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Influence of Actionable Genomic Alterations on Outcomes of Neoadjuvant Immune Checkpoint Inhibition in Resectable Non–Small Cell Lung Cancer

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Abstract

Neoadjuvant immune checkpoint inhibitors (ICIs) have shown improved survival compared with standard chemotherapy in resectable non–small cell lung cancer (NSCLC). However, the role of actionable genomic alterations (AGAs) in shaping response to these therapies remains poorly defined. This study investigates the influence of AGAs on treatment failure (TF) in patients undergoing neoadjuvant ICIs prior to surgical resection.We analyzed tumor molecular profiles from patients with stage I–IIIA resectable NSCLC (AJCC 7th edition) enrolled in a previously reported phase II randomized trial (NCT03158129). Participants received neoadjuvant nivolumab alone (n = 23) or in combination with ipilimumab (n = 21) before surgery. TF was defined as disease progression preventing surgery, confirmed recurrence postoperatively, or death linked to lung cancer or treatment complications. Tumors with AGAs (n = 12) were compared to those without AGAs and non-profiled squamous cell carcinomas (non-AGAs+NP SCC, n = 20).Over a median follow-up of 60.2 months, TF occurred in 34.1% of patients (15/44). Retrospective molecular profiling was available in 47.7% (21/44) of cases, identifying AGAs including EGFR (n = 5), KRAS (n = 2), ERBB2 (n = 1), BRAF (n = 1) mutations, and ALK (n = 2) and RET (n = 1) fusions. Median time to TF in the AGA group was 24.7 months (95% CI: 12.6–40.4), compared with not reached in the non-AGA/NP SCC group. AGAs were associated with a significantly higher TF risk (HR: 5.51; 95% CI: 1.68–18.1), while former or current smoking reduced TF risk (HR: 0.24; 95% CI: 0.08–0.75). Major pathological responses were more likely in the non-AGA/NP SCC group (OR: 4.71; 95% CI: 0.49–45.2), and residual viable tumor burden was higher in AGA tumors (median 72.5% vs 33.0%).NSCLC tumors harboring select AGAs, particularly EGFR mutations and ALK fusions, demonstrate poorer outcomes after neoadjuvant ICI therapy, including higher TF rates, shorter time to failure, and reduced pathological regression. These findings underscore the necessity of pre-treatment molecular profiling to identify patients who may derive limited benefit from chemotherapy-sparing immunotherapy regimens.

Keywords: NSCLC, Immunotherapy, Neoadjuvant therapy, Genomic alterations, Nivolumab, Ipilimumab

Introduction

Neoadjuvant immune checkpoint inhibitors (ICIs) are increasingly recognized as a viable approach in operable non-small cell lung cancer (NSCLC), offering improved pathological response rates relative to conventional chemotherapy [1, 2]. Recent trials—including CheckMate-816, KEYNOTE-671, and AEGEAN—have led to regulatory approval of chemoimmunotherapy in the neoadjuvant and perioperative settings for a subset of patients [3, 4]. Major and complete pathological responses are now considered promising surrogate endpoints for assessing the long-term benefit of these therapies [5]. Nevertheless, the impact of genomic alterations on pathological response and treatment failure (TF) after neoadjuvant ICI therapy remains inadequately understood.

The phase 2 randomized NEOSTAR trial evaluated neoadjuvant ICI therapy with either nivolumab alone or in combination with ipilimumab, followed by surgical resection [6]. Since the trial, molecular profiling has

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become standard practice in early-stage NSCLC. Adjuvant targeted therapies, such as osimertinib for EGFR-mutated NSCLC and alectinib for ALK-rearranged tumors, are now routinely recommended after surgical resection [7-9]. Several phase 3 trials assessing neoadjuvant and perioperative ICIs excluded patients with EGFR or ALK alterations [3, 4, 10, 11], while ongoing studies are investigating perioperative targeted therapy for tumors with actionable genomic alterations (AGAs) [12, 13]. These developments highlight the critical role of molecular testing in planning neoadjuvant and perioperative immune-based treatments.

In NEOSTAR, patients received neoadjuvant ICIs regardless of AGA status because molecular testing was not mandated before treatment. Leveraging long-term follow-up, we identified patients who experienced TF and explored the clinical, pathological, and molecular factors associated with these outcomes, focusing on the influence of select AGAs on treatment failure.

Materials and Methods

Patient population and treatment protocol

Eligible patients had resectable NSCLC classified as stage I–IIIA according to the AJCC seventh edition. Participants were randomized to receive either neoadjuvant nivolumab alone or a combination of nivolumab and ipilimumab prior to surgical resection [6]. The trial's primary endpoint was major pathological response (MPR), defined as ≤10% residual viable tumor in the surgically resected specimen following neoadjuvant therapy [6, 14]. Detailed clinical outcomes have been previously published [6, 15]. Long-term follow-up was conducted to assess survival and recurrence patterns.

Tumor genomic profiling

Pre-treatment molecular profiling was not a requirement for trial participation. For this analysis, post-hoc genomic profiling was performed on all available tumor specimens. The methods used depended on sample availability, type, and quality. When tissue was accessible, DNA-based next-generation sequencing (NGS) was performed in-house at the MD Anderson Cancer Center CLIA-certified Molecular Diagnostic Laboratory, as previously described [16, 17]. This platform evaluated 134 genes for single nucleotide variants (SNVs) and 47 genes for copy number alterations, totaling 146 genes. RNA-based NGS was additionally employed to detect inter- and intragenic fusions across 51 genes.

