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Association of PD-L1 expression with treatment outcomes in patients with *BRAF* mutation–positive melanoma treated with vemurafenib or cobimetinib combined with vemurafenib

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Summary

The prognostic significance of programmed death ligand-1 (PD-L1) on treatment outcomes in patients receiving BRAF with or without MEK inhibitors is not well understood. This retrospective exploratory analysis evaluated the association of tumour PD-L1 expression with progression-free survival (PFS) and overall survival (OS) among 210 patients in the coBRIM trial treated with cobimetinib plus vemurafenib or placebo plus vemurafenib. In the vemurafenib cohort, there was a trend of increased PFS and OS in those with PD-L1⁺ melanoma, with hazard ratios (HRs) (PD-L1⁺ versus PD-L1⁻) of 0.70 (95% CI, 0.46–1.07) and 0.69 (95% CI, 0.42–1.13) for PFS and OS, respectively. However, in patients treated with cobimetinib plus vemurafenib, a similar trend was not observed with HRs (PD-L1⁺ versus PD-L1⁻) of 1.04 (95% CI, 0.66–1.68) and 0.94 (95% CI, 0.57–1.57) for PFS and OS, respectively. The combination cobimetinib plus vemurafenib appears to overcome the poor prognosis associated with low PD-L1 expression.

Significance section

PD-L1 expression in the tumour tissue has been suggested to be directly associated overall survival outcomes in patients receiving treatment with PD-1/PD-L1 blockade in various cancers, including melanoma. Patients with low levels of PD-L1 expression in their tumour tissues may have less favourable survival outcomes. The current study, using data from the coBRIM phase III trial, suggests that combination treatment with a BRAF and MEK inhibitor is equally effective in patients with low or high PD-L1 expression. Thus, cobimetinib plus vemurafenib is a safe and effective option for patients regardless of the expression level of PD-L1 in their tumour tissue.

Key words: cobimetinib, vemurafenib, melanoma, PD-L1, immunohistochemistry, prognosis

Introduction

Escape from immune surveillance is a key “hallmark” of cancer (Hanahan and Weinberg, 2011), and therapeutic approaches that enhance host anti-tumour response have long been used in melanoma. Within the last 5 years, systemic treatment for patients with advanced/metastatic melanoma has shifted from the

decades-long use of cytokines and chemotherapy to combination regimens of BRAF and MEK inhibitors and immune checkpoint inhibitors (Singh and Salama, 2016). While the immunomodulatory mechanisms of agents targeting immune checkpoints are becoming increasingly clear, combination regimens of BRAF and MEK inhibitors also appear to have immunomodulatory functions that may contribute to their activity in melanoma (Ott, 2015; Wilmott et al., 2012).

The programmed cell death protein-1 receptor (PD-1)/programmed death ligand-1 (PD-L1) immune checkpoint has emerged as a key pathway involved in tumour-mediated immune suppression and has been the most recent focus of therapeutic approaches in both haematological malignancies and solid tumours, including malignant melanoma (Obeid et al., 2016). PD-1 is a transmembrane protein that belongs to the CD28 family and is expressed on T and B lymphocytes and natural killer and myeloid cells. Interaction of PD-1 with its ligand PD-L1 can induce programmed cell death and downregulate T-cell function (Obeid et al., 2016). The expression of PD-L1 is induced by multiple pro-inflammatory molecules, including types I and II interferon- γ (IFN- γ), tumour necrosis factor- α (TNF- α), and interleukin (IL)-4 and IL-10, with IFN- γ being the most potent inducer (He et al., 2015; Sznol and Chen, 2013). Malignant melanomas have a highly immunosuppressive tumour microenvironment, characterised by marked elevation of inflammatory mediators and myeloid-derived suppressor cells which may upregulate PD-L1 expression and cooperatively enhance tumour progression (Umansky and Sevko, 2012). Consistent with these findings, PD-L1 expression in the pre-treatment tumour microenvironment has been shown to be associated with improved outcomes to PD-1/PD-L1 blockade in various cancers (Herbst et al., 2014).

