Positive Association Between Location of Melanoma, Ultraviolet Signature, Tumor Mutational Burden, and Response to Anti–PD-1 Therapy

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abstract

PURPOSE Emerging evidence suggests a correlation between the tumor mutational burden (TMB) and the response to programmed cell death-1 protein (PD-1) monotherapy across multiple cancer types. In skin cancers, as high TMB is mostly because of ultraviolet (UV) exposure, we hypothesized a correlation between the primary melanoma cutaneous location according to sun exposure and response to anti–PD-1 monotherapy.

METHODS The aim of this study was to analyze, in advanced melanoma, the relationship between TMB, locations according to sun exposure, and response to PD-1 inhibitors. We conducted a prospective multicentric analysis, by sequencing the most recent metastatic sample before PD-1 inhibitors using FoundationOne assay.

RESULTS One hundred two patients were included, with TMB available for 94 cases. In univariate and multivariate linear regression, TMB was significantly associated with sun-exposed areas of the primary melanoma location and with age (coefficients of the association with log-TMB: non-UV location, –1.05; chronic sun-exposed area, 1.12; P value for the location, < 10^-5; age, 0.021 per year, P value for age, .002). Molecular UV signature present on the metastatic site was associated with higher TMB (P = .003). Melanomas bearing a high TMB had a higher probability of response to PD-1 inhibitors compared with melanomas with a low TMB, with a dose-dependent effect following an exponential curve and a negative odds ratio of 0.40 (95% CI, 0.20 to 0.72, P = .004) between log-TMB and 6-month progression.

CONCLUSION Cumulative sun exposure related to skin location and molecular UV signature present on the metastatic site appear to be relevant biomarkers directly linked to TMB. Because TMB is not yet available to all for routine clinical use, the location of the primary melanoma in a sun-exposed area may play an important role in clinical decisions regarding therapeutic choice.

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INTRODUCTION Immune checkpoint inhibitors (ICls), including programmed cell death-1 protein (PD-1)/programmed cell death-ligand-1 and cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) inhibitors, have profoundly changed the prognosis of patients in multiple cancer types, including advanced melanoma.1,2 PD-1 inhibitors (pembrolizumab and nivolumab) have been approved by the US Food and Drug Administration for metastatic melanoma, and recently in the adjuvant setting in stage III melanoma.3,4 Although response rates (RR) are durable and remarkable, only approximately 35%-45% of patients benefit from these costly drugs, but no positive or negative predictive biomarkers are available to date to guide clinicians in the choice of specific therapies.1,2,5 Tumor mutational burden (TMB, also known as mutation load) has emerged as a seemingly promising predictive biomarker for ICls and is likely to be incorporated into future treatment algorithms for these agents: It reflects the measure of the number of somatic protein-coding base substitution and insertion or deletion mutations occurring in a tumor specimen.6 Several prior studies have reported a positive predictive value on the efficacy of ICls in high TMB tumors across multiple cancers, supporting the fact that neoantigen burden

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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influences sensitivity to ICIs, but this measure is not available worldwide for routine clinical use yet.15–12

In this current study, we analyzed clinical, histologic, and mutational data from patients with advanced melanoma to analyze whether TMB is associated with primary melanoma sun-exposed location, molecular ultraviolet (UV) signature, and response to PD-1 inhibitors.

METHODS

Study Design and Participants
A multicenter prospective study was performed in six academic institutions, hospitals, and cancer centers in France. Patients with advanced melanoma, who underwent a somatic comprehensive genomic FoundationOne assay9 (Foundation Medicine, Cambridge, MA) were prospectively included. Patients’ clinicopathologic characteristics, tumor genomics results, and outcome data were collected. According to the location of the known primary melanoma, we allocated each patient to a group of sun-exposure pattern including chronically sun-exposed area such as head and neck, intermittently sun-exposed area such as trunk, arms, and legs, and sun-protected areas such as feet, soles, toes, genitals, mucosal, and uveal areas. Progression and tumor response to PD-1 inhibitors were assessed according to the RECIST guidelines version 1.1.13 Informed consent was obtained from all patients. The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines.

