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The frequency of core MMR gene alterations in lung cancer and their clinical characterization: A comprehensive study of over 26,000 cases

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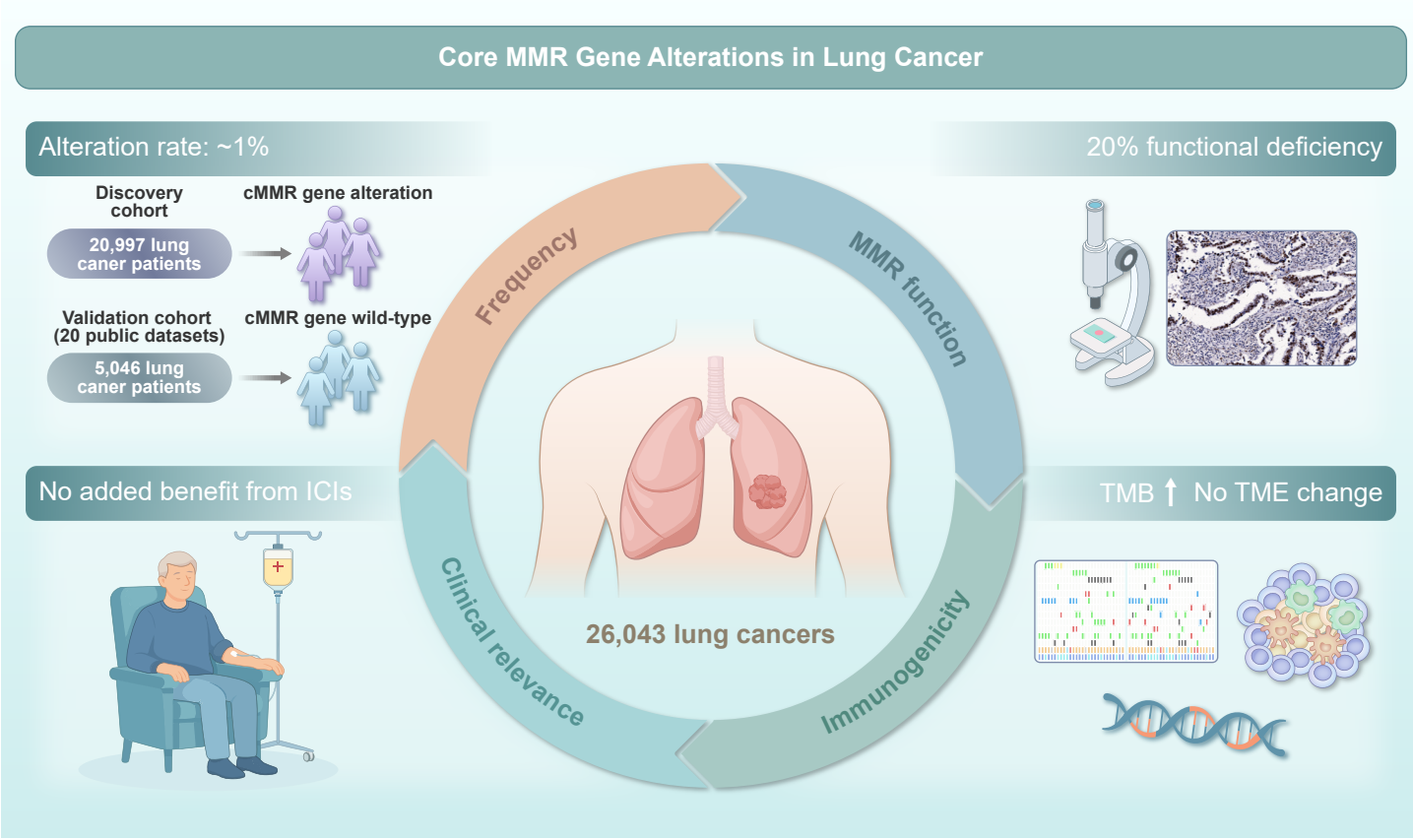
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Core mismatch repair (cMMR) gene alterations are rare in lung cancer (~1%).
- Only ~20% of cMMR gene alterations lead to functional mismatch repair deficiency.
- cMMR gene alterations increase tumor mutation burden but not immune cell infiltration.
- No additional immunotherapy benefit was observed in limited cases with cMMR gene alterations.

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Mismatch repair (MMR) deficiency is a pan-cancer biomarker predictive of immune checkpoint inhibitor (ICI) response, yet its role in lung cancer remains unclear. We analyzed genomic and clinical data of 26,043 lung cancers (20,997 discovery; 5,046 validation) to comprehensively characterize deleterious core MMR gene alterations and their functional and clinical implications. Deleterious core MMR (cMMR) gene alterations were identified in 0.9% and 1.2% patients of the discovery and validation cohort, respectively, with significant enrichment in squamous carcinomas. Functionally, approximately 20% of tumors with cMMR gene alteration exhibited MMR protein loss and/or microsatellite instability-high, which were nearly absent in cMMR wild-type tumors. The tumors with cMMR gene alteration had a higher rate of tumor mutation burden-high compared to their wild-type counterparts (discovery cohort: 73.6% vs. 39.0%, P<0.001; validation cohort: 55.2% vs. 22.9%; P<0.001). Within a limited subset of advanced lung cancer patients receiving ICIs, response rates and survival outcomes were comparable between cMMR gene alteration and wild-type groups. Immune profiling revealed no significant differences in PD-L1 expression or immune infiltration scores between cMMR gene alteration and wild-type tumors. In summary, cMMR gene alterations are rare in lung cancer but confer genomic instability in a subset of cases. Crucially, the majority of these alterations do not lead to functional MMR deficiency or immune microenvironment activation. These findings indicate that cMMR gene alterations alone may be insufficient to guide immunotherapy decisions. Further studies are warranted to clarify the clinical utility of MMR deficiency in lung cancer.

INTRODUCTION

Mismatch repair (MMR) genes are essential tumor suppressors that maintain genomic stability by correcting DNA replication errors. Among these, MLH1, MSH2, MSH6, and PMS2 constitute the core components of the MMR machinery and are known to be most strongly implicated in carcinogenesis and therapeutic responses, particularly immune checkpoint inhibitors (ICIs).[1] In colorectal, endometrial, and gastric cancers, MMR deficiency that predominantly caused by deleterious alterations in these core genes can define distinct clinicopathological features (e.g., poor differentiation, dense lymphocytic infiltration, and favorable prognosis).[2–5] Mechanistically, deleterious alterations of core MMR gene (hereafter referred to as cMMR gene alterations) can induce MMR protein loss, promote somatic hypermutation like microsatellite instability-high (MSI-H), and activate tumor immune microenvironments through neoantigen accumulation.[3,6] These characteristics collectively establish MMR protein loss or MSI-H as key predictors of immunotherapy responsiveness in these cancers.[7,8] The clinical trial has highlighted the significant responses of solid cancers with MMR deficiency to immunotherapy, regardless of the cancer type,[9] which caused considerable interest in investigating MMR in various cancer types.

