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Sunitinib Prolongs Survival in Genetically Engineered Mouse Models of Multistep Lung Carcinogenesis

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Abstract

Non–small cell lung cancer (NSCLC) has a poor prognosis, with substantial mortality rates even among patients diagnosed with early-stage disease. There are few effective measures to block the development or progression of NSCLC. Antiangiogenic drugs represent a new class of agents targeting multiple aspects of tumor progression, including cell proliferation, invasion, migration, and outgrowth of metastatic deposits. We tested the multitargeted angiogenesis inhibitor sunitinib in a novel endogenous mouse model of NSCLC, which expresses a conditional activating mutation in *Kras* with or without conditional deletion of *Lkb1*; both alterations are frequent in human NSCLC. We showed that daily treatment with sunitinib reduced tumor size, caused tumor necrosis, blocked tumor progression, and prolonged median survival in both the metastatic (*Lkb1/Kras*) and nonmetastatic (*Kras*) mouse models; median survival was not reached in the nonmetastatic model after 1 year. However, the incidence of local and distant metastases was similar in sunitinib-treated and untreated *Lkb1/Kras* mice, suggesting that prolonged survival with sunitinib in these mice was due to direct effects on primary tumor growth rather than to inhibition of metastatic progression. These collective results suggest that the use of angiogenesis inhibitors in early-stage disease for prevention of tumor development and growth may have major survival benefits in the setting of NSCLC.

Non–small cell lung cancer (NSCLC) represents about 85% of all lung cancer and frequently has a dismal outcome. Even among early-stage patients, relapse rates are significant. The relapse rate among stage I/II patients is 35% to 67% (1) and nearly all patients who relapse, frequently with metastatic disease, go on to die of their lung cancer. Therefore, identifying targets for early interventions designed to prevent tumor development and suppress early tumor progression could favorably affect the survival rates of NSCLC patients.

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Cytotoxic therapies in the metastatic setting have made marginal improvements in progression-free or overall survival. A recent meta-analysis showed a 1.5-month increase in median survival over best supportive care alone, with no improvement in benefit since the prior analysis in 1995 (2). In the adjuvant setting, multiple large-scale trials have shown a reduction in the risk of relapse of only 5% to 9%, showing that these drugs have little effect in preventing local or distant recurrences (3-6).

Angiogenesis inhibitors represent a new class of drugs geared to interfere with the genesis of tumor vasculature, with the potential to affect not only primary tumor growth but also invasion through the basement membrane to seed cells into the bloodstream, extravasation into tissues, and outgrowth of metastatic deposits, all of which are critical components of tumor progression, relapse, and death.

Several antiangiogenic agents have been developed and are currently in use in cancer therapy for a wide variety of solid tumors. The most extensively studied is bevacizumab (Avastin, Genentech), an antibody that targets vascular endothelial growth factor (VEGF) and has been shown to prolong survival when combined with chemotherapy in metastatic colorectal and NSCLC (5,6).

Newer drugs from this class of agents include multitargeted small-molecule tyrosine kinase inhibitors that block one or more receptors of VEGF. One of the most well-characterized agents is sunitinib (Sutent), a small-molecule tyrosine kinase inhibitor that targets several molecules important for angiogenesis and metastatic progression. Sunitinib has high potency against VEGFR1, VEGFR2, and VEGFR3 and therefore can inhibit other ligands in addition to VEGF, such as placental growth factor, a ligand of VEGFR1, which can rescue vessel growth in the setting of VEGF inhibition (7). Sunitinib also targets platelet-derived growth factor receptor (PDGFR), which is a critical growth factor for pericytes, which in turn are important for vessel formation (8-10). Like bevacizumab, sunitinib has shown antiangiogenic effects *in vitro* that include inhibition of human umbilical endothelial cell proliferation, migration, and capillary tube formation (11) as well as antiangiogenic effects *in vivo*, such as decreased microvessel density and direct inhibition of xenograft growth of some human tumor cell lines (12).

Sunitinib has already been shown to be a clinically effective agent for metastatic renal cell carcinoma and metastatic gastrointestinal stromal tumors, where it has already been Food and Drug Administration approved for clinical use (reviewed in refs. 13,14). In NSCLC, activity has been shown recently in a phase II trial of previously treated patients with progressive disease (15). In this study, the overall disease control rate was 38.7%, of which 28.6% represented stable disease. However, there was no effect on time to progression or overall survival.

Given the multitude of antiangiogenic activities of sunitinib, these somewhat disappointing trial results, which are typical of many antiangiogenic agents in lung cancer, may speak to our limited understanding of the appropriate setting in which these agents can be effective. Most trials of sunitinib and other antiangiogenic agents have been conducted in the metastatic setting, where even the greatest overall effect on survival has been small despite the multiple potential antitumor activities of these drugs.

Although adjuvant clinical trials using these agents are under way in earlier stages of tumor progression, the length of time required for these trials is long and the ability to measure direct target effects is limited. Traditional mouse models using xenografts of human tumors or orthotopic models involving implantation or preseeding of tumor cells into the bloodstream are also limited in their ability to fully evaluate the effects of antiangiogenic drugs as they do not fully recapitulate all of the steps of cancer progression from abnormal growth to primary tumor development to distant metastases.

Our laboratory has developed several *de novo* mouse models of lung cancer tumor progression that recapitulate the whole spectrum of tumor progression—from dysplasia to tumor development (16-21). More recently, we have developed a mouse model of metastatic lung cancer driven by conditionally activated oncogenic *Kras* and concurrent tumor suppressor *Lkb1* loss that has a >60% penetrance of regional and distant metastasis (18). This model, along with similarly derived models that do not metastasize, like that driven by activated oncogenic *Kras* alone (16,22), provides an ideal setting to fully evaluate the potential of antiangiogenic drugs to inhibit each step of tumor progression and metastatic development and enable rational clinical trial design to take full advantage of defined anticancer activities. In the studies described here, we examined the effect of sunitinib on tumor development, growth, and progression and on survival in endogenous mouse models of nonmetastatic and metastatic NSCLC.