Next-Generation Sequencing–Based Assessment of Genomic Characteristics
TMB, UV signature, and microsatellite instability status were analyzed on the most recent metastatic sample of the patient available before PD-1 inhibitors. DNA was extracted from formalin-fixed, paraffin-embedded tissue sections to perform comprehensive genomic profiling with FoundationOne Assay in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited, New York State–approved laboratory (Foundation Medicine).14 TMB was calculated by counting the number of synonymous and nonsynonymous mutations (muts) on up to 1.2 megabases (Mb) of sequenced DNA, according to an algorithm that extrapolated to the genome as a whole (muts/Mb).9 UV signature was not analyzed in samples with < 10 assessable alterations.

Statistical Analysis
Multiple linear regression models were fit to estimate the TMB according to a set of characteristics of the melanoma and the subject, including the location according to sun exposure. To make the distribution of the TMB consistent with a normal distribution, the TMB was log-transformed (log-TMB). Two patients were excluded because of a TMB equal to zero mut/Mb. Univariate logistic regression analyses were performed to estimate the association between the 3-month and the 6-month progression and the log-TMB. Multivariate analysis was conducted to estimate the association between the 6-month progression and the log-TMB after adjustment for potential confounders. We added a sensitivity analysis excluding mucosal and uveal melanomas or adding combination of anti–CTLA-4 and anti–PD-1. TMB was compared between progressors and nonprogressors using a Mann-Whitney U test. For all statistical analyses, the type 1 error has been set at 5%. All data were analyzed using Rstudio Version 1.0.136 (Rstudio, Inc, Boston, MA).

RESULTS

Patients’ Characteristics
One hundred two patients were prospectively included from six French treatment centers: University Hospital of Bordeaux (n = 32), Gustave Roussy Institute (n = 21), University Hospital of Rennes (n = 18), University Hospital of Clermont-Ferrand (n = 17), Saint-Louis Hospital-University of Paris (n = 11), and University of Lille (n = 3), between
October 2017 and June 2019 (Table 1). Median age at diagnosis was 59.3 years (standard deviation [SD] 14.4), with 57% of patients being male. Most melanomas were superficial spreading melanomas (n = 37; 36%), followed by unknown primary (n = 20; 19.6%) and nodular types (n = 17; 16.8%). Twelve (11.8%) were acral lentiginous melanomas, nine (8.8%) mucosal, five (5%) uveal, one naevocytoid, and one desmoplastic. The v-raf murine sarcoma viral oncogene homolog B1 (BRAF) V600E alterations were detected in 34 cases (34%). 80 patients were treated with anti–PD-1 monotherapy, which was the first-line treatment for 72.5% of them (n = 58). TMB was assessed in 94 cases (DNA extraction failure in 8 cases). Median TMB was 12.4 muts/Mb (SD, 12.6; range, 0-60 muts/Mb). Its distribution is depicted in the Data Supplement. Corresponding metastatic sites are presented in the Data Supplement. All melanomas were considered microsatellite instability–stable.

| TABLE 1. Baseline Characteristics of Study Patients |
|--------------------------------------------------|
| Characteristics | All Patients (N = 102) |
| Age, years | Mean (SD) 59.3 (14.4) |
| Sex, No. (%) | Men 57 (55.9) |
| | Women 45 (44.1) |
| ECOG PS, No. (%) | 0 50 (49) |
| | 1 26 (25.5) |
| | 2 3 (2.9) |
| LDH, No. (%) | Elevated 16 (15.7) |
| Metastatic sites (may have multiple), No. (%) | Brain 35 (34.3) |
| | Lung 36 (35.3) |
| | Liver 30 (29.4) |
| | Skin 50 (49) |
| Evidence of BRAF V600E mutation, No. (%) | 35 (34.3) |
| Tumor type, No. (%) | SSM 37 (36.3) |
| | Unknown 20 (19.6) |
| | Nodular 17 (16.7) |
| | ALM 12 (11.8) |
| | MM 9 (8.8) |
| | UM 5 (4.9) |
| | Others 2 (2.0) |
| Breslow | Mean (SD) 17.8 (15.3) |
| Ulceration, No. (%) | Yes 38 (37.3) |
| Sun exposure, No. (%) | Chronic (face, neck) 6 (5.9) |
| | Intermittent (trunk, arms, legs) 50 (49) |
| | Sun-protected areas 34 (33.3) |
| Prior lines of therapy before PD-1 inhibitors, No. (%) | 0 76 (74.5) |
| | 1 22 (21.6) |
| | > 1 4 (3.9) |

Abbreviations: ALM, acral lentiginous melanoma; BRAF, v-raf murine sarcoma viral oncogene homolog B1; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; MM, mucosal melanoma; PD-1, programmed cell death-1 protein; SD, standard deviation; SSM, superficial spreading melanoma; UM, uveal melanoma.

