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Erlotinib in African Americans with Advanced Non-Small Cell Lung Cancer: A Prospective Randomized Study with Genetic and Pharmacokinetic Analysis

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Abstract

Prospective studies focusing on EGFR inhibitors in African Americans with NSCLC have not been previously performed. In this phase II randomized study, 55 African Americans with NSCLC received erlotinib 150mg/day or a body weight adjusted dose with subsequent escalations to the maximum allowable, 200mg/day, to achieve rash. Erlotinib and OSI-420 exposures were lower compared to previous reports, consistent with CYP3A pharmacogenetics implying higher metabolic activity. Tumor genetics revealed only two EGFR mutations, EGFR amplification in 17/47 samples, 8 KRAS mutations and 5 EML4-ALK translocations. Although absence of rash was associated with shorter time to progression (TTP), disease control rate, TTP, and 1-year survival were not different between the two dose groups, indicating the dose-to-rash strategy failed to increase clinical benefit. Observed low incidence of toxicity and low erlotinib exposure suggest standardized and maximum allowable dosing may be suboptimal in African Americans.

Keywords

EGFR; Erlotinib; African American; Pharmacokinetics; Pharmacogenetics

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CONFLICTS OF INTEREST

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INTRODUCTION

African Americans have a higher incidence and overall mortality of lung cancer compared to Caucasians.[1] The reasons for these disparities have been debated extensively, with arguments including increased susceptibility and more aggressive biologic features for lung cancer in African Americans, increased stage of disease at diagnosis, and less aggressive treatment being offered for early-stage disease.[2–6] Survival analyses of patients participating in cooperative groups clinical trials, which provide for uniform extent of disease and therapy, showed statistically significantly worse survival at one year for African American patients.[7]

This disparity suggests an even more pressing need to develop new anticancer agents to target lung cancer in this patient population, and the low rate of enrollment of African Americans in cancer clinical trials is distressing. Moreover, the development of most novel cancer therapeutic agents occurs exclusive of African Americans. Potential metabolism/drug-disposition, tolerance, and drug target frequency issues for this ethnicity, which may be relevant to the safety and/or efficacy of these agents, are often ignored until the drugs are widely available to the general population.

Interest in molecular targeted therapy for lung cancer has been stimulated by the success of tyrosine kinase inhibitors (TKis) (i.e., gefitinib, erlotinib) in the setting of NSCLC tumors harboring epidermal growth factor receptor (EGFR) mutations (approximately 10% of NSCLC tumors).[8–12] Common *EGFR* activating mutations in NSCLC that are sensitive to TKI therapies include amino acid deletions in exon 19 and the L858R point mutation at exon 21.[8,9] However, erlotinib has been shown to be effective at delaying progression and improving overall survival (OS) as second- and third-line treatment of NSCLC, even in unselected patients, thus its current labeling.[12,13] Clinical benefit from this agent may therefore be mediated by additional factors, including tumor mutations outside *EGFR* mutation hotspots, pharmacokinetics, pharmacogenetics, gender and race.[13,14] Furthermore, the primary toxicity of this agent, skin rash, has been correlated with response and survival.[15]

Recent data suggests pharmacogenetic factors may influence disposition of TKIs. For example, erlotinib is inactivated through *CYP3A4, CYP3A5, CYP1A1* and *CYP1A2* metabolism.[16,17] Ethnic diversity in cytochrome P450 polymorphisms and enzyme activity has been well described, and lower erlotinib exposure in African Americans may be expected due to metabolism from higher *CYP3A4/5* and *CYP1A1/2* expression compared to Caucasians.[18–20] Furthermore, previous reports evaluating genetic diversity in *CYP1A1/2* suggest genotypes less prevalent in African Americans may confer protective effects in never smokers and increased risk of lung cancer in ever smokers.[21,22]

In this first of its kind trial, we aimed to prospectively explore the roles of dosing strategy, smoking, pharmacokinetics, pharmacogenetics, and tumor genetics on outcomes following erlotinib treatment in African Americans with NSCLC.

RESULTS

Patient Characteristics

Fifty-eight patients were consented between April 2006 and July 2011. Prior to receiving treatment, one patient withdrew consent and two patients were removed from study due to deteriorating health status. Pretreatment characteristics for the 55 patients treated on study are presented in Table 1. Prior to the 3-month period for response assessment one patient was removed from the study for evaluation of pulmonary toxicity (day 42). Two additional patients died prior to response assessment (sudden death due to likely pulmonary embolism in one patient and clinical deterioration in the other). The remaining 52 patients were evaluable for the primary endpoint of DCR.

Treatment Toxicity

Fifty-five enrolled patients received therapy. All but 8 patients received 150 mg as starting dose; 6 and 2 patients received 175 and 200 mg starting doses, respectively. Of the 9 patients in Arm B who did not experience rash during the first cycle, 1 started at the maximum dose (200 mg), and 5 others did not receive treatment in cycle 2. All 3 patients in Arm B who were dose escalated started at 150 mg and received maximum escalated doses of 175 mg (n=1) or 200 mg (n=2). Missed doses were minimal overall, especially in cycles 1 and 2, according to patient diaries. Adverse events and treatment-related toxicities experienced during the trial are summarized in the Table 2. The predominant toxicity was rash (69% of patients), followed by anorexia and diarrhea (29% each), and nausea (15%). Overall, toxicities were mild, the majority being of grade 1–2, and only 11 patients (20%) experienced any grade 3 toxicity. Severe events included a grade 5 gastrointestinal hemorrhage from a gastric ulcer one month after beginning therapy, a grade 3 interstitial pneumonitis, a grade 3 dehydration, and a grade 2 bowel obstruction. The patient with interstitial pneumonitis was removed from study; there were no other withdrawals due to drug-related adverse events, and no dose reductions for toxicity.

