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DNA methyltransferase 3A (DNMT3A) mutations and PD-(L)1 blockade efficacy in non-small-cell lung cancer

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Abstract

Background: Despite significant improvements in overall survival with programmed cell death protein (ligand) 1 [PD-(L) 1] inhibition, most patients with metastatic non-small-cell lung cancer (NSCLC) do not respond to immune checkpoint inhibition (ICI). Growing evidence suggests the importance of genomic alterations in modulating anticancer immune response and predicting ICI efficacy. However, the genomic correlates of response to ICI in NSCLC are largely unknown.

Design: Patients with advanced NSCLC treated with ICI and comprehensive genomic profiling from multiple independent cohorts were included. Beta-binomial modelling of sequencing read counts was used to infer mutation clonality. NSCLC samples from Cancer Genome Atlas Program

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(TCGA) and NSCLC cell lines from Cancer Cell Lines Encyclopedia (CCLE) were used for transcriptomic analyses.

Results: Among 1539 NSCLCs, we identified deleterious *DNA methyltransferase 3A* (*DNMT3A*) mutations in 4.7% of cases. Patients with *DNMT3A*-mutant NSCLC had improved response rate (41.7% versus 21.5%, $P < 0.001$), progression-free survival [hazard ratio (HR) 0.61, $P < 0.001$], and overall survival (HR 0.66, $P < 0.01$) with PD-(L)1 blockade, compared with *DNMT3A* wild-type cases. *DNMT3A* mutations had no impact on OS among patients with advanced NSCLC who did not receive ICI (HR 0.88, $P = 0.41$). In examining the impact of *DNMT3A* clonality on immunotherapy outcomes to account for potential clonal hematopoiesis of indeterminate potential contamination, we confirmed that clonal *DNMT3A* mutations were associated with improved outcomes compared with *DNMT3A* wild-type cases. In NSCLC cell lines with pathogenic *DNMT3A* mutations, DNMT3A RNA and protein expression were decreased. In the TCGA, NSCLCs with high versus low DNMT3A expression exhibited lowered expression of pathways involved in innate and adaptive immune response, including interferon- γ (INF γ), major histocompatibility complex (MHC)-II antigen presentation, tumor necrosis factor- α (TNF- α), and PD-1 signaling.

Conclusion: Somatic *DNMT3A* mutations can be detected in a fraction of NSCLCs and are associated with a decreased DNMT3A expression and a favorable immunophenotype, and predict improved ICI efficacy.

Keywords

non-small-cell lung cancer (NSCLC); PD-(L)1 inhibition; predictive biomarkers in immunotherapy response; DNMT3A mutations; comprehensive tumor genomic profiling

INTRODUCTION

The development of immunotherapies has revolutionized the treatment landscape of non-small-cell lung cancer (NSCLC), offering unprecedented therapeutic benefits and significantly improving patient survival.^{1–5} Despite these remarkable advancements, a substantial proportion of lung cancer patients do not respond to immunotherapy, while others experience only transient responses followed by disease progression. This variability underscores the need for robust biomarkers that can reliably predict patient response to immunotherapy in lung cancer. Current biomarkers, such as programmed death-ligand 1 (PD-L1) expression and tumor mutational burden (TMB), have shown promise but are insufficiently accurate in identifying patients who are more likely to benefit from immunotherapy. Therefore, there is still a need to explore additional biomarkers to enhance the precision and efficacy of immunotherapy in lung cancer.^{6,7}

In recent years, growing attention has been directed toward the role of epigenetic alterations in cancer development and treatment response. DNA methylation, a crucial epigenetic mechanism regulated by DNA methyltransferases (DNMTs), has emerged as a key player in shaping the tumor microenvironment and modulating immune responses in cancer.^{8,9} Among the DNMTs, mutations in DNMT3A have garnered significant interest due to their prevalence in various cancer types, including lung cancer.^{10,11} These mutations can lead to

widespread changes in DNA methylation patterns, impacting gene expression profiles and therefore tumor biology.¹⁰

Mounting evidence suggests that DNMT inhibition may influence the tumor immune microenvironment, thereby potentially affecting the efficacy of immunotherapy.⁹ These changes may impair immune recognition and clearance of tumor cells or create an immunosuppressive microenvironment which favors tumor growth and immune evasion. Therefore, understanding the impact of loss-of-function mutations in these genes on immune responses in lung cancer is crucial for identifying novel biomarkers and therapeutic targets that can improve patient outcomes with immunotherapy. Here we report on the association between *DNMT3A* loss-of-function mutations and clinical outcomes to PD-(L)1 blockade in patients with NSCLC and perform correlative analysis to characterize the association between DNMT3A expression and immunophenotype in NSCLC.

METHODS

Study population

All consecutive patients with NSCLC at the Dana-Farber Cancer Institute (DFCI), Memorial Sloan Kettering Cancer Center (MSKCC), and Gustave Roussy Center (GR) whose tumors underwent targeted next-generation sequencing (NGS), and who received treatment with a PD-(L)1 checkpoint inhibitor (alone or in combination with a CTLA4 checkpoint inhibitor) in any line between December 2015 and May 2022 were included in this study. Patients who received chemo-immunotherapy were not eligible. An additional, noncomparative cohort of patients with metastatic NSCLC treated with non-ICI based regimens in any line from DFCI was also included.

