ORIGINAL ARTICLE

BAP1 Deficiency Inflames the Tumor Immune Microenvironment and Is a Candidate Biomarker for Immunotherapy Response in Malignant Pleural Mesothelioma

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ABSTRACT

Introduction: Malignant pleural mesothelioma (MPM) is a rare and universally lethal malignancy with limited treatment options. Immunotherapy with immune checkpoint inhibitors (ICIs) has recently been approved for unresectable MPM, but response to ICIs is heterogeneous, and reliable biomarkers for prospective selection of appropriate subpopulations likely to benefit from ICIs remain elusive.

Methods: We performed multiscale integrative analyses of published primary tumor data set from The Cancer Genome Atlas (TCGA) and the French cohort E-MTAB-1719 to unravel the tumor immune microenvironment of MPM deficient in *BAP1*, one of the most frequently mutated tumor suppressor genes (TSGs) in the disease. The molecular profiling results were validated in independent cohorts of patients with MPM using immunohistochemistry and multiplex immunohistochemistry.

Results: We revealed that *BAP1* deficiency enriches immune-associated pathways in MPM, leading to increased mRNA signatures of interferon alfa/gamma response, activating dendritic cells, immune checkpoint receptors, and T-cell inflammation. This finding was confirmed in independent patient cohorts, where MPM tumors with low BAP1 levels are associated with an inflammatory tumor immune microenvironment characterized by increased exhausted precursor T-cells and macrophages but decreased myeloid-derived suppressor cells (MDSCs). In addition, $BAP1^{low}$ MPM cells are in close proximity to T cells and therefore can potentially be targeted with ICIs. Finally, we revealed that *BAP1*-proficient MPM is associated with a hyperactive mitogen-activated protein kinase (MAPK) pathway and may benefit from treatment with MEK inhibitors (MEKis).

Conclusion: Our results suggest that BAP1 plays an immunomodulatory role in MPM and that *BAP1*-deficient MPM may benefit from immunotherapy, which merits further clinical investigation.

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Introduction

Malignant pleural mesothelioma (MPM) is a rare but aggressive malignancy arising from mesothelial cells of the thoracic pleura.[1] The pathologic cause of MPM is highly correlated with asbestos exposure that induces chronic inflammation. Histologically, MPM is divided into the following three different subtypes: epithelioid (50%–60%), sarcomatoid [(10%), including rare desmoplastic subtype], and biphasic (30%–40%). Patients with epithelioid MPM usually have better survival. Compared with other cancers, MPM harbors a low Tumor Mutational Burden (TMB) and is characterized with high resistance to chemotherapy and predominant prevalence of genetic alterations that inactivate tumor suppressor genes, with *BAP1*, *CDKN2A*, and *NF2* being the most frequently mutated.[2–4] In the past decades, platinum-based chemotherapy has been the first-line treatment for unresectable MPM.[5] In 2021, immune checkpoint inhibitor (ICI)-based immunotherapy (combined antibodies targeting PD-1 [encoded by PDCD1] and anti–CTLA-4) was approved by the Food and Drug Administration (FDA) for advanced MPMs, with encouraging clinical activity compared with standard first-line chemotherapy. Nevertheless, the median life expectancy is one and a half years, with a survival benefit of 4 months only.[6] The heterogeneous response to ICIs in the clinic and the lack of reliable biomarkers challenge the benefit of immunotherapy for patients.[7] Therefore, it is urgent to identify credible biomarkers to improve immunotherapy and personalize treatment.

BAP1 (encoded by *BAP1*) is a nuclear deubiquitinase involved in chromatin modulation, homologous recombination, and programmed cell death.[8–10] Biallelic loss of BAP1 occurs in approximately 60% of patients with MPM, more often in epithelioid subtype,[11–13] with germline BAP1 abnormalities in less than 5% of MPMs.[14] Patients with *BAP1*-mutant MPMs have longer survival than those without somatic or germline mutations and benefit from platinum-based therapy.[15–17] A shred of growing evidence from tumors other than MPM also suggests a role for *BAP1* in immune response. In malignant peritoneal mesothelioma (PeM), *BAP1* haploinsufficiency forms a unique molecular subtype characterized by distinct immunomodulatory gene expression and an inflammatory tumor microenvironment.[18] In uveal melanoma, *BAP1* loss induces T-cell infiltration.[19] Nevertheless, few studies have specifically evaluated the association between BAP1 status and the tumor immune microenvironment (TIME) or immunotherapy response in MPM. In this study, we performed integrative analyses of two MPM cohorts to unravel the immunologic configuration and revealed that loss of BAP1 inflames the TIME and is a candidate biomarker for immunotherapy response.

Material and Methods

Data Retrieval and Processing

The RNA sequencing (RNA-seq) data (FPKM value), copy number variants (CNVs), genomic alterations, and clinical information of the MPM patient cohorts from two published data sets TCGA and the French MPM patient cohort (under accession code E-MTAB-1719), herein referred to as E-MTAB-1719 cohort,[2,20] were downloaded from the UCSC Xena (https://xenabrowser.net/datapages/). RNA-seq data were log2 transformed, and the GISTIC algorithm was used to process the CNV information. Transcriptomic data from immunotherapy-treated murine MPM (GSE63557; GSE117358) and human NSCLC (GSE126044; GSE135222) were downloaded from the Gene Expression Omnibus (GEO). Transcriptomic data from immunotherapy-treated human renal cell carcinoma have been previously reported.[21] Data set from metastatic melanoma treated with checkpoint blockade immunotherapy were

Table 1. Clinical Characteristics of Patients With MPM Used in This Study

Characteristics	Nanjing Cohort (n = 17)	Bern Cohort (n = 98)
Age (y), median	58 (32-69)	69 (44-87)
Sex		
Female (%)	7 (41%)	10 (10%)
Male (%)	10 (59%)	88 (90%)
Mesothelioma subtype		
Epithelioid, n (%)	12 (71%)	66 (67%)
Sarcomatoid, n (%)	0 (0%)	8 (8%)
Biphasic, n (%)	1 (5%)	20 (20%)
Not classified, n (%)	4 (24%)	4 (4%)
Stages		
TNM classification I-II, n (%)	4 (24%)	54 (55%)
TNM classification III-IV, n (%)	8 (47%)	42 (43%)
Not classified, n (%)	5 (29%)	2 (2%)
Neoadjuvant treatment		
Chemotherapy	2 (12%)	30 (31%)
Chemo and radiotherapy	0	33 (34%)
Treatment naive	8 (47%)	32 (33%)
No information	7 (41%)	3 (3%)
Survival, mo, median	N/A	16 mo (0.3-204 mo)

MPM, malignant pleural mesothelioma; N/A, not available.