Materials and Methods

Mouse treatment and tumor processing

All mice were housed and treated in accordance with protocols approved by the institutional care and use committees for animal research at the Dana-Farber Cancer Institute. Transgenic mice expressing conditional mutations in *Kras* were initially generously provided by T. Jacks and *Kras* mice with homozygous deletion of *Lkb1* were generated as previously described (18). Mice were treated with 5×10^6 plaque-forming units of adeno-Cre (purchased from University of Iowa adenoviral core) intranasally at 4 wk of age for *Kras/Lkb1^{L/L}* mice and at 6 wk of age for *Kras* mice. Sunitinib was purchased in capsular form commercially. For assessment of short-term effects on intracellular signaling, *Kras/Lkb1^{L/L}* mice were treated a single dose of sunitinib at 40 mg/kg by oral gavage at 7 wk following adeno-Cre treatment, a time when multiple primary lung tumors are readily visible (18). Mice were sacrificed at time 0, 8, 12, or 24 h following this dose (two mice per time point). For long-term treatment trials, mice (both male and female) were randomized to treatment with either vehicle or sunitinib at 40 mg/kg given by daily oral gavage beginning at 4 wk (for *Kras/Lkb1^{L/L}* mice) or at 16 wk (for *Kras*-alone mice) following adeno-Cre treatment. “Early sunitinib” mice are *Kras/Lkb1^{L/L}* mice treated beginning at 2 wk following adeno-Cre treatment. Mice were sacrificed at the indicated time points or when they became visibly ill. One lung was snap frozen, whereas the other was fixed in 10% formalin. Visual inspection for metastases was done at the time of sacrifice and organs with evidence of metastasis were analyzed and sampled for microscopic examination.

Histology and immunohistochemistry

Paraffin embedding and sectioning at 5 μ m was done by the Core Rodent Histopathology facility at Dana-Farber/Brigham and Women’s Hospital. Sections were stained with H&E or with antibodies against VEGFR2 (Cell Signaling) at 1:200 dilution in EDTA buffer with pressure cooker antigen retrieval, CD31 (BD Biosciences) at 1:40 dilution after 10 min of proteinase digestion for antigen retrieval, or with Ki67 (Vector Laboratories) at 1:2,500 in citrate buffer with pressure cooker antigen retrieval.

Cell lines and proliferation assays

KW-634 and KW-857 are tumor-derived cell lines from *Kras/Lkb1^{L/L}* mice and KW-807 and KW-814 are tumor-derived cell lines from *Kras* mice; both of these also contained mutations in *p53*. All human NSCLC cells tested in proliferation assays were obtained from the American Type Culture Collection. H157 cells were a gift from P. Janne. Cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum at a density of 5,000 per well on 96-well plates, cultured in the presence of drugs or vehicle for 72 h, and subjected to the CCK-8

colorimetric assay (Dojindo Molecular Technologies) in duplicate samples according to the manufacturer's specifications.

Immunoblotting

Cells were grown in RPMI 1640 and treated with sunitinib or vehicle (DMSO) for 12 to 24 h $\pm$ recombinant VEGFA stimulation during the last 30 min of treatment. Whole-cell lysates were prepared in radioimmunoprecipitation assay buffer supplemented with protease and phosphatase inhibitor cocktails (Calbiochem). Protein concentrations were determined using a Coomassie protein assay kit (Pierce) or by quantitating β -actin with densitometric analysis using ImageJ software (NIH; refs. 23,24). Equivalent amounts (30 or 60 μ g) were subjected to SDS-PAGE on 4% to 12% gels (Invitrogen). Antibodies used for Western blotting were from Cell Signaling and include phospho-VEGFR2 (Tyr⁹⁵¹), VEGFR2 (55B11), phospho-AKT (Ser⁴⁷³), phospho-p44/42 mitogen-activated protein kinase (MAPK), PDGFR β (28E1), and β -actin.

Statistical analysis

Comparison of overall survival rates was done using a log-rank analysis and Kaplan-Meier survival curves were generated using GraphPad Prism 5 software. *P* values were calculated using a Mantel-Cox two-tailed test for significance.

Results

To determine whether sunitinib could directly affect the growth of and signaling within tumor cells, the effect of sunitinib treatment on the growth of tumor-derived cell lines from tumors isolated from *Kras/Lkb1* or *Kras*-alone conditional mutant mice was examined (Fig. 1A). Established human cell lines, A549 and H2126 (both LKB1-deficient cell lines), were also tested for comparison. None of these lines showed any significant inhibition of cell proliferation by sunitinib and IC₅₀ for growth inhibition was well over 1 μ mol/L. This contrasts with the IC₅₀ of paclitaxel, which showed an IC₅₀ of 30 to 50 nmol/L in the same lines (Fig. 1B). Evaluation of 102 additional established human NSCLC cell lines for sunitinib sensitivity (listed in Supplementary Table S1) showed only one NSCLC cell line to have an IC₅₀ of <1 μ mol/L. To evaluate whether sunitinib treatment affected intracellular signaling pathways regulating proliferation, immunoblotting of phosphorylated AKT or MAPK was done in these same tumor-derived cell lines and no changes were seen with or without treatment (Fig. 1C).

