Toripalimab plus axitinib in patients with metastatic mucosal melanoma: 3-year survival update and biomarker analysis

Siming Li,1 Xiaowen Wu,1 Xieqiao Yan,1 Li Zhou,1 Zihong Chi,2 Chuanliang Cui,2 Bixia Tang,2,1 Lili Mao,2 Bin Lian,2 Xuan Wang,2 Xue Bai,2 Jie Dai,2 Yan Kong,2 Xiongwen Tang,3,4 Hui Feng,3,4 Sheng Yao,3,4 Keith T Flaherty,5 Jun Guo,1 Xinan Sheng1

ABSTRACT

Background Mucosal melanoma is an aggressive melanoma subtype with poor response to anti-programmed cell death-1 (PD-1) monotherapy. Axitinib in combination with toripalimab, a humanized IgG4 mAb against PD-1, showed a promising response rate in patients with metastatic mucosal melanoma (MM) in a phase Ib study. Here, we report the updated overall survival (OS), duration of response (DoR), and biomarker analysis results.

Methods Patients with advanced MM received toripalimab 1 or 3 mg/kg intravenously every 2 weeks combined with axitinib 5 mg orally twice per day until disease progression or unacceptable toxicity. Tumor programmed cell death ligand-1 (PD-L1) expression, tumor mutational burden (TMB), and gene expression profile (GEP) by messenger RNA sequencing were evaluated for correlation with survival.

Results As of April 2, 2021, the median follow-up was 42.5 months. Among 29 chemotherapy-naive patients with metastatic MM, the median OS was 20.7 months (95% CI 9.7 to 32.7 months); the median progression-free survival (PFS) was 7.5 months (95% CI 3.8 to 14.8 months); and the median DoR was 13.4 months (95% CI 5.5 to 20.6 months). The OS rates of 1, 2, and 3 years were 62.1%, 44.8%, and 31.0%, respectively. Biomarker analysis found that PD-L1 expression and TMB level were not associated with survival benefits. In contrast, a 12-GEP signature correlated with improved PFS (17.7 vs 5.7 months, p=0.0083) and OS (35.6 vs 17.6 months, p=0.039).

Conclusions The 3-year survival update confirmed the antitumor activity and long-term survival benefit of the toripalimab plus axitinib combination in patients with advanced MM. The 12-gene GEP is of value in predicting the outcomes of vascular endothelial growth factor receptor-tyrosine kinase inhibitor and PD-1 blockade combination therapy, but requires further validation.

Trial registration numbers NCT03086174.

INTRODUCTION

Defined as a melanoma subtype originated from a mucous membrane, mucosal melanoma (MM) occurs most commonly in the oral and nasal cavities and gastrointestinal and genitourinary tracts with an occult but aggressive natural disease course and poor prognosis. MM represents a rare subtype of melanoma in the Caucasian population but constitutes the second most common subtype in the Asian population.

MM is genetically distinct from cutaneous melanoma (CM) with higher incidences in KIT and NRAS mutations but a lower rate of BRAF V600 alterations. In general, MM harbors a much lower tumor mutational burden (TMB) than CM, as DNA mutations caused by chronic ultraviolet sun exposure are not a major disease mechanism for MM. Such distinctions at the molecular level may lead to different responses to standard treatment between these two melanoma subtypes. In the past decade, the emergence of immune checkpoint inhibitors (ICIs) represented by antiprogrammed cell death-1 (PD-1) antibody and anticytotoxic T-lymphocyte antigen-4 (CTLA-4) antibody brought enormous advances to the clinical management of CM but less so for MM. Data from clinical trials demonstrated that the overall response rates (ORRs) from PD-1 blockade in MM from both the Asian (0%–13.3%) and Caucasian population (23.3%) were much lower than that of CM (33.7%–43.7%) even. Even the dual inhibition of PD-1 and CTLA-4 pathways yielded limited improvement in response rates (37.1%–43.0%) for patients with MM in the Caucasian population, with a median progression-free survival (PFS) of only 5.8–5.9 months.

Recently, a prospective phase II trial from China randomly assigned 114 patients with metastatic MM to a paclitaxel and carboplatin treatment with or without bevacizumab in the first-line setting.
with bevacizumab in this trial significantly prolonged both the PFS (4.8 vs 3.0 months) and overall survival (OS) (13.6 vs 9.0 months) when compared with chemotherapy alone.\textsuperscript{18} The efficacy of the bevacizumab-containing regimen emphasizes the importance of incorporating an antivascular endothelial growth factor (VEGF) therapy in the therapeutic paradigm for patients with MM.\textsuperscript{4} In vivo studies have also shown that angiogenesis inhibition, specifically simultaneous inhibition of the vascular endothelial growth factor receptor (VEGFR) and PD-1 pathways in a mouse colon cancer model, increased T-cell infiltration and suppressed tumor growth synergistically.\textsuperscript{19}

