Clinica Chimica Acta 533 (2022) 71–78

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Diagnostic performance of early increase in S100B or LDH as outcome predictor for non-responsiveness to anti-PD-1 monotherapy in advanced melanoma

Elisa A. Rozeman [a,b,1], Judith M. Versluis [a,b,1], Ruben Moritz [c], Sofie Wilgenhof [a], Johannes V. van Thienen [a], John B.A.G. Haanen [a,b], Michel M. van de Heuvel [d,e], Christian U. Blank [a,b,f,1], Huub H. van Rossum [c,1,*]

[a] Department of Medical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands
[b] Division of Molecular Oncology & Immunology, Netherlands Cancer Institute, Amsterdam, the Netherlands
[c] Department of Laboratory Medicine, Netherlands Cancer Institute, Amsterdam, the Netherlands
[d] Division of Thoracic Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands
[e] Department of Respiratory Diseases, Radboud University Medical Centre, Nijmegen, the Netherlands
[f] Department of Internal Medicine, Leiden University Medical Center, Leiden, the Netherlands

ARTICLE INFO

Keywords:
Melanoma
Serum tumour marker
S100B
LDH
Anti-PD-1
Response

ABSTRACT

As a subset of advanced melanoma patients derive long-term benefit from anti-PD-1 therapy, early identification of non-responsiveness would enable an early switch to next line therapies. This study assessed if an early increase in S100B or lactate dehydrogenase (LDH) could be predictive for non-responsiveness to anti-PD-1. We retrospectively analysed advanced melanoma patients treated with anti-PD-1 monotherapy. Serum S100B and LDH levels were measured at baseline and before every infusion. Non-response was defined as progression or death at 6 months. Marker cut-offs were defined based on > 95% specificity and feasibility in clinical practice. For validation an independent cohort was analysed. In total, 313 patients were included (166 patients in training cohort, 147 patients in validation cohort). Increase of > 50% in LDH or > 100% in S100B above upper limit of normal at week 6 compared to baseline was determined as criterion to positively test for non-responsiveness. In the validation cohort, obtained specificity of the combination test was > 95% with a positive predictive value of 82%; obtained sensitivity was lower (21%), with a negative predictive value of 55%. Early increase in S100B or LDH is a strong parameter for non-responsiveness to anti-PD-1 in advanced melanoma. Prospective confirmation is needed before clinical implementation.

1. Introduction

Checkpoint inhibitors and targeted therapy have revolutionized treatment for advanced melanoma patients [1]. Although the 3-year overall survival (OS) has improved from 10% a decade ago, when patients could be treated only with chemotherapy, to 50% nowadays with anti-PD-1 based therapies, still the majority of patients does not derive long-term benefit [2]. To maximize treatment benefit for individual patients, there is a strong need for biomarkers that identify patients that will derive durable benefit from checkpoint inhibition and those who will not.

Preferentially, baseline biomarkers would be able to guide first-line therapy. For patients with a BRAF-mutated melanoma, anti-PD-1 monoclonal antibody (mAb) monotherapy (pembrolizumab or nivolumab), combination of ipilimumab (anti-CTLA-4 mAb) plus nivolumab or combination BRAF plus MEK inhibition are all viable treatment options [2]. Unfortunately, the baseline biomarkers that characterise patients with the highest chance of long-term benefit are the same for all treatment modalities [3,4]. These are the patients with low lactate dehydrogenase (LDH) levels, low tumour load, metastases in less than three organs and a good performance status [5,6]. Since anti-PD-1 monotherapy has a favourable toxicity profile, patients with favourable

* Corresponding author at: Department of Laboratory Medicine, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands.
E-mail address: h.v.rossum@nki.nl (H.H. van Rossum).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cca.2022.06.001
Received 8 April 2022; Received in revised form 1 June 2022; Accepted 2 June 2022
Available online 13 June 2022

characteristics often start with pembrolizumab or nivolumab.

With a plethora of new (combination) therapies that might become available within the upcoming years, the choice for the right first-line and subsequent therapies will become even more challenging [7]. Therefore, early identification of non-responsiveness to an initiated therapy is important to enable an early switch to subsequent (combination) therapies when patients are still in the best clinical condition.

Therapy response evaluation by imaging regularly takes place every 12 weeks after initiation of therapy. Approximately 30–40% of patients treated with anti-PD-1 monotherapy are diagnosed with progressive disease at first radiographic evaluation [8,9]. It would be relevant to identify progression earlier by less invasive and less costly diagnostic methods. Therefore, we wondered whether early non-responsiveness could be identified by increases of generally available blood-based tumour markers.

Serum LDH is a well-established biomarker for metastatic melanoma and is part of the AJCC staging criteria since 2001 [10]. High levels of serum LDH are associated with worse prognosis. Baseline LDH is correlated with response to checkpoint inhibition [5,11] and a high LDH level is associated with impaired progression-free survival (PFS) and OS to both immune- and targeted therapy [9,12]. The S100 calcium binding protein B (S100B) is a more sensitive marker in advanced malignant melanoma [13]. It has been shown that elevated serum levels of S100B are associated with higher tumour load and poor survival in melanoma patients treated with chemotherapy [14–17]. Furthermore, it could be a useful marker in detecting recurrence in stage III patients after resection [18,19]. In patients treated with ipilimumab elevated baseline S100B and/or an increase in S100B during treatment is associated with impaired OS [20,21].

The aim of our study was to assess if an early increase in S100B or LDH could be predictive for non-response to anti-PD-1 monotherapy. In our institute, a method and software package called Biomarker Response Characteristic (BReC) plot application was developed to assess the applicability of serum tumour marker changes in the response assessment [22]. For this study we used this application to design and validate biomarker-response based tests that allow an early and accurate detection of non-responsiveness to anti-PD-1 monotherapy in advanced melanoma patients.

2. Methods

2.1. Study design

We retrospectively identified advanced melanoma patients treated with anti-PD-1 monotherapy in the Netherlands Cancer Institute. Patient characteristics, laboratory values and radiologic response data were retrieved from electronic patients records. The study was approved by the institutional review board of our institute (IRBd19049).

2.2. Study population and treatment

All consecutive patients who started treatment with pembrolizumab between June 2014 and August 2016 were included in the training cohort. For the validation cohort, we analysed all consecutive patients that were treated with nivolumab between September 2015 and July 2017 and all extra patients that were treated with pembrolizumab between August 2016 and July 2017.

