RESEARCH ARTICLE

# Prospective Comprehensive Molecular Characterization of Lung Adenocarcinomas for Efficient Patient Matching to Approved and Emerging Therapies

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**ABSTRACT** Tumor genetic testing is standard of care for patients with advanced lung adenocarcinoma, but the fraction of patients who derive clinical benefit remains undefined. Here, we report the experience of 860 patients with metastatic lung adenocarcinoma analyzed prospectively for mutations in >300 cancer-associated genes. Potentially actionable genetic events were stratified into one of four levels based upon published clinical or laboratory evidence that the mutation in question confers increased sensitivity to standard or investigational therapies. Overall, 37.1% (319/860) of patients received a matched therapy guided by their tumor molecular profile. Excluding alterations associated with standard-of-care therapy, 14.4% (69/478) received matched therapy, with a clinical benefit of 52%. Use of matched therapy was strongly influenced by the level of preexistent clinical evidence that the mutation identified predicts for drug response. Analysis of genes mutated significantly more often in tumors without known actionable mutations nominated *STK11* and *KEAP1* as possible targetable mitogenic drivers.

**SIGNIFICANCE:** An increasing number of therapies that target molecular alterations required for tumor maintenance and progression have demonstrated clinical activity in patients with lung adenocarcinoma. The data reported here suggest that broader, early testing for molecular alterations that have not yet been recognized as standard-of-care predictive biomarkers of drug response could accelerate the development of targeted agents for rare mutational events and could result in improved clinical outcomes. *Cancer Discov; 7(6); 596–609. ©2017 AACR.*

*See related commentary by Liu et al., p. 555.*

## INTRODUCTION

Tumor genetic testing is standard of care for patients with non–small cell lung cancer (NSCLC). Lung adenocarcinomas, which account for approximately 50% of lung cancers, are molecularly subclassified and their therapy dictated by the presence of distinct molecular alterations, including *EGFR* mutations and *ALK* or *ROS1* fusions that confer sensitivity to selective kinase inhibitors (1–4). Additional alterations such as $BRAF^{V600E}$, *RET* fusions, and *ERBB2* amplifications are found in smaller subsets of patients, but when present may also predict response to targeted inhibitors that are FDA-approved therapies for other tumor types (5–11). In other patients, defined oncogenic drivers such as *KRAS* and *PIK3CA* mutations are detected, for which preclinical studies have nominated targeted approaches, but the clinical utility of such therapies has yet to be established (12, 13). As a result of advances in DNA sequencing, the prospective molecular analysis of tumors for mutations in hundreds of cancer-associated genes is now feasible using multiplexed assays that use as input small quantities of formalin-fixed paraffin-embedded (FFPE) tissue. With the goal of optimizing treatment selection in patients with advanced cancer and to address the limitations in sensitivity and breadth of previous prospective clinical testing approaches, we developed the Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay, a hybridization capture-based, next-generation sequencing platform (14) for matched tumor: normal sequencing to comprehensively profile somatic alterations in all known cancer genes in solid tumors.

Here, we report our experience with the first 860 patients with recurrent or metastatic lung adenocarcinoma analyzed by MSK-IMPACT, with a focus on defining the fraction of patients for whom such testing influenced treatment selection. Potentially actionable genetic events were stratified into one of four categories based on the level of evidence supporting the utility of the mutation as a predictive biomarker of drug response. Outcome data were collected to determine whether patients were treated with, and benefited from, a therapy chosen on the basis of a specific molecular event present in their tumor and to determine whether the likelihood of receiving a matched therapy correlated with the level of preexistent evidence that the particular genomic event correlated with drug response.

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## RESULTS

### Patient Demographics

A total of 915 tumors from 860 patients with recurrent or metastatic lung adenocarcinoma were profiled using MSK-IMPACT during the study period (Table 1). A new biopsy was not required for participation in this study, and the median duration between collection of the tumor sample and its use for genomic analysis was 28 days (range, 0–3,274 days, the longer intervals reflecting the testing of older samples resected up to several years previously due to a lack of availability of a more recent biopsy in a minority of patients). In 765 (89%) of the patients, tumor tissue ≤1 year old was utilized, with tissue ≤30 days old analyzed in 473 (55%). The mean time from receipt of the tumor sample and matched blood sample in the clinical laboratory to the reporting of MSK-IMPACT results was 17 days. Patients had received a median of one (range, 0–7) systemic treatment prior to MSK-IMPACT testing. At the time of analysis, the median follow-up time from diagnosis of metastatic disease was 13 months (1–196 months), and 239 (27.8%) patients had died.

### Known Mitogenic Drivers Identified by MSK-IMPACT

Potentially actionable somatic alterations, as defined by the OncoKB classification (15), were identified in 747 patients (86.9%; Fig. 1A; Supplementary Table S1). We next compared the frequency of potential actionable alterations in this prospective cohort to that observed in the retrospective Cancer Genome Atlas (TCGA) analysis of untreated lung adenocarcinomas (ref. 16; Fig. 1B). By definition, all patients in the MSK-IMPACT cohort had recurrent or metastatic disease as compared with 3.9% (9/230) with stage IV disease in the TCGA dataset. Moreover, 37.7% (345/915) of tumors used for MSK-IMPACT analysis were collected following treatment with at least one prior systemic therapy, whereas samples from treatment-naïve patients were included only in the TCGA cohort. The MSK-IMPACT dataset therefore represents a clinically aggressive cohort distinct from the TCGA set, which examined primary resection, treatment-naïve tumors only. Notably, the MSK-IMPACT cohort included a higher fraction of patients with activating *EGFR* alterations (27% vs. 11%; $P < 0.001$), but other molecular subsets showed no significant differences. In addition, 5.5% (47/860) had an $EGFR^{T790M}$ mutation, all detected post EGFR-TKI therapy, as compared with 0.4% (1/230) in the TCGA dataset ($P < 0.001$). Factors contributing to the higher frequency of *EGFR*-mutant patients in this cohort may include a referral bias attributable to the availability of $EGFR^{T790M}$-focused clinical trials at our institution during the study period, or differences in patients' demographics, as 32.2% of the MSK-IMPACT patients were never-smokers and 8.4% (72/860 patients) were Asian.

