Science Translational Medicine
AAAS

Frequent and Focal *FGFR1* Amplification Associates with Therapeutically Tractable FGFR1 Dependency in Squamous Cell Lung Cancer

Jonathan Weiss, *et al.*

Sci Transl Med **2**, 62ra93 (2010);
DOI: 10.1126/scitranslmed.3001451

LUNG CANCER

Frequent and Focal *FGFR1* Amplification Associates with Therapeutically Tractable FGFR1 Dependency in Squamous Cell Lung Cancer

Jonathan Weiss,[1]* Martin L. Sos,[1]*† Danila Seidel,[1,2]* Martin Peifer,[1] Thomas Zander,[3] Johannes M. Heuckmann,[1] Roland T. Ullrich,[1] Roopika Menon,[4] Sebastian Maier,[4] Alex Soltermann,[5] Holger Moch,[5] Patrick Wagener,[6] Florian Fischer,[1] Stefanie Heynck,[1] Mirjam Koker,[1] Jakob Schöttle,[1] Frauke Leenders,[1,2] Franziska Gabler,[1,2] Ines Dabow,[1,2] Silvia Querings,[1] Lukas C. Heukamp,[7] Hyatt Balke-Want,[1] Sascha Ansén,[3] Daniel Rauh,[8] Ingelore Baessmann,[9] Janine Altmüller,[9] Zoe Wainer,[10] Matthew Conron,[10] Gavin Wright,[10] Prudence Russell,[11] Ben Solomon,[12] Elisabeth Brambilla,[13,14] Christian Brambilla,[13,14] Philippe Lorimier,[13] Steinar Sollberg,[15] Odd Terje Brustugun,[16,17] Walburga Engel-Riedel,[18] Corinna Ludwig,[18] Iver Petersen,[19] Jörg Sänger,[20] Joachim Clement,[21] Harry Groen,[22] Wim Timens,[23] Hannie Sietsma,[23] Erik Thunnissen,[24] Egbert Smit,[25] Daniëlle Heideman,[24] Federico Cappuzzo,[26] Claudia Ligorio,[27] Stefania Damiani,[27] Michael Hallek,[3,28] Rameen Beroukhim,[29,30,31,32] William Pao,[33] Bert Klebl,[34] Matthias Baumann,[34] Reinhard Buettner,[7] Karen Ernestus,[35] Erich Stoelben,[18] Jürgen Wolf,[2,3] Peter Nürnberg,[8,28] Sven Perner,[4] Roman K. Thomas[1,2,3,7]†

(Published 15 December 2010; Volume 2 Issue 62 62ra93)

Lung cancer remains one of the leading causes of cancer-related death in developed countries. Although lung adenocarcinomas with *EGFR* mutations or *EML4-ALK* fusions respond to treatment by epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) inhibition, respectively, squamous cell lung cancer currently lacks therapeutically exploitable genetic alterations. We conducted a systematic search in a set of 232 lung cancer specimens for genetic alterations that were therapeutically amenable and then performed high-resolution gene copy number analyses. We identified frequent and focal fibroblast growth factor receptor 1 (*FGFR1*) amplification in squamous cell lung cancer ($n = 155$), but not in other lung cancer subtypes, and, by fluorescence in situ hybridization, confirmed the presence of *FGFR1* amplifications in an independent cohort of squamous cell lung cancer samples (22% of cases). Using cell-based screening with the FGFR inhibitor PD173074 in a large ($n = 83$) panel of lung cancer cell lines, we demonstrated that this compound inhibited growth and induced apoptosis specifically in those lung cancer cells carrying amplified *FGFR1*. We validated the FGFR1 dependence of *FGFR1*-amplified cell lines by FGFR1 knockdown and by ectopic expression of an *FGFR1*-resistant allele ($FGFR1^{V561M}$), which rescued *FGFR1*-amplified cells from PD173074-mediated cytotoxicity. Finally, we showed that inhibition of FGFR1 with a small molecule led to significant tumor shrinkage in vivo. Thus, focal *FGFR1* amplification is common in squamous cell lung cancer and associated with tumor growth and survival, suggesting that FGFR inhibitors may be a viable therapeutic option in this cohort of patients.

INTRODUCTION

Oncogenic protein kinases are frequently implicated as potential targets for cancer treatment. For examples, the *ERBB2* amplification in breast cancer is associated with clinical response to antibodies targeting ERBB2 (*1*), and *KIT* or *PDGFRA* (platelet-derived growth factor receptor A) mutations in gastrointestinal stromal tumors lead to sensitivity to the KIT/ABL/PDGFR inhibitor imatinib (*2*). In lung adenocarcinoma, patients with *EGFR*-mutant tumors (*3*–*5*) experience tumor shrinkage and prolonged progression-free survival when treated with epidermal growth factor receptor (EGFR) inhibitors (*6*). Furthermore, *EML4-ALK* gene fusion–positive lung cancers can be effectively treated with anaplastic lymphoma kinase (ALK) inhibitors (*7*, *8*).

However, these alterations almost exclusively occur in the rare adenocarcinomas of patients who never smoked, but are uncommon in squamous cell lung cancer, which is almost invariably associated with smoking (*9*). Although previous studies have reported recurrent genetic alterations in squamous cell lung cancer (*10*), no therapeutically tractable targets have so far been identified. Thus, therapeutic options for squamous cell lung cancer patients remain scarce, because molecularly targeted drugs such as erlotinib, gefitinib, pemetrexed, and cetuximab are either poorly active (*6*, *11*) or contraindicated (for example, bevacizumab) (*12*). These observations emphasize the need for new "druggable" targets in squamous cell lung cancer patients.

RESULTS

To identify therapeutically relevant genome alterations in squamous cell lung cancer, we analyzed 155 primary squamous cell lung cancer specimens using Affymetrix 6.0 SNP (single-nucleotide polymorphism) arrays, which yielded high-resolution genomic profiles (median inter-marker distance <1 kb). To separate driver lesions from random noise, we applied the GISTIC algorithm (*13*, *14*). We identified 25 significant amplification peaks, including the previously described amplification of

SOX2 on chromosome 3q26.33 (Fig. 1A and table S1) (*10*) and 26 significant deletions (fig. S1 and table S1). The second most significant amplification ($q = 8.82 \times 10^{-28}$) peak was identified on 8p12 and included *FGFR1* (fibroblast growth factor receptor 1) as well as *FLJ43582* in each amplified sample (Fig. 1A). This region spanned 133 kb (table S1) and was amplified at high amplitude (four or more copies) in 15 of 155 (9.7%) squamous cell lung cancer specimens (Fig. 1A). Notably, 11 of the tumors with *FGFR1* amplification were from smokers, whereas none of these were from patients who had never smoked (table S2). Ten of the 15 tumors with amplified amounts of *FGFR1* also harbored a mutation in *TP53* (table S2). Moreover, patients who had tumors with *FGFR1* amplification [copy number > 9 in fluorescence in situ hybridization (FISH) analysis] had a nonsignificant trend toward inferior survival compared to patients whose tumors lacked *FGFR1* amplifications (copy number = 2 in FISH analysis) (fig. S2). We next analyzed copy number alterations in lung adenocarcinoma specimens ($n = 77$) and found no significant ($q > 0.25$) amplification (four or more copies; 1.3%) at 8p12 (Fig. 1B).

Finally, we analyzed a publicly available lung cancer SNP array data set (*14*) for the presence of *FGFR1* amplifications (four or more copies); *FGFR1* was amplified in 6 of 581 (1%) nonsquamous cell lung cancers (Fig. 1C). Thus, *FGFR1* amplification is significantly enriched in squamous cell lung cancer when compared to our own adenocarcinoma data set ($P = 0.03$) (table S3) and when compared to a published data set of nonsquamous cell lung cancer ($P < 0.0001$) (Fig. 1C). FISH using an 8p12-specific probe on an independent set of 153 squamous cell lung cancers confirmed the presence of frequent high-level amplification of *FGFR1* in 34 of 153 (22%) patients (Fig. 1D and table S4), 27 of whom were current smokers and none of whom were nonsmokers. We note that FISH is not sensitive to the admixture of nontumoral cells; thus, focal amplification of *FGFR1* is likely to be more frequent in squamous cell lung cancer than as estimated by SNP arrays (table S4) (*15*). We also sequenced the *FGFR1* gene in 94 squamous cell lung cancers and 94 adenocarcinomas and found one mutation ($FGFR1^{P578H}$) in the adenocarcinoma cohort, indicating that *FGFR1* mutations might play only a minor role and might not drive alterations in the pathogenesis of lung cancer (*16*).

