Systematic Review

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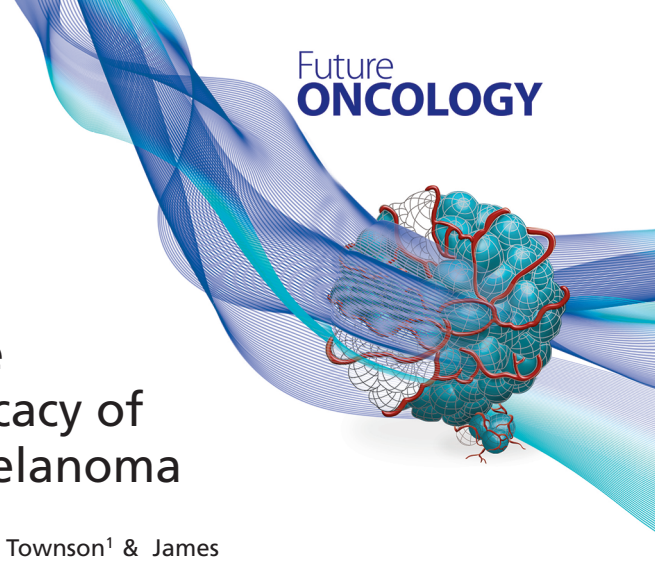

Systematic literature review for the association of biomarkers with efficacy of anti-PD-1 inhibitors in advanced melanoma

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Aim: Summarize the literature assessing biomarkers in predicting efficacy of anti-PD-1 therapy for patients with high-risk unresectable or metastatic melanoma. **Materials & methods:** Relevant studies were identified via a systematic literature review. **Results:** About 334 unique biomarkers or biomarker combinations were identified from 121 citations. Neutrophil-to-lymphocyte ratio was the most frequently studied biomarker, followed by C-reactive protein. Fifty-nine biomarkers were significantly associated with overall survival (OS), 51 with progression-free survival (PFS) and 44 with response. Twenty biomarkers were associated with both OS and PFS; two were associated with OS, PFS and response (MHC-II and tumor mutational burden). **Conclusion:** Numerous biomarkers could potentially predict the efficacy of anti-PD-1-based therapy for melanoma patients. However, confirmatory studies are needed as well as determination of implications for clinical decision-making.

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The improvement in overall survival (OS) of patients with advanced melanoma over the past decade is often attributed to the extensive development of innovative immunotherapies in oncology [1–4]. Immune checkpoint inhibitors (ICI), which reinvigorate antitumor immune responses by targeting inhibitory pathways, have been particularly transformative in this field [5]. Ipilimumab, an anti-CTLA-4 antibody, was the earliest ICI and first approved for the treatment of melanoma in 2011 [6]. Anti-PD-1 and anti-PD-L1 are more recent ICIs that use monoclonal antibodies to competitively bind to the receptor site of PD-1 on T cells and myeloid lineage cells and blocks its activating ligand, PD-L1. In many tumors, including those of melanoma, PD-L1 is often overexpressed allowing tumor cells to elude T-cell-mediated antitumor responses [7].

These anti-PD-1 immune-oncology therapies, including nivolumab and pembrolizumab, have resulted in improved OS outcomes among advanced stage melanoma patients; five-year survival rate increased from 17–41%, overall response rate improved to 42% and disease control rate improved to 65% [8–11]. However, challenges remain in improving overall response and the long-term durability of responses in a subset of these patients with current treatments. Therefore, understanding the criteria that define which patients will benefit from immunotherapy is critical [12,13].

Biomarkers, which have become an integral part of disease prognosis, are now being studied to evaluate their value in predicting the efficacy of immune-oncology drugs [14]. Certain biomarkers, such as PD-L1 expression of formalin-fixed, paraffin-embedded tissues and serum lactate dehydrogenase levels, as well as *BRAF* mutational status are heavily investigated and there is evidence to suggest they may influence individual immune-oncological outcomes among melanoma patients [15–20]. Absolute lymphocyte count has also been described as a biomarker for assessing patient outcomes of those treated with ipilimumab. But to date, there are no established biomarkers that clearly predict clinical benefit from anti-PD-1 therapy in patients with advanced stage melanoma despite numerous

completed clinical trials investigating monotherapy or novel combinations with anti-PD-1 or anti-PD-L1 inhibitors to improve response to treatment [21]. Therefore, the aim of this systematic literature review (SLR) is to review and catalogue any biomarkers that may predict the efficacy of anti-PD-1-based therapy for the treatment of melanoma.