**Table 1. Clinical characteristics of the 860 patients (915 samples) with metastatic or recurrent lung adenocarcinoma profiled by MSK-IMPACT**

| Characteristics | *N* = 860 (%) | Samples (*n* = 915) |
|---|---|---|
| Age | | |
| 18–50 | 122 (14.2) | 134 |
| 51–75 | 615 (71.5) | 652 |
| >75 | 123 (14.3) | 129 |
| Sex | | |
| Men | 354 (41.2) | 369 |
| Women | 506 (58.8) | 546 |
| Smoking status | | |
| Never | 277 (32.2) | 302 |
| Former light (≤15 pack year) | 153 (17.8) | 167 |
| Former heavy (>15 pack year) | 420 (48.8) | 436 |
| Current heavy | 10 (1.2) | 10 |
| Sites of tissue for MSK-IMPACT | | |
| Lung | | 420 |
| Lymph node | | 169 |
| Pleura/pleural fluid | | 110 |
| Other | | 81[a] |
| Liver | | 59 |
| Brain | | 47 |
| Bone | | 29 |

[a]Other: Soft tissue, *n* = 36 (chest wall mass, *n* = 11; epidural tumor, *n* = 7; gluteal mass, *n* = 1; groin, *n* = 1; iliac mass, *n* = 1; ischiorectal mass, *n* = 1; L2 soft-tissue mass, *n* = 1; L5 soft-tissue mass, *n* = 1; pararenal mass, *n* = 1; paraspinal mass, *n* = 3; paratracheal mass, *n* = 1; soft-tissue mass scapula, *n* = 2; pelvic mass, *n* = 2; T1 soft-tissue mass, *n* = 1; T12 soft-tissue mass, *n* = 1; retroperitoneum, *n* = 1), adrenal, *n* = 16; skin/subcutaneous nodule, *n* = 12; pericardium/pericardial fluid, *n* = 6; ascites/omentum, *n* = 4; pancreas, *n* = 2; breast, *n* = 1; colon, *n* = 1; spleen, *n* = 1; uterus, *n* = 1; diaphragm, *n* = 1.

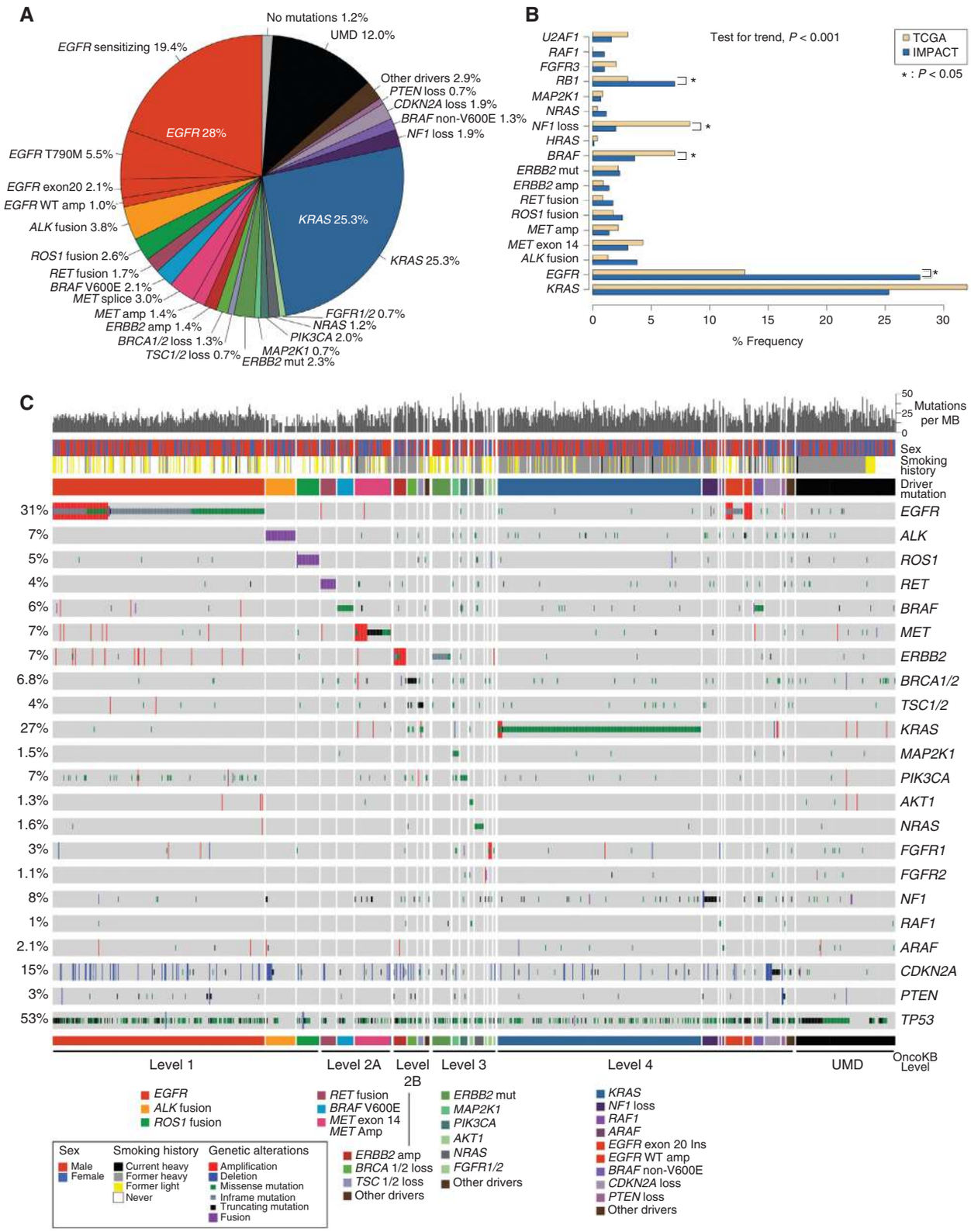



**Figure 1.** Potentially actionable oncogenic drivers identified by MSK-IMPACT testing. **A,** Spectrum of oncogenic drivers assigned to 860 patients with lung adenocarcinoma identified by MSK-IMPACT. **B,** Comparison of selected gene alteration frequencies in the MSK-IMPACT and TCGA cohorts. **C,** Oncoprint of select gene alterations identified by MSK-IMPACT in patients with level 1 to 4 alterations or those with no actionable mutation (UMD).

Conversely, some oncogenic drivers were present at higher rates in the TCGA cohort than in the MSK-IMPACT cohort, including truncating mutations/deletions in *NF1* (8.3% vs. 2%; $P < 0.001$) and *BRAF* mutations (7% vs. 3.6%; $P = 0.042$).

Consistent with prior studies, mutations in *EGFR* and *KRAS* were the most commonly identified oncogenic drivers and were with very rare exception mutually exclusive ($P < 0.0001$; Fig. 1C; refs. 17, 18). Oncogenic fusions in *ALK, ROS1,* and *RET, MET* exon 14 alterations, and $BRAF^{V600E}$ mutations, all of which predict significant clinical benefit to targeted inhibitors of these kinases, were identified in 1.7% to 3.8% of patients. Less common actionable drivers were identified in 110 (12.8%) patients (Fig. 1A) and included RAS/MAPK pathway lesions such as truncating mutations/deletion of *NF1* (16 patients) and known activating mutations in *NRAS* (10 patients; 9 Q61, 1 G13), *HRAS* (1 patient), and *MAP2K1* (also known as *MEK1*; E203K, $n = 1$; K57N, $n = 2$; Q56P, $n = 1$; G128V, $n = 1$; and E102_I103 deletion, $n = 1$; refs. 19, 20). Two patients had hotspot *ARAF* mutations at codon 214 (S214Y, S214P) that have been shown to confer sensitivity to sorafenib (21), and two tumors harbored $RAF1^{S257L}$ mutations, a previously characterized hotspot (21).

