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Scientific Article

Genomic Analyses for Predictors of Response to Chemoradiation in Stage III Non-Small Cell Lung Cancer

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Abstract

Background: Radiation with platinum-based chemotherapy is the standard of care for unresectable stage III non-small cell lung cancer (NSCLC). Despite aggressive treatment, progression-free survival and overall survival remain poor. It is unclear whether any tumor genetic mutations are associated with response to chemoradiation therapy.

Methods: We retrospectively reviewed clinical outcomes of patients with stage III NSCLC treated with definitive radiation who had undergone tumor molecular profiling through a next-generation DNA sequencing platform. Cox proportional hazards model was used to investigate associations between clinical outcomes and genetic mutations detected by next-generation sequencing.

Results: 110 patients were identified with stage III NSCLC and underwent definitive radiation between 2013 and 2017 and tumor molecular profiling. Concurrent or sequential chemotherapy was given in 104 patients (95%). Unbiased genomic analyses revealed a significant association between *AKT2* mutations and decreased local-regional tumor control and overall survival (hazard ratios [HR] 12.5 and 13.7, $P = .003$ and $P = .003$, respectively). Analyses restricted to loss-of-function mutations identified *KMT2C* and *KMT2D* deleterious mutations as negative prognostic factors for overall survival (HR 13.4 and 7.0, $P < .001$ and $P < .001$, respectively). Deleterious mutations in a panel of 38 DNA damage response and repair pathway genes were associated with improved local-regional control (HR 0.32, $P = .049$).

Conclusions: This study coupled multiplexed targeted sequencing with clinical outcome and identified mutations in *AKT2, KMT2C,* and *KMT2D* as negative predictors of local-regional control and survival, and deleterious mutations in damage response and repair pathway

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genes were associated with improved local-regional disease control after chemoradiation therapy. These findings will require validation in a larger cohort of patients with prospectively collected and detailed clinical information.

Introduction

Approximately 30% of patients with non-small cell lung cancer (NSCLC) present with stage III (locally advanced) disease at diagnosis. The standard of care for unresectable stage III NSCLC has been concurrent chemoradiation, based on several randomized clinical trials.[1-3] More recently, the addition of consolidative immunotherapy with durvalumab was associated with improved 2-year progression-free survival and overall survival in stage III patients compared with chemoradiation alone.[4,5] Despite such improvements in outcome, more than 50% of patients still experience progression of disease within 24 months. Among patients who developed progression of disease in the PACIFIC study, 75% to 80% of patients had intrathoracic progression as the first site of progression,[6] which highlights the challenge of local-regional tumor control in this patient population.

Lung adenocarcinoma and squamous cell carcinoma exhibit high rates of somatic mutation. Whole exome sequencing of lung adenocarcinoma and squamous cell carcinoma has revealed recurring somatic mutations in known oncogenes and tumor suppressions.[7,8] However, the functional significance of majority of the somatic mutations remains unknown. It is unclear whether any genetic mutations are associated with local-regional response to radiation and survival after chemoradiotherapy.

Recent years have seen the advent of efforts to expand tumor genomics testing in the clinic, with the goal of identifying mutations that could guide therapy and predict outcome. At Memorial Sloan Kettering Cancer Center (MSKCC), a targeted, next-generation sequencing (NGS) platform named Memorial Sloan Kettering-Integrated Mutational Profiling of Actionable Cancer Targets (MSK-IMPACT) was developed to detect mutations and copy number changes in 468 frequently mutated cancer genes. Since its inception in January 2014, this sequencing platform was rapidly integrated into routine clinical practice.[9] The sequencing test was used in patients with recurrent and metastatic disease, which include a subset of patients who had stage III NSCLC. The sequencing test was also performed in patients with *de novo* diagnosis of stage III NSCLC to aid in diagnosis and assess candidacy for clinical trials. In comparison to untreated primary tumors from the Cancer Genome Atlas Project, primary tumors and distant metastases sequenced on our NGS platform showed strong concordance in identities and frequencies of mutations detected in the Cancer Genome Atlas.[10] We undertook this study to survey the landscape of genetic mutations in stage III NSCLC and use an unbiased approach to investigate any associations between tumor somatic mutations and clinical outcomes after definitive chemoradiation.

