Circulating proteins as biomarkers of response to cancer immunotherapy (Review)

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Abstract. Immune checkpoint inhibitors (ICIs) have revolutionized cancer management; however, durable benefit is observed only in a minority of patients. To overcome the limitations of currently approved tumour tissue-based biomarkers of response, several approaches based on liquid biopsy are currently being developed. Among them, circulating proteins, such as soluble immune checkpoint regulators, cytokines, chemokines, growth factors and cellular modulators, are being increasingly assessed by multiplex technologies that use a low volume of biofluids and offer rapid results. Serum/plasma programmed death-ligand 1, cytotoxic t-lymphocyte-associated protein 4, T-cell immunoglobulin and mucin-domain containing-3, lymphocyte activation gene-3, interleukin (IL)-6 and IL-8 have emerged as potentially useful indicators of early response or resistance to ICIs, particularly when quantified during treatment. However, the optimal timing of on-treatment blood sampling remains to be determined. The current review aimed to present the most important findings on the association between circulating proteins and response to ICIs in solid tumours, and to discuss the position of this biomarker class in the current landscape of biomarkers for ICI therapy.

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Abbreviations: AEs, adverse events; ANG-2, angiopoietin-2; BR, best response; BTLA-4: B- and T-lymphocyte attenuator-4; ctDNA, circulating tumour DNA; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CXCL, C-X-C motif chemokine ligand; DCB, durable clinical benefit; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; ICI, immune checkpoint inhibitor; IFN-γ, interferon γ; IL, interleukin; LAG-3: lymphocyte activation gene-3; NSCLC, non-small cell lung cancer; OS, overall survival; PD-1, programmed cell death-protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; TME, tumour microenvironment; TNBC, triple-negative breast cancer; TTF, time to treatment failure; VEGF, vascular endothelial growth factor

Key words: ICIs, biomarkers, liquid biopsy, circulating proteins, response to therapy

Contents

1. Introduction

Over the past decade, cancer immunotherapy has made progress through the development of monoclonal antibodies, immune modulators, chimeric antigen receptor-T cells and therapeutic vaccines. Targeting immune checkpoint regulator proteins, such as programmed cell death-protein-1 (PD-1), programmed death-ligand 1 (PD-L1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), has markedly improved the survival of patients with melanoma, Merkel's cell carcinoma, head-and-neck cancer, Hodgkin's lymphoma, hepatocellular carcinoma, gastric carcinoma, renal cell carcinoma (1) and non-small cell lung cancer (NSCLC) (2). However, the use of immune checkpoint inhibitors (ICIs) in oncology remains under constraint. A subgroup of patients have shown marked tumour regression in response to ICIs; however, the majority of patients have failed to derive a durable benefit from them (3). Overcoming resistance to ICIs remains

a major issue due to the various underlying mechanisms (4). Another important problem is the association of severe adverse events (AEs) with ICIs, which are often the reason for treatment cessation (5). Finally, the high cost of ICI therapies puts a considerable strain on healthcare systems; the total cost of 1 year of therapy with ICIs has been estimated to be ~$100,000 per patient (6). Therefore, identifying biomarkers of response to ICIs is essential to optimize this therapy, minimize its toxicities and render it affordable to those patients with the greatest probability of responding to treatment (5,7).

2. Current biomarkers for ICI cancer treatment

Translational research has revealed several biomarkers predictive of response to ICIs, primarily derived from the characteristics of tumour cells or of the tumour microenvironment (TME). Tumour tissue PD-L1 levels assessed by immunohistochemistry, tumour mutational load and microsatellite instability/mismatch repair defects have been successfully integrated into approved companion diagnostic assays used for the prescription of ICIs (8-11). However, tissue-based biomarkers have the following limitations: i) They rely on tumour biopsies, which are not always feasible, particularly for metastatic lesions; ii) because of inter-metastatic and intra-tumour heterogeneity and the dynamic nature of the TME, they provide only a partial view of the malignant disease, and cannot capture temporal changes occurring during treatment; iii) tumour tissue biopsy is an invasive procedure, inconvenient for longitudinal monitoring, which is crucial for the early detection of disease progression or severe AEs; and iv) ICIs do not only modulate the intra-tumour immune response, but also influence the systemic immunity, the effective activation of which is critical for durable cancer control and modifications of systemic immunity are reflected in circulating serum/plasma protein concentrations. Current tissue-based biomarkers fall short of capturing the full complexity of the tumour-immune dynamics that dictate treatment outcomes (12).

3. Emerging circulating biomarkers for ICI cancer treatment

To overcome the aforementioned limitations of tumour tissue-based biomarkers, biopsies of body fluids, known as liquid biopsies, are currently being developed. The most frequently sampled biofluid is blood, which has traditionally been used in clinical practice as a source of cells and molecules, the characteristics of which can help in making treatment decisions. Blood sampling is minimally invasive; therefore, it allows repeated sampling for dynamic, real-time monitoring of response and resistance throughout treatment, whereas tissue biopsies are generally performed only once before therapy (13). In immuno-oncology, blood samples are used for the assessment of blood cell counts, immune cell phenotype, and circulating tumour DNA (ctDNA) and protein levels, which may be potential biomarkers of ICI response (14).

Among numerous potential biomarkers derived from blood cell counts, neutrophil-to-lymphocyte ratio is emerging as a strong and cost-effective indicator of poor response to ICIs, and of hyperprogression following treatment initiation (15,16). In addition, the quantity and on-treatment dynamics of peripheral blood lymphocyte subpopulations, such as activated or exhausted T or B cells, which are characterized by specific immunophenotypes, have been shown to be associated with response to ICIs in several studies (17-21). The need for fresh cells and for high-technology equipment (such as multicolour flow cytometers) are some of the obstacles for rapid validation and implementation of these promising circulating biomarkers in the clinic. ctDNA quantification has shown potential to predict the response to ICIs administered in the neoadjuvant setting; however, due to the high variability and low specificity of the assays, this biomarker is still not recommended for clinical decisions (22). In such a situation, circulating protein-based biomarkers have garnered attention based on the efficiency and speed of modern protein assay techniques, which are relatively inexpensive and commonly used in most clinical laboratories. Circulating proteins reflect both tumour-intrinsic characteristics and systemic immune responses (23). Proteomics, using both solid and liquid biopsies, have evolved rapidly over the last decade, providing solutions to complex biomedical problems, due to advances in protein quantification techniques, such as affinity-based methods, mass spectrometry, antibody arrays, fluorescence, PCR or proximity extension assay (24,25). Thus, new strategies have emerged to enable high-plex and high-throughput techniques in this field. A number of novel targeted proteomics technologies, including SomaScan (26-28), Olink (28,29), Luminex (30-32) and Meso Scale Discovery (30,31,33), use low volumes of biofluids while offering high analytical precision and short analysis time (24), compared with the classic mass spectrometry-based proteomics. For all of these reasons, quantification of circulating proteins is becoming an indispensable part of translational studies in cancer immunotherapy (32).