Consistent with prior data, most oncogenic or likely oncogenic PI3K alterations were identified in tumors with a co-occurring higher-level alteration (22, 23). In total, 32 of 860 (3.7%) tumors had activating mutations in the PI3K/AKT/mTOR pathway as their highest-level actionable alteration, including 17 with *PIK3CA* mutations, 6 with inactivating *PTEN* alterations, 3 patients each with truncating *TSC1/TSC2* mutations, 2 $AKT1^{E17K}$ mutations, and 1 $MTOR^{S2215Y}$ mutation. Nine (53%) of the 17 patients with oncogenic *PIK3CA* mutations as their highest level alteration had a co-occurring *KRAS* mutation. Likely inactivating somatic *BRCA1* ($n = 3$, 0.3%) or *BRCA2* truncating mutations ($n = 8$, 0.9%) were deemed level 2B alterations based upon the FDA approval of the PARP inhibitor olaparib in *BRCA*-mutant ovarian carcinomas. Additionally, one patient had a *CD74–NRG* fusion (24) and a second patient a *FGFR3–TACC3* fusion (25) as their highest actionable alteration. An activating exon 9 $KIT^{E490Q}$ mutation, previously described in thymic carcinoma, was present in one patient (26), and one patient had an $ERBB3^{D297H}$ hotspot mutation (27). Although these rare alterations were detected in only a small number of individuals, they highlight the ability of multiplexed sequencing assays to detect rare but potentially actionable drug targets that in the aggregate represent key oncogenic drivers in a small but nontrivial 0.5% of lung cancers.

In total, 239 patients had tumors with co-occurring targetable mutations, with 46 patients having at least three concurrent level 1 to 4 alterations (Supplementary Table S2). Fifty-two patients had co-occurring level 1 to 3 mutations, of which 25 were patients with co-occurring *EGFR* and *PIK3CA* mutations (Supplementary Fig. S1). Notably, only two *EGFR*-mutated patients (out of 214) had a concurrent *KRAS* mutation (level 4). One patient with an $EGFR^{L858R}$ mutation had co-occurring $KRAS^{Q61H}$ and $KRAS^{Q22K}$ mutations. This specimen was collected prior to EGFR-TKI therapy for which the patient received erlotinib for 6 months until progression. The variant allele frequency of $EGFR^{L858R}$ was higher (0.59) as compared with $KRAS^{Q61}$ (0.04) and $KRAS^{Q22}$ (0.03), suggesting subclonal *KRAS*-mutant populations (Supplementary Fig. S2). The second patient had an *EGFR* exon 19 deletion with a $KRAS^{Q61R}$ mutation. This tumor was sequenced following disease progression after prior therapy with erlotinib for 26 months. The allele frequency of the *EGFR* mutation was 0.12 with the coexisting $KRAS^{Q61R}$ mutation (0.35; Supplementary Fig. S3). *KRAS* mutations at codon 61 are rare in comparison with those located at codon 12 in NSCLC and were seen in 12 of 235 (5%) *KRAS*-mutated tumors in the dataset. Eight patients had a $KRAS^{G12}$ mutation concurrent with a level 2B (*BRCA1/2* or *TSC1/2* loss) alteration. Therefore, multiple potentially targetable lesions with different levels of support sometimes coexisted within tumors, highlighting the challenge of defining therapeutic actionability in the setting of more comprehensive tumor profiling. Despite the frequent identification of two or more targetable driver mutations in individual tumors, no patient in this prospective series was treated on a clinical trial that simultaneously targeted two actionable alterations.

## Use of Matched Therapy

Overall, 37.1% (319/860) of patients received a matched therapy guided by their tumor molecular profile, with the likelihood of receiving a matched therapy correlating strongly with the level of evidence that the mutation identified predicts for drug response ($P < 0.0001$; Fig. 2A). Specifically, the majority of patients with level 1 (92%) and level 2A (52%) alterations received matched therapy, whereas only a minority of patients with level 2B (17%), level 3 (25%), or level 4 (2%) alterations received matched therapy (Fig. 2A). In total, 95.3%, 90.9%, and 59.1% of patients with sensitizing *EGFR* mutations, *ALK* fusions, and *ROS1* fusions received matched therapy, with clinical benefit documented in 84.8%, 93.3%, and 84.6% of patients, respectively (Fig. 2B). For patients with level 2A alterations, matched therapy was used for patients with *MET* exon 14 alterations (65.4%), $BRAF^{V600E}$ mutations (55.6%), *RET* fusions (53.3%), and amplification of wild-type *MET* (16.7%). Despite the lower use of matched therapy in patients with level 2A mutations, clinical benefit with the matched targeted agent was substantial in such patients, with 76.5%, 72.7%, 75%, and 50% of patients with *MET* exon 14 alterations, *RET* fusions, $BRAF^{V600E}$ mutations, and amplification of wild-type *MET* deriving clinical benefit, respectively. Ongoing active treatment with chemotherapy or immunotherapy was the most common reason that matched therapy was not used in patients with a *RET* ($n = 7$) or *ROS1* fusion ($n = 6$; Supplementary Fig. S4). There were two patients with *ROS1* fusions (9%) who experienced rapid deterioration and thus did not receive treatment with a *ROS1* kinase inhibitor. Notably, both died after crizotinib had been shown to be active in patients with *ROS1* fusions but prior to its FDA approval for this indication in March 2016. One of these patients had local molecular testing for *EGFR* only and had received three lines of systemic therapy prior to referral, at which time the *ROS1* fusion was detected by MSK-IMPACT. Unfortunately, the patient died of disease 12 days after referral, before *ROS1*-directed therapy could be initiated. No patients with a presumed inactivating alteration in *TSC1/2* or *BRCA1/2* (level 2B)

