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ORIGINAL REPORT

Activity of IPI-504, a Novel Heat-Shock Protein 90 Inhibitor, in Patients With Molecularly Defined Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
IPI-504 is a novel, water-soluble, potent inhibitor of heat-shock protein 90 (Hsp90). Its potential anticancer activity has been validated in preclinical in vitro and in vivo models. We studied the activity of IPI-504 after epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) therapy in patients with advanced, molecularly defined non–small-cell lung cancer (NSCLC).

Patients and Methods
Patients with advanced NSCLC, prior treatment with EGFR TKIs, and tumor tissue available for molecular genotyping were enrolled in this prospective, nonrandomized, multicenter, phase II study of IPI-504 monotherapy. The primary outcome was objective response rate (ORR). Secondary aims included safety, progression-free survival (PFS), and analysis of activity by molecular subtypes.

Results
Seventy-six patients were enrolled between December 2007 and May 2009 from 10 United States cancer centers. An ORR of 7% (five of 76) was observed in the overall study population, 10% (four of 40) in patients who were *EGFR* wild-type, and 4% (one of 28) in those with *EGFR* mutations. Although both *EGFR* groups were below the target ORR of 20%, among the three patients with an *ALK* gene rearrangement, two had partial responses and the third had prolonged stable disease (7.2 months, 24% reduction in tumor size). The most common adverse events included grades 1 and 2 fatigue, nausea, and diarrhea. Grade 3 or higher liver function abnormalities were observed in nine patients (11.8%).

Conclusion
IPI-504 has clinical activity in patients with NSCLC, particularly among patients with *ALK* rearrangements.

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INTRODUCTION

Heat-shock protein 90 (Hsp90) is integral in protein homeostasis and regulates the stability of key proteins involved in oncogenesis, proliferation, and survival through its role as a protein chaperone.[1] Hsp90 is an emerging focus of cancer therapy by virtue of its ability to inhibit multiple vital signaling pathways simultaneously.[2,3] Furthermore, mutated oncoproteins, including epidermal growth factor receptor (EGFR), may preferentially rely on Hsp90 chaperones more than their wild-type counterparts, further increasing the appeal of Hsp90 as a therapeutic target for cancers defined by such mutations.[4-7]

Non–small-cell lung cancer (NSCLC) is a heterogeneous disease that can be subclassified based on driver mutations, specific oncogene alterations that lead to biologic dependence on the driver's signaling pathway, or oncogene addiction. The most common driver mutations in NSCLC appear to involve the genes for *KRAS*, *EGFR*, and anaplastic lymphoma kinase (*ALK*).[8-10] When potent and specific inhibitors are used to block the signal from the driver oncogene, treatment can be extremely effective, as demonstrated in the case of EGFR tyrosine kinase inhibitors (TKIs) in *EGFR*-mutant NSCLC.[11-13] This success may be mirrored with ALK TKI therapy in *ALK*-rearranged NSCLC.[14]

Retaspimycin hydrochloride (IPI-504) is a novel, water-soluble, potent inhibitor of Hsp90. An analog of 17-allylamino-17-demethoxygeldanamycin (17-AAG), IPI-504's potential anticancer activity has been validated in in vitro and in vivo models.[15,16] A phase I/II study of IPI-504 in patients with NSCLC was conducted. In the phase I dose-escalation portion of the study, IPI-504 monotherapy in patients with NSCLC demonstrated a favorable adverse effect profile and evidence of clinical benefit.[17] We therefore conducted this multicenter phase II portion of the study to prospectively assess the efficacy of IPI-504 after EGFR TKI therapy in patients with advanced NSCLC. *EGFR* genotype was mandatory so that differences in activity by mutation status could be observed. We retrospectively assessed other biomarkers to identify groups with differential responses to therapy.

PATIENTS AND METHODS

Study Design and Patients

This was a nonrandomized two-armed phase II clinical trial to assess the objective response rate (ORR) by RECIST (Response Evaluation Criteria in Solid Tumors) 1.0 to IPI-504 monotherapy in patients with advanced NSCLC who either had an activating *EGFR* mutation or were *EGFR* wild-type.[18] Each genotype-defined arm of the trial functioned as a Simon two-stage study with planned interim evaluation after 10 patients and expanded enrollment of an additional 19 patients if there was at least one partial response (PR) or stable disease lasting $\geq$ 3 months, which was achieved for both arms. While available tissue for *EGFR* analysis was mandatory, completed *EGFR* genotype was not required at study entry, thus the trial remained open until both cohorts fully enrolled, which led to overenrollment of the wild-type arm. Secondary aims included describing the safety and progression-free survival (PFS) of the regimen, and examining molecular markers associated with response.

