European Journal of Cancer 173 (2022) 167–177

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Review

Impact of pharmacodynamic biomarkers in immuno-oncology phase 1 clinical trials

Abdulazeez Salawu [a,1], Alberto Hernando-Calvo [a,1], Rachel Y. Chen [b], Daniel V. Araujo [c], Marc Oliva [d], Zhihui A. Liu [a], Lillian L. Siu [a,*]

[a] *Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, Canada*
[b] *Princeton University, New Jersey, USA*
[c] *Department of Medical Oncology, Hospital de Base, Sao Jose do Rio Preto, SP, Brazil*
[d] *Department of Medical Oncology, Institut Català D'Oncologia (ICO) L'Hospitalet, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, Spain*

Received 30 April 2022; received in revised form 20 June 2022; accepted 22 June 2022
Available online 21 July 2022

KEYWORDS
Phase 1 clinical trial;
Pharmacodynamics;
Drug response biomarkers;
Drug development;
Immune system;
Cancer

Abstract ***Background:*** Phase 1 immuno-oncology (IO) trials frequently involve pharmacodynamic (PD) biomarker assessments involving tumour biopsies and/or blood collection, with increasing use of molecular imaging. PD biomarkers are set to play a fundamental role in early drug development of immuno-oncology (IO) agents. In the IO era, the impact of PD biomarkers for confirmation of biologic activity and their role in subsequent drug development have not been investigated.

Methods: Phase 1 studies published between January 2014 and December 2020 were reviewed. Studies that reported on-treatment PD biomarkers [tissue-derived (tissue-PD), blood-based (blood-PD) and imaging-based (imaging-PD)] were analysed. PD biomarker results and their correlation with clinical activity endpoints were evaluated. Authors' statements on the influence of PD biomarkers on further drug development decisions, and subsequent citations of PD biomarker study results were recorded.

Results: Among 386 trials, the most frequent IO agent classes evaluated were vaccines (32%) and PD-(L)1 inhibitors (25%). No PD biomarker assessments were reported in 100 trials (26%). Of the remaining 286, blood-PD, tissue-PD, and imaging-PD data were reported in 270 (94%), 94 (33%), and 12 (4%) trials, respectively. Assessments of more than one PD biomarker type were reported in 82 studies (29%). Similar proportions of blood-PD (9%), tissue-PD (7%), and imaging-PD studies (8%) had positive results that correlated with clinical activity. Results of 22 PD biomarker studies (8%) were referenced in subsequent clinical trials.

Conclusions: Most phase 1 IO studies performed PD biomarker assessments. Overall, positive PD biomarker results were infrequently correlated with clinical activity or cited in subsequent

* *Corresponding author.*
E-mail address: Lillian.siu@uhn.ca (L.L. Siu).
[1] Abdulazeez Salawu and Alberto Hernando Calvo should be considered joint first author.

https://doi.org/10.1016/j.ejca.2022.06.045

trials, suggesting a limited impact on subsequent drug development. With emerging health regulatory emphasis on optimal dose selection based on PD activity, more informative and integrative multiplexed assays that capture the complexity of tumour-host immunity interactions are warranted to improve phase 1 IO trial methodology.

1. Introduction

Increased understanding of the cancer immunity cycle and the success of immune checkpoint inhibitors (ICI) have led to a sustained increase in the clinical development of novel anticancer therapies targeting immune-related pathways (IO agents) [1,2], ushering in an immuno-oncology (IO) era of anticancer drug development [3]. Pharmacodynamic (PD) biomarkers aim to provide early information about proof-of-targeting and downstream effector activity of agents in clinical development [4,5]. They may also identify mechanisms of treatment susceptibility or resistance, provide proof-of-concept for novel combinations, or strategies to overcome resistance [6]. The expectation that PD biomarker utility would make the development of molecular targeted agents (MTA) more efficient led to an increasing number of phase 1 trials in the 1990s and 2000s that performed on-treatment tumour biopsies. However, these biopsies' actual impact on dose selection for subsequent drug development remains uncertain [4,7].

Early phase IO trials may incorporate mandatory PD biomarker sampling in the form of tumour biopsies and/or blood collection. While tumour biopsies provide information on tumour cells and the immune microenvironment, they typically represent only a part of a heterogeneous, multifocal tumour at a particular timepoint. The risk of complications to trial participants and associated financial and logistical burdens limit repeated sampling. Blood sampling, on the other hand, permits tracking PD effects across different timepoints without the risks and logistical constraints associated with biopsies. However, they may not be representative of tumour cell-host immunity interactions, and their interpretation can be confounded by other stimuli such as infection [8,9].

With technological advances, imaging modalities using appropriate radiotracers can provide molecular images based on tumour or immune cell surface receptor expression. Functional magnetic resonance imaging and dynamic contrast-enhanced ultrasound are also able to provide dynamic images of biological processes e.g. blood flow and metabolism that can change in response to treatment [10]. The use of non-invasive blood- and imaging-based biomarkers continues to expand and is expected to augment tissue-based PD assessments [6].

In the IO era, the impact of PD biomarkers on the selection of recommended phase 2 dose (RP2D), or subsequent drug development has not been investigated. This analysis describes the utility of PD biomarkers in phase 1 IO clinical trials and evaluates their impact.

2. Methods

2.1. Data acquisition

Utilising the methodology of our previous review of dose escalation methods in phase 1 trials [11], an updated systematic search of Medline, Embase, Cochrane, and Web of Science databases for all phase 1 trials in human subjects published in the English language between 1st January 2014 and 31st December 2020 was performed (summarised in Supplemental Data Table S1). After the removal of duplicate records, conference abstracts and reviews, all the remaining abstracts were screened by two co-authors (AS and AH).

Immuno-oncology (IO) agents were defined as anticancer agents that target immune-related pathways and organised by their mechanisms of action into ten classes: PD1/PDL1 inhibitors; CTLA4 inhibitors; other ICI (e.g. LAG-3 inhibitors); co-stimulatory monoclonal antibodies (e.g. OX40 agonists); other immunomodulators (e.g. indoleamine 2,3-Dioxygenase 1 (IDO1) inhibitors); anticancer vaccines (including RNA-, DNA-, peptide- and dendritic cell-based agents); oncolytic viruses (e.g. Talimogene Laherparepvec (T-vec)); cytokines (e.g. interleukin-2); bispecific T-cell engagers (BiTEs), and cell therapies (e.g. chimeric antigen receptor T-cell (CAR T-cell) therapies).