Materials & methods

An SLR was performed to identify published clinical trials, observational studies and biomarker studies that met a predefined set of population, interventions, comparisons, outcomes and study design criteria defined in a study protocol. This SLR included patients with high-risk resectable melanoma receiving adjuvant or neoadjuvant therapy, unresectable melanoma or metastatic melanoma with a known biomarker status. The interventions of interest were nivolumab, pembrolizumab or unspecified anti-PD-1, given to patients as monotherapy or in combination with ipilimumab (anti-CTLA-4). Studies were excluded if the intervention was unspecified anti-PD-1 therapy given in combination with unspecified anti-CTLA-4 therapy or if the study population included patients treated with either anti-PD-1, anti-CTLA-4 or combination therapy with no outcomes by treatment group. Outcomes of interest were OS, progression-free survival (PFS) and overall response to anti-PD-1-based therapy by biomarker status. Studies were also excluded if these efficacy outcomes were not available by biomarker status. Additionally, any efficacy outcomes for melanoma patients by exclusively *BRAF*, PD-L1 or lactate dehydrogenase status were not included in this SLR given that these biomarkers have been extensively studied as possible predictive biomarkers for response to anti-PD-1 therapy in melanoma patients [16–20]. Because the aim of this SLR was to identify biomarkers that may predict melanoma patients' response to anti-PD-1-based therapy, only biomarkers measured before treatment were included in this SLR.

Relevant studies were identified by searching MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials through the Ovid platform in March 2020 using search terms related to each intervention, disease area and study design of interest (Supplementary Tables S1–S3). The study design filters recommended by the Scottish Intercollegiate Guidelines Network (SIGN) for MEDLINE and EMBASE were used to identify clinical trials, observational studies and biomarker studies. The intervention terms included terms related to the generic and brand name interventions of interest. Conference proceedings from 2018–2019 were searched for American Association for Cancer Research (AACR), American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO) and Society for Melanoma Research (SMR). The European Union (EU) Clinical Trial Registry was also searched to identify completed clinical trials with available results that were not yet published or indexed in Cochrane, MEDLINE or EMBASE.

Two independent reviewers screened titles, abstracts, conference proceedings and clinical trial registrations using the predefined population, interventions, comparisons, outcomes and study design selection criteria to identify studies of interest. All studies deemed eligible during the title and abstract screening were retrieved as full-texts and were then screened by the same two reviewers. Data from the final list of selected eligible studies were extracted for study characteristics, interventions, patient characteristics and outcomes. Median survival (in months) and hazard ratios (HR) were collected for OS and PFS. It should be noted that HR are more favorable estimates as they can be controlled for confounding variables. Biomarkers reported and information on their diagnostic methods, including biomarker testing method, scoring method, cutoff criteria and biomarker specimen type, were also extracted. Additionally, for studies with plots representing the difference in biomarker levels between responders and non-responders to anti-PD-1 without any reported values, each plot was digitized to obtain the estimated values. Finally, this SLR focuses on outcomes that were reported to be statistically significant, which was defined by a two-sided p-value < 0.05 or a 95% CI for HR that did not include one.

The quality of all included randomized clinical trials was assessed by the same two reviewers using the National Institute for Health and Care Excellence (NICE) instrument [22]. This instrument was used to evaluate six key domains: sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, selective outcome reporting and other sources of bias. The quality of all included nonrandomized single-arm trials and observational studies was assessed using the Newcastle-Ottawa Scale, which was used to evaluate study group and selection, comparability of the groups within studies and the ascertainment of either the exposure or outcomes of interest [23].

During title and abstract screen, full-text screen, data extraction and quality assessment, any disagreements between the two reviewers were resolved by discussion and, if necessary, a third investigator who provided arbitration.

