Supplementary Methods

Genetically Engineered mice

We adapted the transgenic system described by Beard et. al. (1) to knock in a single copy of human *EML4-ALK* fusion variant 1 into the mouse *collagen 1a* locus (Fig. S1A). Successful recombination of the targeting locus was confirmed by PCR. Mouse blastocyst injections resulted in high percentage of chimeras that successfully gave germline transmission. Mice were engineered to simultaneously express a *ccsp-rtTA* allele (2), a transgenic allele encoding a reversed Tet-OP activator under control of the lung type II epithelial cell-specific surfactant protein CCSP promoter. A doxycycline-containing diet (Research Diets, Inc.) was administered to induce EML4-ALK expression in lung type II epithelial cells.

Compounds

TAE684 and WZ4002 were synthesized according to published procedures in Dr. Nathanael Gray's lab (2, 3). The structure of TAE684 was confirmed using liquid chromatography-mass spectrometry and 1H and 13C nuclear magnetic resonance. The synthesized TAE684 was determined to be 98% pure by 1H nuclear magnetic resonance and 99% pure by liquid chromatography-mass spectrometry monitoring at 210 and 254 nm wavelengths. AZD6244 was synthesized by Otava chemicals. NVP-BEZ235, 17-AAG and 17-DMAG were obtained from LC laboratories. Carboplatin was purchased from Sigma. Paclitaxel was purchased from Dana-Farber Cancer Institute's pharmacy. All drugs for *in vitro* studies were dissolved in DMSO, stored at -70°C, and diluted in fresh medium before use.

Antibodies, Western blotting, Immunoprecipitation and Immunohistochemistry

Antibodies recognizing ALK, phospho-ALK (Tyr1604), Akt, phospho-Akt (Ser473), S6, phospho-S6 (Thr389), and PARP were obtained from Cell Signaling Technology. Total ERK1/2 and phospho-ERK1/2 (pT185/pY187) antibodies were purchased from Biosource International. The anti-α-tubulin and Flag-M2 antibodies were purchased from Sigma-Aldrich. Hsc70 (Hsp73) polyclonal, Grp78 (BiP) polyclonal, Hsp70 (Hsp72) monoclonal and Hsp90 monoclonal antibodies were purchased from Assay Designs. Cdc37 (E-4) antibody was purchased from Santa Cruz Biotechnology. Cells were lysed in buffer containing protease and phosphatase inhibitors. Proteins were separated by gel electrophoresis on 4%-12% or 8% polyacrylamide gels, depending on the molecular weight of the protein of interest, transferred to polyvinylidene difluoride membranes, and detected by immunoblotting using an enhanced chemiluminescence system (Perkin-Elmer), as previously described (4). For immunoprecipitation, Flag-M2-agarose beads were used according to the manusfacturer's protocol. The ALK rabbit monoclonal antibody D5F3 was obtained from Cell Signaling Technology (5). Immunohistochemistry was performed as previously described (5).

Cell culture

H3122 cells (4) were maintained in RPMI 1640 (Sigma) supplemented with 10% fetal bovine serum, 100 units/mL streptomycin, and 1 mmol/L sodium pyruvate.

Cell proliferation and growth assays

Growth inhibition was assessed by MTS assay, as previously described (4). NSCLC cells were exposed to drugs alone or in combination for 72 h. All experimental points

were set up in 6 to 12 wells as triplicates. The data were graphically displayed using GraphPad Prism version 5.00 for Windows (GraphPad Software). The curves were fitted using a nonlinear regression model with a sigmoidal dose response. In clonogenic assays, cells were treated with increasing doses of AZD6244 (0.1, 0.3 or 1.0 μM), BEZ235 (0.02, 0.06 or 0.2 μM) or a combination of both in fixed ratio (AZD/BEZ: 0.1/0.02, 0.3/0.06 or 1.0/0.2) for 14 days, and the number of colonies was counted.

LC/MS Analysis of EML4-ALK interactors.

Eluted proteins were denatured by adding RapiGest (Waters, Milford, MA) to a final concentration of 0.1% and reduced by adding dithiothreitol (DTT) to a final concentration of 10 Mm, followed by incubation at 56º C for 30 minutes. Trypsin (2.5 μg) was added and digestion was performed at 37º C overnight. RapiGest was cleaved by adding trifluoroacetic acid (TFA) to a final concentration of 0.5% and precipitated by centrifugation at 14,000 rpm for 10 minutes at room temperature. The resulting peptide solution was desalted by C_{18} chromatography in batch mode. Eluted peptides were concentrated by vacuum centrifugation and solubilized in 500 mM tri-ethyl ammonium bicarbonate. Excess HA peptide was removed by incubating desalted peptide solution with activated thiol sepharose 4B (GE Healthcare) for one hour at room temperature. The supernatant was desalted by C_{18} chromatography; desalted peptides were resuspended in strong cation exchange (SCX) buffer A (25% acetonitrile, 0.1% formic acid) and fractionated by SCX chromatography (in batch mode) using a step gradient elution method with SCX buffer A supplemented with KCl concentrations of 100, 200 and 300 mM.