Lung cancer continues to be a significant global health challenge, ranking as the most common cancer worldwide and the leading cause of cancer-related mortality.[10,11] While immunotherapy has revolutionized treatment paradigms, durable responses remain confined to a minority of patients, underscoring the urgent need for robust predictive biomarkers.[12,13] Although MMR deficiency has been established as a pan-cancer biomarker for immune checkpoint inhibitors,[1,9] it is noteworthy that lung cancer populations were rarely included in these pan-cancer clinical trials. Previous studies have reported that the prevalence of MSI-H or MMR protein loss in lung cancer is 0.4%-1.2% and identified pathogenic germline or somatic MMR mutations as the primary cause of MMR deficiency phenotype. However, these studies had limited sample size, with the included MMR deficiency cases from 6 to 66.[14–18] Currently, the functional penetrance of MMR deficiency, its effects on tumor immune microenvironments, and its predictive utility in immunotherapy are yet to be systematically investigated.

To address these gaps, we conducted a large-scale real-world study integrating genomic and clinical data from 20,997 lung cancer patients, complemented by a validation cohort of 5,046 cases aggregated from 20 public datasets. Leveraging these cohorts, we systematically investigated the prevalence of deleterious alterations in the four core MMR genes (MLH1, MSH2, MSH6, and PMS2) across different lung cancer subtypes. Furthermore, we analyzed the correlation between deleterious cMMR gene alterations and functional MMR deficiency (MMR protein loss or MSI-H), assessed their impact on tumor mutational burden (TMB) and immune microenvironment features [e.g., immune cell infiltration and expression of programmed death-ligand 1 (PD-L1)], and explored the association of cMMR gene alterations with immunotherapy outcomes.

MATERIALS AND METHODS

Patients

Our discovery cohort comprised 20,997 patients with histologically diagnosed lung cancer from Peking University Cancer Hospital and the First Affiliated Hospital of Zhengzhou University between May 2016 and May 2024. These patients all underwent clinically indicated next-generation sequencing (NGS) targeting the four core MMR genes (MLH1, MSH2, MSH6, and PMS2) at Geneplus Biotechnology Co., Ltd (Beijing, China). These genes were selected because they represent the canonical components of the MMR pathway and are the most frequently altered in tumors with mismatch repair deficiency.[1] Furthermore, they are the only MMR genes routinely included in the most clinically used large-panel sequencing assays. In this cohort, 36 patients who received anti-programmed cell death protein 1 (PD-1) -based immunotherapy at the First Affiliated Hospital of Zhengzhou University were prospectively enrolled for efficacy and survival analyses. Demographic data, tumor characteristics (histology, stage, and PD-L1 expression), treatment

history, and longitudinal follow-up data were extracted from electronic medical records. Treatment response was evaluated by radiologists using Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1). Objective response rate (ORR) was defined as the proportion of patients achieving complete response (CR) or partial response (PR). Progression-free survival (PFS) was calculated from the initiation of immunotherapy to disease progression or death from any cause. Overall survival (OS) was measured from treatment initiation to death or the last follow-up. Patients without progression or death were censored at the last confirmed follow-up date.

Our validation cohort initially included 6,648 lung cancer patients from 20 publicly available cohorts, whose data were obtained from cBioPortal (https://www.cbioportal.org) or original publications.[19–33] To address potential sample redundancy—particularly among multiple datasets from the same institution (e.g., MSKCC)—we performed deduplication based on unique case identifiers (Case IDs) associated with each patient. A total of 1,602 duplicate cases were identified and excluded. After deduplication, 5,046 unique lung cancer cases were retained for subsequent analysis. The NGS data (targeted sequencing, whole-exome sequencing, or whole-genome sequencing) covering the four core MMR genes (MLH1, MSH2, MSH6, and PMS2), as well as available clinical annotations, were harmonized across cohorts. Detailed information on the included datasets, including sample sizes before and after deduplication, is provided in Table S1.

NGS and variants calling

For the discovery cohort, tumor-derived genomic DNA or circulating tumor DNA from plasma was used for gene panel sequencing. Different patients received different gene panels based on their clinical characteristics and personal preferences. The gene panels spanned 0.98–1.22 Mb of coding regions (59–1,021 genes depending on different versions). All these panel versions included MLH1, MSH2, MSH6, and PMS2 genes. Sequencing was performed on the Gene+Seq2000 platform with a median coverage depth of >500× for tumor tissues and >3,000× for plasma. Matched samples of peripheral blood mononuclear cells (PBMC) were sequenced to 500× median depth for germline variant filtering.

Raw sequencing reads were aligned to the human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner (BWA v0.1.22). Duplicate reads from PCR amplification were flagged with Picard Mark Duplicates (v2.23.8). To mitigate sequencing errors in tumor/plasma samples, PCR duplicates were further clustered and corrected using a proprietary UID-based error correction algorithm (realSeq v1.0), which simultaneously recalibrated base quality scores. Local realignment around indels and base quality score recalibration (BQSR) was performed using GATK Realigner Target Creator, Indel Realigner, and Base Recalibrator (v3.8).

Somatic SNVs/indels were identified using a dual-caller approach: GATK Mutect2 (v4.2.6) and a proprietary machine learning-based caller (realDcaller v2.1). Hotspot mutations (e.g., KRAS G12V) were re-scanned with an optimized algorithm (NChot v3.0). Structural variants (SVs) were detected by reassembling clipped, unmapped, and discordant paired-end reads using NCsv v1.5.

Identification of deleterious alterations in MMR genes

cMMR gene alterations were uniformly identified in both discovery and validation cohorts through a standardized filtration pipeline to ensure cross-cohort comparability. High-confidence mutations in MLH1, MSH2, MSH6, and PMS2 were selected based on stringent quality thresholds: the tumor tissue-derived variants required sequencing depth ≥ 20× and variant allele frequency (VAF) ≥10%, while the plasma ctDNA variants required sequencing depth ≥ 3,000× and VAF ≥1% (equivalent to 3× absolute coverage).

To prioritize functional impact, truncating variants (nonsense or frameshift mutations) were retained unless located within the terminal 55 base pairs of the penultimate or final exon, as these positions may escape nonsense-mediated decay (NMD) and lack functional domain disruption. Essential splice-site mutations (within ±2 bp of exon-intron boundaries) and missense mutations classified as oncogenic by OncoKB (v4.0) were included. Additionally, structural variants that can disrupt MMR gene integrity (e.g., intragenic rearrangements) were analyzed due to their potential to abrogate protein function. The pathogenic/likely pathogenic germline variants, oncogenic somatic mutations, and structural alterations defined above were aggregated cMMR gene alteration for subsequent analyses.

Microsatellite instability analysis

In the discovery cohort, MSI status was assessed for 151 tumor tissue samples sequenced with large panels (>1,000 genes) using MSIsensor (v0.5), which quantifies MSI by analyzing the proportion of unstable loci across predefined microsatellite regions. Samples were classified as MSI-H if the instability score exceeded the empirically validated threshold of 10%. For the validation cohort, MSI status for 1,028 tumors profiled by MSK-IMPACT sequencing (468 genes) was directly extracted from the published annotations in the original studies.[22,27-30,33]

Immunohistochemical (IHC) assays

MMR protein expression and PD-L1 status were assessed in 89 formalin-fixed, paraffin-embedded (FFPE) tumor specimens from the discovery cohort by immunohistochemistry. Four-micron tissue sections were stained using automated platforms with validated primary antibodies against the MMR proteins: MLH1 (clone GM002, mouse monoclonal; Gene Tech, GT230407), MSH2 (clone RED2, rabbit monoclonal; Gene Tech, GT231007), MSH6 (clone EP49, rabbit monoclonal; Gene Tech, GT219507), and PMS2 (clone EP51, rabbit monoclonal; Gene Tech, GT215907). MMR protein loss was defined as complete loss of nuclear staining in tumor cells for ≥1 MMR protein, with retained expression in adjacent stromal/inflammatory cells serving as internal controls. For PD-L1 evaluation, sections were stained with the 22C3 pharmDx assay (DAKO) and scored as tumor proportion score (TPS): <1% (negative), 1–50% (low), or ≥50% (high). All IHC assays included mandatory controls: embryonic tissue sections as positive controls and PBS-substituted primary antibody slides as negative controls. To minimize bias, two independent pathologists blinded to MMR mutation status scored all slides.

