18. Fayers PM, Aaronson NK, Bjordal K et al. The EORTC QLQ-C30 Scoring Manual, 3rd edition. Brussels: European Organization for Research and Treatment of Cancer 2001.
19. Pinto AC, Ferreira-Santos F, Lago LD et al. Information perception, wishes, and satisfaction in ambulatory cancer patients under active treatment: patient-reported outcomes with QLQ-INFO25. Ecancermedicalscience 2014; 8: 425.
20. Yun YH, Lee MK, Park S et al. Use of a decision aid to help caregivers discuss terminal disease status with a family member with cancer: a randomized controlled trial. J Clin Oncol 2011; 29: 4811–4819.
21. O'Connor A. User Manual—Decisional Conflict Scale. 1993 [updated 2010].
22. Jung KW, Won YJ, Kong HJ et al. Prediction of cancer incidence and mortality in Korea, 2013. Cancer Res Treat 2013; 45: 15–21.
23. Lee YM, Francis K, Walker J, Lee SM. What are the information needs of Chinese breast cancer patients receiving chemotherapy? Eur J Oncol Nurs 2004; 8: 224–233.
24. Repetto L, Piselli P, Raffaele M et al. Communicating cancer diagnosis and prognosis: when the target is the elderly patient—a GIOGer study. Eur J Cancer 2009; 45: 374–383.
25. Fielding R, Hung J. Preferences for information and involvement in decisions during cancer care among a Hong Kong Chinese population. Psychooncology 1996; 5: 321–329.
26. Karani D, Wiltshaw E. How well informed? Cancer Nurs 1986; 9: 238–242.
27. Darwish-Yassine M, Berenji M, Wing D et al. Evaluating long-term patient-centered outcomes following prostate cancer treatment: findings from the Michigan Prostate Cancer Survivor study. J Cancer Surviv 2014; 8: 121–130.
28. Jung K-W, Won Y-J, Kong H-J et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2011. Cancer Res Treat 2014; 46: 109.
29. Ahn HS, Kim HJ, Welch HG. Korea's thyroid-cancer 'epidemic': screening and overdiagnosis. N Engl J Med 2014; 371: 1765–1767.
30. Ahn HY, Park YJ. Incidence and clinical characteristics of thyroid cancer in Korea. Korean J Med 2009; 77: 537–542.
31. Rains SA. Health information seeking and the World Wide Web: an uncertainty management perspective. J Health Commun 2014; 19: 1296–1307.
32. Goh AC, Kowalkowski MA, Bailey DE, Jr et al. Perception of cancer and inconsistency in medical information are associated with decisional conflict: a pilot study of men with prostate cancer who undergo active surveillance. BJU Int 2012; 110: E50–E56.
33. Mitchell AJ, Selmes T. Why don't patients take their medicine? Reasons and solutions in psychiatry. Adv Psychiatr Treat 2007; 13: 336–346.
34. Martin LR, Williams SL, Haskard KB, Dimatteo MR. The challenge of patient adherence. Ther Clin Risk Manag 2005; 1: 189–199.

Annals of Oncology 26: 1980–1987, 2015
doi:10.1093/annonc/mdv255
Published online 2 June 2015

The status of PD-L1 and tumor-infiltrating immune cells predict resistance and poor prognosis in BRAFi-treated melanoma patients harboring mutant BRAF^{V600}

D. Massi[1], D. Brusa[2], B. Merelli[3], C. Falcone[4], G. Xue[5], A. Carobbio[5], R. Nassini[6], G. Baroni[1], E. Tamborini[7], L. Cattaneo[8], V. Audrito[2], S. Deaglio[2,9] & M. Mandalà[3]*

[1]Division of Pathological Anatomy, Department of Surgery and Translational Medicine, University of Florence, Florence; [2]Human Genetics Foundation (HuGeF), Turin; [3]Unit of Medical Oncology, Department of Oncology and Hematology, Papa Giovanni XXIII Hospital, Bergamo; [4]Research Foundation, Papa Giovanni XXIII Hospital, Bergamo, Italy; [5]Department of Biomedicine, University Hospital of Basel, Basel, Switzerland; [6]Unit of Clinical Pharmacology and Oncology, Department of Health Sciences, University of Florence, Florence; [7]Experimental Molecular Pathology, Department of Pathology, National Cancer Institute, Milan; [8]Division of Pathological Anatomy, Papa Giovanni XXIII Hospital, Bergamo; [9]Department of Medical Sciences, University of Turin, Turin, Italy

Received 9 February 2015; revised 6 May 2015; accepted 19 May 2015

Background: BRAF inhibitors (BRAFi) improve survival in metastatic melanoma patients (MMP) but the duration of clinical benefit is limited by development of drug resistance. Here, we investigated whether the expression of programmed death-ligand 1 (PD-L1) and the density of tumor-infiltrating mononuclear cells (TIMC) predict the occurrence of resistance, hence affecting the clinical outcome in BRAFi-treated MMP.

Methods: PD-L1 expression (cutoff 5%) was analyzed by immunohistochemistry with two different antibodies in BRAF^{V600}-mutated formalin-fixed and paraffin-embedded samples from 80 consecutive MMP treated with BRAFi at a single institution. TIMC were evaluated by conventional hematoxylin and eosin staining.