Cytogenetic assessment via fluorescent in-situ hybridization (FISH) was performed selectively, based on tissue availability and clinical judgment, to identify MET amplification and rearrangements in RET, ALK, and ROS1. In certain cases, NGS-based analysis of circulating cell-free DNA from blood samples (liquid biopsy) was performed, capable of detecting SNVs in 70 genes, 19 copy number gains, and six gene fusions [16, 18].

PD-L1 expression was assessed on baseline tumor tissue, when available, using chromogenic immunohistochemistry (clone 28–8, ab205921, 1:100 dilution, Abcam), following previously described protocols [6, 19]. Scoring was conducted independently by two trained pathologists in accordance with International Association for the Study of Lung Cancer guidelines [20]. Membrane staining of tumor cells was used to calculate the tumor proportion score [20].

Molecular analyses, including NGS and FISH, were conducted on samples obtained from primary tumors, surgical resections, or metastatic lesions, collected via bronchoscopic or radiographically guided percutaneous biopsies. Liquid biopsy was performed when feasible, typically at the time of suspected or confirmed disease recurrence or progression, at the discretion of the treating physician.

Study objectives

This exploratory analysis aimed to evaluate the influence of select actionable genomic alterations (AGAs) on treatment failure (TF), major pathological response (MPR), and overall tumor pathological regression in patients receiving neoadjuvant immune checkpoint inhibitors (ICIs). The study also examined subsequent treatment patterns, including ICI-based therapy, targeted therapy, or chemotherapy, following TF after neoadjuvant ICIs. TF was defined as either progression of the primary lung tumor prior to surgery, radiographically or biopsy-confirmed recurrence after surgery, or death due to either primary lung cancer or possibly treatment-related complications, measured from the time of randomization. The database was locked on August 2, 2023.

Statistical analysis

Associations between molecular subgroups (AGAs versus non-AGAs combined with non-profiled squamous

cell carcinomas [NP SCC]) and MPR were analyzed using univariable logistic regression, with odds ratios reported. TF-free survival distributions were estimated with the Kaplan-Meier method. Univariable Cox proportional hazards (PH) regression was employed to assess TF risk in relation to molecular subgroup, baseline clinicopathologic characteristics, and pathologic response, using a landmark analysis at the date of surgery. Multivariable Cox PH models were also applied to estimate TF risk for the AGA and non-AGA/NP SCC groups. As this was an exploratory analysis, formal hypothesis testing was not conducted. All analyses were performed using R (v4.3.3), SAS (v9.4), Microsoft Excel (v2016), and GraphPad Prism (v10.0.3).

Results and Discussion

A total of 44 patients randomized to receive at least one cycle of neoadjuvant ICI were included in this analysis. At a median follow-up of 60.2 months, 15 patients (34.1%) experienced TF. Among these 15 patients, nine deaths occurred: eight were attributable to disease progression or recurrence, and one was potentially treatment-related [6]. One patient experienced disease progression that precluded surgery and subsequently died after receiving additional therapies. Of the 15 TF cases, 13 had documented recurrences, including four locoregional and nine distant recurrences. Postoperatively, eight patients received adjuvant chemotherapy, and six underwent radiotherapy.

Among the 29 patients without TF, one died from a non-cancer cause, one from a non-lung primary malignancy, and two died from unknown causes without documented recurrence of their primary lung cancer **(Figure 1).**