Next, we performed high-throughput cell line screening (*17*, *18*) to determine the activity of the non–isoform-specific FGFR inhibitor PD173074 (*19*) in a collection of 83 lung cancer cell lines (table S5) (*17*, *20*). Of all cell lines tested, four had a half-maximal growth-inhibitory concentration (GI_{50} values) below 1.0 μM (Fig. 2A); remarkably, three of the four sensitive lung cancer cell lines exhibited focal amplification at 8p12 by 6.0 SNP array analysis (Fig. 2B), suggesting that *FGFR1* amplifications are significantly ($P = 0.0002$) associated with FGFR inhibitor activity (Fig. 2A). As expected, *FGFR1*-amplified cells expressed higher amounts of total FGFR1 protein (fig. S3). One (H520) of the three *FGFR1*-amplified cell lines that were sensitive to PD173074 was derived from a squamous cell lung cancer patient (table S5). We next tested whether amplification of *FGFR1* could be linked with sensitivity to FGFR inhibition in an unbiased fashion. Application of a *K*-nearest neighbor–based analysis, followed by leave-one-out cross-validation (*17*), revealed *FGFR1* amplification to be the only genetic predictor of PD173074 sensitivity that retained significance following Bonferroni-based multiple testing correction ($P < 0.05$; table S6). Previous studies indicated that expression of FGFR ligands might contribute to the sensitivity to FGFR inhibitors in lung cancer (*21*). We did not observe elevated amounts of FGF2 in the *FGFR1*-amplified cell lines (fig. S4A), nor did we observe a difference in the expression of FGFR ligands between patients harboring *FGFR1* amplification and those without *FGFR1* amplification (fig. S4B). However, *FGFR1*-amplified cells showed robust phosphorylation of FGFR, suggesting ligand-independent activation, which was further enhanced upon addition of exogenous FGF2 or FGF9 (fig. S4C), compatible with paracrine activation of FGFR1 in *FGFR1*-amplified cells. We next measured induction of apoptosis in *FGFR1*-amplified cells after treatment with PD173074 and found a significant ($P = 0.008$) enrichment of *FGFR1*-amplified lung cancer cells in the group of sensitive cells (Fig. 2C and table S7). Furthermore, FGFR inhibition led to decreased colony formation of *FGFR1*-amplified but not of *EGFR*-mutant cells in soft agar (Fig. 2D), further enforcing the notion that amplification of *FGFR1* drives proliferation of these lung cancer cell lines. Treatment with PD173074 reduced the amounts of phosphorylated FGFR1 (fig. S5) and of the adaptor molecule FRS2 in a dose-dependent manner only in *FGFR1*-amplified cells, but not in the *EGFR*-mutant cell line HCC827 (Fig. 2E). We also observed inhibition of phosphorylation of extracellular signal–regulated kinase (ERK) but not of AKT and S6, indicating that the mitogen-activated protein kinase (MAPK) pathway, and not the phosphatidylinositol 3-kinase (PI3K) pathway, is the major signaling pathway engaged by amplified *FGFR1* (Fig. 2E).

To validate FGFR1 as the critical target of PD173074 in *FGFR1*-amplified lung cancer cells, we ectopically expressed the V561M mutation (*22*) at the gatekeeper position of FGFR1 ($FGFR1^{V561M}$),

[1]Max Planck Institute for Neurological Research, Klaus-Joachim-Zülch Laboratories of the Max Planck Society and the Medical Faculty of the University of Cologne, 50931 Cologne, Germany. [2]Laboratory of Translational Cancer Genomics, Center of Integrated Oncology Köln-Bonn, University of Cologne, 50924 Cologne, Germany. [3]Department I of Internal Medicine and Center of Integrated Oncology Köln-Bonn, University of Cologne, 50924 Cologne, Germany. [4]Institute of Pathology, Comprehensive Cancer Center, University Hospital Tübingen, 72076 Tübingen, Germany. [5]Institute for Surgical Pathology, University Hospital Zurich, 8091 Zurich, Switzerland. [6]Department of Surgery, Weill Medical College, Cornell University, New York, NY 10065, USA. [7]Institute of Pathology, University of Bonn, 53123 Bonn, Germany. [8]Chemical Genomics Center, Max Planck Society, 44227 Dortmund, Germany. [9]Cologne Center for Genomics and Institute for Genetics, University of Cologne, 50931 Cologne, Germany. [10]Department of Surgical Oncology, Peter MacCallum Cancer Centre, Melbourne, 3002 Victoria, Australia. [11]Department of Pathology, St. Vincent's Hospital, Melbourne, 3065 Victoria, Australia. [12]Department of Haematology and Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, 3002 Victoria, Australia. [13]Department of Pathology, Université Joseph Fourier, 38041 Grenoble, France. [14]Institut Albert Bonniot INSERM U823, Université Joseph Fourier, 38042 Grenoble, France. [15]Department of Thoracic Surgery, Rikshospitalet, Oslo University Hospital, 0027 Oslo, Norway. [16]Department of Radiation Biology, Norwegian Radium Hospital, N-0310 Oslo, Norway. [17]Department of Oncology, Radiumhospitalet, Oslo University Hospital, N-0310 Oslo, Norway. [18]Thoracic Surgery, Lungenklinik Merheim, Kliniken der Stadt Köln gGmbH, 51109 Cologne, Germany. [19]Institute of Pathology, Jena University Hospital, Friedrich-Schiller-University, 07743 Jena, Germany. [20]Institute for Pathology Bad Berka, 99438 Bad Berka, Germany. [21]Department for Internal Medicine II, University Clinic Jena, Friedrich-Schiller University, 07740 Jena, Germany. [22]Department of Pulmonary Diseases, University Medical Centre Groningen, 9713 GZ Groningen, Netherlands. [23]Department of Pathology, University Medical Centre Groningen, 9713 GZ Groningen, Netherlands. [24]Department of Pathology, VU University Medical Center Amsterdam, 1007 MB Amsterdam, Netherlands. [25]Department of Pulmonary Diseases, VU University Medical Center Amsterdam, 1007 MB Amsterdam, Netherlands. [26]Department of Medical Oncology, Ospedale Civile, 57100 Livorno, Italy. [27]Department of Haematology and Oncologic Science, University Hospital Bologna, 40138 Bologna, Italy. [28]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, 50931 Cologne, Germany. [29]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02115, USA. [30]Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA. [31]Department of Medicine, Harvard Medical School, Boston, MA 02115, USA. [32]Cancer Program, Broad Institute, Cambridge, MA 02115, USA. [33]Vanderbilt-Ingram Cancer Center, Nashville, TN 37212, USA. [34]Lead Discovery Center GmbH, 44227 Dortmund, Germany. [35]Department of Pathology, Hospital Merheim, Kliniken der Stadt Köln gGmbH, 51109 Cologne, Germany.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: martin.sos@nf.mpg.de (M.L.S.); nini@nf.mpg.de (R.K.T.)

preventing access of the compound to the hinge region of the kinase (*23*) (fig. S6). Expression of FGFR1^{V561M} in *FGFR1*-amplified lung cancer cells abolished PD173074-mediated cytotoxicity and dephosphorylation of FGFR (Fig. 3A), consistent with the notion that FGFR1 is the critical target of PD173074 in *FGFR1*-amplified lung cancer cells. Furthermore, in a panel of 105 biochemically screened kinases, FGFR1 was one of only two kinases strongly inhibited by PD173074 (table S8), recapitulating previous studies (*22*).

