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Identification of Recurrent *FGFR3–TACC3* Fusion Oncogenes from Lung Adenocarcinoma

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Abstract

Purpose: Targetable oncogenic alterations are detected more commonly in patients with non–small cell lung cancer (NSCLC) who never smoked cigarettes. For such patients, specific kinase inhibitors have emerged as effective clinical treatments. However, the currently known oncogenic alterations do not account for all never smokers who develop NSCLC. We sought to identify additional oncogenic alterations from patients with NSCLC to define additional treatment options.

Experimental Design: We analyzed 576 lung adenocarcinomas from patients of Asian and Caucasian ethnicity. We identified a subset of cancers that did not harbor any known oncogenic alteration. We performed targeted next-generation sequencing (NGS) assay on 24 patients from this set with >75% tumor cell content.

Results: *EGFR* mutations were the most common oncogenic alteration from both Asian (53%) and Caucasian (41.6%) patients. No known oncogenic alterations were present in 25.7% of Asian and 31% of Caucasian tumor specimens. We identified a *FGFR3–TACC3* fusion event in one of 24 patients from this subset using targeted NGS. Two additional patients harboring *FGFR3–TACC3* were identified by screening our entire cohort (overall prevalence, 0.5%). Expression of *FGFR3–TACC3* led to IL3 independent growth in Ba/F3 cells. These cells were sensitive to pan-fibroblast growth factor receptor (pan-FGFR) inhibitors but not the epidermal growth factor (EGFR) inhibitor gefitinib.

Conclusions: *FGFR3–TACC3* rearrangements occur in a subset of patients with lung adenocarcinoma. Such patients should be considered for clinical trials featuring FGFR inhibitors. *Clin Cancer Res; 20(24); 6551–8. ©2014 AACR.*

Introduction

The identification of somatic oncogenic alterations has translated into the development of effective targeted therapies for patients with advanced non–small cell lung cancer (NSCLC). Compelling clinical examples include epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangements (1, 2). In both cases, patients with *EGFR*-mutant or *ALK*-rearranged advanced NSCLC are now routinely treated with kinase inhibitors (erlotinib or crizotinib, respectively) as opposed to chemotherapy as their initial systemic treatment for advanced cancer (3–6). Several additional subsets of patients with NSCLC defined by an oncogenic driver genomic alteration, including mutations in *BRAF* and *ERBB2* and rearrangements in *ROS1* and *RET*, are similarly being treated in clinical trials to determine whether kinase inhibitors for these oncogenic proteins may represent an effective treatment approach (7–11).

Therapeutically targetable alterations in NSCLC are found more commonly in patients with lung adenocarcinoma who are never (<100 cigarettes/lifetime) or light (≤10 pack years) former smokers (12–14). However, currently identified genomic alterations do not account for all lung cancers in this patient population. This observation has inspired specific efforts to identify additional oncogenic alterations and has led to the detection

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Translational Relevance

The identification of oncogenic alterations in subsets of patients with non–small cell lung cancer (NSCLC) has accelerated the development of targeted therapies including epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) inhibitors. Although oncogenic alterations are more prevalent in never smokers than smokers with NSCLC, not all cancers from never smokers contain a known alteration. By studying a cohort of lung adenocarcinomas from never smokers without a known oncogenic alteration, we identified recurrent *FGFR3–TACC3* rearrangements. This fusion gene is oncogenic *in vitro* and the transformed cells are sensitive to fibroblast growth factor receptor (FGFR) kinase inhibitors. These findings identify a population of patients with lung cancer who could benefit from treatment with FGFR kinase inhibitors.

of *RET* and *NTRK1* rearrangements (8, 15). Both alterations are detected in 1% to 2% of patients with NSCLC with lung adenocarcinoma that did not contain other common genetic alterations (8, 15). Although relatively small, given that lung cancer is a common disease, this low percentage translates into thousands of patients diagnosed annually. Clinical trials of therapies targeting RET are currently under way and so far have demonstrated encouraging early efficacy in *RET* rearranged patients with NSCLC (16).

In this study, we perform targeted next-generation sequencing (NGS) of lung adenocarcinomas primarily from never or former light smokers whose cancers did not harbor a known genomic alteration. These studies identify a fusion product involving fibroblast growth factor receptor 3 (FGFR3) and transforming acidic coiled-coil 3 (TACC3) in a subset of patients suggesting that patients with NSCLC harboring this alteration should be enrolled in clinical trials evaluating FGFR inhibitors.

