HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2019 January 15.

Published in final edited form as:

Clin Cancer Res. 2018 January 15; 24(2): 334–340. doi:10.1158/1078-0432.CCR-17-1841.

Effects of co-occurring genomic alterations on outcomes in patients with *KRAS*-mutant non-small cell lung cancer

Kathryn C. Arbour[1], **Emmett Jordan**[1], **Hyunjae Ryan Kim**[3], **Jordan Dienstag**[1], **Helena A. Yu**[1,6], **Francisco Sanchez-Vega**[5], **Piro Lito**[1,5], **Michael Berger**[4,5], **David B. Solit**[1,4,5], **Matthew Hellmann**[1,6], **Mark G. Kris**[1,6], **Charles M. Rudin**[1,6], **Ai Ni**[2], **Maria Arcila**[3], **Marc Ladanyi**[4,5], and **Gregory J. Riely**[1,6,*]

[1]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[2]Department of Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[3]Department of Pathology, Molecular Diagnostics Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[4]Marie-Josée & Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[5]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[6]Department of Medicine, Weill Cornell Medical College, New York, NY, USA

Abstract

Background—*KRAS* mutations occur in approximately 25% of patients with non-small cell lung cancer (NSCLC). Despite the uniform presence of *KRAS* mutations, patients with *KRAS*-mutant NSCLC can have a heterogeneous clinical course. Since the pattern of co-occurring mutations may describe different biological subsets of patients with *KRAS*-mutant lung adenocarcinoma, we explored the effects of co-occurring mutations on patient outcomes and response to therapy.

Methods—We identified patients with advanced *KRAS*-mutant NSCLC and evaluated the most common co-occurring genomic alterations. Multivariate analyses were performed incorporating the most frequent co-mutations and clinical characteristics to evaluate association with overall survival as well as response to platinum-pemetrexed chemotherapy and immune checkpoint inhibitors.

Results—Among 330 patients with advanced *KRAS*-mutant lung cancers, the most frequent co-mutations were found in *TP53* (42%), *STK11* (29%), and *KEAP1/NFE2L2* (27%). In a multivariate analysis, there was a significantly shorter survival in patients with co-mutations in *KEAP1/NFE2L2* (HR 1.96, 95%CI 1.33–2.92, p=<0.001). *STK11* (HR1.3, p=0.22) and *TP53* (HR 1.11, p= 0.58) co-mutation status were not associated with survival. Co-mutation in *KEAP1/NFE2L2* was also associated with shorter duration of initial chemotherapy (HR 1.64, 95% CI

*Corresponding Author, Gregory J. Riely, Memorial Sloan Kettering Cancer Center, 300 East 66th Street, NY, NY, 10065, Telephone 646-888-4199, rielyg@mskcc.org.

1.04–2.59, p=0.03) and shorter overall survival from initiation of immune therapy (HR 3.54, 95% CI 1.55–8.11, p=0.003).

Conclusions—Among people with *KRAS*-mutant advanced NSCLC, *TP53*, *STK11*, and *KEAP1/NFE2L2* are the most commonly co-occurring somatic genomic alterations. Co-mutation of *KRAS* and *KEAP1/ NFE2L2* is an independent prognostic factor, predicting shorter survival, duration of response to initial platinum based chemotherapy, and survival from start of immune therapy.

Introduction

Somatic *KRAS* mutations are identified in 25% of patients with non-small cell lung cancers (NSCLC). Patients with these mutations have shorter survival compared to patients with *EGFR*-mutant NSCLC or *KRAS* wild type tumors.[1] Since there is significant clinical heterogeneity in patients with *KRAS*-mutant NSCLC, defining clinically relevant subsets of KRAS-mutant NSCLC is important. In small cohorts, investigators have found specific *KRAS* point mutations (such as G12V and C12R), were associated with poorer outcomes.[2] However in a large retrospective analysis of nearly 700 patients with metastatic disease, no apparent differences in outcome based on *KRAS* mutation subtype were identified.[3]

In patients with lung cancer, the predictive utility of *KRAS* mutations as a marker of response to both targeted therapy and standard cytotoxic chemotherapy has been of particular interest. The presence of a *KRAS* mutation suggests lack of response to *EGFR* tyrosine kinase inhibitors,[4,5] likely because *EGFR* and *KRAS* mutations only rarely occur together. Patients with *KRAS* codon 13 mutations appear to have poorer outcomes with adjuvant cisplatin-based chemotherapy following resection of early stage disease,[6] but in the metastatic setting *KRAS* mutations do not appear to independently predict response or resistance to chemotherapy treatments.[7,8]

While the identification of subsets of NSCLC with oncogenic drivers has transformed the treatment of this disease, these advances have thus far largely been limited to patients with mutations in *EGFR*[9] or oncogenic fusions involving *ALK*[10], *RET*[11], or *ROS1*[12] kinases. There has been progress in the development of compounds that selectively target KRAS G12C, but efforts to specifically target mutant KRAS in the clinic have thus far been largely unsuccessful. Clinical testing of agents targeting downstream pathways, such as MEK and PI3K-AKT, in patients with *KRAS*-mutant tumors has yielded relatively low response rates.[13,14] This may be due to the fact that there is a significant molecular diversity in *KRAS*-mutant tumors compared to other known driver events and it is these underlying mechanisms that drive divergent biologic and clinical behavior.[15] However, even KRAS G12C mutant cell lines exhibit a range of responses to pharmacologic inhibition of KRAS G12C, a finding that further highlights the molecular diversity of *KRAS*-mutant lung cancers.[16]

We hypothesized that broad next-generation sequencing may allow an in-depth description of clinically heterogenous group of patients and offer prognostic and predictive markers based on the presence of co-occurring mutations in patients with *KRAS*-mutant NSCLC. To evaluate this hypothesis, we investigated the effect of commonly co-occurring genomic

alterations, clinical characteristics on survival and treatment response in patients with advanced *KRAS*-mutant NSCLC.

Methods

Patients

Consecutive patients with metastatic or recurrent lung cancers found to have a *KRAS* mutation by next-generation sequencing were included in the analysis. A medical record search was used to identify individuals seen at Memorial Sloan Kettering with a primary tumor diagnosis of lung cancer by ICD-O code who had also undergone hybridization capture-based next-generation sequencing (NGS) testing from January of 2014 to October 2016. The list was then manually reviewed to exclude patients who did not have metastatic or recurrent disease, or a tumor diagnosis of primary lung cancer. Data collection was approved by the MSKCC Institutional Review Board/Privacy Board. Clinical characteristics and treatment course were collected for all patients. Overall survival was defined as the time from date of diagnosis of advanced disease (stage IV or recurrent cancer) until date of death or last follow up.

Genotype Analysis

Tumor and germline DNA were processed to generate bar-coded libraries and subjected to exon capture using custom-designed probes. Matched normal DNA was analyzed simultaneously to identify and filter out germline SNPs. Genomic analysis was performed using the MSK-IMPACT assay[17], a clinical test approved by the New York State Department of Health designed to detect mutations, copy-number alterations, and select fusions involving 341 (version 1), 410 (version 2), or 468 (version 3) cancer-associated genes. Genomic analysis was performed using assay version 1 (341 genes) for 66 samples, version 2 (410 genes) for 250 samples, and version 3 (468 genes) for 14 samples. Normalized mutation burden was calculated as the absolute mutation burden (number of non-synonymous mutations per sample) divided by the genomic coverage for that sample (0.98Mb for version 1, 1.06Mb for version 2, and 1.22Mb for version 3).