Only phase 1 trials involving IO agents (as monotherapy or combination with other IO or MTA) were included. Studies involving any cytotoxic agents or radiotherapy were excluded, as were studies conducted in animals, children, or hematologic malignancies. Full-text manuscripts of all studies that met the eligibility criteria were reviewed to identify all those that performed PD biomarker assessments, as defined by the National Institutes of Health BEST (Biomarkers, EndpointS and other TOOLS) resource [12]. PD biomarker assessments were only evaluated if sampling/assessment was conducted before and after treatment initiation. Samples obtained only at baseline without longitudinal

assessment after treatment initiation were considered predictive biomarkers and excluded.

2.2. Data abstraction and categorisation

Data abstraction and categorisation for each study were performed by at least two of three co-authors (AS, AH, and RC). For studies with discordance in the data abstracted by the initial reviewers, an alternate co-author adjudicated. The data abstraction consisted of three modules. The first module comprised clinical trial characteristics such as the number and class of IO agent(s) evaluated and sample size. The second module comprised the type of PD biomarker assessed and their results. PD biomarkers were categorized into tissue-based PD biomarkers (tissue-PD), blood-based PD biomarkers (blood-PD), and imaging-based PD biomarkers (imaging-PD). The number of patients upon whom PD biomarker assessments were performed and whether these were mandatory were recorded. Data were also collected on the types of tissue- and blood-PD assays performed and classified into genomic, transcriptomic, or proteomic assays. Proteomic assays of immune cells in the tumour microenvironment (tissue immunophenotyping) or circulation (blood immunophenotyping or peripheral blood mononuclear cell (PBMC) assays); as well as serum cytokine and antibody assays were specifically recorded. For imaging-PD assessments, the relevant imaging modality and use of radiotracers were recorded.

Only statistically significant PD assay results (as defined in the study methodology) were considered positive. Positive results were classified into two separate groups – those that demonstrated target engagement or expected biologic activity alone; and those in which positive PD activity showed a statistically significant correlation with clinical activity (based on reported efficacy measures including overall survival, progression-free survival, time-to-progression, or objective response by response evaluation criteria in solid tumours (RECIST v1.0 or 1.1)) [13,14]. For each PD biomarker type, the authors' concluding statements regarding whether the PD assessment results were considered in the RP2D, or further drug development decisions were recorded.

In the third module, all articles that cited the original (index) set of eligible phase 1 IO trials were identified using the Pubmed database. The citing article abstracts were reviewed to include only phase 1b, 2, and 3 clinical trials that evaluated the same IO agent as the index study. Full-text manuscript reviews of the included citing articles were then performed by two co-authors (AS, RC) to identify any PD biomarker references. The number of citing articles and context of the biomarker reference in terms of mechanism(s) of action, dose, and/or schedule were recorded. For agents with regulatory approval by the United States Food and Drug Administration (FDA), reference to PD biomarker in the selection of the approved dose was recorded.

2.3. Statistical methods

Descriptive statistics were used to summarise the characteristics of the phase 1 IO trials and the reported PD. The Cochran–Armitage test for proportional trends was used to evaluate the association between year of publication, number, and type of PD tests performed. The proportions of tissue-, blood- and imaging-PD studies with positive results were compared by Fisher's exact test. All analyses were performed using R software.

3. Results

3.1. Phase 1 IO trials

Database searches identified 17,260 articles including 5,207 duplicates, which were removed. Title and abstract reviews identified another 11,601 articles that did not meet the eligibility criteria. Full-text reviews of the remaining 452 manuscripts led to the exclusion of 66 articles that comprised reports of specific cohorts of phase 1b studies which were already represented in the original set of phase 1 IO trials (Supplemental Data Fig. S1).

A total of 386 phase 1 IO clinical trials (summarised in Supplemental Data Table S2) were included in this analysis. Among these, 276 (72%) evaluated IO agents as monotherapy while 110 (28%) evaluated IO-based combination therapy with other IO agents (n = 54) and non-IO MTA (n = 56). The most frequent IO agent classes evaluated were vaccines and PD-1/PD-L1 inhibitors, comprising 32% and 25%, respectively (Supplemental Data Fig. S2A). There was an increase in the number of phase 1 IO study publications over the period examined, with a notable yearly increase in the number of studies evaluating PD-1/PD-L1 inhibitors (Supplemental Data Fig. S2B).

3.2. Pharmacodynamic biomarker studies

Among 386 phase 1 IO studies, 100 did not report PD biomarker assessments. Blood-PD assessments were the most common type reported in 270 (94%) of the remaining 286 studies (summarised in Supplemental Data Table S3). Tissue-PD assessments were reported in 94 studies (33%), while 12 studies (4%) reported imaging-PD assessments, as summarised in Supplemental Data Table S4 and S5, respectively. Concurrent analysis of more than 1 PD biomarker type was reported in 82 studies (29%), including 71 (25%) where a combination of blood- and tissue-PD biomarker assessments was performed. All three biomarker types were reported in 8 studies (3%) (Fig. 1). Over 80% of PD biomarker studies reported among IO trials evaluating vaccines were blood-based and approximately 15% were tissue-based. In comparison, trials evaluating oncolytic viruses reported similar proportions of blood- and tissue-based PD biomarker assessments (Supplemental Data Fig. S3).

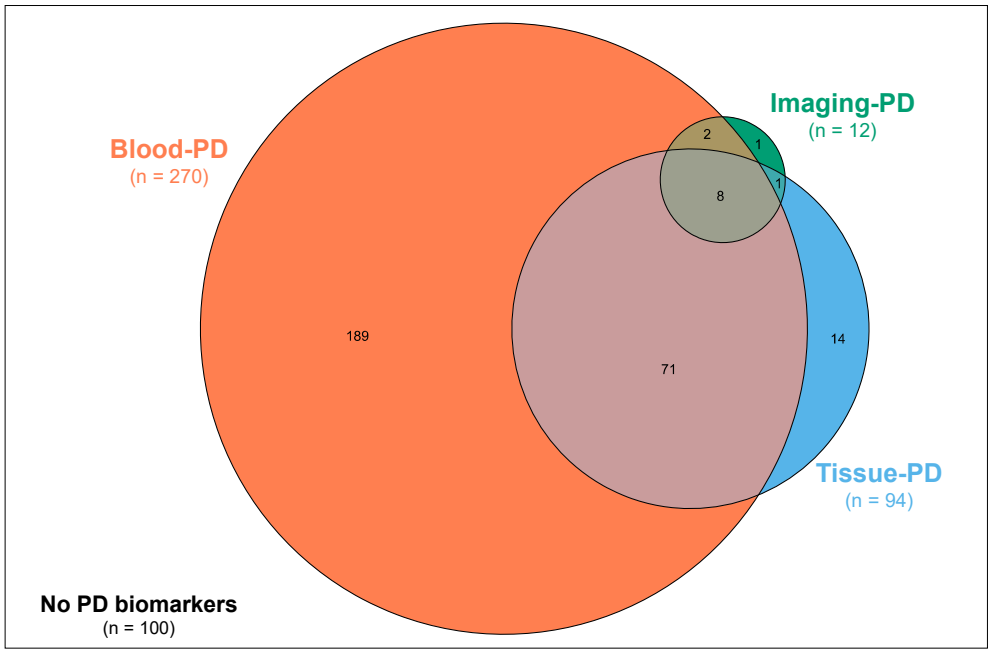

Fig. 1. Pharmacodynamic biomarker assessments by type (tissue-based, blood-based, and imaging-based) reported among phase 1 IO studies (n = 386).

