Phase II Trial of Dasatinib for Patients with Acquired Resistance to Treatment with the Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors Erlotinib or Gefitinib

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Introduction: Dual inhibition of *SRC*- and *EGFR*-dependent pathways may overcome acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) for patients with lung adenocarcinoma with *EGFR* mutations. The *SRC* inhibitor dasatinib demonstrates antitumor activity in gefitinib-resistant cells lines and xenografts. Dasatinib is tolerable for patients with advanced non-small cell lung cancer, and in combination with erlotinib.

Methods: We conducted this phase II study of dasatinib 70 mg twice daily in patients with *EGFR*-mutant lung adenocarcinoma and acquired resistance to EGFR-TKIs. After a protocol amendment based on evolving data about both drugs, patients received dasatinib at a dose of 100 mg daily with continued erlotinib after developing acquired resistance. Enrolled patients either harbored an activating mutation in *EGFR* or experienced clinical benefit with single-agent erlotinib or gefitinib, followed by RECIST documented progression while being treated with an EGFR-TKI.

Results: Twenty-one patients were enrolled, 9 under the original trial design and 12 after the protocol amendments. We observed no complete or partial responses (0% observed rate, 95% confidence interval: 0–18%). The median time to progression was 0.5 months (range, 0.2–1.8 months) in patients treated with dasatinib and 0.9 months (range, 0.4–5 months) for patients treated with dasatinib and erlotinib in combination. Pleural effusions and dyspnea were frequent toxicities.

Conclusions: Dasatinib has no activity in patients with *EGFR*-mutant lung adenocarcinoma with acquired resistance to erlotinib and gefitinib.

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Seventy percent of patients with lung adenocarcinoma harboring mutations in the epidermal growth factor receptor (*EGFR*) gene experience a partial response when treated with EGFR tyrosine kinase inhibitors (TKIs) erlotinib or gefitinib.[1] However, the majority of patients progress within 17 months of the start of the treatment.[2] At least 50% of lung cancer patients with acquired resistance to erlotinib or gefitinib develop a secondary T790M mutation within *EGFR*, and another 10 to 15% of patients demonstrate *MET* amplification.[3–5] Therapies directed against these mechanisms of acquired resistance are desperately needed.

SRC is a nonreceptor tyrosine kinase that demonstrates increased protein levels in *EGFR*-dependent tumors. *SRC* and *EGFR* are proteins capable of mutual phosphorylation that share downstream effectors such as phosphatidylinositol 3-kinase/PTEN/Akt and *STAT* proteins.[6] Because of these functional associations, *SRC* kinase has been proposed as a target to overcome acquired resistance in *EGFR*-mutant tumors.

Preclinical models demonstrate *EGFR*-mutant cell lines containing either L858R (H3255) or exon 19 deletions (PC9 or HCC827) undergo apoptosis when treated with the *SRC* inhibitor dasatinib.[7] Gefitinib-resistant adenocarcinoma cells with T790M (PC9/ZD) or *MET* amplification (HCC827 GR5) undergo cell death when treated with dasatinib.[8] Dasatinib also inhibits tumor growth in HCC827 GR5 nude mouse xenografts.[8] Dasatinib has been studied in patients with advanced solid tumors, with pleural effusions dose-limiting.[9] Dasatinib can be combined with erlotinib in unselected patients with advanced non-small cell lung cancer (NSCLC).[10]

Given its preclinical rationale and early clinical trial results, we conducted a phase II study of dasatinib in patients with *EGFR*-mutant lung adenocarcinoma and acquired resistance to the EGFR-TKIs erlotinib and gefitinib.

PATIENTS AND METHODS

Patients with lung adenocarcinoma meeting consensus criteria for acquired resistance were eligible.[11] All patients

agreed to undergo a repeat tumor biopsy. This protocol was reviewed and approved by the Institutional Review Board at Memorial Sloan-Kettering Cancer Center.

Initial Study Design

Seven days after discontinuing erlotinib or gefitinib therapy, patients began dasatinib at a dose of 70 mg twice daily. Patients were evaluated by computed tomography scan at 4 weeks, 8 weeks, and at 8-week intervals. Modified Response Evaluation Criteria in Solid Tumors (version 1.1) were used to assess response. Toxicities were graded using the National Cancer Institute Common Terminology Criteria of Adverse Events (version 3.0).