Statistical Methods

Survival following diagnosis of stage IV lung cancer and treatment duration was estimated using Kaplan-Meier methodology. Patients were followed until death; patients alive at the end of the study were censored at the time of last available follow up. Univariate group comparisons were performed using log-rank tests. A multivariable Cox proportional hazards model was used to assess the independent effect of co-occurring mutations (*STK11*, *KEAP1/NFE2L2*, and *TP53*), adjusting for age, gender, performance status, and smoking history. Tumor mutational burden normalized by the size of the coding region (MB) captured by sequencing was evaluated as a continuous variable.

Results

Clinical Characteristics

We identified 550 patients with lung cancer and *KRAS* mutations on NGS testing between January of 2014 and October 2016. Of that cohort of patients, 330 had metastatic or recurrent lung cancer. Seventy-two percent of patients had metastatic disease at the time of initial diagnosis (n=240) while 28% (n=90) had recurrent disease. The predominant histology was adenocarcinoma (n=298, 90%). Patient demographics and histology are noted in Table 1. The most common *KRAS* mutation observed was G12C (44%) (Figure 1A).

Co-occurring Mutations

Along with *KRAS,* 377 different genes were mutated in this group of patients. The median number of co-occurring mutations per tumor was 8 (range 0–58). The most frequent mutations were found in *TP53* (41%), *STK11* (28%), *KEAP1* (24%), *RBM10* (16%), and *PTPRD* (15%) (Table 2). Given that genomic alteration in *NFE2L2* elicit similar effects as alterations in *KEAP1* in their effects on the Nrf2 pathway, patients with genomic alterations in either gene were grouped.[18] An additional 3% of patients (n=9) were found to have mutations in *NFE2L2* and therefore 27% of patients were grouped as having either *KEAP1* or *NFE2L2* mutations concurrent with *KRAS*. The three most frequently co-occurring genomic alterations (*TP53*, *STK11*, and *KEAP1*) were selected for further statistical analysis. The distributions of the 3 most frequent co-occurring mutations (*TP53*, *STK11*, and *KEAP1/NFE2L2*) are depicted in a proportional Venn diagram in Figure 1B.

Co-occurring Mutations and Survival

The median follow-up among the 177 patients alive at the data cutoff of January 2017 was 12 months (range 1–114). The median overall survival (mOS) for all patients with *KRAS*-mutant advanced lung cancers in this cohort was 17 months (95% CI: 14–25). Patients with and without concurrent mutation in *TP53* had a similar overall survival (HR 0.9, 95% CI 0.6–1.2, p=0.5). Patients with a concurrent mutation in *STK11* (*KRAS/STK11*) were found to have a shorter overall survival (OS) (HR 1.7, 95% CI 1.1–2.4, p=0.002, Figure 2A). In addition, patients with concurrent mutation in *KEAP1* or *NFE2L2* (*KRAS/KEAP1/NFE2L2*) were also found to have a shorter OS (HR 2.1, 95% CI 1.4–3.1, p<.0001, Figure 2B). A multivariable Cox proportional hazards model was used to assess the independent effect of the most frequently identified co-occurring mutations (*TP53*, *STK11*, *KEAP1/NFE2L2*), adjusting for age, gender, performance status, and smoking history. After adjustment for these clinical variables, only *KEAP1/NFE2L2* was independently associated with shorter OS (HR 1.96, 95% CI 1.3-3-2.92, p<0.001, Table 3)

Initial chemotherapy and co-mutational status

To evaluate the effects of co-mutations on outcomes after chemotherapy, we obtained treatment history of patients who received initial chemotherapy treatment with a platinum agent, pemetrexed, +/− bevacizumab following diagnosis of recurrent or metastatic NSCLC. In a univariate analysis the presence of a co-occurring mutation in *KEAP1/ NFE2L2* was associated with a shorter duration of therapy (HR 1.6 95% CI 1.1–2.4, p=0.008,

Supplemental Figure 1). The presence of either a *STK11* or *TP53* mutation was not associated with a difference in duration of platinum based therapy. A multivariable Cox proportional hazards model (adjusting for *STK11*, *TP53*, *KEAP1/NFE2L2* as well as clinical characteristics of age, gender, performance status, and smoking history, and bevacizumab use), found *KEAP1/NFE2L2* was associated with shorter treatment duration (HR 1.64, 95% CI 1.04–2.59, p=0.03, Supplemental Table 1).

Immunotherapy and co-mutation

To evaluate the effects of co-mutations on outcomes after immunotherapy, we obtained treatment history of 86 patients that underwent therapy with an immune checkpoint inhibitor as monotherapy (nivolumab or pembrolizumab). In a univariate analysis, neither the presence of *TP53*, *SKT11*, nor *KEAP1/NFE2L2* was associated with a difference in duration of immune therapy (Supplemental Figure 2). These results were confirmed in a multivariate analysis adjusting for these three co-mutations as well as, age, gender, performance status, and line of therapy. Patients with a co-occurring mutation in *KEAP1* or *NFE2L2* were found to have a shorter overall survival from the start of immune checkpoint inhibitor in both a univariate (Figure 3) and multivariate analysis (HR 3.54, 95% CI 1.55–8.11, p=0.003, Supplemental Table 3) adjusting for co-mutations as well as, tumor mutation burden, age, gender, performance status, and line of therapy. The presence of a co-occurring mutation in neither *STK11* nor *TP53* was associated with a significant difference in mOS from start of therapy. Tumor mutation burden was associated with difference in overall survival from time of initiation of immunotherapy as patients with higher mutation burdens were found to have longer survival from start of treatment (HR 0.9, 95% CI 0.83–0.99, p=0.025, Supplemental Table 3).

Discussion

We report the landscape of co-occurring genomic alterations in a series of 330 patients with advanced *KRAS*-mutant lung cancer, highlighting the most common events and identifying mutations in the *KEAP1/NFE2L2* pathway as a significant, independent negative prognostic factor for patients with *KRAS*-mutant NSCLC. We go on to demonstrate that this association with poor overall survival is also associated with shorter duration of therapy with platinum-doublet chemotherapy and overall survival after immunotherapy. These data suggest that the observed clinical heterogeneity in patients with KRAS-mutant NSCLC is likely due in part to differences in co-occurring molecular events.

Concurrent mutations are common in patients with *KRAS*-mutant NSCLC, with the most common events in *STK11* and *TP53*. Although *TP53* mutations are common, our analysis concurs with that of others, reporting that concurrent *TP53* mutation in *KRAS*-mutant NSCLC is not prognostic.[19] Multiple prior analyses have reported *STK11* mutations are more frequent in *KRAS*-mutant NSCLC as opposed to *KRAS* wild type tumors.[20] Preclinical data has suggested that loss of *STK11* leads to a more aggressive tumor phenotype.[21] However there has been conflicting evidence whether *STK11* mutations have prognostic or predictive implications.[15,22] Our initial univariate analysis also demonstrated that concurrent *STK11* mutations were associated with shorter overall survival in patients

with advanced *KRAS*-mutant NSCLC. However, when a multivariable analysis was conducted adjusting for concurrent mutations in *KEAP1/NFE2L2*, *TP53* and other clinical variables, concurrent mutation in *STK11* was no longer associated with difference in overall survival.

The differences seen in the univariate analysis compared to multivariable analysis is likely due to significant overlap between patients with *STK11* and *KEAP1* concurrent mutations (also observed by others[15]). Our analysis suggests, however, that it is not the concurrent *STK11* mutation that is associated with adverse outcomes in these patients, but rather the presence of concurrent *KEAP1* or *NFE2L2* mutation. This observation is limited as we evaluated only somatic mutations and not protein expression, and therefore it is possible that we did not capture tumors that have suppressed *STK11* mRNA expression through a mechanism other than *STK11* mutation which were observed in the subsets described by Skoulidis et al.[15] These results highlight the importance of multivariable analyses, incorporating not just clinical features but also genomic features, in order to more accurately describe prognostic features in the genomically complicated landscape of *KRAS*-mutant NSCLC.

