive conclusions, such modern strategies have the potential to identify putative predictive biomarkers of response to aid in patient selection with such immunotherapies.

Tumor infiltrating immune cells and the tumor microenvironment

It is clear that PD-L1 expression is not the sole factor responsible for the prediction of response to PD-1 and PD-L1 therapies. There are instead a complex host of factors in the tumor microenvironment, which collectively influence

immunomodulatory effects to either promote or combat tumor growth. In a study characterizing tumor infiltrating immune cells by gene expression profiling and IHC of colorectal cancer samples, high densities of $CD3^+$ T cells, $CD8^+$ cytotoxic T cells and $CD45RO^+$ memory T cells were associated with decreased tumor recurrence and improved survival [48]. Similarly, strong lymphocytic infiltration has been shown to be associated with improved patient outcomes in other tumor types, including melanoma, lung and bladder cancers [49-51]. While the prognostic value of tumor infiltrating immune cells has shown consistent results in various tumor types, its predictive role in tumor response is still under intense investigation. A study of pretreatment tumor specimens of patients treated with nivolumab showed that tumor PD-L1 expression correlated with the density of infiltrating immune cells including histiocytes and lymphocytes. PD-L1 expression of immune cells also correlated with clinical benefit, but not objective responses [8]. Further studies with various PD-1 and PD-L1 inhibitors will help to verify the predictive role of tumor infiltrating immune cells.

A better understanding of the tumor microenvironment and the interplay between various inflammatory cofactors will also provide further clues as to how we may better predict antitumor responses to immunotherapies. It may be that due to the complexity and adaptive nature of the immune system, a combination of biomarkers will ultimately be required to help guide treatment choice and identify early biomarkers of acquired drug resistance. The challenge now lies in harnessing the application of this myriad of biomarkers and developing optimal strategies to integrate them in a biologically sound and statistically meaningful paradigm to guide the use of immunotherapy **(Figure 1)**.

Response biomarkers for immunotherapy

While antitumor response rates have traditionally been an important and well-established efficacy endpoint in non-immunotherapy clinical trials, its ability to differentiate patients who may derive long term clinical benefit has been put into question in many immunotherapy trials. With immunotherapeutics, patients may appear to have radiological progressive disease using conventional imaging modalities due to pseudoprogression, while others may show delayed responses. Survival curves from immunotherapy trials have consistently shown a persistent tail at the end of the curve, indicating that there is a group of patients who derive durable clinical benefit. As such, there is an urgent need to identify better response biomarkers that more accurately reflect drug effects on tumor **(Table 1)**.

Evolution of imaging criteria

The Response Evaluation Criteria in Solid Tumors (RECIST) criteria has been the most common modality used to define antitumor response and disease control rates in oncology clinical trials. With the advent of immunotherapy, new parameters such as immune-related progression-free survival (irPFS) now need to be addressed. The irPFS accounts for an initial tumor flare due to peritumoral lymphocyte infiltration, which is observed at varying frequencies in different cancers. This causes an apparent increase in the size of tumors on standard contrast CT scans, before eventually leading to a delayed tumor response on subsequent CT scans.

This discrepancy in the types of assessments used to assess immunotherapies and chemotherapies is real, as several large randomized phase III clinical trials

comparing immune checkpoint inhibitors and chemotherapy regimens have demonstrated a significant OS benefit, but minimal or no PFS benefit for immune checkpoint inhibitors [20, 21]. This has led to a separate set of guidelines for the assessment of patients receiving immunotherapy to account for potential early pseudoprogression [52]. As part of the KEYNOTE-001 phase Ib trial, patients with melanoma who were treated with pembrolizumab incorporated tumor assessments using both conventional RECIST v1.1 and immune-related response criteria (irRC) [53]. In this study, of 592 patient who survived 3 or more months, 14% of patients experienced disease progression by conventional RECIST criteria but non-progressive disease by irRC, thus potentially underestimating the benefit with pembrolizumab in these patients. While the irRC system will need further validation in larger patient populations across various tumor types, there is a recognized need for more effective means of response assessment, and the irRC may be a potential option in this area **(Table 2)**.

Molecular imaging as response biomarkers

The current issues with conventional imaging modalities to assess antitumor responses has also led to studies involving radiolabeled antibodies to detect and monitor the immune activity of treatment within tumors [54]. Initial probes involved radionuclides bound to $CD8^+$ or $CD4^+$ T-cells that may be detected by imaging techniques such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT). These novel imaging strategies have however had limited success due to the high non-specific uptake in other organs such as the liver and thymus [55, 56].