Treatment Efficacy

A total of 12 patients had their disease controlled at 3 months; six for each arm. In arm A DCR, TTP, and 1-year OS were 23% (95% CI, 9%–44%), 2.8 months (95% CI, 2.5–3.1), and 30% (95% CI, 13–47%), respectively (Table 3). In arm B DCR, TTP, and 1-year OS were 23% (95% CI, 9%–44%), 2.4 months (95% CI, 1.5–2.7) and 26% (95% CI, 9–42%). When patients were stratified by rash, median TTP for patients with rash (n=38) compared to patients not experiencing rash (n=17) was significantly different (TTP 2.8 vs. 1.8 months, p=0.036). However, median OS was not significantly different (5.2 vs. 5.3 months, p=0.30). Severity of rash (grades 2–3 vs. grade 1) was not associated with differences in outcomes.

Non-compartmental pharmacokinetics

Pharmacokinetic data was obtained from 26 patients who received either 150 mg (n=20) or 175 mg erlotinib (n=5) during cycle 1. Figure 1 displays observed concentration-time profiles for erlotinib and OSI-420 for all patients on cycles 1 and 2. Non-compartmental parameter estimates for both agents are displayed in Table 4 (Table S1 also displays the data

separated by dose level). No associations were observed between rash and erlotinib pharmacokinetics. However, lower mean OSI-420 Cmax was observed in patients with no or mild rash (grades 0,1; 108 ± 87 ng/mL) compared to patients with more severe rash (grades 2,3; 252 ± 182 ng/mL, p-value = .023, 2-tailed t-test). Furthermore, trends were observed where current smokers had overall decreased erlotinib $AUC_{0–24}$ on day 1 of cycle 1 (12.2 ± 6.2 mg/L*hrs, n=11) compared to former/never smokers (17.4 ± 8.9 mg/L*hrs, n=14, Student's 2-tailed t-test $P = 0.12$; see Figure 1 and Supplemental Figure S3). This trend was not apparent beyond cycle 1, but OSI-420 concentrations did trend higher in smokers compared to non-smokers in cycle 2 (see Supplemental Figure S4). No statistically significant associations were observed between erlotinib pharmacokinetics and outcomes, including progression-free and overall survival.

Pharmacogenetics

Erlotinib is metabolized primarily by *CYP3A4/5* and *CYP1A1/2*[16,17] and actively excreted via *ABCB1* and *ABCG2*.[23,24] Genetic polymorphisms in these genes were evaluated in the first 26 patients on study. Additional genotyping was completed for *CYP3A4, CYP3A5,* and *ABCB1* in 16 additional patients. These data are summarized in Table 5.

Thirty of 39 (77%) patients evaluated were homozygous (*1/*1) or heterozygous (*1/*3) for the *CYP3A5* rs776746 A allele, suggesting they had functional expression of *CYP3A5*. Of the 9 patients homozygous for the G allele, only 3 had pharmacokinetic data for both cycles, thus limiting statistical comparison of this polymorphism and erlotinib or OSI-420 pharmacokinetics. Notably, one of the 3 patients with the G/G genotype had no measureable OSI-420 during either cycle 1 or 2, potentially reflecting dysfunctional *CYP3A5* and limited formation of the metabolite. $AUC_{0–24}$ ratios of OSI-420/OSI-774 were low to moderate for the other two (e.g. 0.022 and 0.060 for cycle 1) compared to remaining patients with PK data (range 0.005 – 0.129).

Recently, the *CYP3A4* rs35599367 (*22) polymorphism was shown to associate significantly with pharmacokinetics and dose titration of statins, tacrolimus and cyclosporine.[25–28] Data from these studies suggest this polymorphism, which reduces *CYP3A4* activity, is less prevalent in African Americans compared to other populations studied. Consistent with previous data, all but one of 42 patients evaluated for the rs35599367 polymorphism were homozygous for the major C allele. This patient was a male former smoker with EGFR gene amplification who received 150 mg erlotinib. He was also one of 9 patients homozygous for the *CYP3A5*3* G allele. Pharmacokinetic data was not available for this patient. He achieved grade 1 rash during cycle 1, and his best response was progressive disease.

Erlotinib is also a known substrate of *CYP1A1/2* and multidrug resistance transporters, P-glycoprotein (P-gp, *ABCB1*) and breast cancer resistance protein (*BCRP, ABCG2*). A total of 13 polymorphic variants were evaluated for these genes. Frequencies for these variants were similar to those previously reported (see Table 5).[20] No significant associations were identified with these genes.

We also examined relationships between individual polymorphisms and outcomes from therapy, including rash and response. No trends or significant associations were found.

