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Predictive biomarkers of response to immune checkpoint inhibitors

Biomarcadores predictivos de respuesta a los inhibidores de los puntos de control inmunitario

María Sacramento Díaz-Carrasco[1], Eva González-Haba[2],
Juana Inés García-Soler[1], Alberto Espuny-Miró[1]

[1]Department of Pharmacy, Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Murcia. Spain. [2]Department of Pharmacy, Hospital Gregorio Marañón, Madrid. Spain.

Author of correspondence

María Sacramento Díaz Carrasco
Servicio de Farmacia
Hospital Clínico universitario
Virgen de la Arrixaca
Ctra. Cartagena s/n.
30120 El Palmar (Murcia), Spain.

Email:
msacramento.diaz@carm.es

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Abstract

Objective: The present paper provides a literature review aimed at identifying the tumor-dependent factors capable of influencing a subject's response to immune checkpoint inhibitors, with a special emphasis on those that may act as predictive biomarkers.
Method: A search was performed of the terms *biomarkers*, *PD-1*, *PD-L1*, *CTLA-4*, and *checkpoint* inhibitors in the title and the abstract of the records in the PubMed database. Articles including relevant information on the tumor-dependent factors capable of influencing a subject's response of immune checkpoint inhibitors were selected. Priority was given to studies in humans (clinical trials and reviews) published between January 2015 and June 2019, in English and Spanish.
Results: The literature review exposed the complex relationship that exists between the immune system and tumors. It also revealed that the factors capable of influencing a subject's response to immune checkpoint inhibitors are multiple, heterogeneous and ill understood, which makes it difficult to obtain simple and/or universal predictive biomarkers.
Conclusions: The only biomarkers currently used in clinical practice include the expression of the programmed cell death ligand-1 and microsatellite instability/ deficient DNA mismatch repair, but their usefulness is limited. Tumor mutational burden and gene signatures associated to IFN-γ could become useful biomarkers once determination techniques and cutoff points are systematized.

Resumen

Objetivo: El objetivo del presente trabajo es identificar mediante revisión bibliográfica los factores dependientes del tumor que condicionan la respuesta a los inhibidores de los puntos de control inmunitario, incidiendo especialmente en aquellos que se postulan como posibles biomarcadores predictivos.
Método: Búsquedas en Pubmed con los términos *biomarkers*, PD-1, PD-L1, CTLA-4, *checkpoint inhibitors*, en el título o el *abstract*, seleccionando aquellos que incluyeran información relevante sobre factores tumorales que condicionan la respuesta a los inhibidores de los puntos de control inmunitario. Se priorizaron estudios en humanos (ensayos clínicos y revisiones) publicados entre enero de 2015 y junio de 2019, en idiomas inglés y español.
Resultados: La revisión pone de manifiesto las complejas relaciones entre sistema inmunitario y tumor, con factores que influyen en la respuesta a los inhibidores de los puntos de control inmunitario variados, y aun poco conocidos, lo cual dificulta la obtención de biomarcadores predictivos sencillos y/o universales.
Conclusiones: Actualmente los únicos biomarcadores utilizados en práctica clínica, en algunos escenarios, son la expresión del ligando del receptor de muerte celular programada-1 y la inestabilidad de microsatélites/deficiencias en las enzimas de reparación de los apareamientos erróneos durante la replicación del ácido desoxirribonucleico, aunque su utilidad es limitada. La carga mutacional y las firmas génicas asociadas a interferón gamma se postulan como biomarcadores útiles, una vez sistematizadas las técnicas de determinación y los puntos de corte.

Introduction

In the last few years, immune checkpoint inhibitors (ICIs), anti-cytotoxic T-lymphocyte antigen (CTLA)-4 antibodies and, particularly, anti-programmed cell death-1 receptors (PD1) or their ligand (PD-L1), have been incorporated to the treatment of a variety of tumors, significantly extending survival in a number of patients[1-3]. On the other hand, the above-mentioned therapies are associated with a considerable toxicity profile[4,5].