A Simon two-stage design was employed to calculate an initial sample size of 12. Cohort expansion to 37 patients was planned if one or more partial responses were observed. If 4 or more of the first 12 patients developed grade 3 or 4 pleural effusions, the trial would be stopped.

Amended Design

Coincident with the start of this trial, we observed that patients with acquired resistance who discontinued EGFR-TKIs experienced symptomatic deterioration and accelerated tumor growth with increased [18^F]-fluorodeoxyglucose (FDG) avidity on positron emission tomography scans.[12] After restarting erlotinib or gefitinib in these patients, tumors decreased in size and SUV_{max} on repeat studies, and tumor-related symptoms improved. Given these observations, we now recommend continued erlotinib in patients with acquired resistance while also adding second-line treatment agents and amended this protocol to allow patients to continue erlotinib in addition to beginning dasatinib. In addition, new data indicated that dasatinib 100 mg daily provided similar efficacy with less pleural effusions when used to treat patients with chronic myelogenous leukemia (CML),[13] and we further amended this trial to allow a dose of dasatinib 100 mg daily.

Mutational Analysis

Before initiating dasatinib, all patients underwent tumor biopsies, preferably at a site of growing or new disease. Genomic DNA was extracted from tumor specimens, and all *EGFR* mutations (exon 19 deletions, L858R and T790M substitutions) were identified by mutation-specific polymerase chain reaction-based methods.[14] Tumor specimens were analyzed for *MET* amplification using dual-color fluorescent in situ hybridization with a *MET*-specific gene probe.[5] *MET* amplification was defined as having a *MET*:CEP7 ratio of >2:1.

RESULTS

Dasatinib 70 mg Twice Daily

Nine patients were enrolled under the original trial design. The median age was 68 years, and 66% of patients were women (Table 1). Similar numbers of patients in this cohort harbored exon 19 deletions and L858R mutations in their tumors. One patient had insufficient tissue for analysis (Table 2).

TABLE 1. Patient Characteristics

Characteristic	Dasatinib 70 mg Twice Daily (n = 9)	Dasatinib 100 mg Daily + Erlotinib (n = 12)
Age, median (range)	68 (35–80)	60 (51–73)
Female/male	6/3	7/5
Smoking history		
Never	7	8
Former	1	3
Current	1	1
KPS (median)	80	80
Months (median) treated with EGFR-TKI before developing acquired resistance	16 (8–102)	21 (12–49)

KPS, Karnofsky performance score; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor.

TABLE 2. Molecular Studies of Tumor Specimens

	Dasatinib 70 mg Twice Daily (n = 9)	Dasatinib 100 mg Daily + Erlotinib (n = 12)
Primary *EGFR* mutations		
Exon 19 deletion	4	10
L858R	4	2
Unable to be tested[a]	1	0
Molecular abnormalities found at the time of developing acquired resistance		
T790M + Exon 19 del	2	8
T790M + L858R	2	0
T790M not found + Exon 19 del	2	2
T790M not found + L858R	1	2
T790M unable to be tested[a]	2	0
MET amplification present	0	0
MET amplification absent	4	7
MET amplification unable to be tested[a]	5	5

[a] Unable to be tested due to insufficient tumor tissue.

Patients were treated for a median of 16 months with primary EGFR-TKIs before developing acquired resistance (Table 1). When rebiopsied at the time of study enrollment, 44% (4/9) of patients had developed T790M acquired resistance mutations; none of the patients with adequate tissue for fluorescent in situ hybridization testing exhibited *MET* amplification (0/4 tested) (Table 2).

There were no complete or partial responses observed (0%, 95% confidence interval: 0–34%). All patients progressed within 2 months of starting dasatinib. The median time until progression was 0.5 months (range, 0.2–1.8 months). The median overall survival was 13 months.