Patients were recruited between December 2007 and May 2009 from 10 United States cancer centers. To be eligible, patients had to have stage IIIB (with pleural effusion), or stage IV NSCLC with progression on EGFR TKI therapy at some point in their history; adequate renal, hepatic, and bone marrow function; Eastern Cooperative Oncology Group performance status of 0 to 2; measurable disease by RECIST 1.0; no active or untreated CNS metastases; no significant cardiac conduction abnormalities (based on findings from similar compounds) or ongoing keratoconjunctivitis (based on nonclinical findings with an oral IPI-504 formulation); and either previously defined *EGFR* genotype or sufficient tumor tissue to undergo genotype assessment.[18,19] There was no limit on prior therapies. All patients signed written informed consent and the study was monitored by all local institutional review boards. Funding for the trial was provided by Infinity Pharmaceuticals Inc.

Treatment and Evaluation

Treatment consisted of a 30-minute infusion of intravenous IPI-504 on days 1, 4, 8, and 11 of a 21-day cycle. Therapy continued until progressive disease, intolerable adverse effects, or elective withdrawal. A total of 76 patients were enrolled. The starting dose was 400 mg/m^2 for 75 patients. In April 2009, the dose for patients who were on study (n = 19) was lowered to 225 mg/m^2, due to hepatotoxicities observed at the 400 mg/m^2 dose in a separate trial of IPI-504 in patients with GI stromal tumors,[20] and the last enrolled patient started at a dose of 225 mg/m^2.

All patients were assessed for safety by history, physical examination, blood chemistries, liver function tests, and blood counts, at baseline and before each infusion. Amylase and lipase were assessed if there were symptoms of pancreatitis. Adverse events were graded using the National Cancer Institute Common Toxicity Criteria version 3.0. Slit lamp eye examinations were performed during screening and ECGs were obtained during screening and before and after the first infusion, to evaluate for keratitis and QTc prolongation, respectively. Radiographic evaluation of tumor response by computed tomography scan was performed after every two cycles. All films were reviewed and independently assessed by a central radiology core laboratory.

Patient Tumor Molecular Analyses

Tumor tissue specimens from all patients were assessed for *EGFR* mutations via direct sequencing of exons 18 to 21, using standard methods, at participating institutions' CLIA-certified internal laboratories or else at Genzyme Corporation (Cambridge, MA). A subset of patients also underwent *EGFR* (n = 25), *KRAS* (n = 30), and *BRAF* (n = 5) genotyping analyses with allele-specific amplification-refractory mutation system assays (DxS, United Kingdom) at Infinity Pharmaceuticals, Inc (Cambridge, MA). Patients who underwent successful *EGFR* testing via both methods were classified using the result of the more sensitive assay (allele-specific amplification-refractory mutation system).

Posthoc analyses of other molecular markers of interest were performed in all patients for whom sufficient tissue was available. The primary analyses were performed in a CLIA-certified laboratory at Massachusetts General Hospital and consisted of the SNaPshot assay (Applied Biosystems, Foster City, CA), adapted to detect key oncogenic mutations in *EGFR, KRAS, PIK3CA, BRAF, PTEN, AKT, TP53, NRAS, CTNNB1* (beta-catenin), *APC, KIT, JAK2, NOTCH1,* and *FLT3* (n = 21); and the fluorescent in situ hybridization break-apart assay for detection of *ALK* gene rearrangements, using methods previously described (n = 15).[10,21] Other analyses included genotyping by Oncomap analysis (Dana-Farber Cancer Institute, Boston, MA) covering 1,155 mutations in 114 cancer genes (n = 10). In addition, coding exons for 11 genes (*ALK* exons 20-29, *BRAF* exon 15, *EGFR, ERBB2, HSP90AA1, HSP90AB1, KRAS, MET, NF1, PTEN*, and *STK11*) were sequenced by the Sanger method at Functional Biosciences Inc (Madison, WI; n = 12). Ten potential mutations were then validated by the Sanger method at Genewiz (South Plainfield, NJ).