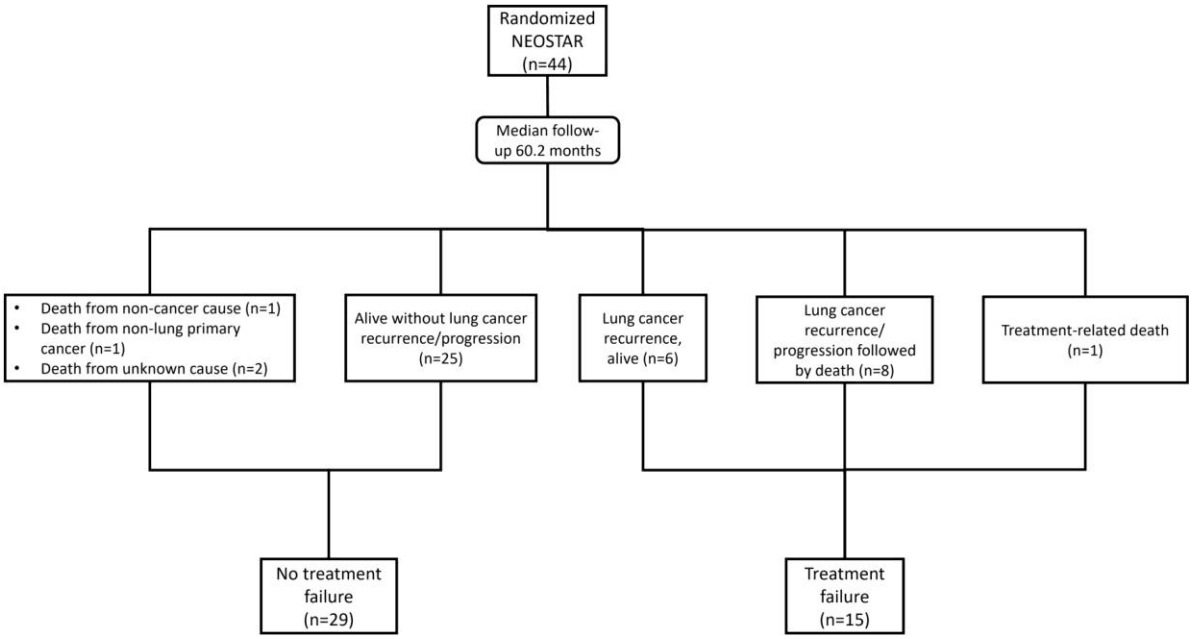


Figure 1. Treatment failure (TF) was defined as either progression of the primary lung tumor in patients who did not undergo surgery, confirmed recurrence of lung cancer after surgery by imaging or biopsy, or death from primary lung cancer or potentially treatment-related complications, measured from the date of randomization. Lung cancer recurrence/progression-free survival was defined as the interval during which no evidence of tumor progression or recurrence was observed on the latest radiographic evaluation. The notation *n* represents the number of patients.

Tumor characteristics of AGA versus Non-AGA/NP SCC groups

Among the 44 evaluable patients, molecular profiling of tumor tissue or blood samples had been performed in 21 patients (47.7%). Of the remaining 23 patients without profiling, 12 had adenocarcinoma and 11 had squamous cell carcinoma at baseline **(Figure 2).**

For exploratory comparisons, patients with tumors harboring actionable genomic alterations (AGAs, n = 12) were analyzed against a composite group consisting of

non-AGAs and non-profiled squamous cell carcinoma cases (non-AGAs+NP SCC, n = 20). This composite group included patients who underwent profiling but lacked AGAs (non-AGAs, n = 9) as well as patients with squamous histology that had not undergone molecular profiling (NP SCC, n = 11) **(Figure 2).**

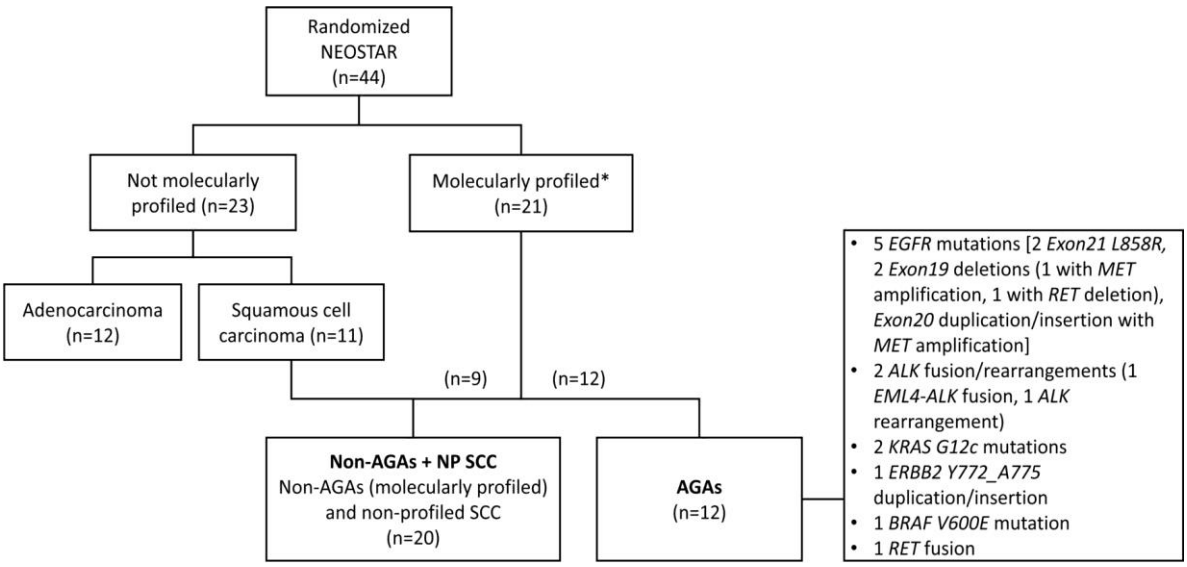


Figure 2. Comparison of patients with tumors containing actionable genomic alterations (AGAs, n = 12) versus the non-AGAs+NP SCC group (n = 20), which comprised tumors that were profiled but lacked AGAs as well as non-profiled squamous cell carcinomas. Tumor histology is reported at baseline**.**

Molecular profiling approaches included DNA-based next-generation sequencing (NGS) panels, RNA-based NGS fusion panels when available, cytogenetic analyses when feasible, and/or circulating cell-free DNA (ccfDNA) assessment via liquid biopsy.

Abbreviations: ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; n, number; NP SCC, non-profiled squamous cell carcinomas.

In the subset of 21 patients who underwent molecular profiling, actionable genomic alterations (AGAs) were identified in 12 individuals, representing 57.1% of the profiled cohort. These alterations included five EGFR mutations (two Exon 21 L858R, one Exon 19 deletion co-occurring with MET amplification, one Exon 19 deletion combined with RET deletion, and one Exon 20 duplication alongside MET amplification), two KRAS G12C mutations, a single ERBB2 Y772_A775dup mutation, one BRAF V600E mutation, two ALK fusions (one EML4-ALK, one other rearrangement), and one KIF5B-RET fusion. TP53 alterations were the most common, present in 57.1% of profiled samples (12/21). Among the AGAs, EGFR mutations were predominant (23.8%), followed by KRAS G12C mutations (9.5%). Additionally, four STK11 mutations were observed, two of which co-occurred with KRAS mutations (one G12C and one Q61H).