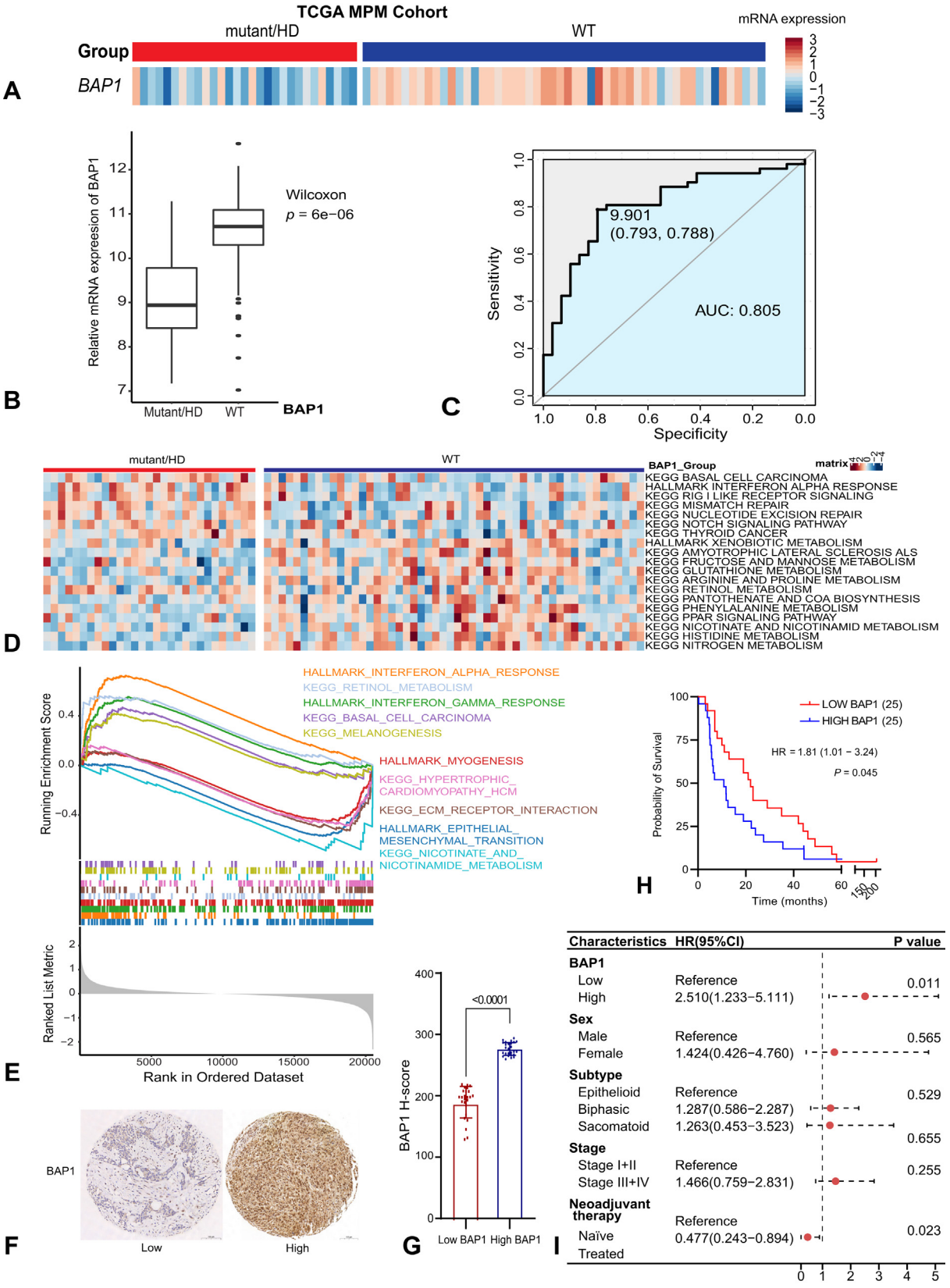

Figure 1. *BAP1* deficiency enriches immune response pathways and predicts favorable survival in MPM. (*A*, *B*) Correlation between genetic alteration and mRNA expression of *BAP1* in the TCGA cohort of patients with MPM (n = 87). Wilcoxon ranked sum test was used for comparison, *p* value less than 0.05 was considered significant. (*C*) ROC curves illustrate that the corresponding mRNA level of *BAP1* is a sensitive marker for predicting its genetic alterations. (*D*) Heatmap of GSVA illustrating

downloaded from https://ddbj.nig.ac.jp/resource/bioproject/PRJEB23709.

Identification of DEGs and Pathway Enrichment Analysis

Patients were classified according to *BAP1* genetic status. The empirical Bayesian approach of the limma R package was applied to identify differentially expressed genes (DEGs) from the gene expression profile between *BAP1*-deficient and *BAP1*-proficient subgroups. The criteria for determining DEGs were set with an adjusted *p* value less than 0.05. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the ClusterProfiler R package.

Immunologic Profiling of the TIME in MPM

The information on the 122 immunomodulators (MHC, immune receptors, chemokines, and immune stimulators) and 33 well-known effector genes of tumor-infiltrating immune cells was according to previous studies.[21–23] Gene signatures of 29 immune cell types and immune-related pathways were based on the previous study.[24] Exhausted and cytotoxic T-cell signatures were scored as the sum of the corresponding gene sets (exhausted: TIGIT, HAVCR2, CTLA4, LAG3, and PDCD1; cytotoxic: GZMA, GZMB, GZMK, IFNG, and IL2). Moreover, the 22 inhibitory immune checkpoints with therapeutic potential and 18 genes associated with T-cell inflammation as the T-cell inflamed score were used to predict the clinical response of ICIs.[25,26] Overall, we dissected the immunomodulatory role of *BAP1* genetic status by evaluating the immunologic pattern of the TIME from the above-mentioned aspects.

Patient Cohorts

Information from two independent cohorts of patients with MPM (Nanjing cohort and Bern cohort) is found in Table 1. The Nanjing cohort comprised 17 patients with MPM who underwent surgery between 2011 and 2020 at The First Affiliated Hospital of Nanjing Medical University. Among these, nine patients have both tumor specimens and adjacent normal pleural tissues. A tissue microarray (TMA) was retrospectively constructed from formalin-fixed, paraffin-embedded samples of these patients based on the protocol by Fedor et al.[27] This study was approved by the institutional review board (No. 2022-SRFA-328). The Bern cohort comprised 98 patients with consecutively resected MPM at Bern University Hospital (Inselspital) between 2003 and 2017. The procedure for processing patient tissue for TMA was approved by the Ethics Commission of the Canton of Bern (KEK Bern 2016-01497). All patients had signed an informed consent for the inclusion of their clinical data and specimens in our research projects.