In addition to evaluating tumor-derived cell lines for phosphoprotein changes, *Kras/Lkb1* mice with established lung tumors were treated with sunitinib for short intervals to assess immediate effects on cell signaling. These mice were treated at 7 weeks following adeno-Cre treatment, a time when multiple primary lung tumors are readily visible (18). Figure 1C (*right*) shows no change in phosphorylation of AKT or of MAPK in whole lung lysate harvested after short-interval treatment of mice with sunitinib, further suggesting that sunitinib has no direct effect on commonly altered pathways in tumor cell growth. VEGF secretion by tumor cells has been postulated to exert a paracrine effect on adjacent endothelial cells to promote tumor angiogenesis (25); however, no differences were found in VEGF secretion by tumor cells treated with sunitinib or in serum from untreated or treated mice harboring *Kras/Lkb1*-driven lung cancers (data not shown). Notably, although inhibition of phosphorylation of VEGFR2 was seen in tumor-containing lung lysate after 24 hours of sunitinib treatment (Fig. 1D), only two cell lines derived from these tumors expressed minimal amounts of VEGFR2 (Fig. 1E) and no PDGFR β . No effect with sunitinib treatment in these cell lines was observed on VEGFR2 phosphorylation (data not shown). Together, these data imply that, in this model, sunitinib targeting may be primarily directed at stromal or endothelial cells within tumors rather than cancer cells themselves.

This absence of direct growth inhibition of tumor cells by sunitinib is consistent with the model of sunitinib action on tumor endothelium, bone marrow–derived endothelial progenitors, and potentially other cells involved in tumor angiogenesis. To examine global effects on tumor progression, longitudinal treatment trials were conducted in these mouse models of lung cancer development to evaluate effects on tumor progression, development of metastases, and overall survival (Table 1).

As previously shown (18), both *Kras* and *Kras/Lkb1^{L/L}* mice developed multiple poorly differentiated carcinomas within the lung (Fig. 2A and C). Histologic examination of tumors from both mouse models showed clear treatment effects of sunitinib within lung tumors, with dying cells visible in even small tumors from *Kras/Lkb1^{L/L}* mice (Fig. 2B) and central necrosis of larger tumors seen in *Kras* mice (Fig. 2D).

Kras mice, which develop only primary lung tumors, develop a mixture of adenomas and adenocarcinomas 16 weeks after adeno-Cre activation of oncogenic *Kras* and die within 24 to 26 weeks following activation (22). We used this model to examine the effects of sunitinib on primary tumor growth. Sixteen weeks after adeno-Cre administration, mice were randomized to receive either placebo treatment or sunitinib at 40 mg/kg by daily oral gavage. Strikingly, at every time point examined, sunitinib-treated mice showed fewer and smaller tumors (Fig. 3). Control mice had multiple visible primary tumors at 18 weeks after adeno-Cre activation of oncogenic *Kras* (Fig. 3A) that became larger and more invasive appearing by 27 weeks (Fig. 3B), in contrast to sunitinib-treated mice that had unremarkable normal lungs at 18 weeks (Fig. 3C) and developed only small tumor foci at 27 weeks (Fig. 3D). Several sunitinib-treated mice were censored at 52 weeks with only small tumor nodules and no evidence of symptoms (Fig. 3E). Overall survival was dramatically prolonged in sunitinib-treated mice compared with control mice (median survival not reached versus median survival of 26 weeks; $P = 0.0003$; Fig. 3F).

Because the described antiangiogenic activities of sunitinib would suggest both effects on primary tumor necrosis as well as on the development of metastases, effects on tumor progression were also examined in *Kras/Lkb1^{L/L}* mice. These mice develop tumors generally within 4 weeks of activation of conditional mutations and die within 8 to 10 weeks, with local and distant metastases (18). *Kras/Lkb1^{L/L}* mice were treated with sunitinib or vehicle starting at either 2 or 4 weeks following adeno-Cre activation of conditional mutations to address whether (a) sunitinib could inhibit the progression of disease in the setting of established primary tumors (4 weeks) and (b) early treatment with sunitinib in the course of tumor progression (2 weeks) could block the development of metastases by chemoprevention. Mice treated with sunitinib showed a similar delay in growth and progression of primary lung tumors as *Kras* mice. Figure 4 shows the progression of small tumor foci seen at 5 weeks after activation of oncogenic mutations (Fig. 4A) to large, invasive-appearing tumors by 9 weeks (Fig. 4C). However, sunitinib-treated *Kras/Lkb1^{L/L}* mice, like sunitinib-treated *Kras* mice, showed fewer and smaller tumors at each stage of tumor development (Fig. 4C and D). Mice treated earlier in the course of tumor progression also showed smaller tumor foci (Fig. 4E) but did not have a greater delay in tumor progression than mice treated later in the course of tumor development. Both sets of sunitinib-treated mice showed a statistically significant prolongation in overall survival (Fig. 4F and G) compared with untreated mice, although the duration of benefit was less than that in *Kras* mice.

Discussion

Sunitinib and other antiangiogenic inhibitors are tantalizing antimetastatic agents because of previously identified *in vitro* inhibition of endothelial cell proliferation, migration and invasion, and neoangiogenesis. Several recent studies have shown that some lung cancer tissue

specimens as well as cell lines express the same VEGF receptors that are expressed on endothelial cells, suggesting that direct blockade of cancer cell growth may be another mechanism of action of angiogenic inhibitors targeting these receptors (26-29). A recent study of global protein phosphorylation in lung cancer also showed a small proportion of lung tumors (2%) to have phosphorylated (activated) PDGFR α in tumor cells compared with surrounding normal lung tissue (30), suggesting a role for activated PDGFR α in tumor cell growth and a possible mechanism for direct inhibition of tumor cell growth by sunitinib.