Methods

Patient selection

An institutional database was queried with the approval of the institutional review board (MSKCC-IRB #16-142) to identify stage III (American Joint Committee on Cancer [AJCC] 7th edition) NSCLC patients treated with definitive radiation therapy from 2013 to 2017 who underwent tumor genetic panel testing between 2014 and 2017. Patients were identified based on International Classification of Diseases (ICD)-9 and ICD-10 codes for malignant neoplasm of the trachea, bronchus, and lung. Patients were excluded if the radiation dose was less than 50 Gy or number of fractions was less than 25 (as these were considered palliative treatment), underwent definitive surgical resection, or were not clinically stage III. The cohort of patients was cross-referenced with MSKCC NGS database and those patients with at least 1 identified tumor genetic abnormality were included.

Tumor genomic testing

Patients were selected and consented for tumor genomic testing to identify genetic alterations that are readily actionable or candidates for clinical trial as part of routine clinical care. Tumor genomic testing was performed with MSK-IMPACT, which is a US Food and Drug Administration (FDA)–approved assay that detects genetic alterations involving 341 (version 1), 410 (version 2), or 468 (version 3) cancer-associated genes.[9] The assay was performed on tissue samples taken either from a primary or metastatic site, depending on the availability of tissue. If multiple samples were taken from the patient, the first sample was chosen for analysis. The definition of a deleterious mutation included either a frameshift, splice site, or nonsense mutation that often leads to a loss of function effect.

Radiation treatment

Simulation was performed with patients in the supine and arms-up position in a customized immobilization

cradle. Four-dimensional (4D)-computed tomography (CT) simulation was included and an internal target volume (ITV) approach was used to account for respiratory motion. The ITV was expanded by 5 to 7 mm to create the clinical target volume, which was then expanded by 5 mm to create the planning target volume. Patients were treated with conventional fractionation with 1.8 to 2 Gy per fraction using either 3-dimensional (3D)-conformal radiation therapy or intensity modulated radiation therapy. Elective nodal radiation (other than the ipsilateral hilar nodes) was not done. After completion of radiation therapy, patients were followed every 3 months for years 1 to 2 and every 6 months for years 3 to 4 in a clinic with surveillance CT scans.

Clinical outcome assessment

Patient clinical outcome was retrospectively collected with institutional review board approval. Patient clinical and tumor characteristics were collected, and response after definitive radiation therapy was assessed based on CT imaging. Local-regional failure after radiation was defined by progression of disease within the radiation field, including irradiated lymph nodes. Distant failure was defined by progression of disease in the contralateral thorax, or outside the thoracic cavity.

Statistical analysis

Cox proportional hazards model was used to assess hazard ratio for local-regional recurrence and death associated with the presence of any or deleterious mutation in each mutated gene. The models have been tested to confirm there is no violation of standard proportional hazards assumptions. *P* values were corrected for multiple hypothesis testing using false discovery rate (FDR) method.[11] Overall and local-regional recurrence-free survivals were estimated using the Kaplan-Meier method. Multivariate Cox regression analysis was performed to estimate associations between factors of interest, including tumor mutational status, age, sex, smoking history, tumor stage, tumor histology, and receipt of chemotherapy. DNA damage response and repair pathway included 48 genes from MSK-IMPACT as previously described.[12,13] All analyses were performed with R software (version 3.6).

Results

Patient characteristics

We identified a total of 110 patients who had stage III NSCLC and underwent tumor genomic testing with MSK-IMPACT (Table 1). The median age was 67 years (range, 29-88). 53% of patients were male and 47% were female. 90% of patients had prior smoking history. 73 patients (66%) had adenocarcinoma, 18 patients (16%) had squamous cell carcinoma, 12 patients (11%) had mixed squamous and adenocarcinoma histology, and 7 patients (6%) had rare histologic types including large cell neuroendocrine carcinoma (2%), sarcomatoid carcinoma of the lung (2%), and combined small cell carcinoma and adenocarcinoma of the lung (1%).

Table 1 Baseline patient clinical and tumor profiling characteristics

Characteristics	No. (%)
Age	
Median (range)	67 (29-88)
Sex	
Male	58 (53%)
Female	52 (47%)
Smoking status	
Current smoker	29 (26%)
Former smoker	70 (64%)
Never smoker	11 (10%)
Stage (AJCC 7th edition)	
IIIA	48 (44%)
IIIB	62 (56%)
Histology	
Adenocarcinoma	73 (66%)
Squamous cell carcinoma	18 (16%)
Mixed adenosquamous	12 (11%)
Other	7 (6%)
Radiation dose (Gy)	
Median (range)	60 (50.4 - 74)
Chemotherapy	
Yes	104 (95%)
Concurrent	83 (75%)
Sequential	21 (19%)
No	6 (5%)
MSK-IMPACT gene panel	
IMPACT-468	40 (36%)
IMPACT-410	55 (50%)
IMPACT-341	15 (14%)
IMPACT sample site	
Primary	47 (43%)
Metastatic	63 (57%)

Abbreviations: AJCC = American Joint Committee On Cancer; MSK-IMPACT = Memorial Sloan Kettering-Integrated Mutational Profiling of Actionable Cancer Targets.