Western Blot Analyses

H1975 (*EGFR* L858R/T790M), HCC827 (*EGFR* del 19), H3122 (*EML4-ALK*), and MGH006 (*EML4-ALK* derived from a patient who was sensitive to the ALK inhibitor PF-02341066) cells were treated with increasing doses of 17-AAG for 24 hours. Western blotting was performed using previously described methods.[22] Membranes were probed with antibodies against P-ALK (Cell Signaling Technology, Beverly, MA), ALK (Cell Signaling Technology), P-EGFR (Biosource, Carlsbad, CA), and EGFR (Santa Cruz Biotechnology Inc, Santa Cruz, CA). Chemiluminescence was detected using the Syngene G:Box camera (Synoptics, Cambridge, UK).

Cell Survival Assays

Cells were seeded at 2,000 cells per well of a 96-well plate. After overnight incubation, the cells were treated in sextuplet with serial dilutions of 17-AAG for 72 hours. Viable cell titer relative to untreated cells was determined using Syto60 assays as previously described.[22]

Statistical Considerations

The primary end point of the study was ORR, calculated as the sum of patients with confirmed CR or PR divided by the number of treated patients. Each arm (*EGFR* mutant and wild-type) was analyzed independently. The study was powered assuming a null ORR of 5% and a target ORR of 20%.

Summary statistics were used to describe safety and included all patients treated with IPI-504. PFS was defined as the time from enrollment to progressive disease or death, censored at the last known follow-up, and was calculated with the Kaplan-Meier method, following the intent-to-treat principle.

RESULTS

Patients

Seventy-six patients were enrolled. *EGFR* genotype analysis did not need to be completed before initiation of treatment; consequently eight patients (10%) with indeterminate genotype were not assigned to either the *EGFR* mutant or wild-type arms. The median age was 64 years (range, 31 to 82 years) and was similar between the genotypes (Table 1). The entire study population had an over-representation of women (63%) and never-smokers (45%), which was even more pronounced among *EGFR* mutants (71% women, 61% never-smokers). The study

Table 1. Demographics by *EGFR*, *KRAS*, and *ALK* Genotype

			EGFR Status (n = 68)				*KRAS* Status (n = 38)				*ALK* Status (n = 15)			
	Total		Wild Type		Mutant		Wild Type		Mutant		Wild Type		Rearranged	
Demographic	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. of patients	76		40		28		26		12		12		3	
Median age, years	64.0		63.0		66.0		61.0		65.0		65.5		48.0	
Range	31-82		31-79		44-82		31-81		52-76		48-76		31-58	
Sex														
Female	48	63	22	55	20	71	17	65	7	58	9	75	1	33
Male	28	37	18	45	8	29	9	35	5	42	3	25	2	67
Race														
Asian	11	14	6	15	5	18	4	15	0		2	17	1	33
Black or African American	4	5	2	5	2	7	2	8	0		0		0	
White	61	80	32	80	21	75	20	77	12	100	10	83	2	67
Smoking status														
Current smoker	0		0		0		0		0		0		0	
Never-smoker	34	45	13	33	17	61	13	50	0		3	25	3	100
Previous smoker	42	55	27	68	11	39	13	50	12	100	9	75	0	
Median time since diagnosis, months	27.5		24.6		37.2		25.7		20.6		28.5		29.7	
Range	8-120		8-120		11-108		10-120		11-71		11-71		10-120	
Histology														
Adenocarcinoma	59	78	31	78	23	82	21	81	10	83	11	92	3	100
Bronchioloalveolar	4	5	2	5	2	7	0		1	8	0		0	
Large cell	2	3	2	5	0		1	4	1	8	0		0	
Squamous	6	8	4	10	1	4	3	12	0		1	8	0	
Unspecified NSCLC	5	7	1	3	2	7	1	4	0		0		0	
Median No. of prior treatment regimens for NSCLC	4.0		4.0		3.0		3.0		3.5		4.0		3.0	
Range	1-11		1-7		1-11		1-6		2-7		2-7		3-5	
Best prior response to EGFR TKI treatment														
CR	1	1	0		1	4	1	4	0		0		0	
PR	18	24	2	5	14	50	3	12	1	8	1	8	0	
Total time on EFGR TKI prior to study, months														
Median	1.8		1.5		10.5		1.7		1.2		1.9		0.0	
Range	0-61		0-25		0-61		0-61		0-16		0-16		0-1	

NOTE. Patients may be counted in more than one column dependent upon molecular analysis.
Abbreviations: EGFR, epidermal growth factor receptor; NSCLC, non–small-cell lung cancer; TKI, tyrosine kinase inhibitor; CR, complete response; PR, partial response.

cohort was also heavily pretreated with a median of four prior systemic regimens and a median time since diagnosis of 27.5 months. Prior EGFR TKI therapy had yielded a 54% response rate and lasted a median of 10.5 months among *EGFR* mutation–positive patients.