More recently, specific radionuclides tagged to anti-CTLA-4 or anti-PD-L1 antibodies have also been investigated. A radiolabeled anti-PD-L1 antibody labeled with ^{111}I demonstrated high sensitivity to anti-PD-L1, with *in vivo* tumor uptake commencing from 24 hours post injection and SPECT imaging showing good contrast between tumor and normal tissue [57]. Similarly, a study involving PET scanning involving a ^{64}Cu-anti-CTLA-4 radionuclide injected into mice showed increased uptake within tumor tissue, indicating that the radionuclide could potentially be used to monitor response during therapy [58]. Nonetheless, such imaging techniques are currently still being analytically validated in early preclinical development, and will require further studies in human subjects before they can be clinically qualified as treatment response biomarkers for immune checkpoint inhibitors.

Immune surveillance as a response biomarker

Regardless of the immunotherapy selected, the centerpiece of immuno-oncology still lies in the host T-cell modulation. The T-cell response is dynamic and dependent on changes in the tumor and its microenvironment, often involving an interplay between positive and negative costimulatory factors, including CTLA-4 and PD-1 [59]. Studies have shown that the monitoring of T-cell receptor (TCR) repertoires both in tumor tissue and peripheral blood samples may yield important insights into both patient response and toxicity.

In clinical trials involving patients with melanoma treated with pembrolizumab, TCR sequencing and clonal quantification was undertaken on tumor samples from responders and progressors to treatment. The assessment of clonality of T-cell repertoire and T-cell infiltration showed distinct clustering between responders

compared to progressors. Patients who progressed on treatment were found to have lower levels of T-cell infiltration and clonality, indicating a more diverse T-cell repertoire, whereas responders tended to have higher median clonality. Analyses of serial tumor biopsies also demonstrated a higher number of significantly expanded T-cell clones in responders versus progressors [25].

In another study involving patients treated with ipilimumab, the serial monitoring of peripheral blood mononuclear cells (PBMCs) was performed and assessed for the frequency of circulating immune cell populations. Increases in $CD4^+$, $CD8^+$ and absolute lymphocyte counts were associated with improved response rates and survival outcomes in patients, suggesting that circulating immune markers could potentially be used as surrogates for tumor response and patient outcomes. While this was an exploratory analysis of patient specimens done retrospectively, these encouraging results warrant further validation in future patient cohorts to explore the role of immune surveillance with blood-based samples as a response biomarker to immunotherapy [60].

The search for robust response biomarkers in immune-oncology will undoubtedly continue to advance, especially as our knowledge expands on the critical interactions between immune checkpoint inhibitors and the tumor microenvironment. While the applicability of these biomarkers may not be as obvious as companion diagnostics to aid patient selection, its utility in accurately identifying responders and progressors will have an important impact in clinical practice to aid treatment decision-making in the field of immuno-oncology.

Future Perspectives

It is conceivable that, in the coming years, a patient with advanced cancer will not only have their tumor interrogated for genetic aberrations, but also have an assessment of their immune response to the cancer, utilising multiple validated biomarker assays and technologies in 'real time' **(Figure 2)**. Patients with tumors that are likely to respond to immunotherapy based on PD-1 and PD-L1 blockade will be treated with immune checkpoint inhibitors, while other patients with tumors that demonstrate little or no immune infiltrate ('cold tumors') will require novel combinatorial immuno-oncology strategies to activate the immune system, for example by stimulating neo-antigen expression, trafficking immune cells into tumors, eliminating immune-suppressive cells, and manipulating the host-tumor microenvironment [61]. These strategies are all being explored pre-clinically, and future work will need to elucidate biomarkers to stratify patients who will most benefit from each strategy. While on therapy, patients will be monitored with improved intermediate endpoint biomarkers, utilising both immunological techniques that monitor the evolution of T-cell repertoires, the dampening of immune-suppression, and the development of immunological memory; and novel functional imaging technologies to gauge their response to treatment. Ongoing prospective longitudinal monitoring utilising liquid biopsies (both for the genomic analysis of circulating tumor DNA and for immune cell profiling) will enable the early identification of biomarkers of resistance, such as signatures of T cell exhaustion, and may enable alternative strategies to be adopted. At time of progression, patients can then be re-evaluated to identify resistance mechanisms to guide further treatment.