Mutations in the *KEAP1/NFE2L2* pathway identify 27% of patients with KRAS-mutant lung cancer as having an independent negative prognostic factor. *KEAP1* and *NFE2L2* mutations have been best described in squamous cell lung cancer. Activation of the NRF2 pathway (through activation of *NFE2L2* or inactivation of KEAP1) was found to be altered in 34% of squamous cell cancers of the lung when evaluated by TCGA investigators.[23] Solis et al. also showed that increased nuclear expression of Nrf2 and decreased or absent expression of KEAP1 in 38% and 46% of patients with squamous cell carcinoma, respectively and less commonly in adenocarcinoma (18%).[24] While low cytoplasmic KEAP1 expression was associated with worse overall survival in squamous cell carcinomas, no association was found between KEAP1 expression and outcomes in patients with adenocarcinoma. That analysis, similar to the TCGA analysis, is heavily weighted towards patients with early stage resected disease as opposed to advanced stage disease which is the focus of our report.

The poor prognosis of patients with concurrent *KEAP1* or *NFE2L2* mutations may be due in part to the observation that activation of the Nrf2 pathway may be associated with resistance to chemotherapy. Mutations in KEAP1 affect the repressive activity of KEAP1, stimulating nuclear accumulation of Nrf2, and induce constitutive expression of cytoprotective enzymes.[25] Cell lines expressing lower levels of KEAP1 or *KEAP1* mutant cells demonstrated greater resistance to cisplatin than cell lines with normal *KEAP1*.[26] Concordant with this, we observed that patients with a concurrent *KEAP1* or *NFE2L2* mutation who were treated with a platinum/pemetrexed combination had more rapid disease progression (as suggested by shorter duration of therapy) compared to *KEAP1* or *NFE2L2* wild type patients. Our analysis was limited to patients with *KRAS*-mutant NSCLC, suggesting a cooperativity between these mutations in *KEAP1* or *NFE2L2* and *KRAS*. Moreover, the frequency of KEAP1/NFE2L2 in KRAS mutant NSCLC is much higher than seen in other lung cancer with other oncogenic drivers.

Treatment with immune check point inhibitors has been a significant advance in the treatment of NSCLC,[27,28] particularly for patients without targetable oncogenic drivers. Despite these advances, single-agent response rates in unselected populations remain relatively low (10–30% in reported clinical trials). Various biomarkers have been reported that may potentially predict response to immune checkpoint inhibitors in solid tumors including PD-L1 expression[29] and tumor mutation burden or neoantigen load[30,31]. We attempted to explore the impact of concurrent genomic alterations in patients who received single agent immune checkpoint inhibitors during the treatment of their disease. Our analysis of clinical benefit with immunotherapy was limited in size, since only a subset of the patients in our larger analysis received this therapy. Keeping these limitations in mind, our analysis suggests that patients with *KRAS*-mutant advanced NSCLC and a concurrent mutation in *KEAP1* or *NFE2L2*, have significantly shorter overall survival from initiation of immune checkpoint therapy. No statistically significant differences in OS from start of therapy were observed based on concurrent *STK11* or *TP53* mutations. Formal response assessment was available on a subset of these patients (data not shown), and no significant difference in response was observed based on concurrent mutation in either *KEAP1/NFE2L2*, *STK11*, or *TP53*.

It is possible that mutations in *KEAP1/NFE2L2* are associated with other features that have been associated with outcomes after immunotherapy, such as PD-L1 expression status which was not available in this series of patients. PD-L1 expression has been reported in *KRAS*-mutant NSCLC and was more frequently observed in smokers,[32] however relationship to concurrent mutation is thus far unknown. Skouldolis et al. described that the cluster of *KRAS*-mutant NSCLC with low *STK11* expression demonstrated a lack of immune system engagement however concurrent mutations in *KEAP1* were also common in this cluster.[15] In a follow up analysis, they reported that patients with concurrent mutations in *STK11* had lower objective response rates to immunotherapy agents,[33] a finding we did not observe in our larger series of patients.

Higher nonsynonymous mutation burden in tumors has been associated with improved objective response, durable clinical benefit, and progression free survival in patients treated with the PD-1 antibody pembrolizumab.[30] More recently, normalized mutation count calculated from results of routine next-generation sequencing was reported to be predictive of response to nivolumab in NSCLC.[34,35] Our multivariable analysis did incorporate normalized tumor mutation count and demonstrated that tumor mutation burden was associated with longer overall survival from initiation of immune checkpoint inhibitor. However, presence of concurrent mutation in *KEAP1* or *NFE2L2* was associated with significantly shorter mOS independent of tumor mutation burden.

While the findings we have described are consistent with other reports analyzing the molecular characteristics of patients with *KRAS*-mutant lung cancer, there are some limitations to our analysis. All patients were identified based on molecular testing at a single institution. Although molecular analysis is offered routinely for all patients, some patients may have had inadequate biopsy specimens, precluding next-generation sequencing analysis. Patients received diverse treatments and therefore the analyses of initial platinum/pemetrexed chemotherapy and immunotherapy involve a much smaller subset of patients.

Duration of therapy was used as a surrogate marker for progression free survival when describing treatment history which has potential pitfalls as patients may discontinue therapy for other reasons (e.g. toxicity) as opposed to progression.

While it has been evident that there is significant clinical heterogeneity in patients with *KRAS*-mutant NSCLC, it is now becoming clear that this clinical heterogeneity is like due to biologic heterogeneity. Skouldolis et al. have previously reported molecular stratification of *KRAS*-mutant lung adenocarcinomas using RNA sequencing expression data from a subset of lung adenocarcinomas in the TCGA. These results were further validated in other small cohorts, however this dataset focused on primarily early stage disease with only a subset of patients analyzed having Stage IV disease (and all of these from the BATTLE-2 clinical trial and therefore platinum refractory).[15]

Our analysis shows that routine next-generation sequencing can not only provide information regarding potential actionable mutations, but also suggest prognostic and predictive features of a patient's cancer by exploring the presence of various co-occurring mutations. Considering any given mutation in the context of other mutations and using multivariate analyses is crucial in evaluating the significance of somatic genetic events. Prospective identification of patients with concurrent *KEAP1* or *NFE2L2* mutations in patients with *KRAS*-mutant NSCLC should be considered as a prognostic factor in clinical trials evaluating therapies for these patients. Our results indicate that patients with concurrent *KRAS* and *KEAP1/NFE2L2* have a clinically distinct behavior and may require stratification in trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Support: This study was funded by the US Department of Health and Human Services through the National Cancer Institute of the National Institutes of Health (P30 CA008748), the DallePezze Family Foundation, and a Stand Up To Cancer – American Cancer Society Lung Cancer Dream Team Translational Research Grant (Grant Number: SU2C-AACR-DT1715). Stand Up To Cancer is a program of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the Scientific Partner of SU2C.

Conflict of Interest Statement: Dr. Riely's institution receives clinical research support from Takeda, Pfizer, Novartis, and Infinity. He has been a compensated consultant for Genentech/Roche.