The high analytical resolution of the 6.0 SNP arrays, together with the large size of our data set, limited the number of candidate genes in the 8p12 amplicon to only two genes, *FGFR1* and *FLJ43582*. A previous study analyzing the 8p12 locus in lung cancer applying lower-resolution techniques suggested *WHSC1L1* to be the relevant oncogene in the 8p12 amplicon (*24*). To test whether genes other than *FGFR1* drive tumorigenesis in the 8p12-amplified tumors, we silenced the genes *WHSC1L1* (*24*) and *FLJ43582* using five different short hairpin RNA (shRNA) constructs in the 8p12-amplified lung cancer cell line H1581. Although silencing of either one of these genes did not inhibit cellular viability (fig. S7), silencing of *FGFR1* strongly reduced the viability of the *FGFR1*-amplified lung cancer cells (Fig. 3B). In light of the focality of the 8p12 amplicon (including *FGFR1* and *FLJ43582*) and the lack of effect of shRNA-mediated knockdown of either *FLJ43582* or *WHSC1L1* in *FGFR1*-amplified cells, our data suggest that *FGFR1* is the relevant target in these cells. Notably, the cell line H1703, which bears a copy number gain at 8p12 and that had been reported to depend on *WHSC1L1* (*24*), was not sensitive to FGFR inhibition (fig. S8). By contrast, H1703 cells depend on PDGFRA for their survival (*25*) because of amplification (copy number >2.8) of the gene encoding this kinase (*26*, *27*). Thus, our data suggest that the gene targeted by the 8p12 amplicon is primarily *FGFR1* and its amplification induces FGFR1 dependency.

Finally, treatment with PD173074 (100 mg/kg, twice a day) resulted in tumor shrinkage in mice engrafted with *FGFR1*-amplified cells (Fig. 3C). This reduction in tumor size was paralleled by reduction in the amounts of phospho-ERK but not of phospho-AKT in immu-

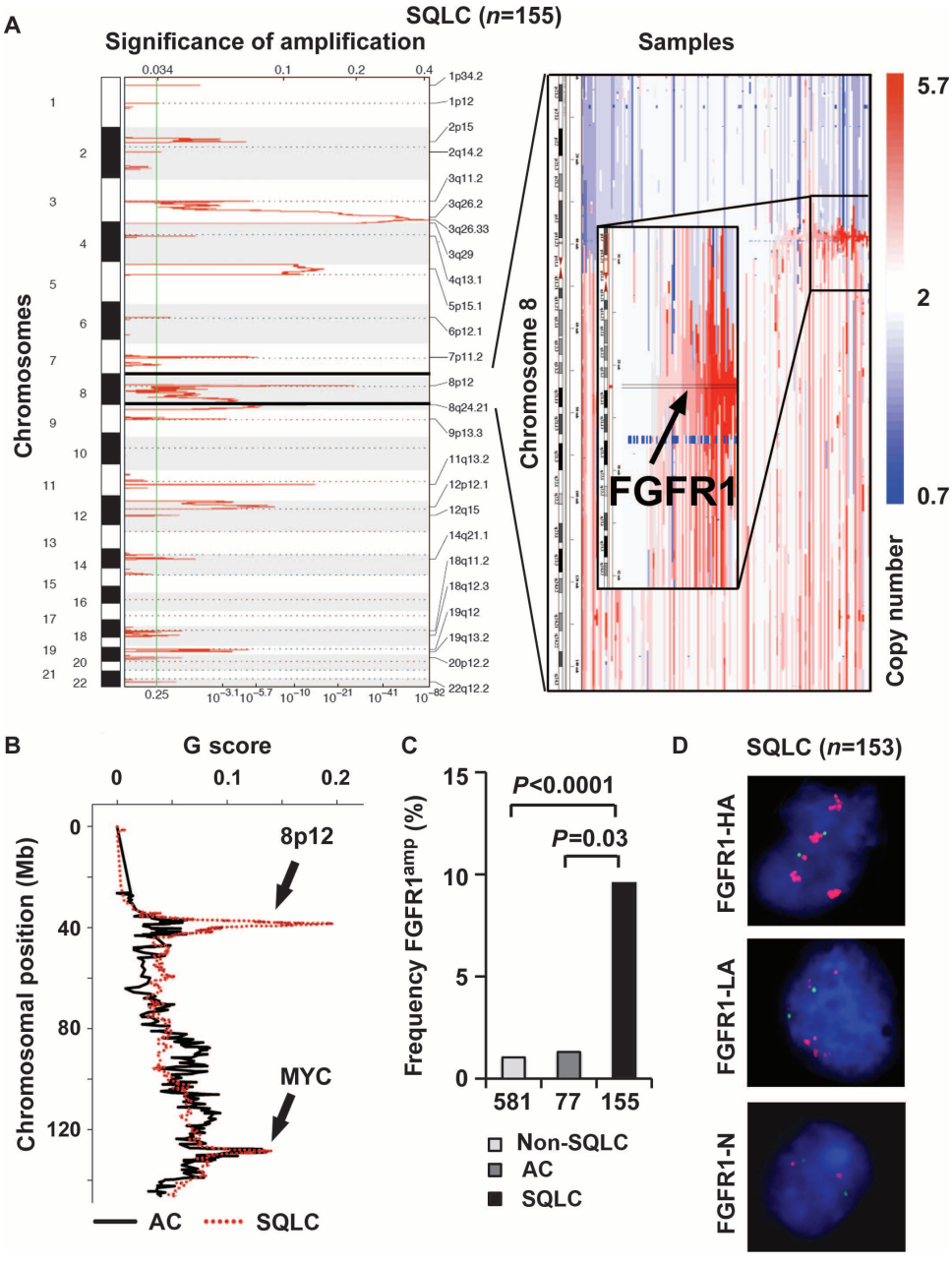

Fig. 1. *FGFR1* is amplified in squamous cell lung cancer (SQLC). (**A**) Left panel: Significant (*14*) [FDR (false discovery rate) value; *x* axis] amplifications across all chromosomes (*y* axis) in SQLC (n = 155) as assessed by GISTIC. Right panel: Copy number alterations (blue, deletion; white, copy number–neutral; red, amplification) at chromosome 8 (*y* axis) across all SQLC samples (*x* axis). Samples are ordered according to focal amplification of *FGFR1*. (**B**) Significant (G score; *y* axis) copy number changes in adenocarcinoma (AC; n = 77) (black line) and SQLC (red dotted line) at chromosome 8. The *q* value for the presence of 8p12 amplification is 8.82×10^{-28} for SQLC and greater than 0.25 for adenocarcinoma. The chromosomal positions of *FGFR1* (8p12) and *MYC* are highlighted (black arrows). (**C**) Frequency of *FGFR1* amplification (% of samples ≥ copy number 4; *y* axis) in non-SQLC from a published data set (*14*), adenocarcinoma, and SQLC. *P* values indicate statistical significance. (**D**) FISH analysis (green, control; red, FGFR1) of 153 SQLC samples (FGFR1-HA: copy number >9; FGFR1-LA: copy number >2 and <9; FGFR1-N: copy number 2). Presented are example images from the three different *FGFR1* amplification groups.

nohistochemical analyses of explanted tumors, validating our in vitro findings that MAPK signaling is the key pathway engaged by amplified *FGFR1* (fig. S9A). Treatment at 50 mg/kg twice a day resulted in only a minimal exposure when compared to the gavage of 100 mg/kg twice a day because of the short half-life of the compound in vivo (fig. S9B). Thus, although we cannot formally exclude inhibitory effects on VEGFR2 (vascular endothelial growth factor receptor 2), the observed tumor regression is likely to be mediated by inhibition of FGFR1. In contrast, xenografted *EGFR*-mutant H1975 cells did not show signs of regression upon PD173074 treatment (fig. S9C). Thus, *FGFR1* amplification leads to FGFR1 dependency in vivo.