2.3. Treatment and response assessments

Patients received pembrolizumab (2 mg/kg or fixed dose 150–200 mg every 3 weeks) or nivolumab (3 mg/kg or fixed dose 240 mg every 2 weeks) in an expanded access program or according to the label after approval. Response was evaluated every 3 months by computed tomography (CT) scan and scored according to immune-related response criteria. When the disease was not measurable on CT, response assessment was performed by PET-CT. Non-responsiveness to anti-PD-1 was defined as progressive disease according to RECIST version 1.1 [23] or death at 6 months after initiation of therapy.

2.4. Design of tumour marker test

The serum tumour markers S100B and LDH were measured before start of therapy (within 3 weeks before start) and within 24 h before every administration of anti-PD-1 therapy. These markers were analysed in routine clinical practice. S100B and LDH were measured on a Roche diagnostics Cobas 6000 system in a continuous and real-time modus, both according to manufacturers' instructions. LDH (supplier code LDHI2) was standardized against IFCC formulation and had an operational analytical coefficient of variation of $\leq$ 2.55%. S100B (supplier code Elecsys S100) was standardized against manufacturer weight-out S100B protein and had an operational analytical coefficient of variation of $\leq$ 6.74%. In the Netherlands Cancer Institute, the applied reference ranges for S100B is $<$ 0.10 μg/L and for LDH $<$ 248 U/L. For follow-up time points a margin of 2 weeks (2–4; 5–7; 8–10; 11–13) was taken as not every infusion was exactly after 2 or 3 weeks. If there was more than one measurement within this period, the latest value was taken.

The training cohort was used to determine cut-offs for a positive test for both markers. The BReC plot application has generated BReC plots at different time points which displays the clinical (non–)response for different cut-offs (Supplemental Fig. 1). Marker cut-offs were defined based on two criteria: specificity for non-response of $>$ 95% for high accurateness and feasibility in daily clinical practice. A test was defined as positive when either of the tumour markers had a value above the upper limit of normal (ULN) and above the defined cut-off. The criterion that the marker had to be above ULN was added to exclude patients with small increases of low biomarker values resulting in large percentage change, as this could potentially lead to more false-positive tests, possibly displaying only biological variation. For different time points the sensitivity, specificity, positive predictive value and negative predictive value of the test with corresponding 95% confidence interval were calculated.

An independent validation cohort was used to validate the tumour marker tests using cut-offs defined in the training cohort. For all patients with a false-positive test the electronic patient record was checked for possible explanations.

2.5. Statistical analysis

Diagnostic performance of the test was obtained using the BReC plot application, the 95% confidence interval of sensitivity and specificity were calculated using binomial distribution [22].

Descriptive statistics were used to report the baseline characteristics of the study population. Differences in baseline characteristics between groups were analysed using the Chi-square test and the Mann-Whitney *U* test. PFS curves were estimated with the Kaplan-Meier method and compared using a log-rank test. Analyses were carried out using SPSS Statistics 25 (IBM, Chicago, USA).

3. Results

3.1. Patients training cohort

In total 166 advanced melanoma patients were included in the training cohort, all treated with pembrolizumab (Fig. 1). We had to exclude 21 patients due to missing data. Of the 145 patients analysed, 55% was male and the majority had an ECOG performance status of 0 or 1. Most patients had M1c disease and 68% received anti-PD-1 not as first-line treatment (Table 1).

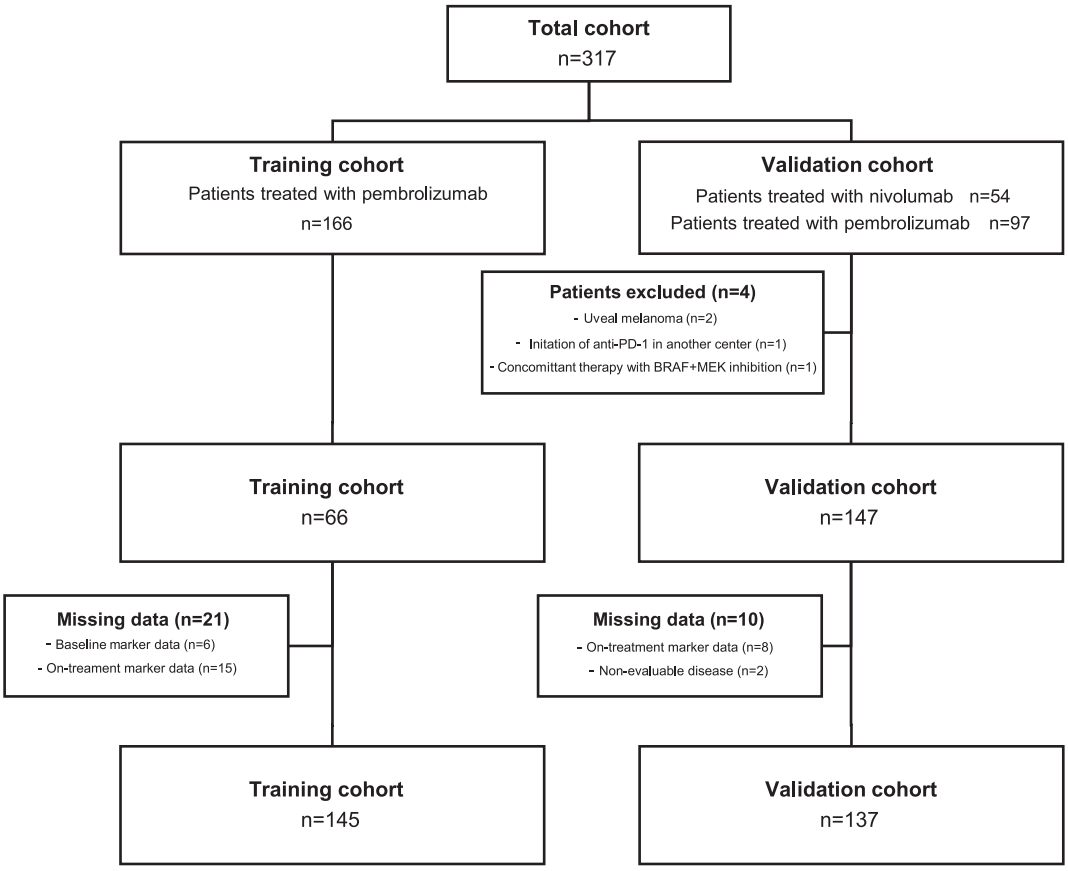

Fig. 1. Flow diagram of patient selection for both the training and validation cohort.