Radiation treatment and clinical outcome

Median radiation dose received was 60 Gy (range, 50.4 Gy-74 Gy), delivered in 1.8 Gy or 2 Gy per fraction. One patient received 50.4 Gy due to bilateral hilar nodal (N3) disease making it difficult to meet lung dose constraints. A total of 104 patients (95%) received chemotherapy either concurrently (75%) or sequentially (19%). No

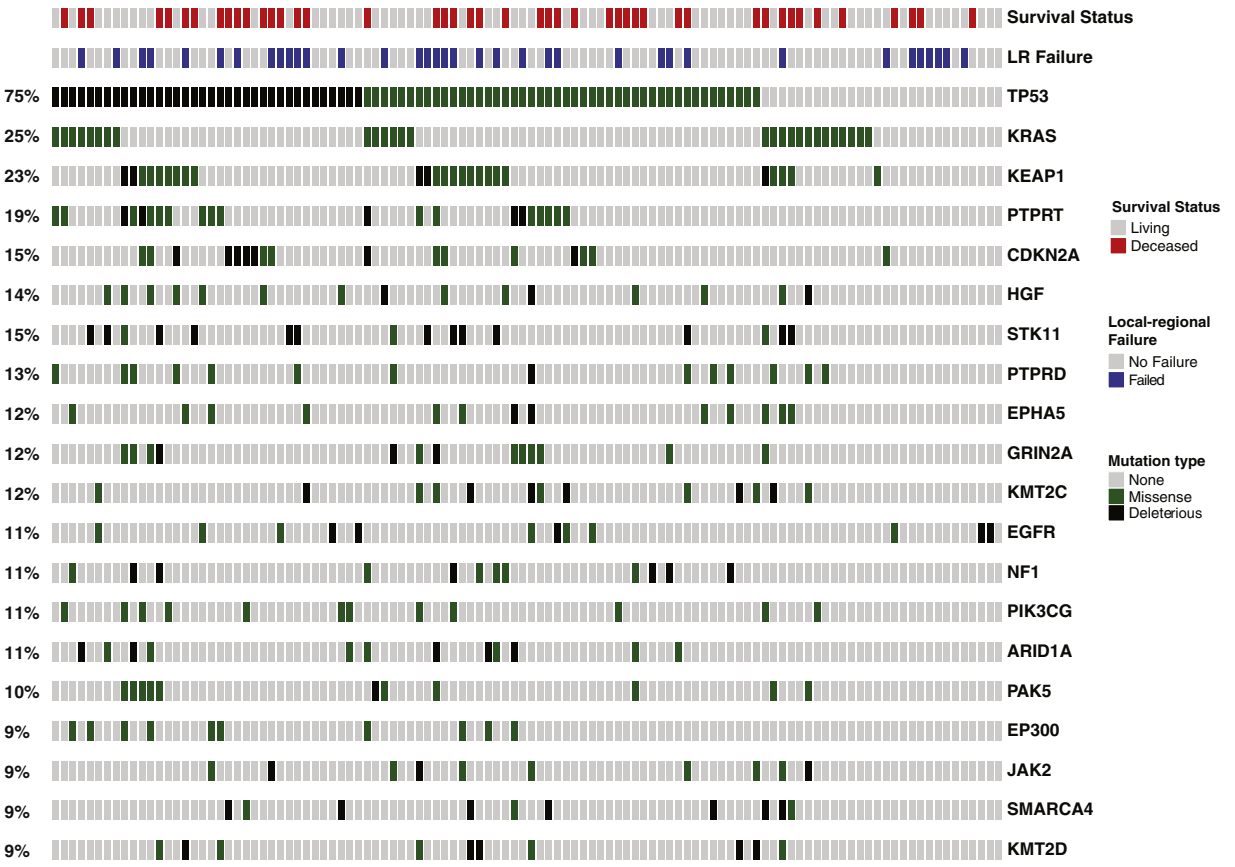

Figure 1 Mutational profile of stage III non-small cell lung cancer. Oncoprint of clinical outcomes (survival status and local-regional failure) and 20 most frequently mutated genes identified by Memorial Sloan Kettering-Integrated Mutational Profiling of Actionable Cancer Targets clinical tumor mutational profiling in 110 patients with stage III non-small cell lung cancer. Genes are ordered based on mutational frequency. Mutational types are color-coded (black, deleterious mutations defined as either frameshift or nonsense mutation; green, missense mutation; gray, no alterations).

patients received adjuvant immunotherapy as it was not standard of care at that time.