Comprehensive tumor genomic profiling

NSCLC samples from DFCI were sequenced with targeted NGS using the OncoPanel platform, which assesses 277 (version 1, April 2013-July 2014), 302 (version 2, July 2014-September 2016), and 447 (version 3, September 2016-present) cancer-associated genes, as previously described.¹² NSCLC samples from MSKCC underwent NGS using the MSK-IMPACT platform, which assesses 341 (version 1), 410 (version 2), and 468 (version 3) cancer-associated genes, as previously described.¹³ NSCLC samples from GR were assessed with FoundationOne CDx, which detects substitutions and indels in 324 genes, as previously described,¹⁴ or institutional whole exome sequencing. TMB was harmonized across platforms as previously described by our group.¹⁵

DNMT3A mutation and clonality assessment

All loss-of-function mutations in *DNMT3A* (including nonsense, frameshift, or splice site) were classified as deleterious. To determine the pathogenicity of missense mutations we carried out an in silico functional analysis using the PolyPhen-2 (Polymorphism Phenotyping v2), Sorting Intolerant from Tolerant (SIFT), and Mutation Assessor prediction tools to determine the functional significance of each missense mutation.^{16–18} Missense mutations of unknown significance predicted to be benign/tolerated were excluded from this analysis. The prediction of *DNMT3A* mutation clonality was carried out with INCOMMON,

a method specifically conceived for the inference of clonality and allele-specific copy number configuration of mutations from tumor-only clinical sequencing.

Transcriptomic analysis from Cancer Cell Lines Encyclopedia (CCLE) and the Cancer Genome Atlas Program (TCGA)

Genomic and transcriptomic data were downloaded from cBioportal (<https://www.cbioportal.org/>) and XENA browser (<https://xenabrowser.net/>). Differential gene expression analysis was carried out using DESeq2 with the following settings: $\text{padj} \leq 0.05$ and $|\text{Log}_2\text{FC}| \geq 0.8$. Pathway and transcription factor (TF) activity were inferred using the R package decoupleR, which is designed for the integrative analysis of transcriptomic data and quantifies the activity of predefined gene sets corresponding to specific pathways and TFs from gene expression data. Immune subtyping was investigated with a method developed at the pan-cancer level that classifies tumors in four different subtypes (immune-depleted and nonfibrotic, immune-depleted and fibrotic, immune-enriched and nonfibrotic, immune-enriched and fibrotic) leveraging a set of 29 microenvironment-related signatures estimated from bulk RNA-Seq (<http://science.bostongene.com/tumor-portrait>; <http://science.bostongene.com/tumor-portrait>).

Clinical outcomes

Overall response rate (ORR) and progression-free survival (PFS) were determined via Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1. PFS was defined as the time from the date of ICI initiation to the date of disease progression or death, whichever took place first. For patients without progression, censoring occurred at the time of their last adequate disease assessment. Overall survival (OS) was defined as the time from the date of immunotherapy initiation to the date of death. For the discovery cohort, RECIST assessment was carried out by a radiologist, who was blinded to *DNMT3A* mutational status. For the validation cohort, RECIST assessment carried out either by a radiologist or by treatment investigator were considered acceptable. A control cohort of patients with metastatic lung cancer who did not receive immunotherapy was included. For this cohort, OS was calculated from the date of diagnosis of metastatic disease to the date of death. For patients who were still alive at the time of data cut-off, censoring occurred at the date of last contact.

Statistical analyses

Differences between continuous variables were evaluated by Wilcoxon signed rank tests and Kruskal—Wallis tests as appropriate, whereas differences between categorical variables were evaluated by Fisher's exact tests. Kaplan—Meier methodology was used to estimate event—time distributions. Log-rank tests were used to assess differences in the event—time distributions, and Cox proportional hazards models were used to generate hazard ratio estimates in both univariate and multivariate models. For multivariable models, the following variables were included: age, sex, Eastern Cooperative Oncology Group (ECOG) performance status (PS), PD-L1, TMB, line of ICI, histology, and history of tobacco use. All *P* values are two-sided and confidence intervals are at the 95% level. Causal mediation analysis was conducted to evaluate whether *DNMT3A* mutation and TMB were independently associated with outcomes to immunotherapy. This approach allowed us to quantify the extent to which the effect of *DNMT3A* mutation on treatment outcomes

was mediated through TMB, while also estimating the direct (non-mediated) effect of *DNMT3A* mutation. We applied parametric regression models to estimate the average causal mediation effect, average direct effect, and total effect. Models were adjusted for relevant clinical covariates including age, smoking status, sex, histology, PD-L1 tumor proportion score (TPS), Eastern Cooperative Oncology Group performance status (ECOG PS), lines of treatment, and institution indicators. Statistical significance was set at $P < 0.05$. Analyses were carried out using R version 4.2.1.

Ethics

The study was conducted in accordance with institutionally approved institutional review board protocols (DF/HCC 02–180). Informed consent was obtained from each patient at each of the participating institutions.

RESULTS

Patient characteristics

Dana-Farber Cancer Institute. A total of 747 patients with metastatic NSCLC who received PD-(L)1 inhibitors at DFCI were used as the discovery cohort. In this cohort, median age was 67 years, 55% were women, 84.9% had a history of tobacco use, 81.5% had adenocarcinoma histology, 36.3% received ICI as first-line therapy, 4.0% received PD-(L)1 + CTLA4 inhibition, and 79.7% had an ECOG PS of 0–1. Median PD-L1 TPS was 50%, and 34.9% of patients had a *KRAS* mutation (Table 1).

Memorial Sloan Kettering Cancer Center and Gustave Roussy Center. An independent cohort of 792 patients who received PD-(L)1 inhibitors at MSKCC and GR was also included as the external validation cohort. In this second cohort, median age was 67 years, 56.8% were women, 84.2% had a history of tobacco use, 98.0% had adenocarcinoma histology, 30.8% received ICI as first-line therapy, 7.7% received PD-(L)1 + CTLA4 inhibition, and 90.9% had an ECOG PS of 0–1. Median PD-L1 TPS in this cohort was 10% and 40.7% of patients had a *KRAS* mutation (Table 1).