IHC and Quantitative Analysis

Multiplexed immunohistochemistry (IHC) (CD8, MSLN, CD163, PD-1, and TCF1) of the Nanjing cohort was performed according to the manufacturer's instructions (Novo-light TSA, 6-Color Multiple IHC Kit, Cat. #H-D110061, WiSee Biotechnology, Shanghai, People's Republic of China) and stained with appropriate antibodies (Supplementary Table 1) using the BOND III automated system (Leica Biosystems, Newcastle, UK). In brief, sections of formalin-fixed, paraffin-embedded blocks were deparaffinized in xylene and rehydrated in ethanol. After microwave antigen retrieval in heated citric acid buffer (pH 6.0) for 10 minutes, endogenous peroxidase activity was blocked by 3% H_2O_2 for 10 minutes, and nonspecific binding sites were blocked by goat serum for 10 minutes. Primary antibodies (Supplementary Table 1) were incubated for 1 hour in a humidified chamber at room temperature, followed by incubation with the corresponding secondary horseradish peroxidase-conjugated polymer. Each target was visualized using fluorescein TSA Plus (1:100). Then, the slide was placed in a heated citric acid buffer (pH 6.0) using microwave antigen retrieval to remove redundant antibodies before the next step. Finally, nuclei were visualized with DAPI, and the sections were coverslipped using an anti-fade mounting medium. Whole TMA slides were scanned and imaged using the

significant (adjusted *p* value < 0.05) differentially altered pathways between *BAP1*-deficient and wild-type MPM tumors. The transcriptomic data of the TCGA cohort (GSE2549 data set) were downloaded from GEO. Wilcoxon ranked sum test was used for comparison. (*E*) GSEA graphs of the molecular pathways most significantly enriched in *BAP1*-deficient MPM compared with *BAP1*-wild-type MPM. The transcriptomic data set GSE2549 was used for GSEA, with Wilcoxon ranked sum test used for comparison. (*F*, *G*) IHC analysis of BAP1 protein level in the Bern cohort. (*F*) Representative images of $BAP1^{high}$ and $BAP1^{low}$, overall magnification: ×15; scale bar: 100 μm. (*G*) The IHC score of BAP1 (whole slide region) in $BAP1^{low}$ (n = 25) and $BAP1^{high}$ (n = 25) subgroups. The score was based on the corresponding protein levels at the 30% top high and low value in the MPM-TMA (n = 82). A two-tailed unpaired *t* test was used for comparison, and *p* value less than 0.05 was considered significant. (*H*) Kaplan-Meier univariate survival analysis based on the upper 30% ($BAP1^{high}$) and lower 30% ($BAP1^{low}$) of BAP1 protein expression in the Bern cohort of patients with MPM (n = 82). Cox regression was used to calculate HRs. (*I*) Multivariate Cox regression analyses of the Bern cohort of patients with MPM (n = 82) based on BAP1 expression, patient sex, histology subtype, staging, and treatment. The *p* value was calculated using the log-rank test. GEO, Gene Expression Omnibus; GSEA, gene set enrichment analysis; GSVA, gene set variation analysis; HR, hazard ratio; IHC, immunohistochemistry; MPM, malignant pleural mesothelioma; ROC, receiver operating characteristic.

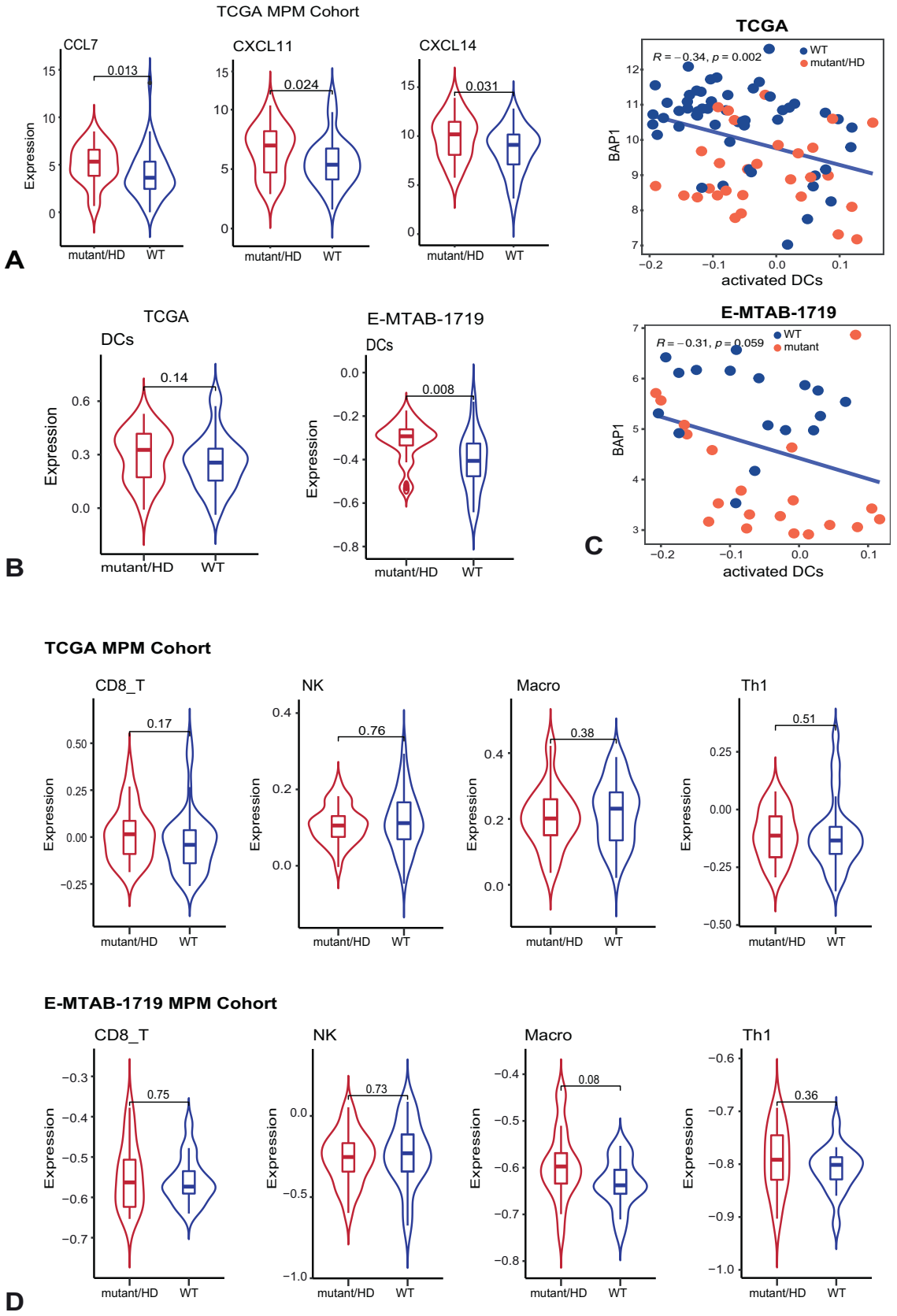

Figure 2. *BAP1* deficiency is correlated with increased immunogenicity in MPM. (*A*) The GSVA gene signature of chemokines (CCL7, CXCL11, CXCL14) are significantly (p value < 0.05) increased in *BAP1*-deficient MPM tumors compared with *BAP1*-wild-type MPM tumors across the TCGA MPM cohort (n = 87). Wilcoxon ranked sum test was used for comparison. (*B*) The GSVA

Pannoramic MIDI platform. IHC of the Bern cohort was similarly conducted. In brief, TMAs were sectioned at 4 mm, deparaffinized, rehydrated, and subsequently stained with appropriate antibodies (Supplementary Table 1) using the automated system BOND RX (Leica Biosystems). Visualization was performed using the Bond Polymer Refine Detection Kit (Leica Biosystems) as instructed by the manufacturer. Images were acquired using PANNORAMIC whole slide scanners.[28]

All IHC images were analyzed in batches using HALO software (version 3.1) to quantify positively stained cell rate (positive cells/total cells, %), positive cell density (positive cell number/tissue area tested, number/mm^2), mean density (integral optical density/positive area), and H-score defined as follows: H-Score = $\sum$(pi × i) = (percentage of weak intensity × 1) + (percentage of moderate intensity × 2) + (percentage of strong intensity × 3). The spatial distribution of T-cells was analyzed as described previously.[29]

Cell Viability Assay

For viability assay, the cells were seeded in 96-well plates (1000–1500 cells/well) as in proliferation assay. After 24 hours, one plate of viable cells was measured representing the time "0" plate and the other plates were brought to drug treatment for up to 5 days. The cell number was then determined by Acid Phosphatase Assay Kit (ab83367; Abcam), and the reported "cell change %" was determined as follows: 100 × (Value-Time 0)/(Baseline-Time 0). Unless otherwise noted, three independent experiments were performed, and a representative result is presented. Error bars are mean ± SD.

