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REVIEW

Prognostic biomarkers in melanoma: a 2023 update from clinical trials in different therapeutic scenarios

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ABSTRACT

Introduction: Over the past decade, significant advancements in the field of melanoma have included the introduction of a new staging system and the development of immunotherapy and targeted therapies, leading to changes in substage classification and impacting patient prognosis. Despite these strides, early detection remains paramount. The quest for dependable prognostic biomarkers is ongoing, given melanoma's unpredictable nature, especially in identifying patients at risk of relapse. Reliable biomarkers are critical for informed treatment decisions.

Areas covered: This review offers a comprehensive review of prognostic biomarkers in the context of clinical trials for immunotherapy and targeted therapy. It explores different clinical scenarios, including adjuvant, metastatic, and neo-adjuvant settings. Key findings suggest that tumor mutational burden, PD-L1 expression, IFN-γ signature, and immune-related factors are promising biomarkers associated with improved treatment responses.

Expert opinion: Identifying practical prognostic factors for melanoma therapy is challenging due to the tumor's heterogeneity. Promising biomarkers include tumor mutational burden (TMB), circulating tumor DNA, and those characterizing the tumor microenvironment, especially the immune component. Future research should prioritize large-scale, prospective studies to validate and standardize these biomarkers, emphasizing clinical relevance and real-world applicability. Easily accessible biomarkers have the potential to enhance the precision and effectiveness of melanoma management.

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Melanoma; biomarker; prognosis; mRNA; gene expression profile; target therapy; BRAF; immunotherapy

1. Introduction

Over the past decade, there has been a significant global increase in melanoma cases, with over 100,000 individuals annually receiving a melanoma diagnosis in the United States alone [1,2]. Meanwhile, a total of 325 000 new melanoma cases and 57 000 deaths were estimated worldwide in 2020 [3], with approximately 3% of new cases lacking an identifiable primary lesion [4]. During this period, two pivotal developments have reshaped the melanoma landscape: the introduction of a novel staging system and the emergence of innovative systemic therapies [5,6]. Particularly in 2017, a shift occurred in patient classification and prognosis with the unveiling of the eighth edition of the American Joint Committee on Cancer (AJCC) classification [5,7]. This revision entailed crucial changes in the T status, including a redefined classification for T1a and T1b melanomas, now employing a reduced cutoff point of 0.8 mm on the Breslow index. In the realm of N status, non-nodal regional disease, encompassing microsatellites, satellites, and in-transit cutaneous metastases, underwent more structured stratification, with adjustments based on the number of tumor-affected lymph nodes [5,7]. These changes had a direct impact on substage migration, holding significant clinical implications, as the staging of melanoma patients not only influences prognosis but also dictates appropriate clinical and surgical interventions, along with eligibility for therapies and clinical trials. Specifically, tumor thickness and ulceration have assumed a more crucial role in predicting melanoma-specific survival (MSS) within the T1 melanoma subgroup, while the mitotic rate was no longer included in the T1 sub-classification [5,7]. Meanwhile, the past decade has witnessed groundbreaking advances in treating stage II, stage III, and stage IV melanoma with systemic therapies, including PD-1 and PD-L1 inhibitors, CTLA-4 inhibitors and agents targeting the BRAF-MEK pathway. Moreover, the PI3K/AKT/NF-κB pathway is dysregulated in up to 43–60% cases of melanoma, contributing to the progression of the disease and suggesting the potential role of PI3K inhibitors as an additive attractive target and may improve treatment outcome in patients with PTEN and/or AKT-mutant melanomas [8] These systemic therapies have ushered in substantial improvements in overall survival (OS), relapse-free survival (RFS), and progression-free survival (PFS), both in adjuvant and metastatic settings [6]. More recently, the neoadjuvant approach has been suggested to be a valid tool to downstage tumors and thus reduce the extent of needed surgery, allowing for further prognostication based on the initial response to therapy [9]. Despite all these remarkable therapeutic breakthroughs, early diagnosis remains paramount in enhancing prognosis [1]. Furthermore, the quest for valid

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Article highlights

- Melanoma management has undergone a transformative shift with targeted therapy and immunotherapy, highlighting the critical need for validated prognostic biomarkers.
- Biomarker investigations in melanoma have extended beyond metastatic and adjuvant settings, with recent insights emerging from studies focused on neo-adjuvant therapy.
- Promising biomarkers, including tumor mutational burden, PD-L1 expression, IFN-γ signature, and immune-related factors, are linked to improved treatment responses in melanoma.
- The translation of these promising biomarker findings from clinical trials into real-life scenarios necessitates thorough validation.
- Validating these discoveries in real-world settings will strengthen their practical applicability and enhance their significance in guiding personalized melanoma treatment strategies.

prognostic biomarkers that furnish precise insights into disease progression, treatment response, and patient outcomes remains ongoing [10]. Prognostic biomarkers offer information about a patient's overall cancer outcome, independent of therapy, while predictive biomarkers shed light on the effects of therapeutic interventions [11,12]. In this landscape, extensive research has been conducted in recent years to enable risk stratification and facilitate personalized medicine approaches for melanoma patients, encompassing both pre-clinical and clinical domains. In the context of melanoma, several types of various prognostic biomarkers have been described, including serologic markers, circulating tumor products, epigenetic markers, signaling pathway-related indicators, and mRNA biomarkers [13]. The critical need for biomarkers is underscored by the fact that the progression of melanoma remains unpredictable. For instance, data from the Dutch registry reveal that 60% of patients diagnosed with stage IV disease had originally presented with stage I or II disease [14]. Moreover, most patients with invasive melanoma are diagnosed with stage IA melanomas, yet 2% of them will still ultimately die of melanoma within 10 years of diagnosis [13,15]. Despite the well-established value of few prognostic factors (i.e. Breslow thickness, ulceration, extent of metastasis, serum lactate dehydrogenase LDH levels), melanoma continues to defy predictability [5]. Alongside the advent of new effective drugs, the dilemma of identifying which patients are likely to experience relapse looms as the pivotal challenge of the next decade [16,17]. This challenge revolves around assessing and ensuring the reliability of biomarkers, as they are much needed across all settings of melanoma care to effectively discern those who will respond to therapy, those at risk of recurrence, and those who stand to benefit from systemic therapy prior to surgery [16,17]. This study aims to provide a comprehensive and up-to-date review of the literature on the role of prognostic biomarkers, focusing specifically on those assessed in clinical trials related to immunotherapy and targeted therapy. The different clinical settings, including adjuvant, metastatic, and neoadjuvant scenarios, will be investigated to present a concise summary of the most used prognostic biomarkers, with a special emphasis on those showing significant promise.

2. Methods

This literature review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A search was conducted across MEDLINE, PubMed, and Scopus using a combination of specific keywords and Medical Subject Heading (MeSH) terms, including 'clinical trial,' 'melanoma,' 'skin,' 'biomarker,' 'prognosis,' 'immunotherapy,' 'targeted therapy,' 'metastatic,' 'adjuvant,' and 'neo-adjuvant,' employing the Boolean term 'AND' to refine the search. The inclusion criteria were as follows: (i) randomized clinical trials involving patients with primary cutaneous melanoma who received targeted therapy or immunotherapy; (ii) a focus on the analysis of prognostic biomarkers; (iii) publication in the English language. Targeted therapies encompassed BRAF-MEK inhibitors, such as dabrafenib +trametinib, encorafenib+binimetinib, and vemurafenib+cobimetinib. Immunotherapies included anti-PD-1 agents (e.g. nivolumab, pembrolizumab, toripalimab), anti-PD-L1 (e.g. atezolizumab), and anti-CTLA4 (e.g. ipilimumab). Regarding the study results, the research approach encompassed investigations with commonly observed primary outcomes of interest in clinical trials, including Progression-Free Survival (PFS), Duration of Response (DOR), Overall Survival (OS), and Response Rate (RR). The review permitted the inclusion of both Kaplan-Meier estimates derived using a Cox proportional hazards model and studies employing logistic regression. The time range of the review was set from 1 January 2011, to 1 October 2023. Exclusions consisted of secondary and tertiary literature sources, such as reviews and textbook chapters, as well as clinical trial reports not specifically focused on the investigation of prognostic biomarkers. Additionally, rarer melanoma subtypes, such as uveal melanoma, were omitted from this review. Two independent investigators (GR, CS) extracted data, with recourse to a third author (VP) in the event of disagreements. The initial screening involved assessing titles and abstracts, followed by a comprehensive review of potentially relevant articles that met the inclusion and exclusion criteria. For each study, the data considered encompassed authorship, journal, year of publication, the cohort of patients or samples analyzed, the biomarker under investigation, and key findings. The risk of bias was assessed according to the RoB 2 Version 2 of the Cochrane risk-of-bias tool for randomized trials [18] The results were categorized based on different clinical settings, namely metastatic, adjuvant, and neo-adjuvant contexts. The Prisma flowchart is depicted in Figure 1.

3. Results

A comprehensive literature search initially yielded 368 records, with 210 duplicates promptly removed. Subsequently, the first phase of literature screening, focused on title and abstract assessment, led to the exclusion of 118 records, leaving 40 full-text records for further eligibility evaluation. After screening based on inclusion criteria, we ultimately incorporated 27 articles into our review. Regarding the classes of patients included in our study, a predominant focus was on the metastatic setting ($n = 16$), followed by adjuvant ($n = 5$) and neoadjuvant ($n = 6$) cases. In the forthcoming sections, we present the key findings for each category, accompanied by respective synoptic tables (Tables 1–3).