References

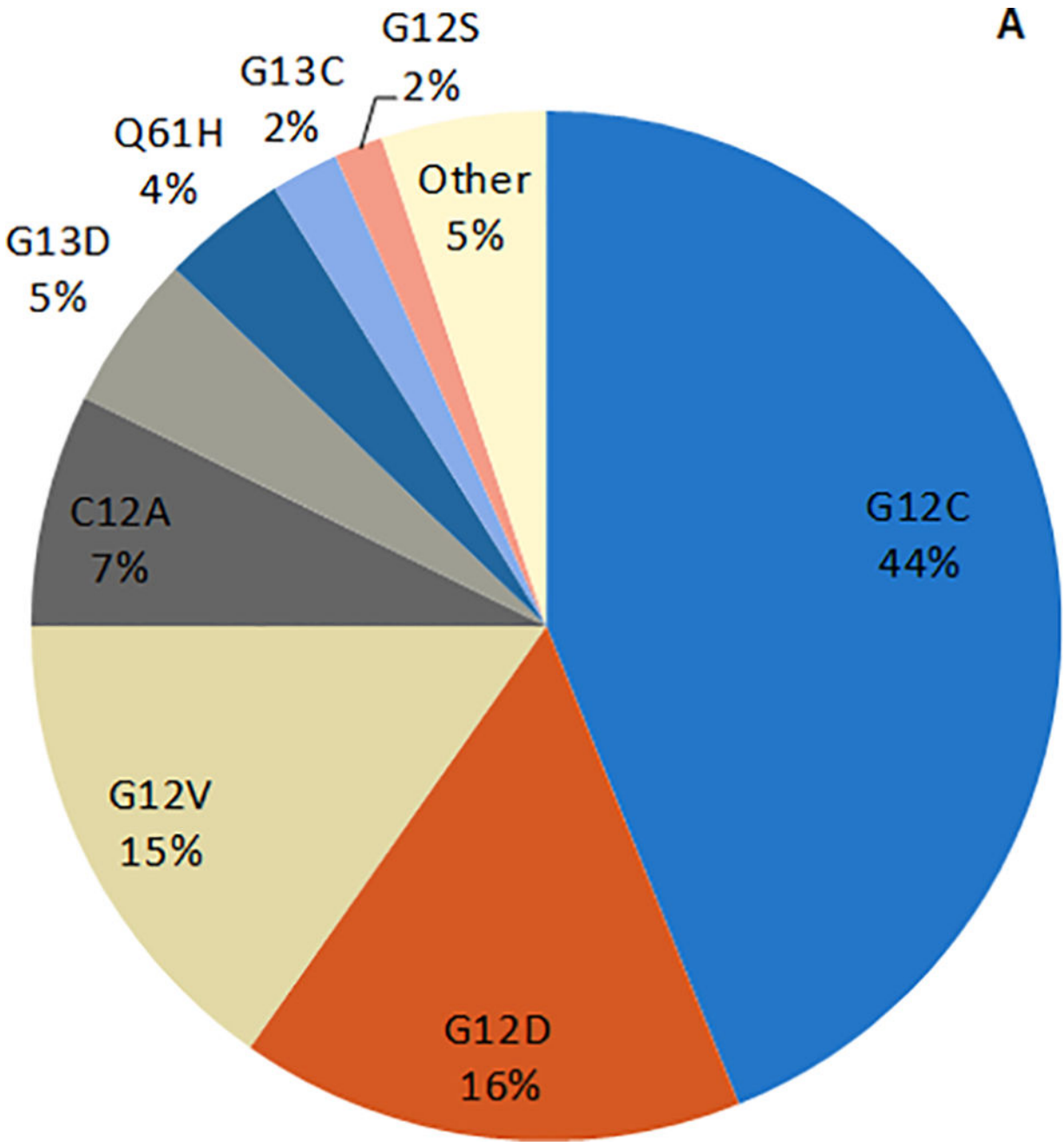
1. Johnson ML, Sima CS, Chaft J, et al. Association of KRAS and EGFR mutations with survival in patients with advanced lung adenocarcinomas. Cancer. 2013; 119(2):356–362. DOI: 10.1002/cncr.27730 [PubMed: 22810899]
2. Villaruz LC, Socinski MA, Cunningham DE, et al. The prognostic and predictive value of KRAS oncogene substitutions in lung adenocarcinoma. Cancer. 2013; 119(12):2268–2274. DOI: 10.1002/cncr.28039 [PubMed: 23526491]
3. Yu HA, Sima CS, Shen R, et al. Prognostic Impact of KRAS Mutation Subtypes in 677 Patients with Metastatic Lung Adenocarcinomas. J Thorac Oncol. 2015; 10(3):431–437. DOI: 10.1097/JTO.0000000000000432 [PubMed: 25415430]

4. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol. 2008; 9(10):962–972. DOI: 10.1016/S1470-2045(08)70206-7 [PubMed: 18804418]
5. Pao W, Wang TY, Riely GJ, et al. KRAS Mutations and Primary Resistance of Lung Adenocarcinomas to Gefitinib or Erlotinib. PLOS Med. 2005; 2(1):e17.doi: 10.1371/journal.pmed.0020017 [PubMed: 15696205]
6. Shepherd FA, Domerg C, Hainaut P, et al. Pooled Analysis of the Prognostic and Predictive Effects of KRAS Mutation Status and KRAS Mutation Subtype in Early-Stage Resected Non–Small-Cell Lung Cancer in Four Trials of Adjuvant Chemotherapy. J Clin Oncol. 2013; 31(17):2173–2181. DOI: 10.1200/JCO.2012.48.1390 [PubMed: 23630215]
7. Rodenhuis S, Boerrigter L, Top B, et al. Mutational activation of the K-ras oncogene and the effect of chemotherapy in advanced adenocarcinoma of the lung: a prospective study. J Clin Oncol. 1997; 15(1):285–291. DOI: 10.1200/JCO.1997.15.1.285 [PubMed: 8996154]
8. Kalikaki A, Koutsopoulos A, Hatzidaki D, et al. Clinical outcome of patients with non-small cell lung cancer receiving front-line chemotherapy according to EGFR and K-RAS mutation status. Lung Cancer. 2010; 69(1):110–115. DOI: 10.1016/j.lungcan.2009.09.010 [PubMed: 19854533]
9. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012; 13(3):239–246. DOI: 10.1016/S1470-2045(11)70393-X [PubMed: 22285168]
10. Camidge DR, Bang Y-J, Kwak EL, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. Lancet Oncol. 2012; 13(10):1011–1019. DOI: 10.1016/S1470-2045(12)70344-3 [PubMed: 22954507]
11. Drilon A, Wang L, Hasanovic A, et al. Response to Cabozantinib in Patients with RET Fusion-Positive Lung Adenocarcinomas. Cancer Discov. 2013; 3(6):630–635. DOI: 10.1158/2159-8290.CD-13-0035 [PubMed: 23533264]
12. Shaw AT, Ou S-HI, Bang Y-J, et al. Crizotinib in ROS1-Rearranged Non–Small-Cell Lung Cancer. N Engl J Med. 2014; 371(21):1963–1971. DOI: 10.1056/NEJMoa1406766 [PubMed: 25264305]
13. Jänne PA, Shaw AT, Pereira JR, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol. 2013; 14(1):38–47. DOI: 10.1016/S1470-2045(12)70489-8 [PubMed: 23200175]
14. Blumenschein GR, Smit EF, Planchard D, et al. A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC). Ann Oncol. 2015; 26(5):894–901. DOI: 10.1093/annonc/mdv072 [PubMed: 25722381]
15. Skoulidis F, Byers LA, Diao L, et al. Co-occurring Genomic Alterations Define Major Subsets of KRAS-Mutant Lung Adenocarcinoma with Distinct Biology, Immune Profiles, and Therapeutic Vulnerabilities. Cancer Discov. 2015; 5(8):860–877. DOI: 10.1158/2159-8290.CD-14-1236 [PubMed: 26069186]
16. Lito P, Solomon M, Li L-S, Hansen R, Rosen N. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. Science. 2016; 351(6273):604–608. DOI: 10.1126/science.aad6204 [PubMed: 26841430]
17. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J Mol Diagn. 2015; 17(3):251–264. DOI: 10.1016/j.jmoldx.2014.12.006 [PubMed: 25801821]
18. Goldstein LD, Lee J, Gnad F, et al. Recurrent Loss of NFE2L2 Exon 2 Is a Mechanism for Nrf2 Pathway Activation in Human Cancers. Cell Rep. 2016; 16(10):2605–2617. DOI: 10.1016/j.celrep.2016.08.010 [PubMed: 27568559]
19. Shepherd FA, Lacas B, Le Teuff G, et al. Pooled Analysis of the Prognostic and Predictive Effects of TP53 Comutation Status Combined With KRAS or EGFR Mutation in Early-Stage Resected Non-Small-Cell Lung Cancer in Four Trials of Adjuvant Chemotherapy. J Clin Oncol Off J Am Soc Clin Oncol. Apr.2017 JCO2016712893. doi: 10.1200/JCO.2016.71.2893

