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Relative Change in S100 as a Biomarker of Survival in Patients With Metastatic Melanoma Treated With Pembrolizumab

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Abstract. *Background/Aim: There is a lack of quality biomarkers of survival for patients with metastatic melanoma treated with immunotherapy. Although the baseline level of S100 has prognostic value, its role during/after therapy in survival is unclear. Patients and Methods: We evaluated patients with metastatic melanoma treated with pembrolizumab with the goal of analysing the relationship between a relative change in S100 level at 12 weeks of immunotherapy and survival. Results: Patients with a relative change in S100 level >145% at 12 weeks of immunotherapy had significantly shorter progression-free (5.1 vs. 18.5 months, p≤0.0001) and overall survival (5.7 vs. 26.3 months, p<0.0001), further confirmed on multivariate analysis with hazard ratio of 32.25 (95% confidence interval=4.78–217.6, p=0.0004) for overall survival. Conclusion: A relative change in S100 level might be useful as a more precise biomarker of survival for patients with metastatic melanoma treated with pembrolizumab.*

Melanoma is cancer that typically arises from the skin and rarely from the mucosa. The incidence of melanoma has increased continuously for the past four decades, which highlights the need to improve its diagnosis and find more effective treatments (1).

The development of immunotherapies such as antibodies to programmed death receptor-1 (PD1) and cytotoxic T-lymphocyte antigen-4 (CTLA4) has resulted in a significant paradigm shift in the treatment of melanoma. Pembrolizumab is a humanized immunoglobulin G4, monoclonal antibody that selectively binds to the PD1 receptor on T-cells, preventing it from binding to its ligand PD-L1 on tumour cells. This interaction prevents tumour tolerance and helps destroy cancer cells. Although immunotherapies offer significant improvements in survival, approximately 60% of patients treated with a PD1 inhibitor monotherapy will not achieve a response. Furthermore, some patients will experience pseudoprogression, which makes it difficult to confirm whether the anti-PD1 immunotherapy treatment actually provided a benefit (2, 3). The inability to correctly assess actual progression can lead to less-than-optimal care for patients and poses a severe financial burden.

Several biomarkers, such as neuron-specific enolase, lactate dehydrogenase (LDH), and S100, have been used for survival analyses and evaluations of disease progression in patients with melanoma, although there are insufficient quality data to determine their use in immunotherapy settings. Hence, there is a crucial need to develop quality survival biomarkers for patients with metastatic melanoma undergoing immunotherapy (4-6).

S100 is a protein that is expressed by glial cells and melanocytes and has been shown to be expressed in patients with brain tumours and melanoma. Among its many functions, S100 can interact with the p53 tumour-suppressor gene (7). High baseline levels of S100 are associated with metastatic disease and shorter survival (8-11). Abusaif *et al.* also showed a good correlation between S100 and a RECIST-confirmed response to vemurafenib (12).

Additionally, although the S100 level does not correlate with fluorodeoxyglucose (FDG)-standardized uptake value in stage III melanoma, it is a strong predictor of disease-free survival (13).

While the baseline level of S100 has shown promise as a survival biomarker, Wagner *et al.* showed in 2018 that early changes in S100 level after several weeks of immunotherapy predicted disease response prior to radiological staging and progression of metastatic disease. Patients with an increase in S100 level greater than 145% had significantly shorter overall survival (OS; $p<0.0001$); this change was determined to be a more precise survival biomarker than both LDH and baseline S100 value for patients with metastatic melanoma (14).