The prognostic significance of PD-L1 expression in patients treated with therapies targeting the BRAF and MEK pathways is not clear. Some preclinical studies suggest that activation of the mitogen activated protein kinase (MAPK) pathway (Yamamoto, et al., 2009) or the phosphoinositide-3 kinase (PI3K)/AKT pathway (Parsa et al., 2009) upregulates PD-L1 expression, and that MEK or PI3K inhibition may reverse this effect (Jiang et al., 2013), whereas others suggest that the effects of BRAF and MEK inhibitors on PD-L1 expression is variable (Atefi et al., 2011).

Pivotal phase 3 studies, including the coBRIM study (ClinicalTrials.gov ID, NCT01689519), have resulted in the adoption of combination treatment with BRAF and MEK inhibitors as standard of care in patients with *BRAF*^{V600} mutation-positive metastatic melanoma (Larkin et al., 2014). Taken together with the potential for this combination regimen to act, in some part, by modulating the PD-1/PD-L1 immune checkpoint, it was of interest to explore the prognostic significance of baseline PD-L1 expression in the targeted treatment setting. Accordingly, we retrospectively evaluated the association of baseline PD-L1 expression in tumour samples from patients who were treated with BRAF and/or MEK inhibitors in the coBRIM study with treatment outcomes.

Results

Patients

Of the 495 patients who were randomized in the coBRIM study, tumour samples from 210 patients (42%) were available for inclusion in the current analysis (cobimetinib combined with vemurafenib, $n = 102$; placebo and vemurafenib, $n = 108$; Figure S1). The most common reason for exclusion from the current analysis was a lack of adequate tissue available for immunohistochemistry (IHC) assays following required testing for *BRAF* mutation status. Overall, the biomarker population had baseline demographics and disease characteristics that were similar to those of the intention-to-treat (ITT) population reported in the primary analysis of coBRIM (Table S1).

Survival outcomes in the ITT and biomarker populations

At the time of data cutoff, the median follow-up duration for the ITT population was 18.5 months (range, 0.5–39.5). In the ITT population, median progression-free survival (PFS) was 12.6 months (95% confidence interval [CI], 9.5–14.7) in the cobimetinib combined with vemurafenib arm and 7.2 months (95% CI, 5.6–7.5) in the placebo plus vemurafenib arm (Figure 1A). The median overall survival (OS) was 22.5 months (95% CI, 20.3–28.8) in the cobimetinib combined with vemurafenib arm and 17.4 months (95% CI, 15.0–19.8) in the placebo plus vemurafenib arm; landmark OS rates at 3 years were 37.4% (95% CI, 30.5–44.2) and 31.5% (95% CI, 24.9–38.2), respectively (Figure 1B). PFS and OS outcomes in the biomarker population for the current analysis were similar to those of the overall ITT population in coBRIM (Figure S2).

PD-L1 expression

The proportion of patients with $\geq 1\%$ PD-L1⁺ immune cells was 59.6% (62.7% and 54.6% in patients treated with cobimetinib combined with vemurafenib and placebo plus vemurafenib, respectively), and the proportion of patients with $\geq 10\%$ PD-L1⁺ immune cells was 14.8% (15.7% and 13.9%, respectively) (Figure 2A). In contrast, few patients (8.1%) had $\geq 1\%$ PD-L1⁺ tumour cells; it was therefore not feasible to break down the patient population into subsets that could be meaningfully correlated with distinct survival outcomes. Thus, we analysed treatment outcomes in patient subgroups defined by the relative proportion of PD-L1⁺ immune cells in their tumour tissue. There were no significant differences in the distribution of baseline characteristics known to be associated with poor prognosis between patients with $< 1\%$ and $\geq 1\%$ PD-L1⁺ immune cells (Table S2). Representative staining of PD-L1⁺ immune cells in

tumour sections is shown in Figure 2B, and PD-L1 RNA expression by proportion of PD-L1⁺ immune cells in tumour sections is shown in Figure 2C.