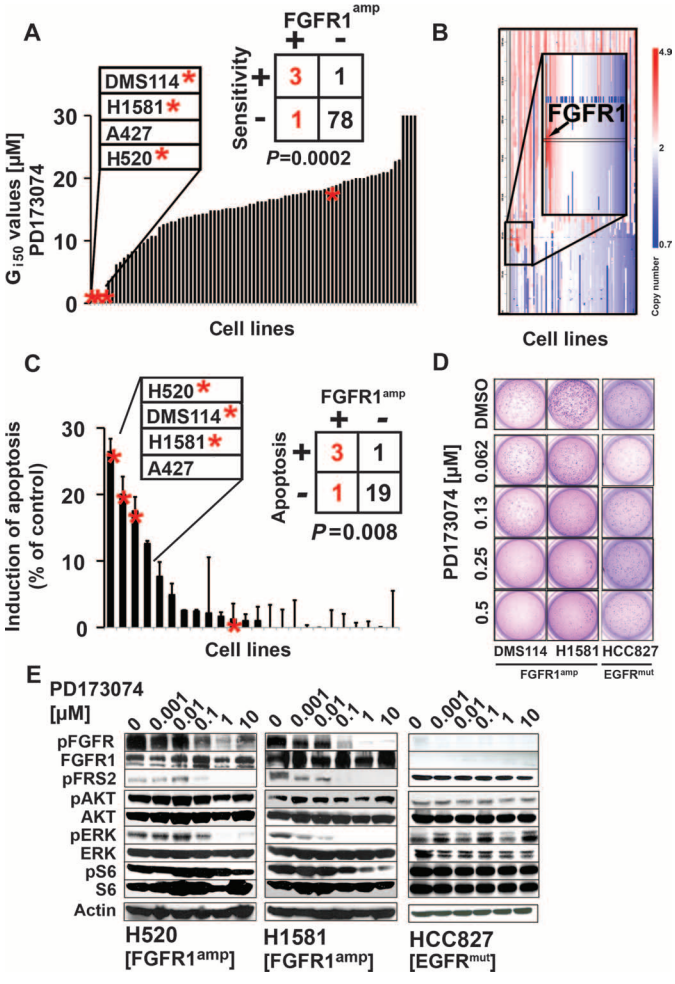

Fig. 2. *FGFR1* amplifications are associated with FGFR inhibitor activity. (**A**) GI_{50} values (*y* axis) of PD173074 across 83 lung cancer cell lines (*x* axis). *FGFR1*-amplified (copy number ≥4) cell lines are marked with asterisks. (**B**) Copy number alterations (*x* axis; blue, deletion; white, copy number 2; red, amplification) on chromosome 8 with a zoom in on 8p12 (*FGFR1* locus is highlighted) across all cell lines (*y* axis). (**C**) Induction of apoptosis (difference between PD173074 at 1 μM and DMSO control after 72 hours; *y* axis) across 24 cell lines (*x* axis; asterisks denote *FGFR1* amplification copy number ≥4) as measured by flow cytometry (after annexin V/PI staining). (**D**) *FGFR1*-amplified cell lines were plated in soft agar and treated with either DMSO (control) or decreasing concentrations of PD173074. (**E**) Phosphorylation of FGFR and of downstream molecules in *FGFR1*-amplified (H1581 and H520) and in *FGFR1* wild-type (*EGFR*-mutant) cells (HCC827) after treatment with PD173074 as assessed by immunoblotting.

DISCUSSION

Here, we have identified frequent high-level amplification of *FGFR1* in squamous cell lung cancer of smokers; this amplification sensitizes the tumors to FGFR1 inhibition. Previous studies in lung cancer cohorts of mixed subtypes and low technological resolution (*24*, *28*) or small size (*10*) have reported occasional amplification of the 8p locus in lung cancer. However, the large size of our sample set was necessary to reveal the high prevalence of this amplicon in squamous cell lung cancer (~10%) in comparison to other lung cancer subtypes (1%). Given the insensitivity of FISH analyses to admixture of nontumoral cells, the true prevalence of this amplification is likely to still be substantively underestimated by SNP arrays and to be up to 20%. We conclude that *FGFR1* amplification is one of the hallmark alterations in squamous cell lung cancer, similar to amplification of *SOX2*. These two alterations were almost completely mutually exclusive (table S9), suggesting an epistatic

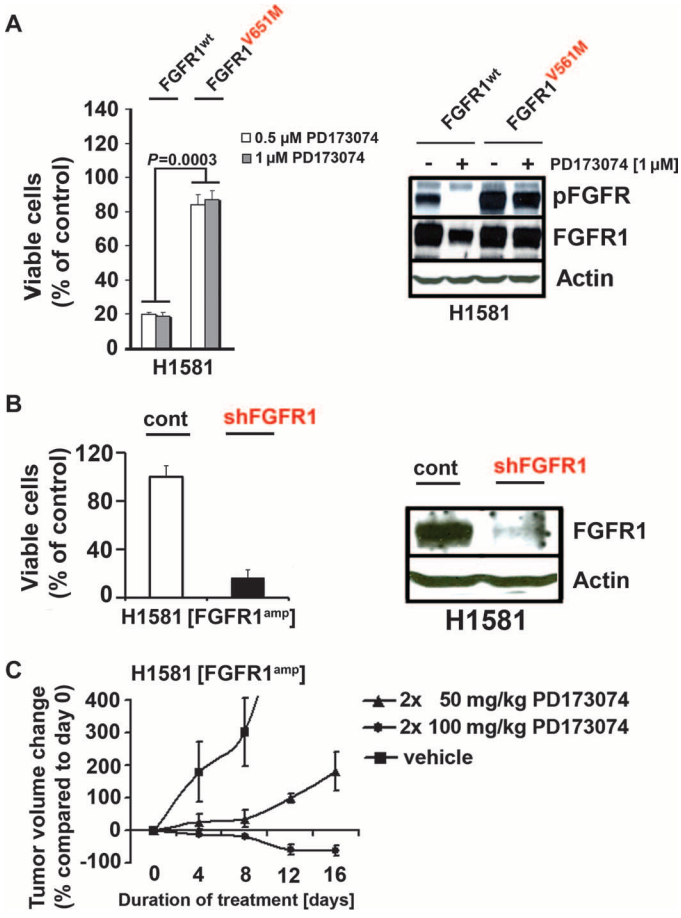

Fig. 3. *FGFR1*-amplified cells are dependent on FGFR1 in vitro and in vivo. (**A**) Left panel: Viability (PD173074 treatment as compared to DMSO control) of *FGFR1*-amplified cells expressing wild-type (wt) or mutant (V561M) *FGFR1* treated with PD173074 [0.5 μM (white bars) and 1.0 μM (gray bars)]. Right panel: Phosphorylation of FGFR in the $FGFR1^{V561M}$ and $FGFR1^{wt}$ cells detected by immunoblotting. (**B**) Left panel: Viability (PD173074 treatment as compared to DMSO control; *y* axis) of H1581 cells after transduction with control shRNA or shRNA targeting FGFR1. Right panel: Silencing of FGFR1 in H1581 cells was confirmed by immunoblotting. (**C**) In mice engrafted with H1581 cells treated with either vehicle or PD173074 (dosage as indicated; *y* axis), tumor volume was measured over time (*x* axis).

relationship. Furthermore, *FGFR1* amplification induced a strong FGFR1 dependency that could be exploited therapeutically, resulting in induction of apoptosis. Thus, *FGFR1* amplification represents an opportunity for targeted therapy in squamous cell lung cancer. We therefore suggest that FGFR1 inhibitors, which are currently in clinical testing in tumor types bearing genetic alterations in FGFR genes (*29*–*31*), should be evaluated in patients with *FGFR1*-amplified squamous cell lung cancer.