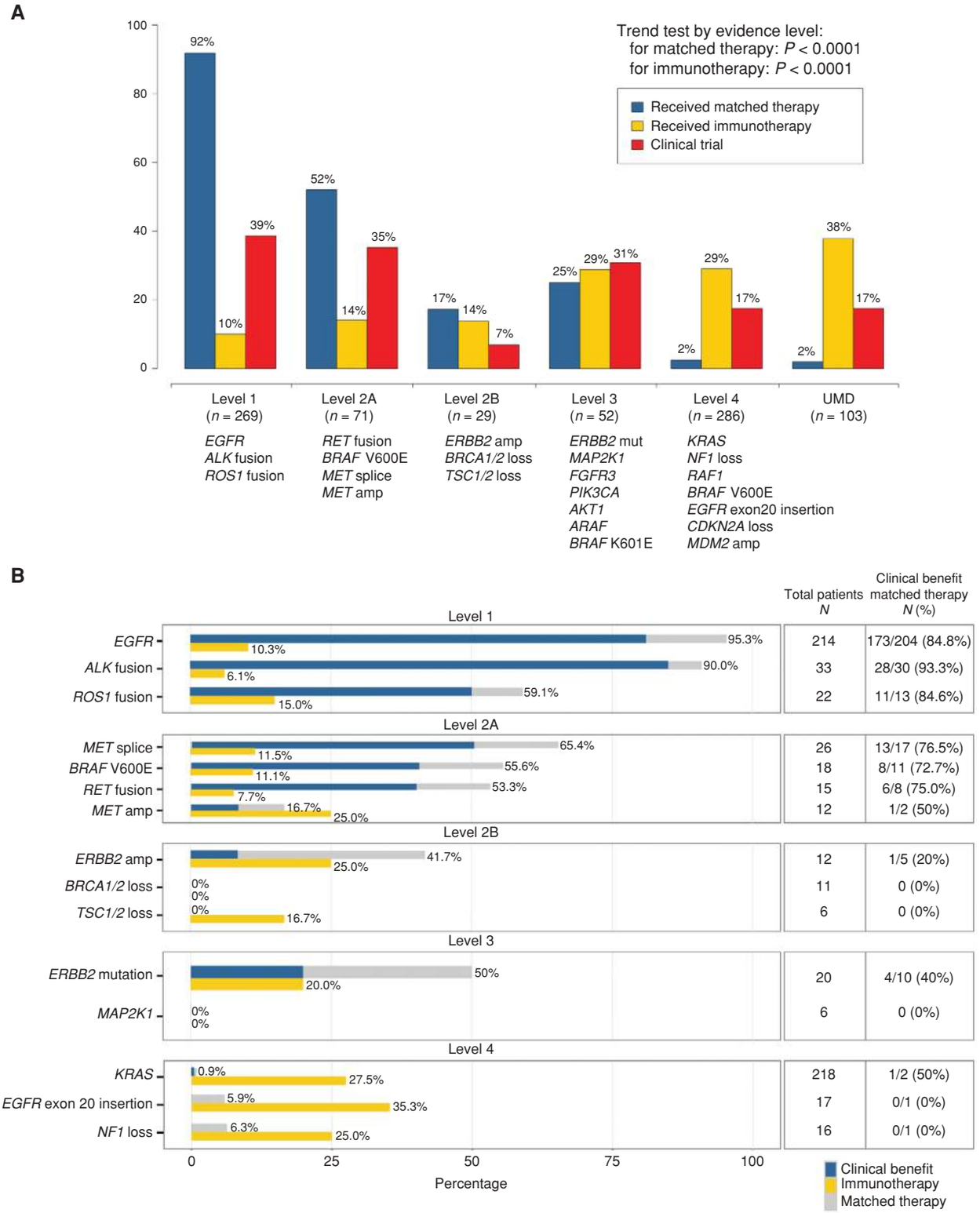



**Figure 2.** A: Use of matched therapy correlates strongly with the level of evidence that the mutation identified predicts for drug response. **A,** Use of matched therapy, immunotherapy, and clinical trial participation in patients with level 1 to 4 alterations or in the UMD cohorts. **B,** Use of matched therapy and immunotherapy and clinical benefit from matched therapy in patients whose tumors harbored alterations in select level 1 to 4 genes.

received matched therapy. Finally, 5 of 12 (42%) patients with *ERBB2*-amplified tumors received matched therapy, of which one demonstrated evidence of clinical benefit (Fig. 2B).

For patients with level 3 and level 4 alterations, matched therapies were typically offered only within the context of a clinical trial. Most notably, the use of matched therapy was also exceptionally rare in patients with *KRAS*-mutant tumors (2/218; 0.9%). Fifty percent of patients with an *ERBB2* mutation received a matched therapy, 40% of whom experienced clinical benefit. Four of 13 patients with a non-V600E *BRAF* alteration (K601E, D594G, T599 duplication, and *SND1–BRAF* fusion) received matched therapy, with none deriving clinical benefit. Three of these patients received single-agent *MEK* or *ERK* inhibitor therapy, whereas the patient with a *BRAF–SND1* fusion received a combination of *BRAF* and *MEK* inhibitors. Two patients with *AKT1*$^{E17K}$ mutations received matched therapy, with one achieving clinical benefit lasting 12 months. No patients with mutations in *MEK1*, *RAF1, ARAF, FGFR3*, or deletions in *CDKN2A* as their highest actionable alterations received matched therapy. Although many of these later patients are still benefiting from chemotherapy or immunotherapy and may receive matched therapy in the future, the lack of clinical trials of targeted agents for these targets and the difficulty of obtaining such drugs for off-indication has been a major impediment to the use of matched therapy in these smaller molecularly defined subsets (Supplementary Fig. S5A–S5E).

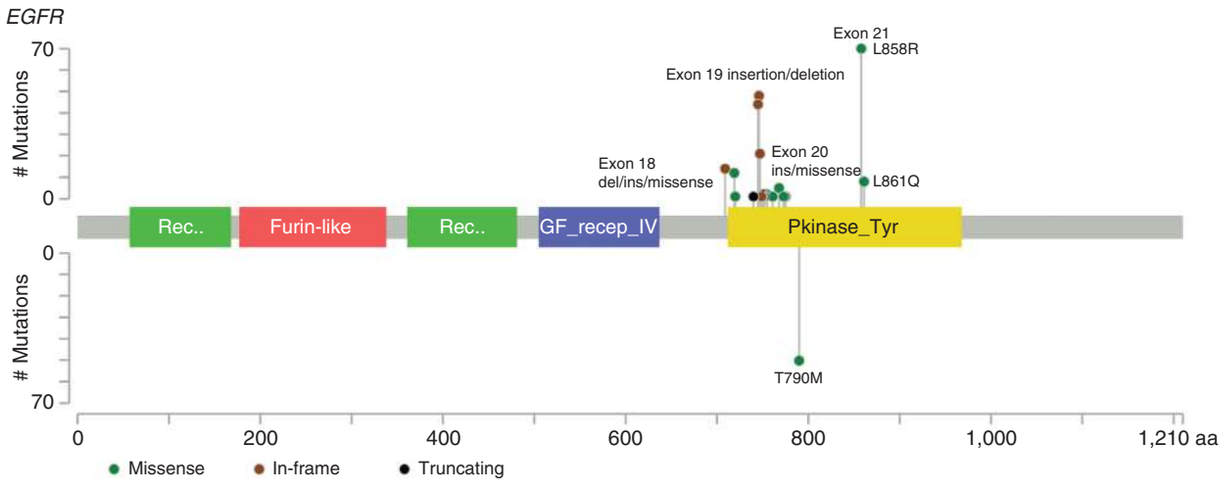
Although patients with level 3 and level 4 alterations were only rarely treated on a genotype-matched therapy clinical trial (7%; 26/373), 19% (70/373) did enroll on a therapeutic clinical study (24.6% and 16.6% for level 3 and level 4 patients, respectively). In most instances (62.9%; 44/70), these patients enrolled on trials of immunotherapy (Supplementary Fig. S6), with an increasingly larger fraction of patients receiving immunotherapy as a standard treatment toward the end of the study period. The data suggest that the low rate of matched therapy use in patients with level 3 and level 4 alterations was not due to a reluctance of such patients to enroll on therapeutic clinical studies, but rather attributable to the lack of compelling matched therapy options available for these patients. Notably, there was an inverse trend toward a higher mutational load in patients with level 3 and level 4 alterations as compared with those with level 1 and level 2 mutations (Supplementary Fig. S7). This was likely attributable to the lower rate of patients who were never-smokers in patients with a level 3 (29.2%; 19/65) and level 4 alteration (12.7%; 39/308) as compared with level 1 (62%; 166/269) and level 2A (45.1%; 32/71) alterations. As higher mutational load in lung cancer has been associated with a greater likelihood of response to immunotherapy (28), and as matched therapy options were limited in patients with level 3 and level 4 alterations, the choice to pursue immunotherapy over a matched therapy in such patients could be considered a rational course of action guided by the clinical sequencing results.