Conclusions

The era of truly personalized immunotherapy is well within reach - heralding further acceleration and improvements in patient care. Building on the significant benefits that have already been demonstrated in multiple tumor types in early to late phase clinical studies, the incorporation of state-of-the-art high-throughput predictive or response biomarkers will improve our ability to select patients for stratified immunotherapy, identifying those who are most likely to respond to treatment while minimizing the risks of immune-related toxicities.

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Executive summary

Predictive biomarkers of response for immunotherapy

- PD-L1 expression has been the most widely studied biomarker for treatment response to anti-PD-1/PD-L1 agents. While high expression of PD-L1 has been correlated to improve patient outcomes, there is currently no consensus for the threshold cut-off to define positivity of PD-L1 expression. Moreover, other tumor and technical factors, including intertumor heterogeneity of PD-L1 expression and the lack of unification in testing, pose further challenges in its role as a predictive biomarker.
- Alternate strategies are currently being explored, including the role of mutational load, neoantigens, non-genomic signatures and tumor microenvironment to further guide treatment choice and identify early biomarkers of acquired drug resistance.
- The complexity and adaptive nature of the immune system may imply that a combination of biomarkers may ultimately be required.

Response biomarkers for immunotherapy

- Conventional tumor assessment by RECIST may be suboptimal due to pseudoprogression and delayed responses to immunotherapy. New criteria such as the irRECIST may help to address these issues.
- Molecular imaging with radionuclides tagged to antibodies like anti-CTLA-4 or anti-PD-L1 may improve monitoring of immune activity within tumors and aid response assessment.
- Immune surveillance with monitoring of the TCR repertoire in both tumor tissue and peripheral blood samples has shown considerable promise and warrants further validation.

Tables and Figures

Supplementary Table 1

Summary of PD-L1 expression and correlation with outcomes in trials involving anti PD-1 and anti PD-L1 antibodies. NA: results not reported or not available in publication.