MATERIALS AND METHODS

Genomic analyses

The tumor specimens analyzed in this study have been collected under local Institutional Review Board approval. All patients gave written informed consent. Genomic DNA was hybridized to Affymetrix 6.0 SNP arrays following the manufacturer's instructions. Raw signal intensities were normalized and modeled with a Gaussian mixture model. Background-corrected intensities were normalized across all arrays of one batch by quantile normalization. Raw copy numbers were calculated by dividing the normalized tumor-derived signal intensities by the mean signal intensities derived from the normal samples hybridized in the same batch. Raw copy number data were segmented by circular binary segmentation and visualized in the integrated genome viewer (IGV) (*32*). GISTIC was performed as described previously (*13*, *14*). The human genome build hg18 was used. Dideoxy sequencing was performed on whole-genome amplified DNA of primary tumors. Cell lines were sequenced with complementary DNA (cDNA). All raw data are publicly available [Gene Expression Omnibus (GEO); GSE25016].

Tissue microarray construction

Tissue microarray slides were obtained from formalin-fixed, paraffin-embedded lung squamous cell carcinoma samples. The tissue microarrays contained samples of a total of 172 patients from the University Hospital Zurich; each of these samples was present in duplicate cores, each core 0.6 mm in diameter (*33*). A second tissue microarray of 22 patients from Weill Cornell Medical Center was obtained, with each sample present in triplicate cores, each core 0.6 mm in diameter. Subsequently, 153 samples were used for FISH analysis.

Gene expression

After RNA isolation, biotin-labeled complementary RNA (cRNA) preparation was performed with Epicentre TargetAmp Kit (Epicentre Biotechnologies) and Biotin-16-UTP (10 mM; Roche Molecular Biochemicals) or Illumina TotalPrep RNA Amplification Kit (Ambion). Biotin-labeled cRNA (1.5 μg) was hybridized to Sentrix whole-genome bead chips WG6 version 2 (Illumina) and scanned on the Illumina BeadStation 500X. For data collection, we used Illumina BeadStudio 3.1.1.0 software. Gene pattern analysis platform (*34*) was used to visualize the normalized data.

FGFR1 amplification FISH assay

A FISH assay was used to detect the *FGFR1* amplification at the chromosomal level on the tissue microarrays. We performed fluorescence signal detection with two probes on chromosome 8. The reference probe is located on a stable region of chromosome 8p23.2 and selected on the basis of SNP array analysis. Only samples where the control bacterial artificial chromosome (BAC) was detectable were used for the determination of the copy number of *FGFR1*. The target probe is located on the *FGFR1* locus spanning 8p11.23 to 8p11.22. We used the digoxigenin-labeled BAC clones CTD 2523O9, which produces a green signal, as reference probe. The target probe was labeled with biotin to produce a red signal with RP11-148D21 BAC clones (Invitrogen). Deparaffinized sections were pretreated with a 100 mM tris and 50 mM EDTA solution at 92.8°C for 15 min and digested with Digest-All III (dilution, 1:2) at 37°C for 14 min; *FGFR1* FISH probes were denatured at 73°C for 5 min and immediately placed on ice. Subsequently, the tissue sections and *FGFR1* FISH probes were co-denatured at 94°C for 3 min and hybridized overnight at 37°C. After hybridization, washing was done with 2× SSC at 75°C for 5 min, and the fluorescence detection was performed with streptavidin–Alexa 594 conjugates (dilution 1:200) and antibodies to digoxigenin–fluorescein isothiocyanate (FITC) (dilution, 1:200). Slides were then counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and mounted. The samples were analyzed under a 63× oil immersion objective with a fluorescence microscope (Zeiss) equipped with appropriate filters, a charge-coupled device camera, and the FISH imaging and capturing software Metafer 4 (Metasystems). The evaluation of the tests was done independently by three experienced evaluators (R.M., S.M., and S.P.). At least 100 nuclei per case were evaluated. The thresholds for assigning a sample to the FGFR1 "high-amplification" group were a copy number of nine. All samples that had a copy number below nine and above two were assigned to the group of "low-amplification" cohort. All the remaining samples were assigned "normal."

Cell lines and reagents

Cell lines were obtained from the American Type Culture Collection (ATCC), the German Resource Centre for Biological Material (DSMZ), or from our own and other cell culture collections and were maintained as described previously. PD173074 was purchased from commercial suppliers, dissolved in dimethyl sulfoxide (DMSO) or vehicle solution, and stored at −20°C.

Cell line screening

Cell line screening was performed as previously described (*17*) with various concentrations of PD173074. Viability was determined after 96 hours by measuring cellular adenosine triphosphate (ATP) content (CellTiter-Glo, Promega). Half-maximal inhibitory concentrations (GI_{50}) were determined with the statistical data analysis software "R" with the package "ic50."

Apoptosis

For determination of apoptosis, cells were seeded in six-well plates, incubated for 24 hours, treated with either DMSO (control) or 1.0 μM PD173074 for 72 hours, and stained with annexin V and propidium iodide (PI). Finally, the cells were analyzed on a FACSCanto flow cytometer (BD Biosciences). The difference between the relative percentage of annexin V/PI–positive cells treated with DMSO and cells treated with PD173074 was determined (induction of apoptosis rate).

Lentiviral RNA interference and retroviral expression

The V561M mutation was introduced into FGFR1 cloned in pBABE-Puro by site-directed mutagenesis. Replication-incompetent retroviruses were produced by cotransfection with the pCL-ampho plasmid in human embryonic kidney (HEK) 293T cells. Hairpin shRNA targeting the different genes was ordered from Sigma. All sequences are given in table S10. Replication-incompetent lentiviruses were produced from pLKO.1-Puro–based vectors by cotransfection with Δ8.9 and pMGD2 in 293T cells as described previously (*35*). After transduction, cells were

selected with puromycin (1.5 μg/ml), and 5 days after selection, cells were counted with trypan blue.

Western blotting

The following antibodies were used for immunoblotting: β-actin (MP Bioscience); phospho-FGFR (Tyr^{653}, Tyr^{654}), phospho-FRS2 (Tyr^{196}), phospho-AKT (Ser^{473}), phospho-S6, S6, AKT, phospho-ERK, and ERK (Cell Signaling Technology); total FGFR1 (Santa Cruz Biotechnology); and horseradish peroxidase (HRP)–conjugated antibodies to rabbit and mouse (Millipore).

Soft agar assay

Cells were suspended in growth media containing 10% fetal calf serum (FCS) and 0.6% agar and plated in triplicate on 50 μl of solidified growth medium (10% FCS; 1.0% agar). Growth medium containing indicated compound concentrations was added on top. Colonies were analyzed with the Scanalyzer imaging system (LemnaTec).

Xenograft mouse models

All animal procedures were approved by the local animal protection committee and the local authorities. Tumor cells (5×10^6) were injected subcutaneously into male nude mice. After the tumors reached a size of at least 50 mm^3, the animals were treated twice daily by oral gavage with PD173074 (15 mg/ml for 50 mg/kg or 30 mg/ml for 100 mg/kg schedule) dissolved in vehicle (sodium lactate) or vehicle detergent alone. Tumor size was monitored by measuring perpendicular diameters as described previously (*17*). For the determination of tumor growth under treatment with PD173074, each experiment presented in the figures compromises the measurement of five different tumors.

Statistical analyses

Tests for statistical significance were either two-tailed *t* tests or Fisher's exact tests. Prediction of compound activity was performed with the KNN algorithm as described previously (*17*). Multiple hypothesis testing was performed with the statistical data analysis software R using *P* value adjustment.

SUPPLEMENTARY MATERIAL

www.sciencetranslationalmedicine.org/cgi/content/full/2/62/62ra93/DC1

Methods

Fig. S1. Significant deletions are observed in squamous cell lung cancer.

Fig. S2. *FGFR1* amplification has no significant impact on overall survival of SQLC patients.

Fig. S3. *FGFR1* amplification correlates with FGFR1 protein expression.

Fig. S4. Expression of FGFR ligands does not correlate with FGFR1 amplification status.

Fig. S5. Treatment of *FGFR1*-amplified cell line H520 with PD173074 leads to dephosphorylation of FGFR1 as measured by immunoprecipitation.

Fig. S6. PD173074 binds inside the ATP-binding pocket of FGFR1.

Fig. S7. Knockdown of genes adjacent to *FGFR1* on 8p12 does not affect cell viability.