Population pharmacokinetics and covariate analysis

The final structural population pharmacokinetic model constructed in this study differed from previously published models. It utilized one-compartment models for both erlotinib and OSI-420, and it included inter-individual variability (IIV) parameters on erlotinib clearance (CL), volume of distribution (V1), and OSI-420 clearance (CLM). A shared ETA approach was used for CL and CLM, as these were observed to be highly correlated (0.86 correlation coefficient). We also included a single inter-occasion variability (IOV) parameter, which was estimated on CL. The final residual error model included a single exponential term for each, erlotinib and OSI-420. The erlotinib first-order absorption rate constant (Ka) was estimated with poor precision (coefficient of variation, CV > 80%) and was therefore fixed to 1.04 h^{-1} based on estimated values from the model and comparison with other reported values.[29,30] Table 6 lists estimated parameter values for the base model. Notable results include high precision for all base structural model parameters (less than 16% CV), except V2 which had a 62.8% CV. However, IIV_{CL} was estimated with relatively poor precision with 80.8% CV and a lower confidence interval (CI) boundary of 0.005. Also, while the shared ETA scale factor had moderate precision (37.0% CV), the base model bootstrap CI included zero (−148.10, 16.11). Ultimately, when re-evaluating the shared ETA in the covariate model, it maintained acceptable precision (56.5 CV%), and its bootstrap confidence interval no longer contained zero. The shared ETA scale factor was therefore retained in the model to address the high correlation between CL and CLM.

Covariate analysis was conducted to identify sources of variability between patients. Several covariates were evaluated for effect on model parameters, including age, weight, sex, smoking status, lean body mass (LBM), pharmacogenetics, and co-medications. Only LBM was found to significantly reduce the objective function value (OBJ). Only 2 patients received a *CYP3A4/5* inhibitor (voriconazole) or inducer (phenytoin) during therapy. Ten patients received *CYP1A2* inducers (6 omeprazole, 5 insulin, 1 received both). Several patients received *CYP1A1/2* and *CYP3A4/5* substrates (the most common of these were acetaminophen and oxycodone). There were no evident relationships between co-medications and pharmacokinetics in this study. The population PK parameter estimates in bootstrap analyses were comparable with those generated using the original data set, indicating acceptable accuracy and stability of the structural and covariate models (see Table 6). One exception to this was IIVCL, for which the bootstrap median was approximately 6-fold higher compared to the model estimated value for the original dataset (0.13 vs. 0.02, respectively). Again, as for the base model, the standard error was relatively high for this estimate (127.1% CV), and the confidence interval had a lower boundary near zero (0.001).

Tumor Genetics

Tissue in sufficient quantities for evaluation of one or more molecular aberrations was available from 53 patients. Tumor genetic data is summarized in Table 7. *EGFR* exon 19 insertions or deletions and exon 21 mutations were successfully evaluated in 45 patients, and only one patient had an exon 19 short in-frame deletion. Notably, an additional *EGFR*

(T790M) mutation[31] was present in this patient at a biopsy obtained at progression after two years on erlotinib. A second patient had an *EGFR* R776L mutation. Seventeen of 47 patients (36%) scored positive for *EGFR* gene amplification (defined as 4–10 *EGFR* gene copies in >20% of the tumor cells independent of *EGFR* to *CEP7* ratio). Interestingly, the *ALK* gene was translocated in 5 of 39 patients evaluated (13%), and one additional patient had *ALK* amplification (>10 copies). *KRAS* mutations were identified in 8 of 46 patients (17%); of these *KRAS* mutations, 6 were of the G12 type[32], and two were of uncertain significance (C/T genotypes in the 5'-UTR, i.e., −9 and −6 positions).

There were no clear associations between DC and absence (5 patients) or presence (7 patients) of most molecular abnormalities (see Table 7). Notably, four of the five patients with *ALK* translocation had progressive disease as best response and the fifth was not evaluable due to death before a restaging scan could be performed. Four of these patients were current smokers.

DISCUSSION

African Americans suffer from a heavier burden of NSCLC compared to other racial and ethnic groups within the U.S., and standard treatment regimens produce less favorable outcomes in African Americans for reasons that are not clear. The approval of *EGFR* tyrosine kinase inhibitors has yielded increased availability of treatment choices for patients with NSCLC, although there is paucity of prospective data regarding the benefits of these choices for African Americans. To address this gap we set out to prospectively evaluate the efficacy and toxicity of erlotinib at standard doses and with an alternative dose-to-rash strategy, as well as to characterize drug disposition, pharmacogenetics, and tumor/host factors as potential contributors to resistance for these agents in African Americans with NSCLC.

Erlotinib-related rash has been heralded as a potentially predictive factor for erlotinib activity. In theory, higher dosing leading to higher erlotinib exposure may lead to higher *EGFR* inhibition and pharmacodynamic effects, such as rash and tumor response in patients with *EGFR* driven tumors. Interestingly, rash was not correlated with erlotinib exposure, although it did directly associate with OSI-420 exposure. Absence of rash was correlated with shorter TTP in our study, although presence of rash was not associated with improvements in overall survival.

