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Circulating biomarkers as predictors of response to immune checkpoint inhibitors in NSCLC: Are we on the right path?

Calogera Claudia Spagnolo [a,1], Francesco Pepe [b,1], Giuliana Ciappina [a], Francesco Nucera [c], Paolo Ruggeri [c], Andrea Squeri [a], Desirèe Speranza [a], Nicola Silvestris [a], Umberto Malapelle [b,2], Mariacarmela Santarpia [a,*,2]

[a] Medical Oncology Unit, Department of Human Pathology "G. Barresi", University of Messina, Messina 98122, Italy
[b] Department of Public Health, University of Naples Federico II, Via S. Pansini, Naples 80131, Italy
[c] Respiratory Medicine Unit, Department of Biomedical Sciences, Dentistry and Morphological and Functional Imaging (BIOMORF), University of Messina, Messina 98122, Italy

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ABSTRACT

Immune checkpoints inhibitors (ICIs) have markedly improved the therapeutic management of advanced NSCLC and, more recently, they have demonstrated efficacy also in the early-stage disease. Despite better survival outcomes with ICIs compared to standard chemotherapy, a large proportion of patients can derive limited clinical benefit from these agents. So far, few predictive biomarkers, including the programmed death-ligand 1 (PD-L1), have been introduced in clinical practice. Therefore, there is an urgent need to identify novel biomarkers to select patients for immunotherapy, to improve efficacy and avoid unnecessary toxicity. A deeper understanding of the mechanisms involved in antitumor immunity and advances in the field of liquid biopsy have led to the identification of a wide range of circulating biomarkers that could potentially predict response to immunotherapy. Herein, we provide an updated overview of these circulating biomarkers, focusing on emerging data from clinical studies and describing modern technologies used for their detection.

1. Introduction

Lung cancer remains the leading cause of cancer deaths worlwide, with an estimated 1.8 million deaths every year (Sung et al., 2021). Over the last decade, the management of lung cancer, mainly of non-small cell lung cancer (NSCLC), that accounts for most lung cancer cases (about 85%), has been radically improved with the introduction for clinical use of both molecularly targeted therapy and immunotherapy.

While targeted therapies represent the standard of care in NSCLC patients with driver mutations, immune checkpoint inhibitors (ICIs) are currently the cornerstone for treatment of those patients with non-oncogene addicted NSCLC (Singh et al., 2023a, 2023b). These agents include monoclonal antibodies specifically directed against inhibitory molecules, the so-called immune checkpoints, including the programmed cell death 1 (PD-1) and its ligand (PD-L1) and the cytotoxic T lymphocyte antigen-4 (CTLA-4), that are able to restore T-cell activity againts cancer cells and enhance antitumor immunity. In several trials conducted in patients with metastatic NSCLC, anti-PD-1/PD-L1 agents, either alone or in combination with chemotherapy or with anti-CTLA-4 agents, have demonstrated to improve survival outcomes compared to chemotherapy alone. ICIs have also shown notable efficacy for the treatment of locally-advanced NSCLC and, more recently, they have been assessed in the early-stage disease with positive results leading to the approval of some anti-PD-1/PD-L1 drugs both in the adjuvant and the neoadjuvant setting (Lazzari et al., 2023; Shields et al., 2021).

Unfortunately, despite significant and durable responses observed in some patients, the majority derive only limited clinical benefit from ICIs, and some can even experience an early disease progression (Goh et al., 2023; Indini et al., 2021). Moreover, also in those patients initially responding to these agents, resistance can finally occur during the course of treatment, limiting their long-term use. Hence, in parallel with an increasing number and clinical indications of ICIs approved, a great

* Correspondence to: Medical Oncology Unit, Department of Human Pathology "G.Barresi", University of Messina, V. Consolare Valeria 1, Messina 98125, Italy.
E-mail address: mariacarmela.santarpia@unime.it (M. Santarpia).
[1] These authors equally contributed to this work and share the first author position.
[2] These authors equally contributed to this work and share the last author position.

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amount of studies have been carried out to identify effective predictive factors, including molecular biomarkers, that could be useful to select patients most likely benefiting from immunotherapy. The quest for predictive biomarkers has become a huge priority to optimize the use of ICIs and improve clinical efficacy in NSCLC patients. Currently, few biomarkers, including PD-L1 expression, tumor mutational burden (TMB), and microsatellite instability (MSI), as assessed by different assays, (Goh et al., 2023) have been approved by Food and Drug Adiministration (FDA) and implemented in clinical practice to predict response to immunotherapy in patients with solid tumors. A positive association between high PD-L1 expression level on tumor cells [defined as Tumor Proportion Score (TPS), assessed by immunohistochemistry] and response to ICIs, primarily when used as monotherapy in the first-line setting of NSCLC, has been widely demonstrated (Borghaei et al., 2015; Oitaben et al., 2022; Rizvi et al., 2015). Furthermore, the TMB, defined as the total number of somatic mutations per megabase detected in tumor genome, has also been correlated with response to immunotherapy in different cancer types, including NSCLC, with high TMB (10 muts/Mb by FoundationOne CDx assay) tumors having better outcomes compared to tumors with low TMB (Cristescu et al., 2018; Hofman et al., 2019; Kowanetz, et al., 2017). This can be explained by the presence of more neoantigens enhancing the antitumor immune response (Hellmann et al., 2018; Hellmann et al., 2019).

Nevertheless, the assessment of tumor tissue biomarkers in clinical practice has some important limitations: a) the availability and accessibility to tumor biopsies, since the existing companion diagnostic assays require testing on tissue samples; b) the heterogeneity of tumor, that can lead to a great variability in the evaluation of PD-L1 expression, TMB or MSI, or other biomarkers, depending by the section of the tissue biopsy analyzed; c) other potential underlying molecular mechanisms that can be involved in ICI response and may not be evaluated (Hofman et al., 2019; Liu et al., 2018; Oitaben et al., 2022). In order to bypass these limitations, research has focused on the identification of novel biomarkers, in particular those derived from liquid biopsy, that have been largely investigated in the setting of targeted therapies for metastatic NSCLC. Liquid biopsy refers to different tumor-derived components that can be analyzed, including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), extracellular vesicles (EVs) and micro-RNAs (miRNAs). The advantages of liquid biopsy include the minimal invasiveness to obtain tumor samples (mainly blood), the possibility to perform parallel testing of several biomarkers within a single sample and to monitor tumor genomic changes over the course of treatment (Ignatiadis et al., 2021; Oitaben et al., 2022; Santarpia et al., 2018). In early-stage NSCLC, liquid biopsy, particularly ctDNA, offers the potential to assess minimal residual disease (MRD) in patients undergoing radical surgery and perioperative therapies in order to identify patients at higher risk of disease recurrence with the aim of personalize treatment decisions.

Moreover, because of their crucial role in antitumor immune response, other potential, emerging predictive biomarkers to immunotherapy include pro-inflammatory mediators, such as cytokines and chemokines, and immune cells (Goh et al., 2023; Indini et al., 2021; Oitaben et al., 2022).

The current review provides an updated overview of emerging circulating biomarkers with a potential role in predicting ICI therapy benefit in patients with advanced and early-stage NSCLC. We further describe the different methods used for their identification, as well as strengths and weaknesses of each biomarker that can limit the implementation in routine clinical practice.

2. Role of ICIs in the treatment of NSCLC

Immune checkpoint inhibitors (ICIs) are monoclonal antibodies that inhibit the so-termed immune checkpoints, thereby improving the response of immune cells and restoring the antitumor activity of cytotoxic T-lymphocytes to recognize and kill tumor cells (Pardoll, 2012). Among the immune checkpoints used by the tumor to evade host immune system, the most known are PD-1/PD-L1 and CTLA-4, that have been exploited for therapeutic approaches in NSCLC. Recent studies have found novel immune checkpoints that can be successfully inhibited by targeted agents, such as the Lymphocyte Activation Gene-3 (LAG-3), T-Cell Immunoreceptor With Ig and ITIM Domains (TIGIT), T-Cell Immunoglobulin and Mucin-Domain Containing-3 (TIM-3), and NK Group 2 Member A (NKG2A), with encouraging results in NSCLC (Cho et al., 2022; Herbst et al., 2022; Mamdani et al., 2022; Niu et al., 2022).

The first breakthrough in the use of ICIs in the treatment of NSCLC was the approval of the PD-1 inhibitor nivolumab as second-line therapy for patients with advanced NSCLC. In two landmark randomized trials, nivolumab showed superior objective response rate (ORR) and overall survival (OS) when compared to docetaxel in patients with advanced NSCLC following progression on platinum-based chemotherapy (Borghaei et al., 2015; Brahmer et al., 2015). Afterwards, other anti-PD-1/PD-L1 agents, including pembrolizumab and atezolizumab were approved by the FDA for the same indication (Herbst et al., 2016; Rittmeyer et al., 2017). The success of ICIs in second-line setting has been the pioneer for their use as upfront treatment of advanced NSCLC. Several phase III clinical trials showed durable responses and improvement in OS with ICIs or ICI plus platinum-based chemotherapy compared to chemotherapy alone, leading to a rapid expansion of first-line treatment options for patients with advanced NSCLC not harboring sensitizing *EGFR* mutations or *ALK* translocations (Gandhi et al., 2018; Hellmann et al., 2019; Herbst et al., 2020; Paz-Ares et al., 2021; Paz-Ares et al., 2018; Reck et al., 2016; Sezer et al., 2021; Socinski et al., 2018). Currently, these options include different immunotherapeutic agents directed against the PD-1/PD-L1 pathway, used as single agents for NSCLC patients with PD-L1 TPS $\geq$ 50%, or as combination regimens with chemotherapy or with chemotherapy and anti-CTLA-4 drugs in the majority of patients, regardless of PD-L1 expression (Singh et al., 2023b) (Table 1).

Based on remarkable results in the metastatic setting, the use of ICIs in NSCLC has subsequently expanded to unresectable stage III and, more recently, to early-stage NSCLC. In the randomized phase III trial PACIFIC comparing durvalumab with placebo in patients with locally advanced NSCLC who had non-progressive disease following concurrent chemoradiotherapy, durvalumab showed superior progression-free survival (PFS) and OS, with durable efficacy (Antonia et al., 2017; Spigel et al., 2017). In a phase III trial comparing atezolizumab with best supportive care (BSC) in patients with resectable stage IB-IIIA NSCLC following complete surgical resection and adjuvant platinum-based chemotherapy, atezolizumab was associated with higher disease-free survival (DFS) (Felip et al., 2021). Similar results were observed in the phase III PEARLS/KEYNOTE-091, that evaluated the efficacy of pembrolizumab versus placebo in completely resected, pathologically confirmed stage IB-IIIA NSCLC patients, showing a significant prolonged DFS in the overall population treated with pembrolizumab (O'Brien et al., 2022). Based on these findings, FDA approved, on October 2021, adjuvant atezolizumab in patients with stage II-IIIA and positive PD-L1 expression, without sensitizing *EGFR* mutations, following platinum-based chemotherapy and, on January 2023, pembrolizumab for adjuvant treatment following resection and platinum-based chemotherapy for stage IB-IIIA NSCLC (FDA accessed on 22 November 2023). Recently, a notable efficacy of neoadjuvant or perioperative immunotherapy, in terms of improvement of pathological complete response (pCR), major pathological response (MPR) and event-free survival (EFS), has emerged from several phase II and III clinical trials testing immunotherapy, chemo-immunotherapy, or dual ICIs (Lazzari et al., 2023). Based on positive results from the Checkmate 816 trial, on March 2022, FDA approved nivolumab with platinum-doublet chemotherapy for patients with resectable NSCLC in the neoadjuvant setting (Provencio et al., 2022) (FDA accessed on 22 November 2023). On October 2023, pembrolizumab with platinum-containing chemotherapy also received approval by FDA as neoadjuvant treatment, with continuation of pembrolizumab as post-surgical adjuvant treatment for resectable stage

Table 1
FDA/EMA approved ICIs in the neoadjuvant, adjuvant and first-line settings.

Target	Drug	Indication	Drug(s) Combined	Pivotal study	Line of therapy	U.S. FDA approval	EMA approval
PD-1	Pembrolizumab	Metastatic NSCLC without *EGFR* or *ALK* mutations and TPS ≥ 50%	-	Keynote-024 NCT02142738	First	October 2016	December 2016
		Metastatic squamous NSCLC	Platinum and either paclitaxel or nab-paclitaxel	Keynote-407 NCT02775435	First	October 2018	January 2019
		Metastatic non-squamous NSCLC	Platinum and pemetrexed	Keynote-189 NCT02578680	First	August 2018	July 2018
		Locally advanced (no possible definitive chemoradiation) or metastatic NSCLC without *EGFR* or *ALK* mutations and TPS ≥ 1%	-	Keynote-042 NCT02220894	First	April 2019	-
		Neoadjuvant/ adjuvant treatment for resectable stage II, IIIA, or IIIB (N2) NSCLC	Platinum- doublet chemotherapy (neoadjuvant) Pembrolizumab single agent (adjuvant)	Keynote-671 NCT03425643	Neoadjuvant/ adjuvant	October 2023	-
		Adjuvant treatment for stage IB (T2a ≥4 cm), II, or IIIA NSCLC following resection and platinum-based chemotherapy	Platinum-doublet chemotherapy	Pearls/Keynote-091 NCT02504372	Adjuvant	January 2023	September 2023
	Nivolumab	Metastatic or recurrent NSCLC without *EGFR* or *ALK* mutations	Ipilimumab and two cycles of platinum-doublet chemotherapy	CheckMate-9LA NCT03215706	First	May 2020	November 2020
		Metastatic NSCLC without *EGFR* or *ALK* mutations and PD-L1 ≥ 1%	Ipilimumab	CheckMate-227 NCT02477826	First	May 2020	-
		Neoadjuvant treatment for resectable NSCLC	Platinum-doublet chemotherapy	CheckMate-816 NCT02998528	Neoadjuvant	March 2022	March 2023[§]
	Cemiplimab	Locally advanced (no possible definitive chemoradiation) or metastatic NSCLC without *EGFR* or *ALK* mutations and TPS ≥ 50%	-	Empower-Lung1 NCT03088540	First	February 2021	May 2021
		Locally advanced (no possible definitive chemoradiation) or metastatic NSCLC without *EGFR*, *ALK* or *ROS-1* mutations	Platinum-based chemotherapy	Study 16113/ Empower-Lung3 NCT03409614	First	November 2022	February 2023[#]
PD-L1	Atezolizumab	Advanced non-squamous NSCLC	Bevacizumab, paclitaxel and carboplatin	IMpower 150 NCT02366143	First	December 2018	January 2019
		Metastatic non-squamous NSCLC without *EGFR* or *ALK* mutations	Nab-paclitaxel and carboplatin	IMpower 130 NCT02367781	First	December 2019	July 2019
		Metastatic NSCLC with PD-L1 expression[*] without *EGFR* or *ALK* mutations	–	IMpower 110 NCT02409342	First	May 2020	March 2021
		Adjuvant treatment of stage II to IIIA NSCLC whose tumors have PD-L1 expression ≥ 1%	Platinum-doublet chemotherapy	IMpower010 NCT02486718	Adjuvant	October 2021	April 2022
	Durvalumab	Unresectable stage III NSCLC who have not progressed following platinum- based chemoradiotherapy	-	Pacific NCT02125461	First	February 2018	September 2018
		Metastatic NSCLC without *EGFR* or *ALK* mutations	Tremelimumab and platinum-based chemotherapy	Poseidon NCT03164616	First	November 2022	June 2023
CTLA-4	Ipilimumab	Metastatic or recurrent NSCLC without *EGFR* or *ALK* mutations	Nivolumab and 2 cycles of platinum-doublet chemotherapy	CheckMate-9LA NCT03215706	First	May 2020	November 2020
		Metastatic NSCLC without *EGFR* or *ALK* mutations and PD-L1 ≥ 1%	Nivolumab	CheckMate-227 NCT02477826	First	May 2020	-
	Tremelimumab	Metastatic NSCLC without *EGFR* or *ALK* mutations	Durvalumab and platinum-based chemotherapy	Poseidon NCT03164616	First	November 2022	June 2023

Abbreviations: ALK: anaplastic lymphoma kinase; EGFR: epidermal growth factor receptor; EMA: European Medicines Agency; NSCLC**:** non-small cell lung cancer; PD-L1: Programmed death-ligand 1; ROS-1: Proto-Oncogene 1 Receptor Tyrosine Kinase; TPS: tumor propotion score; U.S. FDA: United States Food and Drug Administration.

[*] PD-L1 stained ≥ 50% of tumor cells (TC ≥ 50%) or PD-L1 stained tumor-infiltrating immune cells (IC) covering ≥ 10% of the tumor area (IC ≥ 10%).

[#] The EMA indication is limited to patients with locally advanced (no possible definitive chemoradiation) or metastatic NSCLC without EGFR or ALK mutations and PD-L1 expression ≥ 1%.

[§] PD-L1 espression ≥ 1%.