The median follow-up time was 15.3 months (range, 0.8-58.7 months) from the end of RT. Median overall survival of all patients was 24.7 months (Figure E1A). One-year and 2-year overall survival rates were 80% and 51%, respectively. In total, 36 patients developed local-regional failure, 69 patients developed distant metastases, and 22 patients had both local-regional and distant failures. Median time to local-regional failure was 40.3 months (Figure E1B). Median time to developing distant metastasis was 12.0 months.

Tumor molecular profiling

A total of 110 samples were profiled using MSK-IMPACT during the study period; 47 samples were collected from the primary site and 63 samples were collected from a metastatic site including lymph node. Among metastatic sites, the most common site of collection was lymph node (76%), followed by brain (8%), bone (5%), and liver (5%). Among different gene panels used, 50% of the tumor samples were profiled with the 410-gene panel, 40% of samples used the 468-gene panel, and the remaining 15% of samples used the 341-gene panel (Table 1). The average sample sequencing coverage was 778×, which provided sensitivity to detect mutations at low allele frequencies.

The most frequently mutated genes were *TP53* and *KRAS,* with mutations found in 75% and 25% of the tumors, respectively (Fig. 1). For actionable mutations in adenocarcinoma, 18% of tumors had *EGFR* mutations and *ALK* mutations were found in 11% of adenocarcinoma tumors. We also found frequent mutations in tumor suppressor genes aside from *TP53,* including *KEAP1* (23%), *CDK2NA* (19%), *STK11* (15%),[14] *NF1* (11%), and *SMARCA4* (9%).[14-20]

Genomic analyses for mutations associated with differential response after chemoradiation

Unbiased genomics analyses revealed somatic mutations that were associated with differential local-regional disease control and survival after definitive