TMB calculation

TMB was quantified in 177 tumor tissue samples from the discovery cohort sequenced with large panels (>1,000 genes). For these samples, TMB was defined as the total number of nonsynonymous somatic mutations—including single nucleotide variants (SNVs) and small insertions/deletions (indels)—per megabase (Mb) of coding regions, normalized to the panel size. TMB values were reported as mutations per Mb (mut/Mb), with a threshold of ≥10 mut/Mb classifying tumors as TMB-high (TMB-H). In the validation cohort, TMB values were directly retrieved from original publications or cBioPortal (https://www.cbioportal.org) using comparable definitions (nonsynonymous SNVs/indels per Mb). To ensure cross-cohort consistency, TMB normalization was recalculated for validation cases based on panel size when raw mutation counts were available.

RNA sequencing and immune microenvironment profiling

Bulk RNA sequencing (RNA-seq) data from 1,097 tumors in the validation cohort were harmonized through a standardized preprocessing pipeline. First, raw transcriptomic profiles were filtered to retain protein-coding genes expressed in ≥10% of samples and merged across datasets using HUGO gene symbols. To address technical variability, batch effects were corrected using the limma R package (v3.56.2), followed by log2(x-min(x)+1) transformation for variance stabilization. To evaluate the immune landscape, the ESTIMATE algorithm was applied to calculate global immune and stromal scores, which quantify overall immune infiltration and stromal compartment activity. Subsequently, the CIBERSORT deconvolution method was employed to estimate the relative proportions of 22 immune cell subsets, including T cell lineages, B cell lineages (naive/memory), polarized macrophages (M0/M1/M2), dendritic cells (activated/resting), natural killer cells, granulocytes (eosinophils, neutrophils).

Mutational signature analysis

Mutational signatures were profiled in the validation cohort to elucidate the genomic processes associated with cMMR gene alterations. Samples with ≥ 50 somatic single nucleotide variants (SNVs) were selected to ensure robust signature deconvolution. Using the deconstructSigs R package (v1.9.0), nonsynonymous and silent exonic SNVs were mapped to the 30 reference muta-

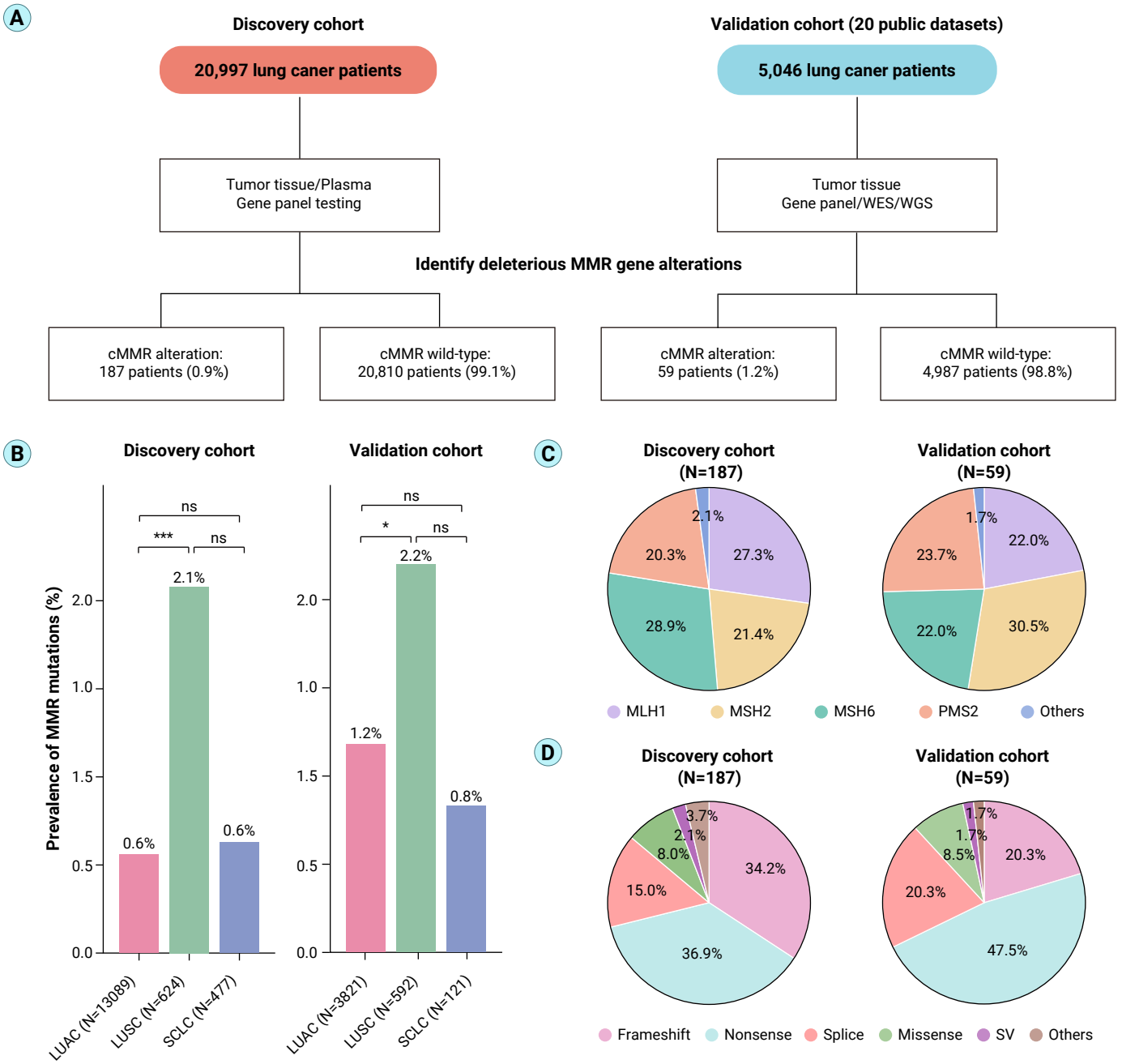

Figure 1. Prevalence and histologic distribution of cMMR gene alterations in lung cancer (A) Study design flowchart for the discovery (N = 20,997) and validation (N = 5,046) cohorts. (B) Frequency of cMMR gene alterations across different histologic subtypes in the discovery and validation cohorts. (C) Distribution of cMMR gene alterations in the four MMR genes (MLH1, MSH2, MSH6, and PMS2) in the discovery and validation cohorts. (D) Mutation types of the four MMR genes in the discovery and validation cohorts. Abbreviations: LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; SCLC, small cell lung cancer. P values were calculated using chi-square tests; "ns" indicates non-significance ($P \geq 0.05$), *, $0.01 < P < 0.05$.

tional signatures cataloged in COSMIC (v3.3; https://cancer.sanger.ac.uk/signatures/). For each tumor, signature contributions were quantified as the proportion of mutations attributed to each COSMIC signature.