II-IIIB (N2) NSCLC (Table 1). Given these findings, several other trials are ongoing to assess the efficacy of ICIs administered in the perioperative setting as part of a multimodality therapeutic approach (Lazzari et al., 2023). Despite their large clinical use in NSCLC patients, the molecular and immunological mechanisms underlying sensitivity or resistance to ICIs remain to be fully elucidated, thus highlighting the urgent need for reliable predictive biomarkers. In this context, emerging blood-based biomarkers have been described, and others are currently under investigation, that may be useful to select those patients most likely benefiting from immunotherapy.

3. Circulating biomarkers and role of liquid biopsy in their evaluation

In the era of precision medicine, liquid biopsy has been rapidly implemented in clinical practice to personalize treatment of patients with different tumor types (Kwapisz, 2017). As commented above, with the term "liquid biopsy" a plethora of different analytes, including circulating cell-free DNA (cfDNA), circulating tumor derived nucleic acids (ctDNA), extracellular vesicles (EVs), circulating tumor cells (CTCs), and small RNAs shed in torrent blood, are grouped. (Malapelle et al., 2021; Rolfo et al., 2018) **(**Fig. 1**)**

Moreover, liquid biopsy-derived biomarkers may be also collected from alternative biological fluids (e.g. saliva, urine, sperm, tears and cerebrospinal fluid) covering with the term liquid biopsy all biological matrix where analytes may be isolated and purified (Crowley et al., 2013). Noteworthy, liquid biopsy testing is clinically approved by international societies that recommend its use when gold standard tissue specimen is inadequate for molecular analysis (Han et al., 2017; Hirsch et al., 2010; Kimura et al., 2007; Wu et al., 2015). As regards, cfDNA purified from plasma consists of the only liquid biopsy-derived biomarker available in routine diagnostic staging. (Han et al., 2017; Kimura et al., 2007). Clinical trials established the role of cfDNA guiding the clinical treatment of NSCLC prior any treatment when tissue specimen is unavailable both for morphological and molecular evaluation. In addition, plasma is also approved for detecting *EGFR* acquired resistance mutation (p.T790M) after first-line therapy with first- or second-generation TKIs (Mok et al., 2017; Mok et al., 2009). Recently, *KRAS* p.G12C hot spot mutations have radically modified the clinical paradigm for the management of NSCLC patients. In fact, small molecules specifically and irreversibly inhibiting *KRAS* p.G12C are investigated (Janne et al., 2022; Skoulidis et al., 2021). Of note, Sotorasib was approved by the FDA to treat NSCLC patients that harbor p.G12C detectable in tissue or plasma specimens (Skoulidis et al., 2021). Interestingly, as previously commented, liquid biopsy is able to dynamically evaluate tumor molecular profile thanks to a less invasive sampling approach and a fully comprehensive molecular assessment overcoming tissue specimen heterogeneity (Malapelle et al., 2022). Despite of these pivotal aspects, liquid biopsy is affected by some challenging like as high rate of false negative cases, not easily managing pre-analytical procedures and clonal hematopoiesis (Bonanno et al., 2022; Rijavec et al., 2019). In this scenario, highly sensitive technologies are required to successfully carry out molecular analysis from low representative circulating biomarkers in torrent blood. Among these, digital polymerase chain reaction (dPCR) and next generation sequencing technologies (NGS) play a pivotal role in the molecular analysis of these analytes (Desai and Lovly, 2023; Malapelle et al., 2017). To date, several commercially available assays are adopted for molecular testing of blood-based biomarkers. Among them, FDA approved as companion diagnostic test two plasma based NGS assays (Guardant360 and Foundation One Liquid CDx) enabling the evaluation of actionable driver gene mutations in NSCLC patients (Chan et al., 2022). Conversely, European Medicine Agency (EMA) established companion diagnostic biomarkers listing all druggable biomarkers that can be analyzed with several validated assays (Orellana Garcia et al., 2021). Circulating biomarkers from liquid biopsy are not currently used in clinical practice to stratify NSCLC patients based on their sensitivity to ICIs. However, a

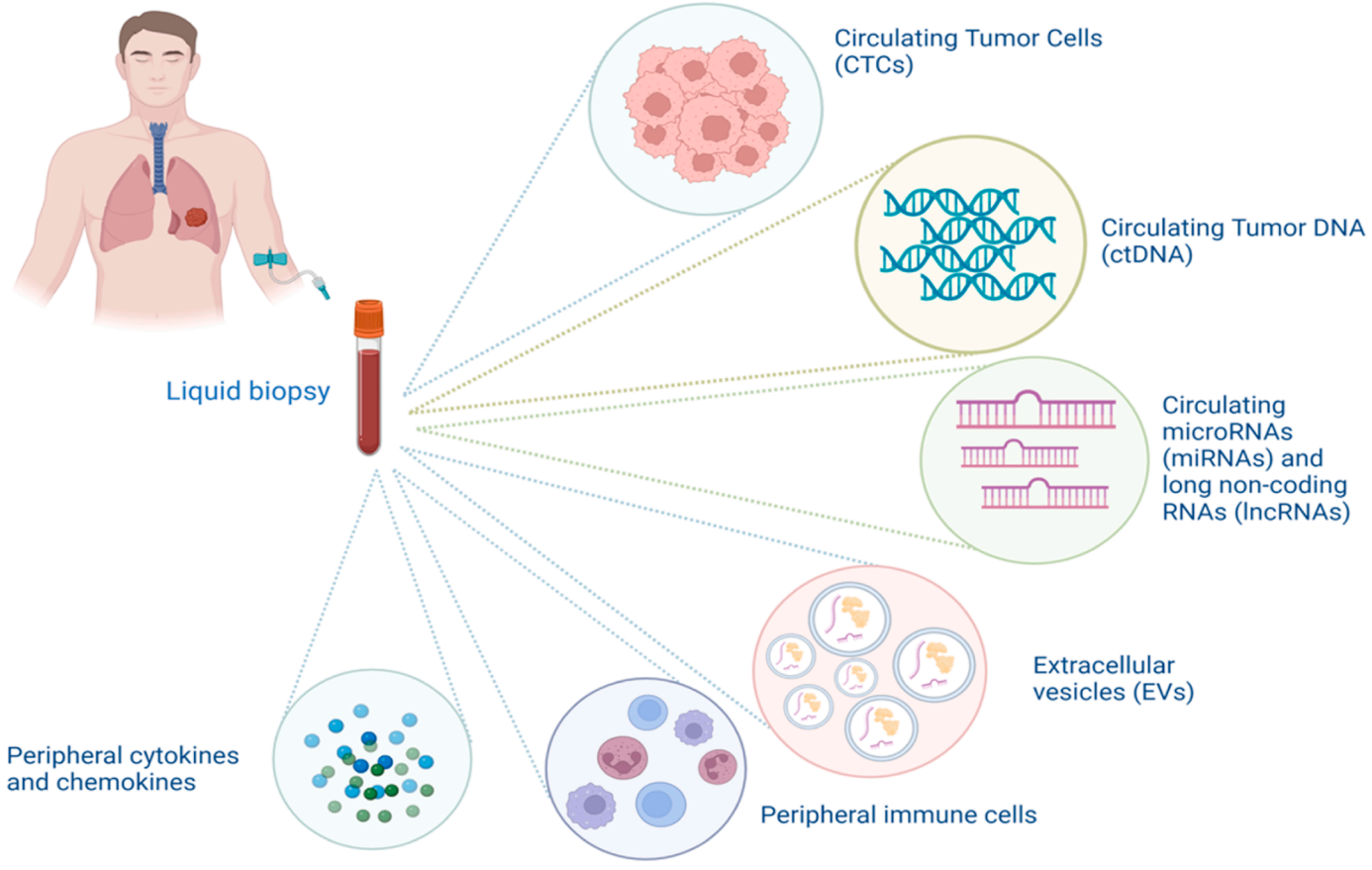

Fig. 1. In clinical practice liquid biopsy has been rapidly implemented. In NSCLC, liquid biopsy can be used to better stratify patients in order to select optimal therapy, avoiding invasive procedures. The main analytes that have demonstrated a potential predictive role of response to immunotherapy in NSCLC are circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating microRNAs (miRNAs) extracellular vesicles (EVs), peripheral immune cells and peripheral cytokines and chemokines.

large number of studies are ongoing to evaluate their prognostic and predictive role in different settings of disease. Particularly, cfDNA has recently emerged as a potential biomarker that could be used to predict and monitor the response to ICI therapy in metastatic- and early-stage-NSCLC (see below).

4. Circulating Tumor DNA (ctDNA)

Overall, ctDNA represents a small fraction (5.0–10.0%) of cfDNA directly and/or indirectly relapsed in blood by tumor cells. Shedding mechanisms are dependent from several factors including tumor burden, tumor stage and other unknown mechanisms impacting on this point. (Bettegowda et al., 2014; Markus et al., 2022). Of note, ctDNA length ranges from 50 to 150 bp being characterized by shorter DNA fragments than non-tumoral cfDNA and it may be specifically detected in plasma on the basis of this parameter or by evaluating tumor-related driver alterations (Markus et al., 2022). In accordance with low ctDNA amount in peripheral blood, ultra-sensitive methods are recommended for ctDNA molecular profiling. In this list, NGS RT-PCR and dPCR are routinely adopted to identify clinically valuable molecular alterations in diagnostic routine patients (Rolfo et al., 2021). Interestingly, ctDNA is actually considered the most promising biomarker to predict ICIs response in NSCLC patients. A prospective series of clinical patients were analyzed to investigate how ctDNA levels impact on immune-mediated response (Cabel et al., 2017). A comprehensive series of melanoma and colorectal cancer patients treated with nivolumab or pembrolizumab in monotherapy with undetectable ctDNA levels after 8 weeks post-treatment demonstrated a statistically significant benefit from anti-PD-1 drugs in terms of PFS (HR = 10.2; 95% CI: 2.5–41, $p < 0.001$) and OS (HR= 15; 95% CI: 2.5–94.9, $p = 0.004$). Interestingly, RT-PCR based systems [bidirectional pyrophosphorolysis-activated polymerization PCR (Bi-PAP) or digital droplet PCR (ddPCR) were adopted to measure mutant allele frequency (MAF) levels in previously detected cancer related genes while a target NGS approach, able to simultaneously cover hot spot regions in 39 cancer related genes, was approached when molecular records were unavailable at basal setting (Cabel et al., 2017).

The rationale behind the implementation of ctDNA as prognostic and/or predictive biomarker in lung cancer patients receiving ICIs could rely on the correlation between ctDNA plasma levels and clinical efficacy (Goldberg et al., 2018). A longitudinal series of ctDNA samples from 28 advanced NSCLC patients under ICI treatment showed that a >50% decreasing level of ctDNA in comparison with baseline samples significantly correlated with high OS rate (HR = 0.17, p = 0.007) (Goldberg et al., 2018). In a similar experience, Anagnostou et al., evaluated ctDNA decreasing rate and T-cell expansion in a prospective series of 24 lung cancer patients (Anagnostou et al., 2019). They customized a targeted error-correction sequencing system (TEC-Seq) aimed to detect low frequency molecular alterations in 12 cancer related genes to monitor molecular response in NSCLC patients treated with ICIs. Statistically relevant lower PFS and OS were demonstrated in NSCLC patients with stable cfDNA levels (14 out of 19 NSCLC patients with a median MAF of 1.87%) than cfDNA decreasing rate (7 out of 14 NSCLC patients with with a median MAF of 0.34%) (5.2 vs. 14.5 and 8.4 vs. 18.7 months; HR= 5.36; 95% CI: 1.57–18.35; p = 0.007 and HR= 6.91; 95% CI: 1.37–34.97; p = 0.02, respectively); similarly, T cell receptor (TCR) molecular analysis also revealed that T-cell expansion level mimic ctDNA variations in accordance with clinical response to ICIs (Anagnostou et al., 2019). These results lay the basis for a clinical trial (NCT04203095) investigating the pivotal role of cfDNA as early predictor of clinical benefit from ICIs. A low pass whole genome sequencing system revealed an early cfDNA clearance in responder lung cancer patients (8 out of 11, 72.7%) compared with the non-responder group (7 out of 8, 87.5%). (Zheng et al., 2020).

As we have commented, appealing data are coming from long-term analyses of trials with immunotherapy in the locally advanced setting and from recent results of trials with perioperative immunotherapy. In the early-stage disease, the use of reliable biomarkers is urgently needed to identify patients at higher risk of relapse and who may or not benefit from neo/adjuvant treatment strategies. Moreover, modifications in cfDNA could have the potential to precede radiologic relapses, theraby anticipating the administration of effective treatments. On a series of 218 peripheral blood specimens from unresectable stage IIB-IIIB NSCLC patients, Moding *et al.* highlighted that undetectable ctDNA level in NSCLC patients after concomitant chemoradiation (CRT) correlated with a major clinical benefit in terms of freedom from progression (FFP) (0% and 100% in undetectable and detectable baseline ctDNA NSCLC patients, respectively). In addition, NSCLC patients showing measurable ctDNA level under ICI treatment also had 0% of FFP at 12 months in comparison with 87.5% for NSCLC patients with undetectable ctDNA ($p< 0.0001$, HR = 84.4, 95% CI: 12.3–579.9), demonstrating its prognostic value. Another important findings was that in those patients with undetectable ctDNA after the CRT, there was not a statistically significant difference in FFP between patients receiving consolidation immunotherapy and those who did not receive treatment. This evidence may suggest it could be a biomarker to be used by clinicians to spare a subgroup of patients from consolidation therapy thereby avoiding potential toxicities. Of note, an ultra deep NGS based approach (CAPP-Seq), enabling to detect a minimum MAF level of 0.001% starting from 10 ng of input material, was used in this study.

In the work by Anagnostou et al., in the cohort of patients with early-stage NSCLC, all tumors with a major or partial pathologic response to neoadjuvant nivolumab demonstrated a molecular response pattern of elimination of ctDNA (Anagnostou et al., 2019).

In the phase III CheckMate 816 trial comparing neoadjuvant nivolumab plus platinum-based chemotherapy versus chemotherapy, the analysis of ctDNA was performed by using a tumor-guided personalized ctDNA panel for whole-exome sequencing (ArcherDX Personalized Cancer Monitoring). In an exploratory analysis, clearance of ctDNA, defined as presurgery change from detectable levels of ctDNA before cycle 1 to undetectable ctDNA before cycle 3, was higher in the nivolumab arm and correlated with higher EFS and pCR in both treatment arms, suggesting it may be an early predictor of favorable outcomes (Forde et al., 2022). In the phase II NADIM trial of neoadjuvant nivolumab plus platinum-based chemotherapy in resectable stage IIIA or IIIB NSCLC, baseline ctDNA levels, assessed by hybridization capture–based TruSight Oncology 500 ctDNA NGS assay on a NovaSeq sequencer (Illumina), were associated with differences in PFS and OS. Overall, 18 of 27 patients (67%) in the experimental group and 4 of 9 patients (44%) in the control group were ctDNA negative after neoadjuvant treatment (Provencio et al., 2023).

Liquid biopsy plays a crucial role in detecting specific molecular hallmarks (molecular alterations, epigenetic modifications) able to predict sensitivity to ICIs. It has been shown that *PTEN* or *STK11* pathogenetic mutations early detectable in baseline liquid biopsy samples drastically impacted on disease progression in lung cancer patients treated with ICIs (HR = 8.9, p = 0.09 for PTEN; HR = 4.7, p = 0.003 for STK11) (Guibert et al., 2019; Pavan et al., 2021). Moreover, transversion mutations (conversion between purine and pyrimidine) in *KRAS* and *TP53* correlated with improved clinical outcome (Pavan et al., 2021). Of note, in a study using a wide NGS assay (Guardant360® test; Redwood City, CA 94063, United States), co-occurring *KRAS/STK11* and *KRAS/STK11/TP53* molecular alterations decreased clinical benefit in terms of OS (HR =10.936, 95% CI: 2.337–51.164, p=0.002; HR =17.609, 95% CI: 3.777–82.089, P<0.001, respectively) (Pavan et al., 2021). In the same direction, Zhu *et al.* demonstrated lower OS in patients under atezolizumab treatment harboring *KEAP1/NFE2L2* functional impacting mutations in comparison with wild-type patients (Zhu et al., 2021). Remarkably, ctDNA based methylation profile should be investigated to define functional activity of cancer-related genes. Aberrant methylation signatures frequently occur in cancer cells distinguishing them from normal cell population. In addition, methylation

patterns are specifically investigated to track tumor evolution and tumor origin from non-neoplastic cells (Goh et al., 2023; Keller et al., 2021). To date, it has been certainly established the identification of specific methylation profiles in genes playing a pivotal role in cancer development for NSCLC patients clinically sensitive to ICIs (Cho et al., 2020; Duruisseaux et al., 2018). In fact, two distinct experiences on tissue-based analysis highlighted statistically significant unmethylated status of *FOXP1* and hypomethylation status of *CYTIP* and *TNFSF8* in NSCLC patients who clinically responded to ICIs (Cho et al., 2020; Duruisseaux et al., 2018). In this scenario, a prospective analysis of liquid biopsy samples from 64 NSCLC patients integrating genomic mutational signature (*EGFR, KRAS, TP53, PI3KCA* and *BRAF*, *c-Met*, amplification of *HER2*, and MSI status) and methylation profile revealed a new strategy to predict OS in a real-world series of NSCLC cases (Guo et al., 2020). Recently, TMB has been investigated to selectively identify NSCLC patients responding to ICIs. Blood TMB (bTMB) has been widely explored in a large number of trials (Ba et al., 2021; Marabelle et al., 2020; Sholl et al., 2020). Interestingly, TMB score is calculated adopting a wide NGS assay integrating genomic analysis with statistics algorithms able to measure the number of non-synonymous mutations/megabases. (Buttner et al., 2019; Pepe et al., 2021). In this scenario, FDA approved the Foundation One Liquid CDx panel, integrating hotspot analysis in 394 cancer related genes, MSI status and score, for molecular analysis of liquid biopsy specimens from NSCLC patients (Woodhouse et al., 2020). The Foundation One Liquid CDx panel was approached to calculate bTMB in two retrospective clinical trials (POPLAR and OAK) focused on the identification of novel biomarkers able to predict response to ICIs on liquid biopsies (Fehrenbacher et al., 2016; Rittmeyer et al., 2017). By

Table 2
List of the main technical approaches available to evaluate liquid biopsy derived biomarkers.