Treatment outcomes and PD-L1 expression

There was a trend towards improved objective response rates in patients with higher proportions of PD-L1⁺ immune cells in the placebo plus vemurafenib arm, with a lower objective response rate in the IC0 subgroup (<1% PD-L1⁺ immune cells) relative to other subgroups (34.7% vs 58.1%–61.5%; Table 1). Objective response rates were similar across subgroups defined by proportion of PD-L1⁺ immune cells in the cobimetinib combined with vemurafenib arm.

Among patients treated with placebo and vemurafenib, there was a trend toward improved survival outcomes in patients whose tumours had higher proportions of PD-L1⁺ immune cells. PFS and OS were improved in patients whose tumour sections had $\geq 1\%$ PD-L1⁺ immune cells (IC1, 2 and 3) compared with patients whose tumour sections had <1% PD-L1⁺ immune cells (IC0 [PD-L1⁻]; reference), with hazard ratios (HRs) of 0.70 (95% CI, 0.46–1.07) and 0.69 (95% CI, 0.42–1.13), respectively (Figures 3A and 3B). This difference was more pronounced when the PD-L1 threshold for differentiating the subgroups in the biomarker population was increased to $\geq 10\%$ of PD-L1⁺ immune cells. Survival was improved in patients whose tumour sections had $\geq 10\%$ PD-L1⁺ immune cells (IC3) compared with patients whose tumour sections had <10% PD-L1⁺ immune cells (IC0, 1 and 2; reference), with HRs of 0.47 (95% CI, 0.22–0.89) and 0.50 (95% CI, 0.19–1.07) for PFS and OS, respectively (Figures 3C and 3D).

A similar analysis among patients in the biomarker population treated with cobimetinib combined with vemurafenib did not show a difference in survival outcomes at either the 1% threshold or the 10% threshold for PD-L1⁺ immune cells in the tumour sample. Among patients treated with cobimetinib combined with vemurafenib, survival outcomes in those whose tumour sections had $\geq 1\%$ PD-L1⁺ immune cells (IC1, 2 and 3) were similar to those of patients who had PD-L1⁻ tumours (IC0; reference), with HRs of 1.04 (95% CI, 0.66–1.68) and 0.94 (95% CI, 0.57–1.57) for PFS and OS, respectively (Figure 4A, B). Key baseline prognostic factors including ECOG PS, disease stage and serum LDH were comparable between patient subgroups with <1% and $\geq 1\%$ PD-L1⁺ immune cells in each treatment arm (Table S3). Likewise, PFS and OS in patients whose tumour sections had $\geq 10\%$ PD-L1⁺ (IC3) immune cells were similar to that of patients whose tumour sections had <10% PD-L1⁺ immune cells (IC0, 1 and 2; reference), with HRs of 0.81 (95% CI, 0.40–1.47) and 0.83 (95% CI, 0.38–1.60) for PFS and OS, respectively (Figure 4C, D).

Discussion

PD-L1 expression has previously been shown to be a favourable prognostic factor for patients with metastatic melanoma (Obeid et al., 2016; Taube et al., 2012). In the current analysis, vemurafenib-treated patients whose tumour sections had $\geq 10\%$ PD-L1⁺ immune cells attained longer PFS and OS than patients whose tumour sections had $< 10\%$ PD-L1⁺ immune cells, although this result should be interpreted with caution because of the small number of patients with $\geq 10\%$ PD-L1⁺ immune cells. In contrast, patients treated with cobimetinib plus vemurafenib had similar PFS and OS, regardless of the relative proportions of PD-L1⁺ immune cells in their tumour sections. These data suggest that the addition of cobimetinib to vemurafenib may overcome the poor prognosis associated with low PD-L1 expression that was evident in the placebo plus vemurafenib arm. Overall, patients treated with cobimetinib combined with vemurafenib had superior outcomes compared with patients receiving vemurafenib only.