4. Most explored circulating protein biomarkers for ICI cancer treatment

PD-L1. In addition to the established value of tumour tissue PD-L1 as a biomarker for ICI treatment (34), numerous researchers have recently become interested in the response-predictive potential of soluble PD-L1 (sPD-L1). Okuma *et al* (35) demonstrated that 59% of patients with NSCLC with low baseline sPD-L1 levels (<3.357 ng/ml) achieved complete or partial response to nivolumab compared with only 25% of patients with high sPD-L1 levels (>3.357 ng/ml) as summarized in Table I, which lists circulating immune checkpoint proteins and their associations with immunotherapy response. The latter group also exhibited shorter time to treatment failure (TTF) and overall survival (OS) compared with the former group. Costantini et *al* (36) observed that sPD-L1 concentration after 2 months of treatment was significantly higher in non-responders, with a median value of 67.64 pg/ml, compared with 32.94 pg/ml in responders; however, the authors did not observe a significant difference in baseline sPD-L1 levels between the two groups (Table I). Similarly, Zizzari *et al* (37) showed that the mean concentration of sPD-L1 after 3 months of treatment with nivolumab was lower among responders (1.70±0.06 pg/ml) than among non-responders (57.00±12.00 pg/ml). Incorvaia *et al* (38) showed contradictory results in metastatic renal cell cancer, where high baseline sPD-L1 levels (>0.66 ng/ml) were associated with longer progression-free survival (PFS) after

Table I. Circulating immune checkpoints as potential biomarkers of response to immunotherapy.

A, PD-1

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Zizzari *et al*, 2020	NSCLC	n=22	Nivolumab	ELISA (LUMINEX xMAP kit)	N/A	N/A	At 3 months of treatment: R: 31 pg/ml; NR: 53 pg/ml	R: Decrease between baseline and 3 months of treatment	(37)
Incorvaia *et al*, 2020	Metastatic RCC	n=21	Nivolumab	In-house ELISA kit (DYNABIO S.A.)	2.11 ng/ml	R: 13.25 ng/ml; all patients: 2.00 ng/ml	At 4 weeks of treatment: R: 1.23 ng/ml	R: Decrease between baseline and 4 weeks of treatment	(38)
Incorvaia *et al*, 2023	Melanoma	n=41	Nivolumab or pembrolizumab	In-house ELISA kit (DYNABIO S.A.)	11.24 ng/ml	R: 10.3 ng/ml; NR: 16.6 ng/ml	N/A	N/A	(43)
Machiraju *et al*, 2021	Melanoma	n=42	Ipilimumab + nivolumab	ELISA kits (LifeSpan BioSciences, Inc. and Abcam)	167 pg/ml	R: 149 pg/ml; NR: 459 pg/ml	N/A	N/A	(42)

B, PD-L1

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Zizzari *et al*, 2020	NSCLC	n=22	Nivolumab	ELISA (LUMINEX xMAP kit)	N/A	R: 1.7 pg/ml; NR: 57.12 pg/ml	At 3 months of treatment: R: Lower concentration	N/A	(37)
Okuma *et al*, 2018	NSCLC	n=39	Nivolumab	ELISA (PDCD1LG1 kit; CLOUD-CLONE CORP)	3.357 ng/ml	R: <3.357 ng/ml; NR: >3.357 ng/ml	N/A	N/A	(35)
Incorvaia *et al*, 2020	Metastatic RCC	n=21	Nivolumab	ELISA kit (DYNABIO S.A.)	0.66 ng/ml	R: 1.09 ng/ml; all patients: 0.64 ng/ml	At 4 weeks of treatment: R: 0.73 ng/ml	R: Decrease between baseline and 4 weeks of treatment	(38)

Table I. Continued.

B, PD-L1

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Zhou *et al*, 2017	Melanoma	n=73	Ipilimumab	ELISA kit (R&D Systems, Inc.)	1.4 ng/ml	R: <1.4 ng/ml; NR: >1.4 ng/ml	N/A	R: Increase (>1.5-fold) between baseline and 5 months of treatment	(40)
Zhou *et al*, 2017	Melanoma	n=21	Pembrolizumab	ELISA kit (R&D Systems, Inc.)	N/A	N/A	N/A	R: Increase (>1.5-fold) between baseline and 5 months of treatment	(40)
He *et al*, 2023	Colorectal cancer	n=40	Sintilimab	ELISA kit (Abcam)	24.2 pg/ml	N/A	N/A	NR: Increase between baseline and cycle 4	(39)
Boschert *et al*, 2020	HNSCC	n=20	Durvalumab or nivolumab +/- ipilimumab	ELISA kit (R&D Systems, Inc.)	N/A	R: 740.2 pg/ml; NR: 94.76 pg/ml	N/A	N/A	(41)
Costantini *et al*, 2018	NSCLC	n=43	Nivolumab	ELISA kit (Abcam)	33.97 pg/ml	N/A	At 2 months of treatment: R: 32.94 pg/ml; NR: 67.64 pg/ml	N/A	(36)

C, CTLA-4

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Zizzari *et al*, 2020	NSCLC	n=22	Nivolumab	ELISA (LUMINEX xMAP kit)	N/A	N/A	At 3 months of treatment: R: 61 pg/ml; NR, 86 pg/ml	N/A	(37)
Leung *et al*, 2014	Melanoma	n=14	Ipilimumab	ELISA kit (Thermo Fisher Scientific, Inc.)	200 pg/ml	R: 2,417 pg/ml; NR: 208 pg/ml	N/A	N/A	(44)

Table I. Continued.

C, CTLA-4

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Pistillo *et al*, 2019	Melanoma	n=113	Ipilimumab	ELISA kit (Thermo Fisher Scientific, Inc.)	200 pg/ml	R: 440.4 pg/ml; stable: 268.5 pg/ml; NR: 108.4 pg/ml	N/A	R: Increase during treatment	(45)

D, LAG-3

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Zizzari *et al*, 2020	NSCLC	n=22	Nivolumab	ELISA (LUMINEX xMAP kit)	N/A	N/A	N/A	NR: Increase between baseline and 3 months of treatment	(37)
Machiraju *et al*, 2021	Melanoma	n=48	Pembrolizumab	ELISA kits (LifeSpan BioSciences, Inc. and Abcam)	148 pg/ml	NR: 186 pg/ml; R: 85 pg/ml	N/A	NR: Increase during treatment	(42)

E, TIM-3

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Zizzari *et al*, 2020	NSCLC	n=22	Nivolumab	ELISA (LUMINEX xMAP kit)	N/A	N/A	At 3 months of treatment: R: 5.4 ng/ml; NR: 10 ng/ml	N/A	(37)

Table I. Continued.