Circulating biomarker	Technical approach	Platform (N° genes)	Application (N° patients)	Reference
ctDNA	RT-PCR, dPCR	7500 Fast Real-Time PCR System (Life technologies); QX100 system (Biorad)	Early ICIs response monitoring (N=15 NSCLC)	Cabel et al., 2017
	NGS	HiSeq2500 (Illumina) (N= 24 genes)	Early ICIs response monitoring (N=28)	Goldberg et al., 2018
	NGS	HiSeq2500 (Illumina) (N= 12 genes)	ICIs response monitoring (N=24)	Anagnostou et al., 2019
	NGS	NextSeq 500 (Illumina) (N= 36 genes)	Prediction of response to ICIs (N=97)	Guibert et al., 2019
	NGS	HiSeq2500 (Illumina) (N= 73 genes) Ion S5 plus (59 genes)	Prediction of response to ICIs (N=103)	Pavan et al., 2021
	Methylation array	Methylation EPIC Array 850,000 CpG sites	Predictive role of methylation pattern in patients undergoing ICI treatment (N=18)	Cho et al., 2020
	Methylation array	EPIMMUNE (863 904 CpG sites)	Predictive role of methylation pattern in patients undergoing ICI treatment (N=142)	Duruisseaux et al., 2018
	NGS+Methylation array	Custom panel (90 genes) MethylMiner kit	Prognostic and predictive role of methylation pattern in patients undergoing ICI treatment (N=64)	Guo et al., 2020
	NGS	HiSeq2500 (Illumina) (N= 68 genes)	Predictive role of bTMB in patients undergoing ICI treatment (N=136)	Chae et al., 2019
	NGS	HiSeq2500 (Illumina) (N= 500 genes)	Prediction response to ICIs by evaluating bTMB (N=66)	Aggarwal et al., 2020
	NGS	HiSeq4000 (N= 270 genes)	Early prediction of ICIs response integrating mutation analysis and bTMB (N=99)	Nabet et al., 2020
CTCs	Cell counting	DAPI+/CD45- staining	PD-L1 expression on CTCs to predict response to ICIs (N=96)	Guibert et al., 2019
	Cell counting	CellSearch system (Menarini)	PD-L1 expression on CTCs to predict response from ICIs (N=39)	Dall'Olio et al., 2021
	Cell counting	DAPI+/CD45- staining	Prognostic role of CTCs in patients undergoing ICI treatment (N=24)	Nicolazzo et al., 2016
	Cell counting	DAPI+/CD45- staining	Predictive role of CTCs in ICIs administration (N=97)	Janning et al., 2019
Circulating biomarker	**Technical approach**	**Platform (N° miRNAs analyzed)**	**Application (N° patients)**	**Reference**
	NGS	NextSeq 500 (Illumina) (N=7)	Predictive role of miRNAs in patients undergoing ICI treatment (N=20)	Halvorsen et al., 2018
	RT-qPCR	Custom Taq Array MicroRNA Card (Thermo Fisher Scientific) (N=24)	Prognostic role of miRNAs in patients undergoing ICI treatment (N=140)	Boeri et al., 2019
	RT-qPCR	7900HT Fast Real-Time PCR System (Applied Biosystems) (N=10)	Predictive role of miRNAs in patients undergoing ICI treatment (N=80)	Fan et al., 2020
miRNAs	RT-qPCR	ViiA 7 Real-Time PCR System (Applied Biosystems) (N=6)	Prognostic and predictive role of miRNAs in patients undergoing ICI treatment (N=69)	Monastirioti et al., 2022
	NGS	HiSeq2500 (Illumina) (N=2)	Predictive role of miRNAs in patients undergoing ICI treatment (N=43)	Costantini et al., 2018
	NGS	HiSeq4000 (Illumina) (N=3)	Predictive role of miRNAs in patients undergoing ICI treatment (N=30)	Peng et al., 2020
	ELISA (Exosomal PD-L1, ExoPD-L1	Invitrogen human PD-L1 ELISA Kit	Predictive role of ExoPD-L1 in patients undergoing ICI treatment (N=149)	Wang et al., 2022
EVs	ELISA	SimoaTM PD-L1 Reagent Kit (Quanterix Corp, Lexington, MA).	Prognostic role of Exo PD-L1 in patients undergoing ICI treatment(N=21)	Yang et al., 2021
	ELISA	DB7H10, ELISA Quantikine PD-L1/ B7H1 Human/Cynomolgus Monkey, R&D Systems	Prognostic role of ExoPD-L1 in combination with CTCs in NSCLC (N=54)	Eslami et al., 2024

CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; ELISA, enzyme-linked immunosorbent assay; EV, extracellular vesicles; ICIs, immune checkpoint inhibitors; miRNAs, micro-RNAs; NGS, next generation sequencing; NSCLC, non-small cell lung cancer; RT-qPCR, quantitative reverse transcription polymerase chain reaction.

considering a bTMB cut-off of ≥16 mutations/Mb, NSCLC patients under atezolizumab treatment showed a clinical benefit in terms of PFS in comparison with the control group (Gandara et al., 2018). Of note, the authors also highlighted an improvement in PFS and ORR extending molecular stratification to NSCLC patients treated with anti-PD1/PD-L1 drugs (bTMB ≥ 6 mutations/Mb) (Wang et al., 2019). Moreover, bTMB was matched with tTMB in 56 NSCLC patients by using OncoScreen (Burning Rock Biotech, Guangzhou, China) panel. Bioinformatic pipeline set up a bTMB cut-off (≥11 mutations/Mb) demonstrating a statistically significant benefit (PFS = 5.8 months versus. 2.0 months, p = 0.0029) in NSCLC patients bTMB positive in comparison with low bTMB patients (Chen et al., 2021).

Remarkably, bTMB calculation was also affected by heterogeneous technical cut-off among clinical trials investigating the predictive role of bTMB to ICIs. In a phase 3 randomized clinical trial investigating the clinical outcome of NSCLC patients treated with durvalumab plus tremelimumab, bTMB ≥ 20 was selected to stratify NSCLC patients exhibiting a positive OS (21.9 months; 95% CI: 11.4–32.8) versus chemotherapy (median OS = 10.0 months; 95% CI: 8.1–11.7) (Kim et al., 2022; Rizvi et al., 2020). In this context, encouraging results were acquired by the implementation of bTMB in clinical stratification of NSCLC patients sensitive to ICIs but opening challenges like as the lack of standardized technical parameters and bioinformatic pipelines reduce the availability of bTMB in the diagnostic setting (Chae et al., 2019). To overcome these limitations, the integration between bTMB and ctDNA is currently under investigation. Aggarwal *et al.* observed a statistically significant PFS improvement (HR=0.24 p<0.001) and OS (HR=0.31 p =0.009) in NSCLC patients that harbor bTMB ≥ 16 mutations/Mb with concomitant wild-type status in clinically relevant genes (*STK11/KEAP1/PTEN* and *HER2* exon 20) modulating first-line ICI therapy response (Aggarwal et al., 2020). In another study, the authors developed a multiparametric model integrating bTMB score and normalized ctDNA levels carried out by CAPP-Seq technology and WES approach, respectively, to predict response to ICIs in a retrospective series of lung cancer patients (Nabet et al., 2020) **(**Table 2**).**

At the sight of these relevant data, further investigations are required to harmonize analytical procedures for the clinical implementation of blood-based biomarkers in the management of NSCLC patients.

5. Circulating Tumor Cells (CTCs)

Overall, CTCs originate from primary lesions and may be counted in the peripheral blood at very low percentage (1–10 CTCs per 10 mL of blood or ~1–100 CTCs per 10^9 blood cells). It has been observed that CTCs are released in blood as a primary mechanism to promote metastatic invasion (Hou et al., 2013; Williams, 2013). Of note, CTCs harbor specific molecular hallmarks (in terms of genomic, transcriptomic, and proteomic profile) able to distinguish CTCs from non-tumoral cells (Manjunath et al., 2019). Genomic and proteomic information may play a pivotal role in guiding diagnostic, prognostic and therapeutical strategies for tumor patients (Capuozzo et al., 2023). Particularly, CTCs amount at baseline was investigated as a prognostic biomarker in NSCLC patients treated with ICIs (Sim et al., 2018). In a prospective series of 96 NSCLC patients, baseline PD-L1 expression on CTCs was successfully carried out in 93.0% of patients showing a positive signal in 83.0% of cases vs 41.0% of matched tissue samples (Guibert et al., 2018). Interestingly, high CTC number was observed in patients exhibiting the worst clinical outcome (HR= 1.06, p = 0.03 for OS; HR= 1.05, p = 0.02 for PFS) (Guibert et al., 2018). Dall'Olio *et al.* stratified 39 NSCLC patients who received ICI therapy in accordance with CTCs detectability in matched blood specimens (no CTC detectable, positive CTC and PD-L1-negative CTC patients) (Dall'Olio et al., 2021). Results highlighted a different outcome across these groups with a median OS of 2.2 months, (95% CI: 0.8–3.6), 3.7 months, (95% CI: 0.1–7.5 (95% CI: 0.13–0.83; p =.019) and 16.0 months, (95% CI: 2.2–29.8 HR= 0.17; 95% CI: 0.06–0.45; p <0.001) in PD-L1 negative, positive and CTCs not detectable patients, respectively (Dall'Olio et al., 2021). Moreover, PD-L1+ CTC counting also revealed to be a prognostic stratification tool for NSCLC patients. By adopting a statistically significant technical cut-off for measuring CTC in blood (≥1%), a PFS <6 months (p = 0.04) was observed in the "non-responders" group (Guibert et al., 2018). In a similar experience, stable CTC levels in a longitudinal series of plasma specimens at different timing points (baseline, 3 months and 6 months post-treatment) highlighted a statistically significant worse clinical outcome (Nicolazzo et al., 2016). Another study by Janning et al., focused on the negative predictive value of CTCs after first- or second-line ICI therapy in NSCLC patients. Briefly, PD-L1+ CTCs increasing levels were identified in non-responder patients treated with ICIs compared with responders that harbored stable or decreasing PD-L1+CTCs levels (n = 11; p = 0.001) (Janning et al., 2019). As previously described, the most critical issue for the implementation of CTCs analysis in the clinical management of NSCLC patients is represented by the heterogeneous expression of PD-L1, the heterogeneous concentration of CTCs in peripheral blood across tumor patients, and the lack of standardized procedures enabled to isolate and molecularly characterize CTCs (Goh et al., 2023; Strati et al., 2023) **(**Table 2**).**

6. Circulating MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are small non-coding oligonucleotides (<200 nucleotides) with regulatory capacity, that can be found, in addition to tumor tissues, free in body fluids or inside extracellular vesicles, such as exosomes, where they are protected from degradation (Oitaben et al., 2022). Through their complementary binding to messenger RNA (mRNA), they can modulate the translation process. The expression patterns of miRNAs in cancer cells are very different from those of normal cells, which can explain many of the abnormal processes in tumors such as tumor growth, angiogenesis, or immune evasion (Baek et al., 2008). Tumor-derived miRNAs have been shown to affect the composition of the tumor microenvironment (Suzuki et al., 2015), and this may be essential in the crosstalk between tumor cells and immune cells (Kuninty et al., 2016). For example, miR-34a has been shown to suppress PD-L1 expression via p53 regulation (Cortez et al., 2016), miR-200b and miR-200c, via the miR-200/ZEB1 axis, negatively regulate the process of Epithelial–mesenchymal transition (EMT) and metastasis (Chen et al., 2014). MiR-146a (Lu et al., 2010), and miR-155 (Kohlhaas et al., 2009) play a critical role in the development of lymphocytes Tregs, while miR-223 participates in the process of myeloid cell proliferation and differentiation (El Gazzar, 2014).

Based on this evidence, several studies have analyzed the role of circulating miRNAs in patients with NSCLC in order to identify whether some of them could represent positive or negative predictive factors for the response to ICI therapy (Boeri et al., 2019; Fan et al., 2020; Halvorsen et al., 2018; Monastirioti et al., 2022; Rajakumar et al., 2022).

Halvorsen et al. identified a sequence of seven microRNAs (miR-215–5p, miR-411–3p, miR-493–5p, miR-494–3p, miR-495–3p, miR-548j-5p and miR-93–3p) in the serum of 51 NSCLC patients treated with nivolumab that were significantly associated to an increased OS (Halvorsen et al., 2018). Boeri et al., in their study, analyzed a panel of miRNAs, defined as miRNA signature classifier (MSC), composed of several miRNAs (miR-16–5p, miR-451a, miR-486–5p and miR-92a-3p, miR-126–5p, miR −15b-5p, miR-221–3p and miR-30b-5p) in combination with PD-L1 expression in 140 advanced NSCLC patients treated with ICIs, showing that an intermediate- or low-risk levels in MSC and/or PD-L1 expression of 50% were correlated to higher ORR (p = 0.0024), and both PFS and OS (p < 0.0001) while an high risk level was associated to a reduction in survival (Boeri et al., 2019). Fan et al. identified a panel of miRNAs (miR-93, miR −138 to 5p, miR-200, miR-27a, miR-424, miR-34a, miR-28, miR-106b, miR-193a-3p, miR-181) significantly related to a better response to nivolumab in patients with NSCLC (Fan et al., 2020). However, in another study by Monastirioti et al., high miR-200 levels were associated with reduced OS

(Monastirioti et al., 2022).

Costantini et al., also found that advanced NSCLC patients treated with nivolumab characterized by a down-expression in miRNA-320b and −375 showed a significant clinical benefit (i.e., complete or partial response). They also showed that miRNA-320b was associated with proliferation genes such as MYC, TUBB1, and miRNA-375 with immune-related genes such as JAK2, TGF-β2, the Wnt/β-catenine (FZD4, FZD8), and the Hippo pathway (YAP1), which are all known to be involved in ICIs resistance mechanisms (Costantini et al., 2018).

In a study by Peng et al., evaluating 30 advanced NSCLC patients treated with immunotherapy, three types of exosomal miRNAs were examined: hsa-miR-320d, hsa-miR-320c, and hsa-miR-320b. Patients who progressed during treatment had high baseline levels of all three miRNAs. Moreover, they found that all patients with response to anti-PD-1 had downregulation of hsa-miR-125b-5p (Peng et al., 2020) **(**Table 2**)**.

The above-mentioned results support that circulating miRNAs could be used as non-invasive biomarkers for predicting the response to ICI treatment in NSCLC patients and for disease management in clinical practice. However, some limitations must be considered, such as the lack of consensus in isolation methods, the heterogeneity of the studies, and the use of small cohorts of patients, raising the need for external validation.

6.1. Circular RNAs

Some circular RNAs (circRNAs) which belong to a class of regulatory RNAs characterized by a covalently closed circular structure, have recently been identified and studied (Awasthi et al., 2018; Kristensen et al., 2019). They are generated by the "backsplicing" of exons in the precursor mRNA (Kristensen et al., 2019) and are able to modulate the activity of miRNAs, functioning as a sponge for the latter (Awasthi et al., 2018). An example is the circRNA CDR1as/ciRS-7 which contains 74 binding sites for miR-7 (Awasthi et al., 2018). Some studies have revealed the potential effects of circRNAs in various biological processes, such as cardiovascular disease (Li et al., 2020), immunity (Liu et al., 2019), and cancer (Goodall and Wickramasinghe, 2021). In particular, a recent clinical and preclinical study showed that an increased expression of a circRNA, circHMGB2 (hsa_circ_0071452), in surgical lung samples from operated NSCLC patients represented an independent indicator of poor prognosis in these patients. *In vivo*, in mouse models, circHMGB2 has been shown to modify the tumor microenvironment contributing to the depletion of antitumor immunity, resulting in a reduced response to anti-PD-1 therapy. *In vitro*, this effect was seen to be related to the sponging of circHMGB2 against miR-181a-5p, thus inactivating the type 1 interferon response (Zhang et al., 2022).