Results: Forty-six and 34 patients received vemurafenib and dabrafenib, respectively. Membranous expression of PD-L1 was detected in 28/80 (35%) of patients. At multivariate analysis, absence of tumoral PD-L1 staining [odd ratio (OR) 10.8, 95% confidence interval (CI) 2.7–43.3, $P < 0.001$] and the presence of TIMC (OR 6.5, 95% CI 1.7–24.3, $P < 0.005$)

*Correspondence to: Dr Mario Mandalà, Unit of Medical Oncology, Department of Oncology and Haematology, Papa Giovanni XXIII Cancer Center Hospital, Piazza OMS 1, Bergamo 24100, Italy. Tel: +39-035-2673687; Fax: +39-035-2674985; E-mail: mariomandala@tin.it

were associated with a better response to treatment. Median progression-free survival (PFS) and overall survival were 10 and 15 months, respectively. By multivariate assessment, PD-L1 expression [hazard ratio (HR) 4.3, 95% CI 2.1–8.7, $P < 0.0001$] and absence of TIMC (HR 2.5, 95% CI 1.4–4.7, $P < 0.002$) correlated with shorter PFS. PD-L1 overexpression (HR 6.2, 95% CI 2.8–14.2, $P < 0.0001$) and absence of TIMC (HR 3.1, 95% CI 1.5–6.5, $P < 0.002$) were independent prognostic factors for melanoma-specific survival.

Conclusion: Our results provide the first proof-of-principle evidence for the predictive and prognostic relevance of PD-L1 immunohistochemical expression and density of immune cell infiltration in $BRAF^{V600}$-mutated MMP treated with BRAFi.

Key words: melanoma, PD-L1, immune cell infiltration, BRAF inhibitors, resistance, prognosis

introduction

At the disseminated stage, melanoma is an incurable disease. For several years, according to the results of nonrandomized clinical trials and meta-analyses, the standard of care of metastatic melanoma patients (MMP) was limited to the administration of a single cytotoxic agent, such as dacarbazine, temozolomide or fotemustine [1].

The understanding of the genetic heterogeneity underlying melanoma has revolutionized treatment options for MMP. The discovery that ~40%–50% MMP harbor BRAF-activating mutations, predominantly at codon 600, has led to the unprecedented identification of a truly actionable molecular target, which eventually resulted in the development of BRAF inhibitors (BRAFi). Seminal clinical trials have shown that treatment of MMP with two different BRAFi (vemurafenib, dabrafenib) is associated with improved response rate (RR), progression-free survival (PFS) and overall survival (OS) compared with conventional chemotherapy [2, 3]. However, while clinical responses to BRAFi may be dramatic, with some patients maintaining remission for several months or years, the median duration of response is between 6 and 7 months [2–4].

In addition to their established molecular mechanism of action, growing evidence suggests that the therapeutic efficacy of BRAFi relies on additional factors that affect the tumor–host interactions, including the enhancement of melanoma antigen expression and the increase in immune response against tumor cells [5]. Consistently, preclinical data show that oncogenic BRAF contributes to immune evasion, and that targeting this mutation may increase the melanoma immunogenicity [6]. Within the first 2 weeks of therapy, the expression of immunomodulatory molecules on the tumor cell surface, such as programmed death-ligand 1 (PD-L1 or B7-H1) and programmed death-1 (PD-1) in T lymphocytes, are increased [5]. Data *in vitro* or from animal models propose PD-L1 as a potential mechanism that favors BRAFi resistance through the modulation of host immune responses [7]. However, demonstration of this hypothesis in the clinical setting is lacking.

Recently, we showed that PD-L1 expression is increased in metastatic melanomas when compared with primary lesions and that PD-L1 expression behaves as a negative prognostic factor in MMP [8]. However, our retrospective study had two potential limitations: (i) the patient cohort, being treated over a wide time span (20 years), was heterogeneous in terms of treatment and (ii) PD-L1 immunohistochemical (IHC) evaluation was carried out in both primary and metastatic tissue samples.

With regards to the PD-L1 expression in the context of melanoma, four distinct groups of tumors were identified and described as having the presence of both PD-L1 and tumor-infiltrating immune cells (TIMC), presence of TIMC without PD-L1, PD-L1 expression without TIMC or absence of both TIMC and PD-L1 expression [9]. These findings raise important questions regarding the function and specificity of TIMC in $PD\text{-}L1^{-}$ tumors and the biological basis for tumors lacking TIMC. In the latter group, it is not clear whether the lack of T-cell infiltration reflects the absence of tumor antigen-specific responses or an as-yet unidentified process that excludes TIMC from the microenvironment.

In the present study, we have evaluated, in a homogeneous series of MMP treated with BRAFi, the association of tumoral PD-L1 IHC expression and the density of TIMC with RR, PFS and OS. Results provide the first proof-of-principle clinical evidence of the predictive and prognostic relevance of PD-L1 IHC expression and density of immune cell infiltration in $BRAF^{V600}$-mutated MMP receiving BRAFi.

materials and methods

patients

Cohort characteristics are reported in Supplementary Materials and Methods (SMM).

tissue samples

Detailed protocols are reported in SMM.

DNA extraction from FFPE tissues and BRAF mutation detection

Detailed protocols are reported in SMM.

immunohistochemistry

Immunohistochemistry was carried out on 4-μm-thick sections with two antibodies: the murine anti-human B7-H1 mAb (clone 5H1) with a concentration of 2 μg/ml according to a previously described protocol [10, 11], with slight modification [8], and the rabbit mAb clone E1L3N (dilution 1:50). Further detailed protocols are reported in SMM.

case evaluation

Detailed protocols are reported in SMM.

statistical analysis

All melanoma patients satisfying eligibility criteria and treated with BRAFi were considered for analysis. RR was defined as the proportion of patients with complete response or partial response. PFS was defined as the time from the beginning of BRAFi to first appearance of progressive disease or death for any cause; patients known to be alive and without progressive

disease at the time of analysis were censored at their last available follow-up assessment. PFS and ORR were based on RECIST, v1.1 [12]. OS was defined as the time from the beginning of BRAFi to the date of death from any cause or the date of the last follow-up.

