Phase 2 Study of Pemetrexed and Itraconazole as Second-Line Therapy for Metastatic Nonsquamous Non–Small-Cell Lung Cancer

Charles M. Rudin, MD, PhD, Julie R. Brahmer, MD, Rosalyn A. Juergens, MD, PhD, Christine L. Hann, MD, PhD, David S. Ettinger, MD, Rosa Sebree, BS, Ruth Smith, RN, Blake T. Aftab, PhD, Peng Huang, PhD, and Jun O. Liu, PhD

Introduction: Preclinical studies have suggested that the oral antifungal agent itraconazole specifically inhibits proliferation, migration, and tube formation of endothelial cells. Itraconazole has potent antiangiogenic activity and enhances the efficacy of cytotoxic chemotherapy in multiple primary xenograft lung cancer models. On the basis of these data, we performed an exploratory clinical study, assessing the efficacy of itraconazole with cytotoxic chemotherapy in the treatment of patients with advanced lung cancer.

Methods: The study enrolled patients with progressive nonsquamous non–small-cell lung cancer after one prior cytotoxic therapy for metastatic disease, randomized 2:1 to intravenous administration of pemetrexed 500 mg/m^2 on day 1, with or without itraconazole 200 mg orally daily, on a 21-day cycle. Outcome measures included percent progression-free at 3 months, progression-free survival, overall survival, and observed toxicity.

Results: A total of 23 patients were enrolled; the study was stopped early because of increasing use of pemetrexed in the first-line setting. At 3 months, 67% of the patients on itraconazole plus pemetrexed were progression-free versus 29% on the control arm of pemetrexed alone ($p = 0.11$). Median progression-free survivals were 5.5 months (itraconazole) versus 2.8 months (control) (hazard ratio = 0.399, $p = 0.089$). Overall survival was longer in patients receiving itraconazole (median 32 months) versus control (8 months) (hazard ratio = 0.194, $p = 0.012$). There were no evident differences in toxicity between the study arms.

Conclusion: Itraconazole is well tolerated in combination with pemetrexed. Consistent with our preclinical data, daily itraconazole administration is associated with trends suggestive of improved disease control in patients receiving chemotherapy for advanced lung cancer.

Key Words: Itraconazole, Antiangiogenic, Lung cancer.

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Johns Hopkins University, Baltimore, Maryland.

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Address for correspondence: Charles M. Rudin, MD, PhD, Professor of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, David H. Koch Cancer Research Building 2, Room 544, 1550 Orleans Street, Baltimore, MD 21231. E-mail: rudin@jhmi.edu

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Lung cancer is the leading cause of cancer-related deaths in the United States, and is responsible for more deaths than colon, breast, and prostate cancers combined.[1,2] More effective treatments for this disease are critically needed. Solid tumor growth and progression is dependent on tumor-associated angiogenesis.[3] Tumor expression and circulating levels of angiogenic factors have been correlated with aggressive tumor growth, predilection for metastasis, and prognosis in a wide array of solid tumors, including non–small-cell lung cancer (NSCLC).[4–6] The only currently approved antiangiogenic therapy for lung cancer is the monoclonal antibody, bevacizumab, directed against the vascular endothelial growth factor (VEGF). A landmark phase III clinical study, the Eastern Cooperative Oncology Group (ECOG) 4599, randomized 878 patients with advanced nonsquamous NSCLC to a standard chemotherapy doublet with or without bevacizumab.[7] This study demonstrated a statistically significant improvement in both progression-free survival and overall survival in favor of the bevacizumab-containing arm.

Although the ECOG 4599 study indicated the potential for antiangiogenic therapy to improve outcome in solid tumor patients, there were major limitations in these data as well. First, the absolute improvements in progression-free and overall survival were modest (1.7 and 2 months, respectively). Second, there were significant toxicities attributable to bevacizumab, with 15 treatment-related deaths on the bevacizumab arm (as opposed to 2 on the control arm; $p = 0.001$), including multiple fatal hemoptyses. The rates of hypertension, proteinuria, bleeding, neutropenia, febrile neutropenia, thrombocytopenia, hyponatremia, rash, and headache were significantly higher for bevacizumab ($p < 0.05$ for each). Third, the financial cost of bevacizumab, given the limited efficacy and significant toxicity, was seen by many as excessive, with an incremental cost-utility ratio compared with chemotherapy alone of more than $500,000 per quality-adjusted year of life.[8] A final concern came from a confirmatory trial—the AVAstin in Lung (AVAiL) study, which enrolled 1043 patients randomized to cisplatin and gemcitabine with or without bevacizumab.[9] This study failed to demonstrate a statistically significant difference in survival.[9] In summary, although evidence is strong that angiogenesis is critical to tumor growth, less toxic, less cost-prohibitive, and more effective therapies are needed.