Multiple lines of evidence point to the involvement of the BRAF/MEK pathway in the regulation of the host anti-tumour response in melanoma (Ott, 2015; Wilmott et al., 2012; Sumimoto et al., 2006; Khalili et al., 2012; Schilling and Paschen, 2013). Small translational studies have shown that treatment with BRAF inhibitors reduces the number of myeloid-derived suppressor cells and induces tumour infiltration of CD4⁺ and CD8⁺ lymphocytes, with the degree of infiltration correlating with tumour size reduction and necrosis in post-treatment biopsies (Wilmott et al., 2012; Schilling and Paschen, 2013). Preclinical models suggest that inhibition of the MAPK pathway with BRAF or MEK inhibitors increased levels of melanoma-derived antigens, which is associated with improved recognition by antigen-specific T lymphocytes (Boni et al., 2010). In patients with metastatic tumours of advanced melanoma treated with a BRAF inhibitor, increased expression of melanoma-derived antigens and PD-L1 and increased CD8⁺ T cell infiltration was observed during treatment (Frederick et al., 2013). Notably, levels of melanoma-derived antigens were downregulated and CD8⁺ cell numbers were decreased at the time of tumour progression and restored upon initiation of combined BRAF/MEK inhibition (Frederick et al., 2013). In addition, it has been shown that in tumours of patients treated with BRAF inhibitors, the number of CD8⁺ tumour-infiltrating T cells was increased when patients were treated with combined BRAF and MEK inhibition (Ott, 2015; Cooper et al., 2013).

Because few patients (8%) had $\geq 1\%$ PD-L1⁺ tumour cells, analyses were limited to patient subgroups defined by the relative proportion of PD-L1⁺ immune cells. The low proportion of patients with PD-L1⁺ tumour cells may be at least partly due to use of the SP142 antibody clone in IHC assays. In the Blueprint PD-L1 IHC Assay Comparison Project, assays using the SP142 antibody clone consistently exhibited fewer PD-L1⁺ tumour cells than assays using 22C3, 28-8 or SP263 antibody clones (Hirsch et al., 2017). However, the Blueprint study was restricted to samples from patients with non-small cell lung cancer, and caution should be taken in applying those learnings to other tumour types. A recent study

found that PD-L1 staining in melanoma samples was similar between the different antibody clones (Sunshine et al., 2017). While PD-L1 may be expressed on both tumour cells and tumour-infiltrating immune cells in melanoma, recent data suggest that expression of PD-L1 on immune cells may be more important as a predictive/prognostic factor than tumour cell expression in melanoma (Herbst et al., 2014; Taube et al., 2012; Kluger et al., 2017; Taube et al., 2015).

These data suggest that the effects of targeted inhibition of the MAPK pathway on the tumour immune microenvironment are complex and may vary depending on the specific components targeted and the degree to which they are inhibited. There appears to be a net positive effect on host anti-tumour response from the combined inhibition of BRAF and MEK (Ott, 2015; Frederick et al., 2013; Cooper et al., 2013). Although further research is needed to elucidate the effects of combined BRAF and MEK inhibition on PD-L1 expression and the overall host anti-tumour response, these exploratory data suggest that concomitant inhibition of BRAF and MEK may overcome the negative prognostic effect of PD-L1 expression and provide a rationale for exploring either a dual-combination approach with BRAF and MEK inhibition or a triple-combination approach with BRAF, MEK and PD1/PD-L1 inhibition in patients with advanced melanoma.

Methods

coBRIM was a phase 3 randomized, double-blind study. Detailed methods have been previously described (Larkin et al., 2014). Briefly, eligible adults with advanced/metastatic melanoma that was confirmed positive for *BRAF*^{V600} mutation were randomised 1:1 to receive first-line treatment with cobimetinib combined with vemurafenib or placebo plus vemurafenib. Treatment was administered in 28-day cycles and continued until disease progression, unacceptable toxicity, or withdrawal of consent. The primary endpoint was investigator-assessed PFS.