High TMB Is Associated With Older Age
Advanced-age patients had higher TMB. The coefficient of age with log-TMB in a multivariate regression model was 0.021 (95% CI, 0.008 to 0.034; P = .002; Table 2). Sex was not associated with TMB (P = .21). BRAF V600E alterations were not statistically correlated with TMB (P = .38).

High TMB Is Associated With Sun-Exposed Location
The distribution of TMB according to the melanoma location is shown in Figure 1. The mean TMB was 4 muts/Mb (SD, 2.1) for sun-protected areas, 13.6 muts/Mb (SD, 10.9) for intermittently sun-exposed areas, and 37.2 muts/Mb (SD, 16.0) for chronically sun-exposed areas. In univariate analysis, log-TMB was associated with sun-protected areas with a −0.95 coefficient (95% CI, −1.32 to −0.57; P < 10−5) and chronically sun-exposed areas with a 1.28 coefficient (95% CI, 0.59 to 1.98; P < 10−5). After adjustment on age, sex, and BRAF mutational status in a multivariate analysis, log-TMB was significantly associated with location according to sun exposure (sun-protected areas: coefficient = −1.05, 95% CI, −1.43 to −0.67; chronically sun-exposed areas: Table 2. Clinical Determinants of TMB in Advanced Melanoma in Multivariate Analysis |
| Characteristics | Coefficient* (95% CI) | P |
| Location (ref = intermittent sun-exposed area) | < 10−8 |
| Sun-protected | −1.05 (−1.43 to −0.67) |
| Chronic sun-exposed | 1.12 (0.45 to 1.79) |
| Sex (men) | 0.22 (−0.13 to 0.56) .21 |
| Age (per year) | 0.021 (0.008 to 0.034) .002 |
| BRAF V600E mutation | 0.13 (−0.25 to 0.52) .49 |

Abbreviations: BRAF, v-raf murine sarcoma viral oncogene homolog B1; TMB, tumor mutational burden.
*Coefficient of the multivariate linear regression between log-TMB and clinical characteristics of the melanoma and the patient.
†Including trunk (n = 23), arm (n = 6), and leg (n = 19).
‡Including mucosal (n = 7), uveal (n = 4), foot (n = 7), toes (n = 7), sole (n = 4), and genital (n = 1).
§Continuous variable.
When a clinical picture of the melanoma scar was available (Fig 2), we observed various signs of past UV exposure, presumably involved in melanoma genesis: pigmentation heterogeneities, sun-induced freckling (lentigines), wrinkles, sagging, and presence of actinic keratoses. Notably, the skin surrounding low TMB melanoma scars were typically devoid of sun damage (Figures 2A and 2B vs 2C and 2D).

**TMB Is Associated With Molecular UV Signature**

The tumor DNA canonical mutational signature secondary to UV-exposure was available for 30 melanomas whose TMB reached at least 10 muts/Mb, as shown in the Data Supplement. UV signature was associated with higher log-TMB (coefficient 1.29, 95% CI, 0.48 to 2.10, \( P = .003 \)).