Statistical analysis

Categorical variables were tested between groups using the chi-square test or Fisher's exact test where appropriate. Continuous variables were tested between groups with a t-test or Mann-Whitney U test, where appropriate. Survival outcomes, including progression-free survival (PFS) and overall survival (OS), were estimated using the Kaplan-Meier method, with between-group differences assessed by the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were derived from univariate Cox proportional hazard models. All statistical tests were two-sided, with $P < 0.05$ considered significant. Data processing and statistical analyses were conducted using SPSS 20.0 (IBM Corp) or R 4.3.1 (survival and survminer packages), with visualization implemented in GraphPad Prism 9.0.

RESULTS

Prevalence and clinicopathologic characteristics of cMMR gene alterations in lung cancer

A total of 20,997 lung cancer patients (discovery cohort) underwent targeted region sequencing, including the four MMR genes closely related to tumorigenesis (Figure 1A). Pathogenic germline variants, oncogenic somatic mutations, and somatic structural alterations were identified in 0.9%

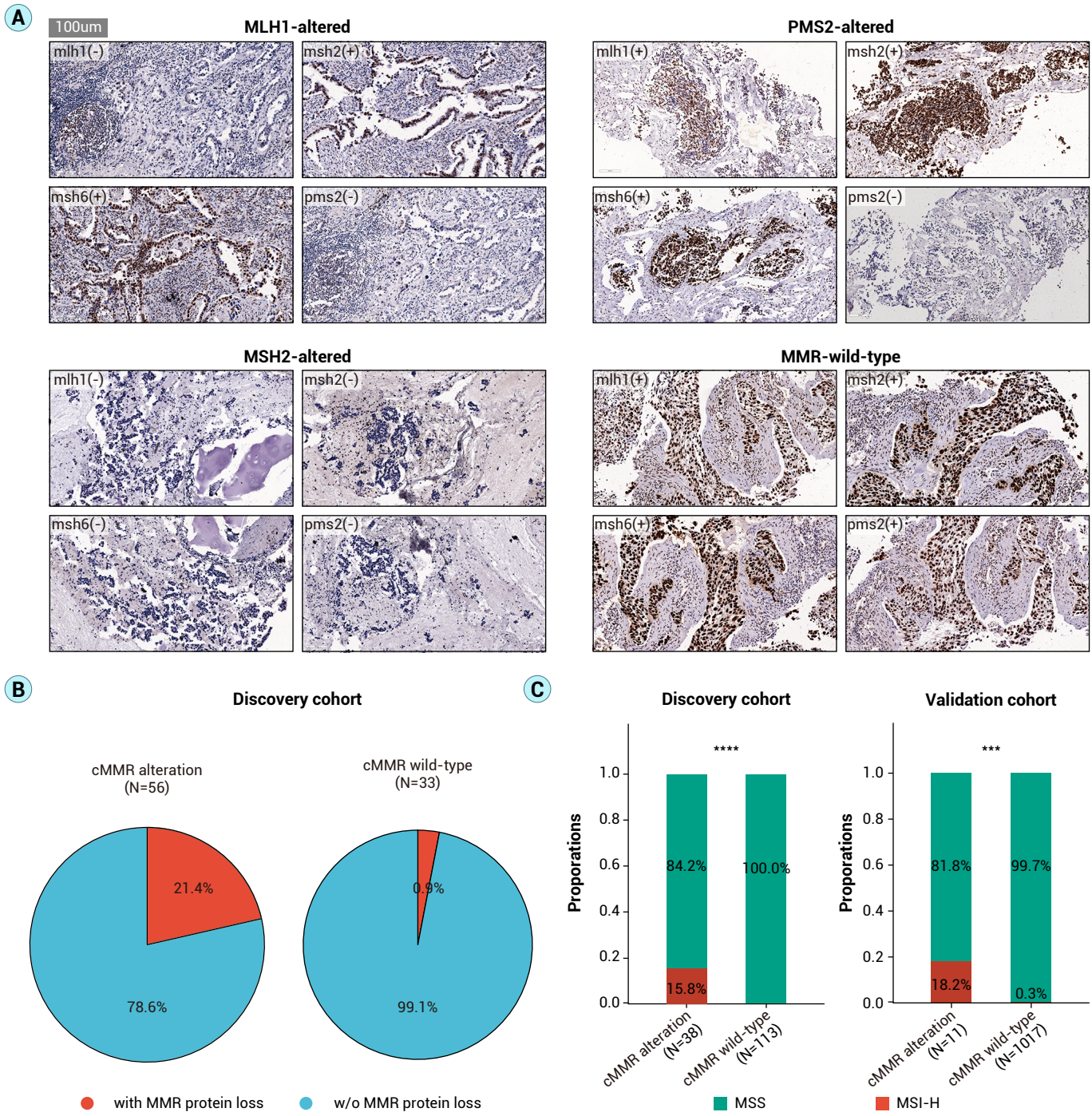

Figure 2. Functional consequences of cMMR gene alterations in lung cancer (A) Representative immunohistochemical staining of MMR proteins in the cMMR gene alteration and wild-type lung cancers. Scale bars: 100 μm. (B) Frequency of MMR protein loss (absent staining in nucleus) in the cMMR gene alteration and wild-type lung cancers from the discovery cohort. (C) Prevalence of high microsatellite instability (MSI-H) in the cMMR gene alteration and wild-type lung cancers from the discovery and validation cohorts. Abbreviations: MSI-H, microsatellite instability-high; MSS, microsatellite stable. ***, P < 0.001; ****, P < 0.0001 (chi-square or Fisher's exact test).

(187/20,997) of cases (Figure 1A). Subgroup analysis revealed a significant histology-specific distribution, with lung squamous carcinomas (LUSC) exhibiting the highest prevalence (2.1%), which was significantly higher than that observed in lung adenocarcinomas (LUAD) (2.1% vs. 0.6%, P < 0.001) (Figure 1B). In contrast to the MLH1/MSH2 predominance in colorectal cancer, lung cancers demonstrated a near-uniform distribution of alterations across all four MMR genes (Figure 1C). Moreover, truncating mutations (nonsense and frameshift) accounted for 68% of alterations, exceeding missense, splice-site, and structure variants (Figure 1D).

An independent validation cohort comprising 5,046 patients from 20 public datasets (Table S1) revealed a comparable cMMR gene alteration frequency in lung cancer (59/5,046; 1.2%) (Figure 1A). Consistent with the discovery cohort, LUSC demonstrated the highest cMMR gene alteration rate (2.2%; Figure 1B). Furthermore, the MMR genes distribution and mutation type profiles in the validation cohort closely mirrored those observed in the discovery cohort (Figures 1C-D). Additionally, tumors with cMMR gene alteration showed no significant differences from cMMR wild-type tumors in sex distribution (male: 51.8% vs. 48.0%; P = 0.6) or smoking history (73.9% vs. 80.4%; P = 0.4) (Table S2). While not statistically significant, tumors with cMMR gene alteration exhibited a trend toward more advanced disease (stage III-IV: 33.3% vs. 20.6%; P = 0.07) (Table S2).