Patients and Methods

Patients

Tumor specimens from patients with NSCLC were obtained at the time of surgical resection and flash-frozen in liquid nitrogen. The initial discovery cohort of patients were limited to never (<100 cigarettes/lifetime) or former light (<10 pack years) smokers, but subsequently included 61 samples from former or current smokers whose tumors did not contain a known oncogenic alteration. Patients were not eligible if they had received prior chemotherapy and/or radiotherapy. Frozen tumor specimens were fixed in Optimal Cutting Temperature (OCT)–embedding compound (Tissue-Tek OCT Compound; Sakura Finetek), sectioned and a representative section stained with hematoxylin and eosin, and reviewed for both histology and tumor content by a staff pathologist. All patients provided written informed consent and the studies were approved by the Dana-Farber Cancer Institute (Boston, MA) Institutional Review Board (IRB).

RNA and DNA extraction

Each frozen tissue sample was sectioned using a microtome to obtain between 10 and 15 mg of tissue for RNA and DNA extraction. RNA was extracted using TRizol (Ambion) according to the manufacturer's instructions and subsequently purified using the RNeasy Mini Kit (Qiagen). DNA was extracted from frozen tissue using the DNeasy Blood and Tissue Kit (Qiagen) and quantified using a standardized PicoGreen fluorescence assay (Invitrogen). RNA concentrations were measured using a NanoDrop ND-2000 Spectrophotometer (Thermo Scientific) or alternatively RiboGreen fluorescent assay (Invitrogen) and the RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies).

Genonomic profiling

All tumors were first genotyped to determine whether they contained a previously known oncogenic alteration. Point mutations or small insertions and deletions were investigated by RT-PCR carried out using the QuanTitec Reverse Transcription Kit (Qiagen). Primers were specifically designed for the tyrosine kinase domain of *EGFR* and *HER2* and the serine/threonine-protein kinase domain of *BRAF*. A DNA-based PCR was used to analyze *KRAS* exons 2 and 3. All primer sequences are available upon request. The amplicons were analyzed by direct sequencing and/or run on the Agilent 2100 Bioanalyzer (Agilent Technologies) using the DNA1000 Kit. The DNA 1000 Kit allows an easy separation of fragments with sizes ranging from 25 to 1,000 bp aiding in the detection of small insertions/deletions and point mutations. Point mutations were identified following digestion with the endonuclease Surveyor (Surveyor Mutation Detection Kit; Transgenomic), which specifically cuts portions of mismatched DNA as previously described (17).

To screen for potential fusion oncogenes, we performed RT-PCR using primers homologous to sequences flanking previously characterized gene rearrangements. Whenever available, we used published primer sequences to detect known rearrangements; otherwise, we designed and optimized our own oligonucleotides (7, 8, 15, 18, 19). In Supplementary Table S1 the specific RT-PCR primers used to screen known rearrangements are listed. The presence of PCRs products was determined on 2% agarose gels and the identified amplified fragments were treated with ExoSapIT (Affymetrix) and subjected to direct sequencing.

Next-generation DNA sequencing

Library construction was performed as previously described using 50 to 200 ng of DNA sheared by sonication to approximately 100 to 400 bp prior to end-repair, dA

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addition and ligation of indexed, Illumina sequencing adaptors (20). Enrichment of target sequences (3,320 exons of 182 cancer-related genes and 37 introns from 14 genes recurrently rearranged in cancer representing approximately 1.1 Mb of the human genome) was achieved by solution-based hybrid capture with a custom Agilent SureSelect biotinylated RNA baitset (20). The selected libraries were sequenced on an Illumina HiSeq 2000 platform using 49 × 49 paired-end reads. Sequence data from genomic DNA were mapped to the reference human genome (hg19) using the Burrows–Wheeler Aligner and were processed using the publicly available SAMtools, Picard, and Genome Analysis Tool kit (21, 22). Genomic rearrangements were detected by clustering chimeric reads mapped to targeted introns.