**Negative Association Between TMB and Progression**

For response to PD-1 inhibitors analysis, 22 patients were excluded. Nine did not receive any systemic treatment, two received PD-1 inhibitors as an adjuvant treatment, and 11 received a combination of CTLA-4 and PD-1 inhibitors. Low TMB was strongly associated with progression to PD-1 inhibitors at 3 and 6 months in cutaneous melanomas (3-month mean TMB from progressors vs nonprogressors: 10.2 vs 18.32 muts/Mb, \( P = .02 \); 6-month mean TMB from progressors vs nonprogressors: 10.6 vs 19.6 muts/Mb, \( P = .01 \), Fig 3). An inversely exponential relationship was observed when plotting the estimated association between the 6-month progression and the TMB against the centers of the quartiles of the TMB (Data Supplement).
A unit increase in log-TMB, ie, a multiplication of TMB by 2.7 (because \( \exp(1) \approx 2.7 \)), changed the odds of 3-month progression by 0.47 (95% CI, 0.26 to 0.79, \( P = .007 \)) and 6-month progression by 0.45 (95% CI, 0.24 to 0.77, \( P = .006 \)) in a univariate analysis. These results were confirmed by multivariate analysis, after adjusting for sex, age, BRAF mutation, and treatment line, with a significant negative association of log-TMB with 6-month progression (OR = 0.40, 95% CI, 0.20 to 0.72, \( P = .004 \)), as shown in Table 3. When excluding uveal and mucosal melanoma from the nonexposed group, we found similar results (OR = 0.36, 95% CI, 0.16 to 0.69, \( P = .005 \), Data Supplement). When including patients receiving a combination of CTLA-4 and PD-1 inhibitors, the negative association was even stronger (\( P = .0009 \)). A direct association between 6-month progression and location according to sun exposure was then analyzed. The association did not reach significance, with an OR of 0.21, 95% CI, 0.02 to 1.38 for sun-protected sites, and 1.15, 95% CI, 0.37 to 3.73 for UV-chronic areas compared with intermittently sun-exposed areas.

### DISCUSSION

Our study identifies a strong association between TMB and cumulative sun exposure related to skin location in advanced melanoma tumors. The TMB in cutaneous melanoma is high compared with other nonmelanoma tumors because of the mutagenic effects of UV exposure.\(^{16-19}\) We found, to our knowledge, for the first time that both the UV molecular signature present on the metastatic site and the sun exposure on the primary location of the melanoma were associated with higher TMB. This suggests that melanoma metastases carry the UV molecular signature present at their primary site of origin, which is a matter of importance. Similarly, BRAF or neuroblastoma RAS viral oncogene homolog mutations are preserved between primary and metastatic melanoma, explaining why the initial melanoma is used for BRAF analysis before treatment.\(^ {20} \) Therefore, one may hypothesize that when TMB measure is not available, the molecular UV signature on the primary melanoma or various signs of past UV exposure have to be considered.

We also analyzed TMB according to histologic subtypes. Although acral, uveal, or mucosal melanomas had different genetic alterations, and tumor behaviors, TMB values were similar between subgroups varying between 0 and 9 muts/Mb. These results are in accordance with those reported in the literature.\(^ {21-24} \) as uveal melanomas had the lowest TMB and the poorest RR to PD-1 inhibitors (overall survival 3.6% and median progression-free survival 2.6 months).\(^ {25} \) In acral and mucosal melanomas, where the RR is lower compared with other melanoma subtypes,\(^ {26} \) higher degrees of aneuploidy and lower numbers of mutagenic drivers may allow a biologic situation where TMB does not need to be

![FIG 2. TMB and clinical signs of sun exposure. Photographs of the observable clinical signs of sun damage surrounding the scars of primary melanoma excision of four patients: (A) melanoma of the external side of the right foot; TMB 2 muts/Mb DNA; progression disease at 6-month follow-up, (B) melanoma of posterior side of the right calf; TMB 1 mut/Mb DNA; progression disease at 6-month follow-up, (C) melanoma of the upper back; TMB 45 muts/Mb; complete response at 6-month follow-up, and (D) melanoma of the posterior part of the neck; TMB 44 muts/Mb DNA; complete response at 6-month follow-up. Absence of sun-damaged skin (*). Sun-induced freckling (black arrow). muts/Mb, mutations per megabases; TMB, tumor mutational burden.](image)
high for tumorigenesis and propagation.27 In our study, TMB was higher in chronically sun-exposed areas compared with intermittently sun-exposed sites. Recent works showed that desmoplastic melanoma, known to be a UV-related melanoma, had a high mutational burden.

**TABLE 3.** Association of Characteristics of Patient and Tumor With 6-Month Progression in Patients With Advanced Melanoma Receiving Anti–PD-1 Monotherapy in Multivariate Analysis

| Patients and Tumors’ Characteristics | 6-Month Progression | \( \text{OR (95% CI)} \) | \( P \) |
|--------------------------------------|---------------------|-----------------|-----|
| Log-TMB* (n = 71)                    |                     |                 |     |
| Sex (men)                            | 0.61 (0.20 to 1.82) | .38             |     |
| Age (per year)*                     | 0.95 (0.88 to 1.01) | .14             |     |
| BRAF V600E mutation                 | 2.32 (0.59 to 11.12) | .25             |     |
| Treatment line (second or higher)    | 0.32 (0.07 to 1.31) | .12             |     |

Abbreviations: BRAF, v-raf murine sarcoma viral oncogene homolog B1; PD-1, programmed cell death-1 protein; TMB, tumor mutational burden.