After vacuum concentration, SCX flow-through and eluates were individually loaded onto a precolumn (100 μm I.D.; packed with 4 cm POROS 10R2, Applied Biosystems) at a flow rate of 4 μL/min for 15 minutes using a NanoAcquity Sample Manager (20 μL sample loop) and UPLC pump (Waters). After loading, the peptides were gradient eluted (1-30% B in 45 minutes; A=0.1% aqueous formic acid, B=0.1% formic acid in acetonitrile) at a flow rate of ~100 nL/min to an analytical column (50 μm I.D. packed with 12 cm Monitor 5 μm C18 from Column Engineering, Ontario, CA), and introduced into an LTQ-Orbitrap XL mass spectrometer (ThermoFisher Scientific) by electrospray ionization (spray voltage = 2200V). The mass spectrometer was programmed to operate in data dependent mode, such that the top 8 most abundant precursors in each MS scan (detected in the Orbitrap mass analyzer, resolution = 60,000) were subjected to MS/MS (CAD, electron multiplier detection, collision energy = 35%, isolation width = 3.0 Da, threshold = 10,000). Dynamic exclusion was enabled with a repeat count of 1 and a repeat duration of 30 seconds.

Database Searching. Orbitrap data files were directly accessed and converted to .mgf using in-house software (6). Files were searched using Mascot version 2.2.1 against a human protein subset of the NCBI nr database. Search parameters specified a precursor ion mass tolerance of 25 ppm, a product ion mass tolerance of 0.6 Da, variable deamidation (NQ, -1 Da) and oxidation (M, +16 Da). False discovery rates (FDR) were evaluated by performing a search with a reverse database and were calculated using the formula: estimated FDR = reverse database identifications/ forward database identifications. Protein list were filtered by removing common lab contaminants (such as trypsin and keratin). “Unique peptides” reported in the “protein report” files correspond to peptides (accounting for variable modifications) with a score below a 1% FDR cut-off.

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4. Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res. 2008;14:4275-83.

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Supplementary Figure Legends

Supplementary Figure S1. Expression of human *EML4-ALK* fusion gene in mouse lung epithelial cells leads to rapid oncogenesis. A. Targeting construct for creating the *EML4-ALK* single copy knock-in transgenic mouse. Expression of the *EML4-ALK* transgene is under direction of the doxycycline inducible Tet-op promoter.

B. Induction of EML4-ALK expression by doxycycline causes rapid development of lung adenocarcinoma. Tumor formation is confirmed by MRI imaging (top left), and histology (top middle). Expression of EML4-ALK is demonstrated by immunohistochemical staining with an anti-ALK antibody (top right). Withdrawal of doxycycline leads to complete tumor regression (bottom left), restoration of normal lung structure (bottom middle), and loss of EML4-ALK fusion expression (bottom right). Scale bar=100μm. **C.** Body weight changes in EML4-ALK mice after administration of a doxycycline-containing diet and subsequent TAE684 treatment. Mouse body weights were documented weekly. Littermate controls were measured at 3 weeks after doxycycline induction, n=5; bitransgenic mice were measured at 3.5 weeks after doxycycline induction, n=17; and at 7 weeks after doxycycline induction, n=10. Six mice from the group that had received 7 weeks of a doxycycline-containing diet were then treated with TAE684, and body weights were documented after 3.5 weeks and 7 weeks of treatment. **D,** Kaplan-Meier survival analysis of *EML4-ALK*-driven lung cancer mouse model. *EML4-ALK* and *CCSP-rtTA* bitransgenic mice were fed with a doxycycline-containing diet beginning at 4-6 weeks of age. X-axis indicates time after doxycycline induction.

Supplementary Figure S2. Cross-species analysis of molecular profiles of *EML4-ALK* and *EGFR*-driven lung cancer. **A**, Unsuperivsed clustering of mouse lung tumor samples harboring *EML4-ALK* rearrangement, or *EGFR-L858R* or *EGFR-Del19* mutations. **B**, Unsuperivsed clustering of human lung cancer patient tumor samples harboring *EML4-ALK* rearrangement, or *EGFR-L858R* or *EGFR-Del19* mutations. **C**, Genes from *EML4-ALK* tumor samples with fold change > 2 and an

FDR *p*-value < 0.05 when compared to *EGFR* tumors were considered components of up or downregulated signatures. Significant correlation for these molecular signatures is observed between mouse and human data sets upon cross-species GSEA analysis.

Supplementary Figure S3. PET-CT scans of EML4-ALK tumor-bearing mice before and after WZ4002 or TAE684 treatment. Levels of ^{18}F-FDG uptake are indicated by color scales as shown in the figure. Signals in the mouse lung regions before treatment are outlined in white. Top, baseline signal before treatment, showing signals of lung of a EML4-ALK tumor bearing mouse; middle, same mouse was treated with 2 doses of WZ4002 as a negative control; bottom, mouse was treated with 2 doses of TAE684.

Supplementary Figure S4. **The amino acid sequence of A**, EML4; **B**, ALK; **C**, HSP70 protein 5; and **D**, HSP70 protein 8. The residues detected by mass spectrometry are shown in red characters. The protein sequence included in EML4-ALK variant 1 fusion proteins are underlined.