We conducted a phase Ib combination study of axitinib, a VEGFR tyrosine kinase inhibitor, with toripalimab, a humanized immunoglobulin G4 monoclonal antibody against PD-1, to treat patients with metastatic MM (ClinicalTrials.gov).\textsuperscript{20} This was the first study testing the combination of immunotherapy and VEGFR-targeting therapy in treatment-naïve patients with advanced MM. In the first analysis as of December 19, 2018, the combination demonstrated a manageable safety profile and showed promising antitumor activity (ORR 48.3%, median PFS 7.5 months).\textsuperscript{20} Based on the results, the combination of toripalimab plus axitinib for the treatment of MM was granted the orphan-drug and fast-track designation by the US Food and Drug Administration (FDA) for the first-line treatment of MM. A global phase III trial of toripalimab in combination with axitinib versus pembrolizumab for the first-line treatment of patients with advanced MM is planned. Nevertheless, the median OS and the median duration of response (mDoR) was not mature by the cut-off date in the first report.\textsuperscript{20} Here we report the 3-year survival data and updated biomarker analyses.

**METHODS**

**Patients and study design**

This was a phase Ib, single-center, open-label, two-part (part A dose escalation, and part B cohort expansion) clinical trial (ClinicalTrials.gov). Eligible patients with metastatic melanoma (part A) or pathologically confirmed metastatic MM (part B) with at least one measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1 at baseline, Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate organ and bone marrow function were enrolled. Exclusion criteria included history of autoimmune diseases, ongoing infections, or prior PD-1 checkpoint inhibitor therapy.

**Treatment and end points**

Details regarding the trial designs in parts A and B were provided in the original publication.\textsuperscript{20} Axitinib (5 mg) was given orally twice per day and toripalimab was intravenously infused at 1 or 5 mg/kg every 2 weeks (online supplemental figure 1) until disease progression or unacceptable toxicity. Responses were evaluated by investigators using both RECIST V.1.1 and immune-related RECIST (irRECIST). Patients who initially developed progressive disease (PD) per RECIST V.1.1 were allowed to continue therapy if the investigator considered patients to be benefiting from the treatment per irRECIST. The primary endpoint was safety, tolerability, and evaluation of dose-limiting toxicity of the combination treatment. The secondary endpoints included the pharmacokinetic profile and immunogenicity of toripalimab in the combination study, antitumor activity (ORR, disease control rate, DoR, PFS, and OS), and the status of antiprogrammed cell death ligand-1 (PD-L1) and other biomarkers as well as their correlations with clinical efficacy.

**PD-L1 expression analysis by immunohistochemistry (IHC)**

Fresh or archival tumor biopsy samples were obtained from each patient before treatment initiation. PD-L1 expression was assessed by IHC staining using an anti-PD-L1 antibody (clone SP263, Ventana) on a Ventana (Tucson, Arizona, USA) autostainer by certified pathologists.\textsuperscript{21} PD-L1 positive expression was defined as the presence of membrane staining of any intensity in ≥1% of tumor cells or the presence of PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥1% of tumor area occupied by tumor cells, associated intratumoral cells, and contiguous peritumoral stroma.

**Whole-exome sequencing (WES) and TMB analysis**

WES was performed using the Sure-Select Human All Exon V6 kit (Agilent, Santa Clara, California, USA) on tumor tissue sections and matched peripheral blood samples. Genomic alterations were assessed, which included microsatellite stability status, single-nucleotide variants, insertions/deletions (indels), copy number variants, and gene rearrangement and fusions.

The TMB was determined by analyzing somatic mutations, including coding base substitution and indels per million base pairs. The TMB\textsuperscript{High} group was defined as TMB of ≥6 mutations per million base pairs (Mbp), according to the original publication.\textsuperscript{20}

**Messenger RNA (mRNA) expression profile analysis**

Tumor biopsy tissues were used to isolate mRNA, followed by complementary DNA synthesis, then sequencing on the NovaSeq platform (Illumina, San Diego, California, USA). The relative abundance of each annotated transcript was recorded as transcripts per million and log2 transformed before analysis. Expression panels included inflammation signature (IL-6, CXCL1, CXCL2, CXCL3, CXCL8, and PTGS2),\textsuperscript{22} angiogenesis signature (VEGFA, KDR, ESM1, PECAM1, ANGPTL4, and CD34),\textsuperscript{22} and interferon gamma (IFN-γ) signature (IDO1, CXCL10, CXCL9, HLA-DRA, STAT1, and IFNG).\textsuperscript{23} A 12-gene expression signatures of eight immune-related genes (CD274/PD-L1, CXCRL6, CD27, CXCL9, IDO1, TIGIT, PDCD1LG2/PD-L2, and LAG3) and four angiogenesis-related genes (ANGPTL5, ANGPTL6, CD34, and KDR) were derived from panel\textsuperscript{22 23} with known association clinical benefits and were selected based on the best differential fit (responder vs non-responder).
The abundance of RNA transcripts of selected genes was loaded into the logistic regression model to best fit coefficients to achieve the best receiver operating characteristic performance. The mean expression of the genes composing the signature was calculated to obtain a gene expression profile (GEP) score for the expression signature of each sample. The GEP cut-off of 450 was chosen so that the ORRs were 100% in the GEP high group and 0% in the GEP low group.