*Continuous variable.
optimizing the choice of first-line treatment, which is currently restricted to administration mode (oral vs intravenous delivery) or side-effect preferences. This predictive biomarker might even one day avoid wide re-excisions in patients with thick melanomas who are predicted long-term responders to adjuvant or neoadjuvant anti–PD-1 therapy.

In conclusion, we showed a specific and significant relationship between the location of the melanoma according to sun exposure and TMB, turning the location into an easy-to-use proxy of the TMB criterion. Furthermore, our study suggests that melanomas bearing a high TMB have a higher probability of response to immunotherapy compared with melanomas with a low TMB, with a dose-dependent effect following an exponential curve. Although it remains to be confirmed on larger settings, clinical criteria such as signs of sun exposure around the initial scar, or UV molecular signature, may be valuable for clinical decision making in conjunction with TMB assessment.

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REFERENCES

1. Hodi FS, Chiarion-Sileni V, Gonzalez R, et al: Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol 19:1480-1492, 2018
2. Robert C, Ribas A, Schachter J, et al: 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 20:1299-1301, 2019
3. Weber J, Mandala M, Del Vecchio M, et al: Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. N Engl J Med 377:1824-1835, 2017
4. Eggermont AMM, Blank CU, Mandala M, et al: Adjuvant pembrolizumab versus placebo in resected stage III melanoma. N Engl J Med 378:1789-1801, 2018
5. Larkin J, Chiarion-Sileni V, Gonzalez R, et al: Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 381:1535-1546, 2019
6. Snyder A, Makarov V, Merghoub T, et al: Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 371:2189-2199, 2014
7. Chalmers ZR, Fabrizio D, Connelly CF, et al: Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 9:15, 2017
8. Riaz N, Havel JJ, Makarov V, et al: Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell 171:934-949.e15, 2017
9. Hugo W, Zaretsky JM, Sun L, et al: Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. Cell 165:35-44, 2016
10. McGranahan N, Furness AJS, Rosenthal R, et al: Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 349:1483-1489, 2015
11. Zehir A, Benayed R, Shah RH, et al: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 23:703-713, 2017
12. BBS, Novartis, Pfizer
13. Eroglu Z, Zaretsky JM, Hu-Lieskovan S, et al: High response rate to PD-1 blockade in desmoplastic melanomas. Nature 553:347-350, 2018
14. Wu Y, Xu J, Du C, et al: The predictive value of tumor mutational burden on efficacy of immune checkpoint inhibitors in cancers: A systematic review and meta-analysis. Front Oncol 9:1161, 2019
15. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45:228-247, 2009
16. Frampton GM, Fichtenholtz A, Otto GA, et al: Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 31:1023-1031, 2013
17. Zehir A, Benayed R, Shah RH, et al: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 23:703-713, 2017
18. Matincentre I, Campbell PJ: Somatic mutation in cancer and normal cells. Science 349:1483-1489, 2015
19. Alexanderov LB, Nik-Zainal S, Wedge DC, et al: Signatures of mutational processes in human cancer. Nature 500:415-421, 2013
20. Hodis E, Watson IR, Kryukov GV, et al: A landscape of driver mutations in melanoma. Cell 150:251-263, 2012
21. Nagore E, Roche K, Budden T, et al: Chronic UV damage of the stroma improves melanoma survival. Cancer Res 79, 2019 (13 suppl; abstr 2022)
22. Boursault L, Haddad V, Vergier B, et al: Tumor homogeneity between primary and metastatic sites for BRAF status in metastatic melanoma determined by immunohistochemical and molecular testing. PLoS One 8:e70826, 2013
23. Kim, Zhang B, Kong BY, et al: Distinct genomic features in a retrospective cohort of mucosal, acral and vulvovaginal melanomas. J Am Acad Dermatol 10.1016/j.jaad.2019.07.017 [epub ahead of print on July 12, 2019]
24. Hayward NK, Wilmott JS, Waddell N, et al: Whole-genome landscapes of major melanoma subtypes. Nature 545:175-180, 2017