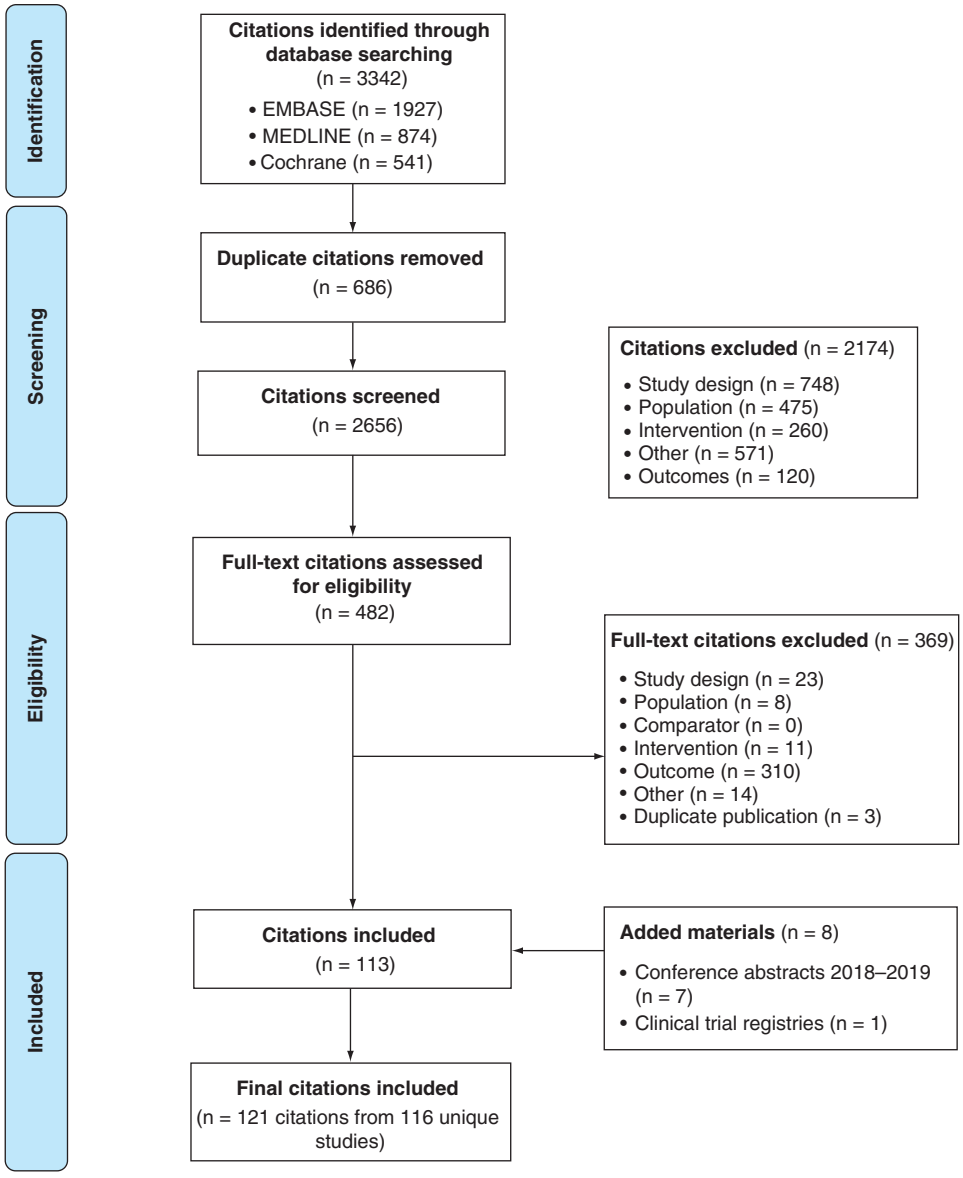

Figure 1. Preferred reporting items for systematic reviews and meta-analyses diagram.
Created using a template from [24].

Results

Study selection

The database search was conducted in March 2020 and 3342 abstracts were identified from MEDLINE, EMBASE and Cochrane Register of Controlled Trials. Of those citations, 2860 were excluded (686 duplicate publications, 748 for study design, 475 for population, 260 for intervention, 120 for outcomes and 571 for other reasons). Of the 482 citations that progressed to the full-text screening stage, 369 were excluded (three duplicate publications, 23 for study design, eight for population, 11 for intervention, 310 for outcomes and 14 for other reasons). Eight additional records were identified from conference proceedings and registry search. Overall, a total of 121 citations corresponding to 116 unique studies were included in this SLR (Figure 1).