However, the optimal cut-off value for change in S100, the optimal change in S100 level at 12 weeks of immunotherapy (when most centres perform follow-up evaluations), and the relationship between S100 level and

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Table I. *Characteristics of patients receiving immunotherapy (N=38).*

Characteristic	Subgroup	All patients	S100 change		*p*-Value
			≤145%	>145%	
	Total	38 (100)	29 (76.3)	9 (23.7)	-
Gender, n (%)	Male	29 (76.3)	22 (75.9)	7 (77.8)	0.91
	Female	9 (23.7)	7 (24.1)	2 (22.2)	-
Age, years	Average (±SD)	63.4 (11.1)	64.8 (10.7)	58.9 (11.9)	0.21
Initial melanoma, n (%)	Metastatic	3 (7.9)	1 (3.4)	2 (22.2)	0.07
	Nodal disease	14 (36.8)	12 (41.4)	2 (22.2)	0.29
	Local disease only	16 (42.1)	13 (44.8)	3 (33.3)	0.54
	Not specified	5 (13.2)	3 (10.3)	2 (22.2)	0.36
Metastatic site (most common), n (%)	Lymph nodes	23 (60.5)	16 (55.2)	7 (77.8)	0.22
	Skin/subcutaneous	21 (55.2)	17 (58.6)	4 (44.4)	0.45
	Lung	20 (52.6)	16 (55.2)	4 (44.4)	0.57
	Liver	9 (23.7)	5 (17.2)	4 (44.4)	0.09
	Brain	5 (13.9)	4 (14.8)	1 (11.1)	0.83
	≥3	25 (65.8)	17 (58.6)	8 (88.9)	0.09
BRAF mutation, n (%)	Positive	13 (34.2)	9 (31.1)	4 (44.4)	0.46
	Negative	24 (66.7)	20 (68.9)	5 (55.6)	-
Additional therapy, n (%)	Target therapy	9 (23.7)	6 (20.7)	3 (33.3)	0.43
	Chemotherapy	14 (36.8)	11 (37.9)	3 (33.3)	0.80
S100 level, μg/l	Baseline	0.27 (0.54)	0.29 (0.61)	0.17 (0.19)	0.94
	Final	0.61 (1.7)	0.13 (0.35)	2.16 (3.09)	**0.00004**

BRAF: B-Rapidly accelerated fibrosarcoma gene; SD: standard deviation. Bold *p*-values denote statistical significance.

progression-free survival (PFS) are still unknown. We aimed to determine the relationships between the relative change in S100 level at 12 weeks of immunotherapy and OS and PFS.

Patients and Methods

We conducted a retrospective study at the Clinical Hospital Centre Zagreb of patients with metastatic melanoma who received either four or five initial cycles of immunotherapy with the PD1 antibody pembrolizumab from March 2017 to August 2018. Follow-up examinations continued until the end of July 2019.

All patients underwent FDG-positron emission tomography–computed tomography (PET/CT) and an evaluation of S100 levels both before and 12 weeks after the initiation of pembrolizumab immunotherapy.

Patients received 2 mg/kg pembrolizumab every 3 weeks in the outpatient clinic. The cut-off value for S100 was set at 145%, as suggested by Wagner and colleagues (14). S100 analysis in serum was performed using an electrochemiluminescence method with a Cobas 6000 (e601) analyser (Roche Diagnostics GmbH, Mannheim, Germany).

OS was defined as the time from the start of immunotherapy to death or loss of contact. PFS was defined as the time from the start of immunotherapy to clinical or radiological progression, or death.

Due to the number of patients and the results of the Kolmogorov–Smirnov test, Mann–Whitney *U*-tests were performed to compare data between the two S100 change groups. The relationships between categorical variables were determined using the chi-squared test. The Kaplan–Meier method and Cox proportional hazard model, including univariate and multivariate analyses, were used for the survival analysis. Statistical analyses were performed using Statistica 12.5 (StatSoft, Inc., Tulsa, OK, USA) and MedCalc Statistical Software, version 19 (MedCalc Software bvba, Ostend, Belgium).

The Ethics Committee of Clinical Hospital Centre Zagreb approved this research (Class 8.1-19/253-5, 02/21 AG).