Trial name/Trial ID	Phase	Trial regimen	Tumor type	PD-L1 assay	PD-L1 cutoff	ORR in PD-L1 negative cohort	ORR in PD-L1 positive cohort	Main Toxicities	Median follow up	PFS	OS	
Nivolumab												
NCT00441337	1	Nivolumab, dose escalation	anoma (26%), prostate (21%), RCC (3%), NSCLC (15%), CRC (3	Not specified	gative, intracytoplasmic, membranc	0/5	3/4 (75%) (membranous)	phopenia (26%), Fatigue (15%), musculoskeletal (1	NA	NA	NA	
NCT00730639	1	Nivolumab, various doses (1,3,10 mg/kg q2w)	anoma (35%), prostate (6%), RCC (11%), NSCLC (41%), CRC (H	1 monoclonal antibod	5%	0/17	9/25 (36%)	Rash (12%), Diarrhea (11%), Pruritus (9%)	NA	NA	NA	
NCT01176461	1	Nivolumab, various doses (1,3,10 mg/kg q2w)	Melanoma	Not specified	5%	6/32 (19%)	8/12 (67%)	Fatigue (23%), Pruritus (13%), Rash (17%)	20 m	NA	NA	
CheckMate 037	3	Nivolumab 3mg/kg q2w vs chemotherapy	Melanoma	Dako	5%	20.3% (95% CI: 11.3 to 32.2%)	43.6% (95% CI: 30.3 to 57.7)	Fatigue (25%), Pruritus (16%), Diarrhea (12%)	8.4 m	4.7 m	6m 48%	
CheckMate 066	3	Nivolumab 3mg/kg q2w vs chemotherapy	Melanoma	Dako	5%	33.1% (95% CI: 25.2 to 41.7)	52.7% (95% CI: 40.8 to 64.3)	Fatigue (20%), Pruritus (17%), nausea (17%)	8.9m	5.1m	10.8m	
CheckMate 063	2, single arm	Nivolumab 3mg/kg q2w	Squamous NSCLC	Dako	5%	7/51 (14%)	6/25 (24%)	tigue (33%), Nausea (15%), Decreased appetite (19	8.0m	1.9m	8.2m	
CheckMate 017	3	Nivolumab 3mg/kg q2w vs Docetaxel	Squamous NSCLC	Dako	1%	17% (95% CI: 8 to 29)	17% (95% CI: 9 to 29)					
					5%	15%(95% CI: 8 to 25)	21% (95% CI: 10 to 37)	igue (16%), decreased appetite (11%), asthenia (10	NA	3.5m	9.2m	
					10%	16% (95% CI: 9 to 26)	19% (95% CI: 8 to 26)					
CheckMate 057	3	Nivolumab 3mg/kg q2w vs Docetaxel	Non-Squamous NSCLC	Dako	1%	9% (95% CI: 5 to 16)	31% (95% CI: 23 to 40)					
					5%	10% (95% CI: 6 to 17)	36% (95% CI: 26 to 46)	tigue (16%), nausea (12%), decreased appetite (10	NA	2.3m	12.2m	
					10%	11% (95% CI: 6 to 17)	37% (95% CI: 27 to 48)					
CheckMate 025	3	Nivolumab 3mg/kg q2w vs Everolimus	RCC	Dako	1%	27.4 months (95% CI: 21.4 to not estimable	OS 21.8 months (95% CI: 16.5 to 28.1)*	Fatigue (33%), nausea (14%), pruritus (14%)	NA	4.6m	25m	ORR not g
					5%	24.6 months (95% CI: 21.4 to not estimable	21.9 months (95% CI: 14.0 to not estimable					
Checkmate 026	3	volumab 3mg/kg q2w vs platinum-doublet chemothera	NSCLC	Dako	1%	1/6 (14%)	9/32 (28%)					
					5%	3/20 (15%)	8/26 (31%)	Fatigue (29%), rash (19%), nausea (14%)	5.1	3.6m	19.4m	
					10%	3/26 (12%)	8/20 (40%)					
Checkmate 141	3	Nivolumab 3mg/kg q2w vs chemotherapy	HNSCC	Dako	1%	9/73 (12.3%)	15/88 (17%)					
					5%	12/107 (11.2%)	12/54 (22.2%)	Fatigue (14%), nausea (8.5%), rash (7.6%)	5.1	2.0m	7.5m	
					10%	12/118 (10.2%)	12/43 (27.9%)					
Pembrolizumab												
KEYNOTE 001	1	brolizumab, various doses (2,10 mg/kg q3w, 10mg/kg q	NSCLC cohort	22C3 (Merck)	50%	16.5%(95% CI: 10 to 25)	45.2%(95% CI: 33 to 57)	igue (19%), Pruritus (11%), Decreased appetite (11	10.9m	3.7m	12m	
KEYNOTE 024	3	Pembrolizumab 200mg q3w vs chemotherapy	NSCLC	22C3 (Merck)	50%	NA	44.8% (95% CI: 36.8 to 53.0)	Diarrhoea (14.3%), fatigue (10.4%), pyrexia (10.4%)	11.2m	10.3m	NR	
Atezolizumab												
NCT01375842	1	Atezolizumab, various doses	Advanced solid tumors	Not specified	5% (in TIL)	1/28 (4%)	4/28(14%)	tigue (24%), Decreased appetite (12%), Nausea (12	NA	18w	NA	
NCT02108652	2	Atezolizumab 1200 mg q21d	Urothelial carcinoma	Not specified	5% (in TIL)	8% (95% CI: 3 to 15)	27% (95% CI: 19 to 37)	tigue (30%), Nausea (14%), Decreased appetite (12	11.7m	2.1m	11.4m	
Durvalumab												
NCT01693562	1/2	Durvalumab 10mg/kg q2w	Urothelial carcinoma	Ventana	25% (in either TIL or tumor)	0/14 (95% CI: 0 to 23%)	46% (95% CI: 27 to 66%)	tigue (13%), Diarrhea (10%), Decreased appetite (8	4.3m	NA	NA	

Table 1

Summary of predictive and response biomarkers in treatment with immune checkpoint inhibitors