With regard to the dose to rash strategy employed in this study, 18 of the 27 enrolled patients in Arm B experienced rash during the first cycle, and one additional patient achieved rash beyond cycle 1. However, only 3 of the 9 potential patients for dose escalation received higher than standard doses of erlotinib. Five patients were not treated on cycle 2, and 1 patient had started cycle 1 at the maximum allowable dose of 200 mg. Two of the 3 patients who were dose escalated also reached the maximum allowable dose of 200 mg without rash, and further escalation was not permitted per protocol design and IND restrictions at the time the study was initiated.

The available PK data shows erlotinib exposure in patients receiving 175 mg tended to be similar to that of patients receiving the 150 mg dose (see Table S1). This is likely a function of the relatively small number of patients receiving the 175 mg dose level, the relatively small difference between the doses, and the wide variability observed with TKI therapies. The data also show that toxicities were overall mild in this group of patients, similar to those reported in other studies using either 21 day cycles or continuous 150 mg erlotinib doses.in 81 Caucasian and 1242 Asian NSCLC patients[12,33] However, in these previous studies, dose reductions due to adverse events were more frequent (14% and 23%, respectively) compared to no required dose reductions in our study. Although we did not monitor plasma drug levels in patients beyond cycle 2, noncompliance was low according to patient dose diaries. Collectively, this information suggests that further dose escalation may have been tolerated and that inclusion of plasma AUC for dose escalation decisions (even beyond the 200 mg level) may have been a more productive strategy.

Pharmacogenetic factors more prevalent within the African American population that are associated with altered *CYP1A* and *CYP3A* activities are well documented and likely alter PK of drugs available for treatment of NSCLC, such as erlotinib. Pharmacogenetic factors observed in our study population were in fact suggestive of high *CYP3A* activity, consistent with most published literature. Only 2% and 26% of subjects harbored the most functionally relevant poor metabolizing genotypes, *CYP3A4*22* T allele (rs35599367) and *CYP3A5*3* A/A genotype (rs776746), respectively. Similarly low prevalence of *CYP1A1/2* alleles with reduced enzymatic activities were also observed in our study population. Only 17% of 24 subjects carry the CYP1A2*1K allele (rs2069536), and on the contrary 50% of 16 subjects carry the CYP1A2*1F allele (rs762551) which is highly inducible (http://www.cypalleles.ki.se/, accessed 11 December 2013).

While overall non-compartmental single dose and steady state PK data in this study was generally similar to that reported in other studies[30,34], estimated erlotinib clearance from nonlinear mixed-effects modeling was higher in our study (6.22 ± 1.52 L/hr) compared to those reported in any other study (range of population estimates for CL/F, 3.29–5.82 L/hr)[13,30,34–36]. Notably, the highest population estimate of CL/F in the previous studies was obtained by Kraut and colleagues, where erlotinib was combined with docetaxel, a known inducer of *CYP3A* enzyme activity[37,38].

African Americans also have a historically higher incidence of tobacco smoking,[39] which is associated with higher erlotinib clearance and has therefore been argued to contribute to the observed outcomes disparities.[39,40] Despite randomization in our study, an unequal distribution of smokers was observed between the control and experimental arms of the study. Current smokers comprised 48% and 29% of the experimental Arm B and Control Arm A, respectively. Pharmacokinetics analysis within the limits of the small number of patients evaluated (n = 26) did show differences (albeit not statistically significant) between current- and former/never-smokers (Figure 1). These data may indicate the intent to increase erlotinib exposure in patients treated on the experimental arm was further inhibited by an increased proportion of smokers randomly assigned to that arm.

Outcomes data indicated DCR at 3 months was similar to what would be expected with standard erlotinib doses in a population of previously treated patients with largely *EGFR* wild type tumors.[41,42,13] As reported by others[43] the prevalence of *EGFR* mutations was low in this population with high prevalence of tobacco use. However, a surprisingly high number of patients were found to have *ALK* translocation and could have benefitted from now clinically available *ALK* inhibitors. [44]

This is the first study to prospectively evaluate tumor genetics, pharmacogenetics, erlotinib pharmacokinetics, rash, and clinical outcomes in African Americans with NSCLC receiving single agent TKI therapy. A relatively high incidence of tumor genetics (*KRAS* mutations, *EML4-ALK*) associated with erlotinib resistance was found. As with other ethnicities, assessment of tumor genetics prior to treatment is recommended. Furthermore, smoking history and pharmacogenetic factors consistent with high erlotinib metabolism are predominantly present in African Americans. The observed apparent higher erlotinib clearance and mild toxicity data suggest that standard erlotinib dosing in African American patients may be associated with lower than desired exposure, and would support the consideration of higher doses from the outset as well as the ability to escalate beyond 200 mg. Overall, the data from this study supports the hypothesis that African Americans may have, on average, increased erlotinib metabolism compared to other patient populations. A larger study directly comparing erlotinib PK in African Americans and non-African Americans receiving the same dose and confirming compliance with drug monitoring will be necessary to better quantify this potential difference.