7. Extracellular vesicles (EVs)

Extracellular vesicles (EVs) are membrane-bound nanoparticles released by cells into the extracellular space and blood (Goh et al., 2023). EVs are secreted by almost all cells in the human body and are strongly implicated in cell-to-cell communications (Moller and Lobb, 2020). To ensure these cellular communication mechanisms, EVs transport various "cargoes", such as proteins, lipids, RNA and DNA. Through these mediators, EVs are responsible for regulating some mechanisms involved in carcinogenesis, such as angiogenesis and cell proliferation (Fabbiano et al., 2020). Several studies have demonstrated that tumor cells are also able to release EVs into the surrounding microenvironment and into circulation, and that this could be a mechanism implicated in both metastasis and response to treatments (Ghoroghi et al., 2021). EVs are classified into three types, based on their biogenesis and size: exosomes (40–160 nm), microvesicles (100–1000 nm), and apoptotic bodies (1–5 μm) (Mullen and Movia, 2023). Exosomes are the most represented and best characterized EVs. The role of EVs in the mechanisms of response to immunotherapy in patients with NSCLC, both in early- and advanced-stages, is still controversial. In particular, it seems that two cargoes are most implicated in the response to ICIs, exosomal PD-L1 (ExoPD-L1) and the above described exosomal miRNAs (Goh et al., 2023). ExoPD-L1 can be secreted by both healthy and tumor cells, and has a higher immunosuppressive effect than other extracellular PD-L1, like free-form PD-L1 (soluble PD-L1, sPD-L1). Infact, ExoPD-L1 interacts with TCR through major histocompatibility complex (MHC) class-I, mimicking the effect of PD-L1 present on the surface of tumor cells (Daassi et al., 2020). Due to its immunosuppressive role, some evidence has identified ExoPD-L1 as a mechanism of resistance to anti-PD-1/anti-PD-L1 treatment (Ye et al., 2021). Preclinical studies have shown that high ExoPD-L1 levels at baseline, before starting treatment with ICIs, predict worse outcomes in terms of PFS, OS and ORR. On the other hand, the behavior of ExoPD-L1 during immunotherapy treatment in patients with NSCLC appears to be correlate with the response. It seems that patients with increased ExoPD-L1 levels after starting the treatment with ICIs, have better response rates (Oitaben et al., 2022). A study conducted by Yang et al. evaluated the behavior of ExoPD-L1 in 33 NSCLC patients treated with ICIs and demonstrated that a 1.86-fold increase of ExoPD-L1 levels after two months of therapy correlated with improved PFS, OS and ORR (Yang et al., 2021). Interestingly, the combination of multiple biomarkers could increase the predictive role of response to treatment with ICIs. A study by Eslami et al. evaluated the combination of CTCs, ctDNA and EVs as predictive factors in 54 patients with metastatic NSCLC. High PD-L1 expression in small EVs (PDL1+ sEV) was correlated with OS (HR = 1.14, 95% CI: 1.03–1.26, p = 0.016), but not with PFS (HR = 1.08, 95% CI: 0.99–1.18, p = 0.095). They also conducted an interaction analysis suggesting that PD-L1+ sEV correlation with PFS changed in function of CTC presence/absence (p interaction = 0.036). In conclusion, the combination analysis highlighted a worse prognosis for patients with CTCs and high PD-L1+ sEV levels (HR = 7.65, 95% CI: 3.11–18.83, p < 0.001) (Eslami et al., 2024) **(**Table 2**).** Given these results, the recognition of high levels of PD-L1 expression on exosomes as a mechanism of resistance to immunotherapy opens new scenarios to try to overcome this resistance. In this regard, different techniques are being evaluated (extracorporeal circulation techniques, pharmacological inhibition of secretion, anti-exoPD-L1 antibodies, etc.) (Ye et al., 2021). In conclusion, data relating to the role of exosomal vesicles as predictive factors are still limited but promising. Combining these circulating biomarkers with others tumor characteristics can likely increase the predictive power. Further studies on larger patient samples are necessary.

8. Peripheral immune cells

The effectiveness of immunotherapy is mainly correlated to the host immune system response able to mediate an anti-tumor effect and deregulation of the immune system can significantly impact on immunotherapy efficacy. For these reasons, a growing number of studies is focusing on the evaluation of several peripheral immune cells as predictive biomarkers of response and toxicity ICI treatment (Goh et al., 2023; Oitaben et al., 2022)

8.1. Innate immune cells

Neutrophils are the main innate immune cells that compose the tumor microenvironment (TME) of NSCLC and can be involved in both an anti-tumorigenic or pro-tumorigenic activity, based on their polarization and phenotypes (Aloe et al., 2021). Some evidence showed that persistent neutrophilia and increased neutrophil-to-lymphocyte ratio (NLR≥ 5) were correlated with poor prognosis in patients with NSCLC (Anderson et al., 2021; Jiang et al., 2019; Jin et al., 2020). Russo et al., showed that increased NLR in patients with metastatic NSCLC treated with nivolumab, represented an independent prognostic factor

correlating with decreased OS and PFS. Conversely, decreased platelet to-lymphocyte ratio (PLR <200) was correlated with increased PFS, OS, ORR, and DCR (Russo et al., 2020). In addition, Sun et al. have found that higher NLR in patients with resectable NSCLC treated with neo-adjuvant chemotherapy alone or in combination with ICI (nivolumab, camrelizumab, or tislelizumab) was also correlated with decreased ICI response and PFS (Sun et al., 2022). Another study by Ayers et al. reported that increased NLR in metastatic NSCLC patients treated with ICIs, including nivolumab, atezolizumab, or pembrolizumab, was correlated with a poor OS (Ayers et al., 2021). In the same study, a mild anemia (<12 g/dl) was correlated with improved OS, independently of the NLR (Ayers et al., 2021). Interestingly, a decreased NLR ratio at baseline in NSCLC patients treated with ICIs was shown to be correlated to an increased risk of development of irAEs (Pavan et al., 2019). Similarly, a more recent study has shown that in NSCLC patients treated with ICIs, baseline NLR was negatively correlated with the risk of development of irAEs (Gao et al., 2023). Different studies have evaluated the potential role of natural killer (NK) cells in predicting clinical outcomes to ICIs, with contrasting results. Some studies have shown that NK cell blood levels were negatively correlated to ICI treatment response (Pettinella et al., 2023), and, in particular, in NSCLC patients treated with nivolumab they were negatively correlated to survival outcomes (Ottonello et al., 2020). Conversely, there are several studies showing that NK cell blood levels are positively correlated to NSCLC patient outcomes. Indeed, decreased blood NK cell levels were correlated to poor OS and PFS in NSCLC patients treated with ICIs (Mazzaschi et al., 2020; Tenuta et al., 2023), interestingly decreased blood NK cell levels were mainly decreased in NSCLC patients with sarcopenia, compared to NSCLC patients without sarcopenia (Tenuta et al., 2023). Another study enrolling patients with NSCLC or small cell lung cancer (SCLC), has shown that increased blood levels of NK cells at baseline were correlated with improved PFS (Li et al., 2021).

8.2. Adaptative immune cells

Various studies have evaluated the role of peripheral adaptative immune cells, such as CD8+ T cells, CD4+ T cells, and regulatory T cells, in the modulation of the individual's response to ICI treatment (Goh et al., 2023). Non-small cell lung cancer patients treated with nivolumab and with increased blood levels of both CD4+ and CD8+ T cells at baseline were characterized by an improved OS (Ottonello et al., 2020). In addition, an improved OS was reported in NSCLC patients characterized by lower baseline expression of PD-1 on CD8+ T cells (Ottonello et al., 2020). Another study reported improved responses for NSCLC patients treated with anti-PD-1 inhibitors showing an enhanced proliferation of PD1+ CD8+ T cells within the first two cycles of treatment (Kamphorst et al., 2017). Of note, the proliferating PD1+ CD8+ T cells were characterized by the expression of a particular phenotype including HLA-DR+, CD38+, Bcl-2 low, CD28, CD27 and ICOS (Kamphorst et al., 2017). Similarly, Kim et al. have found that the proliferation of the PD1+ CD8+ T cells phenotype occurring one week after anti-PD-1 treatment initiation correlated to an improved response (Kim et al., 2019). More recently, another study has shown that proliferation activity of PD1+ CD8+ T cells (expressed through Ki67) was positively correlated to PFS in NSCLC patients treated with ICIs (Wu et al., 2023). Ferrara et al. have found that NSCLC patients treated with ICIs and characterized by increased blood levels of senescent CD8+ T cell population (showing low proliferation and expressing CD28-, CD57+, and KLRG+), were correlated with decreased PFS, OS and reduced responses (Ferrara et al., 2021).

Regulatory T cells (Tregs) belong to the CD4+ T cells and have a key role in immune system modulation by preventing autoimmune response through their immune-suppressive activities (Goh et al., 2023). NSCLC patients who had decreased blood levels at baseline of Tregs showed improved responses to ICI combination therapy. In addition, increased baseline CD4+/CD8+ T cell ratio was correlated to improved PFS to ICI treatment (Li et al., 2021). Koh et al. have shown that in advanced NSCLC patients treated with an anti-PD-1 (pembrolizumab or nivolumab), increased blood levels of Tregs (expressing CD25+ FOXP3+ CD4+) one week after treatment initiation were correlated to a significant improved PFS and OS (Koh et al., 2020). Regarding the correlation of immune cells with immune-related toxicities, a recent study by Gao et al. has shown that total blood lymphocyte levels were positively correlated with an increased risk of development of immune-related adverse events (irAEs) (Gao et al., 2023).

9. Peripheral cytokines and chemokines

Cytokines and chemokines are pro-inflammatory mediators that can be found in several body compartments such as lung tissue, sputum, bronchoalveolar lavage (BAL), and serum. These are produced and secreted by both immune cells such as macrophages, neutrophils, NK cells and T-, B-lymphocytes and structural cells including lung structural cells such as lung epithelial cells, endothelial cells and lung fibroblasts. They can bind their coupled receptors activating several intracellular signaling pathways modulating different cellular functions such as growth cell, activation and recruitment of immune cells, but also inducing acute and chronic pro-inflammatory status (Goh et al., 2023; Nucera et al., 2021), Currently, there are several literature data showing that serum levels of pro-inflammatory mediators such cytokines and chemokines are correlated with response to ICI treatment in NSCLC patients.

9.1. Cytokines

Non-small cell lung cancer patients characterized by decreased IL-6 serum levels after ICI treatment had increased PFS compared with those patients showing increased or invariable IL-6 serum levels after ICI treatment (Keegan et al., 2020). Another study has analyzed baseline IL-6 serum levels in patients with advanced NSCLC treated with PD-1/PD-L1 inhibitors, showing that decreased IL-6 serum levels were negatively correlated with ORR, DCR, PFS and OS (Kang et al., 2020). Similarly, other data showed that serum IL-6 levels in metastatic NSCLC patients receiving second line anti-PD-1 monotherapy were negatively correlated with PFS (Kauffmann-Guerrero et al., 2021). Finally, a recent study has shown that baseline serum IL-6 levels in NSCLC patients treated with ICIs were negatively correlated with PFS (Hu et al., 2023). Also, data regarding the predictive role of serum interferon-γ (IFN-γ) are contrasting. Costantini et al. have shown that IFN-γ levels from NSCLC patients receiving nivolumab were not correlated with ICI response (Costantini et al., 2018). However, Hirashima et al. found that NSCLC patients receiving ICI treatment with decreased serum levels of IFN-γ were characterized by poor response and early disease progression (Hirashima et al., 2019). In accord with these results, Kauffmann-Guerrero et al. showed that increased serum levels of IFN-γ in NSCLC patients were characterized by a durable response to ICI treatment (Kauffmann-Guerrero et al., 2021). Boutsikou et al. have evaluated several pro-inflammatory mediators of NSCLC patients treated with pembrolizumab or nivolumab, showing that increased serum levels of IFN-γ, tumor necrosis factor α (TNF-α), IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 and CXCL8 were associated with enhanced response and improved OS (Boutsikou et al., 2018). Finally, another study demonstrated that baseline serum levels of TNF-α in NSCLC patients treated with nivolumab were negatively correlated to ICI response (Oyanagi et al., 2019), whereas increased TNF-α and IL-6 serum levels after ICI treatment, were correlated to decreased PFS (Hu et al., 2023).

9.2. Chemokines

So far, studies evaluating serum chemokines and clinical outcomes to ICIs in NSCLC have reported contrasting results. Sanmamed et al. have shown that NSCLC patients who had a good response to treatment with

nivolumab or pembrolizumab showed decreased serum CXCL8 levels compared to those who had a poor response, that were characterized by increased or stable serum CXCL8 levels. Moreover, an early decrease (2–4 weeks) of CXCL8 serum levels was correlated to an improved OS in NSCLC patients (Sanmamed et al., 2017). Similarly, there are other data showing that NSCLC patients with increased CXCL8, CXCL10 or CXCL12 serum levels have decreased OS, PFS or DCB with ICI therapy (Agullo-Ortuno et al., 2020; Hu et al., 2023; Oyanagi et al., 2019; Wang et al., 2021; Xu et al., 2023). Moreover, Harel et al. found that NSCLC patients treated with ICI showing a poor OS, were characterized by increased serum levels of CXCL8 and CXCL10 at baseline (Harel et al., 2022). Conversely, another study has shown that increased serum levels of CXCL9 and CXCL10 were significantly correlated with improved response and PFS with ICIs (Eltahir et al., 2021).

10. Future perspectives and conclusions

Liquid biopsy has shown to be an important tool that is increasingly becoming part of clinical practice. In NSCLC patients there is often a technical difficulty to obtain tumor tissue, which implies invasive diagnostic procedures to collect material adequate for molecular testing. Immunotherapy has recently revolutionized survival outcomes in patients with NSCLC, although the range of responses obtained is still very broad. So far, the only tumor characteristic that has shown to be a reliable, useful predictive biomarker in clinical practice is PD-L1 expression. However, even in the subgroup of PD-L1-positive patients, response rates to ICIs are highly variable and also PD-L1-negative patients can present clinically significant responses. Moreover, it has been widely proven that tumor PD-L1 expression is inducible and dynamic over time and location. Therefore, it is necessary to carry out a more accurate stratification, for which the use of effective biomarkers can play a crucial role. In this rapidly evolving scenario, circulating biomarkers emerge as promising tools to optimize the clinical management of patients with various tumor types. Particularly, several studies are currently evaluating the role of biomarkers in predicting immunotherapy benefit in the curative setting, in early-stage NSCLC, with the possibility to identify those patients with high risk of recurrence after surgery that may need more intense therapeutic approaches. However, despite the encouraging role played by these biomarkers, there are still several critical issues

that drastically limit on their widespread diffusion in clinical practice. First of all, the complexity of antitumor response, in which many components of TME are deeply involved through multiple interactions. Then, the lack of standardized technical approaches and of data interpretating criteria both for each individual biomarker or their possible combinations, also remains an opening challenge. Furthermore, as previously described, the amount of data is still on a limited number of patients and the hypothesis of phase III studies, specifically designed to evaluate the effectiveness and application of these methods, is recommended to further implement these biomarkers in diagnostic routine practice.

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Figure created in BioRender.com (accessed on January 10th, 2024).

References

Aggarwal, C., Thompson, J.C., Chien, A.L., Quinn, K.J., Hwang, W.T., Black, T.A., Yee, S. S., Christensen, T.E., LaRiviere, M.J., Silva, B.A., Banks, K.C., Nagy, R.J., Helman, E., Berman, A.T., Ciunci, C.A., Singh, A.P., Wasser, J.S., Bauml, J.M., Langer, C.J., Cohen, R.B., Carpenter, E.L., 2020. Baseline Plasma Tumor mutation burden predicts response to pembrolizumab-based therapy in patients with metastatic non-small cell lung cancer. Clin. Cancer Res 26 (10), 2354–2361.

Agullo-Ortuno, M.T., Gomez-Martin, O., Ponce, S., Iglesias, L., Ojeda, L., Ferrer, I., Garcia-Ruiz, I., Paz-Ares, L., Pardo-Marques, V., 2020. Blood predictive biomarkers for patients with non-small-cell lung cancer associated with clinical response to nivolumab. Clin. Lung Cancer 21 (1), 75–85.

Aloe, C., Wang, H., Vlahos, R., Irving, L., Steinfort, D., Bozinovski, S., 2021. Emerging and multifaceted role of neutrophils in lung cancer. Transl. Lung Cancer Res 10 (6), 2806–2818.