20. Matsumoto S, Iwakawa R, Takahashi K, et al. Prevalence and specificity of LKB1 genetic alterations in lung cancers. Oncogene. 2007; 26(40):5911–5918. DOI: 10.1038/sj.onc.1210418 [PubMed: 17384680]
21. Ji H, Ramsey MR, Hayes DN, et al. LKB1 modulates lung cancer differentiation and metastasis. Nature. 2007; 448(7155):807–810. DOI: 10.1038/nature06030 [PubMed: 17676035]
22. Facchinetti F, Bluthgen MV, Clain GT, et al. Abstract 2250: STK11 mutations in non-small cell lung cancer (NSCLC): descriptive analysis and prognostic significance. Cancer Res. 2016; 76(14 Supplement):2250–2250. DOI: 10.1158/1538-7445.AM2016-2250
23. Network TCGAR. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012; 489(7417):519–525. DOI: 10.1038/nature11404 [PubMed: 22960745]
24. Solis LM, Behrens C, Dong W, et al. Nrf2 and Keap1 Abnormalities in Non–Small Cell Lung Carcinoma and Association with Clinicopathologic Features. Clin Cancer Res. 2010; 16(14):3743–3753. DOI: 10.1158/1078-0432.CCR-09-3352 [PubMed: 20534738]
25. Padmanabhan B, Tong KI, Ohta T, et al. Structural Basis for Defects of Keap1 Activity Provoked by Its Point Mutations in Lung Cancer. Mol Cell. 2006; 21(5):689–700. DOI: 10.1016/j.molcel.2006.01.013 [PubMed: 16507366]
26. Ohta T, Iijima K, Miyamoto M, et al. Loss of Keap1 Function Activates Nrf2 and Provides Advantages for Lung Cancer Cell Growth. Cancer Res. 2008; 68(5):1303–1309. DOI: 10.1158/0008-5472.CAN-07-5003 [PubMed: 18316592]
27. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. N Engl J Med. 2016; 375(19):1823–1833. DOI: 10.1056/NEJMoa1606774 [PubMed: 27718847]
28. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non–Small-Cell Lung Cancer. N Engl J Med. 2015; 373(17):1627–1639. DOI: 10.1056/NEJMoa1507643 [PubMed: 26412456]
29. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res Off J Am Assoc Cancer Res. 2014; 20(19):5064–5074. DOI: 10.1158/1078-0432.CCR-13-3271
30. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science. 2015; 348(6230):124–128. DOI: 10.1126/science.aaa1348 [PubMed: 25765070]
31. Snyder A, Makarov V, Merghoub T, et al. Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. N Engl J Med. 2014; 371(23):2189–2199. DOI: 10.1056/NEJMoa1406498 [PubMed: 25409260]
32. Calles A, Liao X, Sholl LM, et al. Expression of PD-1 and Its Ligands, PD-L1 and PD-L2, in Smokers and Never Smokers with KRAS-Mutant Lung Cancer. J Thorac Oncol. 2015; 10(12):1726–1735. DOI: 10.1097/JTO.0000000000000687 [PubMed: 26473645]
33. Skoulidis F, Elamin Y, Papadimitrakopoulou V, et al. MA04.07 Impact of Major Co-Mutations on the Immune Contexture and Response of KRAS-Mutant Lung Adenocarcinoma to Immunotherapy. J Thorac Oncol. 2017; 12(1):S361–S362. DOI: 10.1016/j.jtho.2016.11.402
34. Carbone DP, Reck M, Paz-Ares L, et al. First-Line Nivolumab in Stage IV or Recurrent Non–Small-Cell Lung Cancer. N Engl J Med. 2017; 376(25):2415–2426. DOI: 10.1056/NEJMoa1613493 [PubMed: 28636851]
35. Stephens, P. Identification of novel modulators of response to immune checkpoint blockade in lung cancer patients through the marrying of clinical and genomic data; AACR; April 1–7th 2017; Washington, DC. Abstract SY40-02

Statement of Translational Relevance

While *KRAS* mutations identify the largest group of patients with oncogene-driven non-small cell lung cancer (NSCLC), patients with *KRAS*-mutant NSCLC have a heterogenous clinical course. We used the results of next-generation sequencing of tumors from patients with *KRAS*-mutant NSCLC to describe the pattern of co-occurring mutations and explore clinical outcomes. In a multivariable analysis, we identified a molecular subtype of *KRAS*-mutant NSCLC, with co-mutations in *KEAP1/NFE2L2* in which patients had a significantly shorter overall survival than other patients with *KRAS*-mutant NSCLC. Patients with concurrent mutations in *KRAS* and *KEAP1 / NFE2L2* had a shorter duration of therapy with platinum-based chemotherapy than other patients with *KRAS*-mutant lung cancer. These clinical findings align with prior pre-clinical work showing that mutations in *KEAP1* or *NFE2L2* result in activation of the Nrf2 pathway inducing constitutive expression of cytoprotective enzymes thus conferring resistance to platinum agents. Mutations in *KEAP1/NFE2L2* were also associated with decreased overall survival from start of immune checkpoint inhibitors, independent of tumor mutational burden. Since our results indicate that patients with *KEAP1* or *NFE2L2* mutations occurring in the context of *KRAS*-mutations have a worse clinical course than other patients with *KRAS*- mutant NSCLC, we recommend that this information be captured as part of clinical trials evaluating therapy for these patients.