Comparison of clinical and pathological characteristics revealed that patients with AGAs tended to be younger than 65 years and were more frequently never-smokers. In contrast, the non-AGAs+NP SCC group contained a higher proportion of patients with squamous cell carcinoma histology. No meaningful differences were observed between the two groups with respect to sex, race, clinical stage, or baseline PD-L1 tumor expression **(Table 1).**

Table 1. Clinicopathological characteristics of AGAs and non-AGAs+NP SCC patients**.**

	AGAs(n=12)	Non-AGAs + NP SCC(n=20)
Age		
< 65	7 (58%)	5 (25%)
≥ 65	5 (42%)	15 (75%)

Gender		
Female	4 (33%)	7 (35%)
Male	8 (67%)	13 (65%)
Smoking status		
Never smoker	5 (42%)	2 (10%)
Former/current smoker	7 (58%)	18 (90%)
Race		
White	9 (75%)	17 (85%)
Non-white	3 (25%)	3 (15%)
Stage		
Stage I/II	9 (75%)	15 (75%)
Stage IIIA	3 (25%)	5 (25%)
Histology*		
Squamous	1 (8%)	16 (80%)
Non-squamous	11 (92%)	4 (20%)
PD-L1†		
<1 %	6 (67%)	8 (67%)
≥1 %	3 (33%)	4 (33%)

Demographic information for patients with tumors harboring actionable genomic alterations (AGAs) or patients with tumors not harboring AGAs or non-profiled squamous cell carcinomas (non-AGAs+NP SCC). The percentages for each characteristic are rounded to the nearest whole number to total 100%.

*Tumor histology at baseline.

†Pretreatment tumor PD-L1 expression status (n=21) on available samples by clone 28–8, Abcam3 23; the PD-L1 expression status of three patients in AGAs and eight patients in non-AGAs+NP SCC were unavailable.

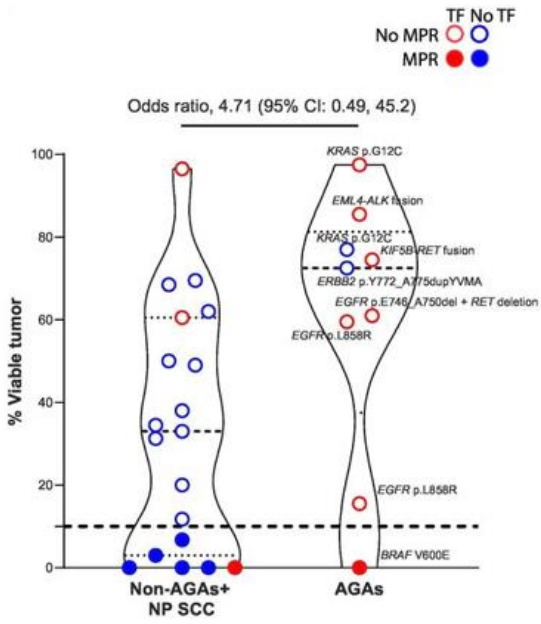
PD-L1programmed death-ligand 1

Clinicopathological and molecular profiles of patients experiencing treatment failure

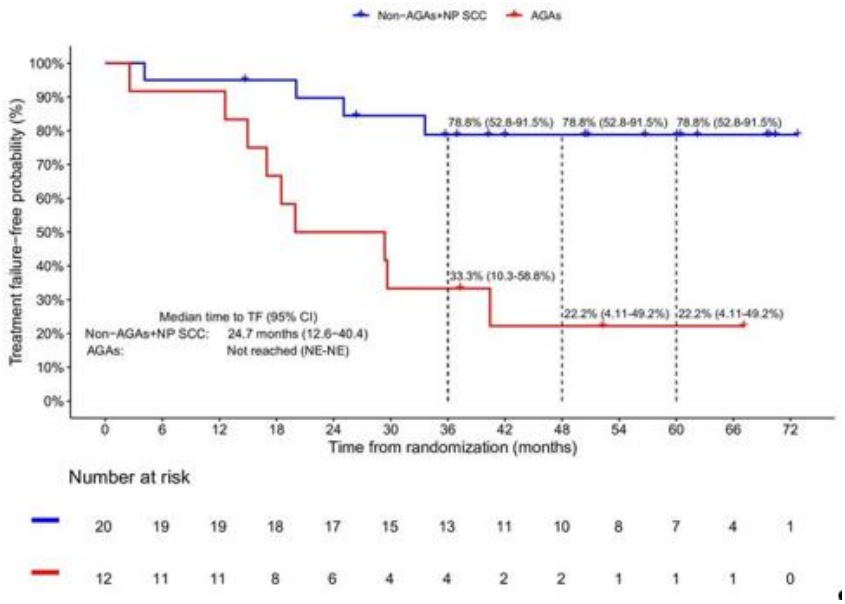
Among patients who underwent on-trial surgical resection (n = 37), 10 (27.0%) developed treatment failure (TF). In contrast, TF was observed in 5 of 7 patients (71.4%) who either did not have surgery or were resected off-trial. Across all 15 TF cases, actionable genomic alterations (AGAs) were detected in 9 patients (60%).

The incidence of TF was notably higher in tumors harboring AGAs compared with the combined non-AGAs and non-profiled squamous cell carcinoma (NP SCC) group, occurring in 75% of AGA cases (9/12) versus 20% (4/20) in the non-AGA+NP SCC cohort. Within the TF group, EGFR mutations were predominant, including two Exon 21 L858R variants, one Exon 19 deletion with MET amplification, one Exon 19 deletion with RET deletion, and one Exon 20 duplication accompanied by MET amplification. Other AGAs observed were a single EML4-ALK fusion, a KIF5B-RET fusion, a KRAS G12C mutation, and a BRAF V600E mutation. Among six profiled squamous tumors, one carried an ALK rearrangement while the remaining five lacked AGAs.