DNMT3A mutations are associated with PD-(L)1 blockade efficacy in patients with NSCLC

To determine whether there was any specific mutation enriched in responders versus nonresponders, we first carried out an unbiased gene mutation enrichment analysis in the DFCI cohort and identified *DNMT3A* mutations (*DNMT3A*-mut) as one of the genes enriched in responders to immunotherapy (Figure 1A). The mutational landscape of *DNMT3A* in the discovery cohort is summarized in the Supplementary Figure S1, available at <https://doi.org/10.1016/j.annonc.2025.06.003>. As expected, mutations in *EGFR* and *STK11* were instead more common in nonresponders. We next sought to investigate the clinicopathological features and outcomes to immunotherapy in patients with versus without *DNMT3A*-mut. First, we compared baseline characteristics according to *DNMT3A* mutation in this cohort. Patients with *DNMT3A*-mut (7.5%) and *DNMT3A*-wt (92.5%) NSCLC were balanced in terms of sex, history of tobacco use, ECOG PS, histology, PD-L1 expression, distribution of driver mutations, and line of therapy for ICI (Supplementary Table S1, available at <https://doi.org/10.1016/j.annonc.2025.06.003>). However, patients with

DNMT3A-mut were older (median age 70.5 versus 66 years, $P = 0.02$), and had a higher median TMB (11.8 versus 9.9 mut/Mb, $P = 0.02$). In terms of clinical outcomes to ICI, there was a significantly higher ORR (37.5% versus 21.9%, $P = 0.01$), longer median PFS (8.9 versus 3.2 months, HR 0.65, $P = 0.004$) and median OS (21.0 versus 12.0 months, HR 0.68, $P = 0.02$) among *DNMT3A*-mut compared with *DNMT3A*-wt NSCLCs (Figure 1B–D). Importantly, *DNMT3A* mutation was confirmed to be an independent predictor of improved PFS and OS in multivariable Cox regression models, after adjusting for relevant clinical and molecular variables (Supplementary Figure S2, available at <https://doi.org/10.1016/j.annonc.2025.06.003>).

To validate these findings, we analyzed an independent cohort of patients with metastatic NSCLC from MSKCC and GR with *DNMT3A* mutation status available. Again, patients with *DNMT3A*-mut and *DNMT3A*-wt NSCLC were well balanced in terms of baseline clinicopathological characteristics, except for TMB, which was higher among patients with *DNMT3A*-mut ($P = 0.001$) (Supplementary Table S2, available at <https://doi.org/10.1016/j.annonc.2025.06.003>). Also in this cohort, *DNMT3A*-mut was associated with significantly higher ORR (56.2% versus 21.1%, $P = 0.01$), and longer median PFS (15.7 versus 2.8 months, HR 0.48, $P = 0.01$) and OS (55.3 versus 14.5 months, HR 0.46, $P = 0.03$) (Supplementary Figure S3, available at <https://doi.org/10.1016/j.annonc.2025.06.003>). Multivariable Cox regression for the validation cohort is shown in Supplementary Figure S4, available at <https://doi.org/10.1016/j.annonc.2025.06.003>. The mutational landscape of *DNMT3A* in the validation cohort is summarized in the Supplementary Figure S5, available at <https://doi.org/10.1016/j.annonc.2025.06.003>.

To further validate these results, we also carried out a pooled analysis of these two cohorts and confirmed significantly higher ORR (41.7% versus 21.5%, $P < 0.001$), and longer median PFS (9.2 versus 2.9 months, HR 0.61, $P < 0.001$) and OS (29.6 versus 13.3 months, HR 0.66, $P < 0.01$) to ICI among *DNMT3A*-mut versus *DNMT3A*-wt NSCLCs (Figure 2A–C). *DNMT3A*-mut also retained an association with improved PFS and OS in multivariable Cox regression in this combined cohort (Figure 3); univariate analyses are shown in Supplementary Figure S6, available at <https://doi.org/10.1016/j.annonc.2025.06.003>. Baseline clinicopathological characteristics of patients from the combined cohort are summarized in Supplementary Table S3, available at <https://doi.org/10.1016/j.annonc.2025.06.003>.

Given that *DNMT3A* mutation status predicts clinical outcomes independently of TMB and because *DNMT3A*-mut in our cohort was associated with higher TMB, we investigated whether this represents a significant direct effect or is mediated through TMB. To address this question, we conducted causal mediation analysis examining the relationship between *DNMT3A* mutational status, harmonized TMB Z-scores and clinical outcomes. The analysis controlled for potential confounders including age, smoking status, sex, histology, PD-L1 TPS, ECOG PS, lines of treatment, and institution indicators and confirmed an independent association between *DNMT3A*-mut and improved ORR, PFS, and OS to immunotherapy, independently from TMB (Supplementary Table S4, available at <https://doi.org/10.1016/j.annonc.2025.06.003>).

Lastly, to dissect the predictive versus prognostic impact of *DNMT3A* mutations, we examined a control cohort of patients with metastatic NSCLC who did not receive immunotherapy and found no difference in OS (19.1 versus 23.7 months, HR 0.88, $P = 0.41$) according to *DNMT3A* mutation status, indicating that *DNMT3A* mutations are likely predictive rather than prognostic (Supplementary Figure S7, available at <https://doi.org/10.1016/j.annonc.2025.06.003>).

Assessment of DNMT3A mutational clonality and impact on ICI efficacy

Because *DNMT3A* mutations have been reported as a common driver of clonal hematopoiesis of indeterminate potential (CHIP),^{11,19,20} and CHIP mutations can be captured using tumor-tissue-only DNA sequencing,²¹ we next aimed to understand whether a fraction of these mutations was subclonal and therefore potentially representative of CHIP. We deployed INCOMMON, a model that infers clonality and allele-specific copy number configurations in tumor-only clinical targeted sequencing.²² Because MSK-IMPACT has paired blood sequencing to subtract CHIP mutations, we applied INCOMMON only to the DFCI and GR cohorts and noted that 2.3% of patients had subclonal *DNMT3A*-mut ($n = 19$), potentially reflecting either CHIP or subclonal cancer mutations. Representative cases of clonal and subclonal *DNMT3A*-mut as assessed by INCOMMON are shown in Figure 4A. As expected, median age was higher in patients with subclonal mutations (71.5 years) compared with patients with clonal *DNMT3A*-mut (68 years) and those with *DNMT3A*-wt genotype (66 years) ($P = 0.04$) (Supplementary Table S5, available at <https://doi.org/10.1016/j.annonc.2025.06.003>), as the development of CHIP mutations increases with age.¹⁹ There were no other differences across these subgroups, except for harmonized TMB, which was higher among patients with clonal or subclonal *DNMT3A*-mut compared with *DNMT3A*-wt cases ($P = 0.02$). In examining clinical outcomes to ICI by *DNMT3A* clonality, we confirmed that patients with clonal mutations, representing somatic cancer-associated mutations, had improved ORR, PFS, and OS compared with patients without *DNMT3A*-mut (Figure 4B–D). Importantly, there was no difference in OS according to *DNMT3A* clonality among patients who did not receive PD-(L)1 blockade ($P = 0.5$) (Supplementary Figure S8, available at <https://doi.org/10.1016/j.annonc.2025.06.003>).