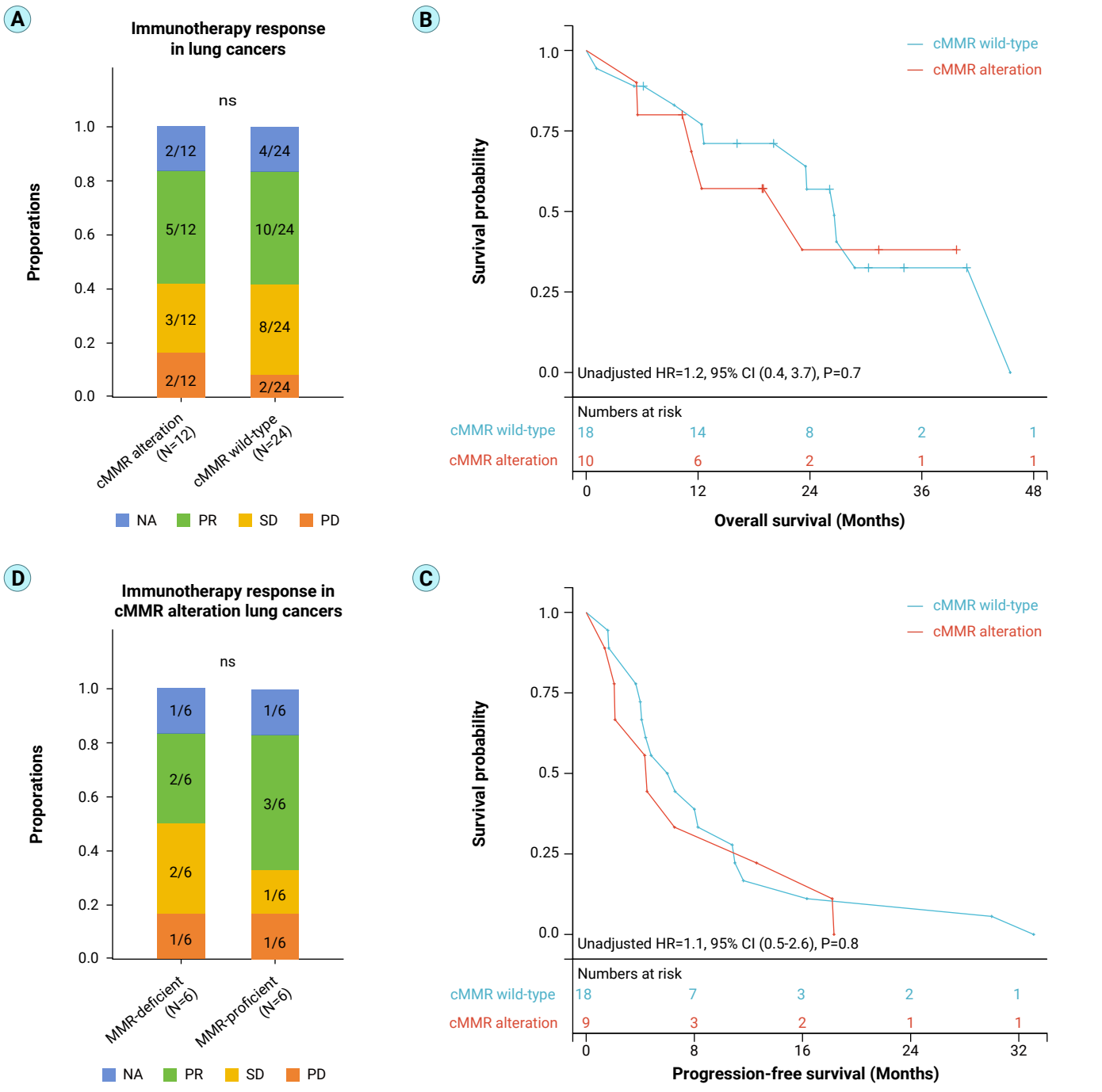

Figure 3. cMMR gene alterations and immunotherapy response in lung cancer (A) Objective response rate in patients with cMMR gene alteration versus wild-type lung cancer treated with immune checkpoint inhibitors. (B-C) Kaplan-Meier survival curves comparing overall the survival (OS) and the progression-free survival (PFS) between the cMMR gene alteration and wild-type lung cancers. Hazard ratios (HR) were derived from univariate Cox proportional hazards models. (D) Subgroup analysis of patients with cMMR gene alteration stratified by functional status: MMR-deficient (MMR protein loss and/or MSI-H) vs. MMR-proficient (intact MMR protein and MSS). Abbreviations: MSI-H, microsatellite instability-high; MSS, microsatellite stable; NA, not available; PR, partial response; SD, stable disease; PD, progressive disease. Statistical significance was assessed using chi-square or Fisher's exact tests (Panel A, D) and log-rank tests (Panels B-C); ns, not significant ($P \geq 0.05$).

cMMR gene alterations and MMR functional deficiency in lung cancer

In Lynch syndrome-associated tumors, cMMR gene alterations typically lead to dysfunctional MMR activity characterized by MMR protein loss or the presence of MSI-H.[34] However, since lung cancer is not traditionally included in the Lynch syndrome tumor spectrum, it remains uncertain whether cMMR gene alterations in lung cancer led to similar consequences. To address this, we performed IHC staining for MMR proteins on FFPE tumor samples from 89 lung cancer patients in the discovery cohort. Among the tumors with cMMR gene alteration, 21.4% (12/56) exhibited loss of at least one of the four MMR proteins, while only 0.9% (1/33) of the cMMR wild-type tumors had MMR protein loss (Figures 2A-B).

We then examined the relationship between cMMR gene alterations and MSI status in lung cancer. In the discovery cohort, among 151 cases with available MSI data, the tumors with cMMR gene alteration showed a significantly higher prevalence of MSI-H compared to the cMMR wild-type tumors (15.8%, 6/38 vs. 0.0%, 0/113; $P < 0.001$) (Figure 2C). In the validation cohort, MSI data were available for 1,028 lung cancer cases. Approximately 18.2% (2/11) of tumors with cMMR gene alteration in this cohort were classified as