Anagnostou, V., Forde, P.M., White, J.R., Niknafs, N., Hruban, C., Naidoo, J., Marrone, K., Sivakumar, I.K.A., Bruhm, D.C., Rosner, S., Phallen, J., Leal, A., Adleff, V., Smith, K.N., Cottrell, T.R., Rhymee, L., Palsgrove, D.N., Hann, C.L., Levy, B., Feliciano, J., Georgiades, C., Verde, F., Illei, P., Li, Q.K., Gabrielson, E., Brock, M.V., Isbell, J.M., Sauter, J.L., Taube, J., Scharpf, R.B., Karchin, R., Pardoll, D. M., Chaft, J.E., Hellmann, M.D., Brahmer, J.R., Velculescu, V.E., 2019. Dynamics of tumor and immune responses during immune checkpoint blockade in non-small cell lung cancer. Cancer Res 79 (6), 1214–1225.

Anderson, R., Blidner, A.G., Rapoport, B.L., 2021. Frontiers in pharmacology: review manuscript targeting of the neutrophil as an adjunctive strategy in non-small cell lung cancer. Front Pharm. 12, 676399.

Antonia, S.J., Villegas, A., Daniel, D., Vicente, D., Murakami, S., Hui, R., Yokoi, T., Chiappori, A., Lee, K.H., de Wit, M., Cho, B.C., Bourhaba, M., Quantin, X., Tokito, T., Mekhail, T., Planchard, D., Kim, Y.C., Karapetis, C.S., Hiret, S., Ostoros, G., Kubota, K., Gray, J.E., Paz-Ares, L., de Castro Carpeno, J., Wadsworth, C., Melillo, G., Jiang, H., Huang, Y., Dennis, P.A., Ozguroglu, M., Investigators, P., 2017. Durvalumab after chemoradiotherapy in stage iii non-small-cell lung cancer. N. Engl. J. Med 377 (20), 1919–1929.

Awasthi, R., Singh, A.K., Mishra, G., Maurya, A., Chellappan, D.K., Gupta, G., Hansbro, P. M., Dua, K., 2018. An overview of circular RNAs. Adv. Exp. Med Biol. 1087 3–14.

Ayers, K.L., Ma, M., Debussche, G., Corrigan, D., McCafferty, J., Lee, K., Newman, S., Zhou, X., Hirsch, F.R., Mack, P.C., Liu, J.J., Schadt, E.E., Chen, R., Li, S.D., 2021. A composite biomarker of neutrophil-lymphocyte ratio and hemoglobin level correlates with clinical response to PD-1 and PD-L1 inhibitors in advanced non-small cell lung cancers. BMC Cancer 21 (1), 441.

Ba, H., Liu, L., Peng, Q., Chen, J., Zhu, Y.D., 2021. The relationship between blood-based tumor mutation burden level and efficacy of PD-1/PD-L1 inhibitors in advanced non-small cell lung cancer: a systematic review and meta-analysis. BMC Cancer 21 (1), 1220.

Baek, D., Villen, J., Shin, C., Camargo, F.D., Gygi, S.P., Bartel, D.P., 2008. The impact of microRNAs on protein output. Nature 455 (7209), 64–71.
Bettegowda, C., Sausen, M., Leary, R.J., Kinde, I., Wang, Y., Agrawal, N., Bartlett, B.R., Wang, H., Luber, B., Alani, R.M., Antonarakis, E.S., Azad, N.S., Bardelli, A., Brem, H., Cameron, J.L., Lee, C.C., Fecher, L.A., Gallia, G.L., Gibbs, P., Le, D., Giuntoli, R.L., Goggins, M., Hogarty, M.D., Holdhoff, M., Hong, S.M., Jiao, Y., Juhl, H.H., Kim, J.J., Siravegna, G., Laheru, D.A., Lauricella, C., Lim, M., Lipson, E.J., Marie, S.K., Netto, G.J., Oliner, K.S., Olivi, A., Olsson, L., Riggins, G.J., Sartore-Bianchi, A., Schmidt, K., Shih l, M., Oba-Shinjo, S.M., Siena, S., Theodorescu, D., Tie, J., Harkins, T.T., Veronese, S., Wang, T.L., Weingart, J.D., Wolfgang, C.L., Wood, L.D., Xing, D., Hruban, R.H., Wu, J., Allen, P.J., Schmidt, C.M., Choti, M.A., Velculescu, V. E., Kinzler, K.W., Vogelstein, B., Papadopoulos, N., Diaz Jr., L.A., 2014. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci. Transl. Med 6 (224), 224ra224.
Boeri, M., Milione, M., Proto, C., Signorelli, D., Lo Russo, G., Galeone, C., Verri, C., Mensah, M., Centonze, G., Martinetti, A., Sottotetti, E., Pastorino, U., Garassino, M. C., Sozzi, G., 2019. Circulating miRNAs and PD-L1 tumor expression are associated with survival in advanced NSCLC patients treated with immunotherapy: a prospective study. Clin. Cancer Res 25 (7), 2166–2173.
Bonanno, L., Dal Maso, A., Pavan, A., Zulato, E., Calvetti, L., Pasello, G., Guarneri, V., Conte, P., Indraccolo, S., 2022. Liquid biopsy and non-small cell lung cancer: are we looking at the tip of the iceberg? Br. J. Cancer 127 (3), 383–393.
Borghaei, H., Paz-Ares, L., Horn, L., Spigel, D.R., Steins, M., Ready, N.E., Chow, L.Q., Vokes, E.E., Felip, E., Holgado, E., Barlesi, F., Kohlhaufl, M., Arrieta, O., Burgio, M. A., Fayette, J., Lena, H., Poddubskaya, E., Gerber, D.E., Gettinger, S.N., Rudin, C.M., Rizvi, N., Crino, L., Blumenschein Jr., G.R., Antonia, S.J., Dorange, C., Harbison, C. T., Graf Finckenstein, F., Brahmer, J.R., 2015. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N. Engl. J. Med 373 (17), 1627–1639.
Boutsikou, E., Domvri, K., Hardavella, G., Tsiouda, D., Zarogoulidis, K., Kontakiotis, T., 2018. Tumour necrosis factor, interferon-gamma and interleukins as predictive markers of antiprogrammed cell-death protein-1 treatment in advanced non-small cell lung cancer: a pragmatic approach in clinical practice. Ther. Adv. Med Oncol. 10, 1758835918768238.
Brahmer, J., Reckamp, K.L., Baas, P., Crino, L., Eberhardt, W.E., Poddubskaya, E., Antonia, S., Pluzanski, A., Vokes, E.E., Holgado, E., Waterhouse, D., Ready, N., Gainor, J., Aren Frontera, O., Havel, L., Steins, M., Garassino, M.C., Aerts, J.G., Domine, M., Paz-Ares, L., Reck, M., Baudelet, C., Harbison, C.T., Lestini, B., Spigel, D.R., 2015. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N. Engl. J. Med 373 (2), 123–135.
Buttner, R., Longshore, J.W., Lopez-Rios, F., Merkelbach-Bruse, S., Normanno, N., Rouleau, E., Penault-Llorca, F., 2019. Implementing TMB measurement in clinical practice: considerations on assay requirements. ESMO Open 4 (1), e000442.
Cabel, L., Riva, F., Servois, V., Livartowski, A., Daniel, C., Rampanou, A., Lantz, O., Romano, E., Milder, M., Buecher, B., Piperno-Neumann, S., Bernard, V., Baulande, S., Bieche, I., Pierga, J.Y., Proudhon, C., Bidard, F.C., 2017. Circulating tumor DNA changes for early monitoring of anti-PD1 immunotherapy: a proof-of-concept study. Ann. Oncol. 28 (8), 1996–2001.
Capuozzo, M., Ferrara, F., Santorsola, M., Zovi, A., Ottaiano, A., 2023. Circulating tumor cells as predictive and prognostic biomarkers in solid tumors. Cells 12 (22).
Chae, Y.K., Davis, A.A., Agte, S., Pan, A., Simon, N.I., Iams, W.T., Cruz, M.R., Tamragouri, K., Rhee, K., Mohindra, N., Villaflor, V., Park, W., Lopes, G., Giles, F.J., 2019. Clinical implications of circulating tumor DNA tumor mutational burden (ctDNA TMB) in non-small cell lung cancer. Oncologist 24 (6), 820–828.
Chan, H.T., Chin, Y.M., Low, S.K., 2022. Circulating tumor DNA-based genomic profiling assays in adult solid tumors for precision oncology: recent advancements and future challenges. Cancers (Basel) 14 (13).
Chen, L., Gibbons, D.L., Goswami, S., Cortez, M.A., Ahn, Y.H., Byers, L.A., Zhang, X., Yi, X., Dwyer, D., Lin, W., Diao, L., Wang, J., Roybal, J., Patel, M., Ungewiss, C., Peng, D., Antonia, S., Mediavilla-Varela, M., Robertson, G., Suraokar, M., Welsh, J. W., Erez, B., Wistuba, I.I., Chen, L., Peng, D., Wang, S., Ullrich, S.E., Heymach, J.V., Kurie, J.M., Qin, F.X., 2014. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat. Commun. 5, 5241.
Chen, X., Fang, L., Zhu, Y., Bao, Z., Wang, Q., Liu, R., Sun, W., Du, H., Lin, J., Yu, B., Chen, S., Zhou, J., Zhou, J., 2021. Blood tumor mutation burden can predict the clinical response to immune checkpoint inhibitors in advanced non-small cell lung cancer patients. Cancer Immunol. Immunother. 70 (12), 3513–3524.
Cho, B.C., Abreu, D.R., Hussein, M., Cobo, M., Patel, A.J., Secen, N., Lee, K.H., Massuti, B., Hiret, S., Yang, J.C.H., Barlesi, F., Lee, D.H., Ares, L.P., Hsieh, R.W., Patil, N.S., Twomey, P., Yang, X., Meng, R., Johnson, M.L., 2022. Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study. Lancet Oncol. 23 (6), 781–792.
Cho, J.W., Hong, M.H., Ha, S.J., Kim, Y.J., Cho, B.C., Lee, I., Kim, H.R., 2020. Genome-wide identification of differentially methylated promoters and enhancers associated with response to anti-PD-1 therapy in non-small cell lung cancer. Exp. Mol. Med 52 (9), 1550–1563.
Cortez, M.A., Ivan, C., Valdecanas, D., Wang, X., Peltier, H.J., Ye, Y., Araujo, L., Carbone, D.P., Shilo, K., Giri, D.K., Kelnar, K., Martin, D., Komaki, R., Gomez, D.R., Krishnan, S., Calin, G.A., Bader, A.G., Welsh, J.W., 2016. PDL1 Regulation by p53 via miR-34. J. Natl. Cancer Inst. 108 (1).
Costantini, A., Julie, C., Dumenil, C., Helias-Rodzewicz, Z., Tisserand, J., Dumoulin, J., Giraud, V., Labrune, S., Chinet, T., Emile, J.F., Giroux Leprieur, E., 2018. Predictive role of plasmatic biomarkers in advanced non-small cell lung cancer treated by nivolumab. Oncoimmunology 7 (8), e1452581.
Cristescu, R., Mogg, R., Ayers, M., Albright, A., Murphy, E., Yearley, J., Sher, X., Liu, X. Q., Lu, H., Nebozhyn, M., Zhang, C., Lunceford, J.K., Joe, A., Cheng, J., Webber, A.L., Ibrahim, N., Plimack, E.R., Ott, P.A., Seiwert, T.Y., Ribas, A., McClanahan, T.K., Tomassini, J.E., Loboda, A., Kaufman, D., 2018. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science 362 (6411).
Crowley, E., Di Nicolantonio, F., Loupakis, F., Bardelli, A., 2013. Liquid biopsy: monitoring cancer-genetics in the blood. Nat. Rev. Clin. Oncol. 10 (8), 472–484.
Daassi, D., Mahoney, K.M., Freeman, G.J., 2020. The importance of exosomal PDL1 in tumour immune evasion. Nat. Rev. Immunol. 20 (4), 209–215.
Dall'Olio, F.G., Gelsomino, F., Conci, N., Marcolin, L., De Giglio, A., Grilli, G., Sperandi, F., Fontana, F., Terracciano, M., Fragomeno, B., Tober, N., Manferrari, G., Brocchi, S., Golfieri, R., Fiorentino, M., Ardizzoni, A., 2021. PD-L1 expression in circulating tumor cells as a promising prognostic biomarker in advanced non-small-cell lung cancer treated with immune checkpoint inhibitors. Clin. Lung Cancer 22 (5), 423–431.
Desai, A., Lovly, C.M., 2023. Challenges in the implementation of ultrasensitive liquid biopsy approaches in precision oncology. J. Immunother. Cancer 11 (6).
Duruisseaux, M., Martinez-Cardus, A., Calleja-Cervantes, M.E., Moran, S., Castro de Moura, M., Davalos, V., Pineyro, D., Sanchez-Cespedes, M., Girard, N., Brevet, M., Giroux-Leprieur, E., Dumenil, C., Pradotto, M., Bironzo, P., Capelletto, E., Novello, S., Cortot, A., Copin, M.C., Karachaliou, N., Gonzalez-Cao, M., Peralta, S., Montuenga, L.M., Gil-Bazo, I., Baraibar, I., Lozano, M.D., Varela, M., Ruffinelli, J.C., Palmero, R., Nadal, E., Moran, T., Perez, L., Ramos, I., Xiao, Q., Fernandez, A.F., Fraga, M.F., Gut, M., Gut, I., Teixido, C., Vilarino, N., Prat, A., Reguart, N., Benito, A., Garrido, P., Barragan, I., Emile, J.F., Rosell, R., Brambilla, E., Esteller, M., 2018. Epigenetic prediction of response to anti-PD-1 treatment in non-small-cell lung cancer: a multicentre, retrospective analysis. Lancet Respir. Med 6 (10), 771–781.
El Gazzar, M., 2014. microRNAs as potential regulators of myeloid-derived suppressor cell expansion. Innate Immun. 20 (3), 227–238.
Eltahir, M., Isaksson, J., Mattsson, J.S.M., Karre, K., Botling, J., Lord, M., Mangsbo, S.M., Micke, P., 2021. Plasma proteomic analysis in non-small cell lung cancer patients treated with PD-1/PD-L1 blockade. Cancers (Basel) 13 (13).
Eslami, S.Z., Cortes-Hernandez, L.E., Sinoquet, L., Gauthier, L., Vautrot, V., Cayrefourcq, L., Avoscan, L., Jacot, W., Pouderoux, S., Viala, M., Thomas, Q.D., Lamy, P.J., Quantin, X., Gobbo, J., Alix-Panabieres, C., 2024. Circulating tumour cells and PD-L1-positive small extracellular vesicles: the liquid biopsy combination for prognostic information in patients with metastatic non-small cell lung cancer. Br. J. Cancer 130 (1), 63–72.
Fabbiano, F., Corsi, J., Gurrieri, E., Trevisan, C., Notarangelo, M., D'Agostino, V.G., 2020. RNA packaging into extracellular vesicles: An orchestra of RNA-binding proteins? J Extracell Vesicles 10 (2), e12043. https://doi.org/10.1002/jev2.12043. Dec; Epub 2020 Dec 28. PMID: 33391635; PMCID: PMC7769857.
Fan, J., Yin, Z., Xu, J., Wu, F., Huang, Q., Yang, L., Jin, Y., Yang, G., 2020. Circulating microRNAs predict the response to anti-PD-1 therapy in non-small cell lung cancer. Genomics 112 (2), 2063–2071.
Fehrenbacher, L., Spira, A., Ballinger, M., Kowanetz, M., Vansteenkiste, J., Mazieres, J., Park, K., Smith, D., Artal-Cortes, A., Lewanski, C., Braiteh, F., Waterkamp, D., He, P., Zou, W., Chen, D.S., Yi, J., Sandler, A., Rittmeyer, A., Group, P.S., 2016. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet 387 (10030), 1837–1846.
Felip, E., Altorki, N., Zhou, C., Csoszi, T., Vynnychenko, I., Goloborodko, O., Luft, A., Akopov, A., Martinez-Marti, A., Kenmotsu, H., Chen, Y.M., Chella, A., Sugawara, S., Voong, D., Wu, F., Yi, J., Deng, Y., McCleland, M., Bennett, E., Gitlitz, B., Wakelee, H., Investigators, I.M., 2021. Adjuvant atezolizumab after adjuvant chemotherapy in resected stage IB-IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase 3 trial. Lancet 398 (10308), 1344–1357.
Ferrara, R., Naigeon, M., Auclin, E., Duchemann, B., Cassard, L., Jouniaux, J.M., Boselli, L., Grivel, J., Desnoyer, A., Mezquita, L., Texier, M., Caramella, C., Hendriks, L., Planchard, D., Remon, J., Sangaletti, S., Proto, C., Garassino, M.C., Soria, J.C., Marabelle, A., Voisin, A.L., Farhane, S., Besse, B., Chaput, N., 2021. Circulating T-cell immunosenescence in patients with advanced non-small cell lung cancer treated with single-agent PD-1/PD-L1 inhibitors or platinum-based chemotherapy. Clin. Cancer Res 27 (2), 492–503.
Forde, P.M., Spicer, J., Lu, S., Provencio, M., Mitsudomi, T., Awad, M.M., Felip, E., Broderick, S.R., Brahmer, J.R., Swanson, S.J., Kerr, K., Wang, C., Ciuleanu, T.E., Saylors, G.B., Tanaka, F., Ito, H., Chen, K.N., Liberman, M., Vokes, E.E., Taube, J.M., Dorange, C., Cai, J., Fiore, J., Jarkowski, A., Balli, D., Sausen, M., Pandya, D., Calvet, C.Y., Girard, N., CheckMate, I., 2022. Neoadjuvant nivolumab plus chemotherapy in resectable lung cancer. N. Engl. J. Med 386 (21), 1973–1985.
Gandara, D.R., Paul, S.M., Kowanetz, M., Schleifman, E., Zou, W., Li, Y., Rittmeyer, A., Fehrenbacher, L., Otto, G., Malboeuf, C., Lieber, D.S., Lipson, D., Silterra, J., Amler, L., Riehl, T., Cummings, C.A., Hegde, P.S., Sandler, A., Ballinger, M., Fabrizio, D., Mok, T., Shames, D.S., 2018. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat. Med 24 (9), 1441–1448.
Gandhi, L., Rodriguez-Abreu, D., Gadgeel, S., Esteban, E., Felip, E., De Angelis, F., Domine, M., Clingan, P., Hochmair, M.J., Powell, S.F., Cheng, S.Y., Bischoff, H.G., Peled, N., Grossi, F., Jennens, R.R., Reck, M., Hui, R., Garon, E.B., Boyer, M., Rubio-Viqueira, B., Novello, S., Kurata, T., Gray, J.E., Vida, J., Wei, Z., Yang, J., Raftopoulos, H., Pietanza, M.C., Garassino, M.C., Investigators, K.-, 2018. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N. Engl. J. Med 378 (22), 2078–2092.
Gao, W., Liu, Q., Zhou, Y., Yang, M., Yu, Y., 2023. The predictive model construction for immune-related adverse events in non-small cell lung cancer patients receiving immunotherapy. Technol. Cancer Res Treat. 22, 15330338231206705.