Together, these findings indicate that, even after removing subclonal mutations that may be representative of CHIP, somatic clonal *DNMT3A* mutations are associated with improved ICI efficacy in NSCLC.

Decreased DNMT3A expression is associated with a distinct immunophenotype in NSCLC

We next asked whether cancer cells with pathogenic *DNMT3A* alterations have decreased DNMT3A expression. We interrogated 140 NSCLC cell lines from the CCLE, to remove the potential confounder of CHIP mutations. We noted that 10 cell lines (7.1%) had *DNMT3A* alterations, including one bi-allelic deletion and nine mutations, of which seven were classified as pathogenic (see Methods section). Cell lines with pathogenic *DNMT3A* alterations had decreased mRNA ($P = 0.06$) and protein abundance compared with *DNMT3A*-wt cell lines ($P = 0.002$) (Figure 5A and B), and there was a linear correlation between DNMT3A mRNA expression and protein abundance (Figure 5C). This further supported the hypothesis that a fraction of lung cancers can have somatic *DNMT3A*

mutations and suggested that pathogenic mutations result in decreased mRNA and protein expression.

Because *DNMT3A* mutations remain a rare event in NSCLC, but these are associated with decreased RNA and protein expression, we next asked the broader question of whether NSCLCs with low (<25th percentile) versus high (≥25th percentile) DNMT3A expression had distinct gene expression profile and immunophenotype. We carried out differential gene expression analysis between these two groups using cell lines from the CCLE, and noted marked lower expression of human leukocyte antigen (HLA) class II genes (HLA-DOA, HLA-DRA, HLA-DPA1, HLA-DPB1), PDCD1LG2 (PD-L2), IL-21R, and IRF8 in NSCLC cell lines with high DNMT3A expression (Supplementary Figure S9, available at <https://doi.org/10.1016/j.annonc.2025.06.003>). Similarly, when we carried out differential gene expression analysis between these two groups using NSCLC samples from the TCGA, there was again a significant lower expression of HLA class II genes (e.g. HLA-DRB1, HLA-DRP1, HLA-DBM, HLA-DQA1), but also CD724 (PD-L1), PDCD1LG2 (PD-L2), B2M, and TMEM173 (STING) among NSCLCs with high versus low DNMT3A expression (Figure 5D). Pathway enrichment analysis also identified significant lower expression of innate and adaptive immune-related pathways, including PD-1 signaling, interferon- γ (INF γ) signaling and response, and major histocompatibility complex (MHC) class II antigen presentation, in NSCLC samples with high versus low DNMT3A expression (Figure 5E). To further characterize the transcriptomic features of these cancers, we investigated cancer-associated pathways inferred from perturbation experiments (PROGENy), and activity of immune- and pathway-related TFs (DoRothEA), and noted marked lower expression of pro-inflammatory pathways including NF κ B, tumor necrosis factor- α (TNF- α), and JAK/STAT, as well as TFs such as NF κ B1, STAT1, and FOS (Supplementary Figures S10 and S11, available at <https://doi.org/10.1016/j.annonc.2025.06.003>) in DNMT3A high versus low NSCLCs. Lastly, to identify potential differences in the immunophenotype of these tumors, we deconvoluted bulk RNA-Seq in immune subtypes and found that DNMT3A^{High} NSCLCs were more likely to be immune deserts compared with DNMT3A^{Low} cases, which instead were more immune enriched (Figure 5F).

DISCUSSION

In this study we elucidated the role of *DNMT3A* mutations in predicting and possibly enhancing the efficacy of PD-(L)1 blockade in patients with NSCLC. DNMT3A and DNMT3B are enzymes belonging to the DNA methyl transferase family and are involved in *de novo* methylation diverting the methyl group from S-adenosylmethionine to a cytosine residue.²³ *De novo* hypermethylation in cancer cells is frequently observed in transcriptional regulatory elements including promoters and enhancers of genes, including tumor suppressor genes (CDKN2A/B), adhesion regulators (CDH1), and immune regulatory elements such as HLA-I.^{24,25} Through comprehensive analysis of large patient cohorts we demonstrated that *DNMT3A* mutations are significantly enriched among responders to immunotherapy, correlating with improved objective response rates, and also longer PFS and OS. The impact of *DNMT3A* mutation on improved outcomes to ICI was consistent across two independent cohorts, underscoring the robustness of DNMT3A as a potential biomarker

for ICI efficacy. Importantly, this effect was not seen among patients who did not receive ICI, suggesting this is a predictive rather than a prognostic biomarker.