Results

Patient characteristics. A total of 38 patients with metastatic melanoma who were treated with pembrolizumab were included in our analysis. Patients were predominantly male (n=29, 76.3%), with an average age of 63.4 years. The most common initial T-stage was 4b (n=9, 23.7%), followed by 3b (n=4, 10.5%), and 13 patients had confirmed nodal disease (34.2%) at the time of initial presentation, most commonly N1 (n=4, 10.5%). Only a minority of patients initially presented with metastatic disease (n=3, 7.9%) (Table I).

When metastatic disease developed, it was most commonly localized to the lymph nodes (n=23, 60.5%) or skin (n=21, 55.2%). The majority of patients had three or more sites of metastases (n=25, 65.8%) and a negative mutation status for B-rapidly accelerated fibrosarcoma (*BRAF*) gene (n=24, 63.1%). Immunotherapy with pembrolizumab was utilized as either a first- (n=24, 63.1%) or second-line of treatment (n=14, 36.9%), with an average of 15.3 cycles applied per patient. Mean baseline S100 values were 0.27 μg/l, increasing to 0.61

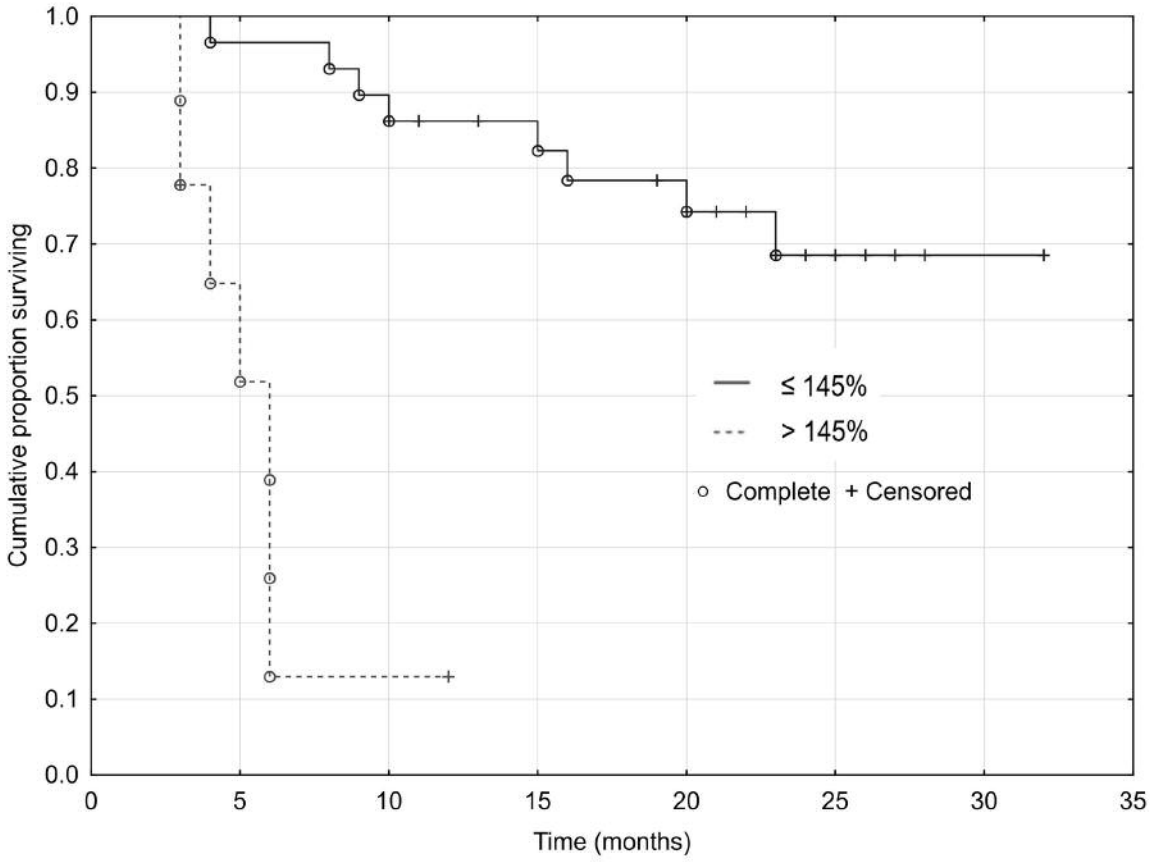

Figure 1. *Overall survival based on the change in S100 level after initial immunotherapy with pembrolizumab (N=38), p=0.0061.*