The huge economic and clinical impact of treatment with ICIs makes the need for further research and a thorough evaluation of the efficiency of these drugs all the more pressing. The main problem lies in the dearth of response-predictive biomarkers able to select those patients who will benefit from the therapy, which would reduce exposure to toxicity in those unlikely to benefit and save costs to the national health system.

Myriads of data are now available on the factors capable of influencing a subject's response to immunotherapy. These factors depend on the patient, the tumor, the environment and other variables including past and present treatments.

The main goal of studies in this field is to come up with immunotherapy response-predictive biomarkers that make it possible to select those patients with the greatest chance of obtaining a clinical benefit so as to maximize the risk-benefit ratio of immunotherapy regimens and reduce unnecessary costs.

The development of immunotherapy-specific biomarkers is a much greater challenge than the development of biomarkers for target-directed therapies. In the latter case, the search for biomarkers focuses on the specific characteristics of tumor cells and the driver mutations that occur in them. In the case of immunotherapy, apart from tumor cell alterations, other factors are involved, such as the characteristics of the tumor microenvironment and the host's immune response, which are equally or even more relevant[6]. Developing these biomarkers represents a significant challenge as it means working in a dynamic, more heterogeneous territory, where markers have a continuous range of expression and there is the added difficulty of having to establish cutoff points.

Against this background, the purpose of the present paper is to carry out a literature review aimed at identifying those tumor-dependent factors that play a role in regulating immune checkpoint inhibitors response, particularly those than may act as potential predictive biomarkers.

Methods

A structured review of articles included in the PubMed database was conducted with a view to identifying the tumor-related factors capable of influencing a subject's response to ICIs. The terms *biomarkers*, *PD-1*, *PD-L1*, *CTLA-4*, and *checkpoint inhibitors* were searched in the title and the abstract of a series of articles, including clinical trials, randomized clinical trials, reviews, systematic reviews and meta-analyses. Papers were required study human subjects; be written in either English or Spanish; and have been published between January 2015 and June 2019. The initial search was later expanded to include references in the selected articles that were considered relevant to the review.

Articles were selected independently by two of the authors. If an article got through the initial screening following a review of its title and abstract, the full text then perused to verify whether it was relevant to the aims of the study. In the event of repetition, articles with the highest degree of topicality, clarity and depth were selected. Extraction of data from the studies was also performed independently by the same two authors.

Results

Fifty references were selected out of a total of 298 articles (31 clinical trials and 267 reviews) on account of their consistency with the purpose of the study (Table 1).

Tumor-related factors that have been associated with the efficacy or effectiveness of ICIs include those that determine the immune characteristics of the tumor's environment; the presence of certain tumor-infiltrating lymphocyte populations; the expression of PD-L1 by the tumor cells or the lymphocytes present in the tumor tissue; the tumor's mutational burden; or the shortage of DNA repair enzymes or microsatellite instability. In addition, gene signatures are being developed that may allow identification of tumors possessing

these characteristics, which may make them more amenable to treatment with ICIs.

Immune profiles of tumor microenvironment: inmunophenotypes

Three immune profiles have been described in connection with the tumor microenvironment[6,7], which are correlated with the subject's response to anti PD1/L1 therapy: a) the inflamed phenotype, which is characterized by infiltration by CD8+ cells and other immune cells (in the tumor parenchyma), accompanied by proinflammatory cytokines; b) the immune-desert phenotype, which does not contain an immune infiltrate and may result from tolerance mechanisms, immune ignorance, or the absence of T cell priming or activation; and c) the immune-excluded phenotype, where CD8+ T cells huddle around the tumor but do not infiltrate it. This may be due to the presence of barriers or vascular factors, or the presence of specific cytokines or inhibitory factors in the stroma.

A tumor located in an immune-desert or immune-excluded phenotype microenvironment is unlikely to respond to immunotherapy with ICIs. Although the inflamed phenotype is usually more likely to elicit a response, its presence does not guarantee that a response will be obtained. In fact, even if effector CD8+ T cells are infiltrated, such cells could be dysfunctional, with an exhausted or even hyperexhausted phenotype. In addition, the infiltrate may contain other immune-suppressive cells, such as regulatory T-cells (Treg), myeloid-derived suppressor cells, suppressor B lymphocytes or cancer-associated fibroblasts. Also, cells in these inflamed tumors may express inhibitory factors that reduce the expression of major histocompatibility complex (MHC) class I molecules (necessary for antigen presentation) or other pathways that decrease sensitivity to anti-tumor immunity[6].