In this study, we showed that pathogenic *DNMT3A* mutations commonly result in reduced RNA and protein expression, and that compared with NSCLCs with low DNMT3A expression, samples with high DNMT3A expression exhibit distinct immunosuppressive profiles, including reduced expression of key immune pathways and reduced expression of HLA class II genes and PD-(L)1/PD-L2. These tumors are also more likely to present as immune deserts, with decreased infiltration of immune cells, as opposed to tumors with low DNMT3A expression, which were instead immune enriched. While the mechanisms underlying this decreased expression are unclear, many of the *DNMT3A* mutations identified in our analysis are predicted loss-of-function events, including nonsense, frameshift, and splice site mutations. These are expected to introduce premature stop codons that can trigger nonsense-mediated mRNA decay (NMD), a well-established mechanism that reduces mRNA transcript levels from mutated alleles. Additionally, some missense mutations may impair transcript stability or promote degradation through altered RNA structure or recognition by RNA-binding proteins. Our observation of reduced DNMT3A mRNA expression in cell lines with pathogenic mutations is consistent with these mechanisms and supports the functional impact of these alterations. Of note, among the limited number of available cell lines, only a subset of DNMT3A-mutmodels demonstrated low DNMT3A expression, suggesting that reduced expression may arise through mechanisms beyond mutational inactivation. This observation raises the possibility that DNMT3A low tumors, independent of mutation status, may represent a broader biologically distinct and potentially targetable subgroup.

Clinically, these findings position *DNMT3A* not only as a predictive biomarker for immunotherapy responsiveness but also as a potential therapeutic target. Strategies aimed at modulating DNMT3A activity or expression could enhance the immunogenicity of NSCLC, thereby improving the efficacy of existing immunotherapies. This is particularly relevant given the limited response rates to PD-(L)1 inhibitors in unselected patient populations and the need for novel approaches to sensitize tumors to immune checkpoint blockade. In this context, DNMT inhibition has been shown to up-regulate MHC-I expression in response to INF γ in murine tumor models of breast cancer, promoting recruitment of CD8 $^{+}$ T cells to the microenvironment of these tumors, which are traditionally less sensitive to ICI.⁹ In addition, a recent study identified KDM1A as a synthetic lethal partner of DNMT3A deletion, and that KDM1A inhibition selectively inhibited liver and lung metastasis in a DNMT3A-KO xenograft model, suggesting that DNMT3A-deficient lung cancers may be susceptible to targeted therapeutic interventions.²⁶ As there are now multiple DNMT inhibitors, including DNMT3A inhibitors, that are being developed as monotherapy or in combination with cytotoxic chemotherapy or PD-(L)1 blockade,^{27,28} a deeper understanding of the mechanisms by which these hypomethylating agents contribute to anticancer tumor biology and immunity will be instrumental to rationally developing novel therapeutic approaches for patients with lung cancer.

This study has limitations to acknowledge, including the retrospective design, the relative rarity of *DNMT3A* mutation in NSCLC, which limited subgroup analysis, and the

lack of paired blood sequencing to identify CHIP mutations in the DFCI and GR cohorts. Nonetheless, by inferring mutation clonality we were able to identify the relative contribution of clonal and subclonal mutations on clinical outcomes to PD-(L)1 blockade in our patient population. This analysis indicated that clonal *DNMT3A* mutations are associated with immunotherapy response, highlighting their potential relevance also in cancer biology, in addition to age-related clonal hematopoiesis. In support of this, *TET2* mutations, which are also commonly seen in CHIP, were not enriched among responders to ICI, suggesting a specific role for somatic, cancer-associated *DNMT3A* mutations in predicting immunotherapy efficacy. Nonetheless, this study highlights the importance of implementing routine paired tumor-blood sequencing to accurately identify potential CHIP alterations and correctly interpret tumor DNA sequencing results. Lastly, this study is limited by the lack of functional validation to determine whether the observed immunologic effects of *DNMT3A* mutations are mediated through direct regulation of specific target genes or broader epigenetic reprogramming. While our transcriptomic analyses suggest associations with lowered expression of key immune-related pathways, the precise downstream targets and mechanisms by which DNMT3A loss influences the tumor immune microenvironment and immunotherapy response remain to be fully elucidated.

In conclusion, our study provides evidence that *DNMT3A* mutations can develop in NSCLC as somatic events, and these could serve as a biomarker for predicting response to PD-(L)1 blockade. Since novel DNMT inhibitors are being developed, blocking DNMT3A could represent a potential therapeutic strategy to augment immunotherapy efficacy, but preclinical investigation is needed to confirm and validate these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DISCLOSURE