MATERIALS AND METHODS

Patient eligibility

The Cancer Institutional Review Boards of The Ohio State University and the University of North Carolina approved this study (clinicaltrials.gov NCT00230126). All patients provided written informed consent prior to study entry. Eligible patients included African Americans 18 years or older with measurable, stage IIIB or IV NSCLC, who had received no more than two chemotherapy regimens. ECOG performance status ≤2, normal organ and marrow function, and tumor tissue samples from archived or fresh biopsies were required. Exclusions included pregnancy, prior treatment with *EGFR* inhibitors, major surgery within 21 days, severe pulmonary insufficiency (pO_2 <90%; CO2 >50mmHg) and uncontrolled inter-current illnesses. Patients receiving HIV combination anti-retroviral therapy were also excluded. Patients were asked to keep a protocol specific diary and to record any medications taken during the study.

Randomization, treatment and response evaluation

Patients were randomized 1:1 into two treatment arms by The Ohio State University Biostatistics Core using fixed-block, non-stratified randomization with a block size of 10. In Arm A patients received standard 150 mg daily doses of oral erlotinib throughout treatment, unless dose reductions were required due to toxicity. At the initiation of this trial, erlotinib had not been fully approved by the U.S. Food and Drug Administration, and the maximal dose tested in clinical studies and allowed by the manufacturer at that point was 200 mg.

Patients in Arm B received initial daily oral erlotinib doses of 150 mg, 175 mg, or the maximum allowable 200 mg based on body weights of <80 kg, 80–90 kg, and >90 kg, respectively. These doses were maintained throughout cycle 1 (28 days) unless reduction was required due to toxicity. For cycle 2, doses remained unchanged for all patients who developed skin rash (grade 1 or greater) during cycle 1, or who started cycle 1 at the maximum study dose of 200 mg daily. For the remaining patients, doses were increased 25 mg weekly until the dose reached 200 mg or until rash was observed. A CONSORT diagram and randomization and dosing scheme are available as supplementary data. Patients completed a daily drug diary to assess compliance, and bottles were returned at the end of each cycle for pill counts. Treatment was available for continuation until disease progression or unacceptable toxicity.

Disease response and progression were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) every 3 cycles (12 weeks).[45] Toxicities were graded according to National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events version 3 (CTCAE v3.0). Erlotinib was withheld for patients developing grade 4 non-hematological toxicity, grade 4 myelosuppression, grade 3 or greater nausea or vomiting unresponsive to maximal antiemetic therapy, grade 3 or greater asthenia/fatigue or diarrhea, and pulmonary insufficiency, and discontinued if symptoms did not resolve after 2 weeks.

Pharmacogenetics

Genomic DNA extracted from peripheral blood mononuclear cells was used in multiplexed analysis of 189 known polymorphisms in drug transporters and metabolizing enzymes as previously described.[46] Additional genotyping was completed for *CYP3A4*, *CYP3A5* and *ABCB1* using standard methods as published previously.[28,47]

Pharmacokinetics

Pharmacokinetics of erlotinib and active metabolite OSI-420 were determined for a subset of patients. Plasma samples were collected up to 24 hours after the initial erlotinib dose on cycle 1, day 1, and again following the first dose on cycle 2, day 1. Both compounds were simultaneously quantified in each plasma sample using a validated liquid chromatography-mass spectrometry assay as previously described.[30]

Non-compartmental pharmacokinetic parameter estimates were generated using WinNonlin v. 5.2 (Pharsight, Mountain View, CA). Population pharmacokinetic modeling and covariate analysis were completed with NONMEM v 7.1.2 using methods similar to those described previously[29,30,35]. A base structural model was built with first order conditional estimation considering proportional and additive residual error models, IIV, and IOV on all estimated parameters. A one compartment model with first order absorption and elimination rate constant was used for parent drug (i.e., erlotinib), parameterized with the use of Ka, CL and V1. The erlotinib model was linked to another one compartment model with first order elimination for active metabolite (i.e., OSI-420), parameterized in terms of metabolite clearance (CLM) and volume of distribution (V2). The model included an estimate for fraction (FMET) of erlotinib converted to OSI-420, which was coded as in equation 1, where FM is an estimated parameter.

$$FMET = \frac{1}{1+\exp(FM)} \quad \text{(eq.1)}$$

For covariate analyses, continuous covariates, including weight, age and LBM, were normalized to median values and evaluated as described in equation 2:

$$\theta_i = \theta_{pop} \times \left(\frac{CON_{ji}}{CON_{j-med}}\right)^{\Theta_j} \times \exp(\eta_i) \quad \text{(eq. 2)}$$

CON_{ji} is the value of the continuous covariate *j* for individual *i*, $CON_{j\text{-}med}$ is the population median value of covariate *j* for all individuals, θ_i is the individual PK parameter estimate, θ_{pop} is the population parameter estimate for the typical individual, and η_i is the IIV parameter. Lean body mass was estimated for 25 patients using equation 3[48]

$$\text{For men : } \mathrm{LBM} = (0.32810^{*}\mathrm{W}) + (0.33929^{*}\mathrm{H}) - 29.5336 \quad \text{(eq. 3.1)}$$

$$\text{For women : } \mathrm{LBM} = (0.29569^{*}\mathrm{W}) + (0.41813^{*}\mathrm{H}) - 43.2933 \quad \text{(eq. 3.2)}$$

where W is body weight in kilograms and H is body height in centimeters. For a male patient who was a bilateral amputee whose record of original height was not available, we used “assumed height” to estimate LBM. This was achieved using linear regression to fit a line for the relationship between height and weight for males.