It must be noted that the previous or concomitant administration of other kinds of therapies (radiotherapy, chemotherapy, targeted therapy...) may result in inflammation of the tumor microenvironment, thereby enhancing sensitivity to ICIs. This has been the basis for the many combination studies currently underway[8-10].

In April 2018, Thorsson *et al.*[11] published the results of a complex immunogenomic analysis of over 10,000 tumors pertaining to 33 different types of cancer. Using data from the *Cancer Genome Atlas* project, the authors identified six immune subtypes as a function of the tumor microenvironment: wound healing, interferon-gamma (IFN-γ)- dominant, inflammatory, lymphocyte-depleted, immunologically quiet, and TGF-β (transforming growth factor beta)-dominant. According to the authors, these immune subtypes have potential prognostic and therapeutic implications for cancer management.

Tumor-infiltrating lymphocytes (TILs)

Infiltration of a tumor by different kinds of immune cells has been associated with a significant prognostic value for different kinds of tumor, such as resected non-small-cell lung cancer (NSCLC)[12] or metastatic NSCLC[13].

Usó *et al.*[12] described the relationship between infiltration by CD4+ and CD8+ cells in different tumor compartments and higher rates of progression-free survival (PFS) and overall survival (OS) in patients with resected NSCLC. Infiltration of the stromal compartment by FOXP3+ cells was associated with a poorer prognosis.

In a meta-analysis published in 2015, Geng *et al.*[13] reported that deep infiltration of tumors by CD8+ and CD4+ T cells was often correlated with higher rates of OS in patients with metastatic lung cancer. Conversely, a high density of Treg lymphocytes (FOXP3+) may be interpreted as a negative prognostic factor.

Other authors have found a correlation between the presence of tumor-infiltrating CD8+ T cells and response to ICI therapy in other types of tumor. For example, in a paper published in *Nature* in 2014, Tumeh *et al.*[14] reported that infiltration of CD8+ T cells into the invasive margin of the tumor can predict the response to anti PD1 treatment in patients with metastatic melanoma.

In 2016, Daud *et al.*[15] went one step further and used multiparameter flow cytometry to analyze populations of tumor-infiltrating CD8+ T cells in patients with metastatic melanoma treated with nivolumab or pembrolizumab.