The proportion of studies per year that reported any PD biomarker assessment showed a significant decrease over time ($p < 0.001$) (Fig. 2A). A similar trend was seen in the proportions of studies per year that reported blood-PD assessments ($p < 0.001$). Trend analysis of the proportions of studies that reported tissue- and imaging-PD assessments per year did not show significant change over time (Fig. 2B). These observed trends in PD biomarker types reported were maintained regardless of whether the studies were evaluating IO agents as monotherapy or in combination (Supplemental Data Fig. S4).

Biopsies were mandated for some or all participants in 29 (31%) of the 94 studies that reported tissue-PD assessments with on-treatment tissue samples obtained from 1,272 (36%) of 3,492 patients enrolled. Of 270 blood-PD biomarker studies, 241 (89%) involved mandatory blood sample collection and 85% (6,165 out of 7,227) of enrolled patients provided samples. About half (47%) of the participants in trials with imaging-PD studies underwent the relevant imaging (Table 1).

3.3. Pharmacodynamic biomarker assays

Most of the reported blood- and tissue-PD biomarker assays used proteomic approaches for the identification and quantification of tumour cells, immune cell subsets, antibodies, or cytokines. Genomic and transcriptomic assays were done in 21 (22%) and 25 (27%) tissue-PD biomarker studies, respectively while immunohistochemistry was the most common assay performed in 85 (90%) of 94 tissue-PD studies (Fig. 3). Almost three-quarters of the tissue-PD studies (67 out of 94) evaluated immune cells in the tumour microenvironment (TME) with all but one of these based on immunohistochemistry (Fig. 3).

Among the blood-PD biomarker studies, 207 (75%) evaluated immune cells in the peripheral circulation. Evaluation of PBMCs, immunophenotyping, and serum cytokines were the most frequently reported, and these PD biomarker assays were the most often combined in the same studies (Fig. 4). Of 30 genomic blood-PD assays, only 2 evaluated tumour cell-derived DNA. The remaining 28 genomic and all 17 transcriptomic blood-PD studies evaluated immune cell-derived nucleic acids (Fig. 4). Positron emission tomography was the most common modality used in 7 out of 12 imaging-PD studies (Supplemental Table S5).

3.4. Pharmacodynamic biomarker study results

Positive results with at least one biomarker type were reported in 209 (73%) of the 286 PD biomarker studies (Table 2). This comprised 192 blood-PD, 59 tissue-PD, and 9 imaging-PD biomarker studies, among which 46 reported positive results for two or more biomarker types, including 5 studies that reported positive results for all three biomarker types (Supplemental Data Fig. S5). There was no significant difference in the proportions of blood-PD, tissue-PD, and imaging-PD biomarker studies that reported positive results (Fisher's exact test $p = 0.60$). Among 61 studies with positive blood-PD results that also performed tissue-PD assays, 45 (74%) had concordant results. Conversely, there were 51 studies with positive tissue-PD results that performed concurrent blood-PD assays, among which 88% (n = 45) had concordant results.

