Title:

Relevance of MIA and S100 serum tumor markers to monitor BRAF inhibitor therapy in metastatic melanoma patients.

Running title:

MIA and S100 in monitoring iBRAF therapy in melanoma

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Abstract

BRAF V600 mutation has been reported in more than 50% of melanoma cases and its presence predicts clinical activity of BRAF inhibitors (iBRAF). We evaluated the role of MIA, S100 and LDH to monitor iBRAF efficiency in advanced melanoma patients presenting BRAF V600 mutations. This was a prospective study of melanoma patients harboring the BRAF V600 mutation and treated with iBRAF within a clinical trial (dabrafenib) or as part of an expanded access program (vemurafenib). MIA, S100 and LDH were analyzed in serum at baseline, and every 4-6 week during treatment. Eighteen patients with melanoma stage IIIc-IV were enrolled with 88.8% of response rate to iBRAF. Baseline concentrations of all the tumor markers correlated with tumor burden. MIA and S100 concentrations decreased significantly one month after the beginning of treatment and, upon progression, their concentrations increased significantly above the minimum levels previously achieved. MIA levels lower than 9 µg/L one month after the beginning of treatment and S100 concentrations lower than 0.1 µg/L at the moment of best response were associated with improved progression-free survival. In conclusion, MIA and S100 are useful to monitor response in melanoma patients treated with iBRAF.

Keywords:

Melanoma; BRAF; therapy; S100; MIA; tumor marker.

1 Introduction[1]

Metastatic melanoma is among the most aggressive and treatment-resistant human cancers. Its incidence is increasing worldwide and it is the first cause of mortality among skin cancers, with 20,100 deaths reported in Europe in 2008 [1]. Until very recently treatment results for advanced melanoma were dismal, with median overall survival ranging between just 6 and 10 months [2]. However, the advances in the knowledge of the pathogenesis of melanoma have allowed to develop new strategies to treat this disease [3]. One of such advances was the discovery that more than 50% of cutaneous melanomas carry mutations in the serine-threonine protein kinase B-RAF (BRAF) gene [4]. Approximately 90% of these mutations result in the substitution of valine to glutamic acid at codon 600 (BRAF V600E), although other mutations have also been described (e.g., BRAF V600K or BRAF V600R) [4]. These mutations lead to a constitutive activation of BRAF increasing the RAF/MEK signaling pathway that controls proliferation, cell survival and invasion [5]. BRAF inhibitors (iBRAF) can block this overactivated pathway [6] and induce dramatic clinical responses in these patients [6-9], even though these responses are rarely complete nor durable [8, 10]. BRAF mutation analysis is now used in routine practice to identify patients with advanced melanoma that could benefit from treatment with iBRAF [11].

Serum LDH is the only marker recommended by the American Joint Committee on Cancer (AJCC) staging system to classify patients with metastatic melanoma in a more advance stage (M1c), whenever high activities are observed [12]. However LDH sensitivity and specificity is low. Other tumor markers employed are Melanoma Inhibitory Activity (MIA) and S100 protein [13-15]. MIA is a small soluble protein of 11 kDa secreted by malignant melanoma cells [16]. S100 is a 21 kDa dimeric acidic calcium binding protein composed of 2 subunits, α or β, thus producing 3 different types of proteins, being the αβ isoform the one that is expressed by melanoma cells [17]. S100 is expressed in approximately 98% of malignant melanocytic tumors [18] and it is one of the best immunohistochemical markers to screen the presence of this tumor. MIA and S100 are elevated in advanced melanoma [19] and can be useful as prognostic factors in stage III and IV disease [14, 20].

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[1] Abbreviations: BRAF: serine-threonine protein kinase B-RAF; iBRAF: BRAF inhibitor; MIA: Melanoma Inhibitory Activity.

Formerly, a major obstacle to develop tumor markers to monitor clinical response in melanoma was the moderate efficacy of the available therapies. However, the development of new treatments has opened the opportunity to reanalyze the utility of these tumor markers as prognostic factors and to follow-up patients during therapy. For this reason, the aim of the present work was to analyze the utility of S100, MIA an LDH as prognostic factors and to monitor patients treated with iBRAF.