Toxicity

IPI-504 was generally well-tolerated. Most adverse events were grades 1 or 2; nine patients (12%) had dose reductions for toxicity, while 11 (14%) discontinued therapy for adverse events. The most commonly reported adverse events (regardless of relationship to drug) were fatigue, nausea, diarrhea, vomiting, cough, anorexia, and joint/muscle aches (Table 2). About one third of patients had transient, nontoxic purple-colored urine due to renal clearance of an IPI-504 chromometabolite. In terms of laboratory abnormalities, AST, ALT, and alkaline phosphatase elevations were common (49%, 41%, and 62%, respectively), but grade 3 or greater elevations were infrequent (9%, 7%, and 5%, respectively). No grade 3 or 4 bilirubin elevation was noted. Three patients died while on study. Two patients died of complications from pneumonia, including sepsis and respiratory distress, which were considered possibly related to study drug; both had renal failure and/or elevations in ALT and AST. The third patient died of respiratory failure, which was considered unrelated to study drug.

Response and Molecular Analyses

Sixty-eight patients (89%) had successful *EGFR* genotype analyses, with 28 patients (37% of the 76 enrolled) assigned to the *EGFR* mutation–positive arm and 40 (53%) to the wild-type arm. Of the *EGFR* mutant patients, 17 (61%) had exon 19 deletions, six (21%) had the exon 21 L858R point mutation, one (4%) had exon 20 insertions, and four (14%) had two mutations each (two patients with exon 18 G719S and exon 21 L861Q mutations; two with exon 19 deletion and exon 20 T790M mutations). Note that both patients with a T790M mutation were genotyped from biopsies obtained after *EGFR* TKI treatment. Reasons for indeterminate *EGFR* genotype included insufficient tumor tissue or poor quality of available tissue. Thirty-eight patients (50%) underwent *KRAS*

Table 2. Most Frequent Adverse Events Regardless of Causality

Parameter	Patients With Any Event No.	%	Grade 1 or 2 Event No.	%	≥ Grade 3 Event No.	%
MedDRA preferred term						
Fatigue	44	57.9	41	53.9	6	7.9
Nausea	43	56.6	41	53.9	6	7.9
Diarrhea	40	52.6	37	48.7	8	10.5
Vomiting	28	36.8	25	32.9	6	7.9
Cough	24	31.6	24	31.6	2	2.6
Urine color abnormal	22	28.9	22	28.9	0	0.0
Anorexia	19	25.0	18	23.7	4	5.3
Arthralgia	19	25.0	17	22.4	2	2.6
Myalgia	19	25.0	18	23.7	1	1.3
Headache	19	25.0	19	25.0	0	0.0
Abdominal pain	18	23.7	18	23.7	1	1.3
Constipation	18	23.7	18	23.7	2	2.6
Dyspnea	18	23.7	15	19.7	6	7.9
Back pain	16	21.1	16	21.1	0	0.0
Infusion site pain	15	19.7	15	19.7	0	0.0
Dehydration	14	18.4	11	14.5	3	3.9
Musculoskeletal chest pain	13	17.1	11	14.5	3	3.9
Pyrexia	12	15.8	12	15.8	0	0.0
Vision blurred	12	15.8	12	15.8	0	0.0
Insomnia	12	15.8	12	15.8	0	0.0
Dizziness	12	15.8	12	15.8	0	0.0
Liver function tests*						
Any abnormality†	52	68.4	43	56.6	9	11.8
Alkaline phosphatase	47	61.8	43	56.6	4	5.3
AST	37	48.7	30	39.5	7	9.2
ALT	31	40.8	26	34.2	5	6.6
Total bilirubin	3	3.9	3	3.9	0	0.0

*Maximum post-baseline grade, based on laboratory values.
†Any abnormality includes abnormalities in alkaline phosphatase, AST, ALT, and/or total bilirubin.

mutation testing and 12 (16%) had a mutation; 15 (20%) underwent *ALK* rearrangement testing and three (4%) were positive (Appendix Fig A1, online only). Demographics of the *KRAS* mutation–positive patients were notable for a positive smoking history, and those of the *ALK*-rearranged patients were notable for young age, male predominance, and never-smoking (Table 1).