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23. Furney SJ, Turajlic S, Stamp G, et al: Genome sequencing of mucosal melanomas reveals that they are driven by distinct mechanisms from cutaneous melanoma. J Pathol 230:261-269, 2013
24. Furney SJ, Turajlic S, Stamp G, et al: The mutational burden of acral melanoma revealed by whole-genome sequencing and comparative analysis. Pigment Cell Melanoma Res 27:835-838, 2014
25. Algazi AP, Tsai KK, Shoushtari AN, et al: Clinical outcomes in metastatic uveal melanoma treated with PD-1 and PD-L1 antibodies. Cancer 122:3344-3353, 2016
26. Mignard C, Deschamps Huvier A, Gillibert A, et al: Efficacy of immunotherapy in patients with metastatic mucosal or uveal melanoma. J Oncol 2018:1908065, 2018
27. Turajlic S, Furney SJ, Lambros MB, et al: Whole genome sequencing of matched primary and metastatic acral melanomas. Genome Res 22:196-207, 2012
28. Shain AH, Garrido M, Botton T, et al: Exome sequencing of desmoplastic melanoma identifies recurrent NFKBIE promoter mutations and diverse activating mutations in the MAPK pathway. Nat Genet 47:1194-1199, 2015
29. Boussemart L, Johnson A, Schrock AB, et al: Tumor mutational burden and response to programmed cell death protein 1 inhibitors in a case series of patients with metastatic desmoplastic melanoma. J Am Acad Dermatol 80:1780-1782, 2019
30. Da Silva IP, Wang KYX, Wilmott JS, et al: Distinct molecular profiles and immunotherapy treatment outcomes of V600E and V600K BRAF-mutant melanoma. Clin Cancer Res 25:1272-1279, 2019
31. Russo D, FPoizeau, MDinulescu et al. Skin photoaging around the site of occurrence of primary melanoma as a clinical predictive biomarker of response to PD-1 inhibitors. Ann Oncol 31, 2020 (suppl 4; abstr 1133P)
32. Russo D, S Dalie, O Deneure, et al. Differential gradients of efficacy of immunotherapy according to the sun-exposure pattern of the site of occurrence of primary melanoma: A multicenter prospective cohort study (MELBASE). J Clin Oncol 39, 2020 (suppl 15; abstr e21546)
33. Goodman AM, Kato S, Bazhenova L, et al: Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 16:2598-2608, 2017
34. Lee JS, Ruppin E: Multomics prediction of response rates to therapies to inhibit programmed cell death 1 and programmed cell death 1 ligand 1. JAMA Oncol 5:1614-1618, 2019
35. Cristescu R, Mogg R, Ayers M, et al: Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science 362:eaar3593, 2018
36. Riaz N, Morris L, Havel JJ, et al: The role of neoantigens in response to immune checkpoint blockade. Int Immunol 28:411-419, 2016
37. Fischner A, Battke F, Hadaschik D, et al: Tumor mutation burden and circulating tumor DNA in combined CTLA-4 and PD-1 antibody therapy in metastatic melanoma-results of a prospective biomarker study. J Immunother Cancer 7:180, 2019
38. Samstein RM, Lee CH, Shoushtari AN, et al: Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 51:202-206, 2019
39. Addeo A, Banna GL, Weiss GJ: Tumor mutation burden—From hopes to doubts. JAMA Oncol 5:934-935, 2019
40. Addeo A, Banna GL, Weiss GJ: Tumor mutation burden-From doubts to concerns-In reply. JAMA Oncol 5:1809, 2019
41. Parikh K, Huether R, White K, et al: Tumor mutational burden from tumor-only sequencing compared with germline subtraction from paired tumor and normal specimens. JAMA Netw Open 3:e200202, 2020
42. Yarchoan M, Hopkins A, Jaffee EM: Tumor mutational burden and response rate to PD-1 inhibition. N Engl J Med 377:2500-2501, 2017
43. Offin M, Rizvi H, Tenet M, et al: Tumor mutation burden and efficacy of EGFR-tyrosine kinase inhibitors in patients with EGFR-mutant lung cancers. Clin Cancer Res 25:1063-1069, 2019