**Statistical analysis**
Safety and efficacy analyses included all patients who received at least one dose of the study treatment. The ORR and its 95% exact CI were determined by the Clopper and Pearson method. PFS and OS were plotted using the Kaplan-Meier method, with medians and corresponding two-sided 95% CIs reported. A p value of <0.05 was considered statistically significant. Statistics analyses were performed using SAS V.9.4 or GraphPad Prism software (GraphPad Software, San Diego, California, USA).

**RESULTS**

**Patient population**
A total of 33 patients with advanced melanoma were enrolled in the study from April 25, 2017, to April 2, 2018 (online supplemental figure 2 and table 1). The majority of patients (31 of 33 patients) were naive to systemic chemotherapy. Among 31 treatment-naive melanomas, 2 were of unknown primary and 29 were pathologically confirmed MMs. By the cut-off date of April 2, 2021, 3 patients remained on the study treatment, 1 patient discontinued treatment due to an adverse event (AE), and 29 patients discontinued treatment due to PD. No new treatment-related AEs emerged during the 28 months since the previous report by the cut-off date of December 19, 2018. Incidences of permanent discontinuation due to AE and the use of corticosteroids remained unchanged from the previous report.

**Updated antitumor activity**
Among the 29 chemotherapy-naive patients with pathologically confirmed MM, one patient, who previously experienced a partial response (PR) as the best response, responded further and became a confirmed complete response (CR) assessed by both RECIST and irRECIST. The overall responses per RECIST included 1 CR, 13 PRs, 11 stable diseases (SDs), and 4 PDs (figure 1A,B). The overall responses per irRECIST included 1 CR, 14 PR, 10 SD, and 4 PD. The ORR by RECIST and irRECIST were 48.3% (95% CI 29.4% to 67.5%) and 51.7% (95% CI 32.5% to 70.6%), respectively. The mDoR was immature in the first report. As of April 02, 2021, the mDoR per RECIST was 13.4 months (95% CI, 5.5 to 20.6 months). The median PFS per RECIST was 7.5 months (95% CI, 3.8 to 14.8 months). The PFS rates of 1, 2, and 3 years per RECIST were 41.4% (95% CI 23.65% to 58.27%), 13.8% (95% CI 4.35% to 28.61%), and 10.3% (95% CI 2.63% to 24.30%), respectively (figure 2A,B).

**Updated OS**
By the cut-off date of the first report, 10 of 29 chemotherapy-naive patients had died and the median OS was not reached. During the additional 28-month follow-up period after the first analysis, 15 additional OS events were recorded. After a median survival follow-up time of 42.5 months (range, 1.47 to 43.74 months), the OS rates of 1, 2, and 3 years were 62.1% (95% CI 42.06% to 76.90%), 44.8% (95% CI 26.52% to 61.57%) and 31.0% (95% CI 15.56% to 47.91%), respectively (figure 2C). The median OS was 20.7 months (95% CI 9.7 to 32.7 months).

**Biomarker analysis**
We conducted exploratory studies to evaluate the correlation of baseline biomarkers with OS and PFS in the chemotherapy-naive patients with MM. The treatment effects on OS were analyzed across key subgroups (figure 3).

**PD-L1 expression**
As indicated in the first report of this study, PD-L1-positive patients responded with better ORR and PFS than PD-L1-negative patients to the combination therapy. Follow-up results showed no significant differences in PFS and OS between PD-L1-positive and PD-L1-negative patients: median PFS of 13.8 vs 5.9 months (HR 0.71, 95% CI 0.32 to 1.55; p=0.39) and median OS of 29.6 vs 17.8 months (HR 0.83, 95% CI 0.37 to 1.85; p=0.65) (figure 4A,B).

**Tumor mutational burden**
WES of 28 baseline tumors and matched peripheral blood showed that TMB was generally low in patients with MM in this study, with no patients with TMB greater than 20 mutations/Mbp (range 0.5–15.3 mutations/Mbp). A cut-off of the top 20% of TMB in this study (6 mutations/Mbp) and TMB Low (<6 mutations/Mbp) was selected, as suggested by Samstein et al. after a correlation study of TMB value with survival in multiple cancer types. In this follow-up report, no significant differences in PFS and OS were identified between TMBHigh (≥6 mutations/Mbp) and TMBLow (<6 mutations/Mbp) patients: median PFS 14.8 vs 6.7 months (HR 0.80, 95% CI 0.33 to 1.92; p=0.61) (figure 4C) and median OS 29.7 months vs 20.4 months (HR 0.77, 95% CI 0.31 to 1.94; p=0.59) (figure 4D).