3.2. Identification of biomarker test

The cut-off for both markers was based on BReC analyses at week 6 as we expected this time point to be most interesting for early evaluation. Based on the generated BReC plot (Fig. 2), an increase of S100B of > 100% compared to baseline value was determined as criterion to positively test for non-responsiveness. An increase of > 50% in LDH compared to baseline identified the largest proportion of patients with PFS<6 months (Fig. 2).

Next, we assessed the performance of the test for both single markers and the combination of both markers at different time points in terms of sensitivity, specificity, positive predictive value and negative predictive value (Table 2). As expected, sensitivity was higher for the combination test including both markers and this did not hamper specificity. Sensitivity of the combination test was higher at week 6 (33%) compared with week 3 (25%) but was not substantially higher at week 9 or 12. Therefore, and because we would like to diagnose non-responsiveness as early as possible, we decided to validate the combination test at week 6 in an independent cohort. At week 6, the positive predictive value was 93% in the training cohort.

The most common explanation for a false positive test in absent of progression were toxicities, which were found in two of the three patients with a false positive test at week 6 (Supplemental Table 1).

In the training cohort, 29 patients were defined as non-responder to anti-PD-1 therapy based on the test (>50% increase in LDH or > 100% increase in S100B) at week 6. Patients with a positive test had significantly more often brain metastases and less often a BRAFV600 mutation (Table 1). Notably, there was no difference in baseline LDH or S100B levels in patients with a positive or negative test at week 6 (Table 1), neither in patients with or without clinical benefit at 6 months (Supplemental Table 2).

3.3. Validation cohort

Of the 147 patients in the validation cohort, 52 were treated with nivolumab and 95 with pembrolizumab. Ten patients were excluded due to missing data (Fig. 1). The 137 included patients were slightly more often male and 64% had M1c disease. The vast majority received anti-PD-1 as first-line treatment (Table 3). The combination test defined 12% as non-responders. These patients were more often female compared to patients with a negative test, and had more often a BRAF mutation. Again, there was no difference in baseline LDH and S100B levels for patients with a positive or negative test at week 6 (Table 3), neither in patients with or without clinical benefit at 6 months (Supplemental Table 3).

There were no differences in age, gender or disease stage between the training and validation cohort. However, more patients received the anti-PD-1 treatment as first-line therapy within the validation cohort, 73% of patients compared to 32% in the training cohort.

The specificity of the combination test was with 96% still high enough, although sensitivity was with 21% substantially lower than in the training cohort (Table 2). This led to a positive predictive and negative predictive value of 82% and 55%, respectively.

In the validation cohort eight patients with a false positive test were identified, of which three patients at week 6 (Supplemental Table 1). One of these patients had progressive disease at the CT scan at week 12, but after 6 months a partial response, showing both radiological and serological pseudoprogression. Of the other two patients, one had a mixed response and the other patient was treated for symptomatic parathyroid disease in parallel.

3.4. Survival

In the training cohort, median PFS for patients with an increase of >

Table 1
Baseline characteristics for patients in the training cohort with a positive or negative test at week 6. Data are presented as n (%) unless stated otherwise. Significance was tested by Chi-Square test or Mann Whitney U test. IQR: interquartile range; S100B: S100 calcium binding protein B; LDH: lactate dehydrogenase; ULN: upper limit of normal.

Training cohort							
	Total cohort ($n = 145$)		**S100B↑ <100% and LDH↑ <50% ($n = 116$)**		**S100B↑ >100% or LDH↑ >50% ($n = 29$)**		***P*-value**
Age							0.105
Median (IQR)	62	(52–70)	64	(52–71)	57	(52–66)	
Gender							0.359
Male	79	(54)	61	(53)	18	(62)	
Female	66	(46)	55	(47)	11	(38)	
Disease stage							0.483
Stage IV M1a	26	(18)	23	(20)	3	(10)	
Stage IV M1b	15	(10)	12	(10)	3	(10)	
Stage IV M1c	104	(72)	81	(70)	23	(79)	
Brain metastases							0.044
Yes	35	(24)	33	(28)	2	(7)	
No	109	(75)	82	(71)	27	(93)	
Unknown	1	(1)	1	(1)	0		
BRAFV600 mutation							0.045
Yes	66	(46)	48	(41)	18	(62)	
No	79	(55)	68	(59)	11	(38)	
Prior ipilimumab							0.269
Yes	88	(61)	73	(63)	15	(52)	
No	57	(39)	43	(37)	14	(48)	
Prior BRAF ± MEK inhibitor							0.108
Yes	38	(26)	27	(23)	11	(38)	
No	107	(74)	89	(77)	18	(62)	
Prior lines of therapy							0.938
0	47	(32)	37	(32)	10	(35)	
1	51	(35)	42	(36)	9	(31)	
2	39	(27)	31	(27)	8	(28)	
>2	8	(6)	6	(5)	2	(7)	
ECOG performance status							0.052
0	92	(63)	73	(63)	19	(66)	
1	48	(33)	41	(35)	7	(24)	
2	5	(3)	2	(2)	3	(10)	
Baseline S100B							
Median (IQR)	0.13	(0.07–0.37)	0.13	(0.07–0.39)	0.13	(0.07–0.36)	0.923
<ULN	65	(45)	51	(44)	14	(48)	0.837
1–10 ULN	63	(43)	52	(45)	11	(38)	
>10 ULN	16	(11)	12	(10)	4	(14)	
Unknown	1	(1)	1	(1)	0		
Baseline LDH							
Median (IQR)	190	(161–240)	188	(160–238)	197	(165–155)	0.528
<ULN	110	(76)	89	(7)	21	(72)	0.817
1–2 ULN	23	(16)	17	(15)	6	(21)	
>2 ULN	8	(6)	9	(6)	1	(3)	
Unknown	4	(3)	3	(3)	1	(3)	