Table 1. Summary of studies included in the review

Author	Year	Country	Type of study	Sample size	Main findings
Planchard D[1]	2018	Switzerland	Technical document	N/A	Diagnosis, molecular biology. Treatment. Follow-up
Karlsson AK[2]	2017	UK	Meta-analysis	3,628	Efficacy. Tolerability
Escudier B[3]	2019	Europe	Technical document	N/A	Diagnosis. Treatment. Follow-up
Brahmer JR[4]	2018	USA	Technical document	N/A	Management of immunomediated toxicity
Haanen JB[5]	2017	Europe	Technical document	N/A	Toxicity. Management of immunomediated toxicity
Chen DS[6]	2017	USA	Review	N/A	Response-predictive biomarkers
Hegde PS[7]	2016	USA, Switzerland	Review	N/A	Mechanism of action. Response-predictive biomarkers
Joshi S[8]	2019	USA	Review	N/A	Combined strategies
Amin A[9]	2019	USA	Review	N/A	Combined strategies in renal cell carcinoma
Hanna GG[10]	2016	UK	Review	N/A	Combined strategies with radiotherapy
Thorsson V[11]	2018	Belgium, Brazil, Canada, USA, Qatar	Observational study	> 10,000	Prognostic biomarkers
Usó M[12]	2019	Spain	Observational study	122	T-cell subpopulations as prognostic biomarkers
Geng Y[13]	2015	China	Meta-analysis	8,600	Influence of infiltrating T-cells on survival
Tumeh PC[14]	2014	USA, France	Observational study	46	Influence of infiltrating CD8+ T-cells on response to treatment
Daud A[15]	2016	Austria, USA	Observational study	40	Influence of infiltrating CD8+ T-cells on response to treatment
Berger KN[16]	2018	USA	Review	N/A	PD-L1 expression and its relation-ship with response to treatment
Khunger M[17]	2017	USA	Meta-analysis	6,664	PD-L1 expression and its relation- ship with response to treatment
Grizzi G[18]	2017	Italy	Review	N/A	Response-predictive biomarkers
Hansen AR[19]	2016	Canada	Review	N/A	PD-L1 diagnostic tests
Hirsch FR[20]	2017	USA	Diagnostic test validation	N/A	PD-L1 diagnostic tests: clinical and analytical evaluation of different tests
Rimm DL[21]	2017	USA	Diagnostic test validation	N/A	PD-L1 diagnostic tests: performance of different tests
Büttner R[22]	2017	Germany, Canada, Spain, France, Italy, UK	Review	N/A	PD-L1 diagnostic tests: comparison between the different PD-L1-expression tests
Reck M[23]	2016	Germany, Australia, Canada, Spain, Hungary, Ireland, Israel, Japan, UK	Clinical trial	305	Progression-free survival and safety of pembrolizumab with respect to platinum in advanced NSCLC and PD-L1 expression ⌧ 50% of tumor cells
Herbst RS[24]	2016	Brazil, South Korea, Spain, USA, France	Clinical trial	1,034	Survival for pembrolizumab vs. docetaxel in previously treated patients with advanced NSCLC and PD-L1 expression
Powles T[25]	2017	Germany, Spain, Canada, Hungary, Japan, UK, Taiwan	Clinical trial	990	Survival and safety for pembrolizumab as monotherapy and in combination with platinum-based chemotherapy vs. chemotherapy in metastatic urothelial carcinoma
Galsky MD[26]	2018	Australia, Spain, USA, Greece, Japan, Switzerland	Clinical trial	1,213	Survival for atezolizumab in combination with platinum-based chemotherapy vs. chemotherapy in 1st line metastatic urothelial carcinoma
Silva MA[27]	2018	Spain	Observational study	50	Diagnostic tests for PD-L1
Inoue Y[28]	2016	Japan	Observational study	654	Response-predictive biomarkers: number of copies of PD-L1
Paré L[29]	2018	Spain, USA	Observational study	773	Response-predictive biomarkers: PD-1 RNAm and activated CD8+ T-cells
Snyder A[30]	2014	USA	Observational study	64	Genetic basis for anti-CTLA-4 response in melanoma