Pharmacogenetic covariates were dichotomized and evaluated in the model both with heterozygous genotypes included in the homozygous major and minor allele groups (i.e. M/m genotypes were coded both as 0 and 1 with M/M always in the 0 category and m/m always in the 1 category). Similarly, co-medications were dichotomized as 0 (no relevant co-medications) or as 1 (*CYP1A1/2* and *CYP3A4/5* substrates, inhibitors, or inducers). Categorical covariates, including sex, previous smoking status, and current smoking status, were evaluated as in equation 4:

$$\theta_i = \theta_{pop} \times (1 + CAT_{ji} \times \Theta_j) \times \exp(\eta_i) \quad \text{(eq. 4)}$$

CAT_{ji} is the value of the categorical covariate *j* for individual *i*, and Θ_j is an estimated parameter. Using a cutoff of $p \leq .05$ for the likelihood-ratio test, corresponding to a decrease in OBJ of 3.84 or greater, individual covariates were evaluated with forward inclusion, backward deletion, and forward selection followed by backward elimination to finalize the covariate model. Model selection in multivariate analysis was based on reduction of OBJ by ≥ 3.84 for forward inclusion, reduction of OBJ ≥ 6.64 ($P \leq 0.01$) for backward deletion, and reduction in residual error and/or IIV of the evaluated PK parameter.

Accuracy and bias of the base and covariate model estimates were evaluated using the nonparametric bootstrap procedure in the Wings for NONMEM software. The median and

95% CI were computed for all population pharmacokinetic parameters using sets of parameter estimates from 1000 bootstrap runs.

Tumor genetics

Formalin fixed, paraffin embedded tissues were obtained from each patient, and genomic DNA was extracted from sections using standard protocols. Extracted DNA was evaluated for *KRAS* (promoter region and G12, G13 codon positions) and *EGFR* (exons 19 and 21 and codon positions T790 and R776) mutations by PCR. *EML4-ALK* fusion and *EGFR* gene amplification were evaluated by fluorescence in-situ hybridization. Assays were conducted at The Ohio State University Molecular Pathology Core Facility according to previously published methods.[49–51] Additional details on these methods are available as supplement.

Data Analyses

The trial utilized a Simon 2-stage minimax design with arms A and B treated as independent phase II studies. The primary endpoint was disease control rate (DCR = complete response (CR), partial response (PR) or stable disease (SD) using RECIST criteria) at 3 months. Early stopping criteria in each arm was set to DCR probability <25% (p_0) with alpha and beta both equal to 0.10. This equated to a first stage accrual of 11 and total accrual of 26 for each treatment arm. Therefore, if disease control was observed in 3 or more of the first 11 patients treated, the arm would be expanded to accrue 15 additional patients. Furthermore, DCR probability ≥50% (p_1) was required for recommendation of further study. Setting alpha and beta to 0.10, equated to observation of DCR in a minimum of 10 of the 26 patients treated for each arm.

Time to progression (TTP) was calculated from date of the first treatment to disease progression. Patients who had not progressed were censored at the last visit. Deaths occurring prior to documented progression were also censored. Overall survival (OS) was determined from the date of the first treatment to death from any cause. Survival curves were estimated using the method of Kaplan-Meier. Differences between survival curves were assessed by log-rank test. Correlations between clinical outcomes (response, PR+SD vs. PD) and rash (Yes vs. No) and tumor genetics (*KRAS* mutation, *ALK* translocation, and *EFGR* amplification) were studied using Fisher's exact test. Secondary evaluations to identify associations between tumor genetics, pharmacokinetics, pharmacogenetics, smoking status, and outcomes used parametric and non-parametric tests. Analyses were performed with the R statistical system[52] and SAS version 9.2 (SAS Institute).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AUC	area under the concentration vs. time curve
Cmax	maximum observed plasma concentration
CI	Confidence Interval
CL	erlotinib plasma clearance
CR	complete response
DC	disease control
DCR	disease control rate (DCR=CR+PR+SD)
OS	Overall Survival
PD	progressive disease
PR	partial response
SD	stable disease
TTP	Time To Progression

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STUDY HIGHLIGHTS

What is the current knowledge on the topic?

African Americans have a higher incidence of lung cancer and less favorable outcomes from approved therapies compared to other patient populations. The reasons for this are unclear, and prospective studies conducted to better understand these disparities are lacking.

What question this study addressed?

The current study was conducted to determine if an alternative dose-to-rash strategy for the EGFR inhibitor, erlotonib, could produce more favorable outcomes in African Americans with NSCLC.

What this study adds to our knowledge?

Results in 52 evaluable African American patients indicated the dose-to-rash strategy produced minimal toxicity, modest improvement in time to progression, and no change in overall survival. Pharmacokinetic data suggests these patients had relatively high clearance of erlotinib, which is supported by associated pharmacogenetic features. Tumor EGFR genetic abnormalities were rare, and EML4-ALK translocations were more prevalent than expected.

How this might change clinical pharmacology and therapeutics?

Prospective tumor genotyping will improve therapy selection, and pharmacokinetically guided dosing will increase erlotinib exposure and potentially improve outcomes in African Americans with NSCLC.