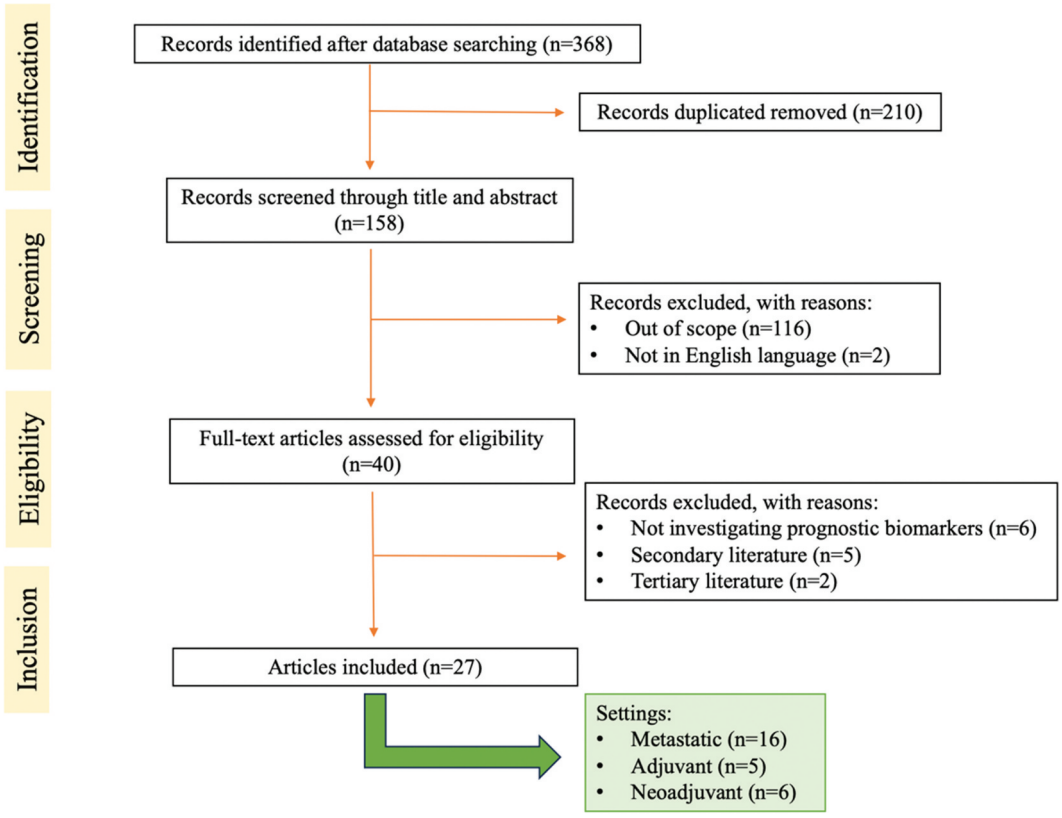

Figure 1. Prisma Flowchart.

3.1. Metastatic setting

In the extensive examination of clinical trials focused on prognostic biomarkers for metastatic melanoma, several significant findings have come to light (Table 1) [19–34]. Dummer and colleagues conducted a retrospective analysis of 366 tumor samples from metastatic patients with BRAF V600E/K mutations treated with targeted therapy in the COLUMBUS trial [19]. Their study revealed that high tumor mutational burden (TMB), PD-L1 expression, and an IFN-γ signature correlated with longer PFS and OS. Conversely, high ErbB2 expression and mutations in the PI3KCA pathway were associated with shorter survival outcomes in the encorafenib+binimetinib arm compared to the vemurafenib arm. The COMBI-MB trial focused on clinical features of BRAF-mutant patients with brain metastasis [20]. It found that a high baseline level of LDH correlated with shorter OS, yet it did not affect PFS. Additionally, patients with a BRAF V600E mutation had a longer PFS (5.9 months) compared to those with BRAF V600D/K/R mutations (4.2 months). Similarly, in the biomarker analysis of the COMBI-i trial for patients with unresectable or metastatic BRAF V600-mutant melanoma, the BRAF V600K mutation, the detection of circulating tumor DNA (ctDNA), and a CD4+/CD8+ ratio higher than the median, correlated with increased PFS in patients treated with spartalizumab, while intratumoral T-cell presence was prognostic across both treatment arms [21]. The pooled analysis by Long *et al*. focusing on the patients receiving dabrafenib+trametinib in the BRF113220, Combi-d, and Combi-v trials showed that patients those with normal LDH and fewer than three organ sites with metastases had the best 1-year PFS (68%) and OS (90%), as well as 2-year PFS (46%) and OS (75%). On the other hand, patients with LDH levels at least two times the upper limit of normal had the poorest 1-year PFS (8%) and OS (40%), and 2-year PFS (2%) and OS (7%) [22]. As for the COBRA trial, it evaluated ctDNA as a prognostic biomarker in metastatic patients treated with chemotherapy, interferon-alpha, and vemurafenib [23]. Their ddPCR (droplet digital polymerase chain reaction) analysis of plasma samples indicated that higher ctDNA levels at baseline and during treatment were associated with a worse prognosis. Similar findings were observed in a single-center, single-arm prospective phase II study of combined treatment with dabrafenib and trametinib, where a decrease in circulating BRAF V600 levels at C1D8 was linked to better disease control, suggesting its potential role as an early biomarker of clinical response [24]. Moreover, baseline circulating BRAF levels were found to correlate with an unfavorable prognosis in metastatic patients enrolled in a randomized phase II trial of vemurafenib+cobimetinib [25]. However, the dynamics of circulating BRAF levels did not correlate with disease survival. Ascierto *et al*. conducted a study in unresectable stage IIIC or stage IV treated with vemurafenib+cobimetinib vs. vemurafenib+placebo in the coBRIM study [26]. They found that high Ki67 expression was associated with a shorter median OS in the vemurafenib group but not in the combination group. No correlation between outcome and pERK and p56 was observed. Extended follow-up of coBRIM revealed that patients treated with vemurafenib+cobimetinib with a normal baseline LDH value and low tumor burden presented longer PFS and OS [27].

Table 1. Metastatic setting.

Trial Name	Study design	Population	Therapy	Biomarkers	Main findings	Ref
Columbus	*Phase III Randomized 3:1*	*Metastatic with BRAF V600E/K mutation n = 344*	*[A] Oral encorafenib 300 mg QD + binimetinib 45 mg BID [B]Oral vemurafenib 300 mg QD*	*TMB, PD-L1, IFN-γ signature, ErbB2, PI3KCA*	*High TMB, PD-L1, and IFN-γ signature associated with longer PFS and OS. High ErbB2 and PI3KCA pathway mutations correlated with shorter survival outcomes in the encorafenib + binimetinib arm*	[19]
COMBI-MB	*Phase II, Non-randomized, 4 arms based on patient characteristics*	*BRAF V600E/D/K/R mutant with symptomatic/asymptomatic brain metastasis with/without prior local brain therapy n = 125*	*Oral dabrafenib 150 mg BID + trametinib 2 mg QD in all 4 arms*	*LDH, BRAF mutation*	*High baseline LDH correlated with shorter OS. Longer PFS was observed in patients with BRAF V600E mutation vs BRAF V600D/K/R mutation*	[20]
COMBI-I	*Phase III Randomized 1:1*	*Unresectable or metastatic BRAF V600-mutant n = 532*	*[A]Intravenous spartalizumab 400 mg every 4 weeks in combination with oral dabrafenib 150 mg BID and trametinib 2 mg QD [B] placebo every 4 weeks in combination with oral dabrafenib 150 mg BID and trametinib 2 mg QD*	*BRAF V600K mutation, ctDNA, CD4 +/CD8+, intratumoral T-cell*	*BRAF V600K mutation, ctDNA and high CD4+/CD8+ ratio correlated with increased PFS in spartalizumab arm. T-cell inflammation was prognostic across the two treatment arms.*	[21]
BRF113220 COMBI-d COMBI-v	*Phase II, Non-randomized, 3 arms Phase III Randomized 1:1 Phase III Randomized 1:1*	*Unresectable or metastatic BRAF V600E/K n = 430 n = 423 n = 704*	*Oral dabrafenib 150 mg BID + trametinib 2 mg QD*	*LDH and organ sites with metastases*	*Normal LDH and fewer than three organ sites with metastases best 1-year and 2-year PFS and OS (90%). LDH levels at least two times the upper limit of normal associated with poorest 1-year and 2-year PFS and OS.*	[22]
COBRA trial	*Prospective study*	*Untreated metastatic with a detectable tumor mutation n = 38*	*Intravenous chemotherapy (temozolomide, vincristine, and lomustine) with interferon-alpha (TOL-IFN) every 4 weeks for the maximum of six cycles followed by IFN maintenance therapy. Oral vemurafenib 960 mg BID was added for patients with BRAFv600 mutated melanomas after two cycles of TOL-IFN.*	*CtDNA*	*CtDNA levels at baseline and during treatment associated with worse prognosis*	[23]
NCT01619774	*Phase II, Non-randomized*	*Unresectable IIIc or metastatic BRAF V600-mutant resistant to BRAFi monotherapy n = 28*	*Oral dabrafenib 150 mg BID + trametinib 2 mg QD*	*Circulating BRAF-RNA*	*Decrease of circulating BRAF- mutant RNA after 1 week of treatment correlated with improved disease control*	[24]
NCT02583516	*Phase II, Randomized*	*Unresectable IIIc or metastatic BRAF V600-mutant n = 70*	*[A] Oral vemurafenib 960 mg BID, days 1 to 28 and 60 mg of cobimetinib QD, days 1 to 21, for each 28-days' cycle [B] Oral vemurafenib 960 mg BID, days 1 to 28 and 60 mg of cobimetinib QD, days 1 to 21, for each 28-days' cycle, during 12 weeks. After that period, patients were treated with both drugs at the same doses indicated previously, but with an intermittent pattern: vemurafenib days 1 to 28 followed by 14 days off and cobimetinib days 1 to 21 followed by 21 days off*	*Circulating BRAF*	*Baseline circulating BRAF correlated with worse prognosis and its dynamics during treatment correlated with clinical response but not with disease survival*	[25]
CoBRIM	*Phase III Randomized 1:1*	*Unresectable IIIc or metastatic BRAF V600-mutant n = 495*	*[A] Oral cobimetinib 60 mg QD on days 1–21 of each 28-day cycle and vemurafenib 960 mg BID on Days 1–28 of each 28-day [B] Oral placebo QD on days 1–21 of each 28-day cycle and vemurafenib 960 mg BID on Days 1–28 of each 28-day*	*Ki67, LDH, tumor burden*	*High Ki67 associated with shorter median OS in the vemurafenib group. Baseline normal LDH and low tumor burden correlated with higher OS and PFS*	[26,27]
BRIM-3	*Phase III Randomized 1:1*	*Unresectable III or stage IV V600E BRAF – mutant n = 675*	*[A] Oral vemurafenib 960 mg BID [B] Intravenous dacarbazine 1000 mg/mΛ2 up to 60 minutes on Day 1 of every 3 weeks*	*Peritumoral CD8 + TILs*	*CD8 + TILs as independent factor of PFS and OS. Neither baseline TILs nor Ki67 predict response to vemurafenib*	[28]