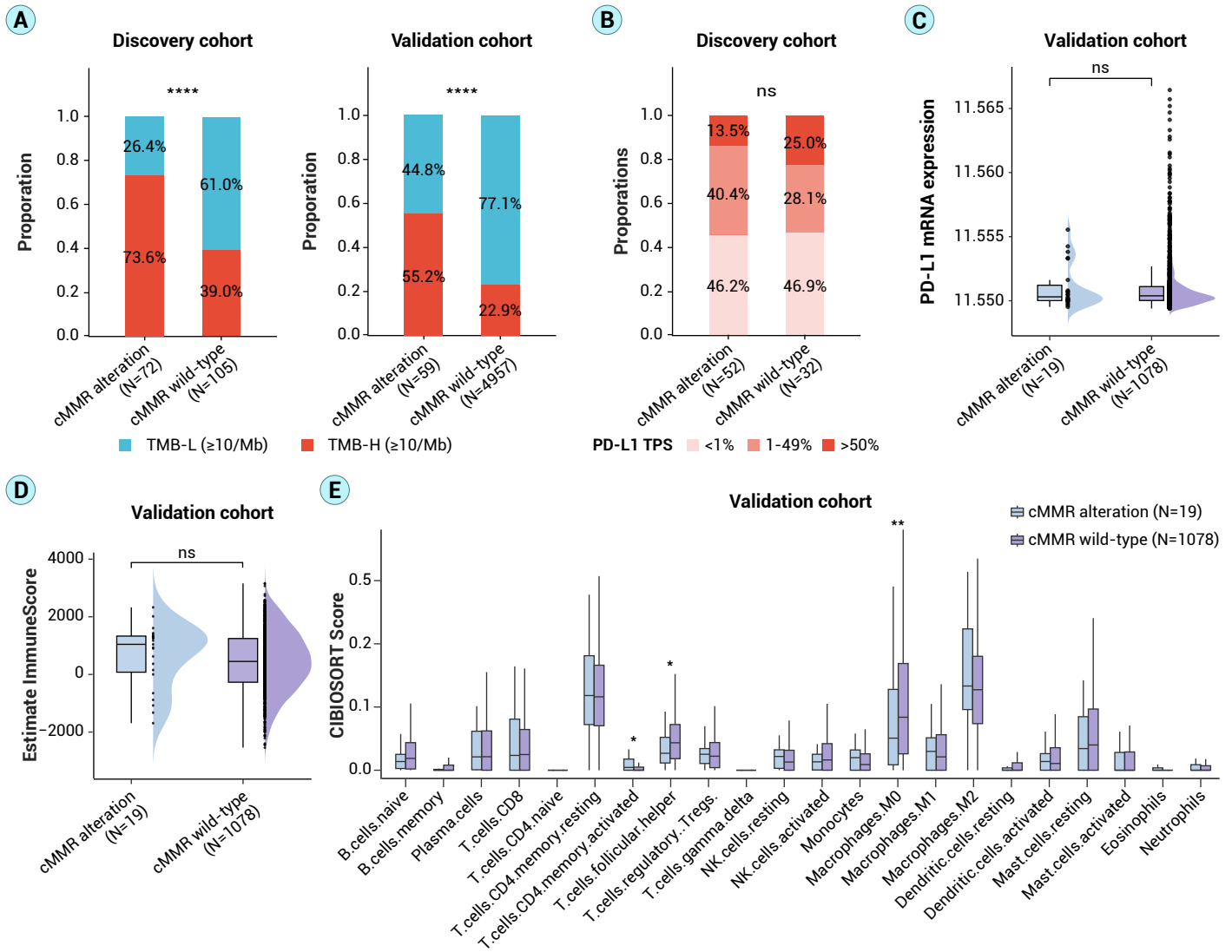

Figure 4. Immunogenicity and immune microenvironment in lung cancer with cMMR gene alteration (A) Prevalence of TMB-H (>10 mutations/Mb) and TMB-L (≤10 mutations/Mb) samples in the cMMR gene alteration and wild-type lung cancers from the discovery and validation cohorts. (B) Comparison of PD-L1 TPS between the cMMR gene alteration and wild-type lung cancers from the discovery cohort. (C) PD-L1 mRNA expression levels in the cMMR gene alteration and wild-type tumors from the validation cohort. (D) Immune infiltration scores (ESTIMATE algorithm) in the cMMR gene alteration and wild-type tumors from the validation cohort. (E) Immune cell composition analyses (CIBERSORT) in the cMMR gene alteration and wild-type tumors from the validation cohort. TMB-H, high tumor mutational burden; TMB-L, low tumor mutational burden; PD-L1, programmed death-ligand 1; TPS, tumor positive score; ns, not significant (P ≥ 0.05). *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001 (Mann-Whitney U, chi-square, or Fisher's exact tests where appropriate).

MSI-H (Figure 2C), a proportion comparable to that observed in the discovery cohort. cMMR gene alterations were also significantly associated with MSI-H in the validation cohort (18.2% vs. 0.3%, P < 0.001) (Figure 2C). The above results demonstrate a strong correlation between cMMR gene alterations, MMR protein loss, and MSI-H in lung cancer.

cMMR cene alterations and response to immune checkpoint inhibition in lung cancer

MMR deficiency (MSI-H or MMR protein loss) serves as a biomarker for immunotherapy efficacy across various cancers.[1,9] Given the strong association between cMMR gene alterations and MSI-H or MMR protein loss in lung cancer, we further explored the relationship between cMMR gene alterations and the response to immune checkpoint inhibition in lung cancer. In the discovery cohort, we identified 12 lung cancer patients with cMMR gene alterations who received ICIs (Table S3). Among them, most received anti-PD-1 therapy with chemotherapy, and one patient received anti-PD-1 monotherapy. These patients were matched with 24 cMMR wild-type lung cancer patients considering the baseline characteristics, including age, sex, histologic subtype, disease stage, smoking history, and number of metastasis sites (Table S4). No significant difference was observed in the objective response rate (CR+PR) between the two groups (5/12 vs. 10/24, P = 0.9) (Figure 3A). The overall survival (unadjusted HR=1.2, 95% CI: 0.4-3.6, P=0.7) and the progression-free survival (unadjusted HR=0.8, 95% CI: 0.5-2.6, P=0.8) also showed no significant difference between the two groups (Figures 3B-C). To account for potential confounding factors, we conducted multivariate Cox regression analyses. After adjusting for PD-L1 tumor proportion score (TPS), TMB, and disease stage, cMMR gene alterations remained unassociated with immunotherapy outcomes (PFS: adjusted HR = 2.0, 95% CI: 0.7–6.1, P = 0.2; OS: adjusted HR = 1.3, 95% CI: 0.4–4.4, P = 0.7) (Table S5).

Given that only a subset of lung cancer cases with cMMR gene alteration exhibits MMR protein loss or MSI-H, we conducted a subgroup analysis, categorizing cases with cMMR gene alteration into the MMR-deficient (those with MMR protein loss or MSI-H) group and the MMR-proficient (those without MMR protein loss or MSI-H) group. However, no significant difference in the objective response rate was observed between the MMR-deficient group and the MMR-proficient group (2/6 *vs.* 3/6). Although the sample size was relatively small, these results suggest that tumors with cMMR gene alteration or the MMR-deficient subgroup are not associated with a better thera-