We performed a high throughput screen for agents with previously unsuspected antiangiogenic activity, using differential inhibition of endothelial cell proliferation as an initial assay.[10] Itraconazole was found to be among the most potent and selective inhibitors of endothelial cell proliferation, with IC_{50} of 0.16 M for human umbilical vein endothelial cells, and minimal if any, antiproliferative effects against multiple nonendothelial controls (IC_{50}s > 100 M). None of the several related antifungal agents had similar activity.

Subsequently, we conducted a series of preclinical analyses of itraconazole, using both in vitro and in vivo model systems.[11] Itraconazole inhibited endothelial cell proliferation in response to known angiogenic drivers, including both VEGF and fibroblast growth factor (FGF), and inhibited phosphorylation of the primary angiogenic receptors for these factors. Itraconazole led to a dose-dependent suppression of VEGF- and FGF-mediated endothelial cell migration and VEGF- and FGF-mediated endothelial tube formation. Oral itraconazole administration to animals bearing primary xenograft NSCLCs resulted in tumor growth inhibition similar to that achieved with cisplatin, and in combination with cisplatin resulted in an even more marked suppression of tumor growth. Use of itraconazole in vivo was associated with tumor hypoxia, as shown by induction of tumor-specific expression of hypoxia inducible factor-1α (HIF-1α), as well as decreased tumor microvessel density, and tumor vascular area.[11]

Taken together, these data support the hypothesis that itraconazole may have substantial promise as a novel antiangiogenic agent. In contrast to bevacizumab, itraconazole is an inexpensive oral agent, off patent, and currently available in a generic formulation. Itraconazole has been safely administered to thousands of patients, including patients receiving high-dosage cytotoxic therapy for allogeneic bone marrow transplantation.[12]

In this study, the first to evaluate the potential anticancer activity of itraconazole in conjunction with standard chemotherapy for patients with lung cancer, we initiated a randomized study of standard-dosage pemetrexed as second-line therapy for nonsquamous NSCLC, given with or without itraconazole at a standard antifungal dosage of 200 mg daily. In the 571 patient phase III trial that supported the U.S. Food and Drug Administration registration of pemetrexed in the second-line setting for metastatic NSCLC, a 3-month event-free survival was approximately 40%, median progression-free survival (PFS) was 2.9 months, and median overall survival was 8.3 months.[13] We hypothesized that the antiangiogenic activity of itraconazole might lead to improved disease control with minimal additional toxicity in patients with recurrent and progressive nonsquamous NSCLC.

MATERIALS AND METHODS

Patient Population

Eligible candidates for this study (NCT00769600) were adults with histologically or cytologically confirmed nonsquamous NSCLC, with progressive disease after only one prior chemotherapy regimen for metastatic disease. Patients with known *EGFR* mutation were allowed to receive prior oral epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor therapy. Eligible patients had an ECOG performance status (PS) of 1 or less, and had adequate bone marrow, renal, and hepatic function. Patients were excluded if they had underlying condition predisposing to bleeding (history of nonchemotherapy-induced thrombocytopenia with bleeding within 1 year, active peptic ulcer, or hemorrhagic esophagitis/gastritis, active immune thrombocytopenic purpura, etc.).

This study was conducted according to the Declaration of Helsinki and with approval from Institutional Review Boards of all participating study sites. All participants provided prior written informed consent.