Using a novel mouse model of endogenous lung cancer development, we showed that tumor cells from these mouse models do not express sunitinib targets at significant levels and no change in activation of VEGFR-mediated downstream signaling or in overall proliferation was seen, implying that there is little, if any, direct effect of sunitinib on tumor cells. Indeed, of 103 human NSCLC lines evaluated, only 1 (<1%) showed any inhibition of cell proliferation by sunitinib, H1703. This line notably contains a focal amplification of the sunitinib target, PDGFR α (data not shown). These data contrast with findings in cell lines or tissues derived from renal cell carcinoma (which express VEGFR proteins) or GIST (which express c-kit and/or PDGFR), where sunitinib efficacy that includes single-agent responses can be attributed to direct effects on tumor cells (reviewed in ref. 13). Instead, our findings are consistent with an indirect role for sunitinib on angiogenic remodeling of the tumor microenvironment that facilitates tumor growth and consistent with the stabilization of disease seen in a clinical trial in patients with NSCLC (15).

Decreases in phosphorylation of VEGFR2 in tumor tissue were seen, which presumably reflects effects on surrounding tumor cells or endothelial cells. However, when changes in VEGFR2, microvessel density, or apoptotic rates within tumors were examined by immunohistochemistry, no differences between treated and untreated mice were seen (data not shown). This likely reflects the unique setting in which lung tumors develop—because they develop within a highly vascularized lung parenchyma, they likely rely primarily on existing vasculature as opposed to neovascularization for initial tumor growth; significant numbers of microvessels reflecting neovascularization were not observed, even in untreated tumors. On a gross level, however, sunitinib-treated mouse tumors displayed histologically the findings typical of tumor imaging of lung cancer patients on sunitinib—specifically, that treatment results in central tumor necrosis within larger tumor foci, which are presumably the most dependent on neoangiogenesis to overcome hypoxic conditions. These results are in concordance with computed tomography observations of decreased density within lesions and dynamic contrast-enhanced magnetic resonance imaging observations of reduced blood flow to lesions in clinical trials of this agent (13).

Both the nonmetastatic (*Kras*) and metastatic (*Kras/Lkb1^{L/L}*) mouse models displayed a striking decrease in the number and volume of lung tumors, implying that the major benefit of sunitinib is through preventing tumor development and blockade of primary tumor growth, perhaps through surrounding bone marrow-derived and stromal cells expressing sunitinib targets, and not via inhibition of metastatic growth. The survival differences between the two mouse models underscore this idea—in a nonmetastatic model where large, primary tumors develop in control mice, there was a more dramatic difference in tumor size and median survival time was not reached. In the metastatic model where survival time seems to be limited by rapid spread of tumor lesions that do not necessarily reach large sizes, median survival is modestly prolonged from 9.1 to 11.7 weeks.

Interestingly, blockade of metastatic development in sunitinib-treated *Kras/Lkb1^{L/L}* mice was not seen—these mice showed an overall similar incidence of metastases with a higher number of metastases per mouse identified in the sunitinib-treated group (data not shown). Increased survival time in the treated mice may have actually allowed for an increase in the number of

macroscopic metastases. Alternatively, some studies have actually suggested that blockade of angiogenesis leads to increased invasive behavior of tumors (31,32).

Mice treated with sunitinib early in the process of tumor development (2 weeks) did not fare better in overall survival compared with mice that started treatment at 4 weeks and even had a slightly reduced median survival (10.7 weeks), although this was not statistically significant. These mice also developed metastases at the same rate as mice that initiated treatment later, consistent with a possibly more “invasive” behavior of sunitinib-treated tumors even when the overall tumor burden is smaller and delayed compared with control mice (Fig. 4). Further studies are required to define the mechanism of sunitinib effects on primary tumor growth versus metastatic spread and to define precise molecular targets of sunitinib action in these models. However, these studies suggest caution in moving forward with adjuvant or maintenance trials with antiangiogenesis inhibitors as a method of blocking metastatic progression. Rather, they suggest that the benefit from angiogenesis inhibitors seen in combination with chemotherapy may be more related to direct synergy with cytotoxic therapies to block primary tumor growth rather than additional effects on preventing the seeding of metastases.

The striking improvement in survival in *Kras* mice underscores the importance of sunitinib action on tumor development and early growth in the absence of metastatic disease. A recent neoadjuvant trial in stage I and II NSCLC using pazopanib, a receptor tyrosine kinase inhibitor with a similar array of targets as sunitinib, for 2 to 6 weeks before resection showed a reduction in tumor volume in 85.7% of evaluated tumors, a notable departure from the relatively static effects of these agents in the metastatic setting (33). Although these early tumor reductions also did not meet Response Evaluation Criteria in Solid Tumors criteria for partial response and would be technically classified as stable disease, given their small initial size, tumor volume is arguably a more precise measure of effect.

The potential benefit of sunitinib or other similar agents in the setting of early tumor development is particularly important for patients with *Kras* mutations. *Kras* mutations are present in 15% to 30% of NSCLC patients and some studies have suggested an association with a history of smoking (18,34,35). The presence of *Kras* mutation has been suggested as a negative predictive factor of benefit from erlotinib or erlotinib in combination with chemotherapy in patients with metastatic disease (35-38). In addition, in a trial of adjuvant therapy where mutation status was prospectively evaluated, patients with *ras* mutations showed no benefit to adjuvant therapy, although a secondary evaluation for interaction of *ras* mutations and benefit was not significant (4). The dramatic survival benefit of sunitinib seen in *Kras* mice supports further evaluation of sunitinib or similar agents in adjuvant and neoadjuvant settings for patients with *Kras* mutations. Studies are ongoing to determine whether the survival benefit of sunitinib is recapitulated in other genetically engineered mouse models of lung cancer development as well. If so, this may point the way toward studies of prevention of tumor development in high-risk populations as well.