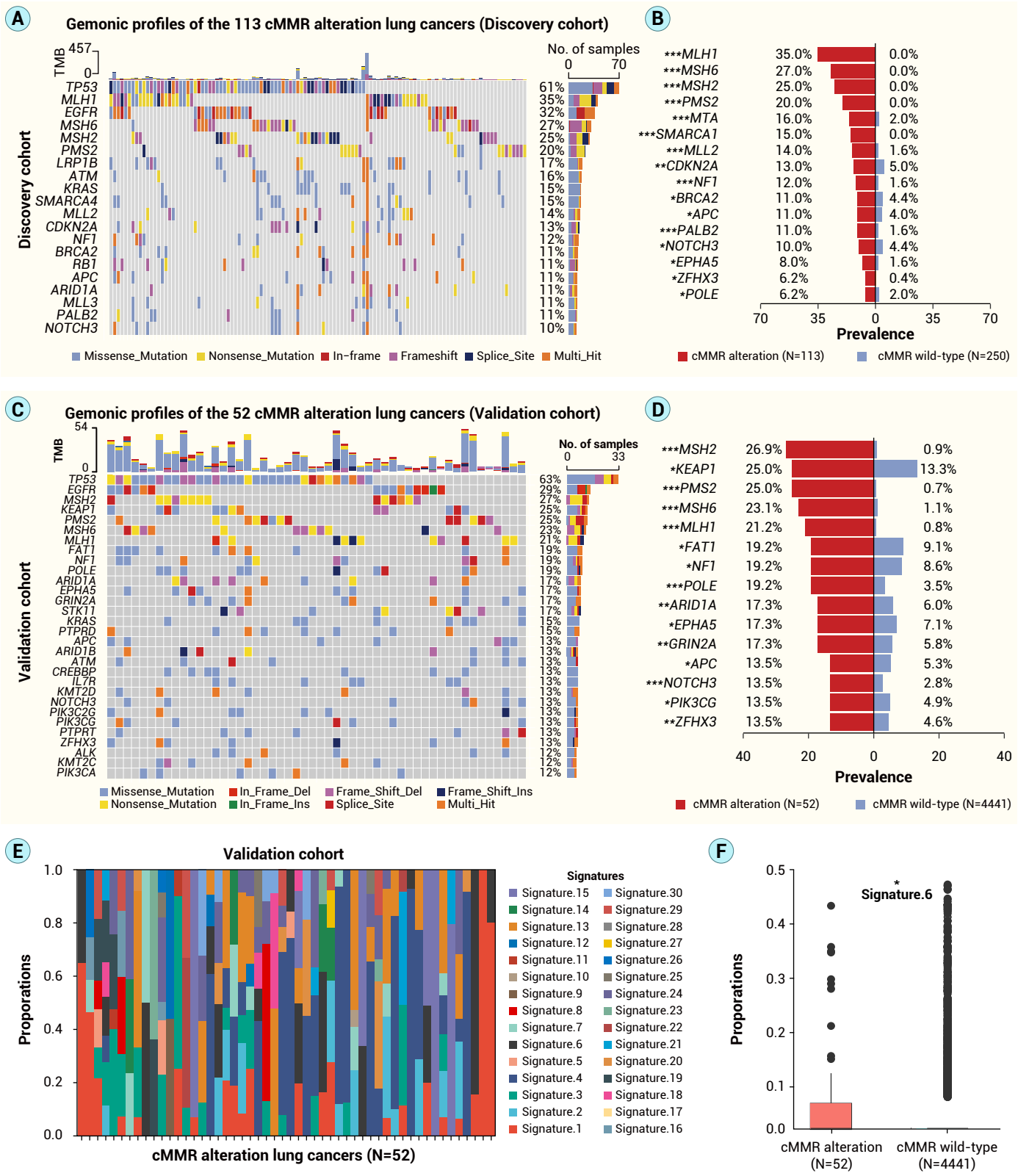

Figure 5. Genomic profile and mutational signature of lung cancer with cMMR gene alteration (A) Somatic mutation waterfall plot of the tumors with cMMR gene alteration from the discovery cohort. Genes are ordered by mutation frequency. (B) Mutated genes with different prevalence in the cMMR gene alteration and wild-type lung cancers from the discovery cohort. Statistical significances were determined by z-test with Benjamini-Hochberg correction for false discovery rate. (C) Somatic mutation waterfall plot of the tumors with cMMR gene alteration from the validation cohort. (D) Mutated genes with different prevalence in the cMMR gene alteration and wild-type lung cancers from the validation cohort. (E) Composition of mutational signatures in the tumors with cMMR gene alteration from the validation cohort. Signature proportions are annotated for each sample. (F) The proportion of mutational signature 6 in the cMMR gene alteration and wild-type lung cancers from the validation cohort. P-values were calculated using the Wilcoxon rank-sum test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

peutic response to immune checkpoint inhibitors.

Immunogenicity and immune microenvironment in lung cancers with cMMR gene alteration

We further explored the reason for the lack of sensitivity to immunotherapy in lung cancer with cMMR gene alteration.[1] In Lynch syndrome, MMR deficiency enhances immunogenicity (e.g., tumor mutational burden) and immunoreactivity (e.g., immune cell infiltration), thus conferring sensitivity to immunotherapy. Therefore, we investigated whether cMMR gene alterations can similarly influence the TMB in lung cancer. In the discovery cohort, 73.6% (53/72) of the tumors with cMMR gene alteration were classified as TMB-high (>10 mut/Mb), in stark contrast to only 39.0% (41/105) in the cMMR wild-type lung cancers ($P < 0.001$). Similar results were found in the validation cohort (55.2% vs. 22.9%; $P<0.001$; Figure 4A), confirming cMMR gene alterations as robust TMB elevators in lung cancer.

Regarding the immune microenvironment, we first estimated PD-L1 tumor-positive scores (TPS) for the 84 cases of lung cancer in the discovery cohort. Surprisingly, we found no significant difference in PD-L1 scores between the cMMR gene alteration and wild-type lung cancers (Figure 4B). In the validation cohort, transcriptomic data were available for 1,097 out of the 5,046 lung cancer samples. Consistent with that in the discovery cohort, neither the PD-L1 mRNA levels nor the immune score was found different between the cMMR gene alteration and wild-type lung cancers (Figures 4C-D). In addition, we calculated the number of various immune cells using CIBERSORT and found that the tumors with cMMR gene alteration exhibited reduced M0 macrophages and follicular helper T cells as well as increased $CD4^+$ memory T cells compared to the cMMR wild-type lung cancers (Figure 4E). However, we did not observe any difference between the two groups in the immune cells known to be associated with anti-tumor immunity, such as $CD8^+$ T cells or NKT cells (Figure 4E). Collectively, these data suggest that although tumors with cMMR gene alteration have an increased tumor mutational burden, this increased burden fails to elicit a corresponding anti-tumor immune response.

Given that cMMR gene alterations are strongly associated with elevated TMB but not with enhanced immune infiltration, we further explored this apparent paradox. We initially hypothesized that impaired antigen presentation might underlie the lack of immune activation. However, transcript levels of genes involved in antigen processing and presentation pathways showed no significant differences between cMMR gene alteration and wild-type groups (Figure S1A). Subsequent differential gene expression analysis identified eleven genes significantly upregulated in tumors with cMMR gene alteration (including IL1A and KLK6; Figure S1B). Both genes have established roles in mediating immune evasion.[35,36] These results suggest that upregulation of immunosuppressive genes may counterbalance the immune-stimulatory effects of high TMB, thereby contributing to an ineffective anti-tumor immune response in tumors with cMMR gene alteration.

Genomic landscape of lung cancer with cMMR gene alterations

To assess the broader genomic impact of cMMR gene alterations, we analyzed mutation frequencies of canonical driver genes in 113 tumors with cMMR gene alteration and 250 matched cMMR wild-type tumors from the discovery cohort. Notably, tumors with cMMR gene alteration exhibited significant enrichment of somatic mutations in DNA repair genes (ATM, BRCA2, PALB2, and POLE), chromatin remodeling genes (SMARCA4 and MLL2), tumor suppressor genes (CDKN2A, NF1, APC, and ZFHX3), and genes related to receptor tyrosine kinase (EPHA5) and NOTCH pathway (NOTCH3) (Figures 5A-B). Validation in the 5,046 lung cancers replicated these associations and confirmed the elevated mutation frequencies of NF1 (OR=2.5, 95% CI: 1.2-5.0), APC (OR=2.8, 1.2-6.1), NOTCH3 (OR=5.4, 2.3-12.0), EPHA5 (OR=2.7, 1.3-5.6), ZFHX3 (OR=3.2, 1.4-7.0), and POLE (OR=6.6, 3.2-13.4) in the tumors with cMMR gene alteration (Figures 5C-D). Critically, this conserved co-mutation pattern across different cohorts suggests a synergistic role of MMR deficiency and these gene mutations in lung carcinogenesis. We then analyzed the mutation signature profile of the tumors with cMMR gene alteration (Figure 5E) and found that signature 6, a signature known to be associated with MMR deficiency in human cancers,[37] was significantly enriched in the tumors with cMMR gene alteration compared to the cMMR wild-type lung cancers (Figure 5F).[33]

DISCUSSION

MMR deficiency is a well-characterized oncogenic mechanism across multiple malignancies, yet its clinicogenomic implication in lung cancer remains poorly understood. Integrating the data from 20,997 cases from the discovery cohort and 5,046 cases from the validation cohort, we demonstrate that cMMR gene alterations occur in approximately 1% of lung cancers, with LUSC showing the highest prevalence of cMMR gene alterations compared with other subtypes. These alterations are associated with MMR protein loss, MSI-H, and TMB-H. Despite exhibiting these genomic hallmarks of immunogenicity, response rates and survival outcomes appeared similar between cMMR gene alteration and wild-type groups in a limited subset of advanced lung cancer patients treated with ICIs. Immune profiling revealed no significant differences in PD-L1 expression or immune infiltration score between the cMMR gene alteration and wild-type groups. These findings suggest that cMMR gene alterations are rare in lung cancer but contribute to genomic instability in a subset of cases. However, they may be insufficient to guide immunotherapy decisions.