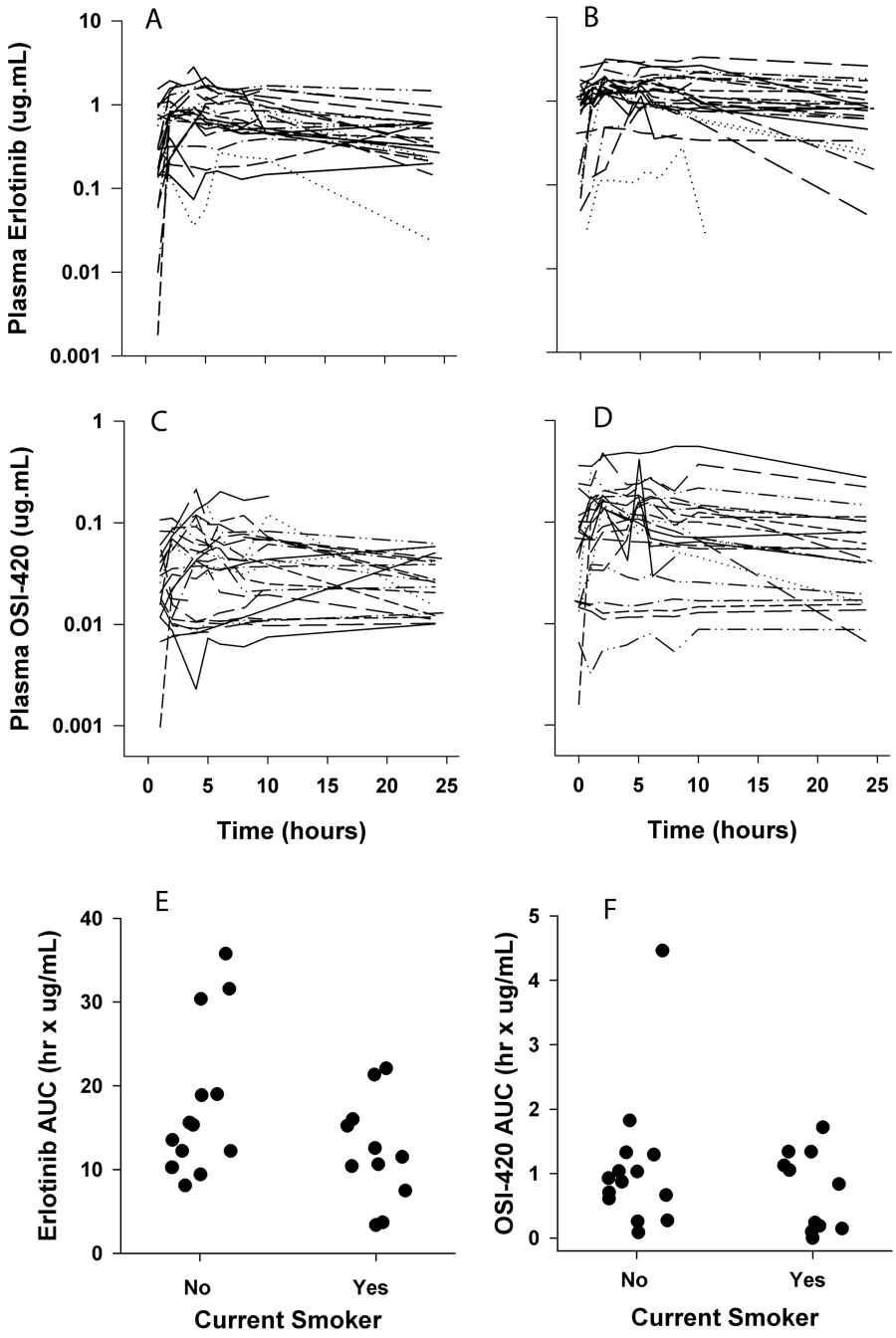

Figure 1. Pharmacokinetics and Impact of Smoking for Erlotinib (OSI-774) and OSI-420

Semi-log concentration vs. time plots of erlotinib (panels A and B) and OSI-420 (panels C and D). Each compound was simultaneously measured with a validated LC/MS/MS assay in patient plasma samples up to 24 hours after the first dose in cycle 1 (panels A and C) and cycle 2 (panels B and D). Dot plots showing smoking status vs. (E) erlotinib (OSI-774) and (F) OSI-420 AUC during cycle 1.