Prediction of Clinical Drug Response

The R package "pRRophetic" was used to predict each patient's response to common clinical chemotherapeutic drugs.[30] The half-maximal inhibitory concentration (IC_{50}) of each sample was calculated by ridge regression. In addition, a 10-fold cross-validation based on the CGP training set was applied to evaluate the prediction accuracy. Here, default options were used for all parameters.

Statistical Analysis

Comparisons between the two groups were made using Student's *t* test or Wilcoxon ranked sum test, depending on the normality as defined by GraphPad Prism 9.5.1. One-way or two-way analysis of variance (ANOVA) was performed to determine the comparisons among more than two groups. Tumor samples within all data sets were divided into two groups based on the best-separation cutoff value to plot the Kaplan-Meier survival curves using the "survminer" and "survival" R packages. Predictive values of categorical variables were assessed using the log-rank test. GraphPad Prism or R software (version 4.0.4, http://www.rproject.org) was used for statistics, and a p value less than 0.05 was considered statistically significant.

Results

BAP1 Plays an Immunomodulatory Role in MPM

BAP1 loss is a key driver of MPM tumorigenesis, but the underlying mechanisms remain poorly understood.[8] We evaluated MPM transcriptomes from the TCGA patient cohort (n = 87) and found that MPM tumors with *BAP1* deficiency (homozygous [biallelic] deletion or mutation), which reduces *BAP1* mRNA transcripts (Fig. 1*A–C*, Supplementary Fig. 1A–C), are enriched for hallmark gene sets involved in mismatch repair (MMR) and nucleotide excision repair (NER) (Fig. 1*D*), consistent with previous reports that *BAP1* mutation causes defects in homologous recombination (HR) repair of double-strand breaks (DSB)[31] and that MMR and NER are compensatory mechanisms of HR.[32] Strikingly, several immune response pathways (e.g., IFNα/γ response) are robustly enriched in *BAP1*-deficient MPM compared with *BAP1*-proficient tumors (Fig. 1*E*, Supplementary Fig. 1D), suggesting that *BAP1* deficiency enhances the immune response in MPM. Supporting this observation, although *BAP1* mRNA levels per se were not prognostic in the TCGA and E-MTAB-1719 cohorts (Supplementary Fig. 1E, F), patients with MPM with low BAP1 protein levels (BAP1^{low}) lived significantly (*p* value: 0.045) longer (31.9 mo versus 17.1 mo) than those with high BAP1 expression (BAP1^{high}) in the independent Bern cohort (n = 82; Fig. 1F–I; Table 1), consistent with previous reports that somatic and germline mutations in *BAP1* are favorably associated with patient survival in MPM.[15,33]

These results indicate that *BAP1* deficiency correlates with an enhanced immune response, suggesting an immunomodulatory role for BAP1 in MPM.

gene signature of the DCs are increased in *BAP1*-deficient MPM tumors compared with *BAP1*-wild-type MPM tumors across the TCGA (n = 87) and E-MATB-1719 (n = 33) MPM cohorts. Wilcoxon ranked sum test was used for comparison. (*C*) Correlation analysis between BAP1 mRNA level and the activated DC gene signatures in the TCGA and E-MATB-1719 cohorts of patients with MPM. (*D*) The gene signatures of the indicated tumor-infiltrating immune cells in *BAP1*-deficient and *BAP1*-wild-type MPM across the TCGA and E-MATB-1719 MPM cohorts. Wilcoxon ranked sum test was used for comparison. CD8_T, CD8$^+$ T cells; DC, dendritic cell; GSVA, gene set variation analysis; Macro, macrophages; MPM, malignant pleural mesothelioma; NK, natural killer cells; T_H1, type 1 T helper cells.