Additional statistical methods are delineated in SSM.

results

patients and treatments

Demographic and clinical characteristics of the cohort are summarized in supplementary Table S1, available at *Annals of Oncology* online. Just over half (53%) were males and the median age was 56 years, ranging from 21 to 86 years. All patients had metastatic disease, over half with M1C disease [56% (44/80)] according to AJCC VII edition. Forty-six patients (58%) received vemurafenib, and the remaining cases received dabrafenib. Three patients received ipilimumab before treatment with BRAFi [two patients PD-L1$^+$ (7%) and one PD-L1$^-$ (2%), respectively]. After the discontinuation of BRAFi, subsequent anticancer therapy was administered to 18 patients (22%). Ipilimumab was administered to five patients PD-L1$^+$ and four patients PD-L1$^-$; the remaining cases received dacarbazine or fotemustine.

PD-L1 expression and tumor-infiltrating immune cells in metastatic melanoma samples

PD-L1 IHC and the tumor-infiltrating mononuclear immune cells expression were evaluated in all consecutive, metastatic melanoma cases. PD-L1 expression on the tumor cell membrane was negative in 51 patients (64%) and positive in 28 patients (35%). For one patient, PD-L1 staining was not feasible due to diffuse melanin pigmentation. Twenty (71%) and 5 (18%) of the PD-L1$^+$ cases were identified in patients with stage M1C and M1a, respectively.

PD-L1 expression was evaluated in the last available metastatic sample before starting BRAFi therapy in 63 patients. In the remaining cases, PD-L1 was evaluated in the primary melanoma sample due to unavailability of metastatic tissue. The median time that the biopsies were taken before starting BRAFi treatment of metastatic disease was 2 months (range 1–6 months).

PD-L1 staining patterns in melanoma tissues were similar with both antibodies; both provided a cell membranous signal, with variable cytoplasmic reactivity (supplementary Figure S1, available at *Annals of Oncology* online). There was a complete concordance for positivity (cutoff 5%) as well as intensity in the 50/80 (63%) cases that were evaluated with both reagents. Consistent with previous observations, PD-L1 expression in the tumor microenvironment was recognized in separated foci mostly at the borders of melanoma cell aggregates in association with an intratumoral immune cell infiltration (interface or peripheral pattern). However, in some cases, melanoma cells were diffusely and strongly positive for PD-L1 in the absence of any inflammatory cell component (diffuse pattern) (Figure 1).

Among 80 MMP, 45 showed absent/focal TIMC, while 34 were associated with at least moderate TIMC among tumor cells. One case was not evaluable due to diffuse melanin pigmentation.

association of PD-L1 expression and TIMC

To explore a potential association between PD-L1 expression and the presence of TIMC, we compared the degree of PD-L1 expression to the intensity of associated immune infiltrates. We found that 35% (28/80) of melanoma samples expressed PD-L1 (mean expression in positive samples, 40%; range, 6%–80%). Interestingly, 30% of the PD-L1$^+$ cases were associated with TIMC positivity compared with 40% of PD-L1$^-$ cases.

correlation between PD-L1 expression and/or tumor-infiltrating mononuclear immune cell density and RR, DFS and OS

Response to treatment at 2 months, available for all included patients, was as follows: 8 (10%) complete response; 40 (50%) partial response; 16 (20%) stable disease and 16 (20%) progressive disease. Results of multivariate logistic model assessing the prognostic effect of the presence of PD-L1 expression are reported in Table 1. At multivariate analysis, PD-L1$^-$ [odd ratio (OR) 10.8, 95% confidence interval (CI) 2.7–43.3, $P < 0.001$] and the presence of TIMC (OR 6.5, 95% CI 1.7–24.3, $P < 0.005$) were significantly associated with a higher probability of responding to treatment (supplementary Table S2, available at *Annals of Oncology* online). At a median follow-up of 9 months, 51 (64%) patients had progressed and 42 (52.5%) had died. Overall, 55 (69%) patients progressed or died. In the whole group, the median PFS and OS were 10 and 15 months, respectively. Figure 2 shows the Kaplan–Meier curves for PFS and OS according to the PD-L1 expression and the presence of TIMC.

At multivariate assessment, PD-L1 expression [hazard ratio (HR) 4.3, 95% CI 2.1–8.7, $P < 0.0001$], as well as the absence of TIMC (HR 2.5, 95% CI 1.4–4.7, $P < 0.002$), were associated with a statistically significant shorter PFS (Table 1).

In the multivariate model, PD-L1 overexpression (HR 6.2, 95% CI 2.8–14.2, $P < 0.0001$), as well as the absence of TIMC (HR 3.1, 95% CI 1.5–6.5, $P < 0.002$), were independent prognostic factors for melanoma-specific survival (Table 1).

Finally, PFS and OS were statistically significant longer in PD-L1$^-$ and TIMC$^+$ patients than in all other combinations [(PD-L1$^+$/TIMC$^-$, as well as those PD-L1$^-$/TIMC$^-$ or PD-L1$^+$/TIMC$^+$ (Figure 3)].

discussion

One of the major issues in exploiting BRAFi for MMP lies in the interpatient degree and duration of response: some patients derive no benefit, others have a dramatic, complete disappearance of all disease, and the remainder is somewhere in between [13]. Hence, there is a clinical need to identify biomarkers that can allow accurate establishment of the best treatment approach in the individual patient with BRAF-mutated melanoma.