Pathologic evaluation of residual viable tumor was performed for 28 of 32 patients across the AGA and non-AGA+NP SCC groups. Patients without AGAs or with NP SCC demonstrated a higher likelihood of achieving a major pathological response (MPR), with an odds ratio of 4.71 (95% CI: 0.49–45.2) relative to the AGA group **(Figure 3a).** Consistently, the median proportion of residual viable tumor was substantially higher in the AGA cohort compared with the non-AGAs+NP SCC group (72.5% vs 33.0%) **(Figure 3a).**



a)



b)

Figure 3. Treatment Failure Outcomes

Pathological Response ***(Figure 3a)***

Analysis of resected tumor specimens revealed marked differences in pathological response between patients with actionable genomic alterations (AGAs, n = 9) and those in the non-AGAs+NP SCC group (n = 19). Major pathological response (MPR), defined as ≤10% residual viable tumor, was considerably more frequent in the non-AGAs+NP SCC cohort. Univariable logistic regression estimated an odds ratio of 4.71 (95% CI: 0.49–45.2) favoring the non-AGAs+NP SCC group. Visualization with violin plots indicated that median residual tumor was substantially higher in the AGA group (72.5%) compared with the non-AGAs+NP SCC group (33.0%). Among patients with AGAs, MPR occurred in only 16.7% of TP53-mutated tumors, and none of the four tumors harboring STK11 mutations achieved MPR. Three of these STK11 mutations were observed in the non-AGAs+NP SCC group, with one patient (33.3%)

experiencing TF; this case also carried a KRAS Q61H co-mutation. The sole AGA patient achieving MPR had a BRAF V600E mutation, developed a solitary brain metastasis 40.4 months post-randomization, received metastasectomy and adjuvant radiotherapy, and remained disease-free at the database cut-off.

Treatment Failure and Survival ***(Figure 3b)***

Patients with AGAs exhibited substantially shorter TF-free intervals. Median time to TF for the AGA group was 24.7 months (95% CI: 12.6–40.4), whereas the median was not reached for the non-AGAs+NP SCC cohort. TF-free survival rates at 36, 48, and 60 months were 33.3%, 22.2%, and 22.2%, respectively, for the AGA group, compared with 78.8% at all three time points for the non-AGAs+NP SCC group.

Univariable Cox regression analyses identified AGAs as a strong predictor of TF (HR: 5.51; 95% CI: 1.68–18.1). Former and current smokers had lower TF risk than never-smokers (HR: 0.24; 95% CI: 0.08–0.75). Notably, 80% of never-smokers who experienced TF carried AGAs (two EGFR, one EML4-ALK, one KIF5B-RET). Squamous histology was associated with decreased TF risk compared with non-squamous tumors (HR: 0.20; 95% CI: 0.06–0.74).

In multivariable analyses adjusting for molecular subgroup and smoking status, AGAs remained an independent predictor of TF (HR: 4.35; 95% CI: 1.24–15.2), while the effect of smoking status was no longer significant (HR: 0.41; 95% CI: 0.12–1.37). The median interval to first systemic retreatment following randomization was 20.2 months (range: 10.7–32.0). Longitudinal treatment timelines for individual patients in the non-AGAs+NP SCC (patients 1–20) and AGA (patients 21–32) groups are depicted in **Figure 5.**