Ba/F3 cell construction and drug treatments

The full-length cDNA of *FGFR3–TACC3* was obtained by RT-PCR amplification directly from tumor RNA. The primers used were 5′-TTGTCGACGCCACCATGGGCGCCCCTGCCTG-3′ (forward) and 5′-TATCTAGAGGGATCTTCTCCATCTTGGAGATGAGG-3′ (reverse). The fusion transcript was then inserted into the pDNRDual vector (BD Biosciences) using *Sal*I and *Xba*I and recombined into a retroviral flag epitope tag containing expression vector PJ1540 (in-house). Retrovirus containing *FGFR3–TACC3* was produced using 293T cells transfected with 3.0 μg of plasmid and p-Ampho packaging vector using FuGene 6 (Roche) according to the manufacturer's instructions. Retroviral infection and culture of Ba/F3 cells were performed using previously described methods (8, 15, 23). Ba/F3 cells were treated with increasing concentrations of BGJ398, ponatinib, and gefitinib and their viability was tested in an MTS assay according to previously established methods (8, 23, 24). Results were plotted in logarithmic scale using GraphPad Prism Version 6.0 (GraphPad Software Inc.) and results were repeated in triplicate.

Western blotting and antibodies

Cells were lysed in NP-40 buffer. Thirty micrograms of total protein was loaded and separated by gel electrophoresis on 4% to 12% SDS Bis–Tris polyacrylamide gels (Invitrogen) and transferred to polyvinylidene difluoride (PVDF)-P membranes (Millipore). Membranes were probed overnight with anti-phosphotyrosine clone 4G10 antibody (Millipore), FGFR3 mAb Y724 (Abcam), anti-α-tubulin Ab and Flag antibody (Sigma-Aldrich). Detection by immunoblotting was performed according to the antibody manufacturer's recommendations.

Results

Patients

Frozen tumor specimens were obtained from 664 patients. We excluded 88 tumors from the analyses because they were of non-adenocarcinoma histology ($n = 56$), had no specified ethnicity data ($n = 6$), had low (< 20%) tumor cell content ($n = 1$), or lacked any clinical data ($n = 25$). A total of 576 patients were analyzed including 463 patients from Asia [Korea ($n = 325$), Japan ($n = 123$); collectively herein referred to as Asian patients] and the United States ($n = 128$). Fifteen of the 128 samples from the United States group were from patients of Asian ethnicity, and therefore included in the Asian patient group. The remaining 113 samples from the United States were from Caucasian patients and are herein referred to as Caucasian patients. The characteristics of the patients are listed in Table 1.

Table 1. Clinical characteristics of the patients cohort by ethnicity

Histology	Caucasian (%)	Asian (%)	Total (%)
ADC	76 (67.2)	455 (98.2)	531 (92.2)
ADC NOS	20 (17.7)	0	20 (3.5)
AIS	14 (12.4)	3 (0.7)	17 (3.0)
MIA	2 (1.8)	4 (0.9)	6 (1.0)
ADC-SCC	1 (0.9)	1 (0.2)	2 (0.3)
Gender			
Female	88 (77.9)	324 (70.0)	412 (71.5)
Male	25 (22.1)	139 (30.0)	164 (28.5)
Smoking			
Never Smoker	71 (62.8)	374 (80.8)	445 (77.3)
Light Smoker	39 (34.5)	31 (6.7)	70 (12.2)
Former/current smoker	3 (2.7)	58 (12.5)	61 (10.6)
Stage			
Early (I–IIIA)	20 (17.7)	362 (78.2)	382 (66.3)
Advanced (IIIB–IV)	1 (0.9)	47 (10.1)	48 (8.3)
N/A	92 (81.4)	54 (11.7)	146 (25.4)

NOTE: Never Smoker, people who smoked <100 cigarettes/lifetime; Light Smoker, people who smoked ≤10 pack years; Former/Current Smoker, people who smoked >10 pack-years.

Abbreviations: ADC, adenocarcinoma; ADC-NOS, adenocarcinoma non-otherwise specified; ADC-SCC, adenosquamous carcinoma; AIS, adenocarcinoma *in situ*; MIA, minimally invasive adenocarcinoma.