The most striking finding of this study is that IHC PD-L1 overexpression, together with the lack of TIMC in metastatic melanoma samples, are associated with resistance and poor prognosis in MMP receiving BRAFi. This conclusion was reached upon testing PD-L1 expression in a cohort of 80 consecutive MMP treated at a single institution, using two validated monoclonal antibodies specific for PD-L1.

The same multidisciplinary team evaluated all enrolled patients. In our center, melanoma patients who do not enter clinical trials are assessed according to standard internal

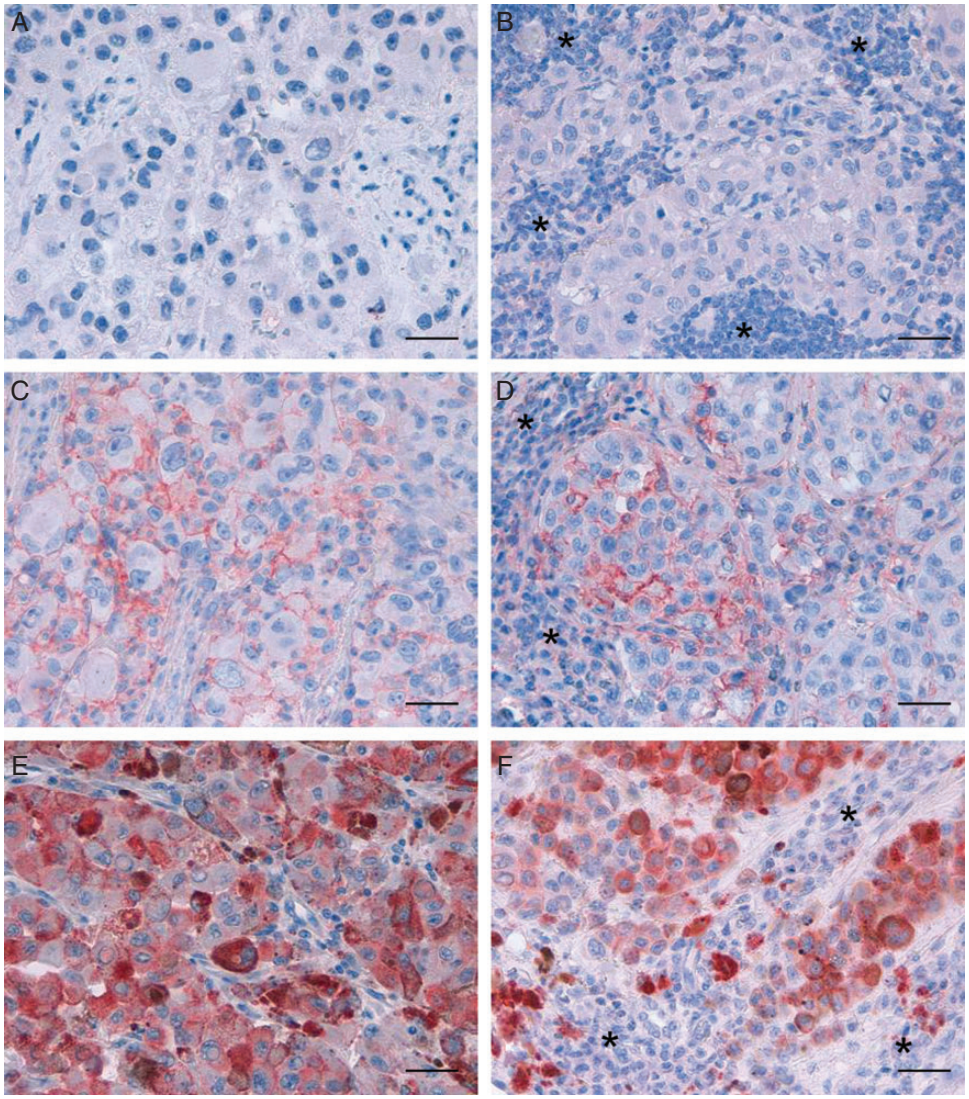

Figure 1. Tumoral PD-L1 expression and immune infiltrates in the tumor microenvironment (mouse anti-human B7-H1 mAb, clone 5H1). (A) PD-L1-negative melanoma metastasis, there are no immune cells in intratumoral location; (B) lack of PD-L1 staining in melanoma cells in association with marked tumor-infiltrating mononuclear cells (TIMC); (C) Mostly membranous PD-L1 staining in melanoma cells in absence of TIMC; (D) PD-L1 expression is recognized mostly at the borders of melanoma cell aggregates in association with an intratumoral immune cell infiltration (interface or peripheral pattern); (E) strong membranous and cytoplasmic staining in aggregates of melanoma cells (diffuse pattern) in absence of TIMC; (F) melanoma cells show diffuse PD-L1 positivity in presence of moderate TIMC infiltration (original magnification ×40, scale bar 50 μm). Asterisks indicate tumor-infiltrating mononuclear cells (TIMC).

guidelines at baseline and upon treatment. Specifically, before starting treatment, all patients underwent total body computed tomographic (CT), ECG and blood laboratory analysis. Physical examination, including dermatological evaluation, was carried out every 4 weeks. The first disease assessment was planned after 8 weeks and thereafter every 12 weeks. The CT scan was anticipated if there was a suspicion of disease progression and/or in the presence of worsening symptoms. Overall, the time schedule for disease assessment was similar for all patients.