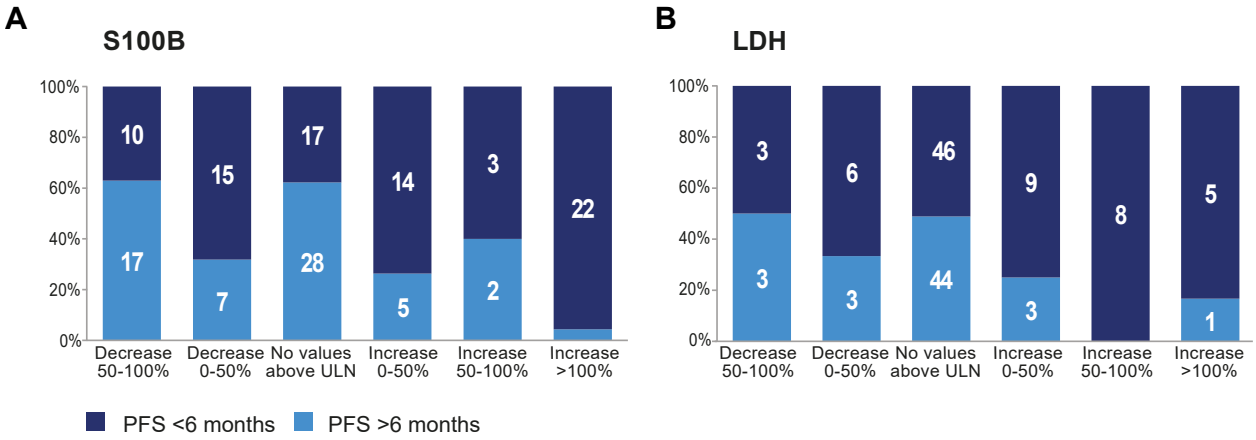

Fig. 2. BReC plots of S100B and LDH for the training cohort at week 6. **A-B.** These two BReC plots are based on S100B and LDH biomarker responses observed after 5–7 weeks of pembrolizumab treatment in the training cohort. Clinical non-response was defined as a PFS < 6 months or death before 6 months after start of therapy. Patients from whom the markers were below ULN (for S100B < 0.10 μg/L and LDH < 248 U/L) at baseline and at week 5–7 were grouped separately. S100B: S100 calcium binding protein B; LDH: lactate dehydrogenase; PFS: progression-free survival.

Table 2
Sensitivity, specificity and predictive values of S100B and LDH in both the training and validation cohort. S100B: S100 calcium binding protein B; LDH: lactate dehydrogenase.

	Number of patients	Sensitivity		Specificity		Positive predictive value		Negative predictive value	
Training cohort									
S100B									
Week 3	146	21%	(13–31)	98%	(91–100)	95%	(74–99)	46%	(37–55)
Week 6	141	27%	(18–38)	98%	(91–100)	96%	(78–100)	50%	(41–59)
Week 9	129	26%	(17–39)	98%	(91–100)	95%	(74–100)	55%	(45–64)
Week 12	133	31%	(21–43)	98%	(91–100)	96%	(78–100)	55%	(46–65)
LDH									
Week 3	137	16%	(9–26)	96%	(88–100)	87%	(60–98)	44%	(35–54)
Week 6	132	15%	(8–25)	98%	(90–100)	92%	(64–100)	45%	(35–54)
Week 9	131	17%	(9–28)	100%	(94–100)	100%	(74–100)	51%	(41–60)
Week 12	126	20%	(11–31)	98%	(90–100)	93%	(68–100)	50%	(40–59)
S100 or LDH									
Week 3	147	25%	(16–35)	97%	(88–100)	92%	(73–99)	46%	(37–56)
Week 6	145	33%	(23–44)	97%	(88–100)	93%	(77–99)	52%	(42–61)
Week 9	135	30%	(20–42)	98%	(91–100)	96%	(78–100)	54%	(45–64)
Week 12	138	35%	(24–47)	97%	(89–100)	93%	(77–99)	55%	(46–65)
Validation cohort									
S100B or LDH									
Week 3	144	16%	(9–27)	94%	(86–98)	75%	(48–93)	52%	(43–60)
Week 6	137	21%	(12–32)	96%	(88–99)	82%	(57–96)	55%	(46–64)
Week 9	127	21%	(11–34)	96%	(88–99)	80%	(52–96)	60%	(50–69)
Week 12	124	22%	(12–35)	96%	(87–99)	80%	(52–96)	61%	(51–71)

100% in S100B or > 50% in LDH at week 6 was 2.4 months (95% confidence interval [CI] 1.8–3.1) while patients with a negative test had a median PFS of 7.0 months (95% CI 4.6–9.3, $p < 0.001$). In the validation cohort, patients with a negative test had an even higher median PFS of 10.3 months (95% CI 5.4–15.20) compared to 2.5 months (95% CI 2.4–2.6) for patients with a positive test ($p < 0.001$, Fig. 3). In the training cohort we observed no significant difference in PFS between patients with or without elevated LDH at baseline (Supplemental Fig. 2A-B), while in the validation cohort patients with a normal baseline had a significantly better PFS than patients with a LDH level above the upper limit of normal (Supplemental Fig. 2C-D).

4. Discussion

Our study shows that an early increase in S100B or LDH is a strong biomarker for early diagnosis of non-responsiveness to anti-PD-1 in advanced melanoma. When using an increase of > 100% in S100B or an increase of > 50% in LDH as cut-off, the specificity of the test is > 95% with a positive predictive value of 93% in the training cohort and 82% in the validation cohort.

As the goal was to guide early-treatment decisions, the test should be very accurate in detecting non-responsiveness and requires high specificity. Some of the patients with a false-positive test had treatment-related toxicities or active comorbidities which might be the explanation for the elevation of S100 and/or LDH levels. Leaving out those patients would increase specificity to > 97% in both cohorts and increase the positive predictive value to 93% in the training cohort and 88% in the validation cohort. One should consider if patients have toxicities or active comorbidities that could influence the S100B and LDH levels when using this test.

Although specificity was high in both cohorts, the positive predictive value in the validation cohort was lower due to the lower number of positive tests. This could be partly explained due to patient selection, as these patients were treated more often with anti-PD-1 as first-line therapy and for most of the patients in the valiation cohort combined ipilimumab plus nivolumab was also a treatment option, since approval was obtained in July 2016 in the Netherlands. The patients selected for anti-PD-1 monotherapy are likely to have less tumour load and thereby less risk on early disease progression. This lower tumor burden might limit the sensitivity of the test in the validation cohort.

In patients with a positive test identifying early non-responsiveness, one might consider an early switch of therapy or addition of other therapies to anti-PD-1, like ipilimumab or targeted therapy. These therapies are potentially more effective when initiated early when patients have a lower tumour load, lower LDH levels and a better performance status [6,24]. As the positive predictive value of the test is not 100%, there is a risk of over-treatment, which can cause unnecessary toxicities and costs in the small fraction of patients with pseudoprogression on anti-PD-1. The incidence of early pseudoprogression is described to be about 5% [25,26], and probably not all of these patients will have an early increase in S100B and LDH as well.