**12-gene gene expression profiling (GEP) score**
RNA sequencing and expression profiling results were available from 24 patients with chemotherapy-naive MM. Three published signatures were compared with clinical outcomes as shown in online supplemental figure 3, including inflammation signature (IL-6, CXCL1, CXCL2, CXCL3, CXCL8, and PTGS2), angiogenesis signature (VEGFA, KDR, ESM1, PECAM1, ANGPTL4, and CD34), and IFN-γ signature (IDO1, CXCL10, CXCL9, HLA-DR, STAT1, and IFNG). However, none of the expression signature scores are significantly different between
The length of the bar represents maximal decrease or minimal increase in target lesion(s). (B) Spider plot. Change in individual tumor burden over time from baseline assessed by investigator per RECIST V.1.1 (n=33). PD, progressive disease; PD-L1, programmed cell death ligand-1; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; TMB, tumor mutational burden.

**Other biomarkers**

Analysis of WES data shows that the prevalence of mutations in MM is relatively low, and no significant differential mutation profiling is observed between responders and non-responders. Baseline-level lactate dehydrogenase, which was deemed as a prognostic predictor for responder (CR+PR) and non-responder (SD+PD). The 12-gene signatures combined eight selected immune-related genes (CD274/PD-L1, CXCR6, CD27, CXCL9, IDO1, TIGIT, PDCD1LG2/PD-L2, and LAG3) with four angiogenesis-related genes (ANGPTL5, ANGPTL6, CD34, and KDR) and were thus selected and evaluated with efficacy. The GEP score value of 450 was used as the cut-off in this study. The GEP cut-off of 450 was chosen so that the ORRs were 100% in the GEP high group and 0% in GEP low group. Patients with a GEP of ≥450 had a statistically significant improvement in PFS (median PFS 17.7 vs 5.7 months: HR 0.28, 95% CI 0.11 to 0.72; p=0.0083) and OS (median OS 35.6 vs 17.6 months: HR 0.40, 95% CI 0.16 to 0.95; p=0.039) when compared with those with GEP of <450.
CM, had no significant impact on PFS and OS in this study (figure 3).

**DISCUSSION**
Establishing guidelines for the treatment of MM has been challenging due to the rarity of the disease. Chemotherapy was demonstrated to be less effective in MM than in CM.\(^{25}\) Antiangiogenic targeted therapy alone has not shown significant improvement compared with chemotherapy in melanoma. A multicenter phase II study of axitinib monotherapy in metastatic melanoma (predominantly CM) showed an ORR of 18.8%, while the median PFS and OS were only 3.8 and 6.6 months, respectively.\(^{26}\)

We are currently conducting a randomized, three-arm, multicenter phase II study (NCT03941795) in patients with advanced MM to compare the efficacy and safety of toripalimab plus axitinib versus axitinib or toripalimab monotherapy in the first-line setting, which would address the individual contribution in treating MM. In the era of immunotherapy, the historical ORR obtained by a PD-1

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| Group          | Parameters       | N  | OS and 95% CI     | P Value |
|----------------|------------------|----|-------------------|---------|
| Total evaluable|                  | 29 | 20.7 (9.7–32.7)   | 0.5579  |
| Age (years)    | ≤50              | 10 | 25.6 (15.1–41.0)  |         |
|                | >50              | 19 | 20.1 (9.7–37.6)   |         |
| Gender         | Male             | 12 | 20.1 (6.7–37.6)   | 0.2740  |
|                | Female           | 17 | 23.8 (6.7–40.1)   |         |
| ECOG           | 0                | 16 | 23.7 (8.9–37.6)   | 0.7790  |
|                | 1                | 13 | 20.7 (6.7–38.5)   |         |
| Stage          | III              | 6  | 14.3 (1.5–38.8)   | 0.5929  |
|                | IV, M1a          | 9  | 32.7 (9.7–NE)     |         |
|                | IV, M1b          | 8  | 18.9 (2.0–40.1)   |         |
|                | IV, M1c          | 6  | 13.7 (2.3–NE)     |         |
| Primary Site   | Nasal or Oral    | 11 | 26.8 (4.4–40.1)   | 0.9688  |
|                | Esophagus        | 6  | 18.7 (8.9–NE)     |         |
|                | Rectum           | 5  | 6.8 (2.3–NE)      |         |
|                | Genital tract    | 7  | 23.8 (6.7–32.7)   |         |
| LDH            | ≥ 280 U/L        | 6  | 8.9 (2.3–37.6)    | 0.1074  |
|                | < 280 U/L        | 18 | 20.4 (9.7–38.8)   |         |
|                | NA               | 5  | 32.7 (8.9–NE)     |         |
| TMB            | High (≥ 6 muts/Mb) | 6 | 29.7 (11.2–NE)    | 0.5856  |
|                | Low (< 6 muts/Mb) | 22 | 20.4 (6.7–37.6)   |         |
| PD-L1          | +                | 19 | 29.6 (2.9–38.5)   | 0.6492  |
|                | -                | 19 | 17.8 (6.7–37.6)   |         |
| GEP            | High (score ≥ 650) | 10 | 35.6 (6.7–NE)     | 0.0389  |
|                | Low (score < 650) | 14 | 17.6 (4.4–26.8)   |         |
|                | NA               | 5  | 10.1 (1.5–NE)     |         |