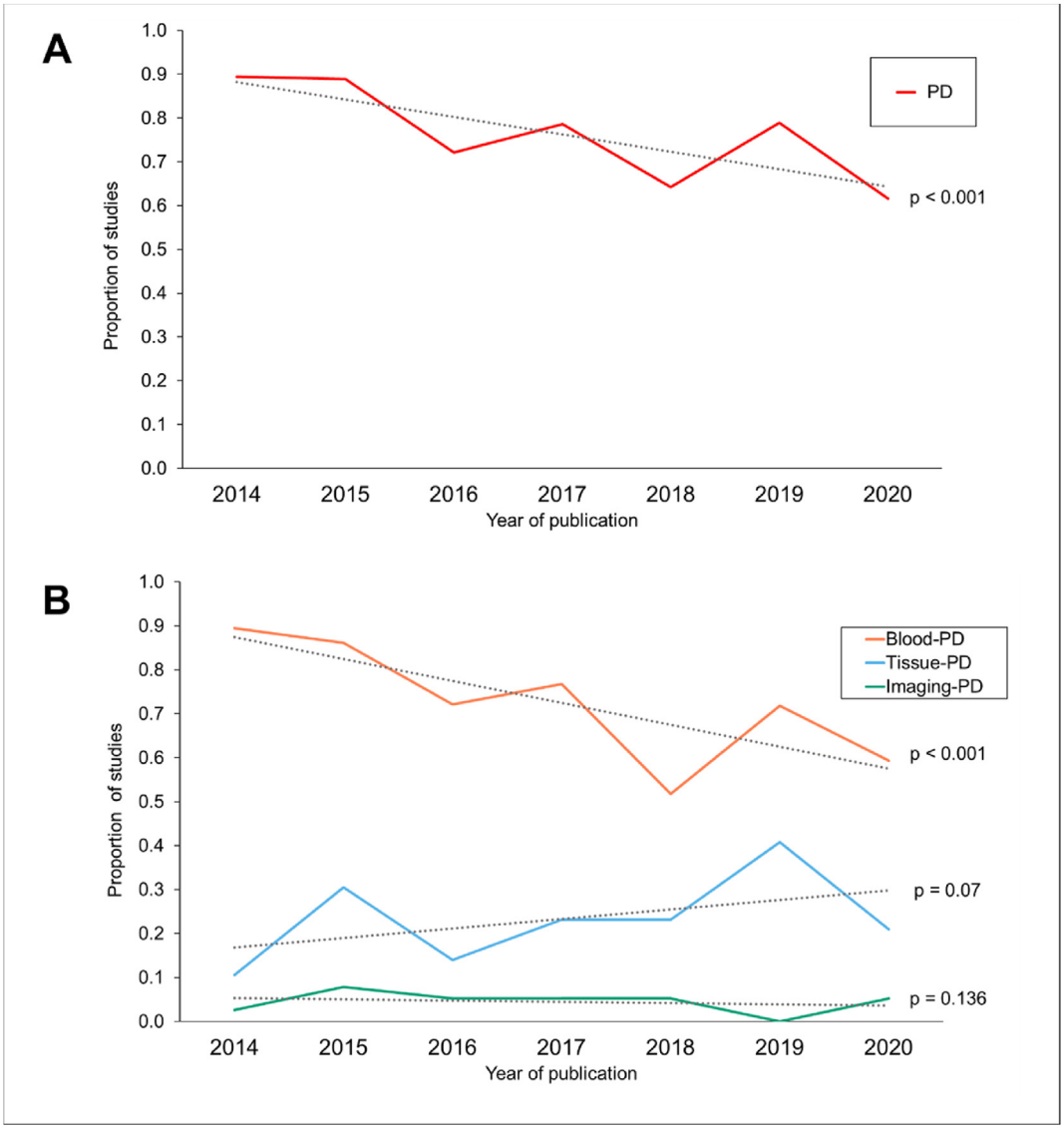

Fig. 2. Phase 1 IO studies by year of publication and (A) proportion that performed any PD biomarkers (red line); and (B) proportions that reported PD biomarkers grouped by type into blood-based (orange line), tissue-based (blue line) or imaging-based (green line). Grey dotted lines represent the trend of proportions with $p < 0.05$ denoting statistical significance by Cochran–Armitage test of proportional trends.

Twenty-eight phase 1 IO trials reported positive PD biomarker results that showed correlation with clinical activity. These comprised 23 (9%) of 270 blood-PD studies, 7 (7%) of 94 tissue-PD studies and 1 (8%) of 12 imaging-PD biomarker studies as summarised in Supplemental Data Table S6. Among these 28 trials, IO agents were evaluated in combination with molecular targeted agents in 3 (11%), or with other IO agents in 9 (32%), while the rest (47%) were monotherapy. Functional *ex vivo* assays of PBMCs for antigen-specific responses were most common among the PD biomarker assays which correlated with clinical activity, and vaccines were the most common class of IO agents evaluated in 15 of the 28 trials (Supplemental Data Table S6).

Authors' statements suggested that PD biomarker results influenced the decision for further drug development

Table 1
Phase 1 IO studies reporting pharmacodynamic biomarkers by sample type.

	Total number of Phase 1 studies	Studies with mandatory PD assessment *n (%)*	Total sample size	Patients involved in PD assessments *n (%)*
Tissue biomarkers	94	29 (31)	3,492	1,272 (36)
Blood biomarkers	270	241 (89)	7,227	6,165 (85)
Imaging biomarker	12	5 (42)	422	198 (47)

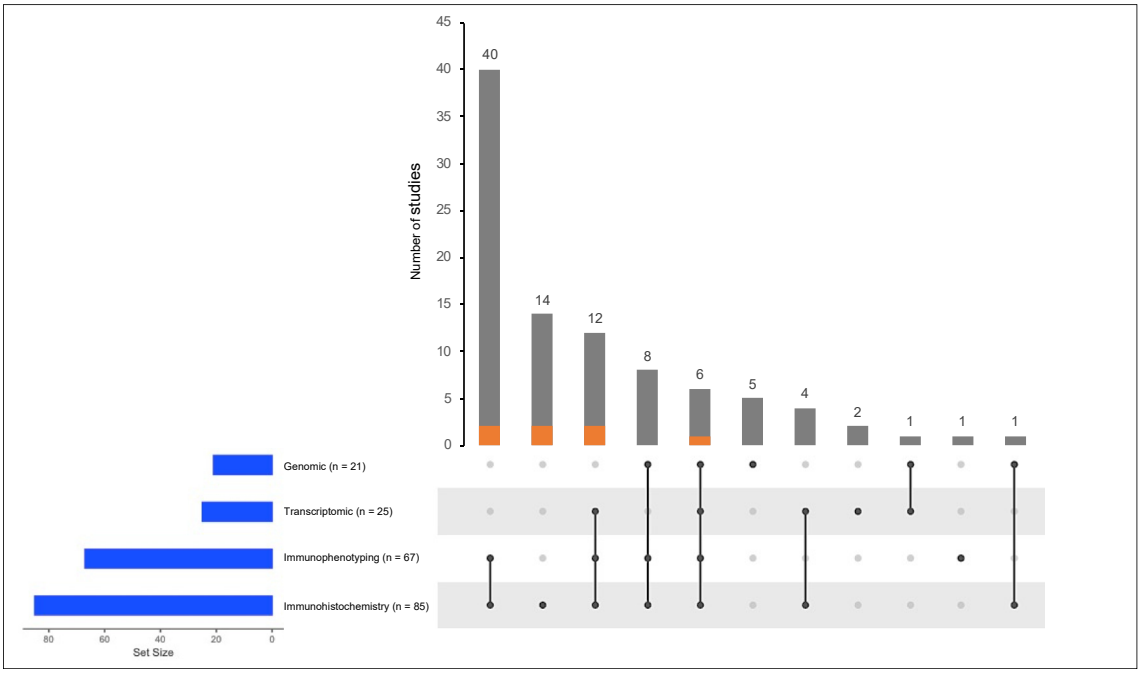

Fig. 3. Upset chart of tissue-based PD biomarker assay types reported in Phase 1 IO trials (n = 94). The blue horizontal bars show the relative number of studies that performed each assay type. Black dots and vertical joining lines represent different combinations of assay types performed concurrently. Grey vertical bars represent the frequency of each assay combination. Overlying orange vertical bars represent the number of studies in each combination group where the PD assay results showed statistically significant correlation with clinical activity.

and/or dose selection in 144 of 270 blood-PD studies (53%). A smaller proportion of tissue-PD biomarker results (38%, n = 36/94) were considered by the study authors in the decision of RP2D and/or further drug development. Five out of 12 imaging-PD studies (42%) suggested a role for their results in decisions for further drug development (Table 2).

3.5. Bibliometric analysis of pharmacodynamic biomarker study results

Of the 286 index PD biomarker studies, 22 (8%) had positive results that were cited in at least one subsequent phase 1b, 2, or 3 clinical trial report (Supplemental Data Table S7). No imaging-PD biomarker results had subsequent citations. Two index studies with positive tissue-PD but not blood-PD biomarker results were cited. The remaining 20 index studies had positive blood-PD biomarker results, including two with concurrent positive tissue-PD biomarker results. Vaccines and anti-PD-1/PD-L1 agents were the most common classes of IO agents evaluated in index studies with subsequent citations of their PD biomarker results (Supplemental Data Table S7).