(*Continued*)

Table 1. (Continued).

Trial Name	Study design	Population	Therapy	Biomarkers	Main findings	Ref
IMspire150	*Phase III Randomized 1:1*	*Unresectable IIIc or metastatic BRAF V600-mutant n = 514*	*[A] Cycle 1 = 28 days: oral vemurafenib 960 mg BID and cobimetinib 60 mg QD on days 1 to 21 followed by vemurafenib 720 mg BID on days 22 to 28, Intravenous atezolizumab 840 mg on day 1 and 15, oral cobimetinib 60 mg QD on days 1 to 21, vemurafenib 720 mg BID on days 1 to 28. From cycle 2 intravenous atezolizumab 840 mg on day 1 and 15, oral cobimetinib 60 mg QD on Days 1 to 21, vemurafenib 720 mg BID on days 1 to 28 [B] Cycle 1 = 28 days: oral vemurafenib 960 mg BID and cobimetinib 60 mg QD on days 1 to 21 followed by vemurafenib 720 mg BID on days 22 to 28, Intravenous atezolizumab 840 mg on day 1 and 15, oral cobimetinib 60 mg QD on days 1 to 21, vemurafenib 720 mg BID on days 1 to 28. From cycle 2 intravenous placebo on day 1 and 15, oral cobimetinib 60 mg QD on days 1 to 21, vemurafenib 720 mg BID on days 1 to 28*	*LDH, TMB, IFN-γ, PD-L1 and CD8 + infiltration*	*High LDH and PD-L1-, high TMB and PD-L1-, strong IFN-γ signature and PD-L1+ and high CD8+ infiltration and PD-L1+ correlated with higher PFS and DOR in atezolizumab vs placebo arm*	[29]
POLARIS-01	*Phase II, single arm*	*Unresectable IIIc or metastatic Refractory to standard therapy n = 128*	*Intravenous toripalimab 3 mg/kg Q2w*	*PD-L1*	*Baseline PD-L1 + correlated with significant better ORR, PFS and OS*	[30]
NCT0268196	*Phase IV, single arm*	*Unresectable III or metastatic n = 150*	*Intravenous Ipilimumab 3 mg/kg*	*Endostatin, OPG, CRP, PARC, GDF15 and Gal3BP*	*Endostatin, OPG, CRP, PARC, GDF15 and Gal3BP higher in non survivors. High Gal3BP and endostatin independently associated with poor 2-year survival*	[31]
NCT00261365	*Phase II Randomized*	*Unresectable stage III or metastatic n = 80*	*[A] Intravenous ipilimumab 3 mg/kg Q3w, 12–48 weeks depending on the response. [B] Intravenous ipilimumab 10 mg/kg Q3w, 12–48 weeks depending on the response*	*IDO, FoxP3; TILs*	*High baseline IDO and FoxP3 and high post-treatment TILs positively correlated with clinical response to ipilimumab*	[32]
Checkmate-064	*Phase II Randomized 1:1*	*Unresectable stage III or metastatic n = 138*	*[A] Intravenous nivolumab 3 mg/kg solution Q2w up to 6 doses followed by intravenous ipilimumab 3 mg/kg Q3w up to 4 doses in induction period followed by nivolumab 3 mg/kg Q2w for a maximum of 2 years [B] Intravenous ipilimumab 3 mg/kg Q3w up to 4 doses followed by intravenous nivolumab 3 mg/kg solution Q2w up to 6 doses in induction period followed by nivolumab 3 mg/kg Q2w for a maximum of 2 years*	*IL-6, CRP, neutrophil/ lymphocyte ratio*	*Higher baseline IL-6, N/L ratio and CRP correlated with shorter OS. On-treatment decrease of IL-6 and CRP at week 13 correlated with better OS*	[33]
Checkmate-066	*Phase III Randomized 1:1*	*Unresectable stage III or metastatic BRAF V600- WT n = 418*	*[A] Intravenous nivolumab 3 mg/kg Q2w + intravenous placebo - matching dacarbazine Q3w [B] Intravenous dacarbazine 1000 mg/m^2 Q3w + intravenous placebo -matching nivolumab Q2w*	*IL-6, CRP and neutrophil/ lymphocyte ratio*	*Higher baseline IL-6, N/L ratio, and CRP correlated with shorter OS.*	[33]
Checkmate-067	*Phase III Randomized 1:1:1*	*Unresectable stage III or metastatic n = 1296*	*[A] Intravenous nivolumab 3 mg/kg Q2w [B] Intravenous nivolumab 1 mg/kg + Intravenous ipilimumab 3 mg/kg Q3w for 4 doses followed by nivolumab 3 mg/kg Q2w [C] Intravenous ipilimumab 3 mg/kg Q3w for 4 doses*	*IL-6, CRP and neutrophil/ lymphocyte ratio*	*Higher baseline IL-6, N/L ratio, and CRP correlated with shorter OS.*	[33]
LCCC1531	*Phase II, single arm*	*Unresectable stage III or metastatic naïve for PD1 -inhibitors n = 27*	*Intravenous pembrolizumab 200 mg Q3w*	*LAT1, IDO1*	*High LAT1 and low IDO1 correlated with worse overall OS. Low baseline C11-AMT PET scan SUVmax correlated with prolonged OS*	[34]

TMB (Tumor Mutational Burden), PD-L1 (Programmed Death-Ligand 1), IFN (Interferon), ErbB2 (Epidermal Growth Factor Receptor 2), PI3KCA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha), ctDNA (Circulating Tumor DNA), Ki67 (Ki-67 Antigen), TILs (Tumor-Infiltrating Lymphocytes), OPG (Osteoprotegerin), CRP (C-Reactive Protein), PARC (Prostatic Acid Phosphatase-Related Chromosomal Marker), GDF15 (Growth Differentiation Factor 15), Gal3BP (Galectin-3-Binding Protein), IDO (Indoleamine 2,3-Dioxygenase), FoxP3 (Forkhead Box Protein P3), IL-6 (Interleukin 6), LAT1 (L-Type Amino Acid Transporter 1), IDO1 (Indoleamine 2,3-Dioxygenase 1), C11-AMT PET (Carbon-11 Alpha-Methyltryptophan Positron Emission Tomography).

Table 2. Adjuvant setting.

Trial Name	Study design	Population	Therapy	Biomarker	Main finding	Reference
CheckMate 238	*Phase III Randomized 1:1*	*Resected stage III/IV n = 906*	*[A] Adj nivolumab 3 mg/kg every 2 wks (four doses) then every 12wsk for 1 year [B] Adj ipilimumab 10 mg/kg every 3 wks (four doses) then every 12wsk for 1 year*	*IFN- γ score TMB*	*High TMB and elevated IFNγ associated with improved RFS and OS in both groups.*	[35]
COMBI-AD	*Phase III Randomized 1:1*	*Resected stage III $BRAF^{V600E-K}$ mutated. n = 870*	*[A] Adj oral dabrafenib 150 mg twice daily + oral trametinib 2 mg once daily [B] two placebos*	*IFN- γ score TMB*	*High IFNγ correlate with prolonged RFS in both groups.*	[36]
AVAST-M	*Phase III Randomized 1:1*	*Resected stage III n = 1343 ($BRAF^{V600E-K}$ mutated n = 303)*	*[A] Adj bevacizumab 7.5 mg/kg i.v. infusion once every 3 weeks for 1 year [B] observation*	*BRAF mutation status*	*Negative correlation between BRAF mutation and OS*	[37]
EORTC1325 KEYNOTE-054	*Phase III Randomized 1:1*	*Resected stage III n = 1019*	*[A] Adj pembrolizumab 200 mg i.v infusion every 3 wks for 1 year [B] placebo*	*ß-blockes baseline therapy*	*Negative correlation between ß-blockers therapy and RFS*	[38]
EORTC1325 KEYNOTE-054	*Phase III Randomized 1:1*	*Resected stage III n = 1019*	*[A] Adj pembrolizumab 200 mg i.v infusion every 3 wks for 1 year [B] placebo*	*Metformin baseline therapy*	*Negative correlation between Metformin therapy and RFS*	[39]

Adj (Adjuvant), wks (weeks), i.v (intravenous), TMB (Tumor Mutational Burden), IFN (interferon)..