In several studies it has been shown that baseline LDH is a biomarker for response and PFS upon anti-PD-1 therapy [5,11]. In the validation cohort, but not in the training cohort, we also found this association. Nowadays, baseline LDH is already used to guide first-line therapy. Since response rates for patients with a high LDH are higher for the combination of ipilimumab and nivolumab compared to anti-PD-1 monotherapy [11], mostly patients with a normal baseline LDH and low tumour load will receive anti-PD-1 as first-line therapy. New (combination) therapies that might come available the upcoming years, will probably also be the most effective in patients with these favourable characteristics. Therefore on-treatment biomarkers will be even more important to detect non-responsiveness in an early stage.

An increase of S100B and LDH after two cycles of anti-PD-1 has been shown previously to predict disease progression, and increasing levels were associated with an impaired OS as well [27]. This study on 152 melanoma patients did not report on sensitivity, specificity or positive predictive value, however, and no association with PFS was reported. A second study including 66 metastatic melanoma patients treated with anti-PD-1 with increased LDH levels at baseline showed that patients with an additional 10% relative increase had a significantly shorter OS compared to patients with ≤ 10% change [28]. In our study not only an association of both markers combined with PFS was reported, we also addressed sensitivity, specificity and positive and negative predictive values of both markers using a cut-off that can be easily used in daily clinic.

Circulating tumor DNA (ctDNA) is also postulated as a non-invasive biomarker for response to immunotherapy in melanoma [29,30]. Lee et al. showed that longitudinal ctDNA profiles were useful to distinguish between pseudoprogression and true disease progression [31].

Table 3
Baseline characteristics for patients in the validation cohort with a positive or negative test at week 6. Data are presented as n (%) unless stated otherwise. Significance was tested by Chi-Square test or Mann Whitney U test. IQR: interquartile range; S100B: S100 calcium binding protein B; LDH: lactate dehydrogenase; ULN: upper limit of normal.

Validation cohort							
	Total cohort ($n = 137$)		**S100B↑ <100% and LDH↑ <50% ($n = 120$)**		**S100B↑ >100% or LDH↑ >50% ($n = 17$)**		***P*-value**
Age							0.068
Median (IQR)	65	(57–74)	66	(58–74)	56	(49–73)	
Gender							0.025
Male	75	(55)	70	(58)	5	(29)	
Female	62	(45)	50	(42)	12	(71)	
Disease stage							0.278
Stage IV M1a	27	(20)	26	(22)	1	(6)	
Stage IV M1b	23	(17)	19	(16)	4	(24)	
Stage IV M1c	87	(64)	75	(63)	17	(71)	
Brain metastases							0.077
Yes	95	(69)	39	(33)	2	(12)	
No	41	(30)	80	(67)	15	(88)	
Unknown	1	(1)	1	(1)			
BRAFV600 mutation							0.025
Yes	62	(45)	50	(42)	12	(71)	
No	75	(55)	70	(58)	5	(29)	
Prior ipilimumab							0.427
Yes	16	(12)	15	(13)	1	(6)	
No	121	(88)	105	(88)	16	(94)	
Prior BRAF ± MEK inhibitor							0.554
Yes	23	(17)	21	(18)	2	(12)	
No	114	(83)	99	(83)	15	(88)	
Prior lines of therapy							0.510
0	100	(73)	86	(72)	14	(82)	
1	31	(23)	29	(24)	2	(12)	
2	6	(4)	5	(4)	1	(6)	
ECOG performance status							0.773
0	83	(61)	72	(60)	11	(65)	
1	39	(29)	34	(28)	5	(29)	
2	15	(11)	14	(12)	1	(6)	
Baseline S100B							
Median (IQR)	0.10	(0.06–0.30)	0.10	(0.05–0.32)	0.15	(0.10–0.26)	0.236
<ULN	68	(50)	62	(52)	6	(35)	0.076
1–10 ULN	52	(38)	41	(34)	11	(65)	
>10 ULN	15	(11)	15	(13)	0		
Unknown	2	(2)	2	(2)	0		
Baseline LDH							
Median (IQR)	194	(161–261)	192	(181–257)	218	(181–271)	0.263
<ULN	94	(69)	82	(68)	12	(72)	0.551
1–2 ULN	31	(23)	26	(22)	5	(29)	
>2 ULN	5	(4)	5	(4)	0		
Unknown	7	(5)	7	(6)	0		

Nonetheless, compared with our test, ctDNA also has some shortcomings: it can only be used in patients with known mutations, and for the 30% BRAF/NRAS wildtype patients next generation sequencing of tumor DNA will be necessary to identify mutations [32], ctDNA analysis is more expensive, and analysis of ctDNA is not yet implemented in all clinics thereby results might not be available early to enable early treatment decisions.

Since our study included patients retrospectively, it is susceptible for selection bias. To minimize this bias, we included all consecutive patients at our centre receiving anti-PD-1 and did not select the patients on baseline characteristics. We used a consecutive cohort as validation cohort, and did not randomly assign patients to one of the cohorts. This implicates that all patients in the validation cohort were treated more recently and in a different treatment landscape, which leads to differences in baseline characticterists and possibly other confounding factors that could lead to differences in test performance. Although, as the treatment landscape in advanced melanoma is evolving rapidly, the characteristics of the group of patients that will be treated with anti-PD-1 monotherapy will change along with it. This makes our validation cohort more representative of the current melanoma patient population, but one should keep this in mind when extrapolating the results to the current patient population.

A limitation of the test itself is, that it was validated for one specific time point. Nevertheless, the test showed also a high specificity at an earlier time point. Sensitivity at week 3 was lower, but the sensitivity and specificity levels at week 6, 9 and 12 were comparable. The moment at week 6 was chosen as it is a clearly earlier evaluation moment (after two cycles of therapy), compared with the first radiographic evaluation that is according to clinical practice generally planned after four cycles of therapy. Hereby an earlier insight in response to the therapy is given, which creates a moment for an earlier intervention when non-responsiveness is predicted.

The cut-offs for both markers were based on identifying the largest proportion of patients with a PFS<6 months. The percentages of a 50% and 100% increase chosen for comparison, were chosen for enabling mental arithmetic, thereby avoiding the necessity of software or calculators for application in clinical practice. This makes our approach preferable over cut-offs defined in an earlier study [27]. As the combination test is based on an OR function, it enables quick results when no fast S100B result is available for example. These two laboratory values are in most centers determined routinely before every anti-PD-1 infusion, so there is no extra burden for the patient and there are no additional costs for this test. A disadvantage of these practical requirements is that not necesasarily the true optimal LDH and S100B criteria are selected and clinical performance might therefore be further improved using more advanced data analysis.