Three IO agents with PD biomarker results that correlated with clinical activity endpoints also had those PD biomarker results cited in subsequent clinical trial reports (Supplemental Data Table S6). They include an autologous CD1c + myeloid dendritic cell vaccine where the presence of *ex vivo* functional tumour antigen-specific T cells in both blood and tissues was associated with longer progression-free survival [15]; spartalizumab, a PD-1 inhibitor where increased CD8 T-cell infiltration of tumour tissue correlated with objective responses [16]; and LV305, a vector-based DNA vaccine targeting the NY-ESO-1 tumour antigen where increased circulating T-cell clonality was associated with better overall survival [17].

Pharmacodynamic biomarker results that were reported in the 22 index phase 1 IO studies (which had positive PD biomarker results, as noted above) were subsequently cited in a total of 31 clinical trials. These subsequent citing trials comprised 22 phase 1b studies (evaluating the index IO agent as part of a novel regimen or combination, or in a specific cohort); seven phase 2 trials, and two phase 3 trials (Supplemental Data Table S7). PD biomarker results from five index phase 1 studies were cited in more than one subsequent clinical trial e.g., dose-dependent reduction in plasma kynurenine levels, a blood-based PD biomarker utilised in a first-in-human study evaluating the oral IDO1 inhibitor, epacadostat [18] was cited in four subsequent clinical trials, all in the context of predicted clinical activity at the doses (where maximal kynurenine reduction was demonstrated) that were chosen for further epacadostat development [19–22].

The number of subsequent citations per year showed an increase from 2014 until 2017, followed by a decline, presumably because there had been less time for

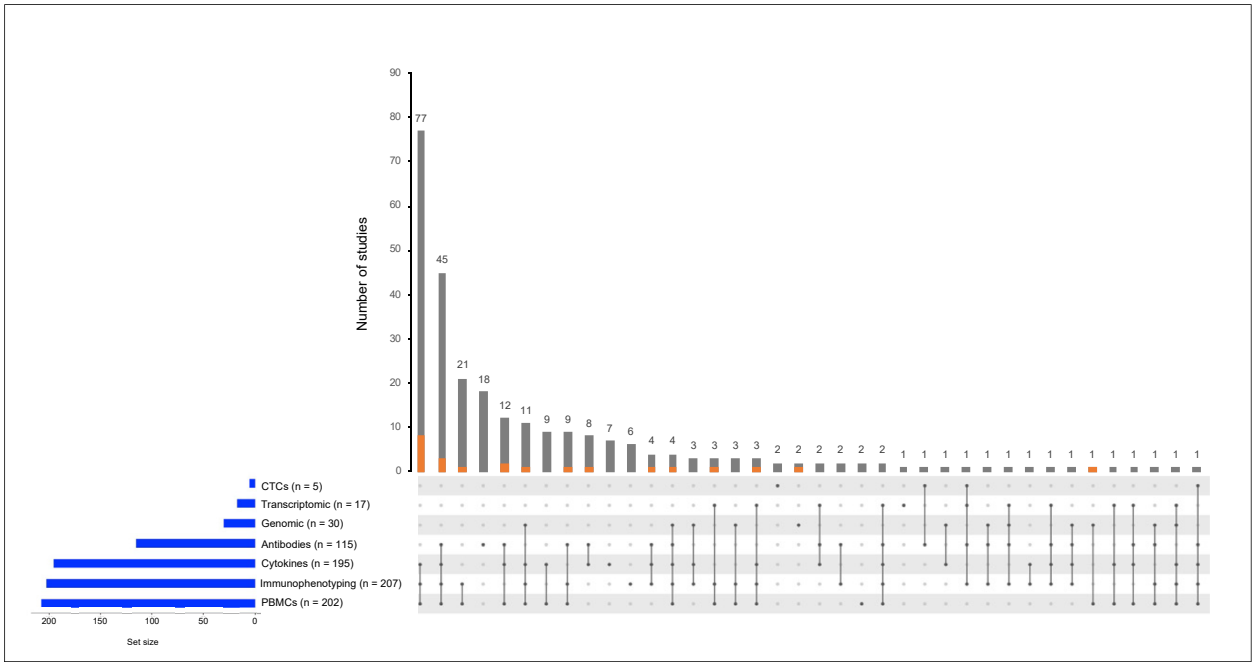

Fig. 4. Upset chart of blood-based PD biomarker assay types reported in Phase 1 IO trials (n = 270). The blue horizontal bars show the relative number of studies that performed each assay type. Black dots and vertical joining lines represent different combinations of assay types performed concurrently. Grey vertical bars represent the frequency of each assay combination. Overlying orange vertical bars represent the number of studies in each combination group where the PD assay results showed statistically significant correlation with clinical activity. *CTCs – circulating tumour cells, PBMCs – peripheral blood mononuclear cells.*

publication of subsequent studies (Supplemental Data Fig. S6). PD biomarker citations were in the context of the mechanism of action in all 31 citing studies, while 16 citations that referenced ten index phase 1 PD biomarker studies were made in the context of the dose and/or schedule of the IO agent that was used in subsequent studies. The PD-1 inhibitor, pembrolizumab, was the only FDA-approved IO agent evaluated in the index studies. Its initial approval in 2014 at a 3-weekly dose of 2 mg/kg was cited in the context of maximum serum target engagement demonstrated at doses >1 mg/kg in the index study [23] (Supplemental Data Table S7).

4. Discussion

To our knowledge, this is the first study to assess the impact of PD biomarkers in the IO era of drug development. Blood- and/or tissue-PD biomarkers were reported in about three-quarters of phase 1 IO studies over the seven-year period. Most patients on these trials (85%) had blood samples drawn for PD biomarker assessments, which is likely reflective of the ease of sample collection. Interestingly, while the proportion of studies that reported any PD biomarker assessments decreased year-on-year, the proportion that performed tissue-PD assessments was stable. The reason for this is unclear. Only 12 studies reported imaging-PD assessments, possibly because the resources required to perform these studies e.g., radiotracer manufacture and imaging facilities are often costly, and may not be widely available at participating trial sites. Furthermore, there is currently a limited number of clinically-validated radiotracers suited to PD analysis of IO agents [24]. This is however expected to change with promising first-in-

Table 2
PD biomarker results reported in Phase 1 IO clinical trials.

	Tissue biomarkers (n = 94)	Blood biomarkers (n = 270)	Imaging biomarkers (n = 12)	≥1 Biomarker type (n = 286)
Negative results	35 (38%)	78 (28%)	3 (25%)	77 (27%)
Positive results	59 (62%)	192 (72%)	9 (75%)	209 (73%)
• *Proof of mechanism/biological activity (no correlation with clinical activity)*	*52 (55%)*	*169 (63%)*	*8 (67%)*	*181 (63%)*
• *Correlation with clinical activity*	*7 (7%)*	*23 (9%)*	*1 (8%)*	*28 (10%)*
Authors stated that PD results influenced decision and dose for further clinical development	36 (38%)	144 (53%)	5 (42%)	153 (53%)

Note: Only statistically significant PD assay results (as defined in the published study methodology) were considered positive.

human study results of novel immuno-PET radiotracers which target CD8 [25] and PD-L1 [26] in the tumour immune microenvironment.