Genomic profiling

All tumors were initially genotyped and divided into two categories based on the findings: those with a known oncogenic alteration and those in which a known oncogenic alteration could not be detected [referred to as pan-wild-type (WT)]. A known oncogenic alteration was detected in 74.3% ($n = 344$) of Asian and 69% ($n = 78$) of Caucasian patients (Table 2 and Fig. 1). All the alterations identified were mutually exclusive with the exception of two cases of concurrent *BRAF* and *KRAS* mutations (Table 2). A subset of this cohort has

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Table 2. Genomic alterations across 576 lung adenocarcinomas grouped by ethnicity

Alteration	Caucasian (%)	Asian (%)	Total (%)
EGFR	47 (41.6)	245 (52.9)	292 (50.7)
ALK	7 (6.2)	36 (7.8)	43 (7.5)
KRAS[a]	15 (13.3)	23 (5.0)	38 (6.8)
ROS1	3 (2.7)	13 (2.8)	16 (2.8)
HER2	2 (1.8)	10 (2.2)	12 (2.1)
RET	1 (0.9)	10 (2.2)	11 (1.9)
BRAF[a]	3 (2.7)	6 (1.3)	9 (1.6)
RIT1	1 (0.9)	1 (0.2)	2 (0.3)
NTRK1	0	1 (0.2)	1 (0.2)
UNKNOWN[b]	35 (31.0)	119 (25.7)	154 (26.7)

[a]Two sample showed a concomitant KRAS and BRAF mutation. An Asian sample has a KRAS G12V mutation together with a G469Del in BRAF. A Caucasian sample harbors a G12A mutation in the KRAS gene and a G469E mutation in BRAF.

[b]The "Unknown" population is the same of the pan-WT reported in the text.

been previously used to identify *RET* and *NTRK1* rearrangements (8, 25). The most common oncogenic alteration in Asian patients was an *EGFR* mutation (detected in 53% of patients). Similarly *EGFR* mutations were the most frequent genomic alteration in Caucasian patients (detected in 41.6% of patients; $P = 0.036$ compared with Asian patients). These *EGFR* mutation frequencies were consistent with prior studies in patients with NSCLC with limited smoking histories (26, 27). Deletions in exon 19 and point mutation in exon 21 (L858R) were the widespread alterations found in *EGFR*, accounting for the 86.3% of all the *EGFR* mutations. Notably, insertion mutations in *EGFR* exon 20 accounted for 5.3% of all the *EGFR* mutations and are recognized to confer primary resistance to available tyrosine kinase inhibitors (28). Point mutations in exon 18 or 21 (not L858R), deletions in exon 18, and insertions in exon 19 together with double point mutations were collectively referred as "Others" in Supplementary Table S2. *KRAS* mutations were found in approximately 7% of tumors from never/light former smokers and more frequently in Caucasian than Asian patients (Caucasian 13.3% vs. Asian 5.0%; $P = 0.003$; Table 2). Moreover, *KRAS* mutational analysis showed variants G12D and G12V to be prevalent among the population in study in agreement with what previous studies of *KRAS* mutations in never/limited former smokers (29). Twelve insertion mutations were identified in *HER2* exon 20 (~2%) and nine *BRAF* mutations, mainly V600E were detected. Two cases harbored alterations in *BRAF* codon G469 and had concomitant *KRAS* mutations. Mutations identified in *HER2*, *KRAS*, and *BRAF* genes are shown in Supplementary Table S3. The incidence of *ALK* rearrangements was similar in the two patient populations and the most frequent *EML4–ALK* variants were variants 1 (E20:A13) and 3 (E20:A6). One rearrangement between *KIF5B* and *ALK* was identified but none involving *TFG–ALK* (30) or the newly described *KLC1–ALK* (31). *RET* fusions were identified in 11 cases (1.9%) of which 10 were *KIF5B–RET* and one was a *CCDC6–RET* rearrangement. *RET* fusions with different partners have been previously identified in papillary thyroid carcinomas (PTC; refs. 32, 33) and some of them have been reported at lower frequencies in lung cancer, such as those involving *CCDC6* (PTC1), *NCOA4* (PTC3), and *TRIM33* (PTC7; ref. 16, 32). Chromosomal translocations involving *ROS1* receptor tyrosine kinase were found in 2.8% of samples involving primarily *CD74* and *EZR* as fusion partners. No fusions with *SLC34A2-*, *LRIG3-*, and *FIG-ROS1* were detected. *CD74* was additionally found fused to the high-affinity nerve growth factor receptor (NTRK1) in a patient of Asian ethnicity (15). The specific *ALK*, *ROS1*, *RET*, and *NTRK1* rearrangements in the two patient populations are detailed in Supplementary Table S4. We also identified two mutations in the recently reported *RIT1* gene, one in the Asian and the other in the Caucasian cohort (34, 35). The first of the two mutations, M90V, occurs in the switch II domain of the gene and is similar to the frequently found