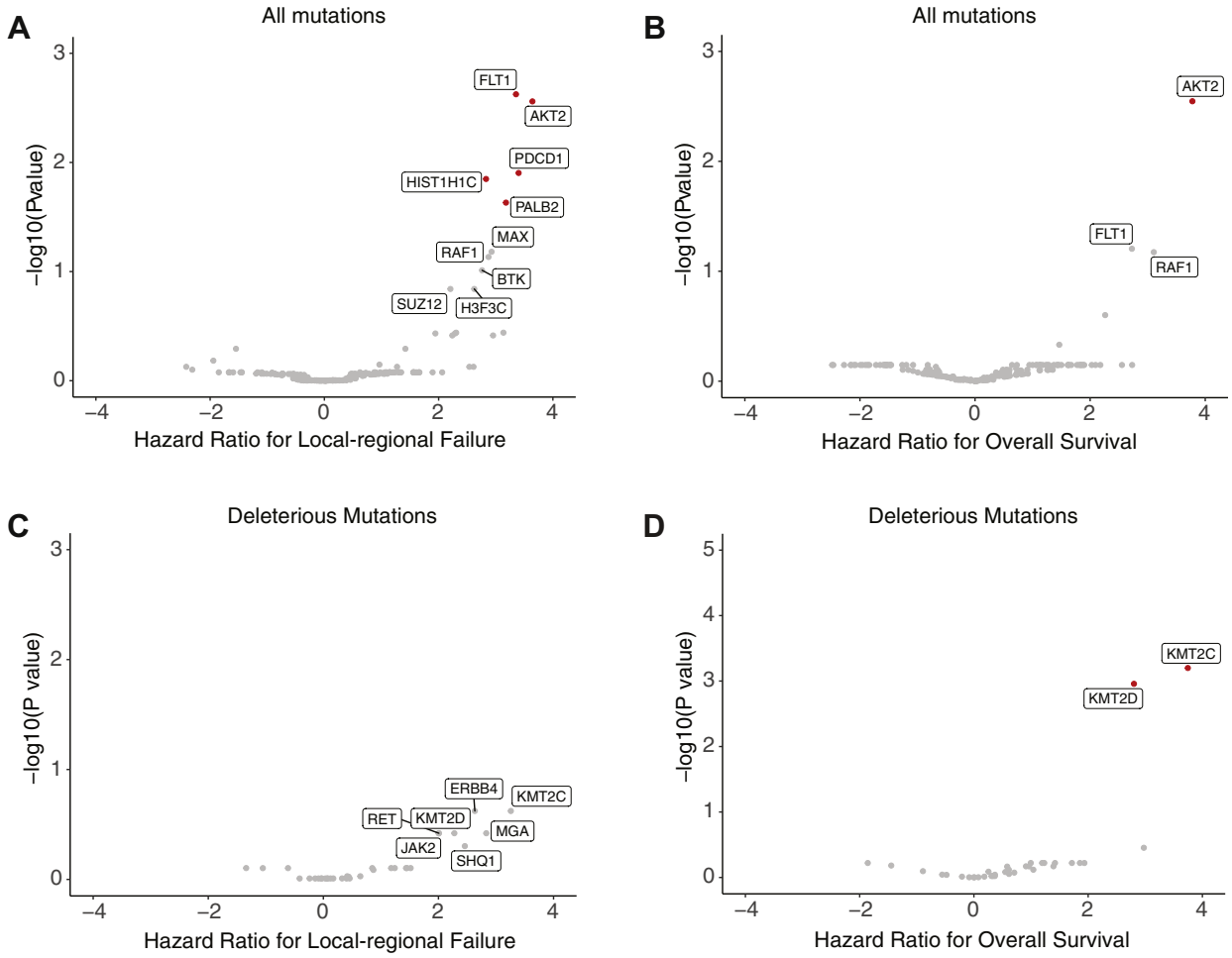

Figure 2 Genomic analyses for mutations associated with differential response after chemoradiation. (A) Volcano plots of mutations associated with differential local-regional failure. Statistical significance (log10-transformed *P* values from log-rank test) was plotted against risk of local-regional failure (log2-fold change of Cox proportional hazard ratios). *P* values were corrected for multiple hypothesis testing. Mutations with significant *P* values after transformation ($>-\log_{10}[0.05]$) were labeled in red. (B) Volcano plots of any mutations associated with differential survival probability. (C) Volcano plots of deleterious mutations associated with differential local-regional failure. (D) Volcano plots of deleterious mutations associated with differential survival probability.

chemoradiation (Figs. 2A and 2B). To investigate the association of loss of function mutations and clinical outcome, we restricted our analysis to include only deleterious mutations. There was no individual deleterious mutation that was associated with worse local-regional disease control (Fig 2C). However, deleterious mutations in *KMT2C* and *KMT2D* were both independently associated with worse overall survival (HR 13.4 and 7.0, $P < .001$ and .001, respectively) (Fig 2D). *KMT2C* and *KMT2D* mutations tended to co-occur (Fisher's exact test, $P = .002$) and mutations in both genes were found in 5 of 13 patients with *KMT2C* mutations and 5 of 10 patients with *KMT2D* mutations.

Somatic mutations in *AKT2* (n = 2) were significantly associated with worse local-regional control (HR 12.5 and 13.7, $P = .003$ and .003, respectively) (Figs. 3A and 3B). Multivariable Cox regression analysis showed that *AKT2* mutations independently increased the likelihood of local-regional failure (Fig. 3C). Two patients with *AKT2* mutations had 2 missense mutations, P115L and W334L. P115L resides between the Pleckstrin homology (PH) domain and the protein kinase domain and W334L is in the protein kinase domain. Patients with deleterious mutations in either *KMT2C* or *KMT2D* exhibited worse local-regional tumor control and survival (Figs. 3D and 3E). Multivariable Cox regression analysis showed *KMT2C* or *KMT2D* mutations are independently associated with worse overall survival after chemoradiation (Fig. 3F).

Association between mutations in DNA damage response and repair genes and local-regional disease control

Because an impairment of DNA damage repair pathways may sensitize tumor cells to DNA damage