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**Figure 2** (A) DoR by RECIST V.1.1. (B) PFS by Response Evaluation Criteria in Solid Tumors V.1.1. (C) OS of 29 patients with chemotherapy-naive mucosal melanoma. Probability of survival is shown at indicated time points. Censored patients are marked with a vertical line in the graph. Numbers of patients at risk at indicated time points are shown below the x-axis. DoR, duration of response; OS, overall survival; PFS, progression-free survival.

**Figure 3** Forest plot. Subgroup analysis of overall survival of 29 patients with chemotherapy-naive mucosal melanoma. The scale is months (0–50) in the forest plot. ECOG, Eastern Cooperative Oncology Group; GEP, gene expression profile; LDH, lactate dehydrogenase; NA, not applicable; OS, overall survival; PD-L1, programmed cell death ligand-1; TMB, Tumor mutational burden.
inhibitor single-agent in MM was only 0%–23.3%,10–12 while the median PFS ranged from 1.9 to 2.8 months and the median OS ranged from 10.3 months to 11.3 months.10–12 The current study is the first to combine immunotherapy with antiangiogenic targeted therapy in treatment-naïve advanced MM. The combination had a tolerable safety profile and showed promising antitumor activity with an ORR of 48.3%, a median PFS of 7.5 months and a median OS rate of 20.7 months. The response was durable as the mDoR was 13.4 months.

As for the dual blockade of CTLA-4 and PD-1 pathways, a pooled analysis showed that among patients with MM who received the combination of nivolumab plus ipilimumab, the ORR (37.1%) and the median PFS (5.9 months) were only slightly improved than CTLA-4 or PD-1 blockade alone,16 while the median OS was not mature. Recently, the phase III CheckMate 067 study released the data from a subgroup analysis of MM.17 The ORRs were 7%, 30%, and 43%, in three arms treated with ipilimumab, nivolumab, and ipilimumab plus nivolumab, respectively. The median OS were 20.2 and 22.7 months in the nivolumab and ipilimumab plus nivolumab arms, respectively, while the median PFS were only 3.0 and 5.8 months. In CheckMate 067, after a minimum follow-up of 5 years in patients with untreated advanced melanoma (predominantly CM), the mDoR was not reached in the nivolumab monotherapy and the nivolumab plus ipilimumab group. The ongoing responses at 5 years were 62% and 61%, respectively.26 In contrast, in a pooled study evaluating the efficacy of anti-PD-1 agents in MM (n=35), the mDoR was 12.9 months.20 The mDoR observed in the current study was 13.4 months, which is similar to the reported anti-PD-1 monotherapy in MM but much shorter than that of CM, reflecting the divergent responses of these two melanoma subtypes to immunotherapy.

The OS result of the axitinib plus toripalimab was comparable to that of ipilimumab plus nivolumab (20.7 months vs 22.7 months). However, there were several major differences between the patients from the current study and the mucosal subgroup from CheckMate 067 that were treated with ipilimumab plus nivolumab. The patients were predominantly Caucasian in CM-067, while all were Asian in the current study. Sixty-eight percent of the patients in CM-067 had stage IV M1C disease, while only 18% of patients from the current study had M1C disease. It remains to be determined in a randomized trial which combination strategy will be the preferred first-line regimen for MM.

Compared with the limited efficacy by anti-PD-1 or axitinib monotherapy, the improved efficacy in this study showed synergistic effects of an antiangiogenic drug with immunotherapy. According to the theory of cancer-immunity cycle, activated T cells need to be trafficked and infiltrated into the tumor, and only when activated T cells overcome local inhibitory factors, in the tumor microenvironment (TME), they can recognize and eliminate tumor cells.29 The use of anti-VEGF-targeted drugs could enhance T-cell infiltration into the tumor and overcome the inhibition from the immune microenvironment. The theory of tumor vasculature normalization also supports this theory. Many studies showed that the use of anti-VEGF-targeted drugs can promote the normalization of tumor vasculature that can increase the infiltration of immune effector cells into tumors and convert the
intrinsically immunosuppressive TME to become immuno
nostimulatory. Thus, combining antiangiogenic therape
ties and immunotherapies might synergistically increase
the effectiveness of immunotherapy.