Predictive biomarker	Clinical relevance	Surrogate response biomarker	Clinical relevance
PDL-1 Expression	Multiple studies have shown impact of PD-L1 expression, at different cutoff, on ORR and OS (Please refer to table 1)	Absolute eosinophils and lymphocytes counts	Increase in ALC in ipilimumab-treated patient is associated with improved OS
Mutational Load	Potential improvement in OS, duration of response and response rate	Circulating $CD4^+$ and $CD8^+$ T Cells	Increase in $CD4^+$ and $CD8^+$ is associated with improved OS
Mismatch repair (MMR) defects	Improved ORR and DCR in colorectal cancer	T cell repertoire	Diversity of T cell has been associated with immune checkpoint blockade clinical response
Neoantigens formation	Associated with higher ORR and survival in ipilimumab-treated melanoma patient	Radiolabeled $CD4^+$ and $CD8^+$ detected by PET or SPECT	High rate of non-specific uptake in non-tumor tissues in preclinical models. No clinical data yet.
Tumor transcriptome analysis	So-called "Innate anti-PD-1 resistance signature" (IPRES), analysed pre-treatment, is associated with upfront treatment resistance	Radiolabeled anti-CTLA-4 antibody detected by PET	Good differential uptake between tumor and non-tumor tissues in animal models. No clinical data yet.
Tumor-infiltrating lymphocytes (TILs)	$CD4^+ICOS^{high}$ and $CD8^+$ T cells predict response to ipilimumab and PD-1 blockade	Radiolabeled anti-PD-L1 antibody detected by SPECT	Significant tumor uptake with good tumor-to-normal tissue ratio in animal model. No clinical data yet.

Table 2
Differences between RECIST and irRECIST

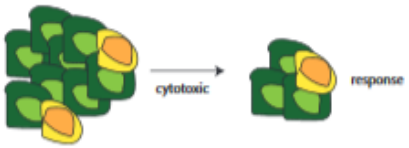
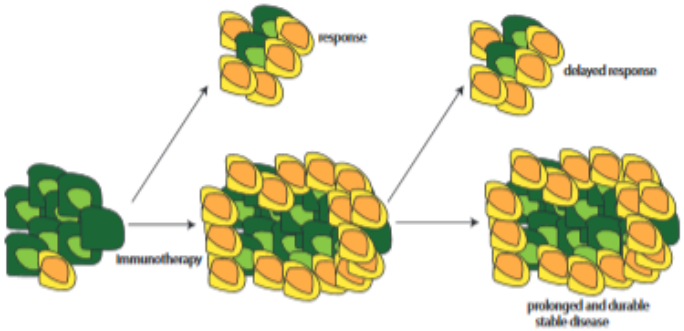
		RECIST criteria (Eisenhauer 2009)		Immune-related RECIST crieteria (Wolchok 2009)	
Background		Ideal for cytotoxics where tumour shrinkage is a measure of activity 		Ideal for the dynamics of the immune-system-tumour interaction - capturing additional response patterns including response after initial increase in tumour burden, and response in the presence of new lesions 	
Principles	Measurement	Unidimensional (longest diameter, LD)		Bidimensional (sum of longest diameter, LD x perpendicular diameter, PD)	
	Measurable lesions	>10mm in LD		>5 x 5 mm (LD x PD)	
	Sum of the measurements	Sum of unilateral measurements of all target lesions		Sum of bidirectional measurements of all targets lesions and all new lesions	
Response Assessment		**CR**	disappearance of all target lesions	**irCR**	disappearance of all lesions
		PR	at least a 30% decrease in the sum of the LD of target lesions from baseline	**irPR**	at least a 50% decrease in tumour burden from baseline
		SD	change in sum of the LD of taget lesions insufficient to be either PR or PD	**irSD**	change in tumour burden insufficient to be either irPR or irPD
		PD	at least a 20% increase in the sum of the LD of target lesions, OR new lesions	**irPD**	at least an increase in tumour burden of 25%
		New lesions	always PD	**New lesions**	incorporated into tumour burden
		Confirmation	2 consecutive time points at least 4 weeks apart	**Confirmation**	2 consecutive time points at least 4 weeks apart in the absence of rapid clinical deterioration

Figure 1

A micro to macro perspective of predictive and response biomarkers for PD-1 and PD-L1 inhibitors. At a cellular level (left panel), specific neoantigens are presented to T-cells that increases immunogenicity of tumors. Surface PD-L1 expression correlates with response to immune checkpoint inhibitors. In the tumor microenvironment (center panel), interactions between tumor cells, T-cells and other immunomodulators further determine whether a patient's tumor will response. Of these, tumor infiltrating cells contributing to T-cell repertoire, and circulating immune cell populations in peripheral blood mononuclear cells may provide insights to early responders or progressors. Interrogation of the tumor on a genomic level through next generation sequencing panels or whole exome/genome sequencing have revealed several signatures that may help identify patients who are more likely to respond to treatment.