Archival or baseline formalin-fixed paraffin-embedded tumour samples were collected from patients in coBRIM, and PD-L1 expression was measured by IHC. Specimens were sectioned at a thickness of 4 µm and were stained for PD-L1 using an anti-human PD-L1 rabbit monoclonal antibody (SP142; Ventana Medical Systems, Tucson, AZ, USA) on an automated staining platform (Benchmark ULTRA; Ventana), followed by signal visualisation using 3,3'-diaminobenzidine tetrahydrochloride (OptiView DAB IHC Detection Kit and OptiView Amplification Kit; Ventana). Specimens were counter-stained with hematoxylin II. Tumour sections stained for PD-L1 were categorised into subgroups defined by threshold levels of staining for PD-L1 positivity based on the proportion of tumour area occupied by PD-L1-expressing tumour-infiltrating immune cells of any intensity (% IC) or the percentage of PD-L1 expressing tumour cells of any intensity (% TC). For % IC, IHC staining was scored as 0, 1, 2 or 3 if <1%, ≥1% to <5%, ≥5% to <10% or ≥10% of cells per area were PD-L1 positive, respectively (Herbst et

al., 2014). For % TC, IHC staining was scored as 0, 1, 2, or 3 if <1%, $\geq 1\%$ to <5%, $\geq 5\%$ to <50%, or $\geq 50\%$ of cells per area were PD-L1 positive, respectively.

Association between survival outcomes (PFS and OS) and categorical subsets of patients defined by PD-L1 expression level were evaluated using univariate Cox proportional hazards and Kaplan-Meier estimates. Analysis of survival outcomes was based on a data cutoff date of 20 June 2016.

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Author contributions

Matthew Wongchenko, Antoni Ribas, Brigitte Dréno, Paolo Ascierto, Grant McArthur, Yibing Yan and James Larkin conceived and designed the study. Antoni Ribas, Brigitte Dréno, Paolo Ascierto, Grant McArthur and James Larkin provided study material or patients. All authors collected, assembled, analysed and interpreted the data, drafted and finalised the manuscript and approved the submitted version.

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Conflict of interest

Matthew Wongchenko is an employee of Genentech and owns stock in ARIAD Pharmaceuticals. Antoni Ribas has is a consultant/advisor for Roche-Genentech, MSD, Novartis and Amgen. Brigitte Dréno reports grants and personal fees from Roche, grants from GSK, and personal fees from BMS. Paolo Ascierto is a consultant/advisor for BMS, Roche-Genentech, MSD, Novartis, Array, Amgen, Merck Serono and Pierre Fabre and reports grants from BMS, Roche-Genentech and Array. Grant McArthur has conducted research funded by Celgene, Genentech-Roche, BMS, Amgen, Merck, Array and Novartis. Jorge Gallo, Isabelle Rooney, Jessie Hsu, Hartmut Koeppel and Yibing Yan are employees of Roche-Genentech and have stock options. James Larkin is a consultant/advisor for Eisai, BMS, MSD, GSK, Kymab, Pfizer, Novartis, Roche/Genentech, Secarna, Pierre Fabre and EUSA Pharma.

References

Atefi, M., von Euw, E., Attar, N. et al (2011). Reversing melanoma cross-resistance to BRAF and MEK inhibitors by co-targeting the AKT/mTOR pathway. *PLoS One* 6, e28973.

Boni, A., Cogdill, A.P., Dang, P. et al. (2010). Selective BRAF^{V600E} inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. *Cancer Res* 70, 5213–5219.

Cooper, Z.A., Frederick, D.T., Juneja, V.R., Sullivan, R.J., Lawrence, D.P., Piris, A., Sharpe, A.H., Fisher, D.E., Flaherty, K.T., and Wargo, J.A. (2013). BRAF inhibition is associated with increased clonality in tumor-infiltrating lymphocytes. *Oncoimmunology* 2, e26615.

Frederick, D.T., Piris, A., Cogdill, A.P. et al. (2013). BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res* 19, 1225–1231.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674.

He, J., Hu, Y., Hu, M., and Li, B. (2015). Development of PD-1/PD-L1 pathway in tumor immune microenvironment and treatment for non-small cell lung cancer. *Sci Rep* 5, 13110.

Herbst, R.S., Soria, J.-C., Kowanetz M. et al (2014). Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 515, 563–567.

Hirsch F.R., McElhinny, A., Stanforth D. et al (2017). PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. *J Thorac Oncol* 12, 208–222.