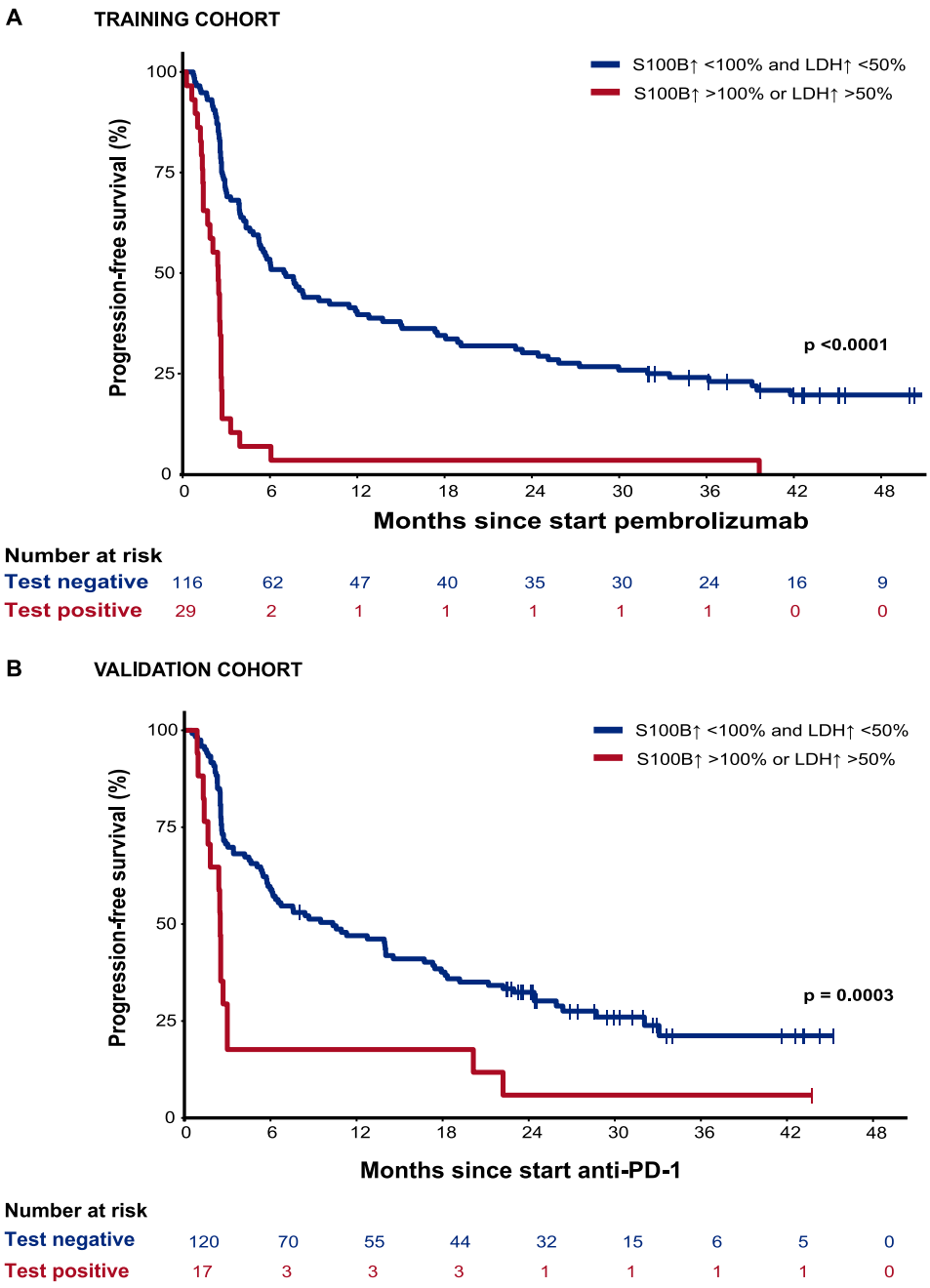

Fig. 3. Progression-free survival curves according to the change in S100B or LDH at week 6 compared to baseline**. A.** Progression-free survival curve for patients with a single positive test compared to patients with a positive combination test in the training cohort. **B.** Progression-free survival curve for patients with a single positive test compared to patients with a positive combination test in the validation cohort. S100B: S100 calcium binding protein B; LDH: lactate dehydrogenase.

In conclusion, an early increase in S100B or LDH is a strong parameter for non-responsiveness to anti-PD-1 in advanced melanoma. This could be an easy, non-invasive, cheap and useful marker to identify patients who may benefit from an early switch to second-line therapy before the first evaluation by imaging that usually is scheduled after three months. Confirmation in a prospective clinical trial is needed before clinical implementation.

CRediT authorship contribution statement

Elisa A. Rozeman: Conceptualization, Formal analysis, Writing – original draft. **Judith M. Versluis:** Formal analysis, Writing – original draft. **Ruben Moritz:** Software, Writing – review & editing. **Sofie Wilgenhof:** Resources, Writing – review & editing. **Johannes V. van Thienen:** Resources, Writing – review & editing. **John B.A.G. Haanen:** Resources, Writing – review & editing. **Michel M. van de Heuvel:** Software, Writing – review & editing. **Christian U. Blank:** Resources, Writing – review & editing. **Huub H. van Rossum:** Conceptualization, Software, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Unrelated with this work, JVvT received teaching fee from MSD (paid to the institute). JBAGH received compensation (all paid to the institute) for advisory roles for Achilles Therapeutics, AIMM, BioNTech, Bristol-Myers Squibb, Gadeta, Immunocore, Ipsen, MSD, Merck Serono,

Molecular Partners, Neogene Therapeutics, Novartis, Pfizer, Roche/Genentech, Sanofi, Seattle Genetics, Third Rock Ventures, and NKI has received research grants from Amgen, BioNTech, BMS, MSD, Novartis. CUB received compensation (all paid to the institute except TRV) for advisory roles for Bristol-Myers Squibb, MSD, Roche, Novartis, GSK, AZ, Pfizer, Lilly, GenMab, Pierre Fabre, Third Rock Ventures; received research funding (all paid to the institute) from Bristol-Myers Squibb, Novartis, NanoString, and declares stockownership in Immagene BV, where he is co-founder. HHvR declares stockownership in Huvaros BV, where he is founder and owner, and stockownership in SelfSafSure Blood Collections BV, where he is co-founder.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2022.06.001.

References

[1] J.J. Luke, K.T. Flaherty, A. Ribas, G.V. Long, Targeted agents and immunotherapies: optimizing outcomes in melanoma, Nat. Rev. Clin. Oncol. 14 (8) (2017) 463–482.

[2] Luke JJ. Comprehensive clinical trial data summation for BRAF-MEK inhibition and checkpoint immunotherapy in metastatic melanoma. Oncologist. 2019.