Besides the combination with immunotherapy in
the current study, antiangiogenic therapy also showed
significant benefits when combined with chemotherapy
in MM. In the phase II study, untreated patients with
advanced MM were 2:1 randomized to receive front-line
carboplatin plus paclitaxel with or without bevacizumab.
Although the ORR was not statistically different, both the
median PFS and median OS were significantly improved
in the combination arm. Although the front-line anti-PD
L1-based immunotherapy remains the preferred approach
for advanced CM without BRAF mutations, incorporating
VEGF-targeting therapy with immunotherapy could
potentially improve the clinical response in patients with
MM.

We also evaluated the predictive values of tumor PD-L1
expression, TMB, and inflammation and angiogenesis
expression signatures for survival. In the first report of
this study, PD-L1-positive expression was associated with
significantly longer PFS. In this updated analysis, the OS
between the PD-L1 and TMB subgroups had no statisti
cally significant differences. The SP263 antibody was
used for PD-L1 IHC staining in the study as it has shown
concordant staining results with other commonly used
PD-L1 IHC antibodies, including 22C3 and 28–8.31

PD-L1 expression has not been a reliable biomarker in
predicting the clinical benefits of ICIs. Several studies
have found no correlation between tumor PD-L1 expres
sion and the clinical efficacy of ICIs, and some patients
with negative PD-L1 expression have also achieved
durable clinical benefit.32 33 Moreover, PD-L1 IHC staining
method has several limitations,34 including the heteroge
neity of PD-L1 expression, no standardized approach for
PD-L1 testing, and the availability of tumor tissues.

TMB is used to quantify the number of somatic muta
tions in human tumors. A higher TMB value correlates
with a higher frequency of neoantigens35 and a more
favorable response to ICIs in certain solid tumors.
However, TMB is not correlated with clinical efficacy of
ICIs in several tumor types, such as breast cancer, glioma,
and prostate cancer. A study performed WES on 294
microsatellite stable tumors (including 151 melanomas)
and concluded that TMB did not have sufficient predic
tive power to distinguish tumor response from PD.36
Prediction incorporating multiple variables, such as TMB,
MHC haplotype and T-cell receptor repertoire, might be
needed.35 On the other hand, MM was demonstrated to
be a low-TMB tumor,37 which may explain the lack of clin
ical efficacy correlation with TMB in this study.

Different from the correlation of a single biomarker
with clinical outcomes, GEP comprehensively describes
the characteristics of TMEs, incorporating multiple path
ways related to antigen presentation, chemokine expres
sion, cytolytic activity, and adaptive immune resistance.23
In the KEYNOTE 001 trial, Ayers et al used an IFN-γ
signature (six genes including IDO1, CXCL10, CXCL9,
HLA-DRA, STAT1, and IFN-γ) and an expanded immune
(18-gene) signature to evaluate the correlation between
gene signatures and clinical outcomes in a cohort of 62
patients with melanoma receiving pembrolizumab mono
therapy. They found that these two sets of gene signatures
were significantly associated with ORR and PFS benefits.23

However, previous GEP studies were primarily focused
on CM, and the application of GEP to predict response to
immunotherapy in MM remains unknown. Furthermore,
unlike anti-PD-1 monotherapy, VEGF/tyrosine kinase
inhibitor and anti-PD-1 combination possibly needs
tailored gene expression signatures specific to the combi
nation regimens to predict response.

The 12-gene expression signatures in the current study
were derived from panels with known association clinical
benefits (McDermott et al22 and Ayers et al23) and were
selected based on the best differential fit (responder vs
non-responder). We also compared three published signa
tures with clinical outcomes as described in the Methods
section and shown in online supplemental figure 3,
including inflammation signature,25 angiogenesis signa
ture,22 and IFN-γ signature.23 None of the expression
signatures could significantly differentiate responders
from non-responders. It is possible that an MM with a low
mutational burden might compromise the predictability of
these signatures. A derived panel to include genes
involved in both immune regulation/inflammation and
angiogenesis might be more suitable to predict the clin
ical response of the combination therapy. The 12-gene
expression signatures of eight immune-related genes
(CD274/PD-L1, CXCR6, CD27, CXCL9, ID01, TIGIT,
PDCD1LG2/PD-L2, and LAG3) and four angiogenesis
related genes (ANGPTL5, ANGPTL6, CD34, and KDR)
were thus selected to construct a logistic regression
model to differentiate patients with different efficacy.22 23
The inflammation and angiogenesis signature GEP scores
were found to be associated with improved ORR and DCR
in the first report of this study. In this updated analysis,
patients with GEP of ≥450 had statistically significant
longer PFS (17.7 vs 5.7 months) and OS (35.6 vs 17.6
months) than those with GEP of <450. To our know
ledge, this is the first study reporting the utility of GEP to
predict not only the ORR and PFS but also OS benefits
in response to the combination of an anti-VEGF therapy
plus an ICI therapy in patients with MM. Nevertheless,
the utility of the 12-gene GEP to predict clinical response
to the combination of axitinib and toripalimab requires
further validation in a prospective randomized trial.