Together, the studies described here show a role for sunitinib in suppressing early tumor progression; suppression of the development and progression of metastases was not shown. The histologic changes suggest an early effect at the level of atypical adenomatous hyperplasia, adenomas, and early adenocarcinomas. The mouse trials accurately recapitulate the human experience with sunitinib in NSCLC and provide a direct example of how interventions in endogenous mouse models of neoplastic progression can help guide the design of rational, more efficacious clinical trials. These studies highlight the importance of targeting the tumor microenvironment (e.g., the tumor-stromal interaction) and provide a strong rationale for targeting tumor development and the progression of early primary tumors for NSCLC treatment. Based on these study results, use of angiogenesis inhibitors in preinvasive disease

or early-stage lung cancer rather than in recurrent or progressive disease has the potential for a major effect on the rates of survival and cure of this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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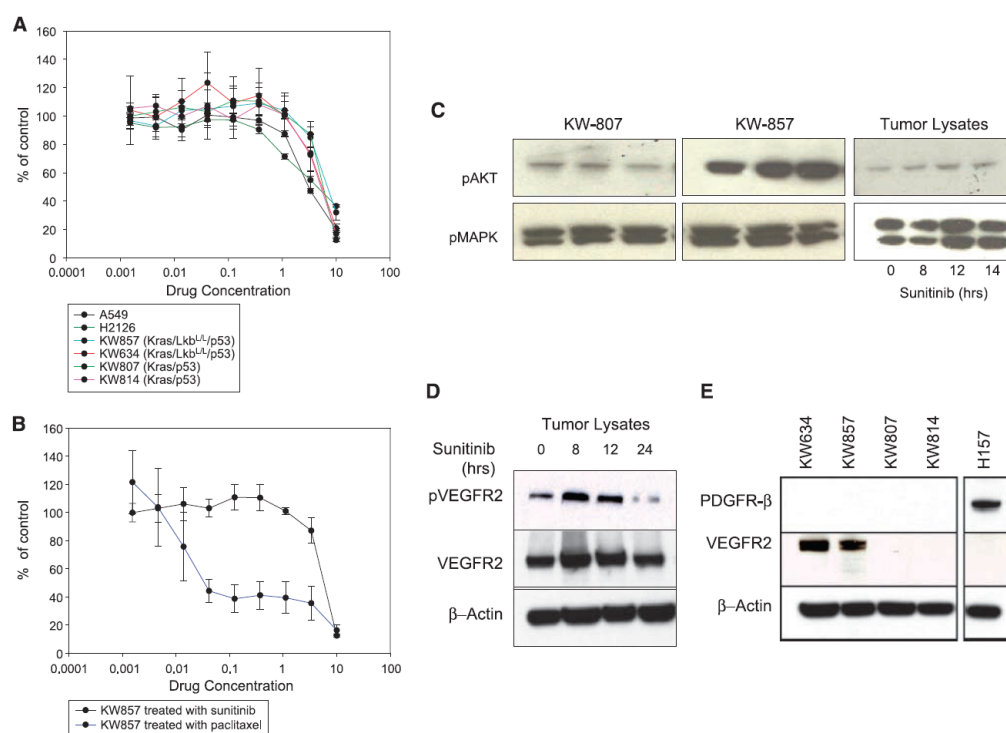
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References

1. van Rens MT, de la Riviere AB, Elbers HR, van Den Bosch JM. Prognostic assessment of 2,361 patients who underwent pulmonary resection for non-small cell lung cancer, stage I, II, IIIA. *Chest* 2000;117:374–9. [PubMed: 10669677]
2. Chemotherapy in addition to supportive care improves survival in advanced non-small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials. *J Clin Oncol* 2008;26:4617–25. [PubMed: 18678835]
3. Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med* 2004;350:351–60. [PubMed: 14736927]
4. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. *N Engl J Med* 2005;352:2589–97. [PubMed: 15972865]
5. Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB–IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. *Lancet Oncol* 2006;7:719–27. [PubMed: 16945766]
6. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol* 2008;26:3552–9. [PubMed: 18506026]
7. Fischer C, Jonckx B, Mazzone M, et al. Anti-PLGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell* 2007;131:463–75. [PubMed: 17981115]
8. O'Farrell AM, Abrams TJ, Yuen HA, et al. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity *in vitro* and *in vivo*. *Blood* 2003;101:3597–605. [PubMed: 12531805]
9. Timar J, Dome B. Antiangiogenic drugs and tyrosine kinases. *Anticancer Agents Med Chem* 2008;8:462–9. [PubMed: 18537529]
10. Potapova O, Laird AD, Nannini MA, et al. Contribution of individual targets to the antitumor efficacy of the multitargeted receptor tyrosine kinase inhibitor SU11248. *Mol Cancer Ther* 2006;5:1280–9. [PubMed: 16731761]
11. Osusky KL, Hallahan DE, Fu A, Ye F, Shyr Y, Geng L. The receptor tyrosine kinase inhibitor SU11248 impedes endothelial cell migration, tubule formation, and blood vessel formation *in vivo*, but has little effect on existing tumor vessels. *Angiogenesis* 2004;7:225–33. [PubMed: 15609077]
12. Mendel DB, Laird AD, Xin X, et al. *In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* 2003;9:327–37. [PubMed: 12538485]
13. Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. *Nat Rev Drug Discov* 2007;6:734–45. [PubMed: 17690708]