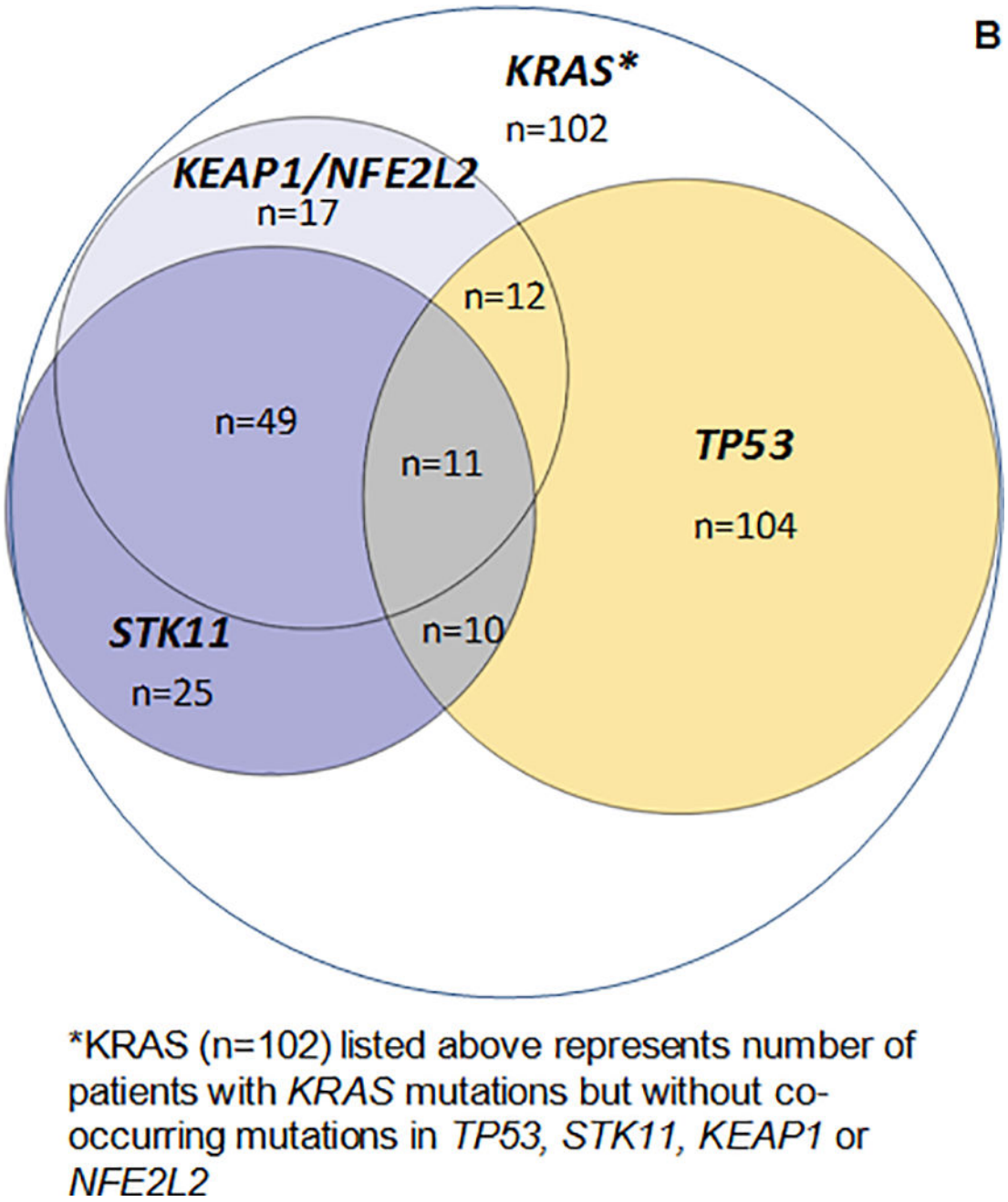
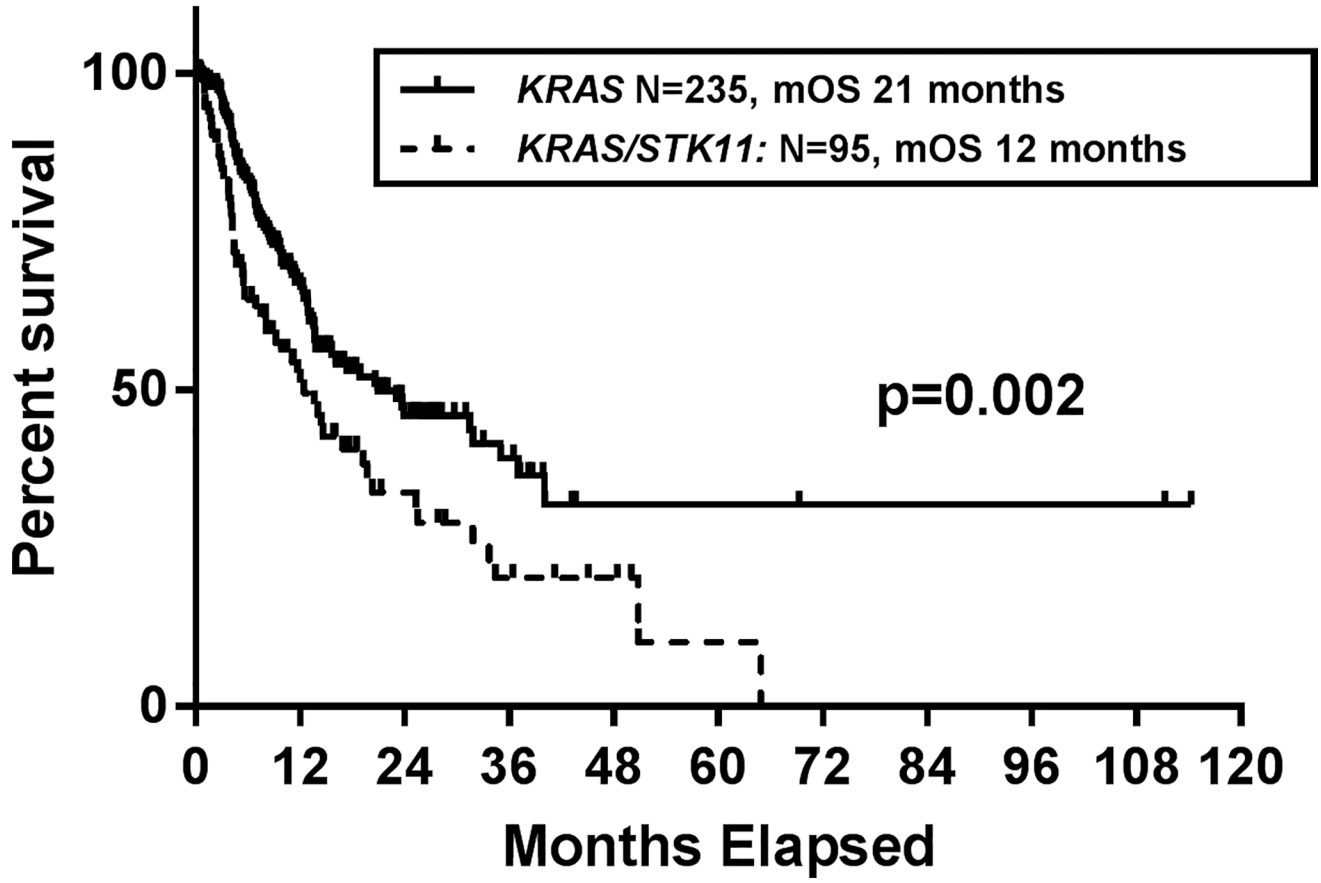