## Clinical Benefit with EGFR Inhibitors in Patients with Uncommon *EGFR*-Mutant Alleles

Not all mutations in a cancer gene have similar oncogenic potential or similarly predict for response to a targeted inhibitor. For most cancer genes, clinical response data are often available only for the most commonly mutated alleles and clinical guidelines for the treatment of patients with rare alleles; even in common cancer genes, data are often based on preclinical drug sensitivities or small case series. Oncogenic *EGFR* alterations known to be predictive of *EGFR* inhibitor sensitivity were identified in 214 (24.9%) patients (Fig. 3; Supplementary Table S3). Consistent with prior studies, exon 21 L858R (70 patients) and exon 19 deletions/insertions (113 patients) were the most common variants (85.5% of cases). Less common variants previously shown to activate *EGFR* kinase activity included L861Q (7), E709_ T710delinsD (6 patients), G719A (4 patients), and exon 18–25 kinase domain duplication (*EGFR*-KDD; 2 patients). Seventeen patients had *EGFR* exon 20 insertions previously shown to confer resistance to EGFR inhibitors such as erlotinib, and one patient had an exon 20 H773R mutation (29, 30). Notably, one patient with an *EGFR* exon 20 insertion (level 4) received erlotinib without response, consistent with prior evidence that such mutations are resistant to this agent (29). Excluding *EGFR* mutations known to confer resistance to erlotinib, nine patients had two or more activating mutations in *EGFR*, and all of these patients had either E709A/K or G719X mutations, alleles previously associated with somatic *EGFR* doublets (31). Overall, 87.3% of patients with sensitizing *EGFR* mutations benefited from the use of an EGFR inhibitor, but we did observe differences in response as a function of the specific *EGFR*-mutant allele present. The rate of clinical benefit was statistically significantly lower in patients with L861Q mutations (43%; $P = 0.039$ vs. L858R; $P = 0.01$ vs. exon 19 deletions) or exon 18 deletions (40%; $P = 0.02$ vs. L858R; $P = 0.005$ vs. exon 19 deletions). These results are consistent with the lower rate of clinical response of tumors harboring L861Q mutations to first-generation TKI therapy reported in prior studies (32, 33) and support the clinical evaluation of afatanib and osimertinib in such patients, as these agents have demonstrated greater potency against this allele (34). Notably, the patient with an L861Q mutation who had the longest duration of clinical benefit received dacomitinib, a second-generation TKI. Consistent with published data (35), both patients with *EGFR* kinase domain duplication (*EGFR*-KDD) treated with erlotinib and afatanib, respectively, derived clinical benefit.

## Unknown Mitogenic Driver Set

In total, 103 patients had tumors for which no level 1 to 4 alteration was identified, and these tumors were therefore designated as unknown mitogenic driver (UMD). To ensure that UMD samples were not enriched for low-purity samples, we generated estimates of their purity and looked for any sequencing reads supporting hotspot mutations. This analysis confirmed that this set of UMD samples was not significantly enriched with low tumor content samples (estimated purity by FACETS analysis of 12%–92%, mean 35% vs. 38%; range, 4%–95% for samples with level 1–4 alterations; Fig. 4A). We then sought to compare the frequency of alterations in these samples to those with a known mitogenic driver, with the goal of nominating additional oncogenic drivers as candidates for future drug development. In comparison with tumors harboring a level 1 to 4 alteration, alterations in *TP53, STK11, KEAP1, KMT2D*, and




| *EGFR* mutations | No. pts | Receive TKI | Clinical benefit TKI | Chemotherapy | Immunotherapy | Clinical trial | Median time on TKI (months) |
|---|---|---|---|---|---|---|---|
| **Exon 21** | | | | | | | |
| L858R | 70 | 66 (96%) | 56^ (89%) | 49 (67%) | 5 (7%) | 23 (33%) | 9 (1–47) |
| L861Q | 7 | 7 (100%) | 3 (43%) | 5 (71%) | 3 (43%) | 2 (29%) | 4.5 (1–33) |
| **Exon 19** | | | | | | | |
| Exon 19 deletion | 112 | 109 (97%) | 100* (94%) | 55 (49%) | 9 (8%) | 46 (41%) | 10 (2–65) |
| Exon 19 insertion | 1 | 1 (100%) | 1 (100%) | 1 (100%) | 1 (100%) | 0 (0%) | 3 (ongoing) |
| L747P | 3 | 3 (100%) | 2 (67%) | 3 (100%) | 1 (33%) | 0 (0%) | 7 (6–15) |
| **Exon 18** | | | | | | | |
| E709_T710delinsD | 6 | 5 (67%) | 2 (40%) | 4 (50%) | 2 (33%) | 2 (33%) | 2 (1.5–22) |
| G719A | 4 | 4 (100%) | 2 (50%) | 4 (100%) | 2 (50%) | 1 (25%) | 5 (2–10) |
| E709K+G719S | 2 | 1 (50%) | 1 (100%) | 2 (100%) | 0 (0%) | 1 (50%) | 12 |
| E709K+G719A | 1 | 1 (100%) | 0 (0%) | 1 (100%) | 0 (0%) | 0 (0%) | 2 (erlotinib)** |
| E709A+G719S | 1 | 1 (100%) | 1 (100%) | 1 (100%) | 0 (0%) | 0 (0%) | 7 (ongoing) |
| **Other** | | | | | | | |
| G719C+S768I | 2 | 2 (100%) | 2 (100%) | 2 (100%) | 1 (50%) | 1 (50%) | 11.5 (3–20) |
| G719S+S768I | 1 | 1 (100%) | 1 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 23 (ongoing) |
| G719A+L861Q | 1 | 1 (100%) | 1 (100%) | 1 (100%) | 0 (0%) | 0 (0%) | 5 (ongoing) |
| G719A+S768I | 1 | 1 (100%) | 1 (100%) | 1 (100%) | 0 (0%) | 1 (100%) | 12 |
| ***EGFR*-KDD** | 2 | 2 (100%) | 2 (100%) | 3 (100%) | 1 (50%) | 1 (50%) | 8 (both pts ongoing) |
| | | | | | | | |
| **Exon 20 insertion** | 17 | 1 (6%) | 0 (0%) | 16 (94%) | 6 (38%) | 4 (25%) | 1.5 |
| **Exon 20 H773R** | 1 | 1 (100%) | 1 (100%) | 1 (100%) | 0 (0%) | 1 (100%) | 6 |

^L858R: 3 patients await imaging
*Exon 19 deletion: 3 patients await imaging
**E709K+G719A: currently receiving afatanib (2 months ongoing)

**Figure 3.** Use of matched therapy and clinical benefit in patients (pts) with known activating mutations in *EGFR*. Top, Frequency of known activating and resistance mutations in *EGFR* identified by MSK-IMPACT. Bottom, Use of matched therapy, chemotherapy, immunotherapy, clinical trial enrollment, and clinical benefit from matched therapy as a function of the specific *EGFR* mutation identified in the patient's tumor.