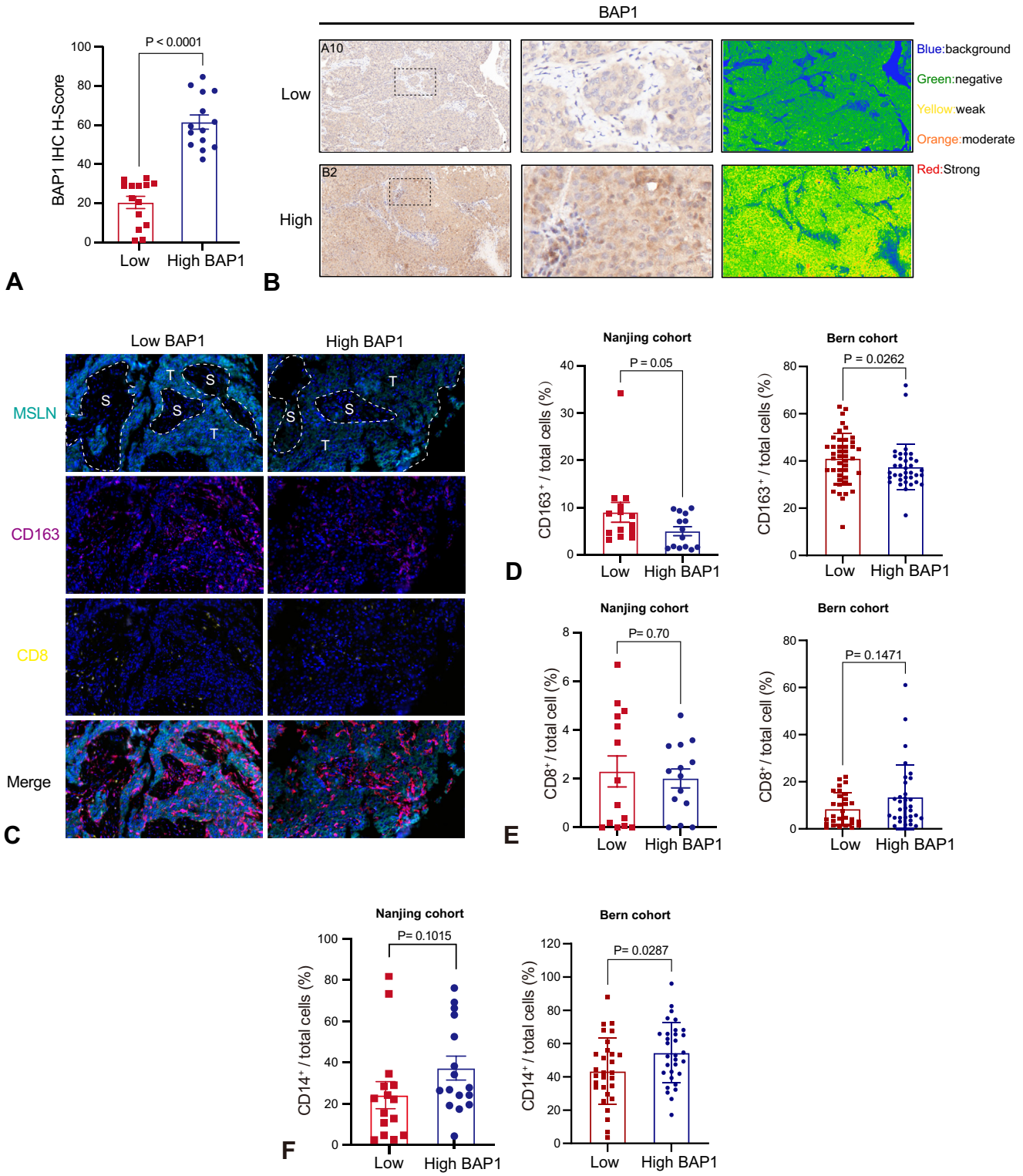

Figure 3. Immune cell infiltration in BAP1^{low} and BAP1^{high} MPM. (*A*) The IHC score of BAP1 in BAP1^{low} and BAP1^{high} subgroups. The stratified score was based on the corresponding protein levels at the median cutoff in the Nanjing cohort (n = 17). A two-tailed unpaired *t* test was used for comparison, and a *p* value less than 0.05 was considered significant. (*B*) Representative IHC images of BAP1 in the BAP1^{low} and BAP1^{high} subgroups. Original slides (left; overall magnification: ×200; scale bar: 50 μm), corresponding magnified regions (middle; scale bar: 20 μm) and the original IHC slides with gradient map visualization (right) are illustrated. Blue, insignificant (background); green, moderate significant; yellow, significant; orange, more significant; red, most significant. Images were acquired and processed using CaseViewer software. (*C*) Representative images and overlay of mIHC staining for tumor (MSLN) and the TIME (CD163, CD8) markers in BAP1^{low} and BAP1^{high} subgroups. Overall magnification: ×200; scale bar: 20μm. (*D*–*F*) Difference in expression of immune cell markers

BAP1 Deficiency Increases Immunogenicity in MPM

Next, we sought to evaluate the immune profiles of *BAP1*-deficient and -proficient MPM. Transcriptomic data from the TCGA and E-MTAB-1719 patient cohorts revealed that several immune cell-attracting chemokines such as the immuno-promoting *CCL7*, *CXCL11*, and *CXCL14* and the chemokine receptors *CCR8* and CXCR1, which are essential for the activation and trafficking of myeloid dendritic cells (DCs) and inflammatory mediators (e.g., macrophages),[34] are significantly up-regulated in *BAP1*-deficient MPM compared with *BAP1*–wild-type MPM (Fig. 2*A*; Supplementary Fig. 2A). Consistent with the finding, the expression of the DC signature is up-regulated in *BAP1*-deficient MPM (Fig. 2*B*) and *BAP1* expression is significantly negatively correlated with the gene signature of activated DCs (Fig. 2*C*), a professional antigen-presenting cell type with the unique ability to induce activation and differentiation of naive T lymphocytes.[35] These results suggest that BAP1 inactivation in MPM may promote DC recruitment and therefore increase immunity. A similar observation has been reported for peritoneal mesothelioma (PeM) and MPM.[18,36] Nevertheless, the gene signature of other immune cell types ($CD8^+$ T cells, NK cell, macrophages, and T_H1 cells) and immunomodulators are not significantly different between *BAP1*-deficient and –wild-type MPM cells (Fig. 2*D*; Supplementary Fig. 2B, C).

These observations prompted us to further investigate the tumor-infiltrating immune cells in two independent cohorts (Nanjing and Bern cohorts; Table 1). We stratified MPM based on BAP1 protein levels (Fig. 1*G*; Fig. 3*A*, *B*), and subsequent multiplex IHC (mIHC) analyses revealed a significant increase of $CD163^+$ M2 macrophages in $BAP1^{low}$ MPM, whereas the abundance of $CD8^+$ T cells is not significantly different between the *BAP1*-low and -high groups (Fig. 3*C*–*E*), consistent with the gene expression results from the TCGA and E-MTAB-1719 cohorts (Fig. 2*D*). In particular and interestingly, $CD14^+$ myeloid-derived suppressor cells (MDSCs), which suppress the function of $CD8^+$ T cells, are less abundant in the $BAP1^{low}$ group compared with $BAP1^{high}$ group (Fig. 3*F*).

These results indicate that BAP1 inactivation in MPM correlates with an increase in DCs and inflammatory macrophages and a decrease in immunosuppressive MDSCs, further supporting the notion that *BAP1* deficiency promotes the immune response in MPM. Our results are consistent with previous findings that inflammatory cytokines and M2-like macrophages are enriched in MPM and are crucial for disease pathogenesis.[37,38] Nevertheless, how BAP1 affects TIME, in particular the crosstalk between BAP1 and T lymphocyte status, remains unclear.

BAP1 Loss Inflames TIME and Is a Candidate Biomarker for Immunotherapy Response

We then investigated whether and how BAP1 modulates T lymphocyte infiltration in MPM. Our findings that *BAP1*-deficient MPM is associated with an enhanced immune response, for example, increased IFN-α/γ gene signatures, proinflammatory chemokines (CCL7, CXCL11, CXCL14), and macrophages (M2) and immunogenicity (activated DCs), but cytotoxic T cells are not significantly altered (Figs. 1–3), suggest that T cell exhaustion may manifest in *BAP1*-deficient MPM. T cell exhaustion has been found to span a spectrum from highly proliferative T cells with stem-like properties ("precursor-exhausted T cells") to T cells with complete loss of effector function and replicative capacity ("terminally exhausted T cells"), and "precursor-exhausted $CD8^+$ T cells," defined by the expression of TCF1, play a key role in mediating the efficacy of cancer immunotherapies.[39] We therefore investigated whether BAP1 status affects "precursor-exhausted T cells" in MPM. Our TMA IHC staining revealed that $BAP1^{low}$ MPM tumors were indeed enriched for precursor-exhausted $CD8^+$ T cells positive for TCF1 and PD-1 ($TCF1^+/PD\text{-}1^+/CD8^+$) (Fig. 4*A*). In support of this finding, *BAP1*-deficient MPM had an up-regulated gene signature of T-cell inflammation (T-cell inflamed score) and exhausted T-cells, but not cytotoxic T-cells (Fig. 4*B*, *C*; Supplementary Fig. 4A), consistent with our IHC results from the Bern cohort (Fig. 3*D*). Moreover, BAP1 inactivation significantly increased the mRNA expression of the immune checkpoint receptors *CD274* (PD-L1), *PDCD1* (PD-1), and *LAG3* (LAG3) in primary TCGA tumors, which are critical mediators of T-cell exhaustion[40] (Fig. 4*D*, *E*). Finally, our mIHC revealed a generally closer proximity between $CD8^+$ T cells and mesothelin-positive ($MSLN^+$) MPM cells in $BAP1^{low}$ compared with $BAP1^{high}$ tumors (Fig. 4*F*, Supplementary Fig. 3A, B). These results indicate that *BAP1*-deficient MPM is associated with an inflammatory TIME characterized by activation of immune checkpoint receptors, increased exhausted T cells, and their proximity to tumor cells.