Table 1 *(cont.).* Summary of studies included in the review

Author	Year	Country	Type of study	Sample size	Main findings
Carbone DP[31]	2017	Germany, Canada, USA, Spain, The Netherlands, Czech Republic, Romania, Switzerland	Clinical trial	423	Survival for nivolumab in first-line treatment of advanced NSCLC in patients with PD-L1 expression > 5%
Hellmann MD[32]	2018	Germany, Australia, Chile, USA, Spain, France,The Netherlands, Italy, Poland, Romania	Clinical trial	1,004	Progression-free survival for nivolumab plus ipilimumab in NSCLC and high mutational burden
Ramalingam SS[33]	2018	Germany, Australia, Canada, Spain, USA	Clinical trial	288	Mutational burden and its relationship with response to low-dose nivolumab plus ipilimumab in first line treatment of advanced or metastatic NSCLC
Ready N[34]	2019	Germany, Australia, Canada, Spain, USA	Clinical trial	252	Progression-free survival for low-dose nivolumab plus ipilimumab in first-line treatment of advanced or metastatic NSCLC, regardless of PD-L1 expression
Rosenberg JE[35]	2016	Germany, Spain, USA, France, The Netherlands, Italy,	Clinical trial	310	Atezolizumab exhibits a better response in patients with platinum-refractory urothelial carcinoma, which higher PD-L1 expression in tumor cells
Balar AV[36]	2017	Germany, Spain, USA, France, The Netherlands, Italy, UK	Clinical trial	119	Atezolizumab exhibits better response and survival rates in first-line treatment of urothelial carcinoma regardless of PD-L1 expression
Cristescu R[37]	2018	USA	Observational study	> 300	Mutational burden, PD-L1 expression and gene expression of inflammation markers as predictive indicators of response to pembrolizumab
Nadal E[38]	2018	Spain	Review	N/A	Update on biomarkers in immuno-oncology
Chan TA[39]	2019	Germany, USA, UK, Switzerland	Review	N/A	Mutational burden, response-related biomarker
Samstein RM[40]	2019	USA	Observational study	1,662	Mutational burden, efficacy-related biomarker
Le DT[41]	2017	USA	Clinical trial	86	MMR deficiency, response-related biomarker
Scarpa A[42]	2016	Europe	Technical document	N/A	Biomarkers
Bonneville R[43]	2017	USA	Observational study	1,139	Microsatellite instability in different tumors
Abida W[44]	2019	USA	Observational study	1,346	dMMR and microsatellite instability in prostate cancer and their impact on response to treatment
Lemery S[45]	2017	USA	Opinion piece	N/A	Therapeutic indication of pembrolizumab based on a biomarker
Viale G[46]	2017	Italy	Review	N/A	MMR deficiency as a response-related biomarker
Schrock AB[47]	2019	USA	Observational study	22	Mutational burden and microsatellite instability as response-predictive biomarkers in patients with metastatic colorectal cancer
Ayers M[48]	2017	USA	Observational study	220	Response-predictive biomarkers, IFN-γ gene expression
Solomon B[49]	2018	Australia	Review	N/A	Response-predictive biomarkers in head and neck squamous cell carcinoma
Fehrenbacher L[50]	2016	Germany, Belgium, South Korea, Spain, USA, UK	Clinical trial	287	Overall survival for atezolizumab vs. docetaxel in previously treated NSCLC patients

dMMR: deficient mismatch repair; IFN-γ: interferon-gamma; MMR: mismatch repair; N/A: not aplicable; NSCLC: non-small-cell lung cancer.

Patients with over 20% of cells with high CTLA4 and PD1 expression levels demonstrated a more favorable response to treatment than those with 20% or less. According to the authors, their data suggest that the relative abundance of partially exhausted CD8+ tumor-infiltrating lymphocytes can predict response to treatment with an anti-PD1 drug. Nonetheless, the technique used in their analysis is difficult to implement in clinical practice.

PD-L1 expression

The role of PD-L1 in the immune response to tumors can be summarized in the following way: the neoantigens expressed on the surface of cancer cells are recognized as extraneous, inducing a tumor-infiltrating lymphocyte-mediated antitumoral response. Part of that response involves the production of IFN-γ which, in turn, gives rise to the expression of PD-L1. The binding of PD-L1 to its receptor (PD1) inhibits the lymphocytes' effector functions[16].

Expression of PD-L1 was the first response-predictive biomarker to be applied in clinical practice as has become a guide for the use of anti-PD1/PD-L1 drugs in certain clinical scenarios. PD-L1 expression is however an imperfect biomarker. Although a high expression of PD-L1 is generally correlated with a greater likelihood of obtaining a response or a clinical benefit[17], a high expression does not in itself guarantee a response, nor is the absence of PD-L1 expression necessarily tantamount to a lack of response. This means that the level of PD-L1 expression cannot be used as a criterion to exclude patients from treatment with anti-PD1/PD-L1 drugs, except in very few cases, associated with the design of pivotal clinical trials. The biomarker's predictive value may also depend on the tumor's histology[17].

The predictive value of PD-L1 expression has been studied at length in the realm of NSCLC, where it has been found to be associated with OS in patients treated with different PD1/PD-L1 inhibitors in monotherapy regimens[18].

The clinical development and approval of the different compounds were based on the results of different diagnostic tests used to quantify PDL1 expression[19]. These tests differed from one another in that they used different antibodies; they studied the marker in different cell types and tissue compartments; and they defined different positive staining thresholds. The use of different tests could result in patients being classified in accordance with disparate criteria and, therefore, in the inclusion of heterogeneous populations in different clinical trials, which would preclude indirect comparisons of the results of different studies. These limitations complicate patient selection in clinical practice.