Ghoroghi, S., Mary, B., Asokan, N., Goetz, J.G., Hyenne, V., 2021. Tumor extracellular vesicles drive metastasis (it's a long way from home). FASEB Bioadv 3 (11), 930–943.
Goh, K.Y., Cheng, T.Y., Tham, S.C., Lim, D.W., 2023. Circulating biomarkers for prediction of immunotherapy response in NSCLC. Biomedicines 11 (2).
Goldberg, S.B., Narayan, A., Kole, A.J., Decker, R.H., Teysir, J., Carriero, N.J., Lee, A., Nemati, R., Nath, S.K., Mane, S.M., Deng, Y., Sukumar, N., Zelterman, D., Boffa, D.J., Politi, K., Gettinger, S.N., Wilson, L.D., Herbst, R.S., Patel, A.A., 2018. Early assessment of lung cancer immunotherapy response via circulating tumor DNA. Clin. Cancer Res 24 (8), 1872–1880.
Goodall, G.J., Wickramasinghe, V.O., 2021. RNA in cancer. Nat. Rev. Cancer 21 (1), 22–36.
Guibert, N., Delaunay, M., Lusque, A., Boubekeur, N., Rouquette, I., Clermont, E., Mourlanette, J., Gouin, S., Dormoy, I., Favre, G., Mazieres, J., Pradines, A., 2018. PD-L1 expression in circulating tumor cells of advanced non-small cell lung cancer patients treated with nivolumab. Lung Cancer 120, 108–112.
Guibert, N., Jones, G., Beeler, J.F., Plagnol, V., Morris, C., Mourlanette, J., Delaunay, M., Keller, L., Rouquette, I., Favre, G., Pradines, A., Mazieres, J., 2019. Targeted sequencing of plasma cell-free DNA to predict response to PD1 inhibitors in advanced non-small cell lung cancer. Lung Cancer 137, 1–6.
Guo, D., Yang, L., Yang, J., Shi, K., 2020. Plasma cell-free DNA methylation combined with tumor mutation detection in prognostic prediction of patients with non-small cell lung cancer (NSCLC). Med. (Baltim.) 99 (26), e20431.
Halvorsen, A.R., Sandhu, V., Sprauten, M., Flote, V.G., Kure, E.H., Brustugun, O.T., Helland, A., 2018. Circulating microRNAs associated with prolonged overall survival in lung cancer patients treated with nivolumab. Acta Oncol. 57 (9), 1225–1231.
Han, B., Tjulandin, S., Hagiwara, K., Normanno, N., Wulandari, L., Laktionov, K., Hudoyo, A., He, Y., Zhang, Y.P., Wang, M.Z., Liu, C.Y., Ratcliffe, M., McCormack, R., Reck, M., 2017. EGFR mutation prevalence in Asia-Pacific and Russian patients with advanced NSCLC of adenocarcinoma and non-adenocarcinoma histology: the IGNITE study. Lung Cancer 113, 37–44.
Harel, M., Lahav, C., Jacob, E., Dahan, N., Sela, I., Elon, Y., Raveh Shoval, S., Yahalom, G., Kamer, I., Zer, A., Sharon, O., Carbone, D.P., Dicker, A.P., Bar, J., Shaked, Y., 2022. Longitudinal plasma proteomic profiling of patients with non-small cell lung cancer undergoing immune checkpoint blockade. J. Immunother. Cancer 10 (6).
Hellmann, M.D., Ciuleanu, T.E., Pluzanski, A., Lee, J.S., Otterson, G.A., Audigier-Valette, C., Minenza, E., Linardou, H., Burgers, S., Salman, P., Borghaei, H., Ramalingam, S.S., Brahmer, J., Reck, M., O'Byrne, K.J., Geese, W.J., Green, G., Chang, H., Szustakowski, J., Bhagavatheeswaran, P., Healey, D., Fu, Y., Nathan, F., Paz-Ares, L., 2018. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N. Engl. J. Med 378 (22), 2093–2104.
Hellmann, M.D., Paz-Ares, L., Bernabe Caro, R., Zurawski, B., Kim, S.W., Carcereny Costa, E., Park, K., Alexandru, A., Lupinacci, L., de la Mora Jimenez, E., Sakai, H., Albert, I., Vergnenegre, A., Peters, S., Syrigos, K., Barlesi, F., Reck, M., Borghaei, H., Brahmer, J.R., O'Byrne, K.J., Geese, W.J., Bhagavatheeswaran, P., Rabindran, S.K., Kasinathan, R.S., Nathan, F.E., Ramalingam, S.S., 2019. Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. N. Engl. J. Med 381 (21), 2020–2031.
Herbst, R.S., Baas, P., Kim, D.W., Felip, E., Perez-Gracia, J.L., Han, J.Y., Molina, J., Kim, J.H., Arvis, C.D., Ahn, M.J., Majem, M., Fidler, M.J., de Castro Jr., G., Garrido, M., Lubiniecki, G.M., Shentu, Y., Im, E., Dolled-Filhart, M., Garon, E.B., 2016. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 387 (10027), 1540–1550.
Herbst, R.S., Giaccone, G., de Marinis, F., Reinmuth, N., Vergnenegre, A., Barrios, C.H., Morise, M., Felip, E., Andric, Z., Geater, S., Ozguroglu, M., Zou, W., Sandler, A., Enquist, I., Komatsubara, K., Deng, Y., Kuriki, H., Wen, X., McCleland, M., Mocci, S., Jassem, J., Spigel, D.R., 2020. Atezolizumab for first-line treatment of PD-L1-selected patients with NSCLC. N. Engl. J. Med 383 (14), 1328–1339.
Herbst, R.S., Majem, M., Barlesi, F., Carcereny, E., Chu, Q., Monnet, I., Sanchez-Hernandez, A., Dakhil, S., Camidge, D.R., Winzer, L., Soo-Hoo, Y., Cooper, Z.A., Kumar, R., Bothos, J., Aggarwal, C., Martinez-Marti, A., 2022. COAST: an open-label, phase ii, multidrug platform study of durvalumab alone or in combination with oleclumab or monalizumab in patients with unresectable, stage iii non-small-cell lung cancer. J. Clin. Oncol. 40 (29), 3383–3393.
Hirashima, T., Kanai, T., Suzuki, H., Yoshida, H., Matsushita, A., Kawasumi, H., Samejima, Y., Noda, Y., Nasu, S., Tanaka, A., Morishita, N., Hashimoto, S., Kawahara, K., Tamura, Y., Okamoto, N., Tanaka, T., 2019. The levels of interferon-gamma release as a biomarker for non-small-cell lung cancer patients receiving immune checkpoint inhibitors. Anticancer Res 39 (11), 6231–6240.
Hirsch, F.R., Wynes, M.W., Gandara, D.R., Bunn Jr., P.A., 2010. The tissue is the issue: personalized medicine for non-small cell lung cancer. Clin. Cancer Res 16 (20), 4909–4911.
Hofman, P., Heeke, S., Alix-Panabieres, C., Pantel, K., 2019. Liquid biopsy in the era of immuno-oncology: is it ready for prime-time use for cancer patients? Ann. Oncol. 30 (9), 1448–1459.
Hou, H.W., Warkiani, M.E., Khoo, B.L., Li, Z.R., Soo, R.A., Tan, D.S., Lim, W.T., Han, J., Bhagat, A.A., Lim, C.T., 2013. Isolation and retrieval of circulating tumor cells using centrifugal forces. Sci. Rep. 3, 1259.
Hu, Y., Li, S., Xiao, H., Xiong, Y., Lu, X., Yang, X., Luo, W., Luo, J., Zhang, S., Cheng, Y., Zhang, L., Dai, X., Yang, Y., Wang, D., Li, M., 2023. Distinct circulating cytokine/chemokine profiles correlate with clinical benefit of immune checkpoint inhibitor monotherapy and combination therapy in advanced non-small cell lung cancer. Cancer Med 12 (11), 12234–12252.
Ignatiadis, M., Sledge, G.W., Jeffrey, S.S., 2021. Liquid biopsy enters the clinic - implementation issues and future challenges. Nat. Rev. Clin. Oncol. 18 (5), 297–312.
Indini, A., Rijavec, E., Grossi, F., 2021. Circulating biomarkers of response and toxicity of immunotherapy in advanced non-small cell lung cancer (NSCLC): a comprehensive review. Cancers (Basel) 13 (8).
Janne, P.A., Riely, G.J., Gadgeel, S.M., Heist, R.S., Ou, S.I., Pacheco, J.M., Johnson, M.L., Sabari, J.K., Leventakos, K., Yau, E., Bazhenova, L., Negrao, M.V., Pennell, N.A., Zhang, J., Anderes, K., Der-Torossian, H., Kheoh, T., Velastegui, K., Yan, X., Christensen, J.G., Chao, R.C., Spira, A.I., 2022. Adagrasib in non-small-cell lung cancer harboring a KRAS(G12C) mutation. N. Engl. J. Med 387 (2), 120–131.
Janning, M., Kobus, F., Babayan, A., Wikman, H., Velthaus, J.L., Bergmann, S., Schatz, S., Falk, M., Berger, L.A., Bottcher, L.M., Pasler, S., Gorges, T.M., O'Flaherty, L., Hille, C., Joosse, S.A., Simon, R., Tiemann, M., Bokemeyer, C., Reck, M., Riethdorf, S., Pantel, K., Loges, S., 2019. Determination of PD-L1 expression in circulating tumor cells of NSCLC patients and correlation with response to PD-1/PD-L1 inhibitors. Cancers (Basel) 11 (6).
Jiang, T., Bai, Y., Zhou, F., Li, W., Gao, G., Su, C., Ren, S., Chen, X., Zhou, C., 2019. Clinical value of neutrophil-to-lymphocyte ratio in patients with non-small-cell lung cancer treated with PD-1/PD-L1 inhibitors. Lung Cancer 130, 76–83.
Jin, J., Yang, L., Liu, D., Li, W., 2020. Association of the neutrophil to lymphocyte ratio and clinical outcomes in patients with lung cancer receiving immunotherapy: a meta-analysis. BMJ Open 10 (6), e035031.
Kamphorst, A.O., Pillai, R.N., Yang, S., Nasti, T.H., Akondy, R.S., Wieland, A., Sica, G.L., Yu, K., Koenig, L., Patel, N.T., Behera, M., Wu, H., McCausland, M., Chen, Z., Zhang, C., Khuri, F.R., Owonikoko, T.K., Ahmed, R., Ramalingam, S.S., 2017. Proliferation of PD-1+ CD8 T cells in peripheral blood after PD-1-targeted therapy in lung cancer patients. Proc. Natl. Acad. Sci. USA 114 (19), 4993–4998.
Kang, D.H., Park, C.K., Chung, C., Oh, I.J., Kim, Y.C., Park, D., Kim, J., Kwon, G.C., Kwon, I., Sun, P., Shin, E.C., Lee, J.E., 2020. Baseline serum interleukin-6 levels predict the response of patients with advanced non-small cell lung cancer to PD-1/PD-L1 inhibitors. Immune Netw. 20 (3), e27.
Kauffmann-Guerrero, D., Kahnert, K., Kiefl, R., Sellmer, L., Walter, J., Behr, J., Tufman, A., 2021. Systemic inflammation and pro-inflammatory cytokine profile predict response to checkpoint inhibitor treatment in NSCLC: a prospective study. Sci. Rep. 11 (1), 10919.
Keegan, A., Ricciuti, B., Garden, P., Cohen, L., Nishihara, R., Adeni, A., Paweletz, C., Supplee, J., Janne, P.A., Severgnini, M., Awad, M.M., Walt, D.R., 2020. Plasma IL-6 changes correlate to PD-1 inhibitor responses in NSCLC. J. Immunother. Cancer 8 (2).
Keller, L., Belloum, Y., Wikman, H., Pantel, K., 2021. Clinical relevance of blood-based ctDNA analysis: mutation detection and beyond. Br. J. Cancer 124 (2), 345–358.
Kim, E.S., Velcheti, V., Mekhail, T., Yun, C., Shagan, S.M., Hu, S., Chae, Y.K., Leal, T.A., Dowell, J.E., Tsai, M.L., Dakhil, C.S.R., Stella, P., Jin, Y., Shames, D.S., Schleifman, E., Fabrizio, D.A., Phan, S., Socinski, M.A., 2022. Blood-based tumor mutational burden as a biomarker for atezolizumab in non-small cell lung cancer: the phase 2 B-F1RST trial. Nat. Med 28 (5), 939–945.
Kim, K.H., Cho, J., Ku, B.M., Koh, J., Sun, J.M., Lee, S.H., Ahn, J.S., Cheon, J., Min, Y.J., Park, S.H., Park, K., Ahn, M.J., Shin, E.C., 2019. The first-week proliferative response of peripheral blood PD-1(+)CD8(+) T cells predicts the response to anti-PD-1 therapy in solid tumors. Clin. Cancer Res 25 (7), 2144–2154.
Kimura, H., Suminoe, M., Kasahara, K., Sone, T., Araya, T., Tamori, S., Koizumi, F., Nishio, K., Miyamoto, K., Fujimura, M., Nakao, S., 2007. Evaluation of epidermal growth factor receptor mutation status in serum DNA as a predictor of response to gefitinib (IRESSA). Br. J. Cancer 97 (6), 778–784.
Koh, J., Hur, J.Y., Lee, K.Y., Kim, M.S., Heo, J.Y., Ku, B.M., Sun, J.M., Lee, S.H., Ahn, J.S., Park, K., Ahn, M.J., 2020. Regulatory (FoxP3(+)) T cells and TGF-beta predict the response to anti-PD-1 immunotherapy in patients with non-small cell lung cancer. Sci. Rep. 10 (1), 18994.
Kohlhaas, S., Garden, O.A., Scudamore, C., Turner, M., Okkenhaug, K., Vigorito, E., 2009. Cutting edge: the Foxp3 target miR-155 contributes to the development of regulatory T cells. J. Immunol. 182 (5), 2578–2582.
Kowanetz, M., Zou, W., Shames, D., Cummings, C., Rizvi, N., Spira, A., Frampton, G., Leveque, V., Flynn, S., Mocci, S., Shankar, G., Funke, R., Ballinger, M., Waterkamp, D., Chen, D., Sandler, A., Hampton, G., Amler, L., Hegde, P., Hellmann, M., 2017. Tumor mutation burden (TMB) is associated with improved efficacy of atezolizumab in 1L and 2L+ NSCLC patients. J. Thorac. Oncol. 12, S321–S322, 2017.
Kristensen, L.S., Andersen, M.S., Stagsted, L.V.W., Ebbesen, K.K., Hansen, T.B., Kjems, J., 2019. The biogenesis, biology and characterization of circular RNAs. Nat. Rev. Genet 20 (11), 675–691.
Kuninty, P.R., Schnittert, J., Storm, G., Prakash, J., 2016. MicroRNA targeting to modulate tumor microenvironment. Front Oncol. 6, 3.
Kwapisz, D., 2017. The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer? Ann. Transl. Med 5 (3), 46.
Lazzari, C., Spagnolo, C.C., Ciappina, G., Di Pietro, M., Squeri, A., Passalacqua, M.I., Marchesi, S., Gregorc, V., Santarpia, M., 2023. Immunotherapy in early-stage non-small cell lung cancer (NSCLC): current evidence and perspectives. Curr. Oncol. 30 (4), 3684–3696.
Li, H., Xu, J.D., Fang, X.H., Zhu, J.N., Yang, J., Pan, R., Yuan, S.J., Zeng, N., Yang, Z.Z., Yang, H., Wang, X.P., Duan, J.Z., Wang, S., Luo, J.F., Wu, S.L., Shan, Z.X., 2020. Circular RNA circRNA_000203 aggravates cardiac hypertrophy via suppressing miR-26b-5p and miR-140-3p binding to Gata4. Cardiovasc Res 116 (7), 1323–1334.
Li, P., Qin, P., Fu, X., Zhang, G., Yan, X., Zhang, M., Zhang, X., Yang, J., Wang, H., Ma, Z., 2021. Associations between peripheral blood lymphocyte subsets and clinical outcomes in patients with lung cancer treated with immune checkpoint inhibitor. Ann. Palliat. Med 10 (3), 3039–3049.
Liu, C.X., Li, X., Nan, F., Jiang, S., Gao, X., Guo, S.K., Xue, W., Cui, Y., Dong, K., Ding, H., Qu, B., Zhou, Z., Shen, N., Yang, L., Chen, L.L., 2019. Structure and degradation of