As "precursor-exhausted $CD8^+$ T cells" play a key role in mediating the efficacy of cancer immunotherapies that act by reactivating exhausted T cells,[41,42] we next investigated whether BAP1 status is predictive of

(CD8, CD163, and CD14) between $BAP1^{low}$ and $BAP1^{high}$ subgroups. The *p* value less than 0.05 was considered significant. IHC, immunohistochemistry; mIHC, multiplex IHC; MPM, malignant pleural mesothelioma; TIME, tumor immune microenvironment.

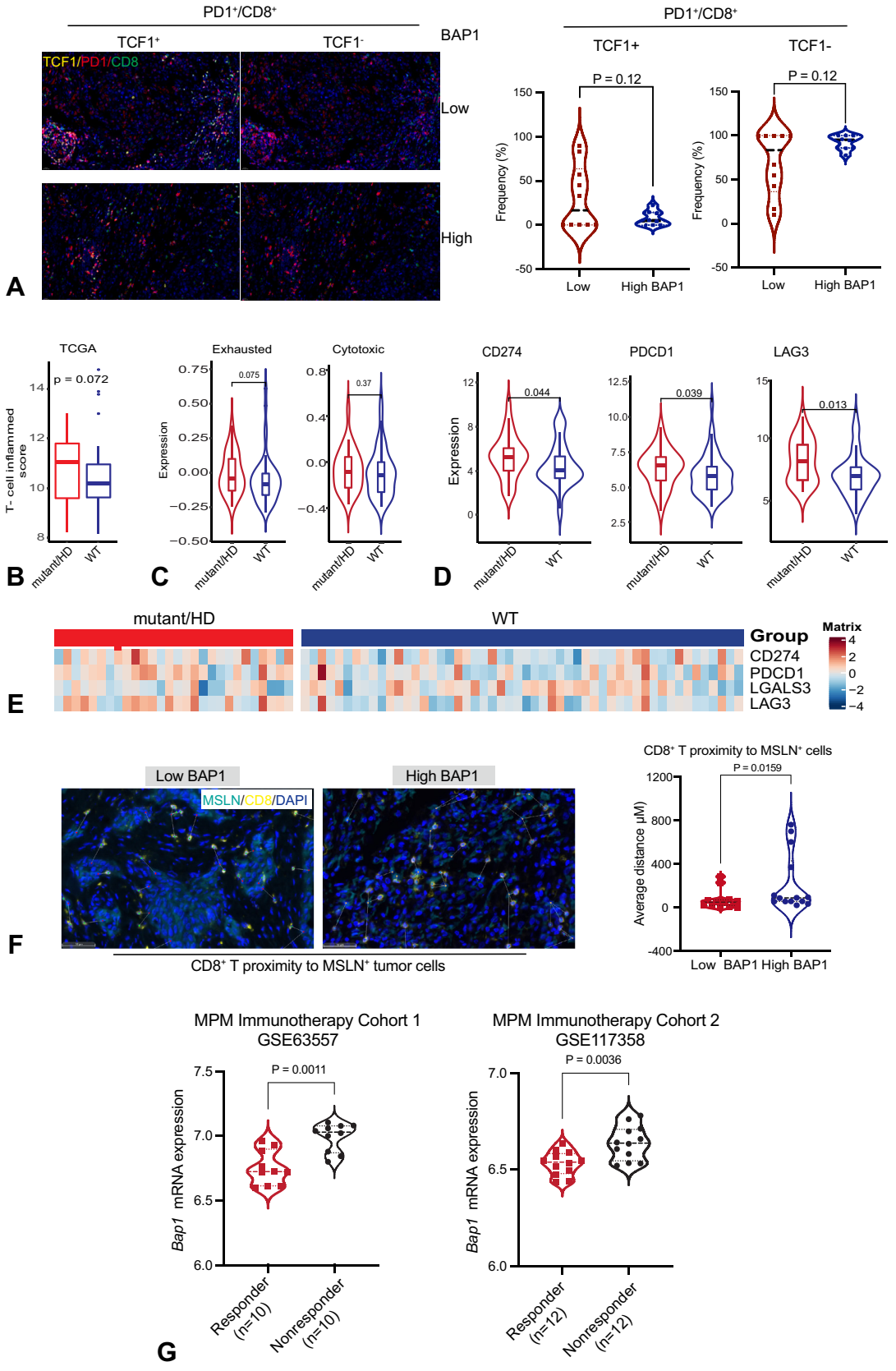

Figure 4. *BAP1*-deficient MPM is characterized by an inflamed TIME and can potentially be targeted by immunotherapy. (*A*) IHC staining (CD8, PD1, and TCF1) and quantification of exhausted T cells ($CD8^+/PD1^+/TCF1^+$) in $BAP1^{low}$ and $BAP1^{high}$ subgroups of the Nanjing cohort. Wilcoxon ranked sum test was used for comparison, and *p* value less than 0.05 was

response to immunotherapy in MPM. Notably, TMB, a valuable predictive biomarker of response to checkpoint inhibitors, is not significantly different between *BAP1*-deficient and -proficient MPM tumors (Supplementary Fig. 4B), consistent with the concept that TMB is not predictive of immunotherapy response in MPM.[43] Importantly, transcriptomic data from two independent cohorts of immunotherapy-treated murine MPM (GSE63557; GSE117358) revealed that *Bap1* expression dichotomized MPM, with ICI responders expressing significantly lower levels of *Bap1* than nonresponders (Fig. 4*G*). Similar results were obtained in patients with melanoma, renal cancer, and NSCLC, in which BAP1 is altered at different frequencies (Supplementary Fig. 5A), and patients whose tumors possess low levels of *BAP1* are associated with a better response to immunotherapy (Supplementary Fig. 5A, B).

Taken together, these results suggest that *BAP1* loss inflames the TIME and that *BAP1* status is a candidate biomarker for ICI response in MPM that warrants further clinical investigation.