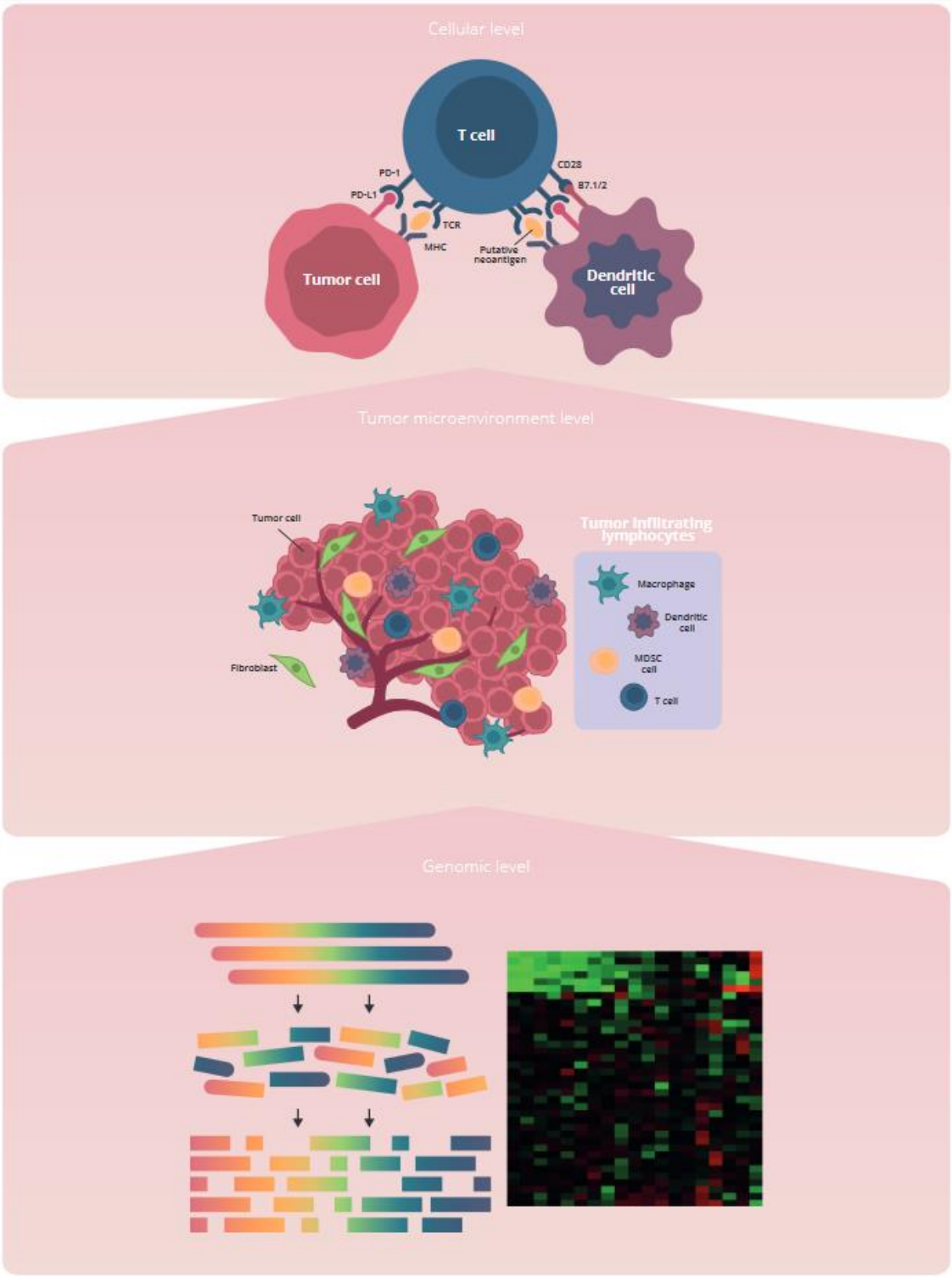
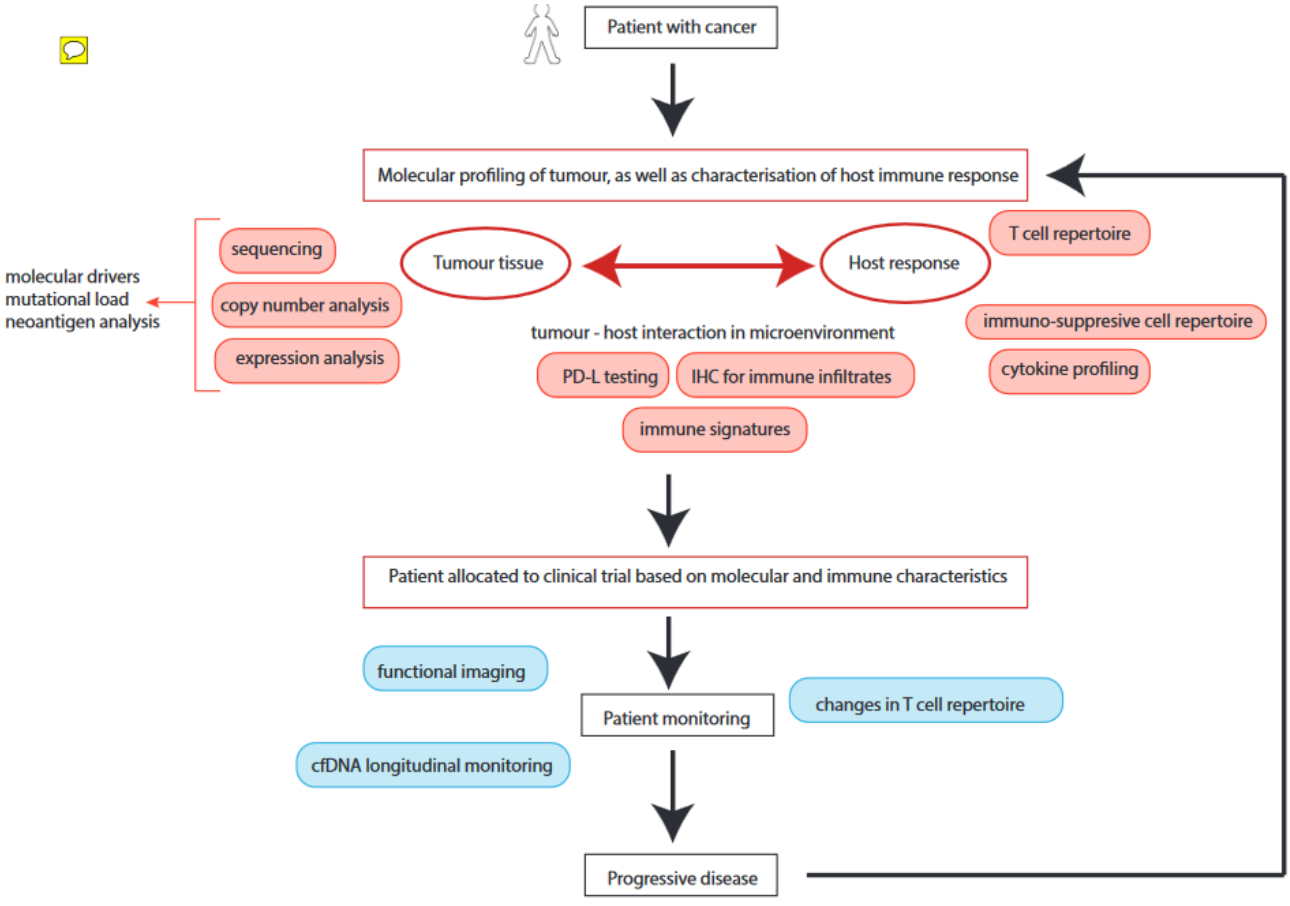

Cellular level
T cell
PD-1
PD-L1
TCR
MHC
CD28
B7.1/2
Putative neoantigen
Tumor cell
Dendritic cell
Tumor microenvironment level
Tumor cell
Fibroblast
Tumor infiltrating lymphocytes
Macrophage
Dendritic cell
MDSC cell
T cell
Genomic level

Figure 2

Future perspective of clinical pathway of patient for PD-1/PD-L1 therapy. Currently, patient selection for PD-1/PD-L1 therapy may or may not be guided by PD-L1 expression levels. We envision that with further understanding of both predictive and response biomarkers to PD-1/PD-L1 therapies, such biomarkers will play a key role in guiding individualization of treatment for patients. Patients who are considered for PD-1/PD-L1 therapy with undergo tumor profiling and also characterization of host immune response. Patients will then be allocated to treatment based on molecular and immune characteristics, with appropriate and timely monitoring of response through functional imaging and immune surveillance. At disease progression, patients could be then re-evaluated to identify resistance mechanisms and consider alternative therapeutic agents.



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**** This paper describes the concept of mutational load and its correlation to PD-1 blockade**

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