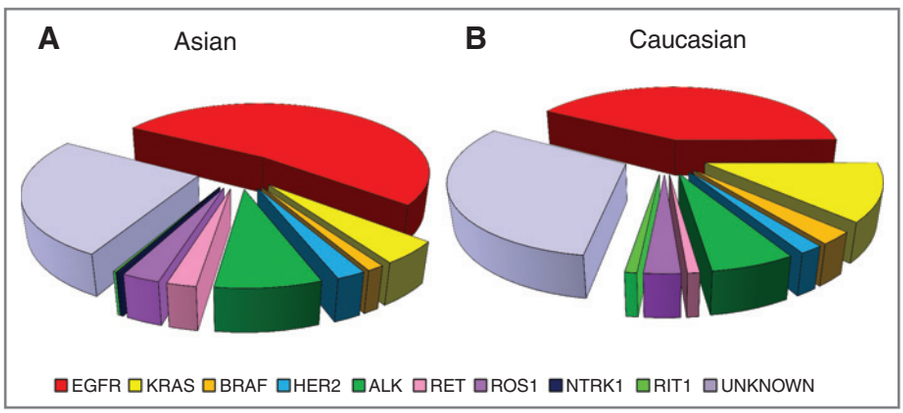

Figure 1. Pie charts demonstrating the distribution of oncogenic alterations in lung adenocarcinoma divided by ethnicity.

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M90I alteration in lung and myeloid malignancies (34, 35). The second one, D51Y, occurs in the switch I domain of *RIT1* and is the second alteration reported in this region of the gene other than one described in subjects with Noonan Syndrome (Supplementary Fig. S1; ref. 36). No oncogenic alteration was detected in 25.7% ($n = 119$) of Asian and 31% ($n = 35$) of Caucasian tumor specimens.

NGS of pan-WT tumor specimens

We next performed targeted NGS on a subset ($n = 24$; 12 Asian and 12 Caucasian patients) of tumors that did not contain any known oncogenic alteration based on our initial analyses (Supplementary Table S5). This subset was chosen because the average tumor content was >75%. In one tumor from a Caucasian patient, a rearrangement fusing exon 17 of the gene *FGFR3* to exon 10 of *TACC3* was detected, similar to that previously described in other tumor types (19, 24, 25, 37, 38). A schematic illustration of the identified *FGFR3–TACC3* fusion is shown in Fig. 2A. A complete list of the variants, copy number alterations, and rearrangements found in these 24 samples is available in the Supplementary Table S5.

Incidence of FGFR3–TACC3 rearrangements in lung adenocarcinoma

We developed specific PCR primers flanking the breakpoint of *FGFR3* (exon 17) and *TACC3* (exon 10) and screened the remaining 130 pan-WT tumor specimens using RT-PCR. We detected *FGFR3–TACC3* fusion transcripts in two additional tumor specimens from the Asian patient cohort (J2-109 and K2-49). Both samples harbored two different fusion variants (Supplementary Fig. S3A): sample J2-109 showed two variants linking exon 17 of *FGFR3* to exon 11 or/and exon 8 of *TACC3* and sample K2-49 showed an amplicon connecting exon 17 of *FGFR3* and exon 11 of *TACC3* and a second one joining together exon 17 of *FGFR3* to exon 4 of *TACC3* (Supplementary Fig. S3B). We further validate these findings by RNA-Seq and exome sequencing for sample J2-109 (Fig. 2B). The hematoxylin and eosin staining of samples J2-109 and CG-42 is shown in Supplementary Fig. S2. We were not able to further