BAP1-Proficient MPM Is Therapeutically Vulnerable to MAPK Inhibitors

We next explored therapeutic vulnerabilities associated with *BAP1*-proficient MPM, which is generally surrounded by an immunosuppressive niche and responds poorly to immunotherapy. Drug sensitivity profiling revealed that *BAP1*-intact MPM exhibited significantly greater sensitivity (low IC_{50}) to several compounds targeting the MAPK-ERK pathway, for example, SB590885 (RAFi), PD 0325901 (MEKi), RDEA-119/Refametinib (MEKi), and CI-1040/PD184352 (MEKi), than *BAP1*-deficient tumors (Fig. 5*A*, *B*). Given that MPM tumors with high MAPK activities (e.g., MAPK pathway reactome) are significantly negatively correlated with the IC_{50} of RAFi/MEKi, such as RDEA119, CI.1040, and SB590885 (Fig. 5*C*), these results suggest that *BAP1*-proficient MPM tumors may feature high MAPK activities. Indeed, gene set enrichment analysis of the TCGA and E-MTAB-1719 patient cohort revealed that *BAP1*-proficient MPM is significantly enriched for KRAS signaling gene signature (Fig. 5*D*) and IHC analysis of the Bern patient cohort confirmed that $BAP1^{high}$ tumors had higher levels of phosphorylated ERK1/2 (p-ERK), the marker of the KRAS-MAPK pathway activity (Fig. 5*E*, *F*). In support of these findings, we confirmed in vitro that *BAP1*-proficient H2052 cells are significantly more sensitive to the MEKi trametinib than *BAP1*-deficient MESO-4 cells and normal mesothelial Met-5A cells (Fig. 5*G*).

These results are in line with our recent finding that MAPK is a promising therapeutic target in MPM[28] and further suggest that clinically viable drugs targeting MAPK-ERK signaling could be prioritized for *BAP1*-intact MPM.

Discussion

Cancer immunotherapy with ICIs that suppress the delivery of co-inhibitory signals to T cells through CTLA-4 and PD1 has revolutionized the clinical management of multiple tumors. Nevertheless, only a few patients affected by an ICI-sensitive tumor type respond to standalone immunotherapy and the biological processes determining an effective outcome are poorly understood.[6,7] Mesothelioma has been found to be non-immunogenic with low TMB and rare antitumor immune cells in the TIME, which limits clinical benefits of ICIs in patients with MPM.[2,37] In this study, we provide evidence that *BAP1*-deficient MPM presents an immune-inflamed TIME and may benefit from immunotherapy. We also reveal that *BAP1*-proficient MPM is endowed with a hyperactive MAPK and therefore can benefit from MAPKi. These findings suggest an immunomodulatory role for BAP1 in MPM and that BAP1 status is a candidate predictive biomarker to dichotomize MPM for immuno- and MAPK-targeted therapy.

BAP1 plays a pivotal role in regulating chromatin modulation, homologous recombination, and programmed cell death.[44] BAP1 is frequently dysregulated in MPM, but whether and how BAP1 status defines a unique subset of MPM vulnerable to cancer treatment remains unclear. Here, our molecular profiling data

considered significant. Overall magnification: ×200; scale bar: 20 μM. (*B*) The mRNA score of T cell-inflamed genes in *BAP1*-deficient and wild-type subgroups in the TCGA MPM cohort. Wilcoxon ranked sum test was used for comparison and *p* value less than 0.05 was considered significant. (*C*) The mRNA score of the exhausted and cytotoxic T cell gene signatures in *BAP1*-deficient and *BAP1*-wild-type MPM in the TCGA MPM cohort. Wilcoxon ranked sum test was used for comparison. (*D*) Violin plot and (*E*) heatmap revealing the mRNA expression of inhibitory immune checkpoints (*CD274*, *PDCD1*, *LAG3*, and *LGALS3*) in *BAP1*-deficient and *BAP1*-wild-type MPM across the TCGA MPM cohort. Wilcoxon ranked sum test was used for comparison. (*F*) Analysis of the spatial distribution between $CD8^+$ T cells and $MSLN^+$ tumor cells. Representative images are illustrated on the left at original magnification ×400. The average distance between $CD8^+$ T cells and $MSLN^+$ tumor cells in $BAP1^{low}$ and $BAP1^{high}$ subgroups is found on the right. Wilcoxon ranked sum test was used for comparison, and *p* value less than 0.05 was considered significant. (*G*) *Bap1* mRNA expression is significantly higher in murine MPM tumors that responded to ICIs (responders) than in murine MPM tumors that did not respond to ICIs (nonresponders). The transcriptomic data sets (GSE63557; GSE117358) from mice with MPM treated with immunotherapy were used for the analysis. A two-tailed unpaired *t* test was used for comparison, and *p* value less than 0.05 was considered significant. ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; MPM, malignant pleural mesothelioma; TIME, tumor immune microenvironment.