Several authors have compared the different diagnostic tests[20,21]. In a literature review published in 2017, Büttner et al.[22] reported high inter-assay agreement and inter-observer reproducibility for three of the platforms available (when measurements were made in tumor cells) (28-8 PharmDx, 22C3 PharmDx and SP263 Assay). For immune cells, a higher level of intra- and inter-assay variability was found. The authors suggested that these three techniques could be interchangeable when used clinically in NSCLC patients. The SP142 Assay was associated with detection of lower levels of PD-L1, even in the tumor cell membranes.

Despite all the shortcomings, there are situations where determination of PD-L1 does influence patient selection. This is the case of pembrolizumab used as first-line therapy for NSCLC with PD-L1 > 50%[23] and in the second line setting with PD-L1 > 1%[24], as this was the inclusion criterion used in the clinical trials that provided these indications. In the context of urothelial cancer, in patients ineligible for platinum-based chemotherapy, initially was approved without PDL1 selection; but preliminary data from two phase III trials have shown lower survival rates when patients with low expression of PD-L1 were treated with pembrolizumab (Keynote 460)[25] and atezolizumab (Mvigor 130)[26] as compared with patients treated with standard chemotherapy. As a result of these findings, both the FDA and the EMA have restricted the use of these drugs as monotherapy for patients with locally advanced or metastatic urothelial cancer who are cisplatin-ineligible and obtained a PD-L1 combined positive score ≥ 10% for pembrolizumab and ≥ 5% for atezolizumab.

Efforts are currently being made to look for alternatives to immunohistochemical tests for the determination of PD-L1 expression. For example, Silva et al.[27] showed the advantages of differentiating between different staining patterns of reactive vs. constitutive PD-L1 expression to improve the biomarker's accuracy. Inoue et al.[28], for their part, in a study of 654 patients with resected NSCLC, suggested an additional or alternative determination of the number of PD-L1 copies to improve the biomarker's predictive value.

Paré et al.[29] established a connection between PD1 expression, measured by a determination of messenger RNA (nCounter platform), and OS and PFS in several tumor types. In this retrospective study, the authors analyzed paraffin-embedded samples of tumors treated with either nivolumab or pembrolizumab and found no correlation between the immunohistochemical determination of PD-L1 and the gene expression of PD1. The conclusion from this study was that the expression of PD1 is more strongly associated with sensitivity to anti-PD1 drugs than any other of the evaluated immune markers. PD1 expression could be associated to the presence of activated CD8+ T cells. It must be taken into consideration however that the subjects in this study were a heterogeneous group of patients with different kinds of tumors. Prospective randomized studies would be required to establish the clinical efficacy and the optimal positivity threshold of PD1 expression.

Tumor mutational burden

A study by Snyder et al.[30], published in 2014, was one of the first analyses to provide tumor mutational burden (TMB) data, based on a whole exome analysis, in patients with metastatic melanoma treated with ipilimumab. These authors found a statistically significant difference in mutational burden between patients obtaining a long-standing clinical benefit (> 6 months) and those with minimal or no clinical benefit. It must be said that no gene was found to be universally mutated in patients showing a sustained clinical benefit. These authors also classified patients into those with a high number of mutations in the exome (> 100 mutations) and those with a lower number and observed statistically significant differences between the OS curves for these two groups of patients. These results made the authors propose TMB as a predictive biomarker capable of identifying immunotherapy-eligible patients. The underlying mechanism proposed is based on a connection between a high mutational burden and an increased number of neoantigens which, on being recognized as extraneous by the T-cells would become more immunogenic and trigger an anti-tumor immune response.

In 2017, the CheckMate 026 trial[31] showed that nivolumab was not superior to chemotherapy in first-line treatment of (squamous and non-squamous) NSCLC, in patients with > 5% PD-L1 expression (primary endpoint), or in patients with > 1% PD-L1 expression (secondary endpoint). The authors performed an exploratory (not pre-specified) subgroup analysis comparing PFS results as a function of TMB. They carried out a whole exome analysis where the mutational burden was considered high if the number of mutations was > 243. In patients with high TMB levels, PFS is longer in the nivolumab group, while patients with low or moderate TMB levels showed a longer PFS with chemotherapy.