Study Design

This was an open-label, randomized two arm phase two study of second-line therapy for metastatic nonsquamous NSCLC. On both arms, patients received standard-dosage on-schedule pemetrexed (500 mg/m^2 intravenously administered on day 1 of a 21-day cycle). Patients on arm A, also received 200 mg itraconazole daily, starting on day 1 of cycle 1. This dosage of itraconazole was chosen for this initial exploratory study based on an expectation that this would be well tolerated because it reflected standard daily dosing recommendations for prolonged treatment of fungal infection. Subjects could remain on therapy indefinitely, until disease progression or intolerable toxicity. Although this study was not completed, the original statistical design called for a total of 112 patients, with the intention to assess the proportion of patients alive and progression-free at 3 months in each arm. The null hypothesis based on historical controls was that 3-month PFS would be approximately 40%. With a target 3-month PFS of 60%, a one-stage Fleming phase II design yielded an α of 0.10 (1-sided) and a power of 0.80 using A'Hern's exact binomial probabilities.[14,15]

Assessments

Safety

Safety assessments included history and physical examinations, vital signs, ECOG PS, adverse event assessment, blood chemistry, and complete blood counts with differential. Safety assessments were performed at screening and at least once in each cycle of therapy, typically on day 1. Adverse event severity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.[16] All adverse effects of grade three or higher were tabulated, regardless of attribution to study drug. Relationships of adverse events to therapy (definitely, probably, possibly, unlikely, or unrelated) were assessed by the principal investigator.

Efficacy

Tumor response was assessed using standard Response Evaluation Criteria in Solid Tumors after every two cycles of therapy.[17] The planned primary endpoint was percentage progression-free on each arm at 3 months. Additional prespecified efficacy variables included progression-free and overall survival assessed by Kaplan–Meier curves, with statistical differences assessed by log rank.

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TABLE 1. Patient Demographics

Demographic Category		Pemetrexed / Itraconazole $n = 15$	Pemetrexed $n = 8$
Median age (range)		60 (49–75)	59 (48–72)
Women, *n* (%)		7 (47)	5 (63)
ECOG PS, *n* (%)	0	6 (40)	4 (50)
	1	8 (53)	4 (50)
	2	1 (7)	0
Smoking history	Never-smoker	4 (27)	3 (37)
	Former smoker	11 (73%)	5 (63%)
	Pack-year, median (range)	25 (0–80)	13 (0–70)
Driver mutations[a]	*KRAS*	3 / 11 (27%)	3 / 8 (37%)
	EGFR	2 / 11 (18%)	2 / 8 (25%)
	Not tested	4	0

[a]One patient on arm A with CTNNB1 mutation.
ECOG, Eastern Cooperative Oncology Group; PS, performance status.

TABLE 2. Grade Three or Higher Adverse Events

	Pemetrexed / Itraconazole ($n = 15$)		Pemetrexed ($n = 8$)	
Toxicity	Grade 3	Grade 4	Grade 3	Grade 4
Lymphopenia	3[a]		2[a]	
Neutropenia		1[a]		
Leukopenia	1[a]			
Cough	1		1	
Dyspnea			1	
Pain				1
Vertigo	1			
Pulmonary embolus				1
Intratumoral bleed		1[a,b]		

[a]Possibly, probably, or definitely therapy-related.
[b]Occurred after discontinuation of study.

RESULTS

Patient Characteristics and Study Drug Dosing

The study was stopped early, after a total of 23 patients had enrolled, because of slow accrual associated with increasingly frequent use of pemetrexed as first-line and/or maintenance treatment of patients with metastatic nonsquamous NSCLC at Johns Hopkins. Fifteen patients were randomized to receive pemetrexed and itraconazole and eight patients to pemetrexed alone. The majority of patients had PS of 1. A summary of patient demographics is shown in Table 1. All patients were started at the planned dosage and schedule. The most common reason for study discontinuation on both arms was disease progression.

Safety and Tolerability

Therapy was well-tolerated on both arms, with the spectrum of adverse effects reflective of the known toxicities of pemetrexed, and disease-related complications of advanced lung cancer. The most common grade three toxicity on both arms was lymphopenia (2 of 8 on pemetrexed, and 3 of 15 on the combination). One patient, on the combination of pemetrexed and itraconazole, experienced transient grade four neutropenia not associated with fever, and grade three leukopenia. Another patient on the combination arm experienced an intratumoral bleed in a large adrenal metastasis within the first 30 days after going off study; this was considered to be possibly therapy-related. All other grade three or higher toxicities were considered not or unlikely to be therapy-related, and are summarized in Table 2. The median number of treatment cycles for patients on the combination of pemetrexed and itraconazole was six (range, 1–35) and the median treatment duration was 4.3 months (range, 1–26) (Fig. 1). The median number of treatment cycles on pemetrexed alone was 3.5 (range, 1–13) and the median treatment duration was 2.7 months (range, 1–9).