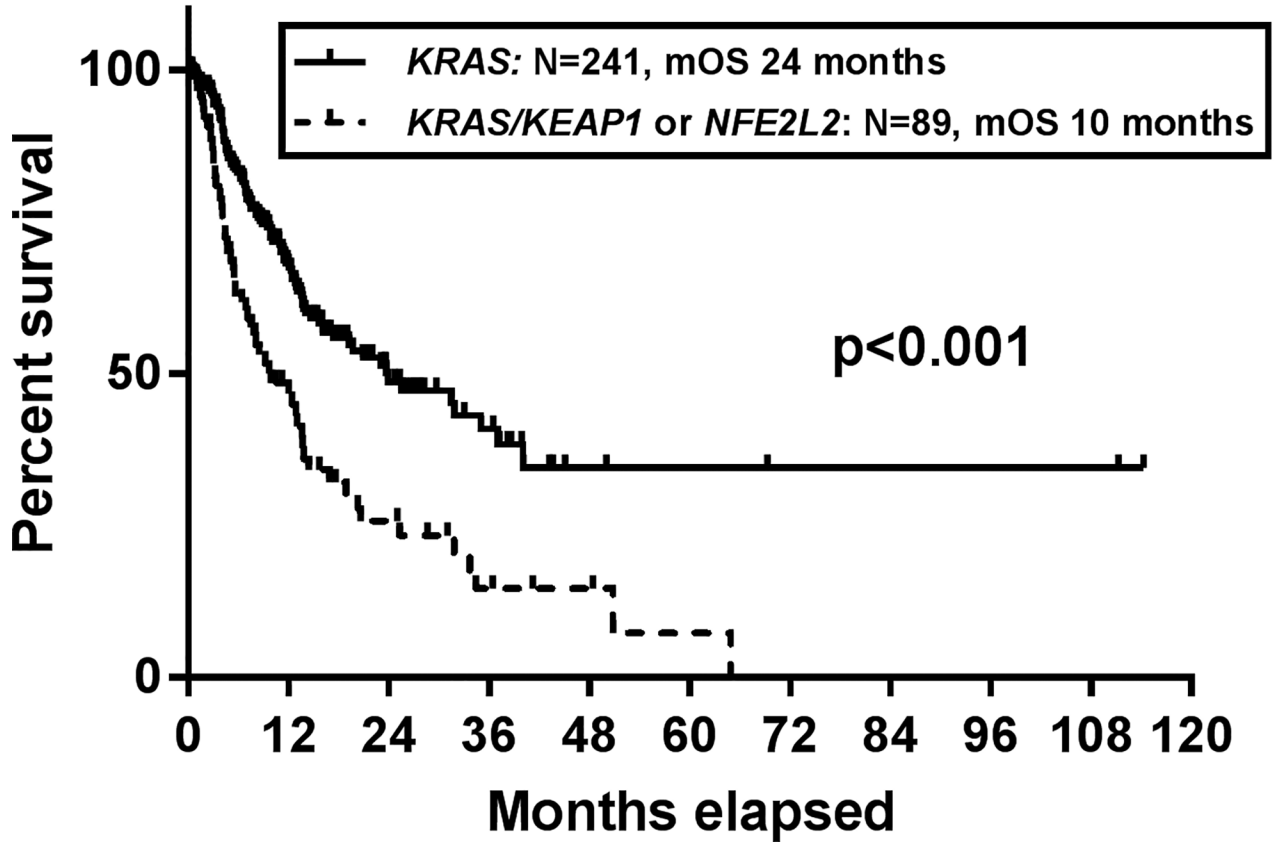
Figure 1.

KRAS genotype analysis. Figure 1A: Type of *KRAS* Codons: *KRAS* point mutations in dataset. Mutations occurring in less than 1% of patients grouped into “other” category. Figure 1B: Distribution of three most frequently co-occurring mutations as depicted in a proportional Venn diagram.

A



B



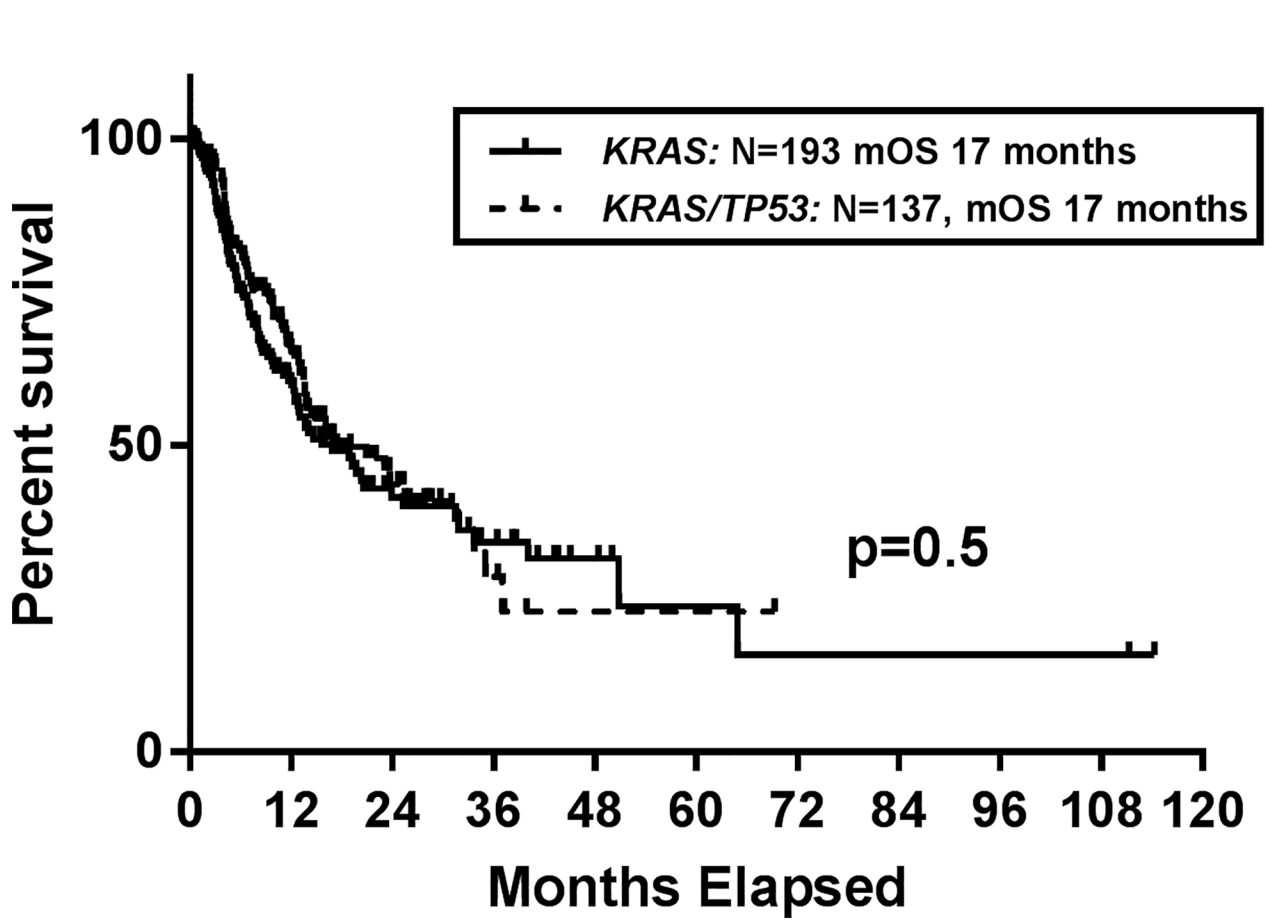
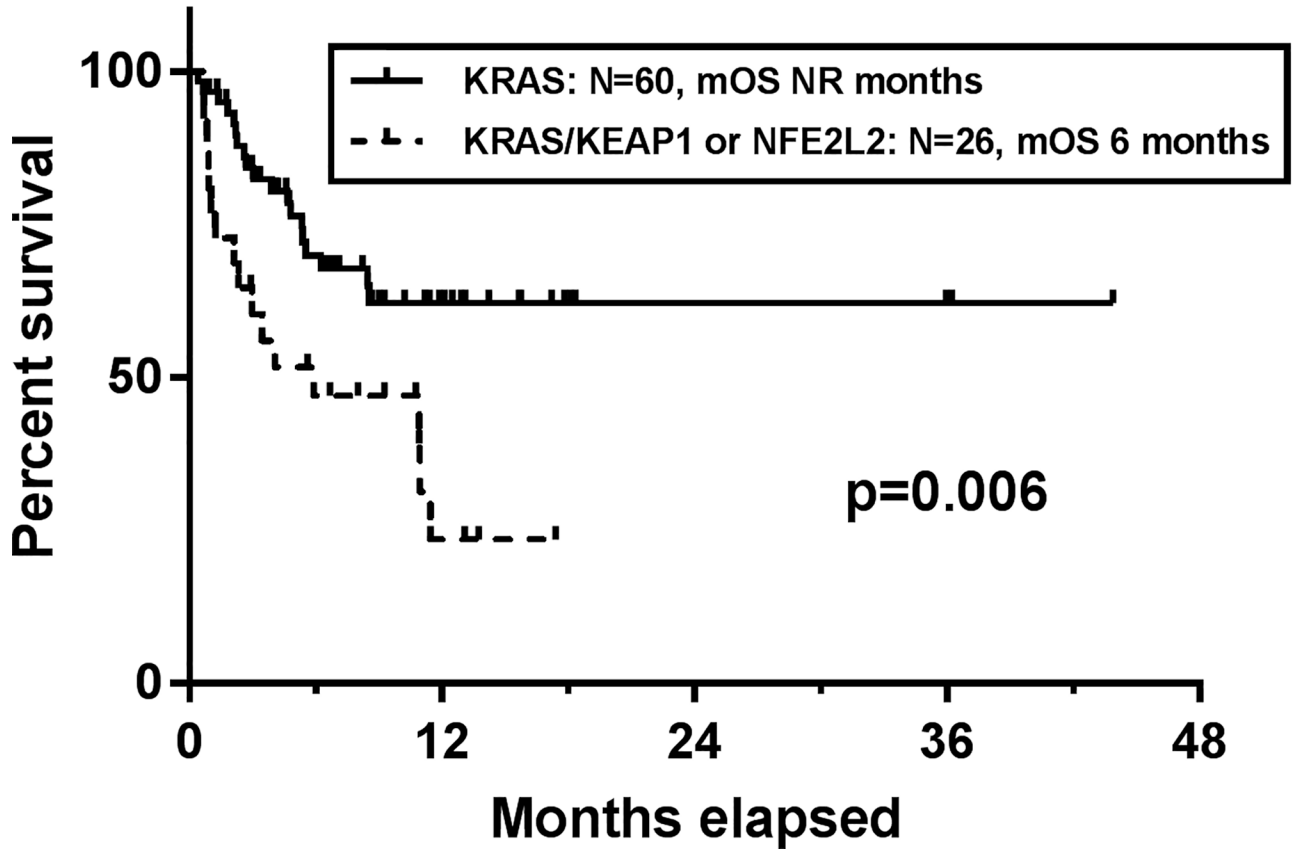