circular RNAs regulate PKR activation in innate immunity. Cell 177 (4), 865–880 e821.
Liu, Y., Dong, Z., Jiang, T., Hou, L., Wu, F., Gao, G., He, Y., Zhao, J., Li, X., Zhao, C., Zhang, W., Tian, Q., Pan, Y., Wang, Y., Yang, S., Wu, C., Ren, S., Zhou, C., Zhang, J., Hirsch, F.R., 2018. Heterogeneity of PD-L1 expression among the different histological components and metastatic lymph nodes in patients with resected lung adenosquamous carcinoma. Clin. Lung Cancer 19 (4), e421–e430.
Lu, L.F., Boldin, M.P., Chaudhry, A., Lin, L.L., Taganov, K.D., Hanada, T., Yoshimura, A., Baltimore, D., Rudensky, A.Y., 2010. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. Cell 142 (6), 914–929.
Malapelle, U., Mayo de-Las-Casas, C., Rocco, D., Garzon, M., Pisapia, P., Jordana-Ariza, N., Russo, M., Sgariglia, R., De Luca, C., Pepe, F., Martinez-Bueno, A., Morales-Espinosa, D., Gonzalez-Cao, M., Karachaliou, N., Viteri Ramirez, S., Bellevicine, C., Molina-Vila, M.A., Rosell, R., Troncone, G., 2017. Development of a gene panel for next-generation sequencing of clinically relevant mutations in cell-free DNA from cancer patients. Br. J. Cancer 116 (6), 802–810.
Malapelle, U., Pisapia, P., Addeo, A., Arrieta, O., Bellosillo, B., Cardona, A.F., Cristofanilli, M., De Miguel-Perez, D., Denninghoff, V., Duran, I., Jantus-Lewintre, E., Nuzzo, P.V., O'Byrne, K., Pauwels, P., Pickering, E.M., Raez, L.E., Russo, A., Serrano, M.J., Gandara, D.R., Troncone, G., Rolfo, C., 2021. Liquid biopsy from research to clinical practice: focus on non-small cell lung cancer. Expert Rev. Mol. Diagn. 21 (11), 1165–1178.
Malapelle, U., Pisapia, P., Pepe, F., Russo, G., Buono, M., Russo, A., Gomez, J., Khorshid, O., Mack, P.C., Rolfo, C., Troncone, G., 2022. The evolving role of liquid biopsy in lung cancer. Lung Cancer 172, 53–64.
Mamdani, H., Matosevic, S., Khalid, A.B., Durm, G., Jalal, S.I., 2022. Immunotherapy in Lung Cancer: Current Landscape and Future Directions. Front Immunol. 13, 823618.
Manjunath, Y., Upparahalli, S.V., Suvilesh, K.N., Avella, D.M., Kimchi, E.T., Staveley-O'Carroll, K.F., Li, G., Kaifi, J.T., 2019. Circulating tumor cell clusters are a potential biomarker for detection of non-small cell lung cancer. Lung Cancer 134, 147–150.
Marabelle, A., Fakih, M., Lopez, J., Shah, M., Shapira-Frommer, R., Nakagawa, K., Chung, H.C., Kindler, H.L., Lopez-Martin, J.A., Miller Jr., W.H., Italiano, A., Kao, S., Piha-Paul, S.A., Delord, J.P., McWilliams, R.R., Fabrizio, D.A., Aurora-Garg, D., Xu, L., Jin, F., Norwood, K., Bang, Y.J., 2020. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 21 (10), 1353–1365.
Markus, H., Chandrananda, D., Moore, E., Mouliere, F., Morris, J., Brenton, J.D., Smith, C.G., Rosenfeld, N., 2022. Refined characterization of circulating tumor DNA through biological feature integration. Sci. Rep. 12 (1), 1928.
Mazzaschi, G., Minari, R., Zecca, A., Cavazzoni, A., Ferri, V., Mori, C., Squadrilli, A., Bordi, P., Buti, S., Bersanelli, M., Leonetti, A., Cosenza, A., Ferri, L., Rapacchi, E., Missale, G., Petronini, P.G., Quaini, F., Tiseo, M., 2020. Soluble PD-L1 and circulating CD8+PD-1+ and NK cells enclose a prognostic and predictive immune effector score in immunotherapy treated NSCLC patients. Lung Cancer 148, 1–11.
Mok, T.S., Wu, Y.L., Ahn, M.J., Garassino, M.C., Kim, H.R., Ramalingam, S.S., Shepherd, F.A., He, Y., Akamatsu, H., Theelen, W.S., Lee, C.K., Sebastian, M., Templeton, A., Mann, H., Marotti, M., Ghiorghiu, S., Papadimitrakopoulou, V.A., Investigators, A., 2017. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N. Engl. J. Med 376 (7), 629–640.
Mok, T.S., Wu, Y.L., Thongprasert, S., Yang, C.H., Chu, D.T., Saijo, N., Sunpaweravong, P., Han, B., Margono, B., Ichinose, Y., Nishiwaki, Y., Ohe, Y., Yang, J.J., Chewaskulyong, B., Jiang, H., Duffield, E.L., Watkins, C.L., Armour, A.A., Fukuoka, M., 2009. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N. Engl. J. Med 361 (10), 947–957.
Moller, A., Lobb, R.J., 2020. The evolving translational potential of small extracellular vesicles in cancer. Nat. Rev. Cancer 20 (12), 697–709.
Monastirioti, A., Papadaki, C., Kalapanida, D., Rounis, K., Michaelidou, K., Papadaki, M. A., Mavroudis, D., Agelaki, S., 2022. Plasma-based microRNA expression analysis in advanced stage NSCLC patients treated with nivolumab. Cancers (Basel) 14 (19).
Mullen, S., Movia, D., 2023. The role of extracellular vesicles in non-small-cell lung cancer, the unknowns, and how new approach methodologies can support new knowledge generation in the field. Eur. J. Pharm. Sci. 188, 106516.
Nabet, B.Y., Esfahani, M.S., Moding, E.J., Hamilton, E.G., Chabon, J.J., Rizvi, H., Steen, C.B., Chaudhuri, A.A., Liu, C.L., Hui, A.B., Almanza, D., Stehr, H., Gojenola, L., Bonilla, R.F., Jin, M.C., Jeon, Y.J., Tseng, D., Liu, C., Merghoub, T., Neal, J.W., Wakelee, H.A., Padda, S.K., Ramchandran, K.J., Das, M., Plodkowski, A. J., Yoo, C., Chen, E.L., Ko, R.B., Newman, A.M., Hellmann, M.D., Alizadeh, A.A., Diehn, M., 2020. Noninvasive early identification of therapeutic benefit from immune checkpoint inhibition. Cell 183 (2), 363–376 e313.
Nicolazzo, C., Raimondi, C., Mancini, M., Caponnetto, S., Gradilone, A., Gandini, O., Mastromartino, M., Del Bene, G., Prete, A., Longo, F., Cortesi, E., Gazzaniga, P., 2016. Monitoring PD-L1 positive circulating tumor cells in non-small cell lung cancer patients treated with the PD-1 inhibitor Nivolumab. Sci. Rep. 6, 31726.
Niu, J., Maurice-Dror, C., Lee, D.H., Kim, D.W., Nagrial, A., Voskoboynik, M., Chung, H. C., Mileham, K., Vaishampayan, U., Rasco, D., Golan, T., Bauer, T.M., Jimeno, A., Chung, V., Chartash, E., Lala, M., Chen, Q., Healy, J.A., Ahn, M.J., 2022. First-in-human phase 1 study of the anti-TIGIT antibody vibostolimab as monotherapy or with pembrolizumab for advanced solid tumors, including non-small-cell lung cancer(☆). Ann. Oncol. 33 (2), 169–180.
Nucera, F., Lo Bello, F., Shen, S.S., Ruggeri, P., Coppolino, I., Di Stefano, A., Stellato, C., Casolaro, V., Hansbro, P.M., Adcock, I.M., Caramori, G., 2021. Role of atypical chemokines and chemokine receptors pathways in the pathogenesis of COPD. Curr. Med Chem. 28 (13), 2577–2653.
O'Brien, M., Paz-Ares, L., Marreaud, S., Dafni, U., Oselin, K., Havel, L., Esteban, E., Isla, D., Martinez-Marti, A., Faehling, M., Tsuboi, M., Lee, J.S., Nakagawa, K., Yang, J., Samkari, A., Keller, S.M., Mauer, M., Jha, N., Stahel, R., Besse, B., Peters, S., Investigators, E.-L.E.P.K.-, 2022. Pembrolizumab versus placebo as adjuvant therapy for completely resected stage IB-IIIA non-small-cell lung cancer (PEARLS/KEYNOTE-091): an interim analysis of a randomised, triple-blind, phase 3 trial. Lancet Oncol. 23 (10), 1274–1286.
Oitaben, A., Fonseca, P., Villanueva, M.J., Garcia-Benito, C., Lopez-Lopez, A., Garrido-Fernandez, A., Gonzalez-Ojea, C., Juaneda-Magdalena, L., Lazaro, M.E., Martinez-Fernandez, M., 2022. Emerging blood-based biomarkers for predicting immunotherapy response in NSCLC. Cancers (Basel) 14 (11).
Orellana Garcia, L.P., Ehmann, F., Hines, P.A., Ritzhaupt, A., Brand, A., 2021. Biomarker and companion diagnostics-a review of medicinal products approved by the european medicines agency. Front Med (Lausanne) 8, 753187.
Ottonello, S., Genova, C., Cossu, I., Fontana, V., Rijavec, E., Rossi, G., Biello, F., Dal Bello, M.G., Tagliamento, M., Alama, A., Coco, S., Boccardo, S., Vanni, I., Ferlazzo, G., Moretta, L., Grossi, F., Mingari, M.C., Carrega, P., Pietra, G., 2020. Association Between Response to Nivolumab Treatment and Peripheral Blood Lymphocyte Subsets in Patients With Non-small Cell Lung Cancer. Front Immunol. 11, 125.
Oyanagi, J., Koh, Y., Sato, K., Mori, K., Teraoka, S., Akamatsu, H., Kanai, K., Hayata, A., Tokudome, N., Akamatsu, K., Nakanishi, M., Ueda, H., Yamamoto, N., 2019. Predictive value of serum protein levels in patients with advanced non-small cell lung cancer treated with nivolumab. Lung Cancer 132, 107–113.
Pardoll, D.M., 2012. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer 12 (4), 252–264.
Pavan, A., Bragadin, A.B., Calvetti, L., Ferro, A., Zulato, E., Attili, I., Nardo, G., Dal Maso, A., Frega, S., Menin, A.G., Fassan, M., Calabrese, F., Pasello, G., Guarneri, V., Aprile, G., Conte, P., Rosell, R., Indraccolo, S., Bonanno, L., 2021. Role of next generation sequencing-based liquid biopsy in advanced non-small cell lung cancer patients treated with immune checkpoint inhibitors: impact of STK11, KRAS and TP53 mutations and co-mutations on outcome. Transl. Lung Cancer Res 10 (1), 202–220.
Pavan, A., Calvetti, L., Dal Maso, A., Attili, I., Del Bianco, P., Pasello, G., Guarneri, V., Aprile, G., Conte, P., Bonanno, L., 2019. Peripheral blood markers identify risk of immune-related toxicity in advanced non-small cell lung cancer treated with immune-checkpoint inhibitors. Oncologist 24 (8), 1128–1136.
Paz-Ares, L., Ciuleanu, T.E., Cobo, M., Schenker, M., Zurawski, B., Menezes, J., Richardet, E., Bennouna, J., Felip, E., Juan-Vidal, O., Alexandru, A., Sakai, H., Lingua, A., Salman, P., Souquet, P.J., De Marchi, P., Martin, C., Perol, M., Scherpereel, A., Lu, S., John, T., Carbone, D.P., Meadows-Shropshire, S., Agrawal, S., Oukessou, A., Yan, J., Reck, M., 2021. First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): an international, randomised, open-label, phase 3 trial. Lancet Oncol. 22 (2), 198–211.
Paz-Ares, L., Luft, A., Vicente, D., Tafreshi, A., Gumus, M., Mazieres, J., Hermes, B., Cay Senler, F., Csoszi, T., Fulop, A., Rodriguez-Cid, J., Wilson, J., Sugawara, S., Kato, T., Lee, K.H., Cheng, Y., Novello, S., Halmos, B., Li, X., Lubiniecki, G.M., Piperdi, B., Kowalski, D.M., Investigators, K.-, 2018. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. N. Engl. J. Med 379 (21), 2040–2051.
Peng, X.X., Yu, R., Wu, X., Wu, S.Y., Pi, C., Chen, Z.H., Zhang, X.C., Gao, C.Y., Shao, Y.W., Liu, L., Wu, Y.L., Zhou, Q., 2020. Correlation of plasma exosomal microRNAs with the efficacy of immunotherapy in EGFR/ALK wild-type advanced non-small cell lung cancer. J. Immunother. Cancer 8 (1).
Pepe, F., Pisapia, P., Gristina, V., Rocco, D., Micheli, M., Micheli, P., Iaccarino, A., Tufano, R., Gragnano, G., Russo, G., De Luca, C., Sgariglia, R., Nacchio, M., Girolami, I., Eccher, A., Russo, A., Troncone, G., Malapelle, U., 2021. Tumor mutational burden on cytological samples: a pilot study. Cancer Cytopathol. 129 (6), 460–467.
Pettinella, F., Lattanzi, C., Donini, M., Caveggion, E., Marini, O., Iannoto, G., Costa, S., Zenaro, E., Fortunato, T.M., Gasperini, S., Giani, M., Belluomini, L., Sposito, M., Insolda, J., Scaglione, I.M., Milella, M., Adamo, A., Poffe, O., Bronte, V., Dusi, S., Cassatella, M.A., Ugel, S., Pilotto, S., Scapini, P., 2023. Plasmacytoid dendritic cell, slan(+)-monocyte and natural killer cell counts function as blood cell-based biomarkers for predicting responses to immune checkpoint inhibitor monotherapy in non-small cell lung cancer patients. Cancers (Basel) 15 (21).
Provencio, M., Nadal, E., Gonzalez-Larriba, J.L., Martinez-Marti, A., Bernabe, R., Bosch-Barrera, J., Casal-Rubio, J., Calvo, V., Insa, A., Ponce, S., Reguart, N., de Castro, J., Mosquera, J., Cobo, M., Aguilar, A., Lopez Vivanco, G., Camps, C., Lopez-Castro, R., Moran, T., Barneto, I., Rodriguez-Abreu, D., Serna-Blasco, R., Benitez, R., Aguado de la Rosa, C., Palmero, R., Hernando-Trancho, F., Martin-Lopez, J., Cruz-Bermudez, A., Massuti, B., Romero, A., 2023. Perioperative nivolumab and chemotherapy in stage iii non-small-cell lung cancer. N. Engl. J. Med 389 (6), 504–513.
Provencio, M., Serna-Blasco, R., Nadal, E., Insa, A., Garcia-Campelo, M.R., Casal Rubio, J., Domine, M., Majem, M., Rodriguez-Abreu, D., Martinez-Marti, A., De Castro Carpeno, J., Cobo, M., Lopez Vivanco, G., Del Barco, E., Bernabe Caro, R., Vinolas, N., Barneto Aranda, I., Viteri, S., Pereira, E., Royuela, A., Calvo, V., Martin-Lopez, J., Garcia-Garcia, F., Casarrubios, M., Franco, F., Sanchez-Herrero, E., Massuti, B., Cruz-Bermudez, A., Romero, A., 2022. Overall survival and biomarker analysis of neoadjuvant nivolumab plus chemotherapy in operable stage iiia non-small-cell lung cancer (NADIM phase II trial). J. Clin. Oncol. 40 (25), 2924–2933.
Rajakumar, T., Horos, R., Jehn, J., Schenz, J., Muley, T., Pelea, O., Hofmann, S., Kittner, P., Kahraman, M., Heuvelman, M., Sikosek, T., Feufel, J., Skottke, J., Notzel, D., Hinkfoth, F., Tikk, K., Daniel-Moreno, A., Ceiler, J., Mercaldo, N., Uhle, F., Uhle, S., Weigand, M.A., Elshiaty, M., Lusky, F., Schindler, H., Ferry, Q., Sauka-Spengler, T., Wu, Q., Rabe, K.F., Reck, M., Thomas, M., Christopoulos, P., Steinkraus, B.R., 2022. A blood-based miRNA signature with prognostic value for