Efficacy

Three Response Evaluation Criteria in Solid Tumors responses were seen, all on the combination arm (response rate 3 of 15 or 20%) (Fig. 1). The duration of response ranged from approximately 1 year to more than 2 years.

The primary objective of this study was percentage progression-free at 3 months, with the underlying hypothesis being that itraconazole administration would be associated with an improvement in 3-month PFS from 40% to 60%. The actual percentage progression-free at 3 months was 67% on the combination of pemetrexed and itraconazole, and 29% on single-agent pemetrexed. Median PFS was 5.5 months on the combination of pemetrexed and itraconazole versus 2.8

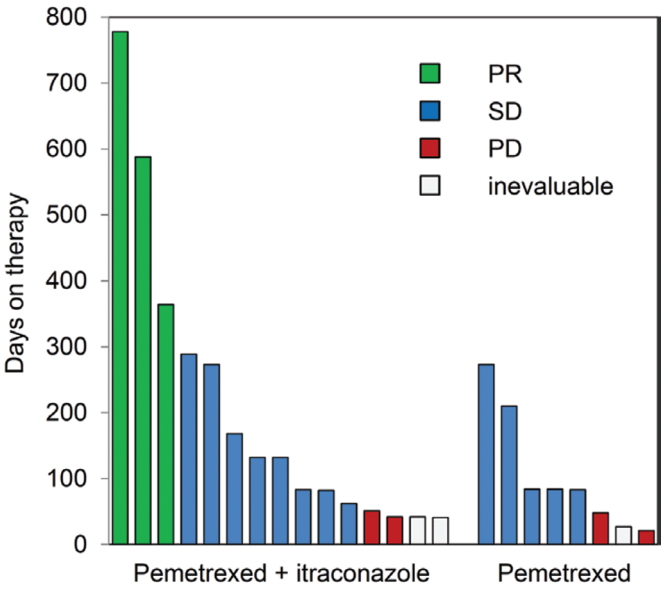

FIGURE 1. Duration of therapy on study. Each vertical bar represents an individual patient on study. Height of the bar represents time on therapy, and color represents Response Evaluation Criteria in Solid Tumors response. PR, partial response; SD, stable disease; PD, progressive disease.

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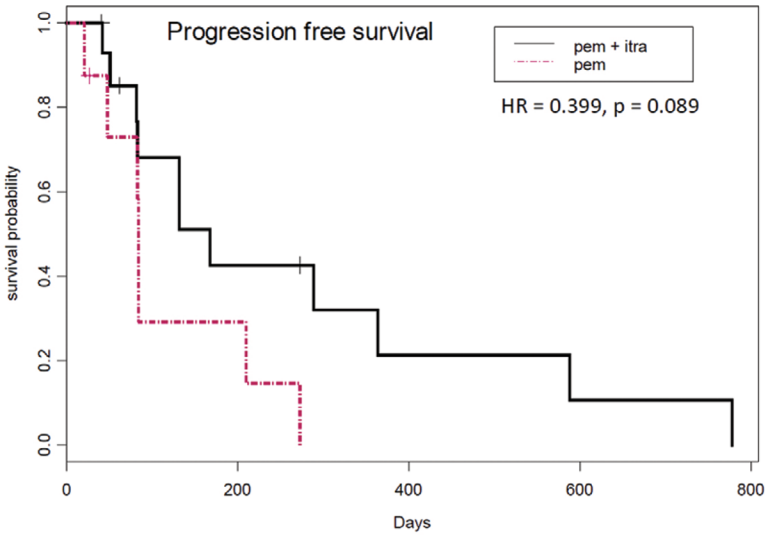

FIGURE 2. Progression-free survival. Kaplan–Meier curves of progression-free survival by study arm are shown. HR, hazard ratio.

months on single-agent pemetrexed (Fig. 2; hazard ratio = 0.399, $p = 0.089$). Median overall survival of patients treated with pemetrexed on this study was 8 months; in contrast, median overall survival of patients treated with the combination of pemetrexed and itraconazole was 32 months (Fig. 3; hazard ratio = 0.194, $p = 0.012$).