In conclusion, this updated report confirms the anti
tumor activity of the combination of toripalimab with
axitinib in patients with advanced MM, including long
term survival benefits. A limitation of the current study
is that the efficacy evaluation was assessed by the inves
tigator in a single-arm study. The clinical efficacy of
the combination therapy as well as the utility of the 12-gene
GEP to predict clinical response is yet to be confirmed by
an independent central radiology review in the phase III trial of toripalimab plus axitinib versus pembrolizumab as first-line treatment for patients with advanced MM (NCT04394975).

**Author affiliations**
1. Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Genitourinary Oncology, Peking University Cancer Hospital and Institute, Beijing, China
2. Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Melanoma and Sarcoma, Peking University Cancer Hospital and Institute, Beijing, China
3. Medical Department, Shanghai Junshi Biosciences Co., Ltd, Shanghai, China
4. Medical Department, TopAlliance Biosciences, Inc, Rockville, Maryland, USA
5. Cancer Center, Massachusetts General Hospital, Boston, Massachusetts, USA

**Acknowledgements**
This study is sponsored by Shanghai Junshi Biosciences. The authors thank the patients who participated in this study and their families.

**Contributors**
JG, XS, KF, and SY performed study conception and design. XS, SL, LZ, XY, ZC, LS, CC, BT, LM, and XWu provided the study materials or patients. JG, XS, SL, XWu, LZ, XY, BT, LM, BL, XWu, XB, YD, YK, XT, HF, and SY contributed to the collection and assembly of data. Data analysis and interpretation were performed by XS and SY. All authors participated in the writing of the manuscript and read and approved the final manuscript. XS, as a guarantor, accepts full responsibility for the overall content.

**Funding**
This work was supported by grants from National Natural Science Foundation of China (81972562 and 82212604), Beijing Municipal Administration of Hospitals’ Ascent Plan (DFL20181101), and Beijing Municipal Science and Technology Commission (Z161100000516062).

**Competing interests**
JG is a member of the advisory board/consultant of MSD, Roche, Pfizer, Bayer, Novartis, Simcere, Shanghai Junshi Bioscience, and Orientgene. KF serves on the board of directors of Luxo Oncology, Clovis Oncology, Strata Oncology, and Vivid Biosciences; on the corporate advisory boards of X4 Pharmaceuticals and PTC Therapeutics; on the scientific advisory boards of Sanofi, Amgen, Asana, Adapimmune, Fent, Aegle, Array BioPharma, Shattuck Labs, Arch Oncology, Teler, Apricity, Oncodeutics, Fog Pharma, Neon Therapeutics, and Tvardi; and as a consultant to Novartis, Genentech, BMS, Merck, Takeda, Verastem, Checkmate, Boston Biomedical, Pierre Fabre, Cell Medica, and Debiopharm. XT, HF, and SY are employed by Shanghai Junshi Bioscience. The rest of the authors have no disclosures of potential conflicts of interest.

**Patient consent for publication**
Not applicable.

**Ethics approval**
This study involves human subjects and was approved by the Peking University Cancer Hospital institutional review board (IRB-OF-07.1-V1.0) and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Subjects gave informed consent to participate in the study before taking part.