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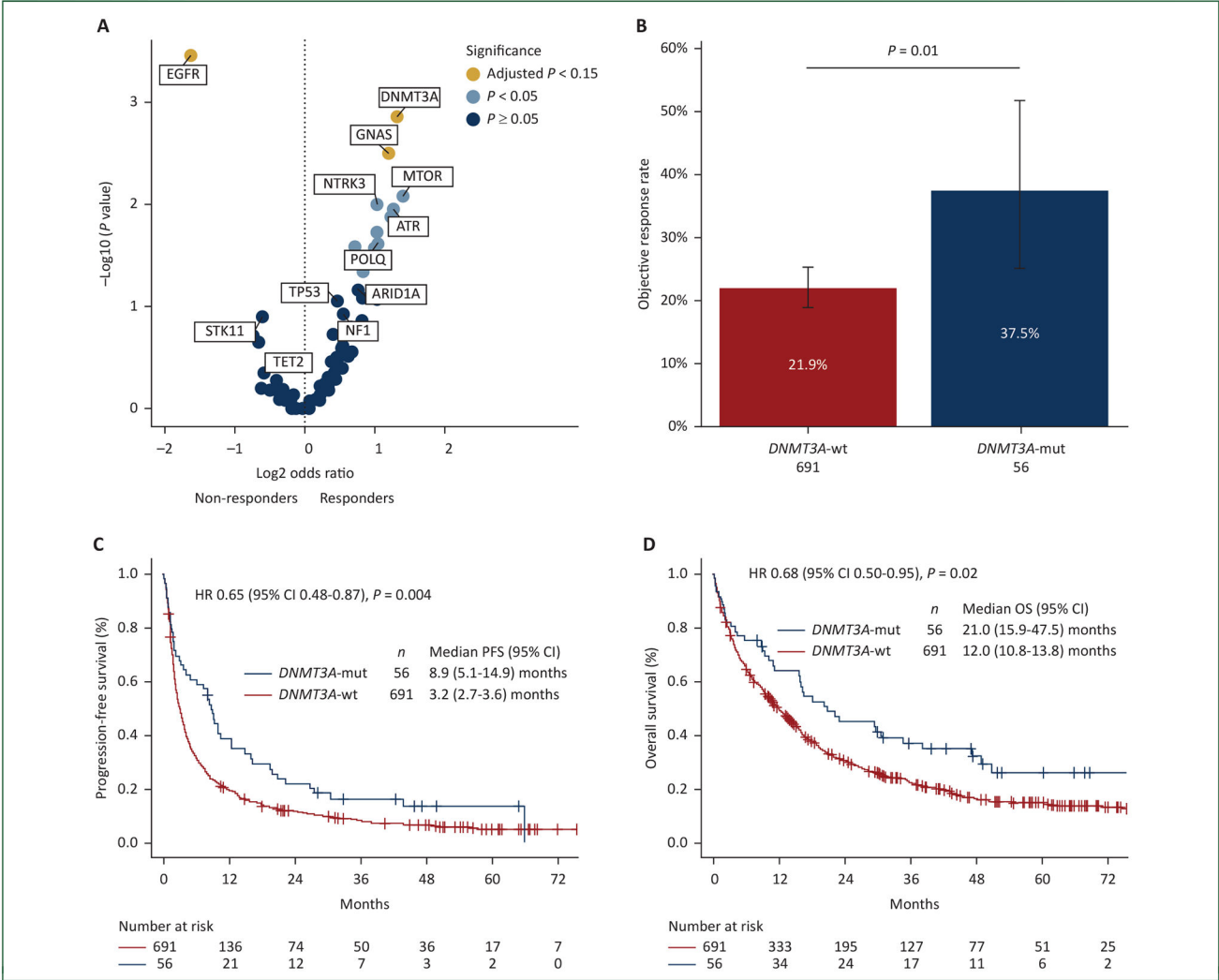


Figure 1. (A) Volcano plot showing gene mutations enriched among responders and nonresponders to programmed death protein (ligand) 1 blockade in the DFCI cohort. (B) Objective response rate, (C) progression-free survival (PFS), and (D) overall survival (OS) to PD-(L)1 blockade according to DNMT3A mutation status. CI, confidence interval; HR, hazard ratio.

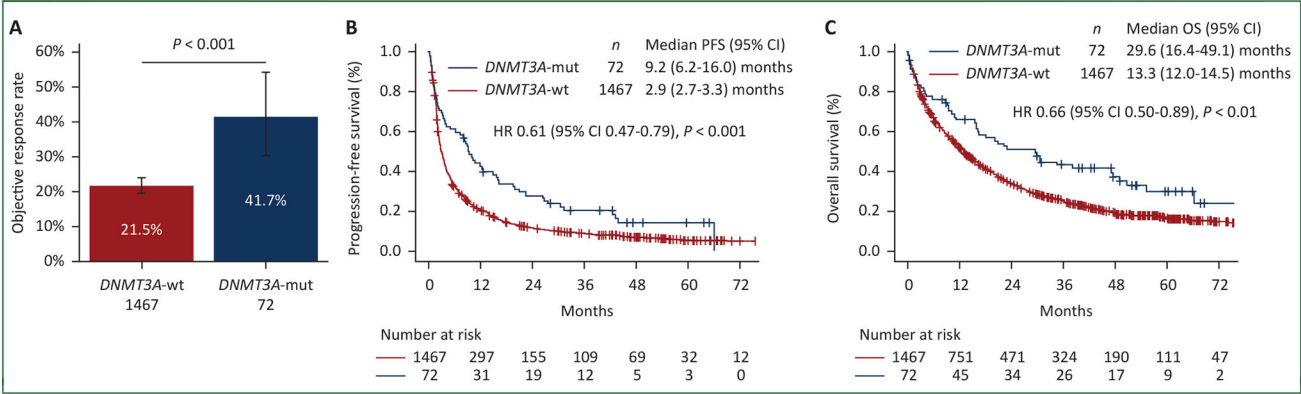


Figure 2. (A) Objective response rate, (B) progression-free survival (PFS), and (C) overall survival (OS) to programmed death protein (ligand) 1 blockade according to DNMT3A mutation status in the combined Dana-Farber Cancer Institute, Memorial Sloan Kettering Cancer Center, and Gustave Roussy Center cohort.
CI, confidence interval; HR, hazard ratio.