14. Goodman VL, Rock EP, Dagher R, et al. Approval summary: sunitinib for the treatment of imatinib refractory or intolerant gastrointestinal stromal tumors and advanced renal cell carcinoma. *Clin Cancer Res* 2007;13:1367–73. [PubMed: 17332278]
15. Socinski MA, Novello S, Brahmer JR, et al. Multicenter, phase II trial of sunitinib in previously treated, advanced non-small-cell lung cancer. *J Clin Oncol* 2008;26:650–6. [PubMed: 18235126]
16. Ji H, Houghton AM, Mariani TJ, et al. K-ras activation generates an inflammatory response in lung tumors. *Oncogene* 2006;25:2105–12. [PubMed: 16288213]
17. Ji H, Li D, Chen L, et al. The impact of human EGFR kinase domain mutations on lung tumorigenesis and *in vivo* sensitivity to EGFR-targeted therapies. *Cancer Cell* 2006;9:485–95. [PubMed: 16730237]
18. Ji H, Ramsey MR, Hayes DN, et al. LKB1 modulates lung cancer differentiation and metastasis. *Nature* 2007;448:807–10. [PubMed: 17676035]
19. Ji H, Wang Z, Perera SA, et al. Mutations in BRAF and KRAS converge on activation of the mitogen-activated protein kinase pathway in lung cancer mouse models. *Cancer Res* 2007;67:4933–9. [PubMed: 17510423]
20. Ji H, Zhao X, Yuza Y, et al. Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors. *Proc Natl Acad Sci U S A* 2006;103:7817–22. [PubMed: 16672372]
21. Li D, Shimamura T, Ji H, et al. Bronchial and peripheral murine lung carcinomas induced by T790M-L858R mutant EGFR respond to HKI-272 and rapamycin combination therapy. *Cancer Cell* 2007;12:81–93. [PubMed: 17613438]
22. Jackson EL, Willis N, Mercer K, et al. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes Dev* 2001;15:3243–8. [PubMed: 11751630]
23. Rasband WS. *Image J* :1997–2008.
24. Abramoff MD, Magelhaes PJ, Ram SJ. Image processing with Image J. *Biophotonics International* 2004;11:36–42.
25. Reinmuth N, Rensinghoff S, Raedel M, et al. Paracrine interactions of vascular endothelial growth factor and platelet-derived growth factor in endothelial and lung cancer cells. *Int J Oncol* 2007;31:621–6. [PubMed: 17671690]
26. Zhang L, Hannay JA, Liu J, et al. Vascular endothelial growth factor overexpression by soft tissue sarcoma cells: implications for tumor growth, metastasis, and chemoresistance. *Cancer Res* 2006;66:8770–8. [PubMed: 16951193]
27. Lee TH, Seng S, Sekine M, Hinton C, Fu Y, Avraham HK, Avraham S. Vascular endothelial growth factor mediates intracrine survival in human breast carcinoma cells through internally expressed VEGFR1/FLT1. *PLoS Med* 2007;4:e186. [PubMed: 17550303]
28. Levina V, Marrangoni AM, DeMarco R, Gorelik E, Lokshin AE. Drug-selected human lung cancer stem cells: cytokine network, tumorigenic and metastatic properties. *PLoS ONE* 2008;3:e3077. [PubMed: 18728788]
29. Giatromanolaki A, Koukourakis MI, Sivridis E, et al. Activated VEGFR2/KDR pathway in tumour cells and tumour associated vessels of colorectal cancer. *Eur J Clin Invest* 2007;37:878–86. [PubMed: 17883421]
30. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* 2007;131:1190–203. [PubMed: 18083107]
31. Blouw B, Song H, Tihan T, et al. The hypoxic response of tumors is dependent on their microenvironment. *Cancer Cell* 2003;4:133–46. [PubMed: 12957288]
32. Du R, Lu KV, Petritsch C, et al. HIF1 α induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell* 2008;13:206–20. [PubMed: 18328425]
33. Altorki N, Heymach J, Guarino M, et al. Phase II study of pazopanib (GW786034) given preoperatively in stage I-II non-small cell lung cancer (NSCLC): a proof-of-concept study. *Annals of Oncology* 2008;19:viii89–viii124.
34. Le Calvez F, Mukeria A, Hunt JD, et al. TP53 and KRAS mutation load and types in lung cancers in relation to tobacco smoke: distinct patterns in never, former, and current smokers. *Cancer Res* 2005;65:5076–83. [PubMed: 15958551]

35. Miller VA, Riely GJ, Zakowski MF, et al. Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib. *J Clin Oncol* 2008;26:1472–8. [PubMed: 18349398]
36. Zhu CQ, da Cunha Santos G, Ding K, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. *J Clin Oncol* 2008;26:4268–75. [PubMed: 18626007]
37. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;23:5900–9. [PubMed: 16043828]
38. Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* 2005;2:e17. [PubMed: 15696205]

**Fig. 1.**

A, cell proliferation assay of tumor-derived cell lines. A549 and H2126 are established human cell lines for comparison with KW-634 and KW-857, derived from *Kras* mice, and KW-807 and KW-814, derived from *Kras/Lkb1^{L/L}* mice. B, *Kras* or *Kras/Lkb1^{L/L}* cell lines or tumor lysates treated with sunitinib at the indicated concentrations were probed for phosphorylated AKT (pAKT) or phosphorylated MAPK (pMAPK). Tumor lysates were whole lung lysates harvested from *Kras/Lkb1^{L/L}* mice treated with a single dose of sunitinib at 7 wk after treatment with adeno-Cre (a time point at which mice have well-developed lung tumors). Mice were sacrificed at indicated time points after administration of sunitinib. D, whole lung lysates harvested as in C were probed for phosphorylated VEGFR2 (pVEGFR2), total VEGFR2, or β-actin. E, *Kras* or *Kras/Lkb1^{L/L}* cell lines probed for total VEGFR2 or PDGFRβ. VEGFR2 panels represent a prolonged (>10 min) exposure. H157 is a human lung cancer cell line previously described to express PDGFRβ used as a positive control.