The combination of rapid disease progression among these initial nine patients, the majority (6/9) of whom developed pleural effusions, prompted revisions to our protocol design, although still 3 patients away from its required sam-

ple size of 12. We decided the protocol revisions were necessary (see Amended Design section) to effectively evaluate our original study hypothesis.

Dasatinib 100 mg Daily + Erlotinib

Twelve more patients were enrolled. The median age was 60 years, and 58% were women (Table 1). The majority (80%) of this cohort had tumors with exon 19 deletions (Table 2). Patients received a median of 21 months of primary EGFR-TKI therapy before the development of acquired resistance (Table 1). At rebiopsy, 75% had T790M (Table 2). *MET* amplification was not identified in the seven specimens tested.

Twelve patients were treated with dasatinib and erlotinib, and no complete or partial responses were observed (0% objective response rate, 95% confidence interval: 0–28%). Patients were treated with dasatinib 100 mg daily and erlotinib for a median of 0.9 months (range, 0.4–5.4 months).

Toxicities

The primary toxicity was the development and/or enlargement of preexisting pleural effusions and dyspnea. Among patients treated with dasatinib alone, three patients required hospitalization for thoracostomy tube placement. One patient receiving dasatinib with erlotinib required a similar intervention. Peripheral and facial edema was also reported.

Fatigue was another significant side effect, whether patients were treated with dasatinib alone or with erlotinib. Patients reported grade 3 fatigue in 2 of 9 (22%) and 2 of 12 (17%) of patients, respectively. Nausea and vomiting (one episode of grade 3 toxicity each) as well as grade 2 diarrhea were reported.

DISCUSSION

In this phase II trial of *SRC* inhibitor dasatinib, with and without erlotinib, in patients with lung adenocarcinoma and acquired resistance to erlotinib, no objective responses were observed. Pleural effusions and dyspnea were the most frequent and significant toxicities. Regardless of whether erlotinib was continued, patients were treated with dasatinib for a median of less than 1 month due to a combination of disease progression and toxicity.

Haura et al.[10] studied dasatinib and erlotinib in unselected patients with advanced lung cancer previously untreated. Two partial responses were reported, one in a patient whose tumor harbored an *EGFR* exon 19 deletion. This response likely reflects solely the effect of erlotinib in a tumor with a sensitizing *EGFR* mutation. Very recently, Johnson et al. also reported the results of a phase II trial of single-agent dasatinib as first-line treatment for unselected patients with advanced NSCLC. Neither *SRC* activity nor mutations in *EGFR* (or *KRAS*) were associated with the modest response rates reported—overall disease control rate of 43% and progression-free survival of 1.36 months.[15]

Pleural effusions were observed in 66% of patients enrolled in this study. The pleural effusions observed were clinically more significant for the patients who received dasatinib 70 mg twice daily—50% of whom required thoracentesis or tube thoracostomy. Only one patient treated with dasatinib 100 mg daily and erlotinib required a tube thoracostomy. Prior studies in patients with CML and advanced solid tumors treated with dasatinib also observed pleural effusions to be dose-related.[9,13] Johnson et al.[15] reported that the presence of pleural effusions in patients with advanced NSCLC before treatment with dasatinib predicted the development of clinically significant effusions during treatment.

In many cases, it was difficult to discern whether these new pleural effusions were dasatinib-related drug toxicity or evidence of progressive cancer. Unlike patients with CML or solid tumors other than lung cancer treated with dasatinib, in whom pleural effusions can easily be recognized as drug toxicity and managed with dose attenuation, diuretics, and corticosteroids without treatment delays, this complication proved very challenging to manage in our patients with lung adenocarcinoma.

The amended trial design also limited interpretation of the study's negative results. We simultaneously changed the dasatinib dosing schedule and added erlotinib to the treatment regimen to incorporate the latest information about both drugs. By making changes to two aspects of the trial design at once, we clouded our ability to discern the true reason (or reasons) for the disappointing results.

We found the combination of dasatinib and erlotinib to be inactive, with significant toxicities. Our study suggests that SRC inhibitors merit no further study in patients with *EGFR*-driven lung adenocarcinoma. Therefore, these patients remain ideal candidates for clinical trials investigating new strategies to overcome acquired resistance.

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