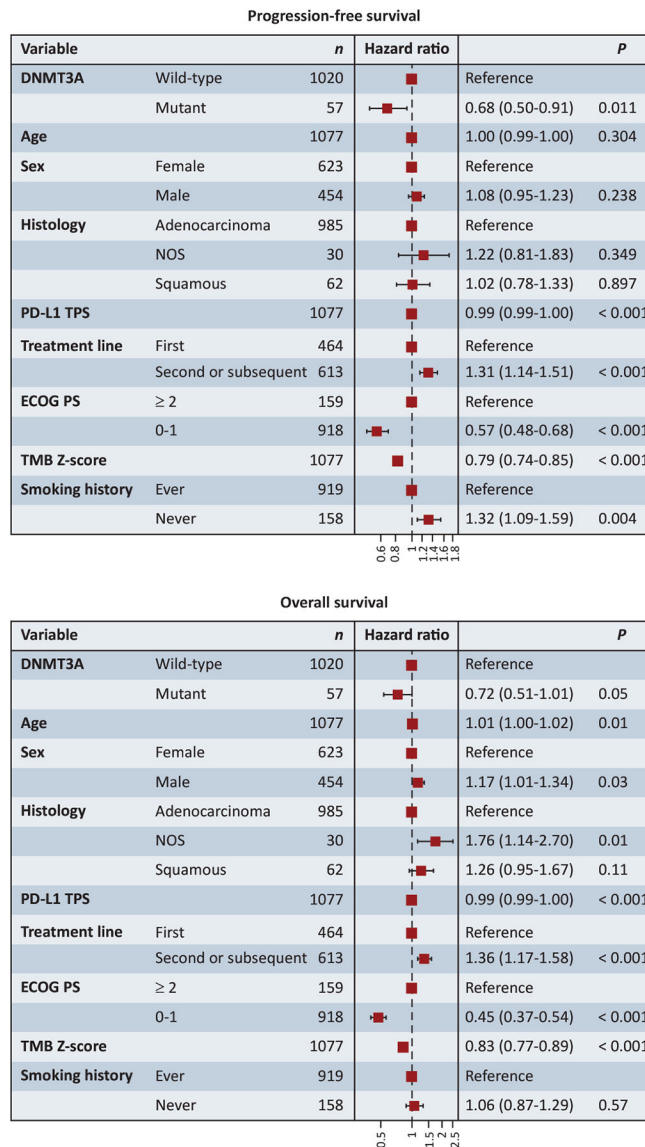
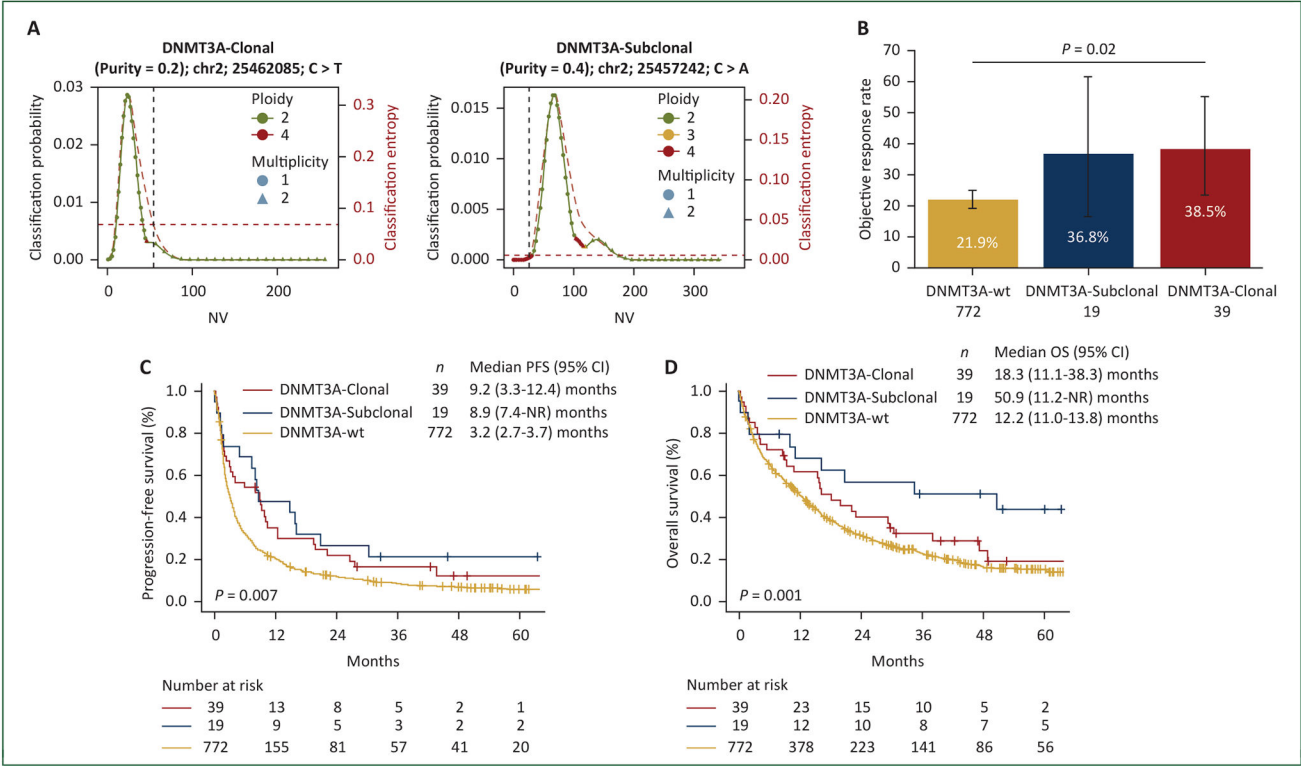
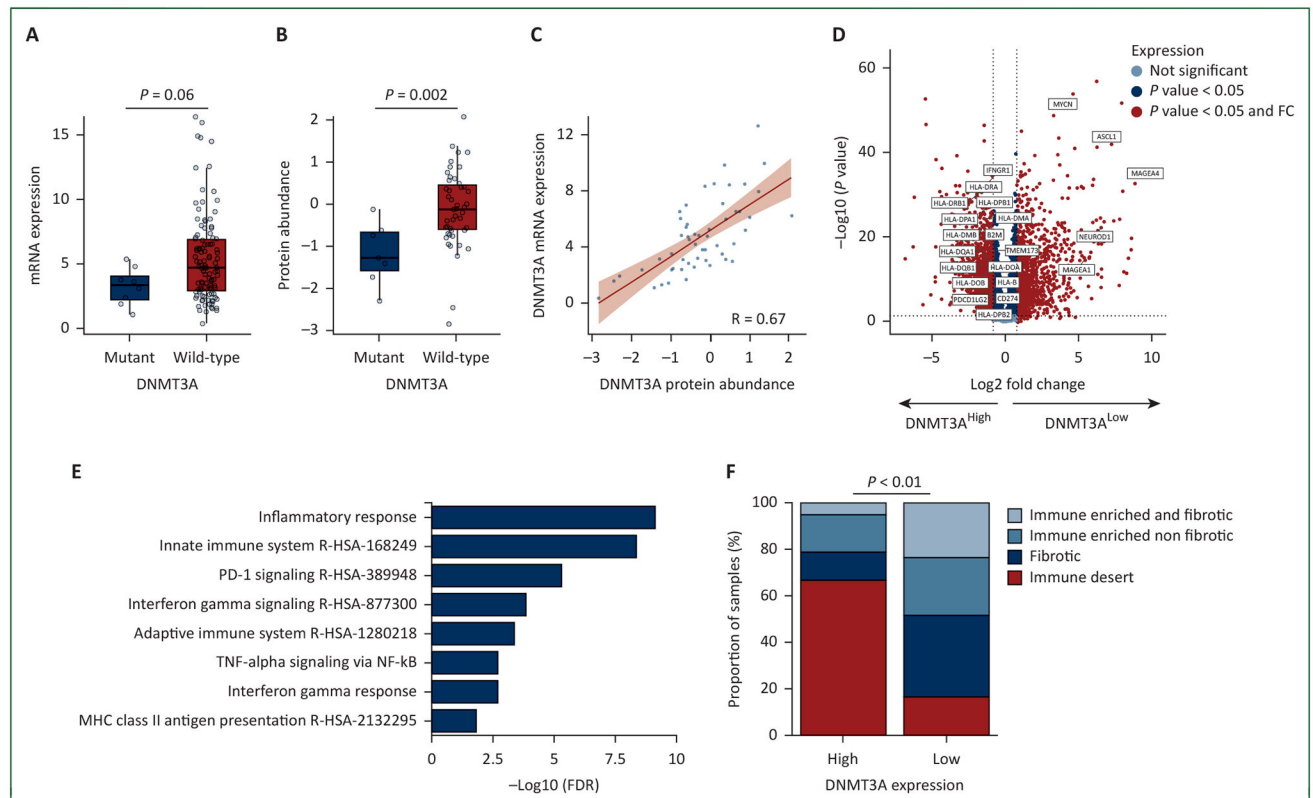