A retrospective tumor sample analysis in the BRIM-3 trial assessed tumor-infiltrating lymphocytes (TILs) and cell proliferation in unresectable stage III and/or IV BRAFV600-mutant patients treated with vemurafenib or dacarbazine [28]. The study revealed that peritumoral CD8+ TILs were a significant prognostic factor but not a predictor of response to vemurafenib. Ki67 was not associated with any objective response. The IMspire150 study enrolled previously untreated BRAF V600 metastatic melanoma patients in a randomized trial of atezolizumab or placebo in combination with vemurafenib+cobimetinib [29]. The study demonstrated that PFS and duration of response (DOR) benefits for atezolizumab compared to placebo were greater in patients with high LDH and PD-L1-, high TMB and PD-L1-, strong IFNγ signature and PD-L1+, and high CD8+ infiltration and PD-L1+. Recursive partitioning analysis identified baseline LDH, TMB, and IFNγ as solid factors associated with PFS in the atezolizumab group. Likewise, the significance of PD-L1 expression was evaluated in the phase II POLARIS-01 trial, where advanced Chinese patients who were unresponsive to standard treatments received anti-PD-1 toripalimab. Those patients with baseline tumor PD-L1 positive expression demonstrated notably improved objective response rates, PFS, and OS [30]. Another phase IV study analyzed serum samples from 56 patients at baseline and during treatment with ipilimumab [31]. This investigation identified high levels of six proteins – endostatin, OPG, C-reactive-protein (CRP), PARC, GDF15, and Gal3BP – as being associated with non-survivors. Notably, high levels of Gal3BP and endostatin were independently linked to poor 2-year survival. In a separate study conducted by Hamid and colleagues, biomarkers in tumor samples were evaluated both at baseline and after the second dose of ipilimumab [32]. This study found that an increase in tumor-infiltrating lymphocytes (TILs) during treatment and elevated basal expression of IDO and FoxP3 positively correlated with a clinical response to ipilimumab [32]. Laino and colleagues also identified IL-6, CRP, and the neutrophil/lymphocyte ratio as prognostic biomarkers, with elevated levels at baseline being associated with shorter OS in patients enrolled in the Checkmate 064–066 and 067 trials, whether they were receiving immune checkpoint inhibitors or chemotherapy [33]. Furthermore, a multivariate analysis of serum samples collected during treatment with sequential administration of nivolumab followed by ipilimumab or vice versa from patients enrolled in the Checkmate-064 study identified IL-6 as a significant prognostic factor for survival. Lastly, the LCCC1531 trial unveiled that high melanoma cell-specific LAT1 expression and low IDO1 expression in baseline tumor biopsies, along with increased tryptophan metabolism detected by C11-AMT PET scan SUVmax, were associated with worse OS in metastatic melanoma patients treated with pembrolizumab [34].

3.2. Adjuvant setting

Numerous biomarkers have undergone evaluation to stratify patients and standardize the assessment of responses to therapies targeting BRAF/MEK or ICI in adjuvant melanoma trials (Table 2) [35–40]. However, as of now, there remains a lack of compelling clinical or biological prognostic factors directly linked to therapeutic responses and overall patient outcomes. One such biomarker under scrutiny is Tumor Mutational Burden (TMB), defined as the total number of somatic mutations per mega base (Mb) in the coding region of a tumor genome. In the CHECKMATE-238 trial [35], researchers examined the baseline levels of several biomarkers in patients receiving adjuvant immunotherapy. Among these, individuals with high TMB and elevated IFNγ levels demonstrated a stronger correlation with positive clinical outcomes when treated with nivolumab or ipilimumab. Conversely, data from the COMBI-AD trial involving 368 patients, as detailed by Dummer *et al*. [36], emphasized the role of TMB through comprehensive DNA sequencing and gene expression analysis (GES) in a substantial patient cohort receiving

Table 3. Neoadjuvant setting.

Trial Name	Phase	Population	Therapy	Biomarker	Main finding	Reference
OpACIN and OpACIN-neo	*Phase Ib Randomized 1:1 And Phase II Randomized 1:1:1*	*Resectable stage III*	*OpACIN:* *[A] Neoadj ipilimumab (3 mg/kg) + nivolumab (1 mg/k) every 3 wks for 2 cycles then Adj ipilimumab + nivolumab for 2 cycles* *[B] Adj-only ipilimumab (3 mg/kg) + nivolumab (1 mg/kg) every 3 wks for 4 cycles* *OpACIN-neo* *[A] Neoadj ipilimumab (3 mg/kg) + nivolumab (1 mg/kg) every 3 wks, for 2 cycles* *[B] Neoadj ipilimumab (1 mg/kg) + nivolumab (3 mg/kg) every 3 wks, for 2 cycles* *[C] Neoadj ipilimumab (3 mg/kg) every 3 wks, for 2 cycles then nivolumab (3 mg/kg) every 3 wks, for 2 cycles*	*IFN- γ score* *TMB*	*High IFN-γ and TMB correlate with better pCR rate*	[41]
OpACIN-neo	*Phase II Randomized 1:1:1*	*Resectable stage III*	*OpACIN-neo* *[A] Neoadj ipilimumab (3 mg/kg) + nivolumab (1 mg/kg) every 3 wks, for 2 cycles* *[B] Neoadj ipilimumab (1 mg/kg) + nivolumab (3 mg/kg) every 3 wks, for 2 cycles* *[C] Neoadj ipilimumab (3 mg/kg) every 3 wks, for 2 cycles then nivolumab (3 mg/kg) every 3 wks, for 2 cycles*	*TLND histopathological aspect*	*Fibrosis in the tumor bed strongly correlated with improved RFS*	[42]
DONIMI	*Phase Ib Randomized 1:1*	*Resectable stage III n = 20*	*Neoadj nivolumab (240 mg iv every 3 wks) ± ipilimumab (ipilimumab 80 mg) with domatinostat stratified according to the IFN-γ score from tumor biopsy.*	*IFN- γ score in tumor tissue*	*High IFN-γ achieved the most favorable pCR with nivolumab single-agent therapy*	[43]
Amaria 2022 NCT02519322	*Phase II*	*Resectable stage III/IV n = 30*	*Neoadj nivolumab (480 mg every 4 wks) + relatinib (160 mg every 4 wks) for 2 doses, then surgery then Adj nivolumab + relatinib for 10 doses.*	*Tumor inflammatory infiltrate*	*Increased memory CD4+ and effector CD8+ T cells and a decrease of M2 macrophages in tumor specimens after treatment correlate with pCR.*	[44]
Amaria 2018 NCT02231775	*Phase II Randomized 1:2*	*Resectable stage III/IV BRAF $^{V600E-K}$ mutated. n = 21*	*[A] Neoadj Oral dabrafenib (150 mg twice daily) + trametinib (2 mg once daily) for 8 wks, then surgery, then Adj dabrafenib + trametinib* *[B] Standard of care (Adj dabrafenib + trametinib)*	*Tumor inflammatory infiltrate*	*Low remodeling of the T-cell population correlate with pCR following neoadjuvant therapy*	[45]

(*Continued*)

Table 3. (Continued).

Trial Name	Phase	Population	Therapy	Biomarker	Main finding	Reference
OpACIN-neo and PRADO	*Phase II*	*Resectable stage III/IV n = 117*	*OpACIN-neo [A] Neoadj ipilimumab (3 mg/kg) + nivolumab (1 mg/kg) for 2 cycles [B] Neoadj ipilimumab (1 mg/kg) + nivolumab (3 mg/kg) for 2 cycles [C] Neoadj ipilimumab (3 mg/kg) × 2 then nivolumab (3 mg/kg) for 2 cycles PRADO: Neoadj ipilimumab + nivolumab*	*LRG1*	*Patients non-responders to neoadjuvant ICI therapy exhibited higher levels of LRG1*	[46]

Neoadj (neoadjuvant therapy), TMB (Tumor Mutational Burden), IFN (interferon), TLND (Therapeutic lymph node dissection), LRG1 (Leucine-rich alpha-2-glycoprotein 1)..

targeted therapy. Notably, there were no significant differences in mutation analysis within the tumor tissue (at baseline and relapse) between those treated with BRAF inhibitors and the placebo group. However, high TMB was associated with a benefit in relapse-free survival (RFS) solely in the placebo group, not in the dabrafenib plus trametinib group. This disparity might be attributed to the heightened immune escape mechanisms triggered by a greater antigenic tumor burden during therapy. Additionally, high expression of IFN-γ GES emerged as an independent prognostic factor for prolonged RFS in both patient groups. Although the AVAST-M trial didn't yield statistically significant results, it did reveal a trend of negative correlation between OS and the presence of BRAF mutations in the observational arm and a positive correlation in the bevacizumab treatment arm for fully resected stage IIB, IIC, or IIIA-C cutaneous melanoma patients [37]. In the domain of genetic mutations, research has shown an upregulation of beta-receptor expression in different types of tumor cells, including melanoma [38]. These receptors play a crucial role in oncogenesis, influencing processes such as apoptosis, inflammation, and DNA repair pathway signaling, as demonstrated in preclinical studies. Additionally, prognostic investigations have delved into the potential benefits of beta-blocker therapy for melanoma patients. A retrospective analysis conducted in the KEYNOTE 054 study [38] revealed that the simultaneous use of beta-blocker therapy did not impact the prognosis in terms of RFS for stage III resectable melanoma patients who were treated with either pembrolizumab or a placebo. Likewise, metformin, known for its potential antitumorigenic effects through cell cycle arrest and proteinase release, may enhance the effectiveness of anti-melanoma treatments, including anti-PD-1 therapies [39]. In the same KEYNOTE cohort, Kennedy *et al.* [40] evaluated the prognostic value of metformin based on BRAF mutation status and found no significant impact on RFS among patients on metformin therapy at baseline.