Study & patient characteristics

Eighty-five of the included studies were biomarker studies and categorized as such given their primary intent of identifying or investigating biomarker profiles associated with survival and response outcomes in melanoma

patients. Two of the included studies were interventional clinical trials and 29 were observational studies primarily investigating the association of treatment outcomes with various clinical factors. Although the main purpose of these clinical trials and observational studies was not explicitly to study biomarkers, they reported subgroup outcomes by patient's biomarker status and; therefore, included in this review. Nineteen of the observational studies and 13 of the biomarker studies included patients treated at multiple institutions. Five studies exclusively enrolled cutaneous melanoma patients, three studies enrolled only uveal melanoma patients and two studies enrolled only cutaneous, mucosal or acral melanoma patients. Twenty three of the included studies were company driven (i.e., sponsored by pharmaceutical companies), 86 were investigator driven (not sponsored by pharmaceutical companies) and four were partially sponsored by pharmaceutical companies. The majority (n = 87) of the included observational studies or biomarker studies that used observational patient data had a Newcastle-Ottawa score of 5 or higher, indicating low or some risk of bias (Supplementary Table S15). The included randomized clinical trials generally had low or unknown risk of bias, mainly due to lack of needed information to assess bias in each domain (Supplementary Table S16).

All included studies evaluated anti-PD-1 monotherapy (nivolumab, pembrolizumab or unspecified) or anti-PD-1 in combination with ipilimumab in high-risk, unresectable or metastatic melanoma patients with specified biomarkers. There was little variation in dosing and schedule in the 39 studies that reported treatment regimens.

Due to the large number of studies in this SLR, there was high variability in terms of patient characteristics. The median age of patients ranged from 49–79 years and the proportion of male patients varied from 13–91%. Race and ethnicity were sparsely reported. Of studies reporting disease stage, the proportion of patients with stage III melanoma varied from 0–71% and the proportion of patients with stage IV melanoma varied from 3–100%. In studies that reported patient's ECOG performance scores, the proportion of patients with an ECOG performance score of 0 ranged from 14–100% and the proportion of patients with a performance score of 1 ranged from 0–68.3%.

Biomarkers identified

Three hundred and thirty-four unique biomarkers or biomarker combinations were identified from the included studies (Supplementary Table S4). The most commonly reported biomarker was neutrophil-to-lymphocyte ratio ([NLR], reported in 19 studies) followed by CRP (reported in nine studies). Absolute neutrophil count neutrophils and relative lymphocyte count were reported in eight studies. All biomarkers included in this SLR were measured prior to treatment and obtained from serum, tissue or stool samples.

Outcomes

Overall survival

Eighty-seven of the included studies evaluated OS, which was generally defined as the time from treatment initiation to death from any cause. Fifty-nine biomarkers were significantly associated with OS for melanoma patients treated with anti-PD-1 monotherapy (Supplementary Table S8 & Supplementary Figure S2). NLR was the most frequently reported (n = 8 studies), with higher NLR exhibiting a significant relationship with lower OS; HR comparing NLR $\geq$5 to NLR <5 ranged from 1.95 in Bartlett 2020 to 2.85 in Capone 2018 (Figure 2) [25,26]. A similar association was seen in patients treated with anti-PD-1 combination therapy (Rosner 2018) [27]. Higher absolute neutrophil count was also significantly associated with lower OS in patients treated with anti-PD-1 monotherapy (n = 2 studies); HR ranged from 1.35 in Bastholt 2019 to 2.04 in Capone 2018 (Figure 2) [26,28]. Among melanoma patients treated with anti-PD-1 in combination with ipilimumab, 22 biomarkers showed significant associations with OS (Supplementary Table S8 & Supplementary Figure S3). Circulating tumor DNA (ctDNA) was reported in two studies, and patients with undetectable ctDNA prior to treatment had higher OS compared with patients with detectable circulating tumor DNA (ctDNA); HR ranged from 0.11 in Lee 2017 to 0.206 in Herbreteau 2018 (Figure 2) [29,30]. This same relationship was reported in patients treated with anti-PD-1 monotherapy in Seremet 2019 [31].