CheckMate 227, the first phase III trial to use TMB for patient selection, demonstrated the usefulness of this biomarker in a prospective way[32]. This trial focuses on first-line treatment of NSCLC and compares three different therapies: ipilimumab plus nivolumab, chemotherapy, and nivolumab as monotherapy. Results show that PFS in patients with high TMB is significantly higher with the nivolumab-ipilimumab combination than with chemotherapy, regardless of the tumor's histology (squamous vs. non-squamous) or PD-L1 expression (≥ 1% vs. < 1%). This analysis determined TMB through a gene panel, using the FoundationOne CDx platform (Foundation Medicine, Cambridge, Massachusetts, USA). TMB was considered elevated if the number of mutations per megabase was > 10, based on the findings of the phase II CheckMate 568 trial[33,34]. This trial, which evaluated the efficacy of nivolumab plus ipilimumab in NSCLC patients, found that the 10 mutations mark was an effective cutoff point to select the patients who are most likely to respond, regardless of their tumor's PD-L1 expression.

The connection between TMB and therapeutic efficacy has been observed in other clinical situations, e.g. the use of atezolizumab to treat urothelial carcinoma[35,36], or the use of pembrolizumab to treat a variety of other tumors[37].

The advantage of using TMB as a predictive biomarker is that it is a quantitative and more reproducible indicator than the immunohistochemical tests used to determine PD-L1 expression. However, it is also associated with a few disadvantages. Firstly, the different trials currently underway to investigate the efficacy of TMB as a biomarker apply disparate methodologies and a different terminology. Moreover, there is no consistency in the positivity thresholds used to determine whether a patient has a high, moderate or low TMB level[38,39]; and cutoff points may be different for different types of tumors[40]. Finally, a molecular analysis of the samples in the CheckMate 227 trial has shown that only 58% of paraffin-embedded samples were eligible for TMB analysis, which raises doubts regarding the possibility of applying these approaches to clinical practice.

Impairment of DNA repair genes/microsatellite instability

Tumors exhibiting deficient mismatch repair (dMMR) during DNA replication present with an exceptionally large amount of somatic mutations in their genome. The data resulting from the phase II trials conducted by Le *et al.*[41] support the hypothesis that a high load of neoantigens arising from mutations in cancers displaying dMMR makes such cancers sensitive to immune checkpoint inhibition, regardless of the nature of the original tumor tissue.

These authors evaluated the efficacy of PD1 inhibition with pembrolizumab in 12 different types of tumors presenting with dMMR. The analysis yielded an objective response rate of 53%, with 21% of complete responses. Estimated 12- and 24-month PFS was 64% and 53%, respectively; and estimated OS after the same two periods was 76% and 64%, respectively. These results are better than expected, taking into consideration the advanced stage of the disease in the patients included in the cohort. The analysis showed no significant differences between patients with colorectal cancer and other patients, or between patients with or without Lynch syndrome. The authors also calculated the percentage of tumors with dMMR in a sample of 12,000 tumors pertaining to 32 different histological types: dMMR was detected in over 2% of endometrium, stomach, small intestine, colon and rectum, cervix, prostate, bile duct and liver adenocarcinomas, as well as in neuroendocrine tumors, non-epithelial ovarian cancer and uterine sarcoma. Given the spectacular results they obtained with anti-PD1 drugs, the authors recommended the performance of dMMR tests in all patients refractory to standard treatment to identify those who could benefit from PD1 inhibition, regardless of their tumor type.

Microsatellites are repetitive nucleotide sequences distributed throughout the genome. These repetitive sequences are highly prone to mutations, which are generally repaired by error-correcting genes. When such genes are inactivated, a phenomenon known as *microsatellite instability* (MSI) occurs. Identification of MSI by a molecular test is a direct proof of the presence of dMMR[42].

Microsatellite instability/dMMR has been described in many solid tumors, sometimes in high proportions. Such is the case of endometrial carcinoma (33%), gastric and colorectal tumors (15%) and ovarian and duodenal cancer (10%)[42]. Bonneville *et al.* identified MSI in 3.8% of over 11,000 samples of 27 different types of tumors (obtained from the *Cancer Genome Atlas* project), and described the phenomenon even in more uncommon tumors such as adrenocortical carcinoma or mesotelioma[43].

