BRIEF REPORT

Pulse Afatinib for *ERBB2* Exon 20 Insertion–Mutated Lung Adenocarcinomas

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ABSTRACT

Introduction: Genomic aberrations involving the erb-b2 receptor tyrosine kinase 2 gene (*ERBB2*) are driver oncogenes in approximately 2% of lung adenocarcinomas. However, the use of daily dosing of ERBB2 tyrosine kinase inhibitors (TKIs)—including afatinib—has been fraught with plasma concentrations that barely achieve preclinical model inhibition, significant patient-reported toxicities, and limited clinical activity. We hypothesized that alternative dosing strategies could improve tolerability and efficacy.

Methods: We profiled lung cancer cell lines against TKIs and retrospectively evaluated the toxicity of and response to pulse afatinib (280 mg once weekly) in lung cancers with *ERBB2* mutations.

Results: An *ERBB2* exon 20 insertion–mutated lung cancer cell line had a 50% inhibitory concentration in response to afatinib that was higher than the reported plasma concentration of afatinib, 40 mg daily. Three patients with advanced *ERBB2*-mutated lung adenocarcinomas were treated with off-label pulse afatinib. The 280-mg weekly dose was well tolerated with no reported rash and minimal diarrhea. One TKI-naive patient achieved a partial response for 5 months and another achieved stable disease for 11 months.

Conclusions: Pulse afatinib at a weekly dosing scheme induced antitumor activity in *ERBB2* exon 20 insertion–mutated lung adenocarcinomas. Future clinical trials of alternative dosing schemes of ERBB TKIs as monotherapy or in combination with other therapies are warranted for *ERBB2*-mutated tumors.

Keywords: Lung cancer; Adenocarcinoma; ERBB2; HER2; Exon 20; Afatinib

Introduction

Somatic mutations of the erb-b2 receptor tyrosine kinase 2 gene (*ERBB2*)—alternatively known as *HER2*—were identified in 1.7% of all lung adenocarcinomas[1] analyzed by The Cancer Genome Atlas. Most *ERBB2* mutations congregate as inframe insertions within exon 20 of this ErbB family member,[2] akin to epidermal growth factor receptor gene (*EGFR*) exon 20 insertion mutations.[3] Preclinical models have consistently demonstrated that ERBB2 exon 20 insertion mutants are transforming in lung models and define oncogene addiction.[2] Dual EGFR/ERBB2 tyrosine kinase inhibitors (TKIs) have been analyzed in these same preclinical models. This line of research disclosed that *ERBB2*-mutated lung adenocarcinomas could be inhibited by irreversible second-generation ERBB TKIs, such as afatinib, dacomitinib, and neratinb, but at concentrations that were close to 100-fold higher than those necessary to inhibit *EGFR* exon 19 deletion– or L858R-mutated models.[4–6]

The dose-limiting toxicities of afatinib, dacomitinib, and neratinib are related to inhibition of wild-type EGFR in the skin and gastrointestinal tract, therefore limiting achievable plasma concentrations in patients to

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nanomolar concentrations that may not fully inhibit ERBB2 exon 20 mutant proteins.[7,8] In as much, clinical trials and case series of afatinib, dacomitinib, and neratinib monotherapy used at their daily dosing schemes have been disappointing to date. The overall response rates (ORRs) to daily dacomitinib, neratinib, and afatinib have been reported at less than 15% to 30%, with short periods of disease control and frequent rash and/or diarrhea requiring dose reductions or discontinuation.[9,10]

We hypothesized that an alternative treatment strategy for irreversible ERRB TKIs, such as intermittent pulsatile doses, could improve tolerability (by decreasing skin toxicities) and efficacy (by achieving intermittent plasma concentrations that would exceed the threshold for antiproliferative inhibition of *ERBB2* exon 20 insertion–mutated lung adenocarcinomas). Here, we report preclinical models that support our hypothesis and retrospectively compile the response and toxicity in three patients with advanced lung adenocarcinoma treated with pulse afatinib at a dose of 280 mg once weekly.