Progression-free survival

Of the 71 included studies that evaluated PFS, PFS was typically defined as the time from treatment initiation to disease progression or death, whichever event occurred first. Fifty-one biomarkers in patients treated with anti-PD-1 monotherapy and 23 biomarkers in patients treated with anti-PD-1 combination therapy were significantly associated with PFS (Supplementary Tables S10–S11 & Supplementary Figures S4–S5). Again, NLR was the

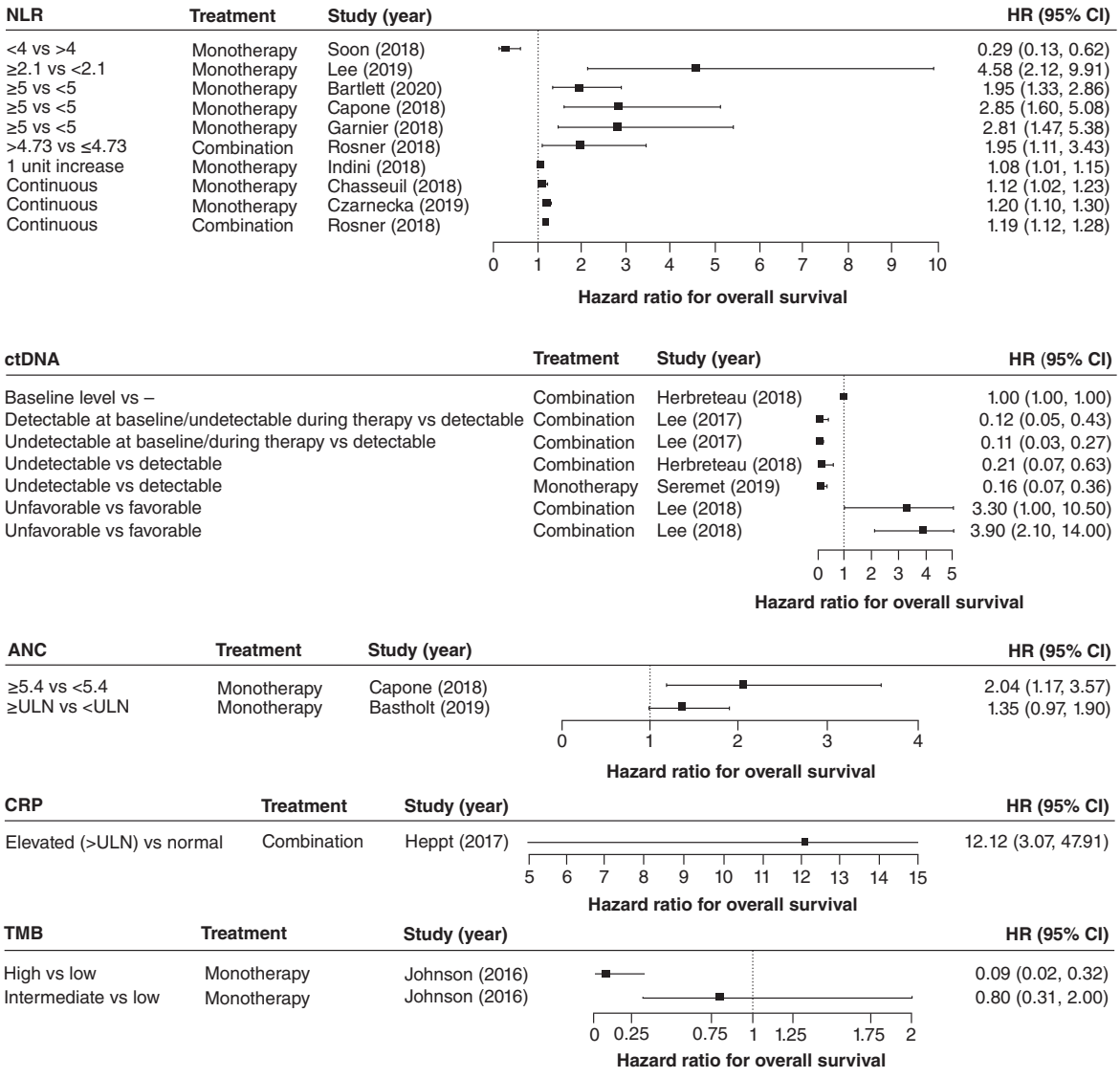

Figure 2. Overall survival for neutrophil-to-lymphocyte ratio, circulating tumor DNA, absolute neutrophil count, C-reactive protein and tumor mutational burden.
ANC: Absolute neutrophil count; ctDNA: Circulating tumor DNA; NLR: Neutrophil-to-lymphocyte ratio; TMB: Tumor mutational burden.