There are several biological explanations that support our clinical data. Suminoto et al. found that oncogenic $BRAF^{V600}$ can lead to immune escape in melanoma [6], and that blocking its activity via MAPK pathway inhibition leads to increased expression of melanocyte differentiation antigens as well as reduction of the secretion of immunosuppressive chemokines such as IL-6/10 [6]. Furthermore, *in vivo* studies showed that after 2 weeks from starting BRAFi treatment, there is an increase in PD-1 expression, coupled with a significant increase in the expression of its ligand PD-L1 within the tumor stroma [5]. Interestingly, *in vitro* studies showed high PD-L1 expression in melanoma cell lines resistant to BRAF inhibition [7]. Overall, the above data support the engagement of a host immune response in regressing melanoma during treatment with BRAFi [5, 14, 15]. This is indirectly confirmed by emerging data strongly suggesting that an increase in the density of tumor-infiltrating lymphocytes within and around metastases in biopsies taken early after commencement of treatment (within 2 weeks) correlates with response to BRAFi [5].

Our clinical results extend these data and support the hypothesis that a more hostile tumor microenvironment, as well as the

Table 1. Multivariate analysis: prognostic factors for PFS and OS

	PFS		OS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex				
Male	1.00 (Ref.)		1.00 (Ref.)	
Female	1.24 (0.71–2.17)	0.447	1.84 (0.95–3.57)	0.071
Age (as continuous)	1.03 (1.00–1.05)	0.021	1.03 (1.01–1.06)	0.012
Stage of disease				
M1a or M1b	1.00 (Ref.)		1.00 (Ref.)	
M1c	2.13 (1.13–0.20)	0.02	2.34 (1.11–4.94)	0.026
PD-L1				
Negative	1.00 (Ref.)		1.00 (Ref.)	
Positive	4.28 (2.10–8.72)	<0.001	6.27 (2.77–14.22)	<0.001
TIMC				
Present	1.00 (Ref.)		1.00 (Ref.)	
Absent	2.59 (1.42–4.73)	0.002	3.11 (1.50–6.48)	0.002

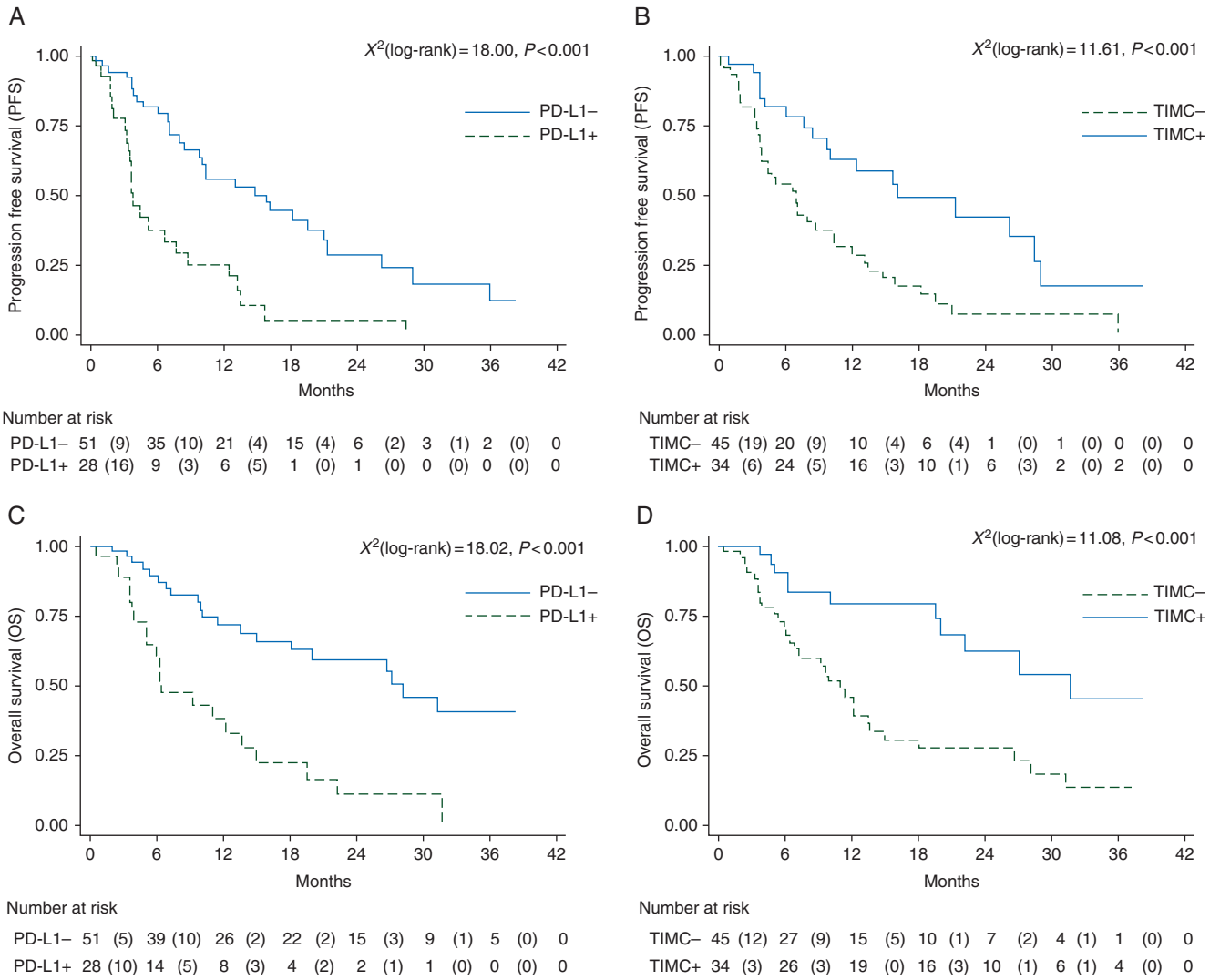

Figure 2. Kaplan–Meier curves showing DFS and OS of metastatic melanoma patients (MMP) according to PD-L1 expression (membrane) and density of tumor-infiltrating mononuclear immune cells (TIMC) in melanoma tissue samples. (A) PFS according to membrane PD-L1 immunohistochemical expression in melanoma tissues (cutoff 5%). (B) PFS according to TIMC density in melanoma tissues. (C) OS according to membrane PD-L1 immunohistochemical expression in melanoma tissues (cutoff 5%). (D) OS according to TIMC density in melanoma tissues (cutoff 5%).