2 Material and methods

2.1. Patients

We included patients treated with iBRAF (dabrafenib or vemurafenib) in our institution within a clinical trial [7] or as part of an expanded access program, respectively. Patients were eligible for inclusion if they had: (i) a confirmed diagnosis of unresectable stage IIIc to IV melanoma, according to the 2009 AJCC classification; (ii) a positive test for the BRAF V600 mutation in tumor biopsy; and (iii) measurable disease by physical examination or imaging studies, according to modified Response Evaluation Criteria in Solid Tumors (RECIST 1.1) [21]. The protocol for the study was approved by the ethics committee (reference 111/2010) and all patients signed written informed consent.

2.2. Study design

Dabrafenib or vemurafenib were administered orally according to the clinical trial and the expanded access program protocols respectively. Evaluation of tumor response was assessed by physical examination and imaging studies using RECIST 1.1 criteria, with the modification that we measured all lesions, and not only target lesions [21]. Best response was defined as the best objective response (stable disease, partial response or complete response) assessed between the first day of treatment to progression, death or last follow-up.

2.3. V600 mutation analysis

Before treatment, the presence of BRAF mutations was confirmed in tumor biopsies from all patients. DNA from tumor cells was isolated using the cobas DNA Sample Preparation Kit (Roche Molecular Systems, Pleasanton, California)[22]. BRAF V600 mutation was determined by real-time polymerase-chain-reaction assay with a kit according to

manufacturer's instructions (Cobas 4800 BRAF V600 Mutation Test, Roche Molecular Systems).

2.4. Sample collection

Blood samples were collected at baseline and sequentially at each visit. After cycle 8 blood samples were collected at the moment of evaluation response (Supplementary Figure 1). Blood samples were centrifuged immediately at 2000 rpm for 10 min and stored at -80ºC until analysis.

2.5. Serum assays

Analytical assays in serum samples were performed in samples corresponding to the dates of baseline, one month (FV), best response (BR) and progressive disease (PD), using commercial kits and following manufacturers' instructions. MIA was determined by a quantitative ELISA kit (Roche, Mannheim, Germany) and S100 was analyzed by an electrochemiluminiscence assay in a Modular E170 analyzer (Roche) [14]. LDH was analyzed using a kit from Roche on a Modular Analytics P800 analyzer (Roche). In this method [23], LDH catalyzes the conversion of pyruvate to L-lactate with the oxidation of NADH to NAD^+. The decrease in absorbance measured at 340 nm with time due to the consumption of NADH is directly proportional to the LDH activity. The optimal cut-off point for each melanoma marker was established by adapting the kit specifications to our laboratory, and were respectively S100=0.1 µg/L; MIA=9 µg/L; and LDH=436 U/L. We used these cut-off to define high and low levels of these tumor markers.

2.6. Statistical analysis

Results are expressed as median and 25th-75th percentile after determining their non-Gaussian distribution with the Kolmogorov-Smirnov and Shapiro-Wilks tests. The non-parametric Kruskal-Wallis and Mann-Whitney U-test tests were applied to compare the levels of the tumour markers. Wilcoxon test was performed to compare changes in serum concentrations of tumor markers during treatment. Progression-free survival and overall survival were respectively measured from the time of iBRAF treatment initiation to time of progression, death or last follow-up and they were analyzed by the Kaplan-Meier method and compared by the log-rank and Breslow tests. A two-tailed P-value ≤ 0.05 was considered to be statistically significant. Statistical analysis was performed with IBM SPSS 20 (Somers, NY, USA).

3 Results

3.1. Patient Characteristics

From April 2011 to April 2013 eighteen patients with melanoma stage IIIc-IV were enrolled in this study. All of them presented BRAF V600 mutation and received treatment with vemurafenib or dabrafenib. Patient characteristics at baseline are summarized in Table 1. The median tumor burden was 53 mm (Q1-Q3: 18-155 mm) and this value was used as the cut-off to classify patients with low and high tumor burden. The median follow up of the patients was 14±6.8 months. The median progression-free survival was 5 months and the response rate was 88.8% (16 of 18 patients), including 5 complete responses and 11 partial responses. At the moment of data analysis 15 of 18 (83.3%) patients had progressive disease.