biomarker most frequently reported to be significantly associated with PFS (n = 6 studies in monotherapy, n = 1 study in combination therapy). As with OS, PFS was shorter in patients with NLR ≥5 compared with patients with NLR <5; HR ranged from 1.73 in Bartlett 2020 to 2.1 in Capone 2018 (Figure 3) [25,32]. In addition, higher CRP was significantly associated with worse PFS (n = 2 studies in monotherapy, n = 0 studies in combination therapy; Figure 3) [29,31,33]. All remaining biomarkers were reported to have a significant relationship with PFS in only one study each.

Response

The final outcome of interest was response to anti-PD-1-based therapy for melanoma patients by biomarker status, which was evaluated in 57 studies. Specifically, this SLR focused on biomarker levels that differed significantly between responders and non-responders to anti-PD-1-based therapy. There was some between study variation in the definitions used to categorize responders and non-responders. Responders were defined as patients experiencing: complete response or partial response; complete response, partial response or stable disease; or complete response, partial response or stable disease for >6 months, while non-responders were defined as patients experiencing:

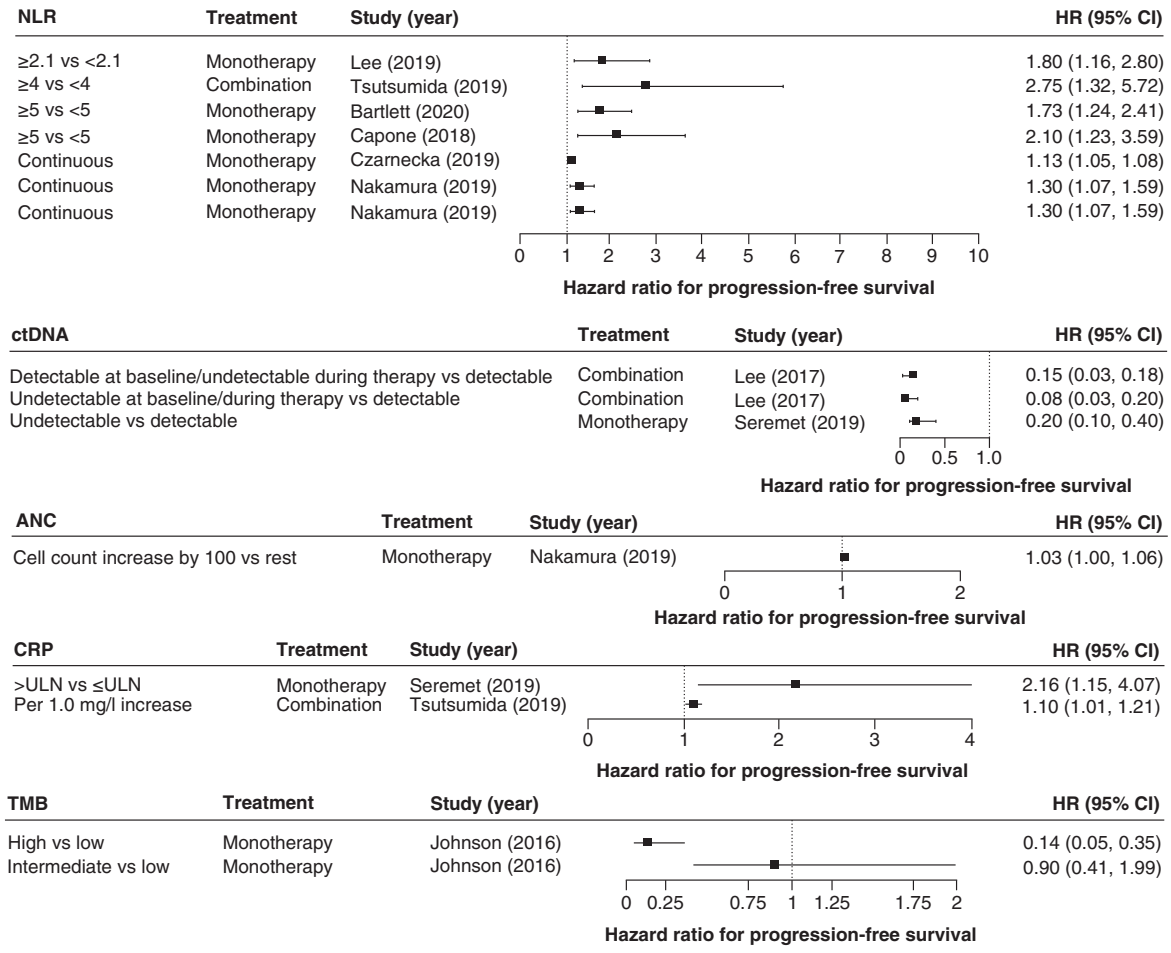

Figure 3. Progression-free survival for neutrophil-to-lymphocyte ratio, circulating tumor DNA, absolute neutrophil count, C-reactive protein and tumor mutational burden..
ANC: Absolute neutrophil count; ctDNA: Circulating tumor DNA; NLR: Neutrophil-to-lymphocyte ratio; TMB: Tumor mutational burden.

progressive disease; progressive disease or stable disease; or progressive disease or stable disease for ≤6 months. The majority of the included clinical trials, observational studies and biomarker studies used the Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.1 to assess tumor response, while six studies used immune-RECIST (iRECIST), immune-related RECIST (irRECIST) or immune-related response criteria (irRC).

Supplementary Table S12 lists the 44 biomarkers that differed significantly between patients who responded to anti-PD-1 monotherapy compared with patients who did not. Responders trended toward significantly higher T-cell markers CD3 and CD8 levels in two studies (Chen and Gopalakrishnan) [34,35]. Among patients treated with anti-PD-1 in combination with ipilimumab, 12 biomarkers differed significantly between responders and non-responders. Each of these 12 biomarkers was only reported to be significant in one study (Supplementary Table S13).