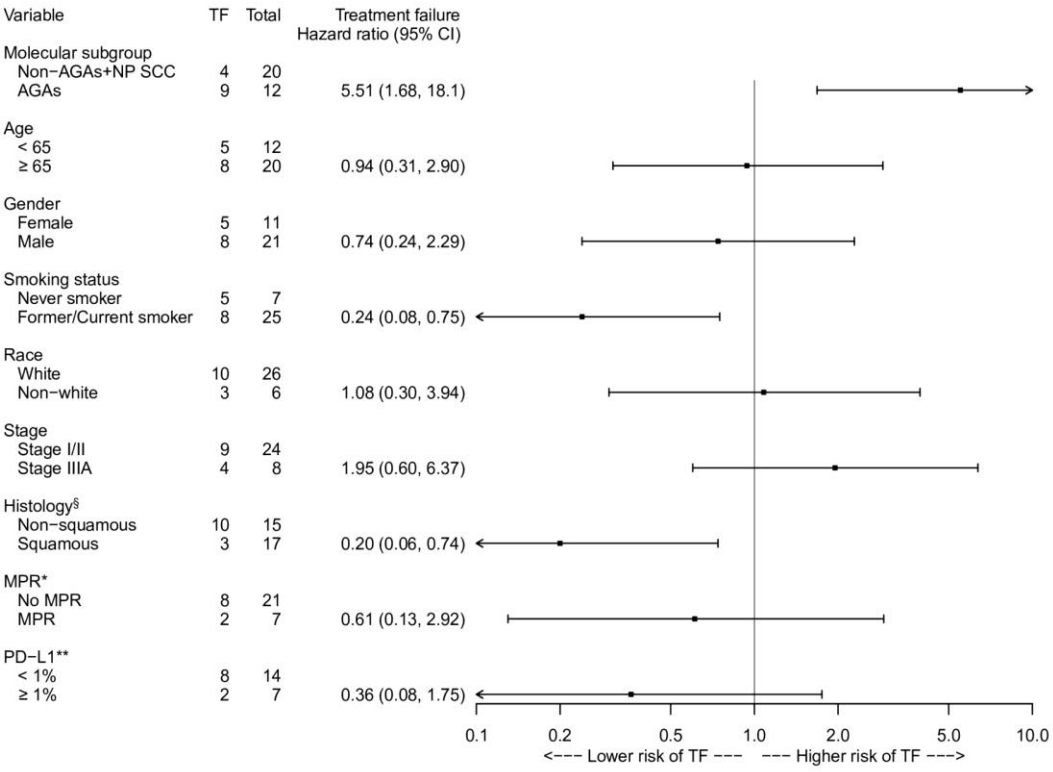


Figure 4. This forest plot displays the relative risk of treatment failure (TF) for each clinical and molecular variable, with hazard ratios (HRs) estimated from univariable Cox proportional hazards models. The HR represents the likelihood of TF in the group of interest compared with the reference group. TF was defined as either progression of the primary lung tumor in patients who did not undergo surgery, radiographic or biopsy-confirmed recurrence following surgery, or death related to primary lung cancer or potentially treatment-

associated complications, counted from randomization. For patients who underwent surgery on trial (n = 28), data were collected at the time of resection. Three patients in the AGA group and one in the non-AGAs+NP SCC group did not have surgery; for these individuals, the interval from surgery to TF was applied. Pretreatment PD-L1 expression was measured on available baseline tumor samples (n = 21) using clone 28-8 (Abcam) [6, 20]. PD-L1 data were missing for three AGA patients and eight non-AGAs+NP SCC patients. Abbreviations: AGAs, patients with actionable genomic alterations; non-AGAs+NP SCC, patients without AGAs or with non-profiled squamous cell carcinoma; MPR, major pathological response (≤10% residual viable tumor in resected specimen); PD-L1, programmed death-ligand 1; TF, treatment failure; HR, hazard ratio.