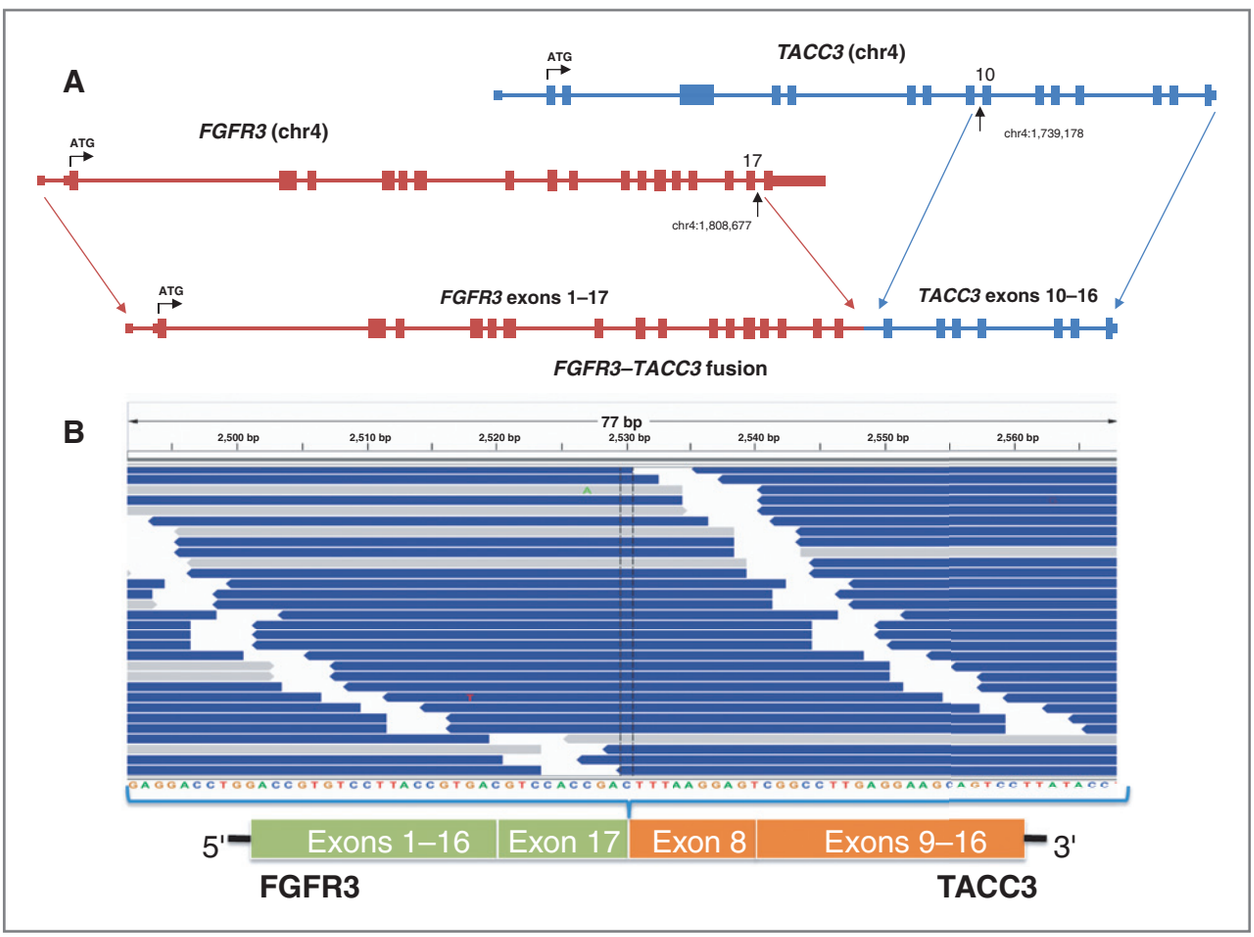

Figure 2. A, schematic representation of the genomic rearrangement from the tumor sample CG-42 harboring the *FGFR3–TACC3* fusion oncogene using the FoundationOne Next-Generation Sequencing Assay. B, *FGFR3–TACC3* fusion in sample J2-109 confirmed by RNA sequencing.