Overall, in patients treated with anti-PD-1 monotherapy, 20 biomarkers were significantly associated with both OS and PFS while only two were found to have a significant relationship with OS, PFS and response (MHC-II and TMB). In patients treated with anti-PD-1 combination therapy, seven biomarkers were significantly associated with both OS and PFS and three biomarkers had a significant relationship with OS, PFS and response (IgG2, Melan-A and MHC-II).

Discussion

In the absence of well-defined biomarkers that predict the efficacy of anti-PD-1-based therapy in melanoma, the goal of this review was to identify biomarkers that have been investigated in high-risk, unresectable or metastatic

melanoma patients treated with anti-PD-1-based therapy and describe associations with OS, PFS or response in this patient population.

This review identified 116 studies investigating the efficacy of anti-PD-1-based therapy by patient's biomarker status. By including clinical trials, observational studies and biomarker studies, this SLR was able to capture a total of 334 unique biomarkers or biomarker combinations that could potentially predict the efficacy of anti-PD-1 therapy in advanced stage melanoma patients. Some biomarkers were consistently reported as having significant associations with OS, PFS or response. However, only a few biomarkers were studied in more than one publication and the more frequently studied biomarkers, such as NLR, ctDNA allele fraction and CRP, have also been shown to be associated with survival outcomes in patients treated with nonanti-PD-1 therapies with various malignancies [36–38]. ctDNA level in particular is a proposed surrogate of tumor burden and pretreatment tumor fraction may predict survival outcomes independent of the cancer therapy received, although the rate of ctDNA shedding varies by tumor [39].

A few biomarkers were reported to be significantly associated with all three efficacy outcomes (OS, PFS and response). In patients treated with anti-PD-1 monotherapy, these biomarkers were MHC-II and tumor mutational burden and in patients treated with anti-PD-1 in combination with ipilimumab, these biomarkers were IgG2, Melan-A and MHC-II. These potential candidates may have predictive value for advanced melanoma patients treated with anti-PD-1-based therapy, although further research is needed to validate these findings.

This SLR involved highly sensitive searches of peer-reviewed literature guided by predefined eligibility criteria, as well as searches of recent conferences and clinical trial registrations to capture completed trials with available results that have not yet been published. Data quality was ensured through involvement of two independent researchers in the study selection and data extraction phases of the project.