F, BTLA-4

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Zizzari *et al*, 2020	NSCLC	n=22	Nivolumab	ELISA (LUMINEX xMAP kit)	N/A	N/A	At 3 months of treatment: R: 1.4 ng/ml; NR: 3.3 ng/ml	N/A	(37)

BTLA-4, B- and T-lymphocyte attenuator-4; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HNSCC, head and neck squamous cell carcinoma; ICI, immune checkpoint inhibitor; LAG-3, lymphocyte activation gene-3; N/A, not available; NR, non-responders; NSCLC, non-small cell lung cancer; PD-1, programmed cell death-protein-1; PD-L1, programmed death-ligand 1; R, responders; ROC, receiver operating characteristic; RCC, renal cell carcinoma; TIM-3, T-cell immunoglobulin and mucin-domain containing-3.

nivolumab treatment. The same study showed a decrease in sPD-L1 (from 1.09 to 0.73 ng/ml) after two cycles of nivolumab among patients with a PFS of >18 months after two cycles of treatment. This conclusion is debatable, as the authors defined high sPD-L1 concentrations as >0.66 ng/ml based on receiver operating characteristic (ROC) curve-derived thresholds, whereas Okuma *et al* (35), applying the same method, reported an optimal threshold of 3.357 ng/ml (Table I). It may be hypothesized that such a difference in the cut-off value could be due to the different methods of sPD-L1 detection: Incorvaia *et al* (38) used a home-made test, whereas Okuma *et al* (35) used a commercially available kit. In addition to this potential cause of difference, the cancer type may also serve a role (namely, renal cancer was the focus of the former study, whereas lung cancer was the focus of the latter).

In patients with mismatch repair-proficient colorectal cancer treated with sintilimab and regorafenib, the baseline and end-of-treatment sPD-L1 levels were not significantly different between patients with durable clinical benefit (DCB) and those without. However, focusing on the change between baseline and fourth treatment cycle, the authors found a notable increase in sPD-L1 levels among patients with progression, whereas no marked change was observed in those who achieved DCB (39) (Table I). Zhou *et al* (40) observed that all patients with high levels of sPD-L1 (>1.4 ng/ml) before anti-CTLA-4 (ipilimumab) treatment experienced progressive disease. In addition, patients with a 1.5-fold increase in sPD-L1 levels after 5 months of treatment were more likely to experience only a partial response to pembrolizumab or to ipilimumab. In contrast to the aforementioned studies, Boschert *et al* (41) reported that higher baseline sPD-L1 levels predicted a better response to ICIs in head-and-neck carcinoma. Notably, the mean sPD-L1 concentration of the responder group was 740 pg/ml vs. 94.76 pg/ml in the non-responder group (Table I).

Taken together, these results indicate that baseline sPD-L1 levels and its dynamics over the first six cycles of anti-PD-1 treatment are worth further studying as biomarkers predictive of response or treatment failure.

PD-1. In melanoma, Machiraju *et al* (42) observed that high baseline soluble PD-1 (sPD-1) concentrations (>167 pg/ml) were associated with resistance to a combination of nivolumab and ipilimumab. Similar results were observed in melanoma by Incorvaia *et al* (43): Significantly lower baseline concentrations were observed among the responders to nivolumab or pembrolizumab compared with the non-responders (10.3 vs. 16.6 ng/ml, respectively). Higher baseline sPD-1 concentrations (>11.24 ng/ml) were also associated with a shorter TTF. A few years earlier, Incorvaia *et al* (38) published contradictory results in metastatic renal cell carcinoma: High baseline sPD-1 levels (>2.11 ng/ml) were associated with improved response and survival. Furthermore, sPD-1 significantly decreased, from 13.25 to 1.23 ng/ml, after two cycles of nivolumab among the responders (38). Notably, the optimal sPD-1 cut-off markedly differed between the two studies: In 2020, it was calculated to be 2.11 ng/ml in a cohort of 22 patients, whereas in 2023 it was estimated to be 11.24 ng/ml in a cohort of 41 patients (38,43) (Table I). Therefore, the results of these

two studies should be interpreted with caution. In NSCLC, Zizzari *et al* (37) reported significantly lower sPD-1 levels at 3 months of treatment with nivolumab in the responder group (31 pg/ml) compared with in the non-responder group (53 pg/ml). Subsequently, by evaluating dynamic changes in sPD-1 during treatment, a lower sPD-1 level after 3 months of treatment was found in responders due to a significant decrease (from 67 pg/ml at baseline to 31 pg/ml at 3 months of treatment) of the protein during this period compared with the pre-treatment values (Table I). Thus, similar to sPD-L1, lower levels of baseline sPD-1 and/or their decrease on treatment may be associated with improved response to ICIs.

CTLA-4. Leung *et al* (44) demonstrated that patients with stage IV melanoma who derived a clinical benefit from ipilimumab had significantly higher baseline serum CTLA-4 (sCTLA-4) levels than patients without clinical benefit (2,417 vs. 208 pg/ml, respectively). Furthermore, the median OS time was 43.2 months for patients with baseline sCTLA-4 >200 pg/ml, whereas it was only 5.9 months for patients with sCTLA-4 <200 pg/ml. Similar results were obtained in a larger study: The mean baseline sCTLA-4 levels for the responder, stable disease and progression group were 440.4, 268.5 and 108.4 pg/ml respectively, and patients with baseline sCTLA-4 levels >200 pg/ml had improved response to ipilimumab and survival (45). Notably, the cut-off of 200 pg/ml was identified by the ROC approach by Leung *et al* (44), whereas it the median sCTLA-4 concentration was used as the cut-off value by Pistillo *et al* (45). The median level of sCTLA-4 in Pistillo *et al* (45) clearly reflected significantly higher sCTLA-4 levels in patients with melanoma than in healthy subjects, in whom sCTLA was in the range of 10-40 pg/ml. sCTLA-4 in melanoma likely originates both from alternative splicing of the CTLA-4 gene in tumour cells and from activated peripheral T cells, particularly regulatory T cells (46). Therefore, sCTLA-4 reflects the global CTLA-4 burden (namely, the availability of the ipilimumab target). In other words, patients with melanoma with >200 pg/ml CTLA-4 have their immune system strongly suppressed by CTLA-4, and administration of ipilimumab provides a stronger relief from this suppression than that in patients with lower sCTLA-4 levels. Zizzari *et al* (37) showed that patients with NSCLC who responded to nivolumab had significantly lower sCTLA-4 levels after 3 months of treatment than non-responders (61 vs. 86 pg/ml, respectively).

Together, the aforementioned data indicate that higher pre-treatment and lower mid-treatment levels of sCTLA-4 could predict a higher probability of response to ipilimumab or nivolumab (Table I).