*PDGFRA* were all significantly more common ($P < 0.05$) in the UMD cohort (Fig. 4B). These findings corroborate the enrichment of *TP53* and *KEAP1* in mitogenic driver–negative samples in the TCGA cohort (16). In contrast to the TCGA set that identified enrichment of *RIT1* mutations in the oncogene-negative set, no *RIT1* mutations were identified within the UMD set. Further analysis identified a number of genes that were statistically enriched in the UMD cohort versus samples with level 1 to 4 mitogenic drivers when subdividing these patients according to smoking history (Fig. 4B). For example, chromatin-modifying genes such as *KMT2C, SETD2,* and *CREBBP* and genes involved in homologous recombination (*MRE11A* and *BRCA2*) were more commonly identified ($P < 0.05$) in patients with a never/former light smoking history within the UMD cohort as compared with level 1 to 4 patient samples. Patients with a former/current heavy smoking history in the UMD cohort had higher rates of *STK11* and *TP53* alterations as compared with those who had a level 1 to 4 driver. Co-occurring mutations in *TP53* and *STK11* are known to be synergistic in tumorigenesis (36), but represented only 27% (18/68) of patients with *TP53* or *STK11* mutations in the former/current heavy

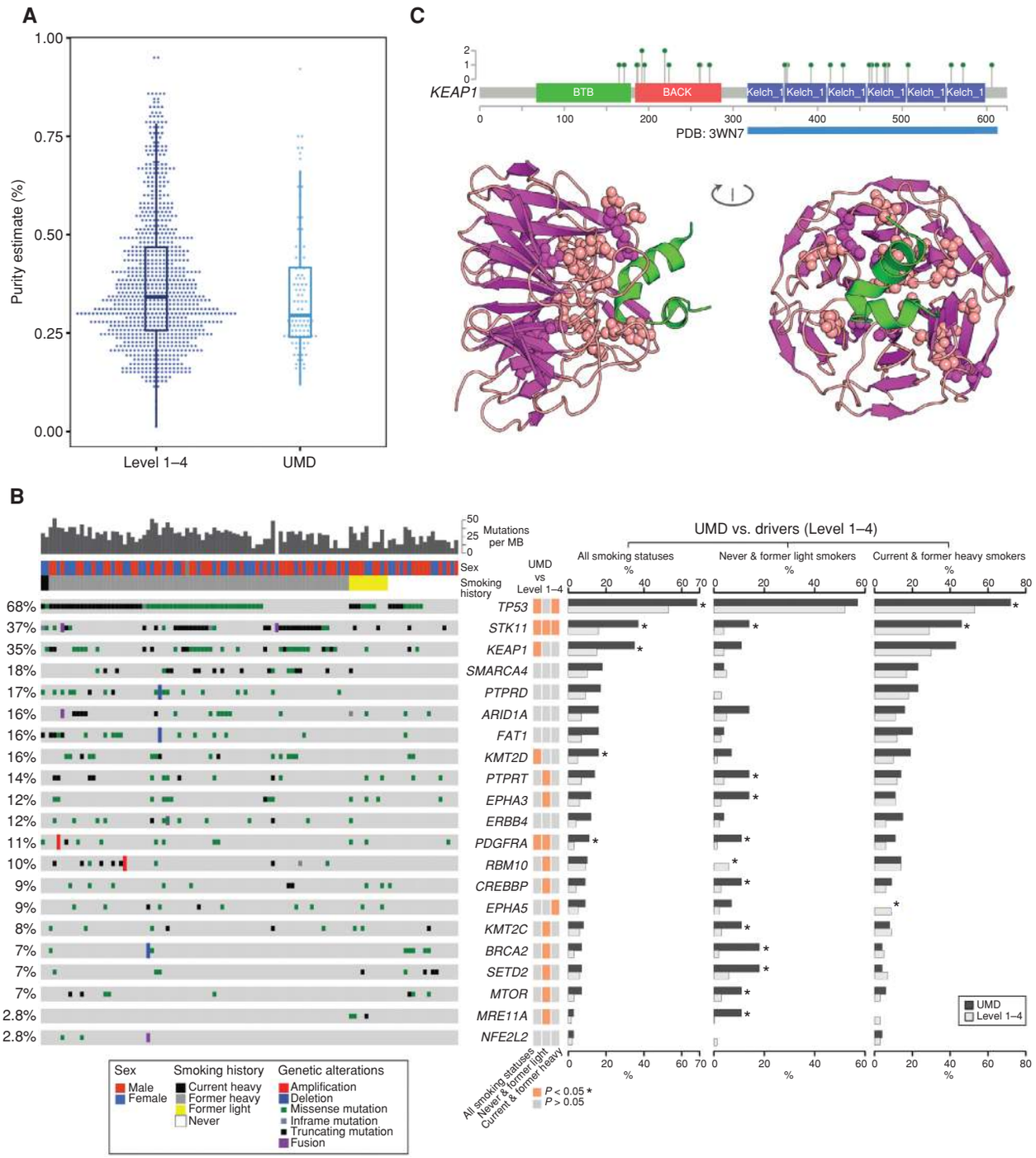



**Figure 4.** Potential driver alterations in the UMD cohort. **A,** Estimated purity analysis by FACETS in samples with level 1 to 4 alterations to the UMD sample set. **B,** Oncoprint of the most common gene alterations in 103 patients with no actionable level 1 to 4 driver mutations with a comparative frequency of select recurrently altered genes in the UMD cohort level 1 to 4 samples according to smoking history. *P* values were calculated using the Fisher exact test **C.** Distribution of missense mutations in KEAP1 detected by MSK-IMPACT (top): x-axis represents amino acid potions and y-axis represents the number of samples mutated. The PFAM domains were also displayed as context. Protein structure analysis revealing that KEAP1 missense mutations identified in patients with lung adenocarcinoma (the ones with side chains displayed) clustered the interaction interface with Nrf2 (nuclear factor erythroid 2-related factor 2). Nrf2 peptide is colored in green.

smoking UMD subset. Although *ERBB4* alterations were identified in the UMD subset, none were hotspot alterations or mutations previously demonstrated to confer sensitivity to HER kinase inhibitors. Furthermore, all *BRCA2* mutations were somatic missense mutations of uncertain significance, and thus further laboratory and clinical studies will be needed to clarify the significance of these findings.