Despite the strengths of this SLR, there are limitations that must be acknowledged. There is a risk of publication bias as some studies fail to be published while others are only published in abstract form, which present limited information. There is also a risk that any recent studies that have been published since 9 March 2020 may not be captured. Additionally, the search and selection of this SLR was restricted to studies published in English, which increases the possibility that non-English publications were not identified. There are also limitations in terms of the included studies that should be noted. In addition to the heterogeneity of the study populations and definitions used to categorize responders and non-responders, there was considerable between-study variation in scoring methods, cutoff thresholds and assays used for the same biomarker. This indicates an unmet need for standardized and validated biomarker definitions and measurements to aid the identification of a subgroup of advanced melanoma patients who may benefit from anti-PD-1 therapy.

Conclusion

This review provides a summary and overview of biomarkers where further research is warranted to identify those patients who may benefit most from anti-PD-1-based therapy for the treatment of advanced melanoma.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2021-0154

Author contributions

Conception/design: E Scherrer, M Lorenzi, I Shui; collection and/or assembly of data: R Rau, M Lorenzi; data analysis and interpretation: E Scherrer, R Rau, M Lorenzi, I Shui, S Townson, J Larkin; manuscript writing: R Rau; final approval of manuscript: E Scherrer, R Rau, M Lorenzi, I Shui, S Townson, J Larkin.

Financial & competing interests disclosure

This study was supported by Merck & Co., Inc. R Rau and M Lorenzi are employees of PRECISIONheor. E Scherrer, I Shui and S Townson are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., NJ, USA and hold shares in Merck & Co., Inc., NJ, USA. J Larkin receives institutional support from and serves as a consultant for MSD. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Summary points

- Improved overall survival (OS) in advanced stage melanoma patients over the past decade has been attributed to the development of immune checkpoint inhibitors, including programmed cell death protein 1 inhibitors (anti-PD-1). Nivolumab and pembrolizumab are approved anti-PD-1 therapies for the treatment of advanced melanoma.
- Challenges remain in improving overall response in a subset of these patients with current treatments and understanding the criteria that define which patients will benefit from immunotherapy is critical.
- We aimed to summarize the literature assessing biomarkers in predicting the efficacy (OS, progression-free survival [PFS] and response) of anti-PD-1 therapy for patients with high-risk unresectable or metastatic melanoma.
- A systematic literature review of Embase, MEDLINE and Cochrane Central Register of Controlled Trials conducted in July 2020 identified 121 citations corresponding to 116 unique studies assessing the relationship of biomarkers to efficacy of anti-PD-1 monotherapy or anti-PD-1 combination therapy with ipilimumab for high-risk unresectable or metastatic melanoma.
- About 334 unique biomarkers or biomarker combinations were identified from the included studies. Neutrophil-to-lymphocyte ratio was the most frequently studied biomarker (n = 19 studies), followed by C-reactive protein (n = 9), absolute neutrophil count, neutrophils and relative lymphocyte count (n = 8).
- In patients treated with anti-PD-1 monotherapy, 59 biomarkers were significantly associated with OS, 51 with PFS and 44 with response. Twenty biomarkers were significantly associated with OS and PFS and two were significantly associated with OS, PFS and response (MHC-II and tumor mutational burden) in these patients.
- In patients treated with anti-PD-1 in combination with ipilimumab, 22 biomarkers showed significant associations with OS, 23 with PFS and 12 with response. Seven biomarkers were significantly associated with OS and PFS and three were significantly associated with OS, PFS and response (IgG2, Melan-A and MHC-II).
- There was considerable between-study heterogeneity in terms of the assays and cutoff thresholds used for the same biomarker. This suggests an unmet need for the standardization of biomarker definitions and measurements to aid the identification of a subgroup of patients who may benefit from anti-PD-1 therapy.
- Results from this systematic literature review suggest that there are numerous biomarkers that could potentially predict the efficacy of anti-PD-1-based therapy for melanoma patients. However, confirmatory studies are needed as well as determination of implications for clinical decision-making.

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- **This biomarker association study conducted immune profiling on melanoma patients treated with anti-PD-1 monotherapy and discovered that responders trended towards higher CD3 and CD8 levels compared with nonresponders.**

35. Gopalakrishnan V, Spencer CN, Nezi L *et al.* Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 359(6371), 97–103 (2018).

- **Authors examined immunohistochemical markers in melanoma patients treated with anti-PD-1 therapy and found responders had significantly higher levels of CD3 and CD8.**

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