Other circulating immune checkpoint regulators. Tumour tissue upregulation of the alternative immune suppressive pathways is one of the mechanisms responsible for acquired resistance to ICIs (47). These modifications can result in the release into circulation of immune checkpoint regulators other than PD-1 or PD-L1. In a previous small study (n=22) on nivolumab-treated patients with NSCLC, non-responders presented higher soluble T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte activation gene-3 (LAG-3) and B- and T-lymphocyte attenuator-4 concentrations than responders after 3 months of treatment (37). Another previous larger study (n=48) on pembrolizumab-treated patients with melanoma showed that the non-responders had significantly higher baseline sLAG-3 levels (186 pg/ml) than patients with stable disease (85 pg/ml). Furthermore, in the resistant patients, sLAG-3 was significantly increased during the first 6 weeks of treatment. These associations between sLAG-3 and response to treatment were not observed in patients treated with ipilimumab (n=23) or with a combination of ipilimumab and nivolumab (n=42) (42) (Table I). The value of sTIM-3 and sLAG-3 quantification for personalized immuno-oncology remains to be determined; however, with advances in the clinical development of TIM-3 and LAG-3 inhibitors such as sabatolimab (48) and relatlimab (49), it is highly probable that LAG-3 will form part of analyte panels to be tested in upcoming clinical trials.

Cytokines. Cytokines, which are essential proteins for communication between immune cells, serve key roles in both the activation and suppression of the immune response. Their blood levels are one of the best reflectors of immune system activity (50).

Interleukins (ILs) are the cytokines most extensively investigated as predictors of immunotherapy response. Several previous studies have shown that high baseline serum IL-6 (sIL-6) concentrations are associated with a poor response to PD-1/PD-L1 blockade. In NSCLC, the sIL-6 cut-off levels were similar across numerous studies, ranging from 11.1 to 13.8 pg/ml (51-54). Patients with sIL-6 above these levels have been reported to have a lower probability of responding to anti-PD-1 drugs as summarized in Table II, which lists cytokines and their associations with immunotherapy response. In squamous NSCLC, an increase in sIL-6 concentrations between baseline and the fifth treatment cycle of nivolumab alone or nivolumab with ipilimumab was observed among non-responders by Parra *et al* (29). In gastric cancer, Qi *et al* (55) showed that sIL-6 levels >13.3 pg/ml after two cycles of chemotherapy and PD-1 blockade were notably associated with a shorter PFS (Table II).

The response to treatments combining ICIs and targeted therapy has been shown to be associated with lower baseline or on-treatment levels of sIL-6 in patients. In patients with hepatocellular carcinoma treated with atezolizumab and bevacizumab, the responder group presented with significantly lower sIL-6 levels at both the baseline and after 6 weeks of treatment compared with in the non-responder group (Table II) (56). In patients with renal carcinoma treated with pembrolizumab combined with axitinib, the baseline sIL-6 levels were significantly lower in responders (8.6 pg/ml) than in non-responders (84.1 pg/ml) (57). Similarly, in a trial of nivolumab and ipilimumab, with or without enzalutamide, in metastatic castration-resistant prostate cancer, lower baseline concentrations of sIL-6 were associated with improved outcomes (58). Finally, two studies conducted in advanced melanoma treated with ipilimumab or nivolumab showed contrasting results regarding sIL-6: Cristiani *et al* (59) showed that low concentrations of sIL-6 (<6.80 pg/ml) were associated with improved PFS and OS, whereas Yamazaki *et al* (60) revealed that the concentrations of sIL-6 were markedly higher in patients with an objective response to nivolumab than in

Table II. Circulating cytokines as potential biomarkers of response to immunotherapy.

A, IL-6

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Jin *et al*, 2024	Various types	n=65	Anti-PD-1/ PD-L1	ELISA kit (Ella system; ProteinSimple; Bio-Techne)	N/A	R: 2,365 pg/ml; NR: 1,769 pg/ml	N/A	N/A	(54)
Cristiani *et al*, 2022	Melanoma	n=24	Nivolumab	ELISA (Cytokine Array I kit; Randox Laboratories, Ltd.)	6.80 pg/ml	N/A	At 2 months of treatment: R <6.80 pg/ml; NR >6.80 pg/ml	N/A	(59)
Yamazaki *et al*, 2017	Melanoma	n=37	Nivolumab	ELISA (Procarta Cytokine Assay kit; Affymetrix; Thermo Fisher Scientific, Inc.)	N/A	R: Higher concentration	N/A	N/A	(60)
Qi *et al*, 2023	Gastric cancer	n=52	Anti-PD-1 + chemotherapy	Multiplex microsphere-based flow cytometric assays (Qingdao kit)	13.33 pg/ml	N/A	After 2 cycles: R <13.33 pg/ml; NR >13.33 pg/ml	N/A	(55)
Yang *et al*, 2023	HCC	n=165	Atezolizumab + bevacizumab	Cytometric bead array (BD Biosciences)	18.49 pg/ml	R: 5.05 pg/ml; NR: 11.56 pg/ml	At 6 weeks of treatment: R: 3.62 pg/ml; NR: 14.15 pg/ml	N/A	(56)
Sang *et al*, 2022	RCC	n=58	Pembrolizumab + axitinib	Cytometric bead array (BD Biosciences)	6.5 pg/ml	R: 8.6 pg/ml; stable: 9,9 pg/ml; NR: 84.1 pg/ml	N/A	N/A	(57)
Shenderov *et al*, 2021	Prostate cancer	n=30	Nivolumab + ipilimumab +/- enzalutamide	ELISA Luminex (R&D Systems, Inc.)	N/A	R: Lower concentration	N/A	N/A	(58)
Parra *et al*, 2024	Squamous NSCLC	n=160	Nivolumab or nivolumab + ipilimumab	Proximity extension assay (Olink Immuno-oncology panel)	N/A	N/A	N/A	NR: Increase between baseline and cycle 5 (week 9)	(29)
Shi *et al*, 2021	NSCLC	n=103	Anti-PD-(L)1	Electrochemi-luminescence immunoassay	13.8 pg/ml	R <13.8 pg/ml; NR >13.8 pg/ml	N/A	N/A	(51)

Table II. Continued.

A, IL-6

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Kauffmann-Guerrero *et al*, 2021	NSCLC	n=29	Nivolumab or pembrolizumab	ELISA Human Cytokine-Inflammation (9-plex) kit; BioVendor]	11.6 pg/ml	R <11.6 pg/ml; NR >11.6 pg/ml	N/A	N/A	(52)
Liu *et al*, 2022	NSCLC	n=45	Nivolumab	ELISA (LEGENDplex kit)	11.15 pg/ml	R <11.15 pg/ml; NR >11.15 pg/ml	N/A	N/A	(53)

B, IL-8

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Kauffmann-Guerrero *et al*, 2021	NSCLC	n=29	Nivolumab or pembrolizumab	ELISA [Human Cytokine-Inflammation (9-plex) kit; BioVendor]	19.6 pg/ml	R <19.6 pg/ml; NR >19.6 pg/ml	N/A	N/A	(52)
Parra *et al*, 2024	Squamous NSCLC	n=160	Nivolumab or nivolumab + ipilimumab	Proximity extension assay (Olink Immuno-oncology panel)	N/A	N/A	N/A	NR: Positive regulation before radiological progression	(29)
Schalper *et al*, 2020	Squamous NSCLC/ non-squamous	n=107/ n=255	Nivolumab	In-house multiplex immuno-fluorescence assay	23 pg/ml	R <23 pg/ml; NR >23 pg/ml	N/A	N/A	(61)
Sanmamed *et al*, 2017	NSCLC	n=19	Nivolumab or pembrolizumab	ELISA kit (BD Pharmingen; BD Biosciences)	>9.2%	N/A	N/A	R: Decrease or increase <9.2% between baseline and 2-3 weeks of treatment; NR: Increase >9.2% between baseline and 2-3 weeks of treatment	(62)

Table II. Continued.