Our study reveals a low prevalence of deleterious cMMR gene alterations in lung cancer (approximately 1% of over 20,000 patients), a finding validated in an independent cohort of 5,046 cases. This prevalence is comparable with prior studies of MSI-H/MMR protein loss rates (typically 0.4%-1.2%) in lung cancers. This prevalence is substantially lower than that observed in tumor types where routine MMR/MSI testing is standard practice, such as colorectal or endometrial cancer.[1] Nevertheless, our study represents the largest systematic analysis of the frequency of cMMR gene alterations in lung cancer to date. We further delineated the histologic specificity of cMMR gene alterations, with LUSC exhibiting the highest frequency of around 2%. Additionally, we observed that tumors with cMMR gene alteration tended to have a more advanced tumor stage. This pattern aligns with the observations in prostate, breast, and endometrial cancers.[38–41] Although recent studies have suggested an association between MMR deficiency and a high smoking rate,[14] similar results were not found in our data, reflecting differences in cohort selection or sample bias across studies. Collectively, given the low prevalence and uncertain therapeutic relevance, routine screening of MMR genes may not be justified in lung cancer populations. Instead, MMR testing could be incorporated into broader genomic profiling conducted for other clinical indications. This opportunistic approach may help accumulate real-world evidence to better delineate the role of MMR deficiency in lung cancer and inform future clinical guidelines.

MSI-H and MMR protein loss are the hallmark consequences of cMMR gene alterations in the Lynch syndrome-associated cancer types.[4] To determine whether similar mechanisms work for lung cancers, we evaluated the functional impact of cMMR gene alterations across both the discovery and validation cohorts. Intriguingly, only approximately 20% of the tumors with cMMR gene alteration exhibited an MSI-H/MMR protein loss phenotype, with a proportion substantially lower than the over 90% prevalence observed in Lynch syndrome-associated malignancies.[4] whereas comparable to that in non-Lynch syndrome cancer types.[40,42] This limited functional penetrance suggests that the genomic alterations of MMR alone may be insufficient to infer pathway inactivation in lung cancer. Since cMMR wild-type tumors rarely showed dMMR/MSI-H, the presence of pathogenic mutations remains a potential indicator of functional deficiency. However, confirmatory functional assessments, such as IHC for MMR protein or PCR-based MSI assays, are still essential when cMMR gene alterations are detected by sequencing. These additional evaluations can more accurately determine MMR status and may help guide immunotherapy decisions in this rare molecular subset.

MSI-H and MMR protein loss are well-established biomarkers predictive of immunotherapy response across different cancers.[9,43] However, some recent case reports have suggested that NSCLC patients with MSI-H or MMR protein loss fail to benefit from treatment with immune checkpoint inhibitors.[44,45] In addition, another retrospective study reported that NSCLC cancer patients with MSI-H (N=10) exhibited a similar response rate after anti-PD-1monotherapy compared to NSCLC without MSI-H.[14] Consistent with these observations, our study, based on a modestly sized cohort of lung cancer patients with deleterious cMMR gene alterations who received

immunotherapy, did not observe a significant improvement in clinical response rates. Although preliminary, these findings suggest a limited predictive value of cMMR gene alterations. However, given the small sample size and retrospective nature of the analysis, prospective validation in larger, well-characterized cohorts is necessary to clarify the predictive relevance of MMR deficiency in this disease context.

The lack of immunotherapy benefit observed in lung cancers with cMMR gene alterations may be attributed to tissue-specific biological constraints. In colorectal cancers, MMR deficiency typically induces substantial remodeling of the tumor immune microenvironment, characterized by increased $CD8^+$ T cell infiltration and PD-L1 upregulation.[44] In contrast, the tumors with cMMR gene alteration in our discovery cohort exhibited comparable immune cell composition and PD-L1 level to their cMMR wild-type counterparts, despite exhibiting elevated TMB. This attenuated immunogenic response may be a result of the intrinsically active baseline immune microenvironment of lung cancers,[46,47] which could mitigate the additive immunogenic effects of neoantigens generated by MMR deficiency. A previous study with similar findings suggested that the lack of improved sensitivity to immunotherapy might result from co-occurring alterations in immune resistance-related genes such as STK11, KEAP1, and JAK1.[14] However, these alterations were not present in our lung cancer cohort with cMMR gene alteration. The reason why the immune system does not respond to the high TMB related to cMMR gene alterations in lung cancers requires further mechanistic studies.

This study has several limitations. Most notably, although we screened over 20,000 lung cancer cases, the number of patients with deleterious cMMR gene alterations who had also received immunotherapy was relatively small. This limited sample size substantially restricts the statistical power of our analyses regarding treatment response and survival outcomes, and the findings should therefore be interpreted with caution. Additional evidences from larger and preferably prospective cohorts will be critical to confirm the clinical relevance of cMMR gene alterations in the context of immunotherapy for lung cancer. Secondly, the selection of different panels for gene sequencing limited the achievement of comprehensive molecular profiling: MSI-H/TMB data were available for only partial cases, potentially obscuring the phenotype-genotype correlations. Finally, this study mainly focused on the clinical associations between cMMR gene alterations, immune-quiescent microenvironments, and co-occurring genomic events, deeper mechanistic insights should be provided by future functional studies in preclinical models.

CONCLUSION

This study presents a large-scale, comprehensive analysis of cMMR gene alterations in lung cancer, demonstrating their low prevalence (~1%). Notably, only approximately 20% of tumors with cMMR gene alteration exhibited MSI-H or MMR protein loss. In a limited subset of advanced lung cancer patients treated with ICIs, response rates and survival outcomes were comparable between cMMR gene alteration and wild-type groups. These findings suggest that cMMR gene alterations alone may be insufficient to guide immunotherapy decisions. Further studies are warranted to clarify the clinical utility of MMR deficiency in lung cancers.

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AUTHOR CONTRIBUTIONS

L.H. and Y.W. conceived and designed the study. Y.P., X.W., and S.L. contributed clinical samples and collected associated clinical data. Q.L. performed immunohistochemical experiments. J.T. and W.C. acquired and curated public datasets for validation. All authors participated in data collation and analysis. The manuscript was drafted by all authors, with critical revisions provided by L.H. and Y.W. All authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

Informed written consent was obtained from all participants. This study was approved by the Research and Ethics Committee of Peking University Cancer Hospital (Approval No: 2023YJZ82).

DATA AND CODE AVAILABILITY

The genomic and clinical data from the discovery cohort are available from the corresponding author upon reasonable request, subject to institutional data sharing agreements and ethical review board approval. The validation cohort data analyzed in this study are publicly accessible as described in the methods section.

SUPPLEMENTAL INFORMATION

It can be found online at https://doi.org/10.59717/j.xinn-med.2025.100163