The ORR to IPI-504 was 7% (five of 76) overall, 10% (four of 40) in *EGFR* wild-type patients and 4% (one of 28) in *EGFR* mutants. The median duration of response was 120 days. Four of the five responses were confirmed with a follow-up scan. The *EGFR* mutation–positive patient with a RECIST PR had an L858R mutation, had previously had a PR to the combination of erlotinib and enzastaurin lasting approximately 8 months, and transitioned directly from erlotinib to IPI-504. Responses were also seen in three (12%) of the 26 *KRAS* wild-type patients, in one (8%) of the 12 patients known to be *ALK* wild-type, and in two (67%) of the three patients with an *ALK* rearrangement (Table 3, Figs 1, 2A). Note that two of the three *KRAS* wild-type responders had *ALK* rearrangement, but the third was confirmed *ALK* wild-type. The single patient that started treatment at 225 mg/m^2 had 5% tumor shrinkage.

At the time of analysis, 35 patients (46%) had a PFS event (progression or death), and 41 (54%) were censored. The estimated median PFS for all patients was 2.86 months (95% CI, 2.43 to 4.18 months), although the three patients with *ALK* rearrangements each received IPI-504 for approximately 7 months (Fig 2B). Additional genetic results from SNaPshot, Oncomap, DxS genotyping, and Sanger sequencing are summarized in Appendix Table A1.

Laboratory assessments of lung cancer models harboring *EGFR* mutations and *ALK* rearrangements confirmed that the *EGFR* mutant models were sensitive to Hsp90 inhibition with 17-AAG, as previously demonstrated, but also revealed that *ALK*-rearranged models were highly sensitive. Indeed, both the stability of the *ALK*-rearranged protein and the viability of the cancer were highly sensitive to Hsp90 inhibition (Fig 3).

DISCUSSION

To our knowledge, this is the first trial of an Hsp90 inhibitor in molecularly defined cohorts of patients with advanced NSCLC. We have demonstrated that IPI-504 is active in NSCLC, with a response rate of 7% (five of 76) in the overall study population, 10% (four of 40) in patients who were *EGFR* wild-type, 4% (one of 28) in *EGFR* mutants with acquired resistance to TKIs, 12% (three of 26) among *KRAS* wild-type patients, and 8% (one of 12) among *ALK* wild-type patients.

Table 3. Efficacy

Parameter	Total No.	%	*EGFR* Status (n = 68) Wild Type No.	%	*EGFR* Status (n = 68) Mutant No.	%	*KRAS* Status (n = 38) Wild Type No.	%	*KRAS* Status (n = 38) Mutant No.	%	*ALK* Status (n = 15) Wild Type No.	%	*ALK* Status (n = 15) Rearranged No.	%
No. of patients	76		40	53	28	37	26	34	12	16	12	16	3	4
Objective response rate (all PRs)	5	7	4	10	1	4	3*	12	0	0	1	8	2	67
RECIST stable disease or better for at least 3 months	18	24	10	25	6	21	4	15	5	42	3	25	3	100
Median PFS, months	2.86		2.86		2.76		2.86		3.91		2.43			
95% CI	2.43-4.18		1.18-5.33		2.40-3.91		1.22-10.20		1.12-4.18		1.13-5.33		Unable to determine	

Abbreviations: PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; PFS, progression-free survival.
*Two of the three *KRAS* wild-type responders were *ALK* rearranged; the third was confirmed *ALK* wild-type.