B, IL-8

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Sanmamed *et al*, 2017	Melanoma	n=29	Nivolumab or pembrolizumab	ELISA kit (BD Pharmingen; BD Biosciences)	N/A	N/A	N/A	R: Decrease between baseline and BR; NR: Increase between baseline and PD	(62)
Cristiani *et al*, 2022	Melanoma	n=24	Nivolumab	ELISA (Cytokine Array I kit; Randox Laboratories, Ltd.)	28.79 pg/ml	N/A	At 2 months of treatment: R <28.79 pg/ml; NR >28.79 pg/ml	N/A	(59)
Sanmamed *et al*, 2017	Melanoma	n=15	Ipilimumab + nivolumab	ELISA kit (BD Pharmingen; BD Biosciences)	N/A	N/A	N/A	R: Decrease between baseline and BR; NR: Increase between baseline and PD	(62)

C, IL-4

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Qi *et al*, 2023	Gastric cancer	n=52	Anti-PD-1 + chemotherapy	Multiplex microsphere-based flow cytometric assays (Qingdao kit)	1.279 pg/ml	N/A	After 4 cycles: R >1.279 pg/ml NR <1.279 pg/ml	N/A	(55)
Haddox *et al*, 2024	Sarcoma	n=57	Eribulin + pembrolizumab	Luminex multiplex (FLEXMAP 3D kit)	N/A	R: Higher concentration	At 1 week: R: Higher concentration	N/A	(63)

Table II. Continued.

D, IL-5

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Zhao *et al*, 2021	Gastric cancer	n=35	Anti-PD-1	Flow cytometry	N/A	R: 7.07 pg/ml; NR: 2.37 pg/ml	BR: 3.78 pg/ml; PD: 4.67 pg/ml	R: Decrease between baseline and BR; NR: Increase between baseline and PD	(64)
Zhao *et al*, 2021	NSCLC	n=40	Anti-PD-1	Flow cytometry	N/A	R: 6.50 pg/ml; NR: 2.43 pg/ml	BR: 2.56 pg/ml; PD: 4.87 pg/ml	R: Decrease between baseline and BR; NR: Increase between baseline and PD	(64)

E, IL-7

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Shenderov *et al*, 2021	Prostate cancer	n=30	Nivolumab + ipilimumab +/- enzalutamide	ELISA (R&D Systems, Inc.)	N/A	R: Lower concentration	N/A	N/A	(58)

F, IL-10

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Yamazaki *et al*, 2017	Melanoma	n=37	Nivolumab	ELISA (Procarta Cytokine Assay Kit; Affymetrix; Thermo Fisher Scientific, Inc.)	N/A	R: Higher concentration	N/A	N/A	(60)
Kim *et al*, 2023	RCC	n=69	Pembrolizumab + axitinib or ipilimumab + nivolumab or pembrolizumab + lenvatinib	Cytometric bead assay (Human Th1/Th2/Th17 CBA kit; BD Biosciences)	4.3 ng/ml	R <4.3 ng/ml; NR >4.3 ng/ml	At cycle 2: R <4.3 ng/ml; NR >4.3 ng/ml	N/A	(65)

Table II. Continued.

G, IL-17

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Shenderov *et al*, 2021	Prostate cancer	n=30	Nivolumab + ipilimumab +/- enzalutamide	ELISA Luminex (R&D Systems, Inc.)	N/A	R: Higher concentration	N/A	N/A	(58)

H, CSF-1

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Parra *et al*, 2024	Squamous NSCLC	n=160	Nivolumab or nivolumab + ipilimumab	Proximity extension assay (Olink Immuno-oncology panel)	N/A	N/A	N/A	NR: Positive regulation before radiological progression	(29)

I, IFN- γ

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Qi *et al*, 2023	Gastric cancer	n=52	Anti-PD-1 + chemotherapy	Multiplex microsphere-based flow cytometric assays (Qingdao kit)	10.79 pg/ml	R: 14.12 pg/ml; NR: 8.99 pg/ml	N/A	N/A	(55)
Zhao *et al*, 2021	Gastric cancer	n=35	Anti-PD-1	Flow cytometry	N/A	R: 6.29 pg/ml NR: 2.81 pg/ml	BR: 3.27 pg/ml; PD: 7.51 pg/ml	R: Decrease from baseline to BR NR: Increase from baseline to PD	(64)
Zhao *et al*, 2021	NSCLC	n=40	Anti-PD-1	Flow cytometry	N/A	R: 8.74 pg/ml; NR: 2.77 pg/ml	BR: 6.27 pg/ml; PD: 8.24 pg/ml	R: Decrease between baseline and BR; NR: Increase between baseline and PD	(64)

Table II. Continued.

I, IFN- γ

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Yamazaki *et al*, 2017	Melanoma	n=37	Nivolumab	ELISA (Procarta Cytokine Assay kit; Affymetrix; Thermo Fisher Scientific, Inc.)	N/A	R: Higher concentration	N/A	N/A	(60)
Haddox *et al*, 2024	Sarcoma	n=57	Eribulin + pembrolizumab	Luminex multiplex (FLEXMAP 3D kit)	N/A	R: Higher concentration	At 1 week: R: Higher concentration	N/A	(63)
Kauffmann-Guerrero *et al*, 2021	NSCLC	n=29	Nivolumab or pembrolizumab	ELISA [Human Cytokine-Inflammation (9-plex) kit; BioVendor]	16.7 pg/ml	R <16.7 pg/ml; NR >16.7 pg/ml	N/A	N/A	(52)

J, TGF-β

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Feun *et al*, 2019	HCC	n=24	Pembrolizumab	ELISA kit (R&D Systems, Inc.)	200 pg/ml	R: 141.9 pg/ml; NR: 1,071 pg/ml	N/A	N/A	(66)

K, CD25

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Carbone *et al*, 2022	Melanoma	n=57	Nivolumab or pembrolizumab	ELISA kit (R&D Systems, Inc.)	N/A	R: 1,348 pg/ml; NR: 451 pg/ml	N/A	N/A	(67)

Table II. Continued.