**Provenance and peer review**
Not commissioned; externally peer reviewed.

**Data availability statement**
Data are available upon reasonable request. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Supplemental material**
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**REFERENCES**
1. Shoushtari AN, Bluth MJ, Goldman DA, et al. Clinical features and response to systemic therapy in a historical cohort of advanced or unresectable mucosal melanoma. *Melanoma Res* 2017;27:57–64.
2. McLaughlin CC, Wu X-C, Jermal A, et al. Incidence of noncutaneous melanomas in the U.S. *Cancer* 2005;103:1000–7.
3. Chi Z, Li S, Sheng X, et al. Clinical presentation, histology, and prognoses of malignant melanomas in ethnic Chinese: a study of 522 consecutive cases. *BMC Cancer* 2011;11:85.
4. Shoushtari AN. Incorporating VEGF blockade into a shifting treatment paradigm for mucosal melanoma. *J Clin Oncol* 2021;39:JCO2003523:867–9.
5. Ferreyra SJ, Turner S, Style JA, et al. Genome sequencing of mucosal melanomas reveals that they are driven by distinct mechanisms from cutaneous melanoma. *J Pathol* 2013;230:261–9.
6. Curtin JA, Busam K, Pinkel D, et al. Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol* 2006;24:4340–6.
7. Kim JH, Jung J, Knecht HH, et al. Oncogenic BRAF fusions in mucosal melanomas activate the MAPK pathway and are sensitive to MEK/PI3K inhibition or MEK/CDK4/6 inhibition. *Oncogene* 2017;36:3334–45.
8. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. *N Engl J Med* 2005;353:2135–47.
9. Hayward NK, Wilmott JS, Waddell N, et al. Whole-genome landscapes of major melanoma subtypes. *Nature* 2017;545:175–80.
10. Tang B, Chi Z, Chen Y, et al. Safety, efficacy, and biomarker analysis of toripalimab in previously treated advanced melanoma: results of the POLARIS-01 multicenter phase II trial. *Clin Cancer Res* 2020;26:4250–9.
11. Si L, Zhang X, Shu Y, et al. A phase Ib study of pembrolizumab as second-line therapy for Chinese patients with advanced or metastatic melanoma. *JAMA Oncol* 2016;2:1282–38.
12. Hamid O, Robert C, Ribas A, et al. Antitumour activity of pembrolizumab in advanced mucosal melanoma: a post-hoc analysis of KEYNOTE-001, 002, 006. *Br J Cancer* 2018;119:670–4.
13. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015;372:320–30.
14. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med* 2016;373:23–34.
15. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med* 2015;372:2521–32.
16. D'Angelo SP, Larkin J, Josan JA, et al. Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: a pooled analysis. *J Clin Oncol* 2017;35:226–35.
17. Shoushtari AN, Waghstaff J, Ascierto PA, Butler MO, et al. CheckMate 067: long-term outcomes in patients with mucosal melanoma. *JCO* 2020;38:10019.
18. Yan X, Sheng X, Chi Z, et al. Randomized phase II study of bevacizumab in combination with carboplatin plus paclitaxel in patients with previously untreated advanced melanoma. *J Clin Oncol* 2021;39:681–9.
19. Yasuda S, Sho M, Yamato I, et al. Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect in vivo. *Clin Exp Immunol* 2013;172:500–6.
20. Sheng X, Yan X, Chi Z, et al. Axitinib in combination with toripalimab, a humanized immunoglobulin G1 monoclonal antibody against programmed cell death-1, in patients with metastatic mucosal melanoma: an open-label phase Ib trial. *J Clin Oncol* 2019;37:2987–99.
21. Scorer P, Scott M, Lawson N, et al. Consistency of tumor and immune cell programmed cell death-1 expression within and between tumor blocks using the VENTANA SP263 assay. *Diag Pathol* 2018;13:47.
22. McDermott DF, Huseni MA, Atkins MB, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. *Nat Med* 2018;24:749–57.
23. Ayers M, Lenceford J, Nebozhyn M, et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest* 2017;127:2930–40.

**ORCID iDs**
Bixia Tang http://orcid.org/0000-0002-3458-461X
Xue Bai http://orcid.org/0000-0003-5203-4080
Sheng Yao http://orcid.org/0000-0003-9988-9937
Xinan Sheng http://orcid.org/0000-0001-9359-0975

8 Li S, et al. J Immunother Cancer 2022;10:e004036. doi:10.1136/jitc-2021-004036

J Immunother Cancer: first published as 10.1136/jitc-2021-004036 on 22 February 2022. Downloaded from http://jitc.bmj.com/ on February 22, 2022 by guest. Protected by copyright.
24 Samstein RM, Lee C-H, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* 2019;51:202–6.

25 Postow MA, Hamid O, Carvajal RD. Mucosal melanoma: pathogenesis, clinical behavior, and management. *Curr Oncol Rep* 2012;14:441–8.

26 Fruehauf J, Lutzky J, McDermott D, et al. Multicenter, phase II study of axitinib, a selective second-generation inhibitor of vascular endothelial growth factor receptors 1, 2, and 3, in patients with metastatic melanoma. *Clin Cancer Res* 2011;17:7462–9.

27 Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2019;381:1535–46.

28 Shoushtari AN, Munhoz RR, Kuk D, et al. The efficacy of anti-PD-1 agents in acral and mucosal melanoma. *Cancer* 2016;122:3354–62.

29 Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 2013;39:1–10.

30 Fukumura D, Kloepper J, Amoozgar Z, et al. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. *Nat Rev Clin Oncol* 2018;15:325–40.

31 Prince EA, Sanzari JK, Pandya D, et al. Analytical concordance of PD-L1 assays utilizing antibodies from FDA-approved diagnostics in advanced cancers: a systematic literature review. *JCO Precis Oncol* 2021;5:953–73.

32 Nishino M, Ramaiya NH, Hatabu H, et al. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. *Nat Rev Clin Oncol* 2017;14:655–68.

33 Topalian SL, Taube JM, Anders RA, et al. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer* 2016;16:275–87.

34 Torlakovic E, Lim HJ, Adam J, et al. "Interchangeability" of PD-L1 immunohistochemistry assays: a meta-analysis of diagnostic accuracy. *Mod Pathol* 2020;33:4–17.

35 Jardim DL, Goodman A, de Melo Gagliato D, et al. The challenges of tumor mutational burden as an immunotherapy biomarker. *Cancer Cell* 2021;39:154–73.

36 Miao D, Margolis CA, Vokes NI, et al. Genomic correlates of response to immune checkpoint blockade in microsatellite-stable solid tumors. *Nat Genet* 2018;50:1271–81.