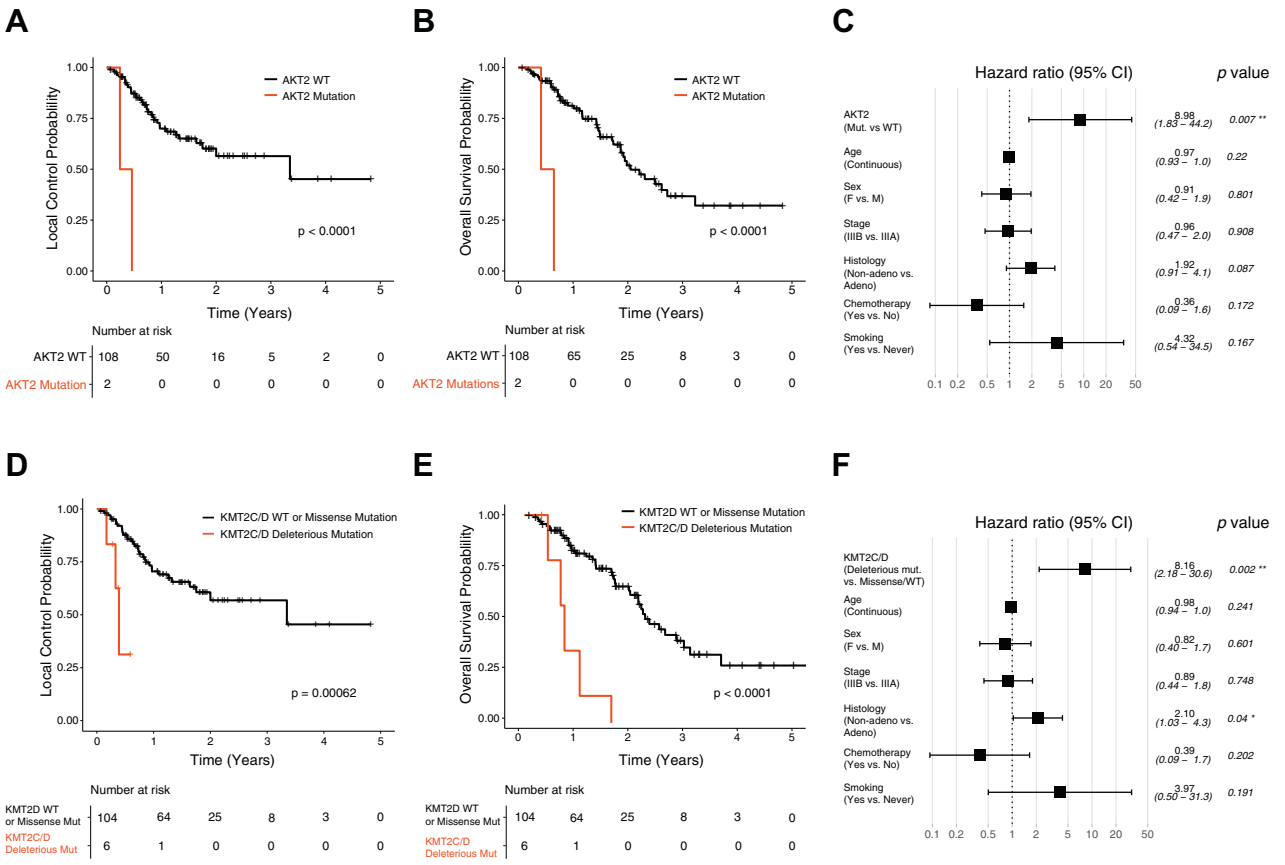

Figure 3 Association of missense mutations in *AKT2* and deleterious mutations in *KMT2C* and *KMT2D* with clinical outcome after definitive chemoradiation. (A) Kaplan-Meier estimate of local-regional control probability for tumors without versus with *AKT2* mutations (n = 2). (B) Kaplan-Meier estimate of overall survival probability for patients with tumor harboring *AKT2* mutations (n = 2). (C) Multivariate analysis of *AKT2* mutation and patient factors associated with local-regional failure. (D) Kaplan-Meier survival curves of local-regional control probability for tumors without versus with *KMT2C* or *KMT2D* mutations (n = 6). (E) Kaplan-Meier survival curves of overall survival probability for patients with tumor containing *KMT2C* or *KMT2D* mutations (n = 6). (F) Multivariate analysis of *KMT2C* or *KMT2D* mutations and patient factors associated with overall survival.

induced by radiation, we investigated the association of mutations in damage response and repair (DDR) genes with outcome after chemoradiation. Tumors were classified based on the presence of mutations in 38 DDR genes included in the MSK-IMPACT panel.[12] Kaplan-Meier analysis revealed similar local-regional control between tumors with wild-type and missense mutations in DDR genes (Fig 4A), and tumors with deleterious mutations in DDR genes had significantly improved local-regional control compared with those with missense mutations or wild-type DDR genes (Fig 4B). There is a nonsignificant improvement in overall survival in patients with deleterious DDR mutations compared with wild type DDR genes (Figure E4B). Frequencies of deleterious mutations in DDR genes ranged from 0% to 3%, and DDR genes from multiple pathways such as homologous recombination, mismatch repair, Fanconi anemia, and nuclear excision repair were represented in the mutated cohort (Fig E4C).