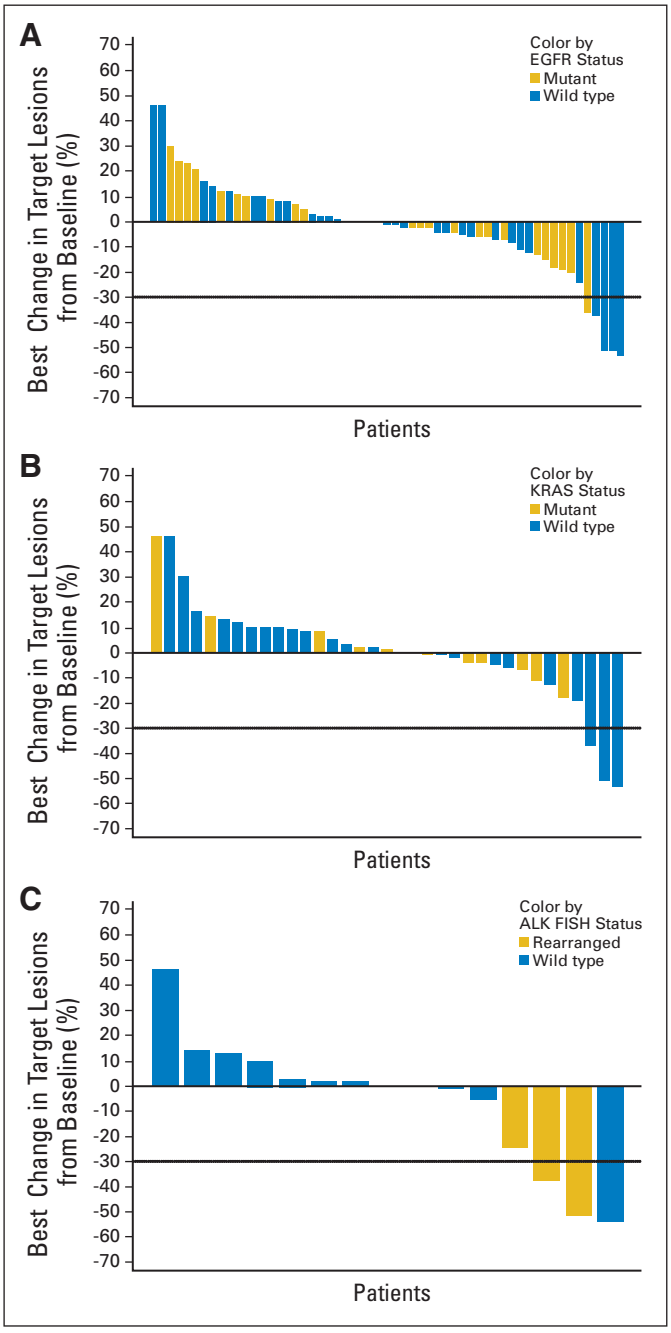

Fig 1. Best percent change in size of target lesions on study. Percent change in measurable tumor at best response is displayed by genotype. (A) Epidermal growth factor receptor (EGFR) mutation status, (B) KRAS mutation status, and (C) ALK rearrangement status.

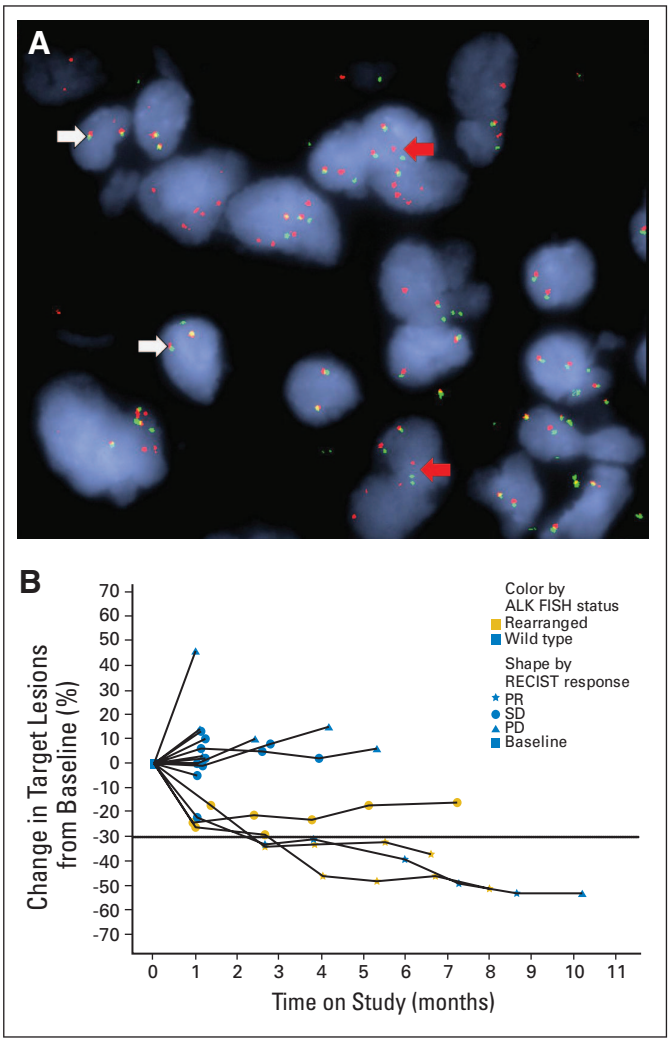

Fig 2. Patients with ALK rearrangements on study. (A) Example of a positive fluorescent in situ hybridization (FISH) break-apart assay in a patient with ALK rearrangement. White arrows indicate the wild-type allele with close proximity of the red and green probes yielding a yellow signal. Red arrows indicate the ALK rearrangement with separated red and green probes. (B) Change in size of target lesions over time for patients tested for ALK rearrangement. RECIST, Response Evaluation Criteria in Solid Tumors; PR, partial response; SD, stable disease; PD, progressive disease.