3.2. Baseline serum S100 and MIA concentrations and LDH activities

Baseline MIA and S100 concentrations above the cut-off were observed respectively in 94.1% and 76.5% of the patients. Conversely, only 35.3% of the patients had LDH activities above the cut-off. The number of patients with high concentrations of MIA or S100 was statistically higher than those with high LDH activities (P=0.002 and P=0.016, respectively). However, none of the patients with high LDH activity had low tumor burden.

The basal median and interquartile range for MIA and S100 concentrations and LDH activities in the patients are shown in Table 2. Baseline concentrations of all the tumor markers correlated with tumor burden. Patients with low tumor burden also had lower median levels of LDH, MIA and S100 than patients with high tumor burden (median MIA: 13 µg/L *vs* 48 µg/L P<0.001; median S100: 0.13 µg/L *vs* 5.1 µg/L; P=0.001; median LDH: 348 *vs* 1169 U/L; P=0.008). All patients with MIA levels higher than 30 µg/L had a tumor burden higher than 53 mm.

3.3. S100, MIA and LDH during iBRAF therapy

Serum concentrations of MIA and S100 decreased, in some cases dramatically, at the moment of the best response and increased after during progression**.** MIA levels already decreased after one month of therapy to a median concentration of 9.5 µg/L (Q1-Q3: 6.9-11.1 µg/L; P=0.002 compared to baseline), similar to the levels observed at the moment of best response (median MIA: 10.5 µg/L; Q1-Q3: 7.6-11.5 µg/L; P=0.001 compared

with baseline). At progression, there was a significant increase in median concentration of MIA (median MIA: 13 µg/L; Q1-Q3: 9.6-27 µg/L), as compared with concentration at best response (P=0.002) (Figure 1A).

Similarly, S100 levels decreased significantly one month after treatment (median: 0.095 µg/L, Q1-Q3: 0.039-0.17 µg/L; P=0.004 related to baseline), similar to the levels observed at the moment of best response (median: 0.07 µg/L; Q1-Q3: 0.05-0.15 µg/L; P=0.002 related to baseline). S100 concentrations also increased significantly at progression (median S100: 0.2 µg/L; Q1-Q3: 0.05-0.8 µg/L), as compared with concentrations measured at best response (P=0.008).

However, median LDH activity did not decrease significantly compared to baseline at the first month of observation (P=0.333 related to baseline), but at the best response (Median LDH: 317 U/L; Q1-Q3: 270-351 U/L; P=0.015 related to baseline). Neither LDH increased significantly at progression (317 U/L; Q1-Q3: 287-952 U/L, P=0.610).

The evolution of MIA, S100 concentrations and LDH activities in patients with either low or high tumor burden are shown in Figure 1B. It can be observed in each group that there was a decrease in the serum concentrations of S100 or MIA during the response to the therapy. This decrease was significant in patients with either low or high tumor burden ($P<0.05$).

3.4. MIA and S100 as prognostic factors during treatment

We analyzed the utility of S100, MIA and LDH as prognostic factors at baseline, one month after starting the therapy, and at the moment of best response.

In relation of the type of response to the iBRAF treatment, patients that have partial response have significant higher basal MIA concentrations (median: 30 µg/L, Q1-Q3: 15-52 µg/L) than patients with complete response (median: 12 µg/L, Q1-Q3: 7-13 µg/L; P=0.026). Neither basal S100 concentrations nor LDH activities could discriminate between patients with partial response to those with complete response.

Using the cut-off previously indicated, none of the biomarkers at baseline have both sensitivity and specificity above 50% to predict progression of the disease. The best of them was LDH which was specific (100%) but with a low sensitivity (42.9%) to predict progression. However, at the first month, MIA had a sensitivity of 81.8 % and a specificity of 100% to predict progression of the disease, much better than LDH, whose sensitivity decreased to 12.5%, and S100 that only had a sensitivity of 58.3% and a specificity of 50%.

Before treatment, none of the tumor markers analyzed showed a significant association with progression-free survival, although there was a trend towards a lower median progression-free survival in patients with increased LDH activities (8 months *vs* 2 months, P=0.08). At the first month, serum MIA concentrations were already a prognostic factor, and those patients with MIA levels below the cut-off have a median progression-free survival longer than those with MIA levels above the cut-off (24 vs. 4 months, P=0.035) (Figure 2). At the moment of best response, LDH, S100 and MIA serum levels, were prognostic factors of treatment efficacy. Patient with high LDH activity had a median progression-free survival significatively poorer than patients with low LDH activities (2 and 13 months, respectively; P=0.042). The median progression-free survival in patients with low or high S100 concentrations were respectively 15 and 3 months (P=0.002). Patients with low MIA at best response also had a trend towards improved median progression-free survival, as compared with patients with high concentrations (8 months *vs* 4 months, P=0.07).