Figure 2.

Associations of co-occurring genomic alterations and *KRAS* with overall survival from time Stage IV diagnosis ***A***, *STK11* ***B***, *KEAP1* or *NFE2L2*, **C**, TP53

A



B

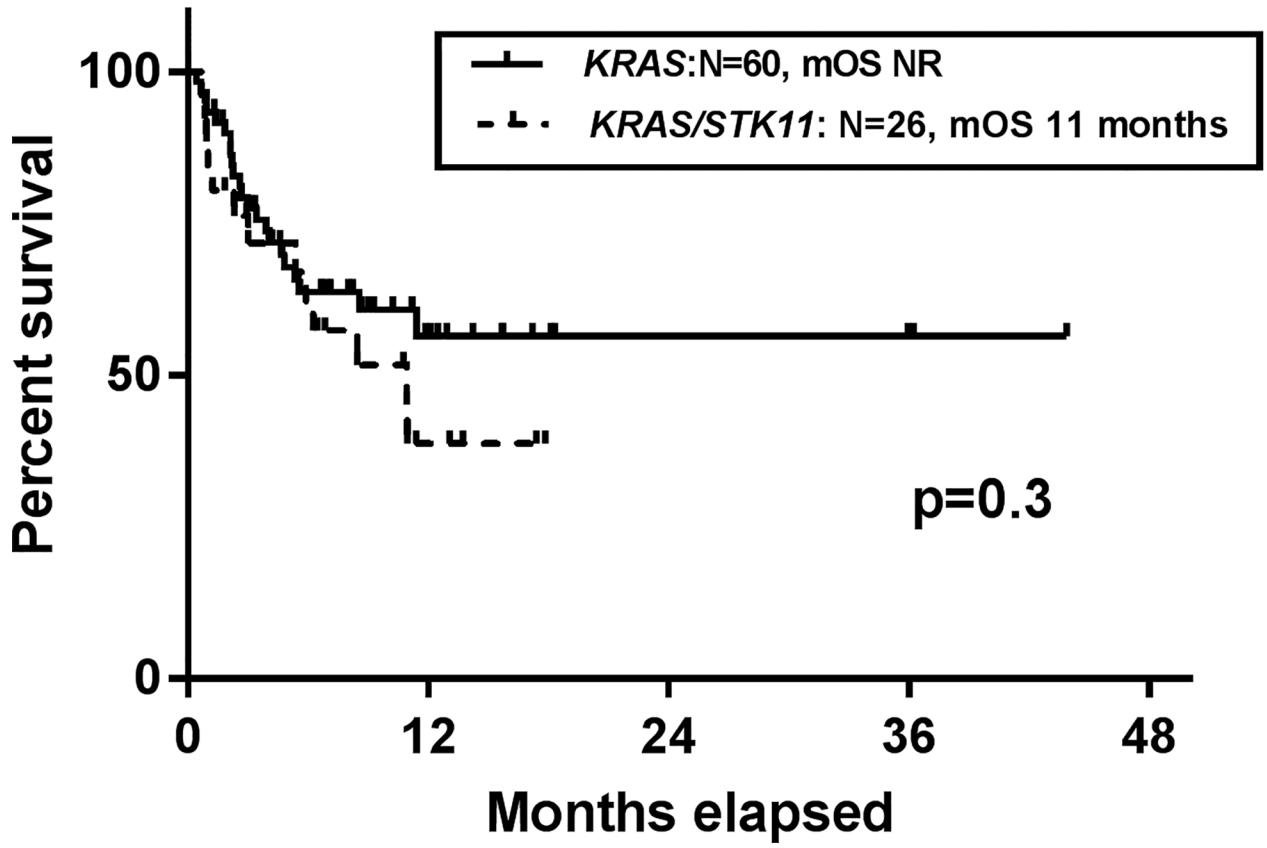


Figure 3.

Overall survival (from time of start of immune checkpoint inhibitor therapy) for treatment of Stage IV disease based on presence of **A**, *KEAP1/NFE2L2* co-mutation and **B,** *STK11* co-mutation

Table 1

Baseline patient characteristics

Characteristic	N=330 (%)
Age at diagnosis (stage IV)	
Median (range)	61 (45–80)
Sex	
Women	195 (59)
Men	135 (41)
KPS (%)	
≥80	241 (73)
<80	72 (22)
Not recorded	17 (5)
Smoking History Category	
Current	37 (11)
Former	271 (82)
Never	22 (7)
Smoker History, pack-years	
Median Pack-year	30
Range	0–135
Unknown	2
Pathology	
Adenocarcinoma	294 (89)
Squamous	11 (3)
Adenosquamous	4 (1)
Other	21 (6)

Table 2

Most Frequent Co-Occurring Mutations Among patients with *KRAS*-mutant NSCLC

Mutation	Frequency (n)
TP53	42% (138)
STK11	29% (95)
KEAP1/NFE2L2	27% (93)
RBM10	16% (52)
PTPRD	15% (50)
SMARCA4	14% (46)
ATM	13% (42)
FAT1	10% (32)
ARID1A	9% (31)
PTPRT	9% (31)

Table 3

Multivariate Analysis of Overall Survival in patients with *KRAS*-mutant NSCLC

Variable	HR (95% CI of HR)	p-Value
TP53 (+ vs −)	1.11 (0.77 to 1.58)	0.581
STK11 (+ vs −)	1.30 (0.86 to 1.97)	0.216
KEAP1/NFE2L2 (+/−)	1.96 (1.33 to 2.92)	<0.001
Gender (male vs female)	1.20 (0.86 to 1.68)	0.284
Age at diagnosis	1.03 (1.01 to 1.05)	0.003
Smoking (former/current vs never)	1.43 (0.86 to 1.68)	0.402
KPS at diagnosis (KPS 80–100 vs <80)	0.88 (0.60 to 1.29)	0.514