Fig. S8. PD173074 is not active in the *PDGFRA*- and *FGFR1*-amplified cell line H1703.

Fig. S9. PD173074 shows antitumor activity in vivo.

Table S1. Significant amplifications and deletions are noted in a subset of 155 SQLC samples.

Table S2. Clinical features and co-occurrent mutations of *FGFR1*-amplified SQLC samples.

Table S3. Significant amplifications and deletions are noted in a subset of 77 adenocarcinoma samples.

Table S4. *FGFR1* amplification is detected using FISH on tumor microarrays.

Table S5. GI_{50} values are not associated with mutation status across the lung cancer cell line panel.

Table S6. KNN algorithm–based scoring predicts PD173074 sensitivity.

Table S7. PD173074 induces apoptosis in *FGFR1*-amplified cell lines.

Table S8. PD173074 has specific activity against two kinases.

Table S9. *FGFR1* and *SOX2* amplification in squamous cell lung carcinoma.

Table S10. Sequences of all shRNA constructs that were used in the study.

References

REFERENCES AND NOTES

1. D. J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga, L. Norton, Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**, 783–792 (2001).
2. M. C. Heinrich, C. L. Corless, G. D. Demetri, C. D. Blanke, M. von Mehren, H. Joensuu, L. S. McGreevey, C. J. Chen, A. D. Van den Abbeele, B. J. Druker, B. Kiese, B. Eisenberg, P. J. Roberts, S. Singer, C. D. Fletcher, S. Silberman, S. Dimitrijevic, J. A. Fletcher, Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J. Clin. Oncol.* **21**, 4342–4349 (2003).
3. W. Pao, V. Miller, M. Zakowski, J. Doherty, K. Politi, I. Sarkaria, B. Singh, R. Heelan, V. Rusch, L. Fulton, E. Mardis, D. Kupfer, R. Wilson, M. Kris, H. Varmus, EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 13306–13311 (2004).
4. J. G. Paez, P. A. Jänne, J. C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F. J. Kaye, N. Lindeman, T. J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M. J. Eck, W. R. Sellers, B. E. Johnson, M. Meyerson, *EGFR* mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
5. T. J. Lynch, D. W. Bell, R. Sordella, S. Gurubhagavatula, R. A. Okimoto, B. W. Brannigan, P. L. Harris, S. M. Haserlat, J. G. Supko, F. G. Haluska, D. N. Louis, D. C. Christiani, J. Settleman, D. A. Haber, Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350**, 2129–2139 (2004).
6. T. S. Mok, Y. L. Wu, S. Thongprasert, C. H. Yang, D. T. Chu, N. Saijo, P. Sunpaweravong, B. Han, B. Margono, Y. Ichinose, Y. Nishiwaki, Y. Ohe, J. J. Yang, B. Chewaskulyong, H. Jiang, E. L. Duffield, C. L. Watkins, A. A. Armour, M. Fukuoka, Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* **361**, 947–957 (2009).
7. M. Soda, Y. L. Choi, M. Enomoto, S. Takada, Y. Yamashita, S. Ishikawa, S. Fujiwara, H. Watanabe, K. Kurashina, H. Hatanaka, M. Bando, S. Ohno, Y. Ishikawa, H. Aburatani, T. Niki, Y. Sohara, Y. Sugiyama, H. Mano, Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer. *Nature* **448**, 561–566 (2007).
8. E. L. Kwak, Y. J. Bang, D. R. Camidge, A. T. Shaw, B. Solomon, R. G. Maki, S. H. Ou, B. J. Dezube, P. A. Jänne, D. B. Costa, M. Varella-Garcia, W. H. Kim, T. J. Lynch, P. Fidias, H. Stubbs, J. A. Engelman, L. V. Sequist, W. Tan, L. Gandhi, M. Mino-Kenudson, G. C. Wei, S. M. Shreeve, M. J. Ratain, J. Settleman, J. G. Christensen, D. A. Haber, K. Wilner, R. Salgia, G. I. Shapiro, J. W. Clark, A. J. Iafrate, Anaplastic lymphoma kinase inhibition in non–small-cell lung cancer. *N. Engl. J. Med.* **363**, 1693–1703 (2010).
9. S. A. Khuder, Effect of cigarette smoking on major histological types of lung cancer: A meta-analysis. *Lung Cancer* **31**, 139–148 (2001).
10. A. J. Bass, H. Watanabe, C. H. Mermel, S. Yu, S. Perner, R. G. Verhaak, S. Y. Kim, L. Wardwell, P. Tamayo, I. Gat-Viks, A. H. Ramos, M. S. Woo, B. A. Weir, G. Getz, R. Beroukhim, M. O'Kelly, A. Dutt, O. Rozenblatt-Rosen, P. Dziunycz, J. Komisarof, L. R. Chirieac, C. J. Lafargue, V. Scheble, T. Wilbertz, C. Ma, S. Rao, H. Nakagawa, D. B. Stairs, L. Lin, T. J. Giordano, P. Wagner, J. D. Minna, A. F. Gazdar, C. Q. Zhu, M. S. Brose, I. Cecconello, U. Ribeiro Jr., S. K. Marie, O. Dahl, R. A. Shivdasani, M. S. Tsao, M. A. Rubin, K. K. Wong, A. Regev, W. C. Hahn, D. G. Beer, A. K. Rustgi, M. Meyerson, *SOX2* is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. *Nat. Genet.* **41**, 1238–1242 (2009).
11. R. Pirker, J. R. Pereira, A. Szczesna, J. von Pawel, M. Krzakowski, R. Ramlau, I. Vynnychenko, K. Park, C. T. Yu, V. Ganul, J. K. Roh, E. Bajetta, K. O'Byrne, F. de Marinis, W. Eberhardt, T. Goddemeier, M. Emig, U. Gatzemeier; FLEX Study Team, Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): An open-label randomised phase III trial. *Lancet* **373**, 1525–1531 (2009).
12. A. Sandler, R. Gray, M. C. Perry, J. Brahmer, J. H. Schiller, A. Dowlati, R. Lilenbaum, D. H. Johnson, Paclitaxel–carboplatin alone or with bevacizumab for non–small-cell lung cancer. *N. Engl. J. Med.* **355**, 2542–2550 (2006).
13. R. Beroukhim, G. Getz, L. Nghiemphu, J. Barretina, T. Hsueh, D. Linhart, I. Vivanco, J. C. Lee, J. H. Huang, S. Alexander, J. Du, T. Kau, R. K. Thomas, K. Shah, H. Soto, S. Perner, J. Prensner, R. M. Debiasi, F. Demichelis, C. Hatton, M. A. Rubin, L. A. Garraway, S. F. Nelson, L. Liau, P. S. Mischel, T. F. Cloughesy, M. Meyerson, T. A. Golub, E. S. Lander, I. K. Mellinghoff, W. R. Sellers, Assessing the significance of chromosomal aberrations in cancer: Methodology and application to glioma. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 20007–20012 (2007).
14. R. Beroukhim, C. H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, J. Barretina, J. S. Boehm, J. Dobson, M. Urashima, K. T. Mc Henry, R. M. Pinchback, A. H. Ligon, Y. J. Cho, L. Haery, H. Greulich, M. Reich, W. Winckler, M. S. Lawrence, B. A. Weir, K. E. Tanaka, D. Y. Chiang, A. J. Bass, A. Loo, C. Hoffman, J. Prensner, T. Liefeld, Q. Gao, D. Yecies, S. Signoretti, E. Maher, F. J. Kaye, H. Sasaki, J. E. Tepper, J. A. Fletcher, J. Tabernero, J. Baselga, M. S. Tsao, F. Demichelis, M. A. Rubin, P. A. Janne, M. J. Daly, C. Nucera, R. L. Levine, B. L. Ebert, S. Gabriel, A. K. Rustgi, C. R. Antonescu, M. Ladanyi, A. Letai, L. A. Garraway, M. Loda, D. G. Beer, L. D. True, A. Okamoto, S. L. Pomeroy, S. Singer, T. R. Golub, E. S. Lander, G. Getz, W. R. Sellers, M. Meyerson, The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899–905 (2010).