μg/l by the first follow-up evaluation (Table I). However, the majority of patients actually exhibited a decrease (n=22, 57.9%) or an increase of ≤145% (n=7, 18.4%) in S100 level.

Patients in both groups had similar demographic characteristics, initial presentation of metastatic disease, and pattern of metastatic disease (Table I).

Relationship of S100 to survival. The patients in our analysis exhibited an average OS of 22.4 months and a PFS of 15.6 months. Although previous authors reported the usefulness of the baseline S100 value (5, 6), we found no association between the baseline level of S100 and OS. S100 values at 12 weeks of immunotherapy were correlated with survival (r=–0.40, $p<0.05$), whereas no correlation was noted for baseline S100 values (r=–0.05, $p>0.05$)

Patients with a relative change in S100 level >145% had a significantly shorter PFS (5.1 *vs*. 18.5 months, $p<0.0001$) and OS (5.7 *vs*. 26.3 months, $p<0.0001$) (Figure 1). Only two patients (22.2%) in the ΔS100>145% group were alive at the time of analysis, compared to 21 patients (72.4%) in the ΔS100 ≤145% group ($p=0.007$), although there was no such difference in the PFS group, possibly due to the small number of patients. There was a trend towards a longer time to metastatic disease in the ΔS100 ≤145% group, but it did not reach statistical significance ($p=0.07$) (Table II). Additionally, patients with a relative change in S100 level >145% had significantly fewer immunotherapy cycles in total (6.2 *vs*. 18.1 cycles, $p=0.0004$).

Complete response (CR) was found in five patients, all of whom had a change in S100 level of ≤145% (17.2%), whereas no patient in the ΔS100 >145% group exhibited a CR, although the difference was not statistically significant.

Using the Cox proportional hazard model, we determined that a relative change in S100 level >145% was associated with a hazard ratio (HR) of 16.09 [95% confidence interval (CI)=2.01-14.97, $p=0.00006$] for OS and an HR of 5.77 (95% CI=2.21-15.06, $p=0.0003$) for PFS. We found no statistically significant relationship between OS and BRAF status, sex, age, initial T- or N-status, additional target therapy or chemotherapy, line of immunotherapy, or presence of brain, liver or lung metastases (data not shown). Unsurprisingly, slower disease progression, defined in our analysis as a time to metastasis longer than the median of 16 months in our population, was associated with a better prognosis (HR=0.23, $p=0.02$). The presence of three or more sites of metastases

Table II. *Relationship between the change in S100 level (baseline to first follow-up) and survival and the time to the presentation of metastatic disease (N=38).*

Patient characteristics		All patients	S100 change ≤145%	S100 change >145%	*p*-Value
Average time to metastatic disease, months	Mean±SD	23.9 (5.1)	27.5 (6.4),	11.7 (5.1)	0.09
	Median	16	18	3	
Progression-free survival, months	Mean±SE	15.6 (1.9)	18.5 (2.2)	5.1 (1.2)	**<0.0001**
	Median	12	16	5	
Progression free at the last follow-up	Frequency (%)	13 (34.2)	12 (41.4)	1 (11.1)	0.09
Overall survival, months	Mean±SE	22.4 (1.9)	26.3 (1.7),	5.7 (0.9)	**<0.0001**
	Median	Not reached	Not reached	6	
Alive at the last follow-up, n (%)	Mean±SD	23 (60.5)	21 (72.4)	2 (22.2)	**0.007**

SD: Standard deviation; SE: standard error; SD: standard deviation. Bold *p*-values denote statistical significance.