L, CD27

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Benhamouda *et al*, 2022	RCC	BIONIKK cohort n=46	Anti-PD-1	ELISA kit (Thermo Fisher Scientific, Inc.)	112.71 U/ml	R <112.71 U/ml; NR >112.71 U/ml	N/A	N/A	(69)
Benhamouda *et al*, 2022	RCC	Colcheck-point cohort n=25	Anti-PD-1	ELISA kit (Thermo Fisher Scientific, Inc.)	91.8 U/ml	R <91.8 U/ml NR >91.8 U/ml	N/A	N/A	(69)
Carbone *et al*, 2022	Melanoma	n=57	Nivolumab or pembrolizumab	ELISA kit (R&D Systems, Inc.)	N/A	N/A	At 1 month of treatment: R: 1,372 pg/ml; NR: 2,493 pg/ml	N/A	(67)

BR, best response; CSF-1, colony stimulating factor 1; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; IL, interleukin; IFN-γ, interferon γ; N/A, not available; NR, non-responders; NSCLC, non-small cell lung cancer; PD, progressive disease; PD-1, programmed cell death-protein-1; PD-L1, programmed death-ligand 1; R, responders; RCC, renal cell carcinoma; ROC, receiver operating characteristic; TGF-β, transforming growth factor β.

patients who experienced disease progression after this treatment (Table II). The main difference between these two studies was the IL-6 quantification time point: IL-6 was detected before treatment in Yamazaki *et al* vs. at 2 months after treatment in Christiani *et al*. With that in mind, the former study results would reflect, as the authors evoked, a stronger antitumour immunity already present before treatment and further stimulated by anti-PD-1 or anti-CTLA-4 treatment. Whereas the results of the latter study may reflect a reduction in the burden of an IL-6-producing tumour. These hypotheses should be verified in future studies by assessment of both the tumour tissue and sIL-6 levels together with the activation status of peripheral lymphocytes.

Similar to sIL-6, serum IL-8 (sIL-8) has mainly been identified as an unfavourable biomarker of response and outcome in immunotherapy studies. Schalper *et al* (61) observed in 107 patients with squamous NSCLC and 255 patients with non-squamous NSCLC that baseline sIL-8 >23 pg/ml was associated with a poor response to nivolumab (61). Kauffmann-Guerrero *et al* (52) reported similar findings in a smaller cohort treated with anti-PD-1, using 19.6 pg/ml as the cut-off value for baseline sIL-8 levels. The majority of studies that have analysed sIL-8 dynamics during treatment with ICIs have shown that an increase in sIL-8 levels compared with baseline levels is associated with a lack of response and/or with progressive disease. For example, Sanmamed *et al* (62) assessed patients with NSCLC treated with anti-PD-1 monotherapy and the results revealed that sIL-8 levels decreased from 20.8 pg/ml at baseline to 6.5 pg/ml at the time of best response (BR), whereas they increased from the baseline level of 15 pg/ml to 51 pg/ml at the moment of progressive disease diagnosis. Similar findings were reported in patients with squamous NSCLC or melanoma treated with nivolumab alone or in combination with ipilimumab (29,59,62) (Table II). Sanmamed *et al* also defined a cut-off (9.2%) of sIL-8 level change between baseline and 2-3 weeks of treatment, which best separated responders from non-responders (Table II).

As part of multi-analyte panels, several other cytokines have been assessed in the serum of patients treated with ICIs. Haddox *et al* (63) detected higher serum IL-4 (sIL-4) levels at baseline and at 1 week of treatment in patients with sarcoma who responded to a combination of eribulin and pembrolizumab. Furthermore, patients with gastric cancer treated with immunochemotherapy who had an increase in sIL-4 after four cycles of treatment (>1,279 pg/ml) also exhibited significantly improved PFS (55). By contrast, serum IL-5 (sIL-5) concentration was decreased between baseline levels (7.07 pg/ml) and time of BR (3.78 pg/ml) in patients with gastric cancer treated with PD-1 blockade; similar dynamics of sIL-5 were observed in a cohort of patients with NSCLC analysed by the same authors (64). Serum IL-7 and IL-17 levels were investigated in prostate cancer, where low and high baseline concentrations, respectively, were associated with an improved response to a combination of nivolumab with ipilimumab, with or without enzalutamide (58). Serum IL-10 (sIL-10) levels below a threshold of 4.3 ng/ml at baseline and after two cycles of ICI was associated with improved response in patients with renal cancer (65). However, in patients with nivolumab-treated melanoma, higher baseline concentrations of sIL-10 were associated with improved response (60) (Table II).

Zhao *et al* (64) showed a decrease in serum interferon γ (sIFN-γ) levels from baseline to time of BR in responders, whereas they increased from baseline to progressive disease in both NSCLC and gastric cancer. In another study on patients with gastric cancer treated with anti-PD-1, patients with low baseline levels of sIFN-γ (<10.79 pg/ml) had a median PFS of 119 vs. 267.5 days for patients with high levels of sIFN-γ (>10.79 pg/ml) (55). Higher sIFN-γ levels were also observed at baseline in melanoma responders treated with nivolumab and in sarcoma responders treated with eribulin associated with pembrolizumab (60,63). Kauffmann-Guerrero *et al* (52) showed contradictory results in NSCLC, where patients with sIFN-γ >16.7 pg/ml at baseline had no response to anti-PD-1 treatment (Table II).

Serum transforming growth factor β has been investigated in patients with hepatocellular carcinoma treated with pembrolizumab. The responders had markedly lower baseline concentrations than the non-responders (141.9 vs. 1,071 pg/ml, respectively) (66). Serum colony-stimulating factor 1 was reported to increase before radiological progression in a cohort of 160 patients with squamous NSCLC treated with nivolumab alone or in combination with ipilimumab (29) (Table II).

The cytokine receptors CD25 and CD27 have been studied in patients treated with PD-1 inhibitors. Patients with melanoma who responded to treatment presented higher baseline serum CD25 levels (1,348 pg/ml) compared with non-responders (451 pg/ml) (67). In the same patients, serum CD27 (sCD27) was found to be significantly lower among the responders after 1 month of treatment (1,372 pg/ml) compared with the non-responders (2,493 pg/ml) (67). Sam *et al* (68) showed, in two large cohorts of patients with melanoma (namely PREDIMEL, n=74 and MelBase, n=210), that sCD27 levels >100 U/ml were associated with resistance to pembrolizumab alone but not when it was combined with anti-CTLA-4 treatment. In metastatic renal cell carcinoma treated with anti-PD-1 therapy, higher circulating sCD27 levels were associated with poorer overall survival across independent cohorts (Colcheckpoint cohort and BIONIKK-like cohort), although the optimal prognostic cutoff varied between studies (91.8 vs. 112.71 U/ml). Despite differences in ROC-defined cut-off values across cohorts, elevated sCD27 was consistently associated with worse survival outcomes in patients with metastatic renal cell carcinoma receiving anti-PD-1 therapy (69) (Table II). Finally, a comprehensive article on the CD27-CD70 axis in cancer immunotherapy has indicated that high blood levels of CD27 are associated with resistance to PD-1/PD-L1 blockade in several types of cancer (68). Thus, quantification of circulating CD27 may serve to select patients for ICI treatment escalation.