Discussion

The current study used an unbiased genomic approach to identify predictors of response to chemoradiation in stage III NSCLC. To date, limited evidence has been published that supports the existence of genetic predictors of radiation response in NSCLC. Prior studies have investigated the effect of individual gene mutations in various stages of NSCLC. In early stage NSCLC, *KRAS* mutations have been reported to correlate with worse local control after stereotactic body radiation therapy (SBRT); however, the studies were limited by sample sizes of 10 to 45 genotyped patients.[21,22] Our institution had reported higher rate of local recurrence after SBRT in tumors harboring *PIK3CA* mutations in 166 tumors of primary lung (93%) or metastatic origin (7%).[23] In addition, mutations in *KEAP1/NFE2L2* (HR 2.19, 95% CI 1.41-3.38) and *STK11* (HR 2.36, 95% CI 1.19-5.08) have been linked to worse overall survival in advanced NSCLC.[24-26]

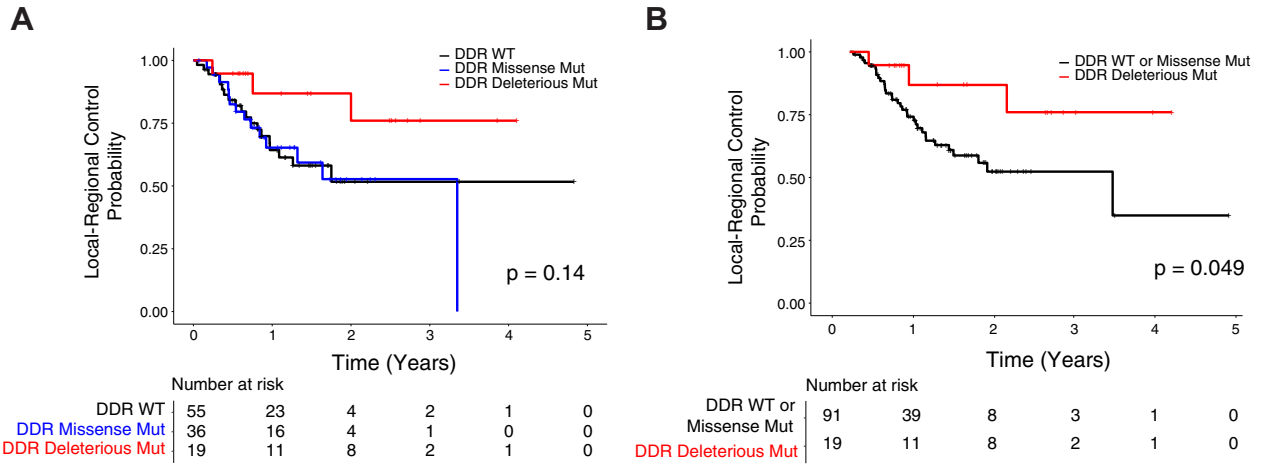

Figure 4 Local-regional disease control in tumors with DNA damage response and repair (DDR) pathway mutations. (A) Local-regional control probability after definitive chemoradiation in patients with wild-type (n = 55) versus missense mutations (n = 36) versus deleterious mutations (n = 19) in 38 DDR genes from Memorial Sloan Kettering-Integrated Mutational Profiling of Actionable Cancer Targets. (B) Local-regional control probability comparing patients with or without deleterious mutations in DDR genes.

Our study identified *AKT2* mutation as a potential predictor of poor response after definitive chemoradiation in stage III NSCLC. Although mutational frequency of *AKT2* was only 1.8%, the 2 patients with tumors containing *AKT2* mutations had significantly worse local-regional control and overall survival after radiation, and this effect is independent from potential confounding factors. *AKT2* encodes 1 of the 3 closely related serine/threonine-protein kinases (AKT1, AKT2, and AKT3) that are expressed in different tissue types and regulate processes critical to oncogenesis including cell survival, proliferation, and metabolism. *AKT2* has been implicated as an oncogene and found to be amplified in 3% to 12% of ovarian carcinoma, breast carcinoma, and pancreatic carcinoma.[27-29] The 2 mutations seen in *AKT2* (P115L and W334L) in our study were both considered pathogenic in the Catalog of Somatic Mutations in Cancer (COSMIC) database. FATHMM scores for P115L and W334 were 0.87 and 0.96, respectively (scores range from 0-1 and values greater than 0.5 are predicted to be pathogenic). It is possible that these particular missense mutations in *AKT2* may lead to more aggressive biology and radioresistant phenotype, though it is difficult to draw firm conclusions from the small number of tumors that harbored such mutations.