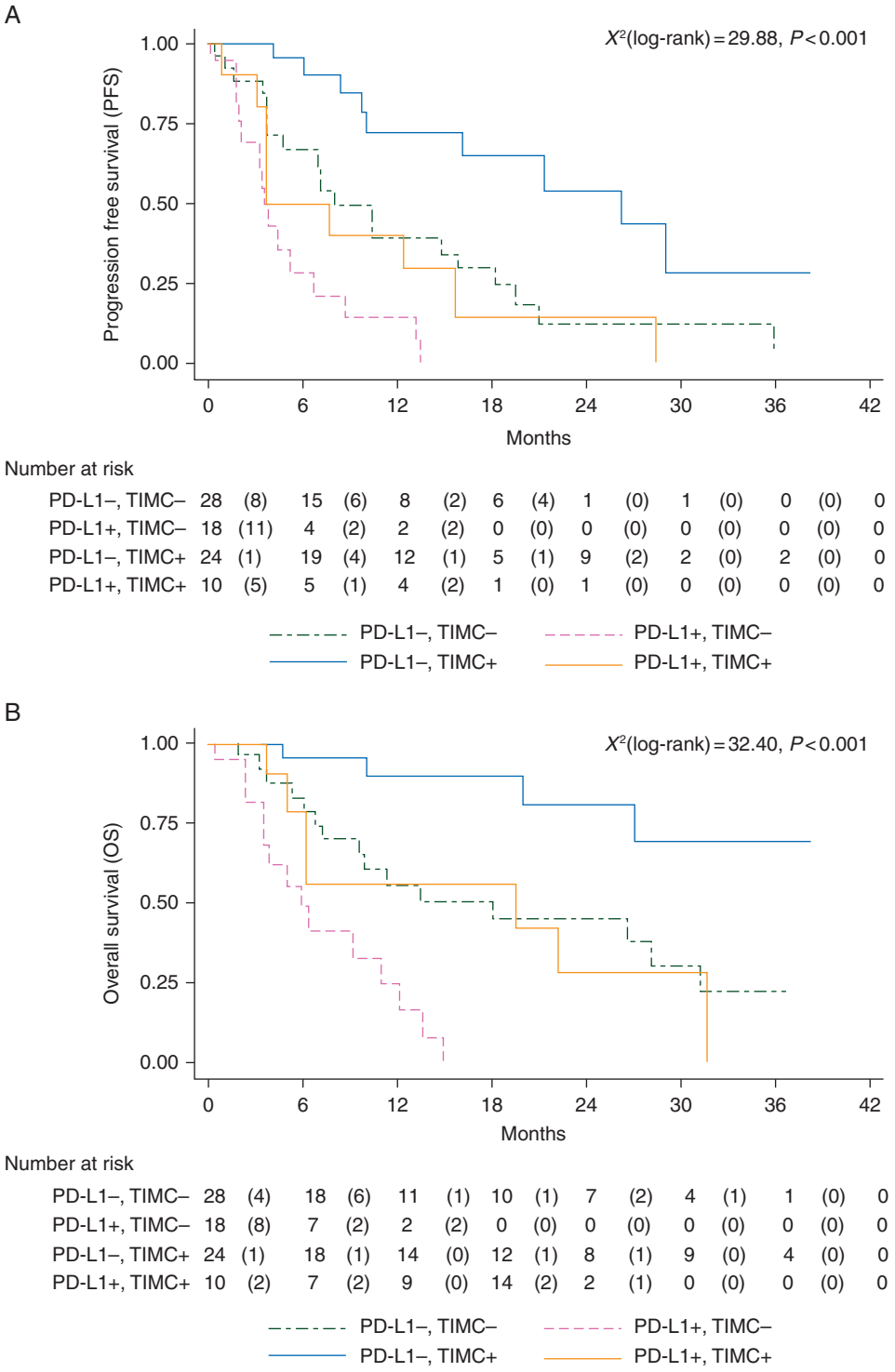

Figure 3. Kaplan–Meier curves showing progression-free survival, PFS (A) and overall survival, OS (B) of metastatic melanoma patients (MMP) according to the combination of PD-L1 membrane expression and density of tumor-infiltrating mononuclear immune cells (TIMC) in metastatic tumor samples.

overexpression of tumor PD-L1, are associated with a worse RR and outcome in $BRAF^{V600}$-mutated MMP receiving BRAFi.

In agreement with previous reports [10, 11], we found that the expression of PD-L1 by tumor cells is a relatively common event in melanoma disease (35% of patients being $PD\text{-}L1^{+}$ in the current series). Furthermore, the finding that 71% of the $PD\text{-}L1^{+}$ cases were identified in patients with stage M1C compared with 18% of positivity in stage M1a patients supports our previous clinical and *in vitro* data suggesting that PD-L1 expression marks a subset of melanomas characterized by a more aggressive disease [8].