Jiang, X., Zhou, J., Giobbie-Hurder A., Wargo, J., and Hodi, F.S. (2013). 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* 19, 598–609.

Khalili, J.S., Liu, S., Rodríguez-Cruz, T. et al (2012). Oncogenic BRAF(V600E) promotes stromal cell-mediated immunosuppression via induction of interleukin-1 melanoma. *Clin Cancer Res* 18, 5329–5340.

Kluger, H.M., Zito, C.R., Turcu, G. et al (2017). PD-L1 studies across tumor types, its differential expression and predictive value in patients treated with immune checkpoint inhibitors. *Clin Cancer Res* 23, 4270–4279.

Larkin, J., Ascierto, P.A., Dréno, B. et al (2014). Combined vemurafenib and cobimetinib in *BRAF*-mutated melanoma. *N Engl J Med* 371, 1867–1876.

Obeid, J.M., Erdag, G., Smolkin, M.E., Deacon, D.H., Patterson, J.W., Chen, L., Bullock, T.N., and Slingluff, C.L. (2016). PD-L1, PD-L2 and PD-1 expression in metastatic melanoma: correlation with tumor-infiltrating immune cells and clinical outcome. *Oncoimmunology* 5, e1235107.

Ott, P.A. (2015). Combined BRAF and MEK inhibition in BRAF^{V600E} mutant melanoma: a synergistic and potentially safe combination partner with immunotherapy. *Ann Transl Med* 3, 313.

Parsa, A.T., Waldron, J.S., Panner A. et al (2007). Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med* 13, 84–88.

Schilling, B., and Paschen, A. Immunological consequences of selective BRAF inhibitors in malignant melanoma: neutralization of myeloid-derived suppressor cells. *Oncoimmunology* 2, e25218.

Singh, B.P., and Salama, A.K.S. (2016). Updates in therapy for advanced melanoma. *Cancers (Basel)* 8, 17.

Sumimoto, H., Imabayashi, F., Iwata, T., and Kawakami, Y. (2006). The BRAF-MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. *J Exp Med* 203, 1651–1656.

Sunshine, J.C., Nguyen, P.L., Kaunitz G.J. et al (2017). PD-L1 expression in melanoma: a quantitative immunohistochemical antibody comparison. *Clin Cancer Res*. doi: 10.1158/1078-0432.CCR-16-1821.

Sznol, M., and Chen, L. (2013). Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. *Clin Cancer Res* 19, 1021–1034.

Taube, J.M., Anders, R.A., Young G.D. et al (2012). Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports and adaptive resistance mechanism of immune escape. *Sci Transl Med*, 4, 127ra37.

Taube, J.M., Young, G.D., McMiller, T.L. et al (2015). Differential expression of immune-regulatory genes associated with PD-L1 display in melanoma: implications for PD-1 pathway blockade. *Cancer Res* 21, 3969–3976.

Umansky, V., and Sevko, A. (2012). Melanoma-induced immunosuppression and its neutralization. *Semin Cancer Biol* 22, 319–326.

Wilmott, J.S., Long, G.V., Howle, J.R., Haydu, L.E., Sharma, R.N., Thompson, J.F., Kefford, R.F., Hersey, P., and Scolyer, R.A. (2011). Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. *Clin Cancer Res* 18, 1386–1394.

Yamamoto, R., Nishikori, M., Tashima, M., Sakai, T., Ichinohe, T., Takaori-Kondo, A., Ohmori, K., Uchiyama, T. B7-H1 expression is regulated by MEK/ERK signaling pathway in anaplastic large cell lymphoma and Hodgkin lymphoma. *Cancer Sci* 100, 2093–2100.

Supporting information

Additional Supporting information may be found in the online version of this article:

Figure S1. Study population.

Figure S2. Comparison of Kaplan-Meier estimates of (A) PFS and (B) OS between the overall ITT population of coBRIM and the biomarker subset (BM).

Table S1. Baseline disease and demographic characteristics (biomarker population).

Table S2. Baseline characteristics by proportion of PD-L1⁺ immune cells.