With regard to overall survival, at baseline only increased LDH activity were significantly associated with decreased overall survival (P=0.002). However, those patients with low MIA concentrations at first response tend to an improved overall survival over patients with high MIA levels, although it was not statistically significant (P=0.06).

4 Discussion

In this study, we report for the first time that MIA, S100 and LDH can be used to monitor response to iBRAF treatment. According to our data, elevated concentrations of LDH, S100 and MIA correlate with the tumor burden assessed by RECIST 1.1 criteria [21]. Previous studies have associated S100 and MIA with tumor load [14, 24, 25], but this is the first time that the association was established using the RECIST 1.1 criteria, which is the current standard for response evaluation in clinical studies [15]. The relationship was more notorious in the case of S100 and MIA, and MIA serum levels higher than 30 µg/L could be associated with high tumor load.

It has been shown that S100 and MIA have better sensitivity than LDH in stage IIIC and IV melanoma [13, 14]. The ESMO-guidelines indicate that serum S100 is the most accurate blood test to follow-up melanoma patients, as it has a higher specificity for progressive disease than LDH [26]. The elevated number of patients with advanced disease and high MIA and S100 concentrations indicated that their measurement before treatment would be of utility in the follow-up of the therapy. Certainly, one of the most

relevant functions of tumor markers is the capability to follow-up treatment efficacy. This is especially important in the case of treatment with iBRAF due to the biphasic response, which is associated in the short term with an important decrease in the tumor burden but it is followed by disease relapse [8, 10]. Low levels of MIA at the beginning of the treatment were associated with complete response. Very importantly, serum S100 and MIA concentrations reflect this type of response to the treatment with iBRAF, as concentrations decreased dramatically during the response and increased later during progression. This could be related with the association observed between tumoral mass and MIA and S100 serum concentrations, as we have mentioned before. MIA is a small secreted protein, while S100 is released as a result of cell damage that affects membrane integrity. S100 has a short half-life, so it can quickly reflect the tumor biology during iBRAF treatment [27].

With the rapidly increasing development of new therapies for melanoma, routine follow-up with imaging studies is becoming increasingly more expensive, and blood tests to evaluate the efficacy of the treatment could reduce the frequency and the cost of imaging studies [15]. S100 and MIA can be useful to follow-up melanoma patients with advanced disease treated with dacarbazine [28]. Also, changes in S100B concentrations are independent predictive factors for clinical outcome during treatment with interleukin-2 [29]. Recently, it was shown that serum S100 can be a useful marker to assess the efficacy of treatment with bevacizumab, an antibody that inhibits vascular endothelial growth factor A [30]. Also, measurement of S100B and LDH during treatment with vemurafenib indicates an initial response [31]. Although iBRAF can induce dramatic responses, most are short-lived, and the disease typically progresses within 5 to 7 months due to the development of multiple resistance mechanisms [10]. We have observed that patients with high LDH baseline levels are associated with poor prognosis when treated with iBRAF. This data agree with the classification in diverse clinical guides and trials of these patients in a more advanced disease stage [12, 32]. LDH and S100 predict independently disease outcome in melanoma patients with distant metastasis [33]. Also, these biomarkers show relevant prognostic information early during treatment. Already at the first month, those patients with high MIA levels showed a poorer prognostic of progression-free disease, while at the moment of best response, those patients with high S100 concentrations had worse prognosis than patients with low concentrations.

However, at baseline, neither S100 nor MIA are of utility in as predictor of progression-free survival [31].

One of the limitations of this study is the low number of patients analyzed. However, these patients were very strictly selected and treated prospectively within two different clinical protocols. Our results strongly suggest that MIA and S100 should be included in future studies of iBRAF in patients with melanoma.

4.1. Conclusions

In this prospective study we have demonstrated that i) baseline concentrations of MIA, S100 and LDH correlated with tumor burden using the RECIST 1.1 criteria; ii) serum concentrations of MIA and S100 changed with the course of the disease, and iii) S100 and MIA levels during response to treatment were of prognostic value, and those patients with high MIA or S100 levels showed a shorter progression-free disease. Our results strongly suggest that MIA and S100 should be included in the follow-up of BRAF inhibitor treatment in patients with melanoma.