3.3. Neoadjuvant setting

In recent investigations involving neoadjuvant trials for stage III melanoma, data are relatively limited due to the novelty of this research avenue (Table 3) [41–46]. In the OPACIN and OPACIN-neo trials, Rozeman *et al.* conducted an extensive biomarker analysis, focusing on patients treated with ipilimumab plus nivolumab [41]. They explored baseline biomarkers as predictors of pathological complete response (pCR) to ICI therapy in a cohort of 65 patients. Notably, individuals with elevated levels of IFN-γ and a high TMB demonstrated a significantly better pCR rate (91%) and RFS after a median follow-up period of 24.6 months. In a separate study [42], participants from the OPACIN-neo trials ($n = 86$) were assessed for clinicopathological characteristics of lymph node dissection (TLND) performed six months after neoadjuvant therapy. A notable finding in this context was the increased percentage of fibrosis in the tumor bed, which strongly correlated with improved RFS, suggesting its potential role as a biomarker of immunotherapeutic response. The DONIMI trial (NCT04133948) [43] represents the first prospective study wherein stage III melanoma patients receiving neoadjuvant immunotherapy

were randomized into different immunotherapy combinations, including nivolumab single-agent therapy, based on their baseline IFN-γ score ($n = 44$). The group with a high IFN-γ score achieved the most favorable pathological response rate with nivolumab single-agent therapy, obviating the need for additional combination treatments. In the framework of new combination neoadjuvant therapies, Amaria et al.. (NCT02519322) examined the use of Relatinib plus nivolumab in resectable III stage or oligometastatic IV stage melanoma [44]. In the study group, patients received this combination therapy before and after surgery. pCR was achieved in more than half of the cases (57%) and in all patients a safety profile was registered. In 70% of patients any pathological response was observed, with a significant free survival rate compared with those without a pathological response in a median follow-up of 2 years. At the same time, pCR was associated with an increase of immune cell infiltration (memory CD4+ and effector CD8+ T cells) and a decrease of M2 macrophages subsets in tumor specimens after treatment. In parallel, neoadjuvant/adjuvant dabrafenib and trametinib was compared to standard adjuvant targeted therapy in resectable III stage and oligometastatic stage IV melanoma. At 12 months, significantly longer event free survival (EFS) and pCR rate were observed in neoadjuvant plus adjuvant arm. Tumor tissue analysis in the neoadjuvant group revealed a lower baseline expression of phosphor-ERK (pERK) in patients who achieved clinical response than in those who did not. Immunohistochemical identification of the immune infiltrate showed no differences in CD8+ T cell concentration by pathological response obtained. Qualitative T cell receptor (TCR) sequencing showed a significant prevalence of the most dominant T cell clone (up to the 20 most dominant clone combination) in case of cPR. Transcriptional profiling of tumor samples showed great upregulation of cytotoxic CD8+ T cells at baseline and early treatment in patients following pCR [45]. Hoefsmit *et al* [46] considered cohorts from the OpACIN-neo ($n = 83$) and PRADO (NCT02977052, $n = 49$) studies to analyze potential biomarkers associated with disease recurrence following neoadjuvant ICI therapy. When comparing baseline soluble factors among patients with stage III melanoma who progressed to stage IV, they found a significant association between the level of leucine-rich alpha-2-glycoprotein 1 (LRG1) and prognosis. Non-responders to neoadjuvant ICI therapy exhibited higher levels of LRG1, which were indicative of a worse prognosis compared to non-responders with lower LRG1 levels.

4. Conclusion

Targeted therapy and immunotherapy have revolutionized the management of melanoma, ushering in significant advancements in patient care [6]. Despite these remarkable strides, the absence of clinically validated prognostic biomarkers remains a major hurdle in tailoring therapeutic decisions to the individual patient [47]. The availability of reliable biomarkers holds the promise of more precisely stratifying patients, assisting clinicians in determining the most suitable therapy for a given clinical scenario. Numerous clinical trials in melanoma, spanning from neoadjuvant to metastatic stages, have incorporated biomarker research into their patient assessments (Table 4). However, the challenge of identifying clinically useful parameters underscores the profound complexity of tumor biology. The initial application of immune checkpoint inhibitors in the metastatic setting paved the way for subsequent investigations into their efficacy and safety in the adjuvant or neoadjuvant contexts. Notably, the combination of nivolumab and ipilimumab has emerged as the most effective treatment for metastatic melanoma patients, irrespective of their BRAF mutation status [48–50]. Nevertheless, this combination therapy is associated with substantial toxicity that may necessitate treatment discontinuation [51]. Targeted therapies employing BRAF and MEK inhibitors have yielded improved overall survival (OS) and progression-free survival (PFS) rates in patients with BRAF mutant advanced melanoma [52–54]. Unfortunately, approximately 15–20% of patients with a BRAF V600E mutation do not respond to therapy. This non-responsiveness can be attributed, in most cases, to the reactivation of the MAPK pathway through various mechanisms, tumor heterogeneity, or the loss of certain suppressor genes. A crucial goal for future research is to identify biomarkers capable of predicting treatment response prior to treatment initiation. In this scenario, the investigation of the tumor mutational burden (TMB) represents a promising tool [55]. Tumors with high TMB have the

Table 4. Main biomarkers investigated in clinical trials.

BIOMARKER	TYPE	CLINICAL TRIALS	REFERENCES
TMB	*Genetic mutation*	*Colombus, IMspire150, CheckMate 238, COMBI-AD, OpACIN and OpACIN-neo*	[18,28,30,31,36]
LDH	*Protein*	*COMBI-MB, BRF113220 COMBI-d, COMBI-v, CoBRIM, IMspire150*	[19,21,25,26,28]
Tissue BRAF V600	*Genetic mutation*	*COMBI-I, AVAST-M*	[20,32]
Circulating BRAF V600,	*Genetic mutation*	*NCT01619774,NCT02583516*	[23,24]
PD-L1	*Protein*	*Colombus, IMspire150, POLARIS-01*	[18,28,29]
IFN- γ signature	*Immune/Inflammatory gene expression*	*Colombus, IMspire150, CheckMate 238, COMBI-AD, OpACIN and OpACIN-neo, DONIMI*	[18,28,30,31,36,38]
ctDNA	*Genetic mutation*	*COMBI-I, COBRA trial*	[20,22]
CD8+ infiltration	*Immune related*	*COMBI-I, BRIM-3, IMspire150*	[20,27,28]
Tumor infiltrated lymphocyte	*Immune related*	*NCT00261365, NCT02519322, NCT02231775*	[31,39,40]
Neutrophil/lymphocyte ratio	*Immune related*	*Checkmate-064, Checkmate-066, Checkmate-067*	[32]
Il-6	*Immune related protein*	*Checkmate-064, Checkmate-066, Checkmate-067*	[32]
CRP	*Inflammatory related protein*	*NCT0268196, Checkmate-064, Checkmate-066, Checkmate-067*	[30,32]