Table III. *The Cox proportional hazard model. Only parameters with statistical significance after univariate analysis are shown (N=38).*

Characteristic	Univariate analysis HR	95% CI	*p*-Value	Multivariate analysis* HR	95% CI	*p*-Value
S100 change >145%	16.09	3.99-56.15	**0.00006**	32.25	**4.78-217.6**	**0.0004**
Metastatic disease at initial presentation	4.79	1.03-22.29	**0.045**	4.08	0.68-24.38	0.12
Time to metastasis >16 months	0.23	0.06-0.84	**0.02**	0.20	**0.04-0.89**	**0.03**
Presence of three or more sites of metastases	4.51	1.01-20.03	**0.048**	1.21	0.22-6.57	0.82

HR: Hazard ratio; CI: confidence interval. Bold *p*-values denote statistical significance. *Corrected by S100 cut-off value, presence of initial metastasis, time to metastasis greater than 16 months, presence of three or more sites of metastases.

was associated with an HR of 4.51 (95% CI=1.01-20.03, p=0.048) and initial presentation of metastatic disease had an HR of 4.79 (95% CI=1.03-22.29, p=0.045).

Hence, we included the four factors that were shown to be significant by the univariate analysis into the multivariate analysis. The multivariate analysis only showed statistical significance for ΔS100 values >145% (HR=32.25, 95% CI=4.78-217.6, p=0.0004) and a time to progression of metastatic disease longer than 16 months (HR=0.20, 95% CI=0.04-0.89, p=0.03) (Table III).

We also aimed to determine whether 145% was the most adequate cut-off value for the change in S100 level with regard to survival. We noticed a dramatic drop-off in OS in patients with a change in S100 level >145% (Figure 2). Interestingly, regardless of whether ΔS100 fell beneath 0% or rose up to 145%, OS (Figure 3) and PFS (Figure 4) appeared to be unaffected.

Discussion

Immunotherapy has caused a revolution in the treatment of malignant melanoma, with a 5-year survival rate of up to 52% for the combination of nivolumab and ipilimumab, and reaching 60% for patients with *BRAF* mutation-positive disease compared to the historical 10-year survival rate of around 10% (15-17). However, one of the main issues with immunotherapy treatment is evaluating its efficacy, as late responses can occur. While the baseline level of S100 is a good biomarker of survival, initial data show that a relative change in S100 level during immunotherapy offers significantly more accurate information regarding survival (14).

We evaluated 38 patients with metastatic melanoma who were treated with pembrolizumab and analysed the relative change in S100 level between baseline and the first evaluation at 12 weeks later. Although Wagner *et al*. showed the value of an early change in S100 after only a few weeks of immunotherapy, we do not usually perform an S100 analysis in the middle of immunotherapy treatment, but only during the evaluation period at 3 months post-therapy (14). However, before our analysis, we did not have the data regarding the value of a relative S100 change at 3-month period.

Most of our patients had either a relative fall in S100 level at 12 weeks (–99% to 0 in 57.9%) or a relative increase of ≤145% (0 to 145% in 18.4%), which was the cut-off value suggested by Wagner *et al*. (14). There were no differences in the baseline characteristics between the groups of patients according to