[3] P.A. Ascierto, R. Dummer, Immunological effects of BRAF+MEK inhibition, OncoImmunology. 7 (9) (2018) e1468955, https://doi.org/10.1080/2162402X.2018.1468955.

[4] E.A. Rozeman, T.J.A. Dekker, J.B.A.G. Haanen, C.U. Blank, Advanced melanoma: current treatment options, biomarkers, and future perspectives, Am. J. Clin. Dermatol. 19 (3) (2018) 303–317.

[5] A. Ribas, O. Hamid, A. Daud, F.S. Hodi, J.D. Wolchok, R. Kefford, A.M. Joshua, A. Patnaik, W.-J. Hwu, J.S. Weber, T.C. Gangadhar, P. Hersey, R. Dronca, R. W. Joseph, H. Zarour, B. Chmielowski, D.P. Lawrence, A. Algazi, N.A. Rizvi, B. Hoffner, C. Mateus, K. Gergich, J.A. Lindia, M. Giannotti, X.N. Li, S. Ebbinghaus, S.P. Kang, C. Robert, Association of pembrolizumab with tumor response and survival among patients with advanced melanoma, JAMA 315 (15) (2016) 1600, https://doi.org/10.1001/jama.2016.4059.

[6] D. Schadendorf, G.V. Long, D. Stroiakovski, B. Karaszewska, A. Hauschild, E. Levchenko, V. Chiarion-Sileni, J. Schachter, C. Garbe, C. Dutriaux, H. Gogas, M. Mandalà, J.B.A.G. Haanen, C. Lebbé, A. Mackiewicz, P. Rutkowski, J.-J. Grob, P. Nathan, A. Ribas, M.A. Davies, Y. Zhang, M. Kaper, B. Mookerjee, J.J. Legos, K. T. Flaherty, C. Robert, Three-year pooled analysis of factors associated with clinical outcomes across dabrafenib and trametinib combination therapy phase 3 randomised trials, Eur. J. Cancer. 82 (2017) 45–55.

[7] D.O. Khair, H.J. Bax, S. Mele, S. Crescioli, G. Pellizzari, A. Khiabany, M. Nakamura, R.J. Harris, E. French, R.M. Hoffmann, I.P. Williams, A. Cheung, B. Thair, C. T. Beales, E. Touizer, A.W. Signell, N.L. Tasnova, J.F. Spicer, D.H. Josephs, J. L. Geh, A. MacKenzie Ross, C. Healy, S. Papa, K.E. Lacy, S.N. Karagiannis, Combining immune checkpoint inhibitors: established and emerging targets and strategies to improve outcomes in melanoma, Front Immunol. 10 (2019;10:453-.), https://doi.org/10.3389/fimmu.2019.00453.

[8] C. Robert, A. Ribas, J. Schachter, A. Arance, J.-J. Grob, L. Mortier, A. Daud, M. S. Carlino, C.M. McNeil, M. Lotem, J.M.G. Larkin, P. Lorigan, B. Neyns, C.U. Blank, T.M. Petrella, O. Hamid, S.-C. Su, C. Krepler, N. Ibrahim, G.V. Long, Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study, Lancet Oncol. 20 (9) (2019) 1239–1251.

[9] J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J.-J. Grob, P. Rutkowski, C.D. Lao, C. L. Cowey, D. Schadendorf, J. Wagstaff, R. Dummer, P.F. Ferrucci, M. Smylie, D. Hogg, A. Hill, I. Márquez-Rodas, J. Haanen, M. Guidoboni, M. Maio, P. Schöffski, M.S. Carlino, C. Lebbé, G. McArthur, P.A. Ascierto, G.A. Daniels, G. V. Long, L. Bastholt, J.I. Rizzo, A. Balogh, A. Moshyk, F.S. Hodi, J.D. Wolchok, Five-year survival with combined nivolumab and ipilimumab in advanced melanoma, NEJM. 381 (16) (2019) 1535–1546.

[10] C.M. Balch, A.C. Buzaid, S.-J. Soong, M.B. Atkins, N. Cascinelli, D.G. Coit, I. D. Fleming, J.E. Gershenwald, A. Houghton, J.M. Kirkwood, K.M. McMasters, M. F. Mihm, D.L. Morton, D.S. Reintgen, M.I. Ross, A. Sober, J.A. Thompson, J. F. Thompson, Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma, J. Clin. Oncol. 19 (16) (2001) 3635–3648.

[11] J. Larkin, P. Ferrucci, R. Gonzalez, L. Thomas, M. Maio, A. Hill, et al., Efficacy of nivolumab plus ipilimumab combination in patients with advanced melanoma and elevated serum lactate dehydrogenase: a pooled analysis, Soc. Melanoma Res. (SMR) Congress. (2016).

[12] C. Robert, J.J. Grob, D. Stroyakovskiy, B. Karaszewska, A. Hauschild, E. Levchenko, V. Chiarion Sileni, J. Schachter, C. Garbe, I. Bondarenko, H. Gogas, M. Mandalá, J.B.A.G. Haanen, C. Lebbé, A. Mackiewicz, P. Rutkowski, P.D. Nathan, A. Ribas, M.A. Davies, K.T. Flaherty, P. Burgess, M. Tan, E. Gasal, M. Voi, D. Schadendorf, G.V. Long, Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma, NEJM. 381 (7) (2019) 626–636.

[13] G. Krähn, P. Kaskel, S. Sander, P.J. Waizenhöfer, S. Wortmann, U. Leiter, et al., S100 beta is a more reliable tumor marker in peripheral blood for patients with newly occurred melanoma metastases compared with MIA, albumin and lactate-dehydrogenase, Anticancer Res. 21 (2B) (2001) 1311–1316.

[14] L.H.M. Smit, C.M. Korse, A.A.M. Hart, J.M.G. Bonfrer, J.B.A.G. Haanen, J.M. Kerst, O.E. Nieweg, G.C. de Gast, Normal values of serum S-100B predict prolonged survival for stage IV melanoma patients, Eur. J. Cancer. 41 (3) (2005) 386–392.

[15] S. Mocellin, G. Zavagno, D. Nitti, The prognostic value of serum S100B in patients with cutaneous melanoma: a meta-analysis, Int. J. Cancer. 123 (10) (2008) 2370–2376.

[16] B. Weide, M. Elsässer, P. Büttner, A. Pflugfelder, U. Leiter, T.K. Eigentler, J. Bauer, M. Witte, F. Meier, C. Garbe, Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis, Br. J. Cancer. 107 (3) (2012) 422–428.