potential to express more neoantigens, which can stimulate the immune response against the tumor and offer insight into the likely therapeutic response. However, conflicting findings regarding the prognostic role of TMB and its relationship with anticancer immunity have been reported. High TMB has been associated with an RFS benefit in patients treated with adjuvant immunotherapy [19,29]. Conversely, different results were observed in trials investigating adjuvant BRAF plus MEK inhibitors versus placebo in the neoadjuvant setting [35]. Another promising tool is represented by circulating tumor DNA (ctDNA), representing the fraction of free DNA found in the bloodstream, released from cancer cells due to cell death. In recent years, ctDNA has been an object of debate within the melanoma research community [56–60]. It enables noninvasive patient monitoring and the specific, sensitive detection of cancer cells. For example, in the COMBI-I clinical trial, ctDNA analysis from blood samples taken at baseline and during treatment revealed that patients achieving complete responses had lower ctDNA levels at baseline compared to those with partial responses or stable disease [21]. Notably, ctDNA held prognostic value for all patients at both baseline and week 8. The COBRA study further underscored the importance of ctDNA, showing that high ctDNA levels at baseline and best overall response were associated with worse prognosis in patients with metastatic cutaneous melanoma treated with chemoimmunotherapy, either alone or in combination with vemurafenib [23]. These findings remained significant even after adjusting for major risk factors, including ECOG performance status, LDH, and M stage. Similar results suggesting the early potential of ctDNA as a clinical response biomarker in melanoma were obtained from trials analyzing circulating BRAF V600 mutant DNA or RNA [24,25]. However, it is essential to conduct more comprehensive investigations in larger populations to better understand the role of ctDNA as a prognostic and predictive biomarker. In addition to the classic markers such as LDH, Ki67, and TMB, which reflect intrinsic tumor characteristics, there is a growing focus on biomarkers that characterize the tumor microenvironment (TME), particularly the immune component. Among serum proteins, elevated levels of C reactive protein levels have been found to be linked to treatment resistance and reduced survival of melanoma patients [61]. The integration of TME into the understanding of tumor development has transformed the approach to cancer treatment [62,63,64–66]. The TME comprises not only normal stroma and tumor cells but also an infiltrating immune component capable of modulating tumor progression. Within the immunosuppressive TME of melanoma, key players include myeloid-derived suppressor cells (MDSCs), T regulatory (Treg) cells, and tumor-associated macrophages (TAMs). These entities collectively impede the body's immune response, fostering tumor growth and progression through various mechanisms. For instance, they release reactive oxygen species (ROS) to suppress NK cell response. Elevated fibroblast levels in the TME lead to increased production of metalloproteinases, which cleave ligands that typically activate receptors on NK cells, thereby hindering T cell activation. Furthermore, the presence of exosomes released by melanoma cells can exacerbate the impairment of NK cell function [67]. Evaluating the immune compartment's features can aid in comprehending tumor evolution and is critical for developing new prognostic tools and enhancing patient stratification for cancer therapy. In the IMSpire trial, authors analyzed factors favoring antitumor immunity, including IFN-γ, PD-L1 expression on tumor-associated immune cells, and CD8+ infiltration [29]. They observed that the PFS and duration of response benefits of atezolizumab versus control were more pronounced in patients with a robust IFN-γ signature and high CD8+ tumor infiltration. However, low PD-L1 expression remained associated with poor prognosis in all cases. In contrast, the POLARIS study found no significant difference in the IFN-γ signature between responders and non-responders in acral and mucosal melanoma [30]. Similarly, the IFN-γ gene expression signature, either alone or in combination with TMB, was strongly linked to prolonged RFS in response to immunotherapy, whether in an adjuvant or single-agent neoadjuvant setting or in adjuvant therapy with BRAF plus MEK inhibitors. In this regard, as previously described, flow cytometry analysis of peripheral blood specimens outlined the impact of cell inflammation subset characterization as a predictor index of tumor regression [64]. Molecular and immunohistochemical identikit of tumor inflammatory infiltrate at baseline and after therapy could predict the type of anti-tumor response among melanoma patients. In the neoadjuvant setting, both immunotherapy and target therapy combination were associated with significantly improved RFS data. After neoadjuvant immunotherapy, T cell inflammatory infiltration in tumor samples (memory CD4+ and effector CD8+ T cells and activated M2 macrophages) could be considered as a predictor of clinical response by the intrinsic mechanism of checkpoint inhibitors stimulating T cell response [44]. Otherwise, in the setting of neoadjuvant targeted therapy no quantitative differences in T-cell infiltration have been shown. In patients who achieved cPR, a lower baseline T-cell remodeling and predominant TCR clonality contribute to anti-tumor response with activation during the MAPK inhibitor-mediated response. This suggests that a predominant tumor clone reactive may preexist at baseline before starting neoadjuvant therapy and predict outcome benefit [45]. These biomarkers hold promise as predictive factors for identifying patients more or less likely to respond to therapies, potentially serving as a decision-making score or algorithm for personalizing treatment schedules. In summary, the outcomes derived from diverse clinical trials highlight the crucial role of biomarker assessment in categorizing melanoma patients and formulating optimal treatment approaches. Notably, the exploration of how these prognostic markers influence drug sequencing, as exemplified by trials like DreamSeq and Secombit [68], is a novel area that has yet to be thoroughly investigated [69]. This research gap highlights the imperative for further investigation to thoroughly gauge the prognostic significance of these potential biomarkers. In this scenario, accessibility and cost-effectiveness are key determinants of practicality. Biomarkers such as BRAF status and LDH

levels, extensively studied for their affordability and easy access, stand out. However, other promising emerging biomarkers encounter hurdles. The extensive monitoring of ctDNA, for example, using techniques like droplet digital PCR (ddPCR), may hinder broad adoption on a larger scale. Ensuring the generalizability of such findings presents challenges, demanding meticulous calibration and well-defined assessment protocols. Overcoming these issues is crucial for integrating into routine clinical practice.

5. Expert opinion

The field of melanoma therapy has experienced a remarkable transformation, primarily driven by the emergence of targeted therapy and immunotherapy, significantly elevating the standard of patient care [1,6]. However, amidst this progress, a persistent challenge looms large: the scarcity of clinically validated prognostic biomarkers. This gap in our understanding hampers the customization of treatment strategies for individual patients, preventing us from fully capitalizing on the potential of these groundbreaking therapies. Despite the plethora of studies conducted in recent years, a recurring issue emerges – reproducibility. Numerous studies, although informative, lack the capacity to influence clinical decision-making [13]. In this scenario, clinical trials, with their structured protocols, can offer a path to establishing clinical reliability (i.e. the selected biomarker can effectively divide the patient population into distinct groups, aiding in the precise selection of therapies). To achieve this, standardized protocols for sample collection and analysis are indispensable. External validation, conducted across different settings, must follow, ensuring that the biomarker's performance remains consistent and reliable. Simultaneously, the concept of clinical functionality is equally essential. A biomarker should not merely be a scientific curiosity; it must enhance patient care. It should contribute valuable information that can guide clinicians in selecting the most suitable therapies. This means that the biomarker needs to provide an advantage, either in terms of improved outcomes or reduced toxicity, to justify its inclusion in clinical practice. In recent years, various biomarkers have been scrutinized, including TMB and ctDNA (Figure 2). TMB, while showing promise in stimulating an immune response, has yielded conflicting findings about its prognostic role. ctDNA offers noninvasive monitoring and sensitive detection, yet its position as a prognostic and predictive biomarker necessitates further exploration. Moreover, increasing attention is being directed toward biomarkers characterizing the tumor microenvironment (TME), with a particular focus on the immune component. This exploration holds immense potential, as clinically validated and utility-driven biomarkers can revolutionize patient stratification, ultimately leading to more precise treatment decisions. Nonetheless, the journey ahead is not without its hurdles. The generalizability of the findings from the mentioned studies remains constrained for various reasons. Firstly,

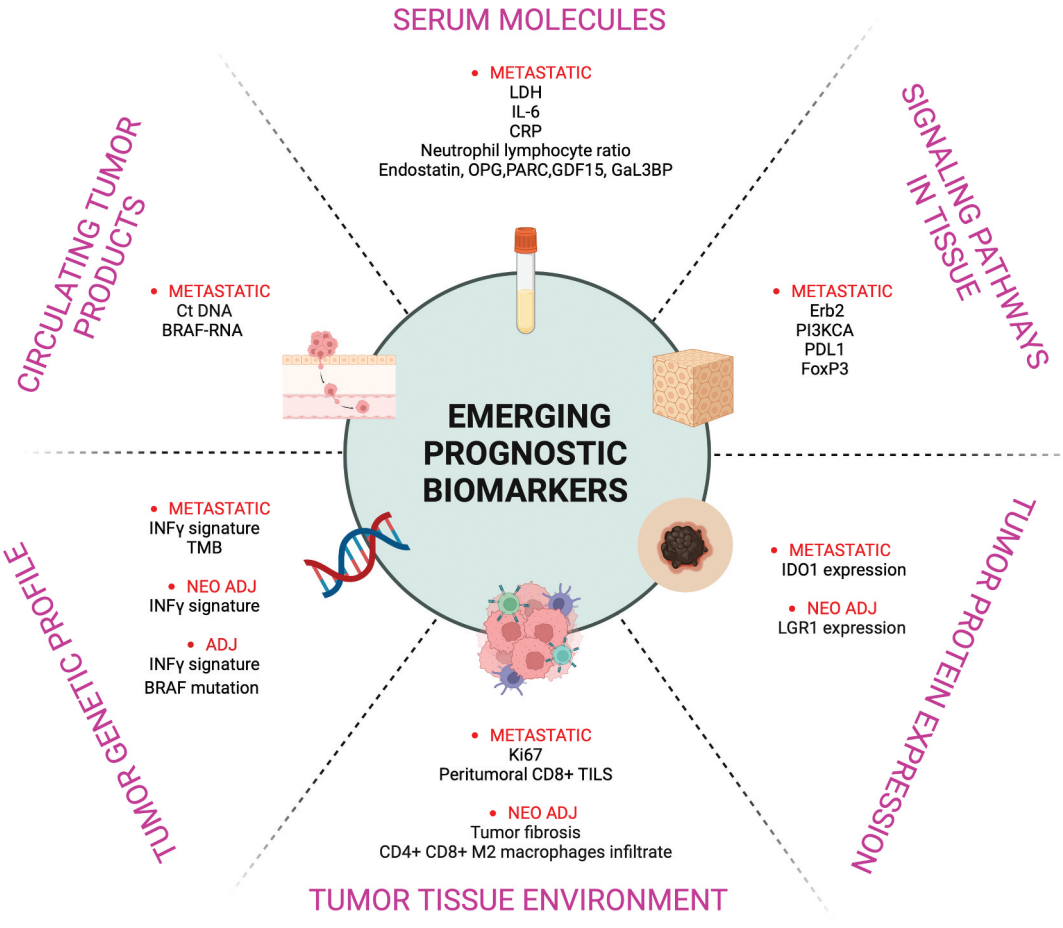

Figure 2. Emerging prognostic biomarkers from clinical trials.

the trial cohorts are notably selected, deviating from real-world scenarios. Secondly, the inclusion criteria's diversity may yield variable outcomes, notably concerning patients with distinct mutation statuses, like BRAF-positive versus BRAF wild-type. Additionally, their independence from other well-known prognostic factors requires further assessment. Implementing these biomarkers in real-world clinical practice is intricate, demanding standardized evaluation and grappling with the complexities of tumor biology. Therefore, the future of melanoma therapy research should prioritize large-scale, prospective studies to validate and standardize these biomarkers, placing strong emphasis on clinical validity and utility. As the field advances, we advocate for a growing emphasis on easily accessible biomarkers [13,17]. Exploring easily accessible biomarkers will be pivotal, making patient monitoring feasible in real-life scenarios. To unlock the full potential of these biomarkers, further research, standardization, and rigorous clinical trials are imperative, ultimately leading to more precise and effective melanoma management.