Chemokines. As with cytokines, serum chemokines can provide information on the mechanisms underlying ICI resistance, often linked to the appearance of immune-related AEs (50). In melanoma, Kasanen *et al* (70) showed a significant increase in three C-X-C motif chemokine ligands (CXCLs), namely CXCL9, CXCL10 and CXCL11, between baseline and 1 month of nivolumab treatment among the responders, while no significant change was observed among the non-responders. In

a larger cohort of patients with ipilimumab-treated melanoma, serum CXCL11 levels <35 pg/ml were associated with response to treatment (71). Similar observations were reported in another study regarding serum CXCL9 (sCXCL9) baseline levels, which were significantly higher in responders to PD-1 inhibition (75 pg/ml) than in non-responders (0.4 pg/ml); sCXCL9 was much higher in responders than in non-responders also after 1 and 2 months of treatment (67). This protein was also studied in patients with hepatocellular carcinoma treated with anti-PD-1. sCXCL9 levels <478 pg/ml were primarily associated with progressive disease, whereas levels exceeding twice the threshold value of 478 pg/ml were associated with improved response to treatment (72). Serum chemokines including CXCL8, CXCL9, CXCL10, CXCL12 and CXCL13 have been evaluated in ICI-treated NSCLC cohorts, with heterogeneous and sometimes conflicting results. While several studies have reported that increased serum levels of CXCL8, CXCL10 or CXCL12 are associated with poorer outcomes (reduced OS, PFS or DCB), other studies have shown that higher CXCL9 and CXCL10 levels are associated with improved response and PFS. In addition, an early decrease in CXCL8 levels during treatment has been associated with better survival outcomes in patients with NSCLC (72). Parra *et al* (29) observed an increase in CXCL13 among non-responding patients with squamous cell lung cancer between baseline and cycle 5 (week 9 of treatment with nivolumab in monotherapy or in combination with ipilimumab). Furthermore, CCL2 was the focus of a study on a cohort of 24 patients with melanoma treated with nivolumab; patients responding to treatment presented with concentrations of >446.72 pg/ml (59). A summary of the reported chemokines and their associations with immunotherapy response is presented in Table III.

Cellular modulators. Among the proteins involved in the regulation of cellular processes, the most investigated ones in terms of their response to ICIs are those involved in mesenchymal cell proliferation and angiogenesis, including fibroblast growth factor (FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and angiopoietin-2 (ANG-2).

Higher baseline concentrations of serum HGF (sHGF) have been observed in patients with melanoma who responded to PD-1 or CTLA-4 blockade and to their combination (73). Similarly, in a cohort including various cancer types, patients who responded to anti-PD-1 or anti-PD-L1 therapy showed significantly higher baseline levels of sHGF compared with the non-responders (2,365 vs. 1,769 pg/ml, respectively) (54). In patients with hepatocellular carcinoma treated with a combination of atezolizumab and bevacizumab, Yang *et al* (74) observed a significant increase in serum FGF-19 concentrations between baseline and the BR time-point, whereas serum ANG-2, together with serum VEGF-D, significantly increased at the time of progression in patients who initially responded to treatment. Cristiani *et al* (59) showed that patients with melanoma with better response to two cycles of nivolumab had serum VEGF levels <413.06 pg/ml at that time-point. Table IV summarizes circulating cellular modulators and their reported associations with response to immunotherapy. These findings indicate that circulating angiogenesis markers are worth further validation in the ICI treatment setting, particularly to indicate development of resistance to ICIs, which could be suppressed by angiogenesis inhibitors.

5. Discussion and perspectives

Circulating proteins are emerging biomarkers for cancer immunotherapy. As shown in the present review, the data to date are heterogeneous; however, some soluble proteins such as PD-L1, IL-6, IL-8, CD27 and angiogenesis markers appear to be particularly promising as biomarkers of response or resistance to ICIs. The expression of ICI targets within the tumour tissue are inherently dynamic, shaped by immune editing, and so is the release into the bloodstream of proteins that are results of these intra-tumour changes. Namely, the presence of various ICI targets in the TME and in the bloodstream suggest the possibility of ligand recycling on tumour and immune cells, representing the dynamic part of the tumour-resident lymphocyte clones. Luoma *et al* (75) demonstrated that tumour-infiltrating $CD8^+$ T cells expand during neoadjuvant ICI treatment in oral cancer, and express elevated cytotoxicity and tumour-resident memory programs. In addition, this treatment induced a systemic immune response, characterized by the expansion of activated T cells enriched in tumour-infiltrating T-cell clonotypes, including both pre-existing and emergent clonotypes undetectable prior to therapy. The ICI-induced boost of both intra-tumour and systemic immunity can generate a plethora of soluble ICI targets potentially reflecting the efficacy of ICI treatment. It should be expected to encounter a great diversity of soluble proteins associated with response to cancer immunotherapy, as various programs such as angiogenesis and tissue injury/remodelling may be activated at both the tumour and systemic levels. Another aspect that can be reflected by circulating proteins is the association between the patient microbiome and the immune system, as recently shown in renal cancer (76). In such situations, the non-invasiveness of blood sampling and ease of serum protein profiling using modern high-plex technologies call for broader implementation of circulating protein assessment in ICI clinical trials.

The majority of studies conducted thus far on predictive circulating protein biomarkers have focused on melanoma and lung cancer. While these studies have yielded promising results, validation across other tumour types is essential to establish the clinical relevance of serum proteomics for cancer immunotherapy. For example, recent and ongoing investigations in triple-negative breast cancer (TNBC) are expected to reveal additional areas of blood protein profiling use in immuno-oncology (77,78). Although circulating proteins are not included in the list of essential biomarkers to assess in cancer immunotherapy trials (79), the number of new trials in the field that include serum protein profiling (particularly cytokines) is expected to increase. Notably, activation of both tumour tissue and cells circulating in peripheral blood is reflected by changes in circulating cytokines and chemokines, as shown, for example, by Stopeck *et al* (80) on a series of 44 metastatic TNBC cases. The studies on circulating proteins in patients with melanoma and lung cancer treated by immunotherapies presented in the current review suggest that monitoring of immunotherapy efficacy can be performed by dynamic assessment of this biomarker type; however, larger cohorts of serum/plasma samples are necessary for

Table III. Circulating chemokines as potential biomarkers of response to immunotherapy.