The most intriguing finding is the posthoc analysis demonstrating that two of three patients known to have *ALK* rearrangements had a PR to IPI-504 and the third patient had stable disease (24% reduction) durable for 7.2 months. This is the first clinical demonstration of activity of an Hsp90 inhibitor in patients with *ALK* rearrangements.

ALK is a member of the insulin superfamily of receptor tyrosine kinases and was initially associated with anaplastic large-cell lymphoma, which commonly has ALK oncogenic signaling mediated by fusion between the *ALK* kinase domain and the partner protein nucleophosmin.[23] More recently, *EML4-ALK* and other rearrangements involving the *ALK* locus have been described in NSCLC as transforming driver mutations conferring sensitivity to therapy with ALK TKIs.[10,14,24] Preclinical models have demonstrated that nucleophosmin *ALK* is a client of Hsp90,[25] and our data indicate that *EML4-ALK* is also a key client (Fig 3).

Overall, the study validates the hypothesis that an Hsp90 inhibitor, by virtue of the chaperone role of Hsp90 for multiple oncoproteins and its pervasive effect on key signaling pathways, has the potential to be an effective cancer therapy against multiple types of oncogene-addicted cancers, including those that have developed resistance to receptor-specific targeted treatments. TKIs that inhibit driver mutations in such cancers have been extremely effective, including imatinib in chronic myelogenous leukemia and gastrointestinal stromal tumors (targets *BCR-ABL* and *CKIT*, respectively), gefitinib and erlotinib in NSCLC (targets *EGFR*), and potentially PF-02341066 in NSCLC (targets *ALK*).[11,14,26,27] Our study confirms that