The molecular mechanisms behind the constitutive oncogenic driven upregulation of PD-L1 have not been clarified yet. In a study using glioblastoma cell lines, Parsa et al. found that the

mutation and loss of *PTEN* was associated with increased PD-L1 expression [16]. However, our preclinical data (supplementary Table S3, available at *Annals of Oncology* online) and those reported in a recent study did not replicate the same findings in melanoma models [17], suggesting that different molecular circuits may be active in melanoma. However, it should be noticed that we did not check for PTEN deletion in our clinical series, leaving room for future studies.

A major challenge in the PD-L1 field concerns the availability of easy-to-use and reliable antibodies for tissue staining. In our study, comparative analysis indicated that both monoclonal antibodies, 5H1 and rabbit clone E1L3N mAb, yield reliable results. When we compared the staining pattern of 5H1 and E1L3N mAb, we found no discrepancies in number of positive cases, but the fully automatic staining procedure of E1L3N mAb represents a clear advantage in routine practice.

Two recent studies found a frequently discordant PD-L1 expression between primary and metastatic samples and between intrapatient metastases [8, 18]. Considering that PD-L1 expression in primary melanoma is unreliable as predictor of PD-L1 expression in distant metastases, we herein selected for analysis the most recent metastatic melanoma tissue specimen, and preferentially large excision biopsies, rather than small core or fine-needle biopsy samples. Nevertheless, the clinical impact of the heterogeneous PD-L1 expression in multiple distant metastases from the same patient remains to be elucidated. When patients evaluated at primary melanoma were excluded from the analysis the prognostic role of PD-L1 remained statistically significant ($P < 0.0001$).

This study has several strengths, including: (i) the novelty of our findings, since this is the first time that a negative immunologic biomarker has been found, and this study adds further evidence to the strict relationship between BRAFi, the immune markers and the microenvironment; (ii) in the present study, patients have been enrolled over the last 4 years, and all have been homogeneously treated with BRAFi, within a single center; (iii) PD-L1 IHC evaluation was carried out mostly (63/80, 80%) in metastatic samples to reduce the potential discordance between primary and metastatic samples [18] and to better reflect the actual PD-L1 biological status of the patient cohort.

We are aware of the limitations of our report, namely: (i) this is a monoinstitutional retrospective study and a validation dataset is needed; (ii) in our patient cohort, no patient received BRAF and MEK inhibitors, which are now known to improve RR, PFS and OS, when compared with BRAFi alone [19–21]; (iii) we did not evaluate the immunophenotype of immune cell infiltration, and the absence of CD8 staining that has been widely used in previous studies in association with PD-L1 expression and could give more quantitative and reliable results regarding the immune infiltrate. Insights on the contribution of the specific immune infiltration phenotype components that correlate with response to BRAFi would certainly be critical in instructing how targeted and immunological treatments may be used in combination for clinical trials.

It is intriguing that, in our study, the degree of TIMC infiltration assessed by simple evaluation of H&E staining tumor sections has prognostic and predictive value, despite a lack of detailed information on the immune phenotype of the infiltrate. The inclusion of TIMC assessment in metastatic samples seems to mirror the efficacy of each individual patient's antitumor immune response. Extension of this observation to clinical practice would require a standardized scoring methodology.

From a schematic point of view, the PD-1/PD-L1 cross-talk could take place in the context of tumor/T-cell or APC/T-cell interactions. In general, the tumor–T-cell context is the most studied. In melanoma models, PD-L1 expression by tumor cells may be geographically associated with infiltrating immune cells and with expression of PD-1 on neighboring lymphocytes. The subgroup of patients with PD-L1 expression, particularly on the CD8 subset, and infiltrating lymphocytes shows better prognosis than the group with PD-L1 expression without infiltrating lymphocytes [10]. However, PD-L1 positivity can be found also in melanoma samples without tumor lymphocyte infiltration and, in this subgroup of patients, it is unclear whether the lack of T-cell infiltration reflects the absence of tumor antigen-specific responses or an unidentified process that excludes lymphocytes infiltration from the microenvironment. Hence, PD-L1 expression should be interpreted in the context of the microenvironment as well as the immune cells phenotype.

Since PD-L1 overexpressing melanoma patients are at higher risk of developing early progression and worse outcome than those who are PD-L1 negative, a different strategy should probably be pursued in these patients. Whether starting with anti PD-1 antibodies may result in a better outcome should be evaluated in *ad hoc* designed studies, since PD-L1 positive melanomas with immune cell infiltration seem to benefit particularly from anti PD-1 antibodies [22, 23].

funding

Associazione Oncologica Bergamasca and Fondazione Ente Cassa di Risparmio di Firenze supported this work. Grant number does not apply.

disclosure

The authors have declared no conflicts of interest.

references

1. Ives NJ, Stowe RL, Lorigan P, Wheatley K. Chemotherapy compared with biochemotherapy for the treatment of metastatic melanoma: a meta-analysis of 18 trials involving 2,621 patients. J Clin Oncol 2007; 25: 5426–5434.
2. 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.
3. Hauschild A, Grob JJ, Demidov LV et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2012; 380: 358–365.
4. Flaherty KT, Puzanov I, Kim KB et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010; 363: 809–819.
5. Frederick DT, Piris A, Cogdill AP et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res 2013; 19: 1225–1231.
6. Sumimoto H, Imabayashi F, Iwata T, Kawakami Y. The BRAF-MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. J Exp Med 2006; 203: 1651–1656.
7. Jiang X, Zhou J, Giobbie-Hurder A et al. The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition. Clin Cancer Res 2013; 19: 598–609.
8. Massi D, Brusa D, Merelli B et al. PD-L1 marks a subset of melanomas with a shorter overall survival and distinct genetic and morphological characteristics. Ann Oncol 2014; 25: 2433–2442.