Methods

Cell Culture, Cell Proliferation Assays, and Reagents

NCI-H1781 (H1781) cells harboring *ERBB2*-G776>VC were purchased from ATCC (Manassas, VA). Cells were maintained in RPMI 1640 medium (Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum and grown at 37°C in a humidified atmosphere with 5% CO_2. These cells were overlaid in 96-well plates, allowed to attach overnight, and then treated with or without two TKIs (erlotinib and afatinib) for 72 hours. Cell viability was determined by a CellTiter 96 Aqueous One solution proliferation kit (Promega, Madison, WI) according to the manufacture's protocol. Experiments were performed in triplicate. Inhibitory proliferation curves and the 50% inhibitory concentration (IC_{50}) were generated using the GraphPad Prism 6 software (GraphPad Software, La Jolla, CA). We used IC_{50} values obtained in identical conditions and previously published[11] for NCI-H3255 (H3255, *EGFR*-L858R) and NCI-H1975 (H1975, *EGFR*-L858R+T790M) to contrast H1781 with an EGFR/ERBB2 TKI-sensitive and TKI-resistant cell line, respectively. Erlotinib and afatinib were purchased from LC Laboratories (Woburn, MA). All reagents were dissolved in DMSO and stored at −80°C.

Tumor and Data Collection

Patient-tumor pairs followed at Beth Israel Deaconess Medical Center with a diagnosis of lung adenocarcinoma were identified through an ongoing institutional review board–approved study.[12] Pathologic data, tumor genotype, toxicity to afatinib, and radiographic parameters were gathered from retrospective chart extraction. Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 was used to fit target and nontarget lesions. Data were collected and managed using REDCap electronic data capture hosted at Beth Israel Deaconess Medical Center.

Afatinib Dosing Choice and Treatment

Afatinib—an irreversible EGFR/ERBB2 TKI—has a label dose of 40 mg daily for advanced *EGFR*-mutated non–small cell lung cancers (equal to a dose of 280 mg in 1 week). For the pulse dose of afatinib in *ERBB2*-mutated non–small cell lung cancers, the clinicians and patients at our center used an off-label dosing scheme of the full weekly dose once weekly (e.g., 280 mg once weekly). The rationale was based on prior experience with pulsatile dosing schemes of the EGFR TKI erlotinib, for which doses exceeding the weekly total dose can be given once weekly.

Results

Preclinical Models

We selected three representative lung adenocarcinoma cell lines to contrast the antiproliferative effects of the reversible EGFR TKI erlotinib and the irreversible EGFR/ERBB2 TKI afatinib. H3255 (*EGFR*-L858R) represents an erlotinib/afatinib-hypersensitive cell, H1975 (*EGFR*-L858R plus T790M) characterizes an erlotinib/afatinib-resistant cell, and H1781 (*ERBB2*-G776>VC) embodies an *ERBB2* exon 20–mutated lung cell line.

Figure 1 depicts the IC_{50} values of dose-dependent proliferation experiments for the aforementioned TKIs. We also plotted, on the basis of patient-level pharmacokinetic data,[7,8] the reported median trough plasma concentration of erlotinib, 150 mg/day (~2000 nM, Fig. 1*A*), or afatinib, 40 mg/day (~60 nM, Fig. 1*B*). H3255 showed an IC_{50} value that is many folds lower than the plasma concentrations of erlotinib or afatinib. H1975 showed an IC_{50} value that exceeds the plasma concentrations of erlotinib or afatinib (Figs. 1*A* and *B*, respectively).

For the *ERBB2*-mutated cell line H1781, the IC_{50} value for erlotinib at 6377 nM is consistent with lack of significant anti-ERBB2 activity of this reversible EGFR TKI (Fig. 1*A*). The IC_{50} value for afatinib at 91 nM was lower than that for H1975 but still higher than the plasma concentration of afatinib, 40 mg daily (Fig. 1*B*). The lower IC_{50} values of all cell lines in response to afatinib compared with in response to erlotinib reflect the potency of this irreversible EGFR/ERBB2 TKI against mutant and wild-type proteins.