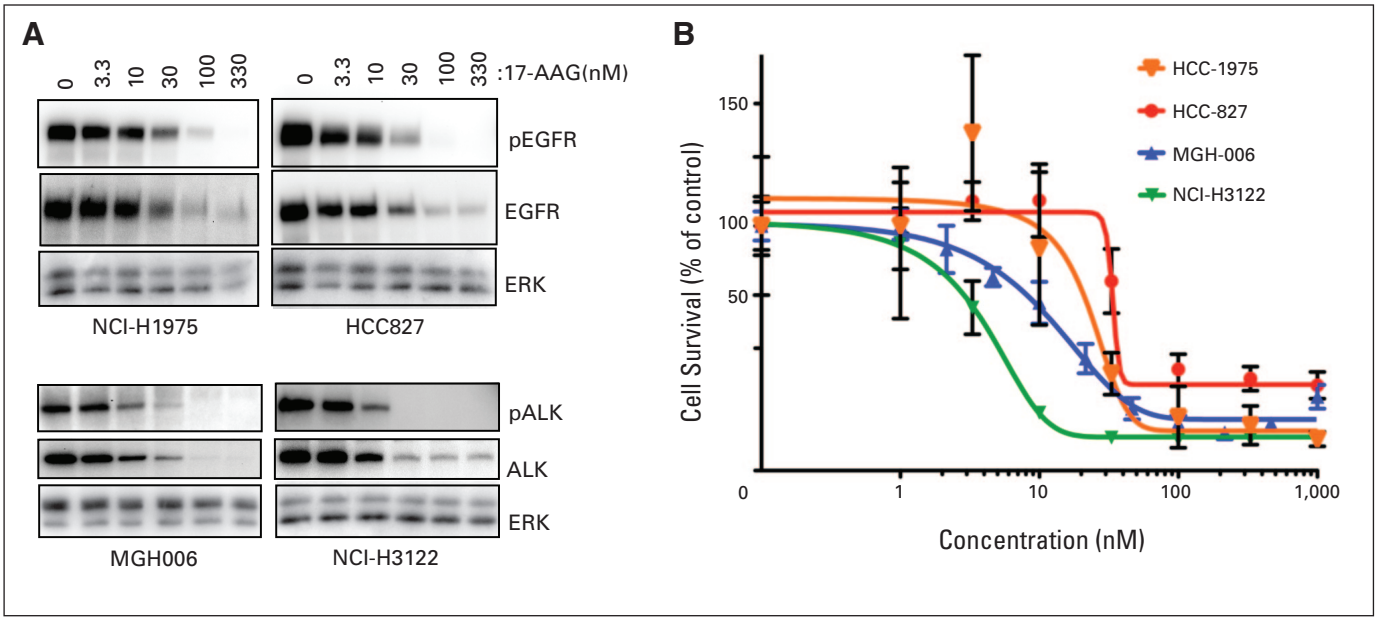

Fig 3. ALK rearranged cancer cell lines are sensitive to heat-shock protein 90 (Hsp90) inhibitors. (A) Cancer cell lines with epidermal growth factor receptor (EGFR) mutations (H1975, L858R/T790M, and HCC827 exon 19 deletion) or *ALK* gene rearrangement (H3122 and MGH-006) were treated with increasing doses of the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) for 24 hours. Protein lysates were probed with the indicated antibodies. (B) NCI-H1975, HCC827, MGH-006, and NCI-H3122 cells were treated with increasing doses of 17-AAG for 72 hours. Total cell viability was determined by Syto60 assay. Data are presented as the percent of viable cells relative to cells grown in the absence of drug.

inhibition of a driver mutation need not be via a receptor-specific molecule in order to be effective.

It is notable that despite extensive preclinical evidence that Hsp90 inhibition, and specifically IPI-504 treatment, leads to effective cell killing and tumor regression in *EGFR* mutation–positive models, including those with acquired resistance to EGFR TKIs, we saw few responses in patients with *EGFR* mutations.[28-30] There could be several reasons for this observation. Our population of patients was particularly atypical in that the median time from diagnosis among patients with *EGFR* mutations was 2 years and 54% had been treated with at least two prior EGFR TKI agents. Since their cancers had become resistant to EGFR TKIs, the biology of their tumors may have changed from being dependent on a single oncogene to a more heterogeneous state. The dose of IPI-504 could also have been a factor in the modest response rate among *EGFR* mutants. Our analyses of cancer cell lines suggest that lower concentrations of Hsp90 inhibitors may be required to downregulate expression of *EML4-ALK* compared with mutant *EGFR*. The dose-response curve for *EGFR* mutant cancers was modestly shifted to the right compared with the dose-response curve for *ALK*-rearranged cancers. This potentially wider therapeutic window may possibly have contributed to the higher response rate observed in the patients with *ALK* rearrangements. Importantly, the lack of acquired resistance to ALK-specific therapy among the patients with *ALK* rearrangements may imply a discrete molecular biology that was more susceptible to Hsp90 inhibition than the patients with *EGFR* mutations, all of whom had previously received and acquired resistance to EGFR TKIs. None of the patients on our trial (regardless of genotype) had previously been treated with ALK-specific inhibitors.

In our study, IPI-504 was generally safe and tolerable, with low rates of grade 3 or higher adverse events. The most common adverse events included nausea, fatigue, and diarrhea, and these were mostly grades 1 and 2. Grade 3 or higher liver function abnormalities were observed in nine patients (11.8%), and drug-related deaths were complicated by patients' underlying lung cancer.

Limitations of our study include its relatively small size and the atypical population studied, which may affect the generalizability of the results. Both the *EGFR* mutation–positive and wild-type cohorts had a long interval since diagnosis, a high number of prior therapies, and a low proportion of smokers. Furthermore, tumor tissue available for genetic analysis was primarily from diagnostic biopsies, before any targeted therapy or development of resistance that might have altered the genetic signature. However, the fact that tumor tissue for genotyping was collected from 100% of participants due to eligibility mandate was important. Not only did this allow us to make specific observations regarding response by *EGFR* genotype, we were able to carefully examine the minority of patients with robust responses, enabling the key observation of activity in *ALK*-rearranged NSCLC. We believe that all studies of targeted therapies should require tissue from all participants. It is not uncommon for studies of novel agents to show activity among only a minority of subjects, and our study effectively illustrates how posthoc molecular analysis of the best responding patients can provide direction for avenues of further research. Of note, we confirmed *ALK* rearrangement in our patients with the current standard break-apart fluorescent in situ hybridization assay that detects rearrangement in chromosome 2, but does not identify the specific variant of *EML4-ALK* present (*EML4* has multiple break points at which it can partner with *ALK*).[31] Therefore, we do not currently know if IPI-504 has a range of expected activity dependent on the oncogenic *EML4-ALK* variant.

In summary, although the predefined end point of 20% response was not observed in either *EGFR*-genotyped cohort, the novel Hsp90 inhibitor IPI-504 has activity in NSCLC, in particular among patients with *ALK* rearrangements. Additional study is required to prospectively evaluate the efficacy of Hsp90 inhibition in patients with *ALK* rearrangements and other oncogenic driver mutations.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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