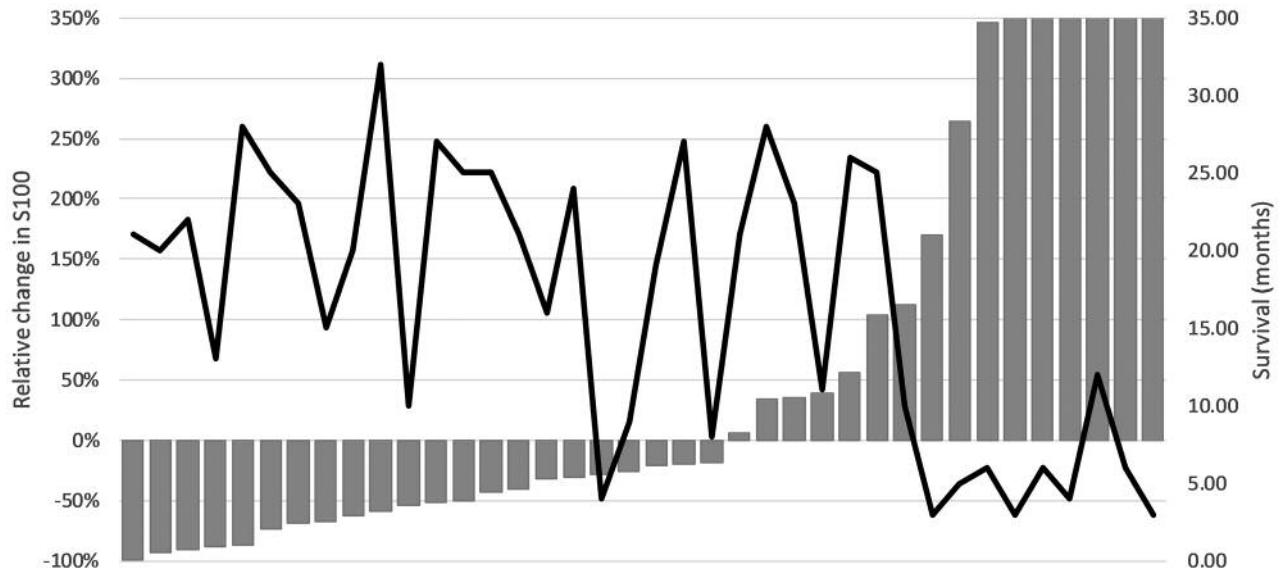

Figure 2. *Relationship between individual change in S100 level (gray columns) after initial immunotherapy with pembrolizumab compared to overall survival (black line) (N=38).*