15. B. A. Weir, M. S. Woo, G. Getz, S. Perner, L. Ding, R. Beroukhim, W. M. Lin, M. A. Province, A. Kraja, L. A. Johnson, K. Shah, M. Sato, R. K. Thomas, J. A. Barletta, I. B. Borecki, S. Broderick, A. C. Chang, D. Y. Chiang, L. R. Chirieac, J. Cho, Y. Fujii, A. F. Gazdar, T. Giordano, H. Greulich, M. Hanna, B. E. Johnson, M. G. Kris, A. Lash, L. Lin, N. Lindeman, E. R. Mardis, J. D. McPherson, J. D. Minna, M. B. Morgan, M. Nadel, M. B. Orringer, J. R. Osborne, B. Ozenberger, A. H. Ramos, J. Robinson, J. A. Roth, V. Rusch, H. Sasaki, F. Shepherd, C. Sougnez, M. R. Spitz, M. S. Tsao, D. Twomey, R. G. Verhaak, G. M. Weinstock, D. A. Wheeler, W. Winckler, A. Yoshizawa, S. Yu, M. F. Zakowski, Q. Zhang, D. G. Beer, I. I. Wistuba, M. A. Watson, L. A. Garraway, M. Ladanyi, W. D. Travis, W. Pao, M. A. Rubin, S. B. Gabriel, R. A. Gibbs, H. E. Varmus, R. K. Wilson, E. S. Lander, M. Meyerson, Characterizing the cancer genome in lung adenocarcinoma. *Nature* **450**, 893–898 (2007).
16. C. Greenman, P. Stephens, R. Smith, G. L. Dalgliesh, C. Hunter, G. Bignell, H. Davies, J. Teague, A. Butler, C. Stevens, S. Edkins, S. O'Meara, I. Vastrik, E. E. Schmidt, T. Avis, S. Barthorpe, G. Bhamra, G. Buck, B. Choudhury, J. Clements, J. Cole, E. Dicks, S. Forbes, K. Gray, K. Halliday, R. Harrison, K. Hills, J. Hinton, A. Jenkinson, D. Jones, A. Menzies, T. Mironenko, J. Perry, K. Raine, D. Richardson, R. Shepherd, A. Small, C. Tofts, J. Varian, T. Webb, S. West, S. Widaa, A. Yates, D. P. Cahill, D. N. Louis, P. Goldstraw, A. G. Nicholson, F. Brasseur, L. Looijenga, B. L. Weber, Y. E. Chiew, A. DeFazio, M. F. Greaves, A. R. Green, P. Campbell, E. Birney, D. F. Easton, G. Chenevix-Trench, M. H. Tan, S. K. Khoo, B. T. Teh, S. T. Yuen, S. Y. Leung, R. Wooster, P. A. Futreal, M. R. Stratton, Patterns of somatic mutation in human cancer genomes. *Nature* **446**, 153–158 (2007).
17. M. L. Sos, K. Michel, T. Zander, J. Weiss, P. Frommolt, M. Peifer, D. Li, R. Ullrich, M. Koker, F. Fischer, T. Shimamura, D. Rauh, C. Mermel, S. Fischer, I. Stückrath, S. Heynck, R. Beroukhim, W. Lin, W. Winckler, K. Shah, T. LaFramboise, W. F. Moriarty, M. Hanna, L. Tolosi, J. Rahnenführer, R. Verhaak, D. Chiang, G. Getz, M. Hellmich, J. Wolf, L. Girard, M. Peyton, B. A. Weir, T. H. Chen, H. Greulich, J. Barretina, G. I. Shapiro, L. A. Garraway, A. F. Gazdar, J. D. Minna, M. Meyerson, K. K. Wong, R. K. Thomas, Predicting drug susceptibility of non–small cell lung cancers based on genetic lesions. *J. Clin. Invest.* **119**, 1727–1740 (2009).
18. U. McDermott, S. V. Sharma, L. Dowell, P. Greninger, C. Montagut, J. Lamb, H. Archibald, R. Raudales, A. Tam, D. Lee, S. M. Rothenberg, J. G. Supko, R. Sordella, L. E. Ulkus, A. J. Iafrate, S. Maheswaran, C. N. Njauw, H. Tsao, L. Drew, J. H. Hanke, X. J. Ma, M. G. Erlander, N. S. Gray, D. A. Haber, J. Settleman, Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 19936–19941 (2007).
19. M. Mohammadi, S. Froum, J. M. Hamby, M. C. Schroeder, R. L. Panek, G. H. Lu, A. V. Eliseenkova, D. Green, J. Schlessinger, S. R. Hubbard, Crystal structure of an angiogenesis inhibitor bound to the FGF receptor tyrosine kinase domain. *EMBO J.* **17**, 5896–5904 (1998).
20. M. L. Sos, S. Fischer, R. Ullrich, M. Peifer, J. M. Heuckmann, M. Koker, S. Heynck, I. Stückrath, J. Weiss, F. Fischer, K. Michel, A. Goel, L. Regales, K. A. Politi, S. Perera, M. Getlik, L. C. Heukamp, S. Ansén, T. Zander, R. Beroukhim, H. Kashkar, K. M. Shokat, W. R. Sellers, D. Rauh, C. Orr, K. P. Hoeflich, L. Friedman, K. K. Wong, W. Pao, R. K. Thomas, Identifying genotype-dependent efficacy of single and combined PI3K- and MAPK-pathway inhibition in cancer. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 18351–18356 (2009).
21. L. Marek, K. E. Ware, A. Fritzsche, P. Hercule, W. R. Helton, J. E. Smith, L. A. McDermott, C. D. Coldren, R. A. Nemenoff, D. T. Merrick, B. A. Helfrich, P. A. Bunn Jr., L. E. Heasley, Fibroblast growth factor (FGF) and FGF receptor-mediated autocrine signaling in non-small-cell lung cancer cells. *Mol. Pharmacol.* **75**, 196–207 (2009).
22. W. Zhou, W. Hur, U. McDermott, A. Dutt, W. Xian, S. B. Ficarro, J. Zhang, S. V. Sharma, J. Brugge, M. Meyerson, J. Settleman, N. S. Gray, A structure-guided approach to creating covalent FGFR inhibitors. *Chem. Biol.* **17**, 285–295 (2010).
23. S. Blencke, B. Zech, O. Engkvist, Z. Greff, L. Orfi, Z. Horváth, G. Kéri, A. Ullrich, H. Daub, Characterization of a conserved structural determinant controlling protein kinase sensitivity to selective inhibitors. *Chem. Biol.* **11**, 691–701 (2004).
24. G. Tonon, K. K. Wong, G. Maulik, C. Brennan, B. Feng, Y. Zhang, D. B. Khatry, A. Protopopov, M. J. You, A. J. Aguirre, E. S. Martin, Z. Yang, H. Ji, L. Chin, R. A. Depinho, High-resolution genomic profiles of human lung cancer. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 9625–9630 (2005).
25. K. Rikova, A. Guo, Q. Zeng, A. Possemato, J. Yu, H. Haack, J. Nardone, K. Lee, C. Reeves, Y. Li, Y. Hu, Z. Tan, M. Stokes, L. Sullivan, J. Mitchell, R. Wetzel, J. Macneill, J. M. Ren, J. Yuan, C. E. Bakalarski, J. Villen, J. M. Kornhauser, B. Smith, D. Li, X. Zhou, S. P. Gygi, T. L. Gu, R. D. Polakiewicz, J. Rush, M. J. Comb, Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* **131**, 1190–1203 (2007).
26. U. McDermott, R. Y. Ames, A. J. Iafrate, S. Maheswaran, H. Stubbs, P. Greninger, K. McCutcheon, R. Milano, A. Tam, D. Y. Lee, L. Lucien, B. W. Brannigan, L. E. Ulkus, X. J. Ma, M. G. Erlander, D. A. Haber, S. V. Sharma, J. Settleman, Ligand-dependent platelet-derived growth factor receptor (PDGFR)-α activation sensitizes rare lung cancer and sarcoma cells to PDGFR kinase inhibitors. *Cancer Res.* **69**, 3937–3946 (2009).
27. A. H. Ramos, A. Dutt, C. Mermel, S. Perner, J. Cho, C. J. Lafargue, L. A. Johnson, A. C. Stiedl, K. E. Tanaka, A. J. Bass, J. Barretina, B. A. Weir, R. Beroukhim, R. K. Thomas, J. D. Minna, L. R. Chirieac, N. I. Lindeman, T. Giordano, D. G. Beer, P. Wagner, I. I. Wistuba, M. A. Rubin, M. Meyerson, Amplification of chromosomal segment 4q12 in non-small cell lung cancer. *Cancer Biol. Ther.* **8**, 2042–2050 (2009).
28. X. Zhao, B. A. Weir, T. LaFramboise, M. Lin, R. Beroukhim, L. Garraway, J. Beheshti, J. C. Lee, K. Naoki, W. G. Richards, D. Sugarbaker, F. Chen, M. A. Rubin, P. A. Jänne, L. Girard, J. Minna, D. Christiani, C. Li, W. R. Sellers, M. Meyerson, Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. *Cancer Res.* **65**, 5561–5570 (2005).
29. J. S. Reis-Filho, P. T. Simpson, N. C. Turner, M. B. Lambros, C. Jones, A. Mackay, A. Grigoriadis, D. Sarrio, K. Savage, T. Dexter, M. Iravani, K. Fenwick, B. Weber, D. Hardisson, F. C. Schmitt, J. Palacios, S. R. Lakhani, A. Ashworth, *FGFR1* emerges as a potential therapeutic target for lobular breast carcinomas. *Clin. Cancer Res.* **12**, 6652–6662 (2006).
30. N. Turner, R. Grose, Fibroblast growth factor signalling: From development to cancer. *Nat. Rev. Cancer* **10**, 116–129 (2010).
31. N. Turner, A. Pearson, R. Sharpe, M. Lambros, F. Geyer, M. A. Lopez-Garcia, R. Natrajan, C. Marchio, E. Iorns, A. Mackay, C. Gillett, A. Grigoriadis, A. Tutt, J. S. Reis-Filho, A. Ashworth, *FGFR1* amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. *Cancer Res.* **70**, 2085–2094 (2010).
32. http://www.broadinstitute.org/igv/.
33. S. Perner, P. L. Wagner, A. Soltermann, C. LaFargue, V. Tischler, B. A. Weir, W. Weder, M. Meyerson, T. J. Giordano, H. Moch, M. A. Rubin, TTF1 expression in non-small cell lung carcinoma: Association with *TTF1* gene amplification and improved survival. *J. Pathol.* **217**, 65–72 (2009).
34. http://www.broadinstitute.org/cancer/software/genepattern/.
35. M. L. Sos, H. B. Rode, S. Heynck, M. Peifer, F. Fischer, S. Klüter, V. G. Pawar, C. Reuter, J. M. Heuckmann, J. Weiss, L. Ruddigkeit, M. Rabiller, M. Koker, J. R. Simard, M. Getlik, Y. Yuza, T. H. Chen, H. Greulich, R. K. Thomas, D. Rauh, Chemogenomic profiling provides insights into the limited activity of irreversible EGFR inhibitors in tumor cells expressing the T790M EGFR resistance mutation. *Cancer Res.* **70**, 868–874 (2010).