A, CCL2

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Cristiani *et al*, 2022	Melanoma	n=24	Nivolumab	ELISA (Cytokine Array I kit; Randox Laboratories, Ltd.)	446.72 pg/ml	R >446.72 pg/ml; NR <446.72 pg/ml	N/A	N/A	(59)

B, CXCL11

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Koguchi *et al*, 2015	Melanoma	n=137	Ipilimumab	ELISA kit (R&D Systems, Inc.)	35 pg/ml	R <35 pg/ml; NR >35 pg/ml	N/A	N/A	(82)
Kasanen *et al*, 2020	Melanoma	n=17	Nivolumab	Proximity extension assay (Olink Proseek Multiplex Inflammation panel)	N/A	N/A	N/A	R: Increase between baseline and 1 month of treatment	(70)

C, CXCL10

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Kasanen *et al*, 2020	Melanoma	n=17	Nivolumab	Proximity extension assay (Olink Proseek Multiplex Inflammation panel)	N/A	N/A	N/A	R: Increase between baseline and 1 month of treatment	(70)

D, CXCL9

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Kasanen *et al*, 2020	Melanoma	n=17	Nivolumab	Proximity extension assay (Olink Proseek Multiplex Inflammation panel)	N/A	N/A	N/A	R: Increase between baseline and 1 month of treatment	(70)

Table III. Continued.

D, CXCL9

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Carbone *et al*, 2022	Melanoma	n=57	Nivolumab or pembrolizumab	ELISA kit (R&D Systems, Inc.)	N/A	R: 75 pg/ml; NR: 0.4 pg/ml	At 1 month of treatment: PR: 396 pg/ml; NR: 124 pg/ml At 3 months of treatment: PR: 591 pg/ml; NR: 74 pg/ml	R: Increase between baseline and 3 months of treatment; NR: Decrease between 1 and 3 months	(67)
Wang *et al*, 2024	HCC	n=123	Anti-PD-1	ELISA kit (R&D Systems, Inc.)	478 pg/ml	R >2X ULC; NR <478 pg/ml	N/A	N/A	(71)

E, CXCL13

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Parra *et al*, 2024	Squamous NSCLC	n=160	Nivolumab or nivolumab + ipilimumab	Proximity extension assay (Olink Immuno-oncology panel)	N/A	N/A	N/A	NR: Increase between baseline and cycle 5 (week 9)	(29)

CXCL, C-X-C motif chemokine ligand; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; N/A, not available; NR, non-responders; NSCLC, non-small cell lung cancer; PD-1, programmed cell death-protein-1; PR, partial response; R, responders; ROC, receiver operating characteristic. ULC, upper limit of the cutoff value.

Table IV. Circulating cellular modulators as potential biomarkers of response to immunotherapy.

A, HGF

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Rossi *et al*, 2022	Melanoma	n=87	Anti-PD-1, anti-CTLA-4, anti-PD-1 + anti-CTLA-4	Proximity Extension Assay technology (Olink Inflammation panel)	N/A	NR: Higher concentration	N/A	N/A	(73)
Yang *et al*, 2023	HCC	n=32	Atezolizumab + bevacizumab	Cytometric Bead Array kit (BD Biosciences)	N/A	N/A	N/A	R: Decrease between baseline and BR	(74)
Jin *et al*, 2024	Cancer	n=65	Anti-PD-1/ PD-L1	ELISA kit (Ella system; ProteinSimple; Bio-Techne)	N/A	R: 2,365 pg/ml; NR: 1,769 pg/ml	N/A	N/A	(54)

B, FGF-19

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Yang *et al*, 2023	HCC	n=32	Atezolizumab + bevacizumab	ELISA kit (R&D Systems, Inc.)	N/A	N/A	N/A	R: Increase between baseline and BR	(74)

C, VEGF-D

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Yang *et al*, 2023	HCC	n=28	Atezolizumab + bevacizumab	Cytometric Bead Array kit (BD Biosciences)	N/A	N/A	N/A	NR: Increase between BR and PD	(74)

D, VEGF

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Cristiani *et al*, 2022	Melanoma	n=24	Nivolumab	ELISA (Cytokine Array I kit; Randox Laboratories, Ltd.)	413.06 pg/ml	N/A	At 2 months of treatment: R <413.06 pg/ml; NR <413.06 pg/ml	N/A	(59)

Table IV. Continued.

E, ANG-2

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Yang *et al*, 2023	HCC	n=28	Atezolizumab + bevacizumab	Cytometric Bead Array kit (BD Biosciences)	N/A	N/A	N/A	R: Decrease between baseline and BR; NR: Increase between BR and PD	(74)

F, LAMP-3

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Parra *et al*, 2024	Squamous NSCLC	n=160	Nivolumab or nivolumab + ipilimumab	Proximity extension assay (Olink Immuno-oncology panel)	N/A	N/A	N/A	R: Increase between baseline and cycle 5 (week 9)	(29)

G, MMP12

First author, year	Cancer	Cohort	Evaluated ICI	Assay method	ROC cut-off concentration	Baseline measurement	Measurement during treatment	Dynamic measurement	(Refs.)
Parra *et al*, 2024	Squamous NSCLC	n=160	Nivolumab or nivolumab + ipilimumab	Proximity extension assay (Olink Immuno-oncology panel)	N/A	N/A	N/A	NR: Positive regulation before radiological progression	(29)

ANG-2, angiopoietin-2; BR, best response; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FGF, fibroblast growth factor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; ICI, immune checkpoint inhibitor; LAMP-3, lysosome-associated membrane protein 3; N/A, not available; NR, non-responders; NSCLC, non-small cell lung cancer; PD, progressive disease; PD-1, programmed cell death-protein-1; PD-L1, programmed death-ligand 1; R, responders; VEGF, vascular endothelial growth factor.

the identification of robust predictors. Therefore, systematic blood sampling before, during and after treatment with any immunotherapy for any cancer type is highly recommended, to constitute biological collections assessable by various proteomics technologies. At present, the data are not sufficient to propose a 'universal' cytokine/chemokine panel that is worth assessing in all cancer types; however, some biofluid proteomics technology providers offer notably large panels that can be applied in biomarker discovery, as has been recently shown in an ancillary study linked to the OXEL trial in TNBC (81).

It may be hypothesized that combining analysis of circulating proteins with other types of biomarkers, both circulating and tissue-based, would provide a more comprehensive and accurate assessment of the likelihood of a patient to respond to ICIs and to have a durable benefit from them. Relying on a single biomarker, or even on a single class of biomarkers, is unlikely to be sufficient in immuno-oncology; instead, composite biomarkers are required to better capture the complexity and dynamic nature of the cancer-immune interplay and its modulation by immunotherapies (9). Circulating proteins should be included as important parameters in this approach. Their assessment is of particular value in limited-resource settings, where combinations of blood protein levels and blood cell counts or their ratios, may generate powerful and affordable biomarkers to tailor treatments with high-cost drugs such as ICIs.

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Authors' contributions

CP and NRR designed and performed the literature search. CP wrote the initial draft of the manuscript. NRR edited the initial draft (including adding text). XD, AG and CA reviewed and edited the manuscript. CP and NRR brought the manuscript it to its final form. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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