Given that PD biomarker studies of IO agents in early clinical development typically aim to demonstrate immunologic activity as proof-of-concept, it was not surprising that most PD assays involved quantification of immune cell subsets in the circulation and/or TME. Many blood-PD biomarker studies also evaluated cytokine levels in serum and/or in response to antigen-specific *ex vivo* stimulation of PBMCs. In comparison, genomic and transcriptomic assays of immune cells e.g., circulating T-cell repertoire or expression profiling of PBMCs to detect activated immune cell sub-populations were under-represented. This may be related to the cost and required expertise for such assays. Additionally, only two studies evaluated circulating tumour-derived DNA (ctDNA) as a PD biomarker. Within the period covered in our study, there was likely a dearth of reliable ctDNA quantification methods with sufficient sensitivity and specificity to be applied to the histologically-diverse patient populations that are typical of phase 1 studies. A recent study using a personalised, histology-agnostic ctDNA detection assay has however demonstrated that early ctDNA kinetics correlate with the likelihood of clinical benefit from pembrolizumab in various cancers [27].

Over half of PD biomarker studies provided some proof-of-targeting and/or biologic activity, but few (under 10%) showed a significant correlation between PD biomarker results and clinical activity. It is worth noting that phase 1 trials are not primarily designed to evaluate clinical activity. Sample sizes are typically small, tumours heterogeneous, participants heavily pre-treated, and some receive sub-therapeutic doses of investigational agents. These factors ultimately lead to a lack of clear discriminators of patient groups with clinical benefit and make the correlation with PD activity challenging.

Many authors stated that PD biomarker results factored in their decision for RP2D or further drug development, presumably based on their assessment that sufficient proof-of-concept and biologic activity had been demonstrated (without evidence of correlation with clinical activity). However, analysis of subsequent citations showed that the results of only 22 PD biomarker studies (8%) were referenced in subsequent trials, among which only ten influenced the dose and/or schedule utilised for subsequent drug development. Notably, only one PD biomarker study was referenced in the context of a dose that was ultimately FDA-approved.

These results have a variety of implications. First, despite the increased availability of blood-PD assays, the requirement of tumour biopsies for research in trial eligibility remains common. This is likely reflective of the current understanding that given an immune-suppressive TME, demonstration of enhanced immunologic activity within tumour tissue is more likely to be a surrogate of clinical activity than that demonstrated in the peripheral circulation alone. However, the performance of tissue-PD biomarkers in our analysis was limited. One possible explanation is that the existent tissue-PD biomarkers being performed are non-informative. Emerging science may therefore help direct the focus to biomarkers that reflect tumour-host interactions more reliably. Novel non-invasive imaging-PD analyses of the TME may also play an important role in future trials, particularly for cancers where tumour biopsies are challenging e.g. glioblastoma, or pancreatic carcinoma.

Secondly, despite optimism that PD analyses would lead to more accurate definition of RP2D without necessarily reaching a maximum tolerated dose (MTD) defined by toxicity events, our findings suggest that current PD biomarkers used in phase 1 trials have a limited impact on IO drug development. Such limited impact was observed for both invasive and non-invasive biomarkers, supporting that the determination of RP2D relying solely on PD biomarkers is not currently ready. Nevertheless, a definite shift away from the paradigm of cytotoxic drug development where the MTD defines the RP2D has recently been signalled by the US FDA with emerging guidelines for future drug development trials. Named 'Project Optimus', the emphasis is to identify a range of doses in phase 1 studies in which evidence of PD activity can be demonstrated. This will be followed by randomised dose-ranging phase 2 trials in which additional PD assessments, toxicity, and clinical activity data will be collected to identify an optimal dose for phase 3 drug development [28]. This concept has long been mandated for non-oncology drug development. More importantly, it raises the essential role that PD biomarkers will play in future oncology drug development, especially for IO agents for whom an MTD is infrequently demonstrated.

In this regard, novel model-based and model-assisted dose escalation designs that are at least in theory, more efficient for optimal dose determination may also see greater representation among phase 1 clinical trials of IO agents [11]. An important consideration of dose escalation designs in dose optimisation, regardless of rule-based, model-based or model-assisted, is to take into account clinical efficacy and toxicity data, as well as pharmacokinetic and PD biomarker data, in totality to determine at the most relevant dose range for further refinement.

PD biomarkers that are true surrogates of biological activity with kinetics linked directly to clinical outcome (such as ctDNA quantification) are warranted for use in these innovative future trial designs. Given the limited impact of current single-plexed PD assays and the complexity of tumour cell-host immunity interactions, bioinformatic models which integrate different tissue-, blood- and imaging-PD results may be needed to overcome the performance of single analytes and improve

dose-finding strategies for specific IO agents [29]. Recent studies utilising an integrated biomarker approach to predict tumour response to IO agents have demonstrated improved performance over single independent biomarkers [30,31]. The future of PD analyses will likely rely on the joint use of different PD biomarkers depending on the expected biologic activity of the IO agent.

There are limitations to our analysis. The first is publication time bias. More recent PD biomarker studies may have subsequent studies whose manuscripts are yet to be published, such that it is not currently possible to fully evaluate their impact on future drug development. Additionally, we attempted to minimise subjective interpretation of the impact of PD results by having two co-authors review each study independently and when needed, a third co-author adjudicated. However, there remains inherent subjectivity in the processes of data abstraction and evaluation. Another limitation is publication bias, as positive results are more likely to be included in the final published manuscript. Thus, the actual number of index phase 1 IO trials that conducted PD biomarker assessments may be greater than that observed in our study, especially if the results were negative. Hence, the proportion of index phase 1 IO trials reporting positive PD biomarker results may have been overestimated.

In conclusion, here we present the first study assessing the impact of PD biomarker analyses in phase 1 IO trials. Our results suggest that in the IO era, most studies perform PD biomarker assessments, most commonly blood-based. Similar proportions of tissue-PD and blood-PD biomarker studies demonstrated proof of mechanism/biologic activity. However, given the complexity of tumour-host immunity interactions, more informative and possibly multiplexed PD analyses will need to be implemented to improve phase 1 IO trial methodology. Moreover, recent changes in the health regulatory landscape suggest that informative PD biomarkers will play an increasingly prominent role in future phase 1 trial design and IO drug development.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Salawu: Conceptualization, Data Curation, Investigation, Formal Analysis, Writing – Original Draft, Visualization, Writing – Review & Editing.