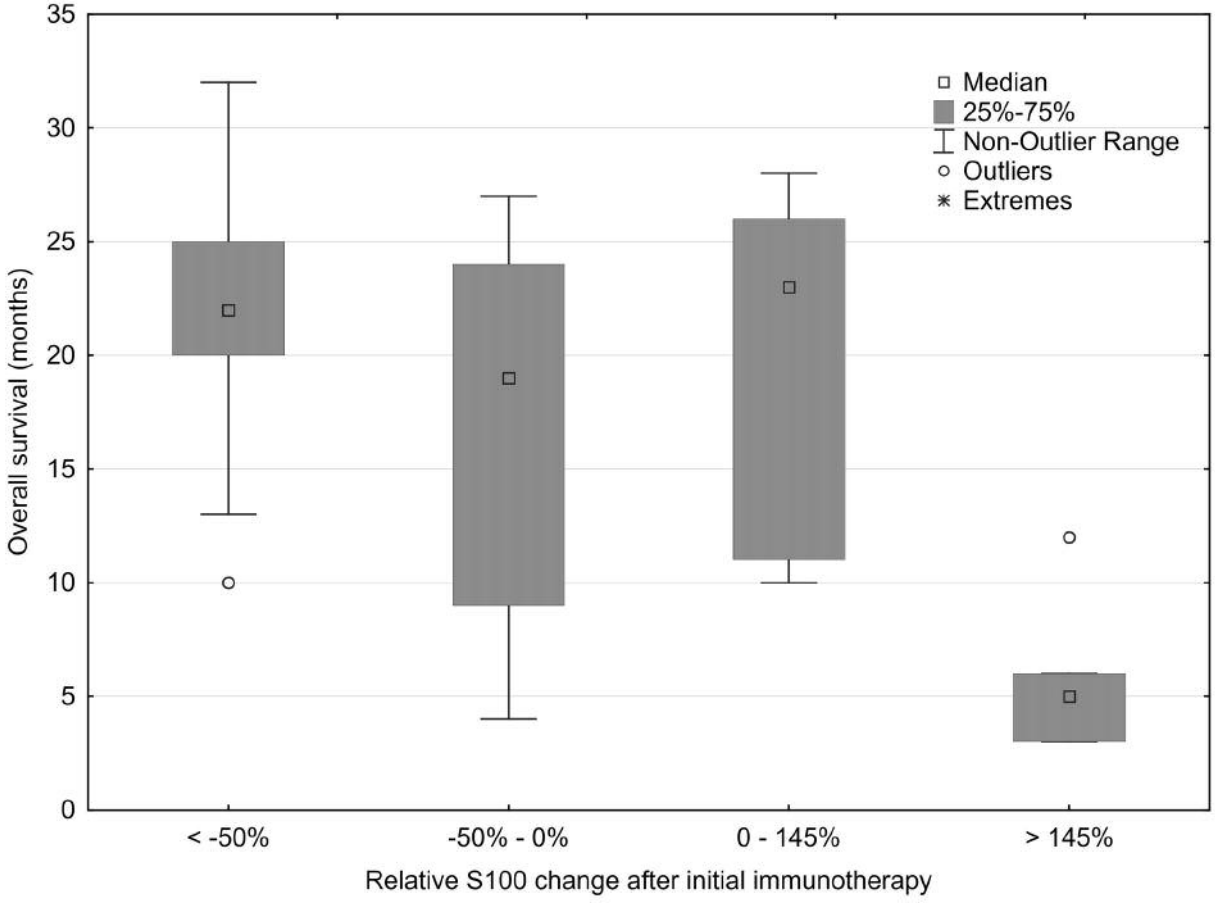

Figure 3. *Relationships between overall survival and different categories of change in S100 level after initial immunotherapy with pembrolizumab (N=38).*