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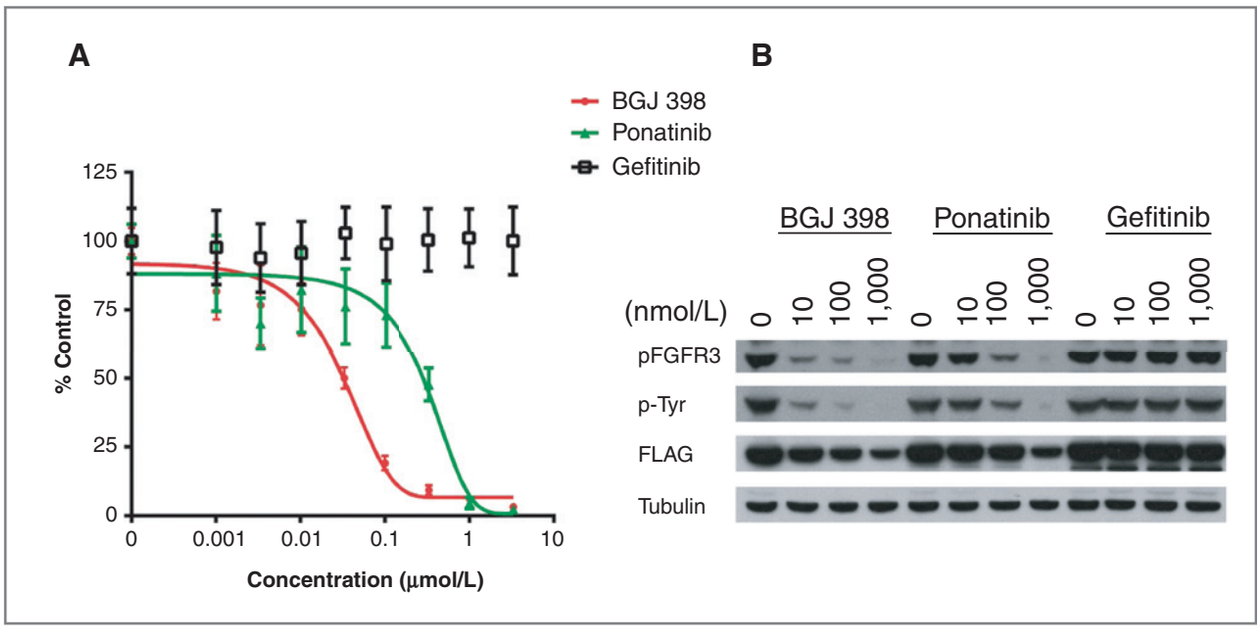

Figure 3. Inhibition of *FGFR* fusion kinase activity following pan-FGFR kinase inhibitors. A, *FGFR3–TACC3* containing Ba/F3 cells were treated at the indicated concentration of BGJ398, ponatinib, and gefitinib and viable cells were measured after 72 hours of treatment and plotted relative to the off-target gefitinib treatment. B, *FGFR3–TACC3* containing Ba/F3 cells were treated with indicated concentration s of BGJ398, ponatinib, and gefitinib for 6 hours. Cells extracts were immunoblotted to detect the indicated proteins.

analyze sample K2-49 because the tissue sample was exhausted.

FGFR3–TACC3 is oncogenic *in vitro* and sensitive to FGFR inhibitors

The full-length *FGFR3–TACC3* transcript from the index patient (CG-42) was cloned using RT-PCR into the pDNR-dual vector and confirmed by direct sequencing to be correct. The expression vector was then introduced into Ba/F3 cells using retroviral infection. The cells were grown in the presence and absence of IL3 and the *FGFR3–TACC3* containing Ba/F3 cells were able to proliferate in the absence of IL3 consistent with oncogenic transformation (data not shown). We further evaluated whether pan-FGFR kinase inhibitors, BGJ398 and ponatinib, could represent a potential therapeutic strategy for patients with *FGFR3–TACC3* NSCLC. In particular, BGJ398 is a selective FGFR inhibitor while ponatinib is a multitargeted agent with FGFR activity. Both BGJ398 and ponatinib, but not the EGFR kinase inhibitor gefitinib, effectively inhibited the growth of the *FGFR3–TACC3* Ba/F3 cells (Fig. 3A). The growth inhibition was mirrored by inhibition of *FGFR3* phosphorylation as shown by p-Tyr and pFGFR3 antibodies (Fig. 3B). The decrease in the fusion protein (as measured by the anti-FLAG antibody) is likely due to degradation as only detected in the FGFR inhibitor but not gefitinib-treated cells (Fig. 3B).