Figure 3. Multivariable Cox regression model for progression-free survival (PFS) and overall survival (OS) in the combined Dana-Farber Cancer Institute, Memorial Sloan Kettering Cancer Center, and Gustave Roussy Center cohort.

PD-L1, programmed death-ligand 1; TPS, tumor proportion score; ECOG PS, Eastern Cooperative Oncology Group performance status; TMB, tumor mutational burden.



**Figure 5.**

Box plots showing differences in DNMT3A (A) mRNA, and (B) protein expression in non-small-cell lung carcinoma (NSCLC) cell lines. (C) Linear correlation between DNMT3A mRNA and protein expression in NSCLC cell lines. (D) Volcano plot of differentially expressed genes between NSCLC with high versus low DNMT3A expression. (E) Pathway enrichment analysis depicting top pathways with reduced expression in NSCLC with high versus low DNMT3A expression in the TCGA dataset. (F) Immune subtype deconvolution using RNA-Seq data from the TCGA dataset of NSCLC with high versus low DNMT3A expression.

Table 1.

Baseline characteristics of patients with non-small-cell lung cancer treated with programmed death protein (ligand) 1 blockade at Dana-Farber Cancer Institute (discovery cohort) and at Memorial Sloan Kettering Cancer Center and Gustave Roussy Center (validation cohort)

	Discovery cohort	Validation cohort
	<i>n</i> (%)	<i>n</i> (%)
Age, median years (range)	67 (25–92)	67 (22–93)
Sex		
Female	411 (55.0)	450 (56.8)
Male	336 (45.0)	342 (43.2)
Smoking history		
Ever	634 (84.9)	666 (84.2)
Never	113 (15.1)	125 (15.8)
Missing		1
Pack-years, median (range)	25 (0–120)	25 (0–193)
Histology		
Adenocarcinoma	609 (81.5)	776 (98.0)
Squamous	88 (11.8)	15 (1.9)
NOS	50 (6.7)	1 (0.1)
PD-L1 TPS, median (range)	50 (0–100)	10 (0–100)
PD-L1 TPS		
<1%	100 (18.2)	220 (41.1)
≥1%–49%	172 (31.3)	95 (17.8)
≥50%	277 (50.5)	220 (41.1)
Not available	198	257
Driver alteration		
KRAS	261 (34.9)	322 (40.7)
EGFR	81 (10.8)	70 (8.8)
BRAF	35 (4.7)	41 (5.2)
MET	22 (2.9)	19 (2.4)
HER2	17 (2.3)	0 (0.0)
ALK	9 (1.2)	3 (0.4)
RET	7 (0.9)	3 (0.4)
ROS1	4 (0.5)	10 (1.3)
NTRK	1 (0.1)	0 (0.0)
None identified	310 (41.5)	324 (40.9)
Harmonized TMB, median (range)	−0.068 (−3.90 to 4.44)	0.014 (−2.81 to 4.56)
TMB, median (range) (DFCI)	9.9 (0–61.7)	
TMB, median (range) (MSKCC)		7 (0–100)
TMB, median (range) (GR)		7.5 (0–100)
Treatment line		
First	271 (36.3)	244 (30.8)
Second or subsequent	476 (63.7)	548 (69.2)

	Discovery cohort	Validation cohort
	<i>n</i> (%)	<i>n</i> (%)
Treatment regimen		
PD-(L)1 blockade	717 (96.0%)	731 (92.3)
PD-(L)1 + CTLA4 inhibition	30 (4.0%)	61 (7.7)
ECOG PS		
0–1	588 (79.7)	720 (90.9)
≥2	150 (20.3)	72 (9.1)
Not available	9	
Institution		
DFCI	747 (100)	
MSKCC		707 (89.3)
GR		85 (10.7)

Ever smoker: >100 cigarettes in the lifetime. Never smoker <100 cigarettes. Only cigarette smokers included here.

DFCI, Dana-Farber Cancer Institute; ECOG PS, Eastern Cooperative Oncology Group performance status; GR, Gustave Roussy Center; MSKCC, Memorial Sloan Kettering Cancer Center; NOS, not otherwise specified; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden; TPS, tumor proportion score.