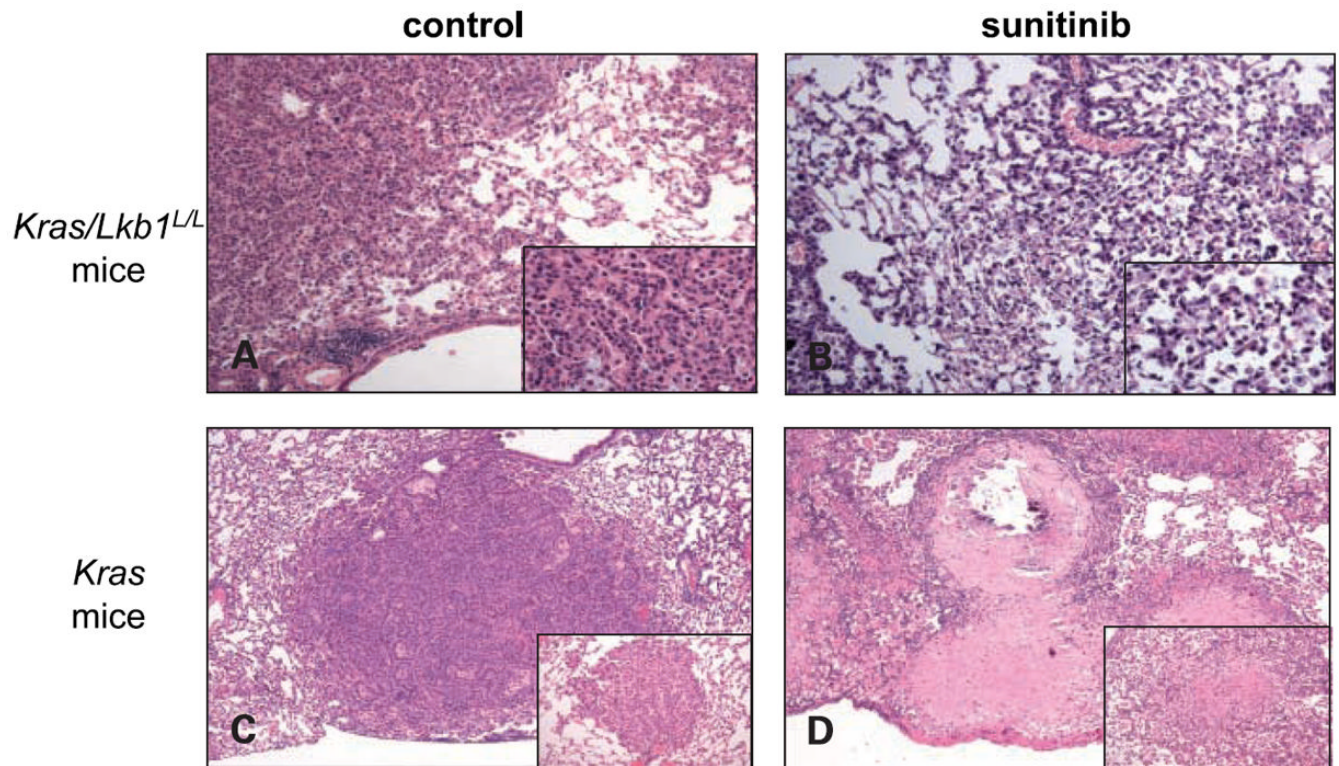


Fig. 2.

Representative H&E-stained paraffin sections of lung tissue from control (A and C) or sunitinib-treated (B and D) mice are shown. A and B, tumors from *Kras/Lkb1^{L/L}* mice treated for 5 wk with sunitinib or vehicle (both sacrificed at ~9 wk after activation of oncogenic mutations by adeno-Cre). C and D, tumors from *Kras* mice treated for 11 wk with sunitinib or vehicle (both sacrificed at 27 wk). Insets are at 10x increased magnification.