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Declaration of interest

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Amabile S, Roccuzzo G, Pala V, et al. Clinical significance of distant metastasis-free survival (DMFS) in melanoma: a narrative review from adjuvant clinical trials. J Clin Med. 2021;10(23):5475. doi: 10.3390/jcm10235475
2. Howlader N, Noone A, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2018. Bethesda, MD: National Cancer Institute; 2021. https://seer.cancer.gov/csr/1975_2018/
3. Arnold M, Singh D, Laversanne M, et al. Global Burden of Cutaneous Melanoma in 2020 and projections to 2040. JAMA Dermatol. 2022 May 1;158(5):495–503. doi: 10.1001/jamadermatol.2022.0160
4. Boussios S, Rassy E, Samartzis E, et al. Melanoma of unknown primary: new perspectives for an old story. Crit Rev Oncol Hematol. 2021 Feb;158:103208. doi: 10.1016/j.critrevonc.2020.103208
5. Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma staging: evidence-based changes in the American Joint Committee on cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017;67(6):472–492. doi: 10.3322/caac.21409
6. Qin Z, Zheng M. Advances in targeted therapy and immunotherapy for melanoma (review). Exp Ther Med. 2023;26(3):416. doi: 10.3892/etm.2023.12115
7. Barreiro-Capurro A, Andrés-Lencina JJ, Podlipnik S, et al. Differences in cutaneous melanoma survival between the 7th and 8th edition of the American Joint Committee on cancer (AJCC). A multicentric population-based study. Eur J Cancer. 2021;145:29–37. doi: 10.1016/j.ejca.2020.11.036
8. Adeleke S, Okoli S, Augustine A, et al. Melanoma-the therapeutic considerations in the clinical practice. Ann Palliat Med. 2023 Nov;12:(6):1355–1372.
9. Bushara O, Tidwell J, Wester JR, et al. The Current state of neoadjuvant therapy in resectable advanced stage melanoma. Cancers (Basel). 2023;15(13):3344. doi: 10.3390/cancers15133344
10. Tonella L, Pala V, Ponti R, et al. Prognostic and predictive biomarkers in stage III melanoma: Current insights and clinical implications. Int J Mol Sci. 2021;22(9):4561. doi: 10.3390/ijms22094561
11. Oldenhuis CN, Oosting SF, Gietema JA, et al. Prognostic versus predictive value of biomarkers in oncology. Eur J Cancer. 2008;44(7):946–953. doi: 10.1016/j.ejca.2008.03.006
12. Roccuzzo G, Gherardi E, Maio M, et al. Immunotherapy in cutaneous melanoma and biologics in psoriatic disease: similarities and differences from a clinical multidisciplinary perspective. Expert Opin Biol Ther. Published online 2024 Feb 29;24(3):125–131. doi: 10.1080/14712598.2024.2326168
13. Ding L, Gosh A, Lee DJ, et al. Prognostic biomarkers of cutaneous melanoma. Photoderm Photoimm Photomed. 2022;38(5):418–434.
14. Zhou C, Louwman M, Wakkee M, et al. Primary melanoma characteristics of metastatic disease: a nationwide cancer registry study. Cancers (Basel). 2021;13(17):4431. doi: 10.3390/cancers13174431
15. Strömberg U, Parkes BL, Holmén A, et al. Disease mapping of early- and late-stage cancer to monitor inequalities in early detection: a study of cutaneous malignant melanoma. Eur J Epidemiol. 2020;35(6):537–547. doi: 10.1007/s10654-020-00637-0
16. Ascierto PA, Avallone A, Bifulco C, et al. Perspectives in immunotherapy: meeting report from immunotherapy bridge (Naples, November 30th-December 1st, 2022). J Transl Med. 2023;21(1):488. doi: 10.1186/s12967-023-04329-7
17. Ribero S, Quaglino P, Roccuzzo G. Predicting progression in very thin melanoma: the challenge of the next decade? Br J Dermatol. 2023;189(4):362–363. doi: 10.1093/bjd/ljad218
18. Higgins JPT, Altman DG, GÃ¸tzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials BMJ. BMJ. 2011;343:d5928. doi: 10.1136/bmj.d5928
19. Dummer R, Pathan N, Deng S, et al. 786O tumor biomarker analysis from COLUMBUS part 1: encorafenib + binimetinib for BRAF V600E/K-mutant advanced or metastatic melanoma. Ann Oncol. 2022;33(7):S902.
20. Wilmott JS, Tawbi H, Engh JA, et al. Clinical features associated with outcomes and biomarker analysis of dabrafenib plus trametinib treatment in patients with BRAF-Mutant melanoma brain metastases. Clin Cancer Res. 2023;29(3):521–531. doi: 10.1158/1078-0432.CCR-22-2581
21. Tawbi HA, Robert C, Brase JC, et al. Spartalizumab or placebo in combination with dabrafenib and trametinib in patients with BRAF V600-mutant melanoma: exploratory biomarker analyses from a randomized phase 3 trial (COMBI-i). J Immunother Cancer. 2022;10(6):e004226. doi: 10.1136/jitc-2021-004226
    - **Description of predictive and prognostic impact of LDH and organ sites with metastases from pooled analysis of three randomized trials of dabrafenib plus trametinib treatment.**
22. Long GV, Grob JJ, Nathan P, et al. Factors predictive of response, disease progression, and overall survival after dabrafenib and trametinib combination treatment: a pooled analysis of individual patient data from randomized trials. Lancet Oncol. 2016;17(12):1743–1754.
23. Mattila KE, Mäkelä S, Kytölä S, et al. Circulating tumor DNA is a prognostic biomarker in metastatic melanoma patients treated with chemoimmunotherapy and BRAF inhibitor. Acta Oncol. 2022 Oct;61:(10):1263–1267.
24. Chen G, McQuade JL, Panka DJ, et al. Clinical, molecular, and immune analysis of dabrafenib-trametinib combination treatment