A potentially targeted approach was utilized in two of the UMD patients: off-label azacitidine, a DNA methyltransferase inhibitor, in a patient with a *KDM5C* frameshift mutation (6 months; stable disease); and everolimus for an $MTOR^{L2383F}$ mutation (1 month; no benefit). In the latter case, the *MTOR* mutation was a novel missense variant of unknown significance. One patient in the UMD cohort without an *EGFR* mutation or amplification had clinical benefit from erlotinib treatment. A tumor biopsy after disease progression revealed an uncharacterized $ERRFI1^{A143D}$ missense mutation as the sole nonsynonymous mutation. *ERRFI1* loss of function causes hyperactivation of EGFR and persistent MAPK signaling, with tumors in mice responsive to gefitinib (37), and it has been shown to accelerate initiation and progression of *EGFR*-mutated lung adenocarcinoma in mice (38). *ERRFI1* mutations were identified in six (0.7%) patients, four of whom had higher-level alterations [*EGFR* exon 19 deletion (2 patients); $KRAS^{G12}$ (2 patients)].

As real-time functional validation of all nonrecurrent somatic missense mutations of unknown significance is not currently feasible, we used *in silico* modeling to identify other potentially functional missense variants in the UMD cohort. *KEAP1*, a negative regulator of *NRF2*, was altered in 34% (35/103) of the UMD cases (10 truncations and 25 missense variants) with *NRF2* mutations noted in 2.9% (3/103). Using 3-D structure-based computational analysis of the KEAP1 protein structure, we found that many of these *KEAP1* mutations cluster in the Kelch domain, which interacts with NRF2 (Fig. 4C). *KEAP1* mutations can induce increased NRF2 accumulation, resulting in chemoresistance through induced expression of cellular antioxidants and xenobiotic detoxification enzymes (39). Targeting NRF2 with inhibitors such as luteolin and brusatol may enhance chemotherapy sensitization (40, 41), but trials testing this hypothesis have yet to be conducted in NSCLC.

## DISCUSSION

Rapid advances in sequencing methodology have made it feasible to prospectively profile increasing numbers of cancer-associated genes using the small quantities of FFPE-derived DNA that are typically available as part of the routine clinical care of patients with advanced cancer. However, the fraction of patients who derive clinical benefit from molecular characterization remains undefined. Here, we report the prospective clinical experience with MSK-IMPACT testing in the first 860 patients with lung adenocarcinoma with a focus on defining the fraction of patients who received a matched therapy and derived clinical benefit from such treatment.

Overall, 37.1% of patients with lung adenocarcinoma who had undergone MSK-IMPACT testing received a matched therapy, with the likelihood of matched treatment correlating strongly with the level of evidence that the mutation identified predicts for drug response. For patients with *EGFR* mutations and *ALK* or *ROS1* fusions (level 1 alterations), 93% received the corresponding matched therapy with 85.8% deriving clinical benefit. Alterations in these genes are now recognized by the FDA as predictive biomarkers of drug response. Of the patients for whom a treatment other than the corresponding matched therapy was chosen, 85% (17/20) remain clinically stable on chemotherapy or immunotherapy. With longer follow-up, we anticipate that most if not all of these patients will ultimately receive matched targeted therapy.

The described patients with *ROS1* fusions identified too late in their clinical course to receive matched therapy illustrate the potential importance of early broad molecular testing that includes genes beyond those recognized as biomarkers by the FDA, in particular those mutations categorized as level 2A, defined here as standard-of-care biomarkers for FDA-approved drugs in patients with lung cancer based on currently accepted practice guidelines such as those issued by the National Comprehensive Cancer Network (NCCN). We found that the proportion of patients with level 2A alterations who received a matched therapy was significantly lower ($P < 0.0001$) than patients with level 1 alterations (93% vs. 52%), but the rate of clinical benefit (76%) was compelling in those who were treated with appropriate matched therapy. This lower use of matched therapy in patients with level 2A alterations was likely attributable to limited access to the corresponding matched therapies, in particular the lack of access to several of the agents outside the context of a clinical trial during at least a portion of the study period. As an example, the clinical efficacy of MET inhibitors in patients with exon 14 *MET* alterations was first reported in August 2015 (42), and the availability of MET inhibitors for patients with activating alterations in *MET* was limited outside of clinical trials prior to this date. A substantial fraction of patients with lung adenocarcinoma are still not screened for alterations in *MET, RET*, and *BRAF*. Notably, 39% (28/71) of patients with level 2A alterations were screened after at least one previous systemic therapy, and the identification of such alterations by MSK-IMPACT testing was more likely to occur in the setting of symptomatic advanced disease, which may have led at least some patients to have been deemed poor candidates for clinical studies of the corresponding matched therapy. As 11.3% (8/71) of the level 2A patients had rapid clinical deterioration and never received treatment with the appropriate matched therapy, broader screening for such alterations in patients with lung cancer at the time of diagnosis may result in improved outcomes.

In contrast to the high uptake of matched therapies in the standard-of-care setting (level 1 and level 2A alterations), only 7.6% (31/407) of patients with level 2B to 4 alterations received a matched therapy. These results are consistent with a prior study that assessed the use of matched therapy in the investigational setting (43). The low frequency of matched therapy treatment in patients with level 2B to 4 alterations was likely not due to a lack of interest by such patients or their physicians in participating in clinical trials, as 17.9% (73/407) of patients with level 2B to 4 alterations were enrolled on a therapeutic study; 70% (51/73) used

immunotherapy/other investigational compounds with 30% informed by the patient's mutational profile. This low frequency of matched therapy use in patients with level 2B to 4 alterations is likely attributable to the lack of compelling matched therapy studies for such alterations and the inability of patients to access matched therapies outside the context of a clinical trial and/or the reluctance of patients/physicians to pursue compassionate use of such treatments. The results were particularly striking for patients with *ERBB2* amplifications and inactivating mutations of *BRCA1/2* and *TSC1/2*, which are standard-of-care predictive biomarkers of response to HER2, PARP, and MTOR inhibitors, respectively, in other cancer types. Based upon this early experience, we have sought to open basket clinical trials that would allow for treatment of patients with such alterations in the context of a clinical study (NCT02201212 and NCT02675829).

With the use of a broader sequencing panel, we often identified multiple potentially actionable targets coexisting within individual tumors. In total, 239 (27.8%) patients had two or more actionable mutations, but not a single such patient received a combination of matched therapies (Supplementary Table S2). Although responses to single-agent targeted therapy are often dramatic in patients with lung adenocarcinoma, intrinsic and acquired resistance continue to be major hurdles in achieving the promise of a precision medicine approach. Given the various signaling pathways involved in oncogenesis and their interdependence through cross-talk signaling and feedback mechanisms, the use of combinations of targeted agents could prevent or delay the emergence of drug-resistant clones. However, the complexity imposed by drug–drug interactions, the potential for increased toxicity, and the need to identify an optimal dose and timing schedule (sequential or coadministration) require that each potential combination be explored in the context of a clinical trial prior to broaching use. This complexity, along with the significant logistical and financial challenges in targeting more than one potentially actionable alteration, suggests to us that novel clinical trial designs will be needed to achieve progress with combination strategies in patients with lung cancer (44, 45).