9. Merelli B, Massi D, Cattaneo L et al. Targeting the PD1/PD-L1 axis in melanoma: biological rationale, clinical challenges and opportunities. Crit Rev Oncol Hematol 2014; 89: 140–165.
10. Taube JM, Anders RA, Young GD et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med 2012; 4: 127ra137.
11. Dong H, Strome SE, Salomao DR et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002; 8: 793–800.
12. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors: European Organisation for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000; 92: 205–216.
13. Salama AK, Flaherty KT. BRAF in melanoma: current strategies and future directions. Clin Cancer Res 2013; 19: 4326–4334.
14. Wilmott JS, Long GV, Howle JR et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res 2012; 18: 1386–1394.
15. Hu-Lieskovan S, Robert L, Homet Moreno B, Ribas A. Combining targeted therapy with immunotherapy in BRAF-mutant melanoma: promise and challenges. J Clin Oncol 2014; 32: 2248–2254.
16. Parsa AT, Waldron JS, Panner A et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 2007; 13: 84–88.
17. Atefi M, Avramis E, Lassen A et al. Effects of MAPK and PI3K pathways on PD-L1 expression in melanoma. Clin Cancer Res 2014; 20: 3446–3457.
18. Madore J, Vilain RE, Menzies AM et al. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. Pigment Cell Melanoma Res 2015; 28: 245–253.
19. Long GV, Stroyakovskiy D, Gogas H et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med 2014; 371: 1877–1888.
20. Robert C, Karaszewska B, Schachter J et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 2015; 372: 30–39.
21. Larkin J, Ascierto PA, Dréno B et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014; 371: 1867–1876.
22. Topalian SL, Hodi FS, Brahmer JR et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443–2454.
23. Tumeh PC, Harview CL, Yearley JH et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515: 568–571.

Annals of Oncology 26: 1987–1993, 2015
doi:10.1093/annonc/mdv252
Published online 10 June 2015

Pazopanib in pretreated advanced neuroendocrine tumors: a phase II, open-label trial of the Spanish Task Force Group for Neuroendocrine Tumors (GETNE)†

E. Grande[1*], J. Capdevila[2], D. Castellano[3], A. Teulé[4], I. Durán[5], J. Fuster[6], I. Sevilla[7], P. Escudero[8], J. Sastre[9], J. García-Donas[10], O. Casanovas[11], J. Earl[12], L. Ortega[13], M. Apellaniz-Ruiz[14], C. Rodriguez-Antona[14], T. Alonso-Gordoa[1], J. J. Díez[15], A. Carrato[1] & R. García-Carbonero[5]

[1]Department of Medical Oncology, Ramón y Cajal University Hospital, Madrid; [2]Department of Medical Oncology, Vall d'Hebron University Hospital and Vall d'Hebron Institute of Oncology (VHIO), Universitat Autònoma de Barcelona, Barcelona; [3]Department of Medical Oncology, I + 12 Research Institute, 12 de Octubre University Hospital, Madrid; [4]Department of Medical Oncology, IDIBELL, Catalan Institute of Oncology L'Hospitalet, Barcelona; [5]Department of Medical Oncology, Instituto de Biomedicina de Sevilla (IBIS) [HUVR, CSIC, University of Seville], Virgen del Rocío University Hospital, Seville; [6]Department of Medical Oncology, Son Espases Hospital, Palma de Mallorca; [7]Department of Medical Oncology, Virgen de la Victoria University Hospital, Malaga; [8]Department of Medical Oncology, Clínico Lozano Blesa University Hospital, Zaragoza; [9]Department of Medical Oncology, Clínico San Carlos Hospital, Madrid; [10]Department of Medical Oncology, Centro Integral Oncológico Clara Campal, Madrid; [11]Tumor Angiogenesis Group, IDIBELL, Catalan Institute of Oncology L'Hospitalet, Barcelona; [12]Department of Medical Oncology Research Laboratory, Ramón y Cajal University Hospital, Madrid; [13]Department of Pathology, Clínico San Carlos Hospital, Madrid; [14]Hereditary Endocrine Cancer Group, Spanish National Cancer Research Center, ISCIII Center for Biomedical Research on Rare Disease (CIBERER) Madrid, Madrid; [15]Department of Endocrinology, Ramón y Cajal University Hospital, Madrid, Spain

Received 22 January 2015; revised 19 May 2015; accepted 26 May 2015

Background: The management of advanced neuroendocrine tumors (NETs) has recently changed. We assessed the activity of pazopanib after failure of other systemic treatments in advanced NETs.

Methods: This was a multicenter, open-label, phase II study evaluating pazopanib as a single agent in advanced NETs (PAZONET study). The clinical benefit rate (CBR) at 6 months was the primary end point. Translational correlation of

*Correspondence to: Dr Enrique Grande, Department of Medical Oncology, Ramón y Cajal University Hospital, Carretera de Colmenar Viejo km. 9100, Madrid 28034, Spain. Tel: +34-91-336-8263; E-mail: egrande@oncologiahrc.com

†2011 ASCO Annual meeting: Poster Session; 2012 ASCO Annual meeting: Poster Session; 2013 ASCO Annual meeting: Poster Session; 2014 ASCO Annual meeting: Abstract; ESMO 2012 Congress Vienna: Oral presentation; European Cancer Congress 2013: Poster Session; Sociedad Española de Oncología Médica (SEOM) 2013: Oral presentation.