36. **Acknowledgments:** We thank M. Meyerson and A. Bass for sharing unpublished data and C. Reinhardt and A. Ullrich for discussion. **Funding:** This work was supported by the Deutsche Krebshilfe (grant 107954 to R.K.T.); the German Ministry of Science and Education (BMBF) as part of the NGFNplus program (grants 01GS08100 to R.K.T. and 01GS08101 to J. Wolf and P.N.); the Max Planck Society (M.I.F.A.NEUR8061 to R.K.T.); the Deutsche Forschungsgemeinschaft (DFG) through SFB (TP6 to R.K.T. and R.T.U.; TP5 to L.C.H. and R. Buettner); the Ministry for Innovation, Science, Research and Technology of the State of Nordrhein-Westfalen (MIWT, 4000-12 09 to R.K.T. and B.K.); and an anonymous foundation to R.K.T. E.B. and C.B. were supported by the PNES INCA grant 2008. B.S. was supported by the International Association for the Study of Lung Cancer Young Investigator Award. W.P. was supported by a grant from the NIH National Cancer Institute. Z.W. was supported by the Royal Australasian College of Surgeons and a Raelene Boyle Scholarship. G.W. was supported by the Australasian Society of Cardiac and Thoracic Surgeons Foundation Grant, Peter MacCallum Foundation Grant, and a private research donation from family and friends of former patients. **Author contributions:** J. Weiss and M.L.S. designed and performed the experiments, analyzed the data, and wrote the manuscript. D.S. designed the experiments and analyzed the data. M.P., T.Z., F.L., and D.R. analyzed the data. J.M.H., R.T.U., R.M., S.M., F.F., S.H., M.K., J. Schöttle, F.G., I.D., S.Q., L.C.H., H.B.-W., I.B., and J.A. performed the experiments and discussed the data. A.S., H.M., P.W., S.A., Z.W., M.C., G.W., P.R., B.S., E.B., C.B., P.L., S.S., O.T.B., W.E.-R., C.L., I.P., J. Sänger, J.C., H.G., W.T., H.S., E.T., E. Smit, D.H., F.C., C.L., S.D., M.H., R. Beroukhim, W.P., B.K., M.B., R. Buettner, K.E., E. Stoelben, J. Wolf, and P.N. contributed critical tumor specimens and contributed to the discussion of the data. S.P. reviewed tumor histology, analyzed the FISH data, and wrote the manuscript. R.K.T. conceived the project, designed the experiments, analyzed the data, and wrote the manuscript. **Competing interests:** R.K.T. receives consulting and lecture fees from Sequenom, Sanofi-Aventis, Merck, Roche, Infinity, Boehringer, AstraZeneca, and ATLAS Biolabs, as well as research support from Novartis and AstraZeneca. J. Wolf is a member of advisory boards of Roche, AstraZeneca, Novartis, Amgen, Bayer, and Merck and has received lecture fees from Roche, AstraZeneca, and Merck. R. Beroukhim receives consulting fees for Novartis Institutes for BioMedical Research. W.P. receives consulting fees from Molecular MD, AstraZeneca, BMS, and Symphony Evolution. H.G. received research support from Eli Lilly, Roche, and Boehringer Ingelheim through the University Medical Center Groningen. The other authors declare that they have no competing interests. **Accession numbers:** All raw data are publicly available (GEO; GSE25016).

Submitted 6 July 2010
Accepted 24 November 2010
Published 15 December 2010
10.1126/scitranslmed.3001451

Citation: J. Weiss, M. L. Sos, D. Seidel, M. Peifer, T. Zander, J. M. Heuckmann, R. T. Ullrich, R. Menon, S. Maier, A. Soltermann, H. Moch, P. Wagener, F. Fischer, S. Heynck, M. Koker, J. Schöttle, F. Leenders, F. Gabler, I. Dabow, S. Querings, L. C. Heukamp, H. Balke-Want, S. Ansén, D. Rauh, I. Baessmann, J. Altmüller, Z. Wainer, M. Conron, G. Wright, P. Russell, B. Solomon, E. Brambilla, C. Brambilla, P. Lorimier, S. Sollberg, O. T. Brustugun, W. Engel-Riedel, C. Ludwig, I. Petersen, J. Sänger, J. Clement, H. Groen, W. Timens, H. Sietsma, E. Thunnissen, E. Smit, D. Heideman, F. Cappuzzo, C. Ligorio, S. Damiani, M. Hallek, R. Beroukhim, W. Pao, B. Klebl, M. Baumann, R. Buettner, K. Ernestus, E. Stoelben, J. Wolf, P. Nürnberg, S. Perner, R. K. Thomas, Frequent and focal *FGFR1* amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci. Transl. Med.* **2**, 62ra93 (2010).