In our analysis limited to deleterious mutations that presumably demonstrate a loss of function phenotype, deleterious mutations in *KMT2C* and *KMT2D* were significantly associated with worse overall survival. KMT2C and KMT2D are members of the histone-lysine N-methyltransferase 2 (KMT2) family of proteins.[30] KMT2 proteins methylate lysine 4 on the histone H3 tail (H3K4) and change chromatin structures to promote genomic accessibility and transcription of specific genes. *KMT2C* and *KMT2D* are 2 of the most frequently mutated genes in cancer and have been described in multiple cancer types including non-small cell[7] and small cell lung cancer,[31] prostate cancer,[32] breast cancer,[33] renal cell carcinoma,[34] and non-Hodgkin lymphoma.[35] Most cancer-associated *KMT2C* and *KMT2D* mutations are frameshift or nonsense mutations, and it is postulated that they function as tumor suppressor genes. In our study, 12% of tumors had mutations in *KMT2C* and 9% had mutations in *KMT2D,* and significant fractions were deleterious mutations (33% of *KMT2C* mutations and 55% of *KMT2D* mutations). Furthermore, mutations in *KMT2D* were previously shown to be associated with worse survival in locally advanced and metastatic NSCLC. In a study of 194 patients with stage III (23%) and stage IV (77%) NSCLC, 34 patients with *KMT2D* mutations had worse survival with lower median overall survival of 10 months compared with 30 months in patients with wild-type *KMT2D*.[36] In the context of prior molecular, sequencing, and clinical studies, our finding supports loss of function mutations in *KMT2C* and *KMT2D* as potential prognostic factors for worse response after chemoradiation in stage III NSCLC.

It has been hypothesized that impaired DNA damage response and repair mechanisms may sensitize the tumor cells to genotoxic therapies such as radiation. There is emerging clinical evidence suggesting mutations in DDR genes can predict better response. Loss-of-function mutations in the *ATM* gene, which plays a central role in DNA damage response, was found in 8 exceptional responders after radiation.[37] In a larger cohort of 95 patients with NSCLC who received radiation therapy to either thoracic or extrathoracic lesions, the 2-year cumulative incidence of local failure was 6.7% for pathogenic *ATM* mutations compared with 19.9% for missense *ATM* mutations.[38] It is likely that mutations in

other DDR genes are also important in determining the response after radiation. The presence of somatic mutations in a panel of 34 DDR genes is associated with improved response after platinum-based chemotherapy in patients with locally advanced and metastatic urothelial carcinoma.[12] Interestingly, our analysis showed that only deleterious mutations, not missense mutations, in DDR genes correlate with improved local-regional tumor control. This is in contrast with the previous study showing any type of mutations in the DDR gene can correlate with improved outcome in urothelial carcinoma.[12] Due to the rare frequencies of individual DDR gene mutations and insufficient number of patients, our study did not have the power to detect a difference in outcome at an individual gene or pathway level.

It is important to address several limitations of the study. First, it is important to keep in mind the exploratory nature of the study due to the limited number of patients available for analysis. Insufficient power due to small patient cohort may limit the detection of many potential genomic predictors with low prevalence. In the stage III NSCLC population, genomic testing is only recently being integrated into more routine practice; therefore, the study cohort did not capture all of the patients with stage III NSCLC treated with definitive radiation at our institution. Patients included in this cohort were chosen for gene panel testing to look for actionable mutations or clinical trial eligibility, and therefore may reflect a bias toward more aggressive disease. Second, the gene panel only includes a select group of up to 468 genes that were previously known to be involved in cancer. It does not include the rest of the exome or noncoding regions of the genome that may be implicated in cancer. Lastly, the study did not account for staging and treatment heterogeneity within the stage III NSCLC cohort. The study included both stage IIIA and IIIB patients who were treated with concurrent or sequential chemotherapy, and a small number of patients (5%) with definitive radiation alone.

Although our study represents a step toward understanding the genomic determinants of chemo-radiation response, more studies are needed to validate our findings. These studies will require a much larger cohort of patients, prospectively collected and detailed clinical information, and deep tumor sequencing to fully investigate the relationship between each mutation and clinical outcome.

Conclusion

The study used an unbiased genomic approach and identified mutations in several genes including *AKT2, KMT2C,* and *KMT2D* as negative predictors of local-regional control and survival in stage III NSCLC patients. Deleterious mutations in DNA damage response and repair pathway genes were associated with improved local-regional control after chemoradiation.

Supplementary Materials

Supplementary material for this article can be found at https://doi.org/10.1016/j.adro.2020.10.027.

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