Hernando-Calvo: Conceptualization, Data Curation, Investigation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing.

Chen: Data Curation, Investigation, Formal Analysis, Writing – Review & Editing.

Araujo: Conceptualization, Formal Analysis, Writing – Review & Editing.

Oliva: Conceptualization, Formal Analysis, Writing – Review & Editing.

Liu: Formal Analysis, Visualization, Writing – Review & Editing.

Siu: Conceptualization, Data Curation, Investigation, Formal Analysis, Writing – Review & Editing, Supervision.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

A. Hernando Calvo: Financial Interests, Personal, Other, Travel, Accommodations, Expenses: Merk Serono; Financial Interests, Personal, Other, Travel, Accommodations, Expenses: Kyowa Kirin International.

D.V. Araujo: Financial Interests, Personal, Other, honoraria: MSD; Financial Interests, Personal, Other, honoraria: Pfizer; Financial Interests, Institutional, Educational Grant: Astellas; Financial Interests, Institutional, Non-financial support: Novartis.

M. Oliva: Financial Interests, Personal, Advisory Role: Bristol Myers Squibb; Financial Interests, Personal, Other, Travel/Accommodation expenses: MSD Oncology; Merck. Financial Interests, Personal and Institutional, Research Grant: GlaxoSmithKline; Financial Interests, Personal, Advisory Role: Merck; Personal and Institutional, Research Grant: Roche.

L.L. Siu: Financial Interests, Institutional, Research Grant: Bristol-Myers Squibb; Financial Interests, Institutional, Research Grant: Genentech/Roche; Financial Interests, Institutional, Research Grant: GlaxoSmithKline; Financial Interests, Institutional, Research Grant: Merck; Financial Interests, Institutional, Research Grant: Novartis; Financial Interests, Institutional, Research Grant: Pfizer; Financial Interests, Institutional, Research Grant: MedImmune; Financial Interests, Institutional, Research Grant: AstraZeneca; Financial Interests, Institutional, Research Grant: Boehringer Ingelheim; Financial Interests, Institutional, Research Grant: Bayer; Financial Interests, Institutional, Research Grant: Amgen; Financial Interests, Institutional, Research Grant: Astellas Pharma; Financial Interests, Institutional, Research Grant: Shattuck Labs; Financial Interests, Institutional, Research Grant: Symphogen; Financial Interests, Institutional, Research Grant: AVID Radiopharmaceuticals; Financial Interests, Institutional, Research Grant: Mirati Therapeutics; Financial Interests, Institutional, Research Grant: Intensity Therapeutics; Financial Interests, Institutional, Research

Grant: Karyopharm Therapeutics; Financial Interests, Personal, Stocks/Shares, An Immediate Family Member: Agios; Financial Interests, Personal, Leadership Role, An Immediate Family Member: Treadwell Therapeutics; Financial Interests, Personal, Advisory Role: Merck; Financial Interests, Personal, Advisory Role: AstraZeneca/MedImmune; Financial Interests, Personal, Advisory Role: MorphoSys; Financial Interests, Personal, Advisory Role: Roche; Financial Interests, Personal, Advisory Role: Loxo; Financial Interests, Personal, Advisory Role: Voronoi; Financial Interests, Personal, Advisory Role: Oncorus; Financial Interests, Personal, Advisory Role: Symphogen; Financial Interests, Personal, Advisory Role: Mirati Therapeutics; Financial Interests, Personal, Advisory Role: GlaxoSmithKline; Financial Interests, Personal, Advisory Role: Seattle Genetics; Financial Interests, Personal, Advisory Role: Treadwell Therapeutics; Financial Interests, Personal, Advisory Role: Arvinas; Financial Interests, Personal, Advisory Role: Navire; Financial Interests, Personal, Advisory Role: Janpix; Financial Interests, Personal, Advisory Role: Relay Therapeutics; Financial Interests, Personal, Advisory Role: Daiichi Sankyo/UCB Japan; Financial Interests, Personal, Advisory Role: Marengo Therapeutics; Financial Interests, Personal, Advisory Role: LTZ Therapeutics.

All other authors have declared no conflicts of interest.

Acknowledgements

A Salawu and A Hernando Calvo have received Hold'em for Life fellowship awards. A Salawu has received an Ashley Miklas fellowship award. A Hernando-Calvo has received a fellowship grant from the Spanish Society of Medical Oncology (SEOM) and CRIS Cancer Foundation. L L Siu holds the BMO Chair in Precision Cancer Genomics.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.06.045.

References

[1] Tang J, Shalabi A, Hubbard-Lucey VM. Comprehensive analysis of the clinical immuno-oncology landscape. Ann Oncol 2018; 29(1):84–91. https://doi.org/10.1093/annonc/mdx755.

[2] Upadhaya S, Neftelino ST, Hodge JP, Oliva C, Campbell JR, Yu JX. Combinations take centre stage in PD1/PDL1 inhibitor clinical trials. Nat Rev Drug Discov 2021;20(3):168–9. https://doi.org/10.1038/d41573-020-00204-y.

[3] Beaver JA, Pazdur R. The wild west of checkpoint inhibitor development. N Engl J Med 2022;386(14):1297–301. https://doi.org/10.1056/NEJMp2116863.

[4] Sweis RF, Drazer MW, Ratain MJ. Analysis of impact of post-treatment biopsies in phase I clinical trials. J Clin Oncol 2016; 34(4):369–74. https://doi.org/10.1200/JCO.2015.63.6126.

[5] Ochoa de Olza M, Oliva M, Hierro C, Matos I, Martin-Liberal J, Garralda E. Early-drug development in the era of immuno-oncology: are we ready to face the challenges? Ann Oncol 2018; 29(8):1727–40. https://doi.org/10.1093/annonc/mdy225.

[6] Gainor JF, Longo DL, Chabner BA. Pharmacodynamic biomarkers: falling short of the mark? Clin Cancer Res 2014;20(10): 2587–94. https://doi.org/10.1158/1078-0432.CCR-13-3132.

[7] Goulart BH, Clark JW, Pien HH, Roberts TG, Finkelstein SN, Chabner BA. Trends in the use and role of biomarkers in phase I oncology trials. Clin Cancer Res 2007;13(22 Pt 1):6719–26. https://doi.org/10.1158/1078-0432.CCR-06-2860.

[8] Nixon AB, Schalper KA, Jacobs I, Potluri S, Wang IM, Fleener C. Peripheral immune-based biomarkers in cancer immunotherapy: can we realize their predictive potential? J Immunother Cancer 2019;7(1):325. https://doi.org/10.1186/s40425-019-0799-2.