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6 References

[1] Forsea AM, del Marmol V, de Vries E, Bailey EE, Geller AC. Melanoma incidence and mortality in Europe: new estimates, persistent disparities. Br J Dermatol 2012; 167:1124-1130.

[2] Jennings L, Murphy GM. Predicting outcome in melanoma: where are we now? Br J Dermatol 2009; 161:496-503.

[3] Curtin JA, Fridlyand J, Kageshita T, et al. Distinct Sets of Genetic Alterations in Melanoma. N Engl J Med 2005; 353:2135-2147.

[4] Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002; 417:949-954.

[5] Smalley KSM. A pivotal role for ERK in the oncogenic behaviour of malignant melanoma? Int J Cancer 2003; 104:527-532.

[6] Flaherty KT, Infante JR, Daud A, et al. Combined BRAF and MEK Inhibition in Melanoma with BRAF V600 Mutations. N Engl J Med 2012; 367:1694-1703.

[7] Hauschild A, Grob J-J, Demidov LV, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. The Lancet 2012; 380:358-365.
[8] Sosman JA, Kim KB, Schuchter L, et al. Survival in BRAF V600-Mutant Advanced Melanoma Treated with Vemurafenib. N Engl J Med 2012; 366:707-714.
[9] Chapman PB, Hauschild A, Robert C, et al. Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. N Engl J Med 2011; 364:2507-2516.
[10] Jang S, Atkins MB. Which drug, and when, for patients with BRAF-mutant melanoma? Lancet Oncol 2013; 14:e60-e69.
[11] Duffy MJ, Crown J. Companion Biomarkers: Paving the Pathway to Personalized Treatment for Cancer. Clin Chem 2013; 59:1447-1456.
[12] Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009; 27:6199-6206.
[13] Garbe C, Leiter U, Ellwanger U, et al. Diagnostic value and prognostic significance of protein S-100beta, melanoma-inhibitory activity, and tyrosinase/MART-1 reverse transcription-polymerase chain reaction in the follow-up of high-risk melanoma patients. Cancer 2003; 97:1737-1745.
[14] Diaz-Lagares A, Alegre E, Arroyo A, et al. Evaluation of multiple serum markers in advanced melanoma. Tumour Biol 2011; 32:1155-1161.
[15] Kluger HM, Hoyt K, Bacchiocchi A, et al. Plasma markers for identifying patients with metastatic melanoma. Clin Cancer Res 2011; 17:2417-2425.
[16] Blesch A, Bosserhoff A-K, Apfel R, et al. Cloning of a Novel Malignant Melanoma-derived Growth-Regulatory Protein, MIA. Cancer Res 1994; 54:5695-5701.
[17] Santamaria-Kisiel L, Rintala-Dempsey AC, Shaw GS. Calcium-dependent and -independent interactions of the S100 protein family. Biochem J 2006; 396:201-214.
[18] Nakajima T, Watanabe S, Sato Y, Kameya T, Shimosato Y, Ishihara K. Immunohistochemical demonstration of S100 protein in malignant melanoma and pigmented nevus, and its diagnostic application. Cancer 1982; 50:912-918.
[19] Deichmann M, Benner A, Bock M, et al. S100-Beta, melanoma-inhibiting activity, and lactate dehydrogenase discriminate progressive from nonprogressive American Joint Committee on Cancer stage IV melanoma. J Clin Oncol 1999; 17:1891-1896.