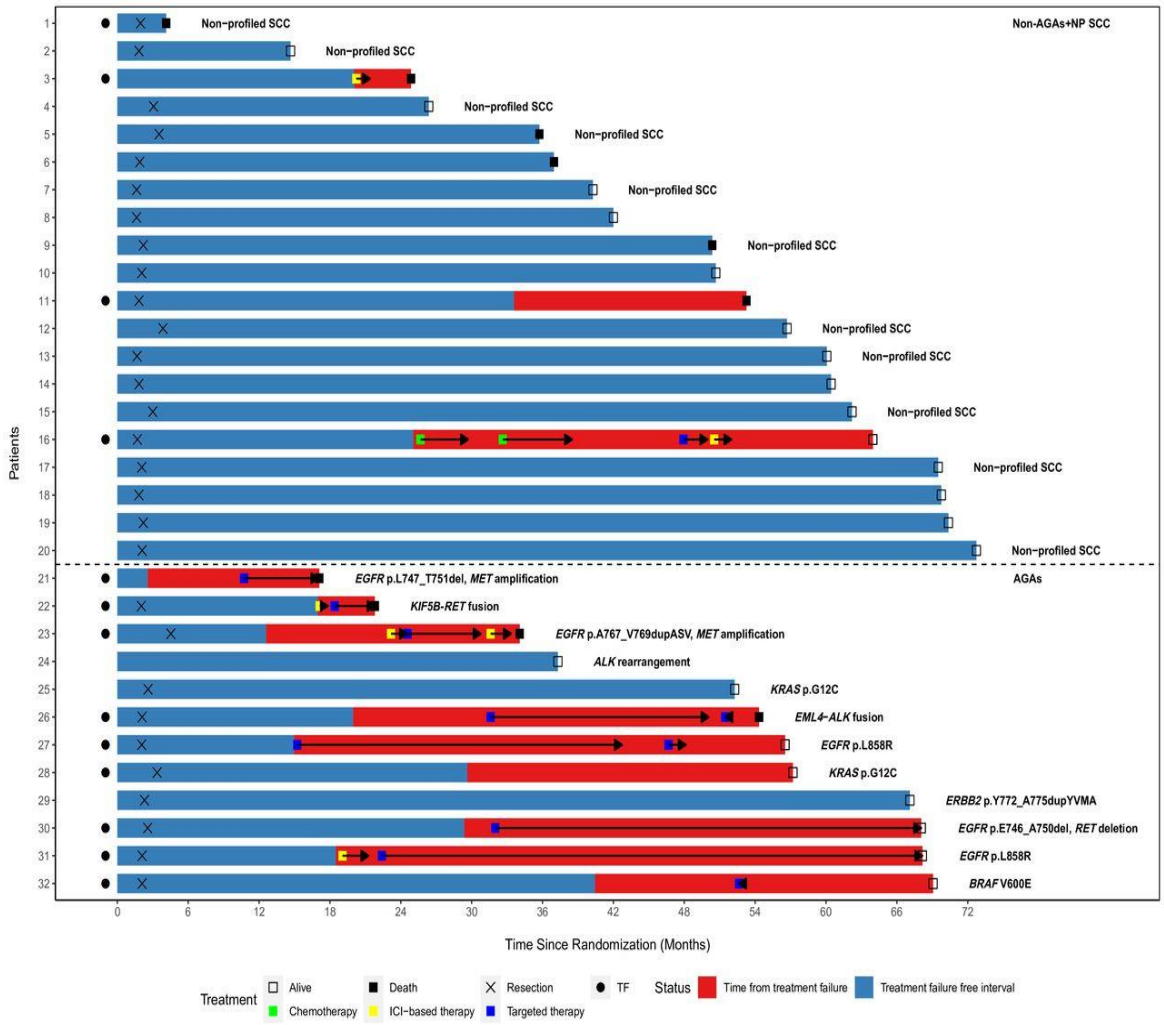


Figure 5. The swimmer plot depicts the duration of treatment failure-free intervals (blue) and post-treatment failure periods (red) for individual patients. Patients without actionable genomic alterations (AGAs) or with non-profiled squamous cell carcinoma (non-AGAs+NP SCC; patients 1–20) are shown alongside patients with tumors harboring AGAs (patients 21–32). Initiation of systemic therapies—including chemotherapy, immune checkpoint inhibitor (ICI)-based therapy, or targeted agents—occurred following the occurrence of treatment failure (TF). Non-profiled adenocarcinoma cases (n = 12) were excluded from this analysis. The timing and type of each patient's subsequent therapies are presented chronologically from the date of randomization. Notably, patient 23 underwent surgical resection off-trial, and patient 24, despite having no disease progression after neoadjuvant therapy, declined curative-intent surgery.

Abbreviations: ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; ICI, immune checkpoint inhibitor.

Post-treatment analyses and outcomes

Sensitivity check

To evaluate the robustness of our findings, a sensitivity analysis was conducted by reclassifying the two KRAS G12C cases into the non-AGAs+NP SCC cohort. In this scenario, the likelihood of achieving major pathological response (MPR) remained higher in the reclassified group than in the AGA group (odds ratio 3.38, 95% CI: 0.35–32.6; **Figure 1a**). Residual viable tumor was notably greater among patients with AGAs compared with the non-AGAs+KRAS G12C group (median 61.0% vs 34.5%). Median time to treatment failure (TF) for AGAs decreased to 19.2 months (95% CI: 2.57–40.4), while the median was not reached for the non-AGAs+KRAS G12C cohort **(Figure 1b).** AGAs conferred a 5.96-fold higher risk of TF relative to this group (95% CI: 1.92–18.5).

Retreatment with immune checkpoint inhibitors

Of 13 patients who experienced TF and received additional therapy, four were treated with ICIs. Only one patient derived durable benefit: disease recurred 18.5 months post-randomization, initial chemoimmunotherapy achieved stable disease for 2.1 months, and molecular profiling revealed TP53 and EGFR L858R mutations. The patient then received osimertinib, achieving disease control lasting 38.5 months from recurrence.

The remaining three ICI-treated patients did not achieve disease control. One patient progressed at 12.6 months, received chemoimmunotherapy without response, and, after identification of an EGFR Exon 20 duplication with MET amplification, was switched to targeted therapy for 6.2 months before progression. Subsequent immunotherapy failed, and the patient died 2.47 months later. Another patient, treated with chemoradiation (no surgery), later progressed locally, began single-agent immunotherapy, but died 4.8 months later. A third patient recurred at 17 months, received one cycle of chemoimmunotherapy without response, harbored a KIF5B-RET fusion, entered a RET TKI trial, and died 4.67 months after retreatment initiation.

Targeted therapy outcomes

Four additional patients received targeted therapy as the primary post-TF intervention. One patient with an EML4-ALK fusion recurred at 20 months and achieved 18.5 months of disease control. Two patients with EGFR mutations (Exon 19 deletion and L858R Exon 21) remained progression-free at 22.8 and 27.5 months, respectively. Another patient with EGFR Exon 19 deletion and MET amplification progressed after chemoradiation and later received targeted therapy but died 6.37 months after initiation. In total, targeted therapy, whether alone or sequentially with other treatments, achieved disease control in 71.4% (5/7) of treated patients.

KRAS and STK11 mutations

Among the two KRAS G12C patients, one carried a co-occurring STK11 mutation and developed locoregional recurrence at 29.6 months, treated with stereotactic body radiotherapy, maintaining disease control for 14.4 months. The second KRAS G12C patient underwent neoadjuvant ICI therapy followed by on-trial surgery and remained disease-free at 52.3 months.

Three additional patients harbored STK11 mutations. One had a concurrent KRAS Q61H mutation and recurred 25.1 months post-randomization. Sequential chemotherapy provided 4.03 and 5.87 months of disease control, followed by 2.03 months on a PARP inhibitor, and finally chemoimmunotherapy with bevacizumab, resulting in stable disease at data cut-off. The two other patients with STK11 mutations alone experienced no disease recurrence.

In this exploratory analysis, we assessed the influence of select tumor genomic alterations on treatment failure (TF) following neoadjuvant immune checkpoint inhibitor (ICI) therapy in patients with resectable non-small cell lung cancer (NSCLC) enrolled in a randomized phase 2 trial. Our results indicate that tumors harboring actionable genomic alterations (AGAs) were associated with higher percentages of residual viable tumor after neoadjuvant ICI therapy and shorter median time to TF compared with tumors lacking these alterations. Collectively, these findings emphasize the value of comprehensive molecular profiling in early-stage NSCLC to inform therapeutic decision-making and optimize clinical outcomes.