[9] Hegde PS, Karanikas V, Evers S. The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. Clin Cancer Res 2016;22(8):1865–74. https://doi.org/10.1158/1078-0432.CCR-15-1507.

[10] Merchant S, Witney TH, Aboagye EO. Imaging as a pharmacodynamic and response biomarker in cancer. Clin Transl Imaging 2014;2(1):13–31. https://doi.org/10.1007/s40336-014-0049-z.

[11] Araujo DV, Oliva M, Li K, Fazelzad R, Liu ZA, Siu LL. Contemporary dose-escalation methods for early phase studies in the immunotherapeutics era. Eur J Cancer 2021;158:85–98. https://doi.org/10.1016/j.ejca.2021.09.016.

[12] FDA-NIH Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD). Bethesda (MD): Food and Drug Administration (US) National Institutes of Health (US); 2016. Available from: https://www.ncbi.nlm.nih.gov/books/NBK402286/.

[13] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45(2): 228–47. https://doi.org/10.1016/j.ejca.2008.10.026.

[14] Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. JNCI: J Natl Cancer Inst 2000;92(3): 205–16. https://doi.org/10.1093/jnci/92.3.205.

[15] Schreibelt G, Bol KF, Westdorp H, Wimmers F, Aarntzen EH, Duiveman-de Boer T, et al. Effective clinical responses in metastatic melanoma patients after vaccination with primary myeloid dendritic cells. Clin Cancer Res 2016;22(9):2155–66. https://doi.org/10.1158/1078-0432.CCR-15-2205.

[16] Naing A, Gainor JF, Gelderblom H, Forde PM, Butler MO, Lin CC, et al. A first-in-human phase 1 dose escalation study of spartalizumab (PDR001), an anti-PD-1 antibody, in patients with advanced solid tumors. J Immunother Cancer 2020;8(1). https://doi.org/10.1136/jitc-2020-000530.

[17] Somaiah N, Block MS, Kim JW, Shapiro GI, Do KT, Hwu P, et al. First-in-Class, first-in-human study evaluating LV305, a dendritic-cell tropic lentiviral vector, in sarcoma and other solid tumors expressing NY-ESO-1. Clin Cancer Res 2019;25(19): 5808–17. https://doi.org/10.1158/1078-0432.CCR-19-1025.

[18] Beatty GL, O'Dwyer PJ, Clark J, Shi JG, Bowman KJ, Scherle PA, et al. First-in-Human phase I study of the oral inhibitor of indoleamine 2,3-dioxygenase-1 epacadostat (INCB024360) in patients with advanced solid malignancies. Clin Cancer Res 2017;23(13): 3269–76. https://doi.org/10.1158/1078-0432.CCR-16-2272.

[19] Long GV, Dummer R, Hamid O, Gajewski TF, Caglevic C, Dalle S, et al. Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. Lancet Oncol 2019;20(8):1083–97. https://doi.org/10.1016/S1470-2045(19)30274-8.

[20] Hellmann MD, Gettinger S, Chow LQM, Gordon M, Awad MM, Cha E, et al. Phase 1 study of epacadostat in combination with

atezolizumab for patients with previously treated advanced non-small cell lung cancer. Int J Cancer 2020;147(7):1963–9. https://doi.org/10.1002/ijc.32951.

[21] Gibney GT, Hamid O, Lutzky J, Olszanski AJ, Mitchell TC, Gajewski TF, et al. Phase 1/2 study of epacadostat in combination with ipilimumab in patients with unresectable or metastatic melanoma. J Immunother Cancer 2019;7(1):80. https://doi.org/10.1186/s40425-019-0562-8.

[22] Mitchell TC, Hamid O, Smith DC, Bauer TM, Wasser JS, Olszanski AJ, et al. Epacadostat plus pembrolizumab in patients with advanced solid tumors: phase I results from a multicenter, open-label phase I/II trial (ECHO-202/KEYNOTE-037). J Clin Oncol 2018;36(32):3223–30. https://doi.org/10.1200/JCO.2018.78.9602.

[23] Patnaik A, Kang SP, Rasco D, Papadopoulos KP, Elassaiss-Schaap J, Beeram M, et al. Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. Clin Cancer Res 2015;21(19):4286–93. https://doi.org/10.1158/1078-0432.CCR-14-2607.

[24] van de Donk PP, Kist de Ruijter L, Lub-de Hooge MN, Brouwers AH, van der Wekken AJ, Oosting SF, et al. Molecular imaging biomarkers for immune checkpoint inhibitor therapy. Theranostics 2020;10(4):1708–18. https://doi.org/10.7150/thno.38339.

[25] Pandit-Taskar N, Postow MA, Hellmann MD, Harding JJ, Barker CA, O'Donoghue JA, et al. First-in-Humans imaging with (89)Zr-Df-IAB22M2C anti-CD8 minibody in patients with solid malignancies: preliminary pharmacokinetics, biodistribution, and lesion targeting. J Nucl Med 2020;61(4):512–9. https://doi.org/10.2967/jnumed.119.229781.

[26] Kist de Ruijter L, Hooiveld-Noeken JS, Giesen D, Lub-de Hooge MN, Kok IC, Brouwers AH, et al. First-in-Human study of the biodistribution and pharmacokinetics of 89Zr-CX-072, a novel immunopet tracer based on an anti-PD-L1 probody. Clin Cancer Res 2021;27(19):5325–33. https://doi.org/10.1158/1078-0432.CCR-21-0453.

[27] Bratman SV, Yang SYC, Iafolla MAJ, Liu Z, Hansen AR, Bedard PL, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nat Cancer 2020;1(9):873–81. https://doi.org/10.1038/s43018-020-0096-5.

[28] Shah M, Rahman A, Theoret MR, Pazdur R. The drug-dosing conundrum in oncology - when less is more. N Engl J Med 2021;385(16):1445–7. https://doi.org/10.1056/NEJMp2109826.

[29] Sholl LM. Biomarkers of response to checkpoint inhibitors beyond PD-L1 in lung cancer. Mod Pathol 2022;35(Suppl 1):66–74. https://doi.org/10.1038/s41379-021-00932-5.

[30] Nabet BY, Esfahani MS, Moding EJ, Hamilton EG, Chabon JJ, Rizvi H, et al. Noninvasive early identification of therapeutic benefit from immune checkpoint inhibition. Cell 2020;183(2):363–76. https://doi.org/10.1016/j.cell.2020.09.001. e13.

[31] Jiang J, Jin Z, Zhang Y, Peng L, Zhang Y, Zhu Z, et al. Robust prediction of immune checkpoint inhibition therapy for non-small cell lung cancer. Front Immunol 2021;12:646874. https://doi.org/10.3389/fimmu.2021.646874.