overall survival in advanced stage non-small cell lung cancer treated with immunotherapy. NPJ Precis Oncol. 6 (1), 19.
Reck, M., Rodriguez-Abreu, D., Robinson, A.G., Hui, R., Csoszi, T., Fulop, A., Gottfried, M., Peled, N., Tafreshi, A., Cuffe, S., O'Brien, M., Rao, S., Hotta, K., Leiby, M.A., Lubiniecki, G.M., Shentu, Y., Rangwala, R., Brahmer, J.R., Investigators, K.-, 2016. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N. Engl. J. Med 375 (19), 1823–1833.
Rijavec, E., Coco, S., Genova, C., Rossi, G., Longo, L., Grossi, F., 2019. Liquid biopsy in non-small cell lung cancer: highlights and challenges. Cancers (Basel) 12 (1).
Rittmeyer, A., Barlesi, F., Waterkamp, D., Park, K., Ciardiello, F., von Pawel, J., Gadgeel, S.M., Hida, T., Kowalski, D.M., Dols, M.C., Cortinovis, D.L., Leach, J., Polikoff, J., Barrios, C., Kabbinavar, F., Frontera, O.A., De Marinis, F., Turna, H., Lee, J.S., Ballinger, M., Kowanetz, M., He, P., Chen, D.S., Sandler, A., Gandara, D.R., Group, O.A.K.S., 2017. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 389 (10066), 255–265.
Rizvi, N.A., Cho, B.C., Reinmuth, N., Lee, K.H., Luft, A., Ahn, M.J., van den Heuvel, M.M., Cobo, M., Vicente, D., Smolin, A., Moiseyenko, V., Antonia, S.J., Le Moulec, S., Robinet, G., Natale, R., Schneider, J., Shepherd, F.A., Geater, S.L., Garon, E.B., Kim, E.S., Goldberg, S.B., Nakagawa, K., Raja, R., Higgs, B.W., Boothman, A.M., Zhao, L., Scheuring, U., Stockman, P.K., Chand, V.K., Peters, S., Investigators, M., 2020. Durvalumab with or without tremelimumab vs standard chemotherapy in first-line treatment of metastatic non-small cell lung cancer: the MYSTIC phase 3 randomized clinical trial. JAMA Oncol. 6 (5), 661–674.
Rizvi, N.A., Mazieres, J., Planchard, D., Stinchcombe, T.E., Dy, G.K., Antonia, S.J., Horn, L., Lena, H., Minenza, E., Mennecier, B., Otterson, G.A., Campos, L.T., Gandara, D.R., Levy, B.P., Nair, S.G., Zalcman, G., Wolf, J., Souquet, P.J., Baldini, E., Cappuzzo, F., Chouaid, C., Dowlati, A., Sanborn, R., Lopez-Chavez, A., Grohe, C., Huber, R.M., Harbison, C.T., Baudelet, C., Lestini, B.J., Ramalingam, S.S., 2015. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol. 16 (3), 257–265.
Rolfo, C., Mack, P., Scagliotti, G.V., Aggarwal, C., Arcila, M.E., Barlesi, F., Bivona, T., Diehn, M., Dive, C., Dziadziuszko, R., Leighl, N., Malapelle, U., Mok, T., Peled, N., Raez, L.E., Sequist, L., Sholl, L., Swanton, C., Abbosh, C., Tan, D., Wakelee, H., Wistuba, I., Bunn, R., Freeman-Daily, J., Wynes, M., Belani, C., Mitsudomi, T., Gandara, D., 2021. Liquid biopsy for advanced NSCLC: a consensus statement from the international association for the study of lung cancer. J. Thorac. Oncol. 16 (10), 1647–1662.
Rolfo, C., Mack, P.C., Scagliotti, G.V., Baas, P., Barlesi, F., Bivona, T.G., Herbst, R.S., Mok, T.S., Peled, N., Pirker, R., Raez, L.E., Reck, M., Riess, J.W., Sequist, L.V., Shepherd, F.A., Sholl, L.M., Tan, D.S.W., Wakelee, H.A., Wistuba, I.I., Wynes, M.W., Carbone, D.P., Hirsch, F.R., Gandara, D.R., 2018. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): a statement paper from the IASLC. J. Thorac. Oncol. 13 (9), 1248–1268.
Russo, A., Russano, M., Franchina, T., Migliorino, M.R., Aprile, G., Mansueto, G., Berruti, A., Falcone, A., Aieta, M., Gelibter, A., Russo, A., Barni, S., Maio, M., Martelli, O., Pantano, F., Iacono, D., Calvetti, L., Quadrini, S., Roca, E., Vasile, E., Imperatori, M., Occhipinti, M., Galvano, A., Petrelli, F., Calabro, L., Pasquini, G., Intagliata, S., Ricciardi, G.R.R., Tonini, G., Santini, D., Adamo, V., 2020. Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and outcomes with nivolumab in pretreated non-small cell lung cancer (NSCLC): a large retrospective multicenter study. Adv. Ther. 37 (3), 1145–1155.
Sanmamed, M.F., Perez-Gracia, J.L., Schalper, K.A., Fusco, J.P., Gonzalez, A., Rodriguez-Ruiz, M.E., Onate, C., Perez, G., Alfaro, C., Martin-Algarra, S., Andueza, M.P., Gurpide, A., Morgado, M., Wang, J., Bacchiocchi, A., Halaban, R., Kluger, H., Chen, L., Sznol, M., Melero, I., 2017. Changes in serum interleukin-8 (IL-8) levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients. Ann. Oncol. 28 (8), 1988–1995.
Santarpia, M., Liguori, A., D'Aveni, A., Karachaliou, N., Gonzalez-Cao, M., Daffina, M.G., Lazzari, C., Altavilla, G., Rosell, R., 2018. Liquid biopsy for lung cancer early detection. J. Thorac. Dis. 10 (Suppl 7), S882–S897.
Sezer, A., Kilickap, S., Gumus, M., Bondarenko, I., Ozguroglu, M., Gogishvili, M., Turk, H. M., Cicin, I., Bentsion, D., Gladkov, O., Clingan, P., Sriuranpong, V., Rizvi, N., Gao, B., Li, S., Lee, S., McGuire, K., Chen, C.I., Makharadze, T., Paydas, S., Nechaeva, M., Seebach, F., Weinreich, D.M., Yancopoulos, G.D., Gullo, G., Lowy, I., Rietschel, P., 2021. Cemiplimab monotherapy for first-line treatment of advanced non-small-cell lung cancer with PD-L1 of at least 50%: a multicentre, open-label, global, phase 3, randomised, controlled trial. Lancet 397 (10274), 592–604.
Shields, M.D., Marin-Acevedo, J.A., Pellini, B., 2021. Immunotherapy for advanced non-small cell lung cancer: a decade of progress. Am. Soc. Clin. Oncol. Educ. Book 41, 1–23.
Sholl, L.M., Hirsch, F.R., Hwang, D., Botling, J., Lopez-Rios, F., Bubendorf, L., Mino-Kenudson, M., Roden, A.C., Beasley, M.B., Borczuk, A., Brambilla, E., Chen, G., Chou, T.Y., Chung, J.H., Cooper, W.A., Dacic, S., Lantuejoul, S., Jain, D., Lin, D., Minami, Y., Moreira, A., Nicholson, A.G., Noguchi, M., Papotti, M., Pelosi, G., Poleri, C., Rekhtman, N., Tsao, M.S., Thunnissen, E., Travis, W., Yatabe, Y., Yoshida, A., Daigneault, J.B., Zehir, A., Peters, S., Wistuba, I.I., Kerr, K.M., Longshore, J.W., 2020. The promises and challenges of tumor mutation burden as an immunotherapy biomarker: a perspective from the international association for the study of lung cancer pathology committee. J. Thorac. Oncol. 15 (9), 1409–1424.
Sim, W.C., Loh, C.H., Toh, G.L., Lim, C.W., Chopra, A., Chang, A.Y.C., Goh, L.L., 2018. Non-invasive detection of actionable mutations in advanced non-small-cell lung cancer using targeted sequencing of circulating tumor DNA. Lung Cancer 124, 154–159.
Singh, N., Jaiyesimi, I.A., Ismaila, N., Leighl, N.B., Mamdani, H., Phillips, T., Owen, D.H., 2023a. Therapy for stage IV non-small-cell lung cancer with driver alterations: ASCO living guideline, version 2023.1. J. Clin. Oncol. JCO2300281.
Singh, N., Jaiyesimi, I.A., Ismaila, N., Leighl, N.B., Mamdani, H., Phillips, T., Owen, D.H., 2023b. Therapy for stage iv non-small-cell lung cancer without driver alterations: ASCO living guideline, version 2023.1. J. Clin. Oncol. JCO2300282.
Skoulidis, F., Li, B.T., Dy, G.K., Price, T.J., Falchook, G.S., Wolf, J., Italiano, A., Schuler, M., Borghaei, H., Barlesi, F., Kato, T., Curioni-Fontecedro, A., Sacher, A., Spira, A., Ramalingam, S.S., Takahashi, T., Besse, B., Anderson, A., Ang, A., Tran, Q., Mather, O., Henary, H., Ngarmchamnanrith, G., Friberg, G., Velcheti, V., Govindan, R., 2021. Sotorasib for lung cancers with KRAS p.G12C mutation. N. Engl. J. Med 384 (25), 2371–2381.
Socinski, M.A., Jotte, R.M., Cappuzzo, F., Orlandi, F., Stroyakovskiy, D., Nogami, N., Rodriguez-Abreu, D., Moro-Sibilot, D., Thomas, C.A., Barlesi, F., Finley, G., Kelsch, C., Lee, A., Coleman, S., Deng, Y., Shen, Y., Kowanetz, M., Lopez-Chavez, A., Sandler, A., Reck, M., Group, I.M.S., 2018. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N. Engl. J. Med 378 (24), 2288–2301.
Spigel, D.R., Edelman, M.J., O'Byrne, K., Paz-Ares, L., Mocci, S., Phan, S., Shames, D.S., Smith, D., Yu, W., Paton, V.E., Mok, T., 2017. Results from the phase iii randomized trial of onartuzumab plus erlotinib versus erlotinib in previously treated stage iiib or iv non-small-cell lung cancer: METLung. J. Clin. Oncol. 35 (4), 412–420.
Strati, A., Economopoulou, P., Lianidou, E., Psyrri, A., 2023. Clinical significance of PD-L1 status in circulating tumor cells for cancer management during immunotherapy. Biomedicines 11 (6).
Sun, X., Feng, Y., Zhang, B., Huang, W., Zhao, X., Zhang, H., Yue, D., Wang, C., 2022. The role of neutrophil-to-lymphocyte ratio in predicting pathological response for resectable non-small cell lung cancer treated with neoadjuvant chemotherapy combined with PD-1 checkpoint inhibitors. Cancer Res Treat. 54 (4), 1017–1029.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 71 (3), 209–249.
Suzuki, H.I., Katsura, A., Matsuyama, H., Miyazono, K., 2015. MicroRNA regulons in tumor microenvironment. Oncogene 34 (24), 3085–3094.
Tenuta, M., Pandozzi, C., Sciarra, F., Campolo, F., Gelibter, A.J., Sirgiovanni, G., Cortesi, E., Lenzi, A., Isidori, A.M., Sbardella, E., Venneri, M.A., 2023. Circulating natural killer cells as prognostic value for non-small-cell lung cancer patients treated with immune checkpoint inhibitors: correlation with sarcopenia. Cancers (Basel) 15 (14).
Wang, Y., Chen, H., Zhang, T., Yang, X., Zhong, J., Wang, Y., Chi, Y., Wu, M., An, T., Li, J., Zhao, X., Dong, Z., Wang, Z., Zhao, J., Zhuo, M., Huang, J., 2021. Plasma cytokines interleukin-18 and C-X-C motif chemokine ligand 10 are indicative of the anti-programmed cell death protein-1 treatment response in lung cancer patients. Ann. Transl. Med 9 (1), 33.
Wang, Y., Niu, X., Cheng, Y., Zhang, Y., Xia, L., Xia, W., Lu, S., 2022. Exosomal PD-L1 predicts response with immunotherapy in NSCLC patients. Clin. Exp. Immunol. 3, 316–322. https://doi.org/10.1093/cei/uxac045.
Wang, Z., Duan, J., Cai, S., Han, M., Dong, H., Zhao, J., Zhu, B., Wang, S., Zhuo, M., Sun, J., Wang, Q., Bai, H., Han, J., Tian, Y., Lu, J., Xu, T., Zhao, X., Wang, G., Cao, X., Li, F., Wang, D., Chen, Y., Bai, Y., Zhao, J., Zhao, Z., Zhang, Y., Xiong, L., He, J., Gao, S., Wang, J., 2019. Assessment of blood tumor mutational burden as a potential biomarker for immunotherapy in patients with non-small cell lung cancer with use of a next-generation sequencing cancer gene panel. JAMA Oncol. 5 (5), 696–702.
Williams, S.C., 2013. Circulating tumor cells. Proc. Natl. Acad. Sci. USA 110 (13), 4861.
Woodhouse, R., Li, M., Hughes, J., Delfosse, D., Skoletsky, J., Ma, P., Meng, W., Dewal, N., Milbury, C., Clark, T., Donahue, A., Stover, D., Kennedy, M., Dacpano-Komansky, J., Burns, C., Vietz, C., Alexander, B., Hegde, P., Dennis, L., 2020. Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. PLoS One 15 (9), e0237802.
Wu, H., Weng, G.Z., Sun, L.N., Pan, Z.C., Zhang, L., Chen, Q., Shi, C.M., 2023. T cell invigoration is associated with the clinical response to anti-PD-1-based immunotherapy in non-small cell lung cancer. Cancer Manag Res 15, 1141–1153.
Wu, Y.L., Zhou, C., Liam, C.K., Wu, G., Liu, X., Zhong, Z., Lu, S., Cheng, Y., Han, B., Chen, L., Huang, C., Qin, S., Zhu, Y., Pan, H., Liang, H., Li, E., Jiang, G., How, S.H., Fernando, M.C.L., Zhang, Y., Xia, F., Zuo, Y., 2015. First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study. Ann. Oncol. 26 (9), 1883–1889.
Xu, Y., Ding, L., Li, H., Peng, Z., Ding, K., Huang, Z., Zhou, Z., Xie, M., Yan, J., Feng, S., Fan, Y., 2023. Serum cytokine analysis in a cohort of advanced non-small cell lung cancer treated with PD-1 inhibitors reveals predictive markers of CXCL12. Front Immunol. 14, 1194123.
Yang, Q., Chen, M., Gu, J., Niu, K., Zhao, X., Zheng, L., Xu, Z., Yu, Y., Li, F., Meng, L., Chen, Z., Zhuo, W., Zhang, L., Sun, J., 2021. Novel biomarkers of dynamic blood PD-L1 expression for immune checkpoint inhibitors in advanced non-small-cell lung cancer patients. Front Immunol. 12, 665133.
Ye, L., Zhu, Z., Chen, X., Zhang, H., Huang, J., Gu, S., Zhao, X., 2021. The importance of exosomal PD-L1 in cancer progression and its potential as a therapeutic target. Cells 10 (11).
Zhang, L.X., Gao, J., Long, X., Zhang, P.F., Yang, X., Zhu, S.Q., Pei, X., Qiu, B.Q., Chen, S. W., Lu, F., Lin, K., Xu, J.J., Wu, Y.B., 2022. The circular RNA circHMGB2 drives immunosuppression and anti-PD-1 resistance in lung adenocarcinomas and

squamous cell carcinomas via the miR-181a-5p/CARM1 axis. Mol. Cancer 21 (1), 110.
Zheng, D., Ni, J., Zhong, Y., Ziliang, Q., Wang, B., 2020. Monitoring early clearance of circulating tumor DNA as an effective tool to predict clinical benefits of immune checkpoint inhibitors in lung cancer patients: the preliminary results from clinical trial NCT04203095. JCO 38 e15056-e15056.
Zhu, H., Xie, D., Yu, Y., Yao, L., Xu, B., Huang, L., Wu, S., Li, F., Zheng, Y., Liu, X., Xie, W., Huang, M., Li, H., Zheng, S., Zhang, D., Qiao, G., Chan, L.W.C., Zhou, H., 2021. KEAP1/NFE2L2 mutations of liquid biopsy as prognostic biomarkers in patients with advanced non-small cell lung cancer: results from two multicenter, randomized clinical trials. Front Oncol. 11, 659200.