for BRAF inhibitor-refractory metastatic melanoma: a phase 2 clinical trial. JAMA Oncol. 2016;2(8):1056–1064.
25. Gonzalez-Cao M, Mayo de Las Casas C, Oramas J, et al. Intermittent BRAF inhibition in advanced BRAF mutated melanoma results of a phase II randomized trial. Nat Commun. 2021;12(1):7008. doi: 10.1038/s41467-021-26572-6
26. Ascierto PA, McArthur GA, Dréno B, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. Lancet Oncol. 2016;17(9):1248–1260.
27. Ascierto PA, Dréno B, Larkin J, et al. 5-year outcomes with Cobimetinib plus vemurafenib in BRAFV600 mutation-positive advanced melanoma: extended follow-up of the coBRIM study. Clin Cancer Res. 2021;27(19):5225–5235.
28. Googe PB, Theocharis S, Pergaris A, et al. Theragnostic significance of tumor-infiltrating lymphocytes and Ki67 in BRAFV600-mutant metastatic melanoma (BRIM-3 trial). Curr Probl Cancer. 2022;46(3):100862. doi: 10.1016/j.currproblcancer.2022.100862
29. Robert C, Lewis KD, Gutzmer R, et al. Biomarkers of treatment benefit with atezolizumab plus vemurafenib plus cobimetinib in BRAFV600 mutation-positive melanoma. Ann Oncol. 2022;33(5):544–555.
30. Tang B, Chi Z, Chen Y, et al. Safety, efficacy, and Biomarker Analysis of Toripalimab in Previously treated advanced melanoma: results of the POLARIS-01 multicenter phase II trial. Clin Cancer Res. 2020;26(16):4250–4259. doi: 10.1158/1078-0432.CCR-19-3922
31. Nyakas M, Aamdal E, Jacobsen KD, et al. Prognostic biomarkers for immunotherapy with ipilimumab in metastatic melanoma. Clin Exp Immunol. 2019;197(1):74–82. doi: 10.1111/cei.13283
32. Hamid O, Schmidt H, Nissan A, et al. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. J Transl Med. 2011;9(1):204. doi: 10.1186/1479-5876-9-204
- **Description of prognostic impact of baseline serum IL-6, CRP and peripheral blood N/L in 3 randomized clinical trials.**
33. Laino AS, Woods D, Vassallo M, et al. Serum interleukin-6 and C-reactive protein are associated with survival in melanoma patients receiving immune checkpoint inhibition. J Immunother Cancer. 2020;8(1):e000842. doi: 10.1136/jitc-2020-000842
34. Oldan JD, Giglio BC, Smith E, et al. Increased tryptophan, but not increased glucose metabolism, predict resistance of pembrolizumab in stage III/IV melanoma. Oncoimmunology. 2023;12(1):2204753. doi: 10.1080/2162402X.2023.2204753
35. Larkin J, Del Vecchio M, Mandalá M, et al. Adjuvant Nivolumab versus ipilimumab in resected stage III/IV melanoma: 5-year efficacy and biomarker results from CheckMate 238. Clin Cancer Res. 2023;29(17):3352–3361. doi: 10.1158/1078-0432.CCR-22-3145
- **Description of potential predictive biomarkers in high-risk melanoma patient treated with immunotherapy.**
36. Dummer R, Brase JC, Garrett J, et al. Adjuvant dabrafenib plus trametinib versus placebo in patients with resected, BRAFV600-mutant, stage III melanoma (COMBI-AD): exploratory biomarker analyses from a randomised, phase 3 trial. Lancet Oncol. 2020;21(3):358–372. doi: 10.1016/S1470-2045(20)30062-0
- **Description of potential predictive biomarkers in high-risk BRAF mutant melanoma patient treated with target therapy.**
37. Corrie PG, Marshall A, Nathan PD, et al. Adjuvant bevacizumab for melanoma patients at high risk of recurrence: survival analysis of the AVAST-M trial. Ann Oncol. 2018;29(8):1843–1852. doi: 10.1093/annonc/mdy229
38. Kennedy OJ, Kicinski M, Valpione S, et al. Prognostic and predictive value of β-blockers in the EORTC 1325/KEYNOTE-054 phase III trial of pembrolizumab versus placebo in resected high-risk stage III melanoma. Eur J Cancer. 2022;165:97–112. doi: 10.1016/j.ejca.2022.01.017
39. Ugurel S, Röhmel J, Ascierto PA, et al. Survival of patients with advanced metastatic melanoma: the impact of MAP kinase pathway inhibition and immune checkpoint inhibition - update 2019. Eur J Cancer. 2020;130:126–138. doi: 10.1016/j.ejca.2020.02.021
40. Kennedy OJ, Kicinski M, Valpione S, et al. Prognostic and predictive value of metformin in the European Organisation for Research and Treatment of Cancer 1325/KEYNOTE-054 phase III trial of pembrolizumab versus placebo in resected high-risk stage III melanoma. Eur J Cancer. 2023;189:112900. doi: 10.1016/j.ejca.2023.04.016
41. Rozeman EA, Hoefsmit EP, Reijers ILM, et al. Survival and biomarker analyses from the OpACIN-neo and OpACIN neoadjuvant immunotherapy trials in stage III melanoma. Nat Med. 2021;27(2):256–263. doi: 10.1038/s41591-020-01211-7
- **Description in large cohort of prognostic impact of high IFN-γ signature and TMB in high risk melanoma patients treated with neoadjuvant different schedules therapies.**
42. Rawson RV, Adhikari C, Bierman C, et al. Pathological response and tumour bed histopathological features correlate with survival following neoadjuvant immunotherapy in stage III melanoma. Ann Oncol. 2021;32(6):766–777. doi: 10.1016/j.annonc.2021.03.006
- **Description of potential histopathological prognostic features after neoadjuvant therapy.**
43. Reijers ILM, Rao D, Versluis JM, et al. IFN-γ signature enables selection of neoadjuvant treatment in patients with stage III melanoma. J Exp Med. 2023;220(5):e20221952. doi: 10.1084/jem.20221952
44. Amaria RN, Postow M, Burton EM, et al. Neoadjuvant relatlimab and nivolumab in resectable melanoma. Nature. 2022;611(7934):155–160. doi: 10.1038/s41586-022-05368-8
45. Amaria RN, Prieto PA, Tetzlaff MT, et al. Neoadjuvant plus adjuvant dabrafenib and trametinib versus standard of care in patients with high-risk, surgically resectable melanoma: a single-center, open-label, randomised, phase 2 trial. Lancet Oncol. 2018;19(2):181–193.
46. Hoefsmit EP, Völllmy F, Rozeman EA, et al. Systemic LRG1 expression in melanoma is associated with disease progression and recurrence. Cancer Res Commun. 2023 [Published 2023 Apr 20];3(4):672–683. doi: 10.1158/2767-9764.CRC-23-0015
47. Pilla L, Alberti A, Di Mauro P, et al. Molecular and immune biomarkers for cutaneous melanoma: Current status and future prospects. Cancers (Basel). 2020;12(11):3456. doi: 10.3390/cancers12113456
48. Hodi FS, Chapman PB, Sznol M, et al. Safety and efficacy of combination nivolumab plus ipilimumab in patients with advanced melanoma: results from a North American expanded access program (CheckMate 218). Melanoma Res. 2021;31(1):67–75. doi: 10.1097/CMR.0000000000000708
49. Asher N, Ben-Betzalel G, Lev-Ari S, et al. Real world outcomes of ipilimumab and nivolumab in patients with metastatic melanoma. Cancers (Basel). 2020;12(8):2329. doi: 10.3390/cancers12082329
50. Yang Y, Jin G, Pang Y, et al. Comparative efficacy and Safety of Nivolumab and Nivolumab plus ipilimumab in advanced cancer: a systematic review and meta-analysis. Front Pharmacol. 2020;11:40. doi: 10.3389/fphar.2020.00040
51. Ramos-Casals M, Brahmer JR, Callahan MK, et al. Immune-related adverse events of checkpoint inhibitors. Nat Rev Dis Primers. 2020;6(1):38. doi: 10.1038/s41572-020-0160-6
52. Sun J, Carr MJ, Khushalani NI. Principles of targeted therapy for melanoma. Surg Clin North Am. 2020;100(1):175–188. doi: 10.1016/j.suc.2019.09.013
53. Corrie P, Meyer N, Berardi R, et al. Comparative efficacy and safety of targeted therapies for BRAF-mutant unresectable or metastatic melanoma: results from a systematic literature review and a network meta-analysis. Cancer Treat Rev. 2022;110:102463. doi: 10.1016/j.ctrv.2022.102463
54. Broman KK, Dossett LA, Sun J, et al. Update on BRAF and MEK inhibition for treatment of melanoma in metastatic, unresectable, and adjuvant settings. Expert Opin Drug Saf. 2019;18(5):381–392. doi: 10.1080/14740338.2019.1607289
55. Addeo A, Friedlaender A, Banna GL, et al. TMB or not TMB as a biomarker: that is the question. Crit Rev Oncol Hematol. 2021;163:103374. doi: 10.1016/j.critrevonc.2021.103374
56. Tivey A, Britton F, Scott JA, et al. Circulating tumour DNA in Melanoma-Clinic Ready? Curr Oncol Rep. 2022;24(3):363–373.
57. Boerlin A, Bellini E, Turko P, et al. The prognostic value of a single, randomly timed circulating tumor DNA measurement in patients with metastatic melanoma. Cancers (Basel). 2022;14(17):4158. doi: 10.3390/cancers14174158

- **Prognostic role of ctDNA in metastatic melanoma.**

58. Marsavela G, McEvoy AC, Pereira MR, et al. Detection of clinical progression through plasma ctDNA in metastatic melanoma patients: a comparison to radiological progression. Br J Cancer. 2022;126(3):401–408. doi: 10.1038/s41416-021-01507-6
59. Gouda MA, Polivka J, Huang HJ, et al. Ultrasensitive detection of BRAF mutations in circulating tumor DNA of non-metastatic melanoma. ESMO Open. 2022;7(1):100357. doi: 10.1016/j.esmoop.2021.100357
60. Zhang J, Qian D, Xu X, et al. Evaluating the diagnostic potentials of circulating tumor DNA against melanoma: a systematic review and meta-analysis. J Oncol. 2022;2022:6233904. doi: 10.1155/2022/6233904
61. Revythis A, Shah S, Kutka M, et al. Unraveling the wide spectrum of melanoma biomarkers. Diagn (Basel). 2021 Jul 26;11(8):1341. doi: 10.3390/diagnostics11081341
62. Pérez-Romero K, Rodríguez RM, Amedei A, et al. Immune landscape in tumor microenvironment: implications for biomarker development and immunotherapy. Int J Mol Sci. 2020;21(15):5521. doi: 10.3390/ijms21155521
63. Llaó-Cid L, Roessner PM, Chapaprieta V, et al. EOMES is essential for antitumor activity of CD8+T cells in chronic lymphocytic leukemia. Leukemia. 2021;35(11):3152–3162. doi: 10.1038/s41375-021-01198-1
64. Nepote A, Avallone G, Ribero S, et al. Current controversies and challenges on BRAF V600K-Mutant cutaneous melanoma. J Clin Med. 2022;11(3):828. doi: 10.3390/jcm11030828
65. Roccuzzo G, Moirano G, Fava P, et al. Obesity and immune-checkpoint inhibitors in advanced melanoma: a meta-analysis of survival outcomes from clinical studies. Semin Cancer Biol. 2023;91:27–34. doi: 10.1016/j.semcancer.2023.02.010
66. Riaz N, Havel JJ, Makarov V, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell. 2017;171(4):934–949.e16.
67. Das A, Ghose A, Naicker K, et al. Advances in adoptive T-cell therapy for metastatic melanoma. Curr Res Transl Med. 2023 Jul;71:(3):103404.
68. Ascierto PA, Mandalà M, Ferrucci PF, et al. Sequencing of ipilimumab plus nivolumab and encorafenib plus binimetinib for untreated BRAF-Mutated metastatic melanoma (SECOMBIT): a randomized, three-arm, open-label phase II trial. J Clin Oncol. 2023;41(2):212–221. doi: 10.1200/JCO.21.02961
69. Atkins MB, Lee SJ, Chmielowski B, et al. Combination Dabrafenib and Trametinib versus combination Nivolumab and Ipilimumab for patients with advanced BRAF -mutant melanoma: the DREAMseq trial—ECOG-ACRIN EA6134. J Clin Oncol. 2023;41(2):186–197.