Finally, in 13.1% of cases, we did not identify any level 1 to 4 alterations. Although broader molecular testing such as whole exome, genome, or transcriptome sequencing may have identified potentially actionable fusions or other alterations in some of these tumors, the majority did harbor mutations in cancer genes that have in laboratory models been shown to contribute to lung cancer pathogenesis, such as alterations in the tumor suppressor genes *TP53* and *STK11*. Although certain targeted agents have been proposed as rational treatments in the setting of several of the genes mutated in the UMD cohort, our review of the literature suggests that such mutations were not compelling biomarkers of drug response in lung adenocarcinoma in these cases. As an example, mTOR is a kinase downstream of STK11/LKB1 and hence mTOR inhibitors have been proposed as a potential therapeutic approach in patients with *STK11* mutations (46). However, no patients with *STK11/LKB1* alterations received an mTOR inhibitor in our cohort. A phase II trial of the mTOR inhibitor everolimus in patients with solid malignancies that harbor *TSC1/2, NF1/2,* or *STK11* mutations was recently initiated (NCT02352844), but we were unable to find any trials testing the utility of *STK11* as a predictive biomarker of response to mTOR inhibition in lung cancer.

In summary, we find that 37.1% of patients with lung cancer who underwent MSK-IMPACT testing received a matched therapy based on their mutational profile. Of these patients, 78.1% (249 patients) derived clinical benefit. Excluding standard-of-care therapy (*EGFR* mutations, *ALK,* and *ROS1* fusions), 14.4% (69/478) of patients with a level 2 to 4 alteration as their highest actionable target received matched therapy, with 52% (36/69) exhibiting clinical benefit. The use of matched therapy was strongly influenced by the level of clinical evidence that the mutation identified predicts for drug response. Our data suggest that the use of matched therapies is limited by a lack of access to FDA-approved drugs in patients with level 2 alterations and by the lack of compelling clinical trials of investigational agents in patients with level 3 and level 4 mutations.

## METHODS

### Patient Selection

All patients had recurrent or metastatic lung adenocarcinoma and were referred for genomic testing from January 2014 to March 2016. Clinical data were collected within the context of a prospective clinical trial (ClinicalTrials.gov, NCT01775072), under an Institutional Review Board–approved protocol allowing genomic testing on patients' tumors. Informed consent was obtained from all participating patients. This study was conducted in accordance with the Declaration of Helsinki.

### Genomic Sequencing

Tumor and germline DNA were processed to generate bar-coded libraries and subjected to exon capture using custom-designed probes (14). The average sequence coverage across all tumors was 615×, providing high sensitivity to detect mutations at low allele frequencies in heterogeneous or low-purity specimens. Matched normal DNA, available for 97% of samples, was analyzed simultaneously to identify and filter out germline SNPs. Genomic analysis was performed using the MSK-IMPACT assay, 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) or 410 (version 2) cancer-associated genes (Supplementary Table S4; ref. 14). Genomic analysis was performed using assay version 1 (341 genes) for 296 samples and version 2 (410 genes) for 619 samples.

### Analysis

We stratified potentially actionable genetic events into one of four levels based on published clinical or laboratory evidence that the mutation in question confers increased sensitivity to standard or investigational therapies. An interactive compendium of the mutations deemed actionable is available at the OncoKB website (15). Level 1 alterations included mutations and fusions that are FDA-approved biomarkers in patients with lung cancer (sensitizing *EGFR* mutations, *ALK* or *ROS1* fusions), whereas level 2A events were alterations that were deemed to be standard-of-care biomarkers for FDA-approved drugs in patients with lung cancer based on currently accepted practice guidelines such as those issued by the NCCN. Level 2B alterations included those that are FDA-approved biomarkers in another cancer indication (e.g., *ERBB2* amplification) but not in

patients with lung cancer. Level 3 included alterations for which compelling clinical evidence links the biomarker to drug response in patients but use of the biomarker is not currently a standard of care in any cancer type (e.g., *ERBB2* mutation). Finally, level 4 alterations were those in which compelling preclinical data associate the biomarker with drug response (e.g., *NF1* loss). Patients with two or more level 1 to 4 oncogenic drivers were grouped with the highest-level actionable driver.

Clinical records for all patients were reviewed to determine whether the patient received a matched targeted therapy or immunotherapy and whether the patient was enrolled on a therapeutic clinical trial. Clinical trials were designated as either matched therapy based on the assigned oncogenic mutational profile, immunotherapy, or "other" if it did not meet the aforementioned criteria. Patients were deemed to have derived clinical benefit if there was a reduction in tumor size on imaging and documented symptom improvement or stable disease on two consecutive imaging scans ≥30 days apart with symptom improvement. All clinical and genomic data are available in electronic form through the cBioPortal for Cancer Genomics (47, 48).

Samples without a potentially actionable mitogenic driver mutation (UMD) were subsequently analyzed for the presence of sequencing reads with nonreference bases at mutational hotspots (27). To guard against false-negative results due to insufficient tumor content, the purity of the UMD samples was also estimated by allelic copy-number analysis using FACETS (49). Cochran–Armitage tests were used to assess the trend in the probability of receiving matched therapy and immunotherapy across the level of evidence categories, followed by Fisher exact tests for pairwise comparisons. The Cuzick trend test was used to assess the trend in the number of mutations as a function of the level of evidence category. Fisher exact tests were used to compare the rates of mutation for each gene between MSK-IMPACT and TCGA patients. All statistical tests were two-sided and a $P$ value $< 0.05$ was considered statistically significant.

## Disclosure of Potential Conflicts of Interest

B.T. Li is a consultant/advisory board for Thermo Fisher Scientific. M.D. Hellmann reports receiving commercial research grants from Genentech and BMS and is a consultant/advisory board member for Genentech, Merck, AstraZeneca, BMS, Janssen, and Novartis. J.E. Chaft is a consultant/advisory board member for Genentech and AstraZeneca. C.M. Rudin is a consultant/advisory board member for Bristol-Myers Squibb, Harpoon Therapeutics, G1 Therapeutics, and Chugai. D.M. Hyman reports receiving commercial research grants from AstraZeneca and PUMA Biotechnology, and is a consultant/advisory board member for Atara Biotherapeutics, CytomX, and Chugai. M. Ladanyi reports receiving a commercial research grant from Loxo Pharmaceuticals, and is a consultant/advisory board member for NCCN/Boehringer-Ingelheim and NCCN/AstraZeneca. G.J. Riely reports receiving commercial research support from Pfizer, Ariad, and Novartis, and is a consultant/advisory board member for Genentech/Roche, NCCN/AstraZeneca, and Novartis. No potential conflicts of interest were disclosed by the other authors.

One of the Editors-in-Chief is an author on this article. In keeping with the AACR's editorial policy, the peer review of this submission was managed by a senior member of *Cancer Discovery*'s editorial team; a member of the AACR Publications Committee rendered the final decision concerning acceptability.

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