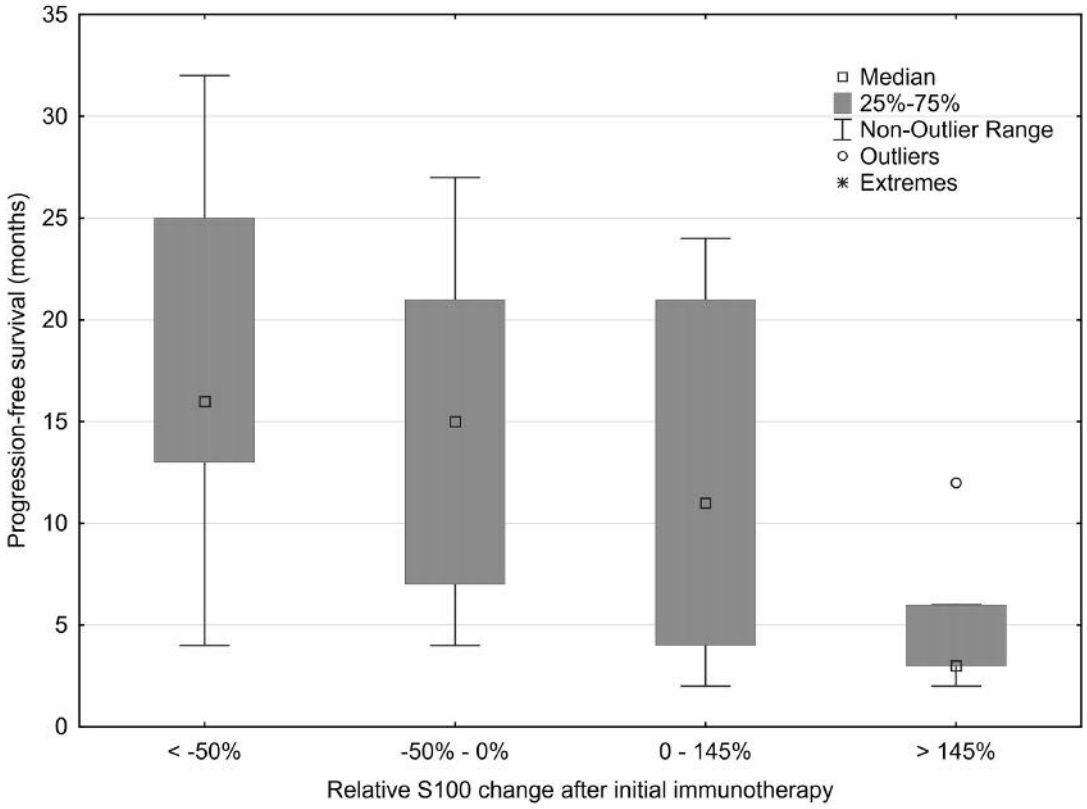

Figure 4. *Relationship between progression-free survival and different categories of change in S100 level after initial immunotherapy with pembrolizumab (N=38).*

change in S100 level (Table I). However, patients with either a decrease or increase of ≤145% in S100 level exhibited significantly longer survival compared to patients with a greater elevation in S100 level (PFS: 15.6 *vs*. 5.1 months and OS: 22.4 *vs*. 5.7 months, respectively, both $p<0.0001$). Almost three quarters of patients in the ΔS100 ≤145% group were alive at the time of this analysis, compared to less than a quarter in the ΔS100 >145% group (Table II). To the best of our knowledge, this is the first study to evaluate the relationship between a change in S100 level and PFS, which offers valuable insight considering that PFS has only been associated with the effects of pembrolizumab and not with subsequent treatments (18).

Along with a relative change in S100 level, we identified a time to metastasis of longer than 16 months, the presence of three or more sites of metastases, and the initial presentation of metastatic disease as potential biomarkers of survival (Table III). However, only the change in S100 level (HR=32.25, $p=0.0004$) and the time from initial presentation to metastatic disease greater than 16 months (HR=0.20, $p=0.03$) were statistically significant in the multivariate analysis. Additionally, 17.2% of patients in the ΔS100 ≤145% group achieved a CR, compared to no patients in the ΔS100 >145% group, although this difference was not statistically significant. Surprisingly, we found no differences in PFS and OS based on the initial S100 value, which contrasts with previous studies. We also evaluated the ideal cut-off value for the change in S100 level but our data suggest that a more significant reduction in S100 is not necessarily associated with a better PFS or OS compared to patients with a relative elevation in S100 level from 0 to ≤145%. This is a surprising insight that implies a complex relationship between the S100 protein and immunotherapy, and requires further analysis (Figures 2-4). However, such data also suggest that a previously reported threshold of 145% change seems valid for a 12-week time frame, which might simplify its clinical use and inform further investigations in this area.

Our research confirms the value of a relative change in S100 level as a more precise biomarker of survival for patients with metastatic melanoma treated with immunotherapy. Such data suggest that a relative change in S100 level might offer insights into the efficacy of immunotherapy and possibly lead to early changes in the choice of therapy, with the goal of prolonging survival in those patients with a poor response to immunotherapy.

Certain limitations of this research must be addressed. We performed a single-centre study, which included a relatively

small group of patients. Due to the retrospective nature of the research, only an association between the observations can be described. Selection bias was probably also present, as only patients with at least two evaluations of S100 level were analysed; such a criterion automatically disqualifies patients who died before a second S100 evaluation and is the most likely reason why we did not see a relationship between baseline S100 level and survival, as well as finding a relatively high HR of 32.25 for OS for patients with an elevation of S100 level >145%.

Conflicts of Interest

The Authors disclose no conflicts of interest pertaining to this study.

Authors' Contributions

LS and MG conceived and designed the analysis, collected data and contributed to the article; KB, KM, and DH performed analysis, collected data, and contributed to the article.

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