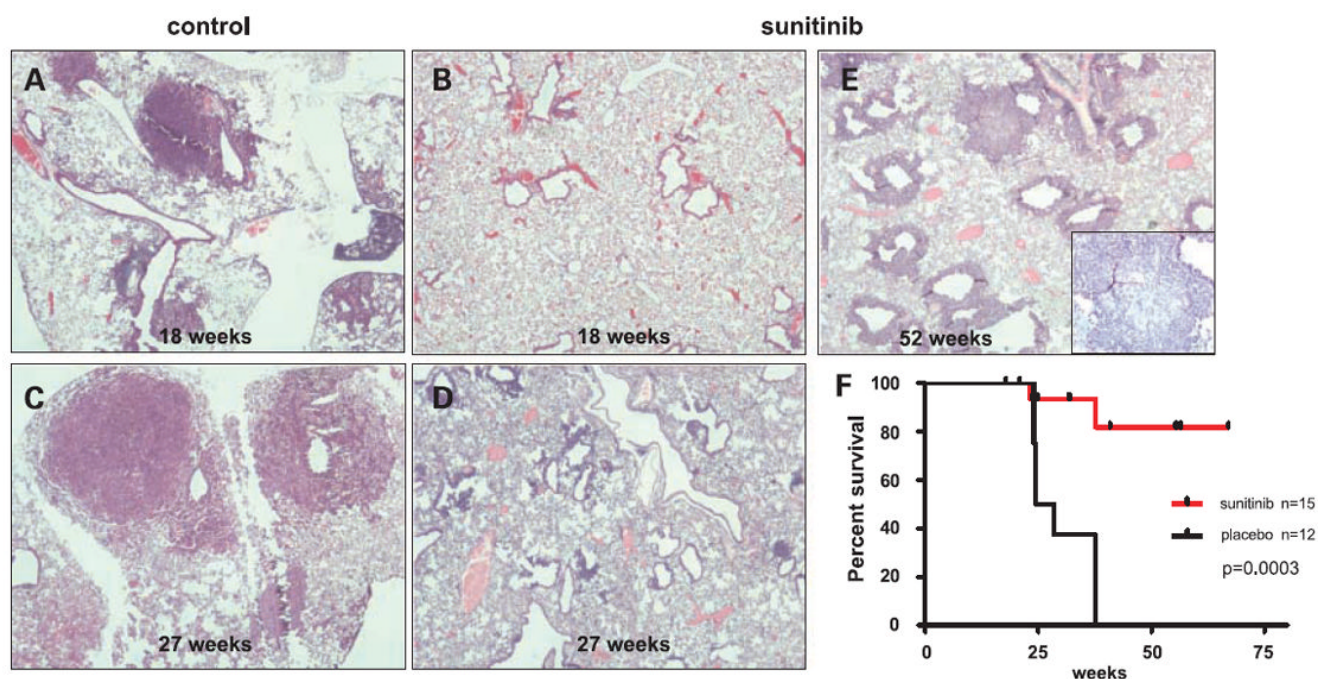


Fig. 3. *A* and *C*, mice treated with vehicle only. *B* and *D*, mice treated with sunitinib starting at 16 wk after adeno-Cre activation of mutations. Mice in *A* and *C* were sacrificed for histologic examination at 18 wk. *E*, a sunitinib-treated mouse censored at 52 wk (inset is x8 magnification). *F*, Kaplan-Meier survival curve of mice treated with sunitinib or placebo. *n* indicates the number of mice in each group used for analysis.

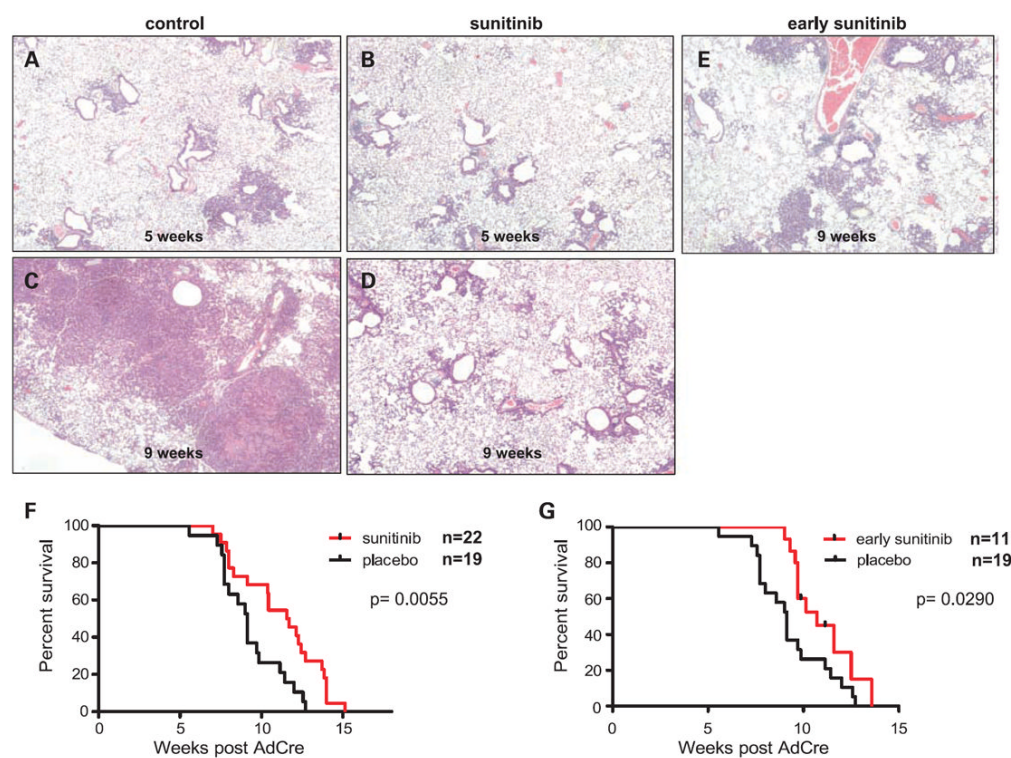


Fig. 4. *A* and *C*, mice treated with vehicle only. *B* and *D*, mice treated with sunitinib starting at 4 wk after adeno-Cre activation of mutations. *E*, a representative mouse treated with sunitinib starting at 2 wk after adeno-Cre activation of mutations. Mice were sacrificed for histologic examination at the time points indicated. Kaplan-Meier survival curves of mice treated with sunitinib at 4 wk (*F*) or 2 wk (*G*) are shown below. *n* indicates the number of mice in each group used for analysis.

Kras/Lkb1^{L/L} (top) or *Kras* (bottom) mice treated with daily sunitinib or placebo were analyzed for overall survival

Table 1

	<i>Kras/Lkb1^{L/L}</i> mice			
	Sunitinib	Placebo	Early sunitinib	Placebo
<i>n</i>	22	19	15	19
Median survival (wk)	11.7	9.1	10.7	9.1
<i>P</i>			0.0055	0.029
Hazard ratio (confidence interval)			0.46 (0.17-0.74)	0.46 (0.2-0.92)
<i>Kras</i> mice				
	Sunitinib	Placebo		
<i>n</i>	15	12		
Median survival (wk)	Not reached	26.4		
<i>P</i>			0.0003	
Hazard ratio (confidence interval)			0.11 (0.16-0.29)	

NOTE: Survival times refer to weeks after activation of oncogenic mutations by adeno-Cre. The first three columns in the top table refer to mice treated beginning at 4 wk after adeno-Cre activation of oncogenic mutations, a time when visible tumors are present. Early sunitinib mice in the second three columns were treated beginning at 2 wk after adeno-Cre activation before visible tumor development. *P* values are calculated from log-rank analysis using a Mantel-Cox two-tailed test. Confidence intervals are shown in parentheses after hazard ratios.