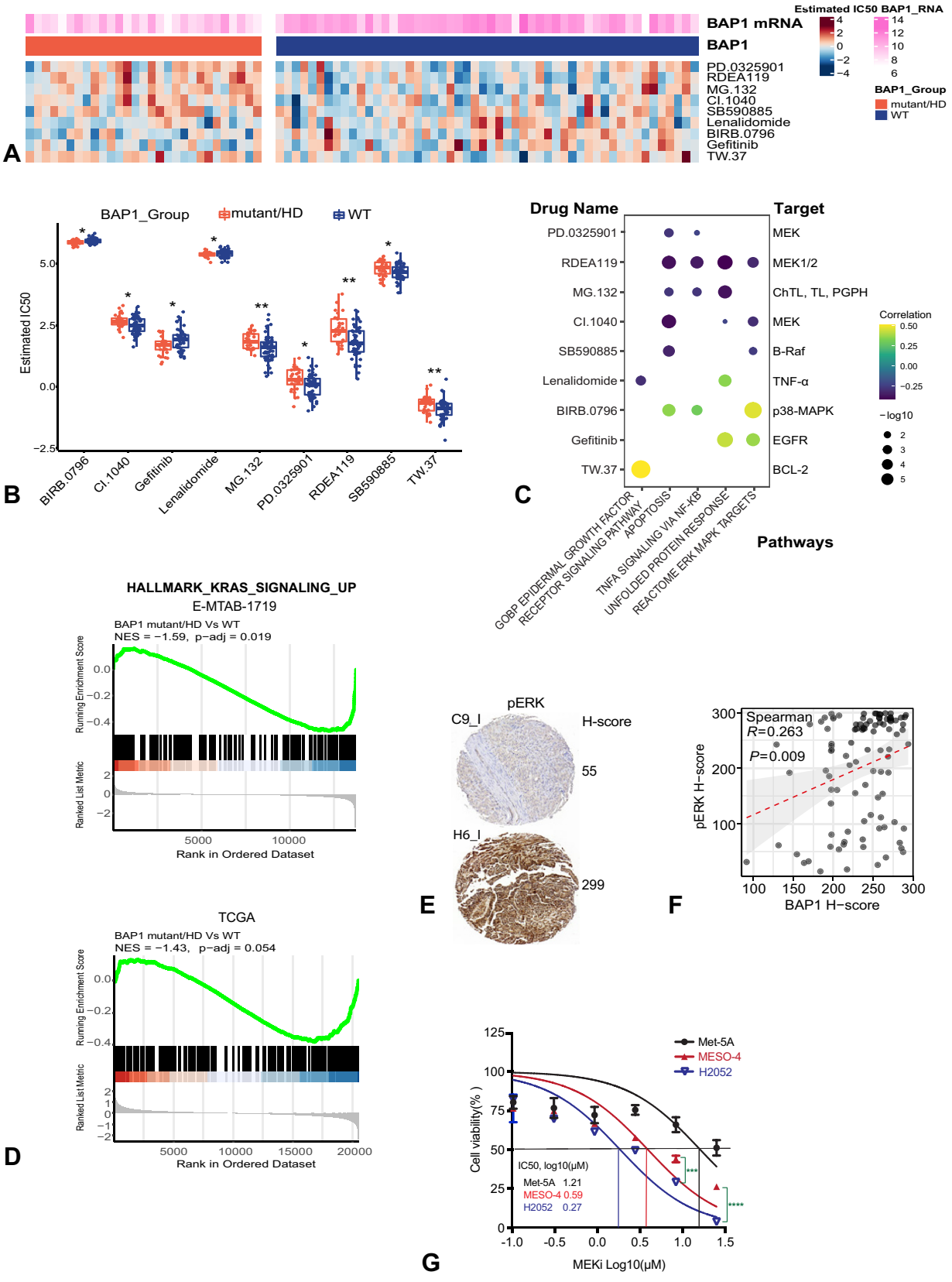

Figure 5. MAPK pathway is a potential therapeutic target in *BAP1*-proficient MPM. (*A*, *B*) Identification of therapeutic drug candidates with their estimated half-maximal inhibitory concentration (IC_{50}) values significantly (p value < 0.05) different in *BAP1*-deficient and *BAP1*-wild-type MPM. Analysis was based on the transcriptomic data set from the TCGA cohort of patients with MPM using the "pRRophetic" R package and Wilcoxon ranked sum test was used for comparison. (*C*) Correlation analysis

revealed that *BAP1*-deficient MPM is enriched for immune-related pathways (e.g., IFN-α/γ response) and strongly correlates with the gene signature of DCs, a professional antigen-presenting cell type that induces the activation and differentiation of naive T lymphocytes,[35] suggesting that BAP1 contributes to antitumor immunity in MPM beyond its function intrinsic to cancer cells.

First, we observed that *BAP1*-deficient MPM exhibited increased expression of several inhibitory immune checkpoints, such as PD-L1, PD-1, and LAG3. Similar observations have been reported in PeM,[18] and combined anti–PD-1 and anti-LAG3 therapy was found to have efficacy against mesothelioma in vitro and in vivo.[45] Further analysis of the immunologic contexture in the TIME revealed that *BAP1*-deficient MPM has an increased infiltration of the $TCF1^+/PD\text{-}1^+/CD8^+$ subpopulation (precursor-exhausted T cells), a hypofunctional state characterized by progressive loss of T cell effector functions and self-renewal capacity and associated with reduced efficacy of ICIs. In support of this finding, $BAP1^{low}$ MPM has increased $CD163^+$ M2 macrophages, a tumor-associated macrophage (TAM) that has been reported to be the most prominent immune cell in MPM[46] and to contribute to immunosuppression by inhibiting T cell activation,[34] a similar finding that has been reported in uveal melanoma.[47] Finally, we revealed that $BAP1^{low}$ MPM cells ($MSLN^+$) are in a closer proximity to $CD8^+$ T cells than $BAP1^{high}$ tumor cells, consistent with our finding that *BAP1* deficiency in MPM increases IFN-α/γ and DCs, which have been found to be able to expand stem-like $CD8^+$ T cells by secreting type I IFNs.[48]

Our finding that *BAP1* deficiency in MPM upregulates immune checkpoints and precursor-exhausted T cells, key mediators for the excellent efficacy of cancer immunotherapies,[41,42] suggests a potential role for BAP1 in predicting immunotherapy response. Indeed, murine MPM with low *Bap1* levels is associated with a better immunotherapy outcome, as are *BAP1*-deficient melanomas. Interestingly, a recent study reported that *BAP1* alterations do not seem to affect PFS or OS of patients with MPM treated with immunotherapy, although the small number of patients ($n = 7$) limits a firm conclusion.[49] Taken together, the various lines of evidence highlight that *BAP1*-deficient MPM has a distinct immunomodulatory gene expression, manifested by immune checkpoint activation, increased M2 macrophages, and T cell exhaustion, which contribute to an immunosuppressive TIME required for tumor development, while sensitizing tumors to ICIs. Future prospective immunotherapy trials are needed to validate these findings.

Several studies have revealed that MEKi can reprogram $CD8^+$ T lymphocytes and enhance the antitumor effect,[50] and we have revealed that *BAP1*-proficient MPM exhibits a sensitive phenotype to MEKi, suggesting that patients with MPM with intact *BAP1* may benefit from TIME reprogramming after MEKi. Interestingly, activation of the MAPK pathway has been observed in MPM,[28] and MEKi in combination with a FAK inhibitor has been evaluated in a clinical trial (NCT01938443) in solid tumors, including mesotheliomas, although *BAP1* status was not considered in this trial. Whether and how the combination of MEKi with ICIs is a valuable therapeutic rationale in *BAP1*-proficient MPM will require further studies.

Our current study has several limitations. First, this is a retrospective analysis and the patient cohorts used were not treated with ICIs; the immunomodulatory and predictive role of BAP1 requires further investigation in other cohorts with real-world clinical outcomes of immunotherapy, allowing prospective assessment of the association between BAP1 status and clinical response to immunotherapy. Second, the antitumor immune milieu in *BAP1*-deficient MPM tumors is largely based on correlational analysis and requires further validation using independent experimental approaches.

In conclusion, we provide multiple lines of evidence that *BAP1* deficiency defines an immune-inflamed phenotype in MPM that could be prioritized for ICI treatment and that *BAP1* status is a candidate biomarker predictive of response to immunotherapies as supported by in vivo mouse data.

CRediT Author Contribution Statement

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between the estimated IC_{50} values of therapeutic drug candidates and pathway enrichment scores using the "GSVA" R package. Note the significant negative correlation between the IC_{50} of the RAFi/MEKi (RDEA119, Cl.1040 and SB590885) and the mRNA score of the Reactome ERK MAPK Targets (MAPK pathway). (*D*) GSEA revealed a significant up-regulation of the KRAS_SIGNALING_UP gene signature in the *BAP1*-wild-type subset compared with *BAP1*-deficient MPM tumors. The transcriptomic data set from the TCGA cohort of patients with MPM was used for GSEA. (*E*, *F*) Correlation analysis of p-ERK and BAP1 protein levels in the Bern cohort. Representative IHC images of pERK are illustrated. (*G*) Cell viability assay of Met-5A and MPM cell lines deficient (MESO-4) or proficient (H2052) in BAP1 treated with MEK inhibitor (trametinib) for 96 hours. Data are presented as mean ± SD ($n = 3$). The IC_{50} is expressed as $\log_{10}$ (μM) in the graph. A two-tailed unpaired *t* test was used for comparison, *** $p < 0.001$, **** $p < 0.0001$. GSEA, gene set enrichment analysis; IHC, immunohistochemistry; MPM, malignant pleural mesothelioma; TCGA, The Cancer Genome Atlas.

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Disclosure

The authors declare no potential conflicts of interest.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100672.

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