[17] E.D. Mårtenson, L.O. Hansson, B. Nilsson, E. von Schoultz, E.M. Brahme, U. Ringborg, J. Hansson, Serum S-100B protein as a prognostic marker in malignant cutaneous melanoma, J. Clin. Oncol. 19 (3) (2001) 824–831.

[18] A.A. Tarhini, J. Stuckert, S. Lee, C. Sander, J.M. Kirkwood, Prognostic significance of serum S100B protein in high-risk surgically resected melanoma patients participating in Intergroup Trial ECOG 1694, J. Clin. Oncol. 27 (1) (2009) 38–44.

[19] M.Q. Mohammed, H.D. Abraha, R.A. Sherwood, K. MacRae, S. Retsas, Serum S100beta protein as a marker of disease activity in patients with malignant melanoma, Med. Oncol. (Northwood, London, England). 18 (2) (2001) 109–120.

[20] T. Gambichler, V. Brown, A.-K. Steuke, L. Schmitz, E. Stockfleth, L. Susok, Baseline laboratory parameters predicting clinical outcome in melanoma patients treated with ipilimumab: a single-centre analysis, J. Eur. Academy of Dermatol. Venereol. JEADV. 32 (6) (2018) 972–977.

[21] V. Damuzzo, S. Solito, L. Pinton, E. Carrozzo, S. Valpione, J. Pigozzo, R. Arboretti Giancristofaro, V. Chiarion-Sileni, S. Mandruzzato, Clinical implication of tumor-associated and immunological parameters in melanoma patients treated with ipilimumab, OncoImmunology. 5 (12) (2016) e1249559, https://doi.org/10.1080/2162402X.2016.1249559.

[22] R. Moritz, M. Muller, C.M. Korse, D. van den Broek, P. Baas, V. van den Noort, J. J. ten Hoeve, M.M. van den Heuvel, H.H. van Rossum, Diagnostic validation and interpretation of longitudinal circulating biomarkers using a biomarker response characteristic plot, Clinica chimica acta; int. j. clin. Chem. 487 (2018) 6–14.

[23] E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij, New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), Eur. J. Cancer. 45 (2) (2009) 228–247.

[24] I. Pires da Silva, T. Ahmed, I.L.M. Reijers, A.M. Weppler, A. Betof Warner, J. R. Patrinely, P. Serra-Bellver, C. Allayous, J. Mangana, K. Nguyen, L. Zimmer, C. Trojaniello, D. Stout, M. Lyle, O. Klein, C.L. Gerard, O. Michielin, A. Haydon, P. A. Ascierto, M.S. Carlino, C. Lebbe, P. Lorigan, D.B. Johnson, S. Sandhu, S.N. Lo, C. U. Blank, A.M. Menzies, G.V. Long, Ipilimumab alone or ipilimumab plus anti-PD-1 therapy in patients with metastatic melanoma resistant to anti-PD-(L)1 monotherapy: a multicentre, retrospective, cohort study, Lancet 22 (6) (2021) 836–847.

[25] F.S. Hodi, W.-J. Hwu, R. Kefford, J.S. Weber, A. Daud, O. Hamid, A. Patnaik, A. Ribas, C. Robert, T.C. Gangadhar, A.M. Joshua, P. Hersey, R. Dronca, R. Joseph, D. Hille, D. Xue, X.N. Li, S.P. Kang, S. Ebbinghaus, A. Perrone, J.D. Wolchok, Evaluation of immune-related response criteria and RECIST v1.1 in patients with advanced melanoma treated with pembrolizumab. journal of clinical oncology : official journal of the american society of, Clin. Oncol. 34 (13) (2016) 1510–1517.

[26] G.V. Long, J.S. Weber, J. Larkin, V. Atkinson, J.-J. Grob, D. Schadendorf, R. Dummer, C. Robert, I. Márquez-Rodas, C. McNeil, H. Schmidt, K. Briscoe, J.-F. Baurain, F.S. Hodi, J.D. Wolchok, Nivolumab for patients with advanced melanoma treated beyond progression: analysis of 2 phase 3 clinical trials. JAMA, Oncol. 3 (11) (2017) 1511, https://doi.org/10.1001/jamaoncol.2017.1588.

[27] N.B. Wagner, A. Forschner, U. Leiter, C. Garbe, T.K. Eigentler, S100B and LDH as early prognostic markers for response and overall survival in melanoma patients treated with anti-PD-1 or combined anti-PD-1 plus anti-CTLA-4 antibodies, Br J Cancer. 119 (3) (2018) 339–346.

[28] S. Diem, B. Kasenda, L. Spain, J. Martin-Liberal, R. Marconcini, M. Gore, J. Larkin, Serum lactate dehydrogenase as an early marker for outcome in patients treated with anti-PD-1 therapy in metastatic melanoma, Br J Cancer. 114 (3) (2016) 256–261.

[29] J.H. Lee, G.V. Long, S. Boyd, S. Lo, A.M. Menzies, V. Tembe, A. Guminski, V. Jakrot, R.A. Scolyer, G.J. Mann, R.F. Kefford, M.S. Carlino, H. Rizos, Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma, Ann Oncol. 28 (5) (2017) 1130–1136.

[30] T. Seremet, Y. Jansen, S. Planken, H. Njimi, M. Delaunoy, H. El Housni, G. Awada, J.K. Schwarze, M. Keyaerts, H. Everaert, D. Lienard, V. Del Marmol, P. Heimann, B. Neyns, Undetectable circulating tumor DNA (ctDNA) levels correlate with favorable outcome in metastatic melanoma patients treated with anti-PD1 therapy, J. Trans. Med. 17 (1) (2019), https://doi.org/10.1186/s12967-019-2051-8.

[31] J.H. Lee, G.V. Long, A.M. Menzies, S. Lo, A. Guminski, K. Whitbourne, M. Peranec, R. Scolyer, R.F. Kefford, H. Rizos, M.S. Carlino, Association between circulating tumor DNA and pseudoprogression in patients with metastatic melanoma treated with anti-programmed cell death 1 antibodies, JAMA oncology. 4 (5) (2018) 717, https://doi.org/10.1001/jamaoncol.2017.5332.

[32] M.R. Girotti, G. Gremel, R. Lee, E. Galvani, D. Rothwell, A. Viros, et al., Application of Sequencing, Liquid Biopsies, and Patient-Derived Xenografts for Personalized Medicine in Melanoma, Cancer Discov. 6 (3) (2016) 286–299, https://doi.org/10.1158/2159-8290.CD-15-1336.