[20] Auge JM, Molina R, Filella X, et al. S-100B and MIA in Advanced Melanoma in Relation to Prognostic Factors. Anticancer Res 2005; 25:1779-1782.
[21] Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45:228-247.
[22] Anderson S, Bloom KJ, Vallera DU, et al. Multisite Analytic Performance Studies of a Real-Time Polymerase Chain Reaction Assay for the Detection of BRAF V600E Mutations in Formalin-Fixed, Paraffin-Embedded Tissue Specimens of Malignant Melanoma. Arch Pathol Lab Med 2012; 136:1385-1391.
[23] Deutsche Gesellschaft für klinische Chemie. Empfehlungen der deutschen Gesellschaft für Klinische Chemie (DGKC). Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. Z Klin Chem Klin Biochem 1972; 10:182-192.
[24] Harpio R, Einarsson R. S100 proteins as cancer biomarkers with focus on S100B in malignant melanoma. Clin Biochem 2004; 37:512-518.
[25] Bosserhoff AK, Kaufmann M, Kaluza B, et al. Melanoma-inhibiting activity, a novel serum marker for progression of malignant melanoma. Cancer Res 1997; 57:3149-3153.
[26] Dummer R, Hauschild A, Guggenheim M, Keilholz U, Pentheroudakis G, Group obotEGW. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23:vii86-vii91.
[27] Ghanem G, Loir B, Morandini R, et al. On the release and half-life of S100B protein in the peripheral blood of melanoma patients. Int J Cancer 2001; 94:586-590.
[28] Djukanovic D, Hofmann U, Sucker A, Rittgen W, Schadendorf D. Comparison of S100 protein and MIA protein as serum marker for malignant melanoma. Anticancer Res 2000; 20:2203-2207.
[29] Schmidt H, Sorensen BS, Nexo E, von der Maase H. S100beta protein in peripheral blood may predict progressive disease during interleukin-2 based immunotherapy in patients with metastatic melanoma. Melanoma Res 2004; 14:211-215.
[30] Kruijff S, Bastiaannet E, Brouwers AH, et al. Use of S-100B to Evaluate Therapy Effects during Bevacizumab Induction Treatment in AJCC Stage III Melanoma. Ann Surg Oncol 2012; 19:620-626.

[31] Abusaif S, Jradi Z, Held L, et al. S100B and lactate dehydrogenase as response and progression markers during treatment with vemurafenib in patients with advanced melanoma. Melanoma Res 2013; 23:396-401.
[32] Agarwala SS, Keilholz U, Gilles E, et al. LDH correlation with survival in advanced melanoma from two large, randomised trials (Oblimersen GM301 and EORTC 18951). Eur J Cancer 2009; 45:1807-1814.
[33] Weide B, Elsasser M, Buttner P, et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. Br J Cancer 2012; 107:422-428.

Figures

Figure 1A. Serum MIA (up), S100 (middle) concentrations and LDH (bottom) activities at baseline (Basal), first evaluation (FV), best response (BR) and progressive disease (PD). Comparison by Wilcoxon test.

Figure 1B. Evolution of the serum concentrations of MIA, S100 and LDH activities at baseline (Basal), first evaluation (FV), best response (BR) and progressive disease (PD) in patients receiving treatment with BRAF inhibitors and classified according to their tumor load. Solid lines correspond to median in each situation. Comparison by Wilcoxon test.

Figure 2. Kaplan-Meier plots representing progression-free survival for patients with advanced melanoma according to MIA and S100 above the cut-off at the moment of first evaluation of treatment (one month) and best response. Cut-off: MIA: 9 µg/L; S100: 0.1 µg/L.

Table 1. Patients baseline characteristics. ULN: Upper limit of the reference range.

Variable	**No (%)**
Age	
Mean years (SD)	51 (11.9)
Gender	
Male	13 (72.2)
Female	5 (27.8)
Stage	
Unresectable IIIc	1 (5.6)
M1a	5 (27.8)
M1b	1 (5.6)
M1c	11 (61.1)
Lactate dehydrogenase	
≤ ULN	11 (61.1)
>ULN	6 (33.3)
Primary site	
Cutaneous	13 (72.2)
Mucosal	1 (5.6)
Unknown	4 (22.2)

Table 2. Basal serum concentrations of MIA and S100 and LDH activities in the patients, and in relation with the tumor burden as measured with the RECIST 1.1 criteria (cut-off: 53 mm). *:P<0.05 related to low tumor burden.

	Median	**Q1-Q3**
MIA (µg/L)		
Total patients	16.8	11.9-48
Low tumor burden	13	10.7-15.5
High tumor burden	48	32.7-70.5*
S-100 (µg/L)		
Total patients	0.43	0.11-5.1
Low tumor burden	0.13	0.06-0.40
High tumor burden	5.1	0.76-17.69*
LDH (U/L)		
Total patients	382	303-1169
Low tumor burden	348	263-373
High tumor burden	1169	435-1553*