DISCUSSION

Antiangiogenic therapy as a strategy for the treatment of solid tumors is attractive in many respects: tumors are tenuously hypoxic and highly dependent on ongoing neovascularization for continued growth, and the endothelial cells and associated stromal elements may be more genetically stable and less likely to develop acquired resistance to targeted therapies than cancer cells. There have been successes in antiangiogenic treatment of cancer, using both targeted monoclonal antibodies and small molecule receptor tyrosine kinase inhibitors.[18] Nonetheless, the progress to date in the development of potent and durable inhibitors of tumor-associated angiogenesis has not lived up to some of the initial exceptionally high expectations.[19]

Most targeted antiangiogenic agents studied in cancer patients to date are highly selective targeted agents directed against key ligand-receptor interactions implicated in cancer-associated angiogenic drive. Like these molecularly targeted agents, itraconazole has minimal if any cytotoxic effect on cancer cells directly, but has potent inhibitory activity against endothelial cell proliferation. Although the mechanism of action of itraconazole as a selective inhibitor of endothelial cell proliferation has not been fully defined, it seems to be a *dirtier* inhibitor, active against multiple angiogenic stimuli, in a variety of contexts. In multiple primary xenograft models of NSCLC, the efficacy of oral itraconazole in suppressing tumor growth was similar to that of cisplatin.[11]

On the basis of this promising but surprising preclinical data, we initiated a clinical trial to explore the efficacy of itraconazole in patients being treated with standard pemetrexed as second-line treatment for metastatic nonsquamous NSCLC.

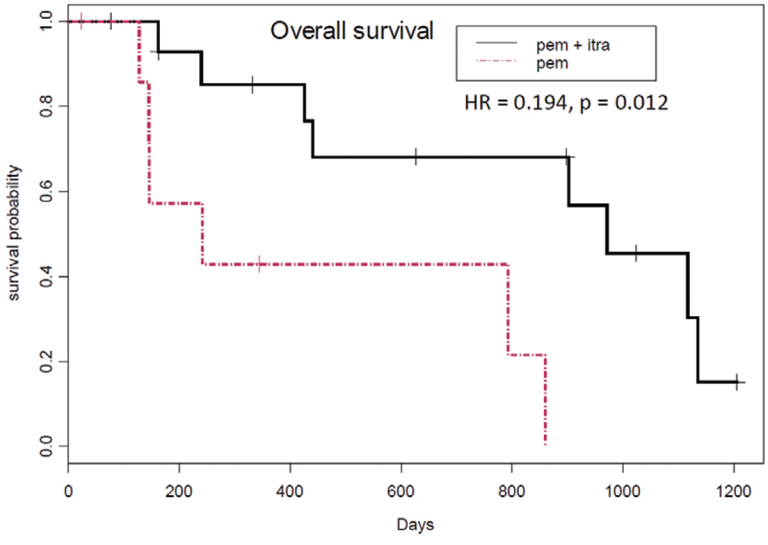

FIGURE 3. Overall survival. Kaplan–Meier curves of progression-free survival by study arm are shown. HR, hazard ratio.

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TABLE 3. Outcome Data Summary

Outcome Measure	Pemetrexed / Itraconazole $n = 15$	Pemetrexed $n = 8$
RECIST response, *n* (%)		
PR	3 (20)	0
SD	8 (53)	5 (62)
PD	2 (13)	2 (25)
Inevaluable	2 (13)	1 (12)
PFS		
Median (mo)	5.5	2.8
HR (*p* value)	0.399 ($p = 0.089$)	
OS		
Median (mo)	32	8
HR (95% CI)	0.194 ($p = 0.012$)	

RECIST, Response Evaluation Criteria in Solid Tumors; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; OS, overall survival.

The control arm of this study, pemetrexed alone, performed essentially as expected, with progression-free and overall survival medians of 2.8 and 8 months, respectively (compared with the expected 2.9 and 8.3 months based on historical control). In contrast, the cohort receiving the addition of itraconazole experienced median progression-free and overall survivals of 5.5 and 32 months, respectively, with the overall survival difference achieving statistical significance.