Table S3. Baseline characteristics by proportion of PD-L1⁺ immune cells and treatment arm.

FIGURE LEGENDS

Figure 1. Kaplan-Meier estimates of (A) PFS and (B) OS in the overall ITT population of coBRIM.

Figure 2. PD-L1 expression. (A) Distribution of patients by subgroups defined by the proportion of PD-L1⁺ immune cells (IC) or tumour cells (TC). (B) Representative staining of PD-L1⁺ immune cells in tumour sections. (C) PD-L1 RNA expression by proportion of PD-L1⁺ immune cells in tumour sections. The subgroups are defined as follows: IC0: <1% PD-L1⁺ immune cells; IC1: ≥1% to <5% PD-L1⁺ immune cells; IC2: ≥5% to <10% PD-L1⁺ immune cells; IC3: ≥10% PD-L1⁺ immune cells. TC0: <1% PD-L1⁺ tumour cells; TC1: ≥1% to <5% PD-L1⁺ tumour cells; TC2: ≥5% to <50% PD-L1⁺ tumour cells; TC3: ≥50% PD-L1⁺ tumour cells.

Figure 3. Survival outcomes in the biomarker population of coBRIM treated with placebo plus vemurafenib. Patient subgroups were defined by various cutoff thresholds of proportion of PD-L1⁺ immune cells, as follows: (A) PFS and (B) OS outcomes by PD-L1⁺ immune cells <1% (IC0) vs ≥1% (IC1, 2 and 3). (C) PFS and (D) OS outcomes by PD-L1⁺ immune cells <10% (IC0, 1 and 2) vs ≥10% (IC3).

Figure 4. Survival outcomes in the biomarker population of coBRIM treated with cobimetinib combined with vemurafenib. Patient subgroups were defined by various cutoff thresholds of proportion of PD-L1⁺ immune cells, as follows: (A) PFS and (B) OS outcomes by PD-L1⁺ immune cells <1% (IC0) vs ≥1% (IC1, 2 and 3). (C) PFS and (D) OS outcomes by PD-L1⁺ immune cells <10% (IC0, 1 and IC2) vs ≥10% (IC3).

Table 1. Objective response rates by proportion of PD-L1⁺ immune cells in tumour sections.

PD-L1 subgroup	Proportion PD-L1–	C + V	P + V
	positive/section		
IC0	<1%	68.4% (26/38)	34.7% (17/49)
IC1	≥1% to <5%	67.6% (23/34)	58.1% (18/31)

IC2	$\geq 5\%$ to $< 10\%$	78.6% (11/14)	61.5% (8/13)
IC3	$\geq 10\%$	62.5% (10/16)	60.0% (9/15)

C, cobimetinib; P, placebo; PD-L1, programmed death ligand-1; V, vemurafenib.

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No. at risk

P + V	248	122	62	44	31	18	1
C + V	247	175	115	81	65	35	1

No. at risk

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P + V	248	194	142	107	84	65	15
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No. at risk

<1% PD-L1 ⁺ immune cells	49	20	13	8	4	1	0
≥1% PD-L1 ⁺ immune cells	59	31	19	13	11	5	0

No. at risk

<1% PD-L1 ⁺ immune cells	49	36	24	19	12	12	2
≥1% PD-L1 ⁺ immune cells	59	48	35	26	25	15	3

No. at risk

<10% PD-L1 ⁺ immune cells	93	41	25	15	9	3	0
≥10% PD-L1 ⁺ immune cells	15	10	7	6	6	3	0

No. at risk

<1% PD-L1 ⁺ immune cells	38	28	21	14	11	6	1
≥1% PD-L1 ⁺ immune cells	64	45	28	19	17	9	0

No. at risk

<1% PD-L1 ⁺ immune cells	38	34	28	23	16	12	4
≥1% PD-L1 ⁺ immune cells	64	54	44	34	28	19	2

No. at risk

<10% PD-L1 ⁺ immune cells	86	61	40	27	22	14	1
≥10% PD-L1 ⁺ immune cells	16	12	9	6	6	1	0

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Title:

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