Our data suggest that ICI monotherapy or chemotherapy-sparing neoadjuvant regimens may not provide maximal benefit for all patients, particularly those whose tumors

harbor actionable driver mutations such as EGFR, RET, KRAS G12C, and ALK. The majority of TF events in our cohort occurred in patients with these AGAs, highlighting the critical importance of pre-neoadjuvant molecular testing and the consideration of targeted therapies for specific genomic subgroups, including non-EGFR alterations. Importantly, patients who experienced recurrence and subsequently received targeted therapy demonstrated durable disease control, consistent with prior evidence in advanced and metastatic NSCLC showing limited immunotherapy responsiveness in tumors with EGFR mutations or ALK fusions [21, 22].

The timing and selection of systemic therapies are increasingly recognized as essential for achieving long-term benefit. Recent trials underscore the advantages of integrating targeted agents in the perioperative setting. For instance, the ADAURA study demonstrated significant improvements in disease-free and overall survival with adjuvant osimertinib in patients with EGFR-mutant resected NSCLC (stage IB–IIIA) compared with placebo [7, 8]. Similarly, the ALINA trial showed that adjuvant alectinib conferred a disease-free survival advantage over chemotherapy in stage IB–IIIA ALK-positive NSCLC, emphasizing the benefit of aligning targeted therapies with specific genomic drivers in the early-stage context [9].

Conversely, the efficacy of neoadjuvant or perioperative ICIs in patients with AGAs remains inconsistent across trials. For example, subgroup analyses of EGFR-mutant patients in the AEGEAN trial evaluating perioperative durvalumab plus neoadjuvant chemotherapy did not demonstrate an event-free survival benefit at 12 or 24 months, with a pathologic complete response (pCR) rate of only 3.8%, markedly lower than the overall population rate of 17.2% [4]. By contrast, data from the IMpower010 trial suggested potential disease-free survival benefits of adjuvant atezolizumab in EGFR-positive patients with PD-L1 ≥1% [23]. Similarly, KEYNOTE-671 reported improved event-free survival in EGFR- and ALK-positive patients receiving perioperative pembrolizumab, though the small sample sizes limit interpretability [24].

The impact of immunotherapy in KRAS-mutant tumors appears more favorable compared with chemotherapy [25]; however, co-mutations such as STK11 may confer resistance [26, 27]. Given our limited cohort, our study cannot draw definitive conclusions regarding the influence of KRAS co-mutations on neoadjuvant ICI efficacy. Nevertheless, these data suggest that the benefit of ICI monotherapy may be restricted in early-stage NSCLC harboring select non-KRAS AGAs. Integrating molecular insights from prior studies, the optimal neoadjuvant treatment strategy for tumors with actionable alterations warrants further investigation through larger, dedicated trials.

Future directions, molecular testing, limitations, and conclusion

Neoadjuvant targeted therapy trials

Ongoing studies are actively investigating the integration of targeted therapies, with or without chemotherapy, in the neoadjuvant management of resectable NSCLC. A phase 2 study of neoadjuvant osimertinib (NCT03433469) reported a major pathological response (MPR) rate of 15% and a partial response rate of 48% in appropriately staged I–IIIA operable patients [28]. The phase 3 NeoADAURA trial (NCT04351555) is currently evaluating osimertinib with or without chemotherapy versus chemotherapy alone in patients with EGFR-mutant resectable NSCLC [29]. Additionally, the NAUTIKA-1 trial (NCT04302025) employs an umbrella design to identify patients with actionable genomic alterations (ALK, ROS1, NTRK, BRAF, RET) and assign them to tailored neoadjuvant therapy strategies prior to surgery [12, 13]. Collectively, these studies will provide essential insights into how tumor molecular profiling can inform individualized perioperative treatment decisions, including the optimal sequencing and combination of immunotherapy, targeted therapy, and chemotherapy.

Importance of comprehensive tumor profiling

Comprehensive molecular characterization remains a cornerstone of precision medicine in NSCLC. Current guidelines recommend evaluation of key driver alterations, including EGFR, ALK, ROS1, BRAF, MET, RET, ERBB2, and KRAS, as well as PD-L1 expression [30]. Despite potential logistical hurdles such as limited tissue availability and long turnaround times, tumor molecular testing should be performed whenever feasible. Accurate profiling enables the selection of targeted therapies with proven efficacy, guides evidence-based treatment planning, and facilitates monitoring of tumor evolution and the emergence of resistance mechanisms.

Study limitations

This study has several notable limitations. Not all patients underwent tumor molecular profiling, reflecting

both the historical context of trial initiation and the evolving landscape of early-stage NSCLC care. Nevertheless, profiling was conducted whenever feasible. Non-profiled squamous cell carcinomas (NP SCCs) were included in the comparison group due to the lower frequency of targetable alterations in this histology, although some may harbor actionable mutations. Furthermore, the study reflects a single-center experience with a limited sample size, underscoring the need for validation in larger, multicenter cohorts to confirm the generalizability of these findings.

Conclusion

Our findings indicate that in resectable NSCLC, the presence of actionable genomic alterations predicts a shorter median time to treatment failure following neoadjuvant immunotherapy. These results suggest that immunotherapy alone may not provide optimal benefit for patients with tumors sensitive to targeted therapies. Incorporating comprehensive molecular profiling into pre-neoadjuvant evaluation is therefore critical for guiding individualized treatment strategies and selecting the most effective perioperative approach for patients with operable NSCLC.

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Conflict of Interest: None

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Ethics Statement: This study involves human participants and was approved by MD Anderson Cancer Center Institutional Review Board 2016-0982 NEOSTAR clinical study protocol, and LAB10-0288, analysis protocol. Participants gave informed consent to participate in the study before taking part.

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