There are important caveats to consider in interpreting these data. First, and most notably, the sample size was small and the estimates of effect sizes are, therefore, highly unstable. The study was stopped after 23 patients because of concerns for study feasibility: increasingly, patients with good PS and newly diagnosed metastatic nonsquamous NSCLC at our center are treated, either with a molecularly targeted agent (in the case of an identified driver mutation), or with a pemetrexed-containing doublet; this study was open only to patients after treatment with a prior platinum-doublet, which could not include pemetrexed. We are currently launching a second randomized phase II study of itraconazole in a more feasible clinical context.

Second, this initial exploratory study did not include correlative analyses documenting antiangiogenic effects in patients treated with itraconazole. Our preclinical work in human tumor xenograft models did convincingly demonstrate marked inhibition of tumor-associated vascularity, with resultant tumor induction of the hypoxia-responsive gene HIF-1α. Our upcoming trial will include serial contrast-enhanced magnetic resonance imaging to assess changes in tumor blood flow. This will be a 70-patient randomized phase II study in the first-line metastatic setting, evaluating a standard regimen of cisplatin and gemcitabine with or without itraconazole, with coprimary endpoints of response rate, and decrease in tumor-associated blood flow.

Despite these inherent limitations, the results from this study are entirely consistent with the strong treatment effects seen in our preclinical modeling of itraconazole in human NSCLC primary xenografts. We believe these new clinical data are both provocative and encouraging and should prompt further clinical evaluation of itraconazole as a novel therapeutic for lung cancer and other solid tumors.

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REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012;62:10–29.
2. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. *CA Cancer J Clin* 2010;60:277–300.
3. Horsman MR, Siemann DW. Pathophysiologic effects of vascular-targeting agents and the implications for combination with conventional therapies. *Cancer Res* 2006;66:11520–11539.
4. Andersen S, Donnem T, Al-Saad S, et al. Angiogenic markers show high prognostic impact on survival in marginally operable non-small cell lung cancer patients treated with adjuvant radiotherapy. *J Thorac Oncol* 2009;4:463–471.
5. Mineo TC, Ambrogi V, Baldi A, et al. Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumour vessel invasion after radical surgery for IB-IIA non-small cell lung cancer. *J Clin Pathol* 2004;57:591–597.
6. Seto T, Higashiyama M, Funai H, et al. Prognostic value of expression of vascular endothelial growth factor and its flt-1 and KDR receptors in stage I non-small-cell lung cancer. *Lung Cancer* 2006;53:91–96.
7. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006;355:2542–2550.
8. Goulart B, Ramsey S. A trial-based assessment of the cost-utility of bevacizumab and chemotherapy versus chemotherapy alone for advanced non-small cell lung cancer. *Value Health* 2011;14:836–845.
9. Reck M, von Pawel J, Zatloukal P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil. *J Clin Oncol* 2009;27:1227–1234.
10. Chong CR, Xu J, Lu J, et al. Inhibition of angiogenesis by the antifungal drug itraconazole. *ACS chemical biology* 2007;2:263–270.
11. Aftab BT, Dobromilskaya I, Liu JO, et al. Itraconazole inhibits angiogenesis and tumor growth in non-small cell lung cancer. *Cancer Res* 2011;71:6764–6772.
12. Robenshtok E, Gafter-Gvili A, Goldberg E, et al. Antifungal prophylaxis in cancer patients after chemotherapy or hematopoietic stem-cell transplantation: systematic review and meta-analysis. *J Clin Oncol* 2007;25:5471–5489.
13. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* 2004;22:1589–1597.
14. Fleming TR. One-sample multiple testing procedure for phase II clinical trials. *Biometrics* 1982;38:143–151.
15. A'Hern RP. Sample size tables for exact single-stage phase II designs. *Stat Med* 2001;20:859–866.
16. Common Terminology Criteria for Adverse Events. Version 3.0. Available at: http://ctep.cancer.gov. Accessed December 1, 2012.
17. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–216.
18. Jayson GC, Hicklin DJ, Ellis LM. Antiangiogenic therapy–evolving view based on clinical trial results. *Nat Rev Clin Oncol* 2012;9:297–303.
19. Kolata G. Hope in the lab: a special report; a cautious awe greets drugs that eradicate tumors in mice. *The New York TImes* 1998;May 03.

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