Discussion

Recent advances in sequencing technologies have led to an increase in the discovery of novel and therapeutically actionable genomic alterations in a broad range of cancers, including those arising in the lung (8, 38, 39). In lung cancer, many of these alterations, including *ALK*, *ROS1*, *RET*, and *NTRK1*, are chromosomal inversions or translocations that would not have been detected using more conventional methods such as Sanger sequencing. In this study, using NGS, we identify recurrent *FGFR3–TACC3* fusions in lung adenocarcinomas from patients who do not harbor any previously identified oncogenic alterations. We used an enrichment strategy and focused our studies on tumors that did not harbor any known oncogenic alterations.

Genomic alterations in *FGFR* family members have been identified in several cancers including glioblastoma multiforme (GBM), breast cancer, bladder cancer, cholangiocarcinoma, prostate cancer, thyroid cancer, oral cancer, and in lung cancer (19, 24, 25, 37, 38, 40). In lung cancer, *FGFR* alterations include *FGFR1* amplifications in squamous cell lung cancer, *FGFR2* and *FGFR3* somatic mutations in both lung adenocarcinoma and lung squamous cell carcinoma, and recently identified *FGFR3–TACC3* fusion genes in lung squamous cell carcinomas (38). This study adds to this growing list by identifying *FGFR3–TACC3* fusions also in lung adenocarcinomas (incidence, 0.5%) and predominantly from never/limited smokers. Unlike other fusion oncogenes found in lung cancer, the fusion here occurs after the *FGFR3* kinase domain. Nevertheless, the function of the partner gene, *TACC3*, is still likely the same as in other fusion proteins, to force dimerization and consequently activation of FGFR3 kinase activity. In our study, two fusions

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were found in never smokers and a third in a smoker of Asian ethnicity (50 pack-year smoker). Interestingly, the fusion found in the smoker subject is the same one most commonly identified in lung squamous cell carcinoma, GBM and bladder cancer (19, 24, 25, 37). The reported fusions occur primarily in two exons of *FGFR3* (17 and 18), preserving almost entirely the tyrosine kinase domain that ends after the first three codons of exons 18, but involving many different portions of the *TACC3* gene (exons 4, 7, 8, 9, 10, and 11). The same variants have been identified in diverse cancers, suggesting that they are not tumor specific. However, it could be possible to detect additional breakpoints by increasing the number of cases tested. We further demonstrate that pan-FGFR inhibitors may be therapeutically effective in such cancers. Several clinical trials of FGFR inhibitors, including with BGJ398 (NCT 01928459, NCT 01975701, NCT01697605, and NCT01004224), are currently under clinical development. Preliminary data of the phase I study of BGJ398 in genetic preselected solid tumors showed a tolerable safety profile and tumor regression in patients with urothelial cell carcinomas, squamous cell carcinoma of the lung, cholangiocarcinomas, and breast cancers (41).

Disclosure of Potential Conflicts of Interest

P.A. Janne is a consultant/advisory board member for Abbot, AstraZeneca, Boehringer Ingelheim, Chugai, Clovis Oncology, Genentech, Merrimack, Pfizer, and Sanofi; has ownership interest (including patents) in Gatekeeper Pharmaceuticals; and reports receiving post-marketing royalties from a Dana-Farber Cancer Institute–owned patent on EGFR mutations, which is licensed to Lab Corp. D. Lipson, L. Young, P.J. Stephens, and V.A. Miller have ownership interest in and are employees of Foundation Medicine. D. Jablons is a consultant/advisory board member for Genentech and Eli Lilly. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Capelletti, S.-I. Park, J. Kim, D.M. Jablons, V.A. Miller, N.I. Lindeman, W.G. Richards
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Capelletti, D. Ercan, P.S. Hammerman, D. Lipson, L. Young, P.J. Stephens, N.I. Lindeman, P.A. Jänne
Writing, review, and/or revision of the manuscript: M. Capelletti, P.S. Hammerman, V.A. Miller, N.I. Lindeman, P.A. Jänne
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Capelletti, P.S. Hammerman, K.J. Munir, P.A. Jänne
Study supervision: J. Kim
Other (sample collection and acquisition and management of patients.): H. Sasaki
Other (provided/processed tissue samples for analyses): K.J. Munir

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