Abida *et al.* found a 3.1% prevalence of MSI in 1,033 patients with prostate cancer and reported that, in some cases, the molecular phenotype was acquired in the course of the development of the disease. Eleven patients with MSI/dMMR were treated with PD1/L1 inhibitors, with promising responses[44].

Use of MSI/dMMR as a predictive biomarker has been incorporated to clinical practice in some specific scenarios. The FDA approved nivolumab for colorectal cancer with MSI in 2017 and more recently approved pembrolizumab, the first drug authorized for use based on a molecular biomarker, regardless of tumour type[45].

Clinical trials are underway on ICIs (on their own and in combination) to explore the predictive role that the presence of dMMR/MSI may play in different solid tumors[46]. A recent study has found TMB to be associated with an additional predictive value in colorectal cancer patients with MSI[47].

Gene signatures or inflammation signatures

Several signatures or platforms of gene expression are being developed, made up of genes associated with the activation of IFN-γ (a T-cell activation mediator). Such platforms, also known as inflammation signatures, are used to carry out a transcriptomic analysis of the expression of genes that may cause the activation of T cells.

In an analysis of gene expression profiles using RNA, from baseline samples obtained from tumors of different etiologies treated with pembrolizumab, Ayers *et al.*[48] identified a signature of 18 genes associated with INF-γ production and T-cell activation, which were correlated with the clinical benefit obtained from the treatment. The results of their pilot study, which analyzed samples from patients with melanoma, were confirmed in a larger cohort of patients with melanoma and other cohorts of patients with solid tumors of different origins such as head and neck[49] and the GI tract. According to the authors, their results demonstrate that an inflamed microenvironment (characterized by active signs of INF-γ, cytotoxic effector molecules, antigen presentation and T cell activating cytokines) is a biological hallmark of ICI treatment-responsive tumors. According to these authors, a small group of genes could identify this biological profile and predict a subject's clinical response of a wide variety of tumors.

In 2016, Fehrenbacher *et al.*[50] published the results of the POPLAR trial, a phase II study comparing atezolizumab with docetaxel in previously-treated NSCLC patients. As part of an exploratory analysis of biomarkers, these authors evaluated their results as a function of a signature of genes associated with T-cell activation, immune cytolytic activity and INF-γ expression. They found that patients with high levels of that activation signature exhibited a better response to treatment with atezolizumab, with higher OS. However, in patients undergoing chemotherapy, having a high or a low level of the signature had no impact on the outcome of the treatment. It must be added that a high expression of this gene signature was associated with PD-L1 expression in tumor-infiltrating immune cells but not in tumor cells.

This signature, which at the beginning included the expression of around 18 different genes, was subsequently restricted to just three genes (PD-L1, IFN-γ and CXCL9), and was shown to be capable of successfully identifying those patients who would benefit from treatment with atezolizumab.

Discussion

The advent of ICIs has made it possible to treat several types of tumor in a more accurate and individualized manner. It is now a priority to understand factors capable of influencing the response and to develop predictive biomarkers to identify those patients that are most likely to benefit from ICIs. The present review is not exempt from limitations given the difficulties inherent in interpreting the results of the different studies, many of them retrospective and presenting preliminary data, and the variability of the clinical scenarios analyzed. Implications for clinical practice are for now limited to the use of PD-L1 and MSI as biomarkers in some specific situations. A systematic survey of accessible factors and of the results obtained in clinical practice may contribute to improving patient selection.

In conclusion, the complexity of the relationship between the immune system and tumour means that the tumoral factors capable of predicting a subject's response to ICIs are extremely varied and scarcely understood. This complicates the development of simple and/or universal predictive biomarkers. The only biomarkers used in current clinical practice are PD-L1 expression and MSI/dMMR, albeit with limited utility. TMB and INF-γ-associated gene signatures may potentially become useful biomarkers once the determination techniques have been systematized and the required cutoff points have been established.

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