Table 1

Patient characteristics

	Number of patients (%)		
	Arm A (fixed dose)	**Arm B (dose adjusted)**	**All (A + B)**
Number of enrolled patients	28	27	55
Men	17 (60.7)	14 (51.9)	31 (56.4)
Age (years)			
Median	63	59	62
Range	29–83	37–96	29–96
Weight (Kg)			
Mean	75.8	72.5	74.2
Standard Deviation	15.2	13.7	14.4
Performance Status			
0	7 (25.0)	6 (22.2)	13 (23.6)
1	14 (50.0)	17 (63.0)	31 (56.4)
2	7 (25.0)	4 (14.8)	11 (20.0)
Histologic cell type			
Adenocarcinoma	15 (53.6)	17 (63.0)	32 (58.2)
Squamous	7 (25.0)	5 (18.5)	12 (21.8)
NSC not otherwise classified	5 (17.9)	2 (7.4)	7 (12.7)
Adenosquamous	0	1	1
Large cell neuroendocrine	1	1	2
Sarcomatoid	0	1	1
Prior Chemotherapy			
No	4 (14.3)	4 (14.8)	8 (14.5)
1 regimen	19 (67.9)	15 (55.6)	34 (61.8)
2 regimens	5 (17.9)	8 (29.6)	13 (23.6)
Previous radiation therapy	11 (39.3)	11 (40.7)	22 (40.0)
Smoking Status			
Current	8 (28.6)	13 (48.1)	21 (38.2)
Former	15 (53.6)	14 (51.9)	29 (52.7)
Never	5 (17.9)	0 (0)	5 (9.1)

Table 2

Treatment Related Toxicities

Toxicity, no. of pts (%)	Arm A (n=28)	Arm B (n=27)	All (n=55)
Any grade 3+ toxicity	5 (18)	6 (22)	11 (20)
Any grade 4/5 toxicity	1 (4)	0	1 (2)
Rash, maximum grade			
Any grade	19 (68)	19 (70)	38 (69)
Grade 1	12 (43)	10 (37)	22 (40)
Grade 2	7 (25)	5 (19)	12 (22)
Grade 3	0	4 (15)	4 (7)
Dermatologic, other than rash*			
Any, grade 1–3	7 (25)	9 (33)	16 (29)
Diarrhea			
Any grade	9 (32)	7 (26)	16 (29)
Grade 1	7 (25)	7 (26)	14 (25)
Grade 2	2 (7)	0	2 (4)
Grade 3+	0	0	0
Other gastrointestinal			
Anorexia, grade 1–2	5 (18)	9 (33)	14 (25)
Anorexia, grade 3	2 (7)	1 (4)	3 (5)
AST elevation	4 (14)	0	4 (7)
Dehydration, grade 1–2	0	1 (4)	1 (2)
Dehydration, grade 3	1 (4)	1 (4)	2 (4)
Hyperbilirubinemia	0	1 (4)	1 (2)
Nausea, grade 1–3**	2 (7)	6 (22)	8 (15)
Xerostomia	0	2 (7)	2 (4)
Other			
Pneumonitis, grade 3	1 (4)	0	1 (2)

* erythema, hyperpigmentation, pruritus, xerodermia.

** only one grade three nausea was recorded

Table 3

Efficacy

	Number of patients (%)		
	Arm A (n=26)	**Arm B (n=26)**	**All (n=52)***
DCR at 3 months	6 (23)	6 (23)	12 (24)
PR	1 (4)	0	1 (2)
SD	5 (19)	6 (23)	11 (22)
PD	20 (77)	20 (77)	40 (78)
	Arm A (n=28)	Arm B (n=27)	All (n=55)**
TTP, months			
Median (95% CI)	2.8 (2.5–3.1)	2.4 (1.5–2.7)	2.6 (1.9– 2.8)
Range	0.6–14.4	0.3–20.2	0.3–20.2
1 year progression free rate (%)	10	4	7
Survival, months			
Median	5.3	5.2	5.3
Range	0.9–37.4	1.3–42.8	0.9–42.8
1 year survival rate (%)	30	26	28

* responses based on evaluable patients

** based on all 55 treated patients

Table 4

Noncompartmental Pharmacokinetic Parameters for Erlotinib and OSI-420 in Cycles 1 and 2

	Cycle 1						Cycle 2					
Parameter* (units)	**Arm A**	**n¥**	**Arm B**	**n**	**Total**	**n**	**Arm A**	**n**	**Arm B**	**n**	**Total**	**n**
Erlotinib (OSI-774)												
AUC_{0-24}** (mg/L*hr)	15.3 (7.5–35.8)	13	12.4 (3.4–31.6)	12	12.6 (3.4–35.8)	25	26.3 (8.9–51.0)	14	21.4 (1.4–71.5)	10	22.9 (1.4–71.5)	24
Cmax£ (mg/L)	0.91 (0.32–2.1)	13	0.79 (0.16–1.9)	12	0.88 (0.16–2.1)	25	1.7 (0.48–3.2)	14	1.6 (0.28–3.1)	10	1.6 (0.28–3.2)	24
CL/F§ (L/hr)	9.8 (4.2–20.1)	13	13.5 (5.5–44.5)	12	12.3 (4.2–44.5)	25	5.7 (2.9–16.8)	14	7.6 (2.1–110)	10	6.6 (2.1–110)	24
Tmax† (hrs)	5 (1–24)	13	4 (1–8)	12	4 (1–24)	25	2 (1–6)	14	2.5 (0–10)	10	2 (0–10)	24
T1/2‡ (hrs)	11.6 (2.1–116)	11	11.2 (4.7–29.9)	9	11.6 (2.1–116)	20	28.4 (5.7–68.5)	14	19.7 (1.7–45.6)	7	22.5 (1.7–68.5)	21
OSI-420ϟ												
AUC_{0-24} (mg/L*hr)	0.93 (0.088–4.5)	13	0.69 (0–1.7)	11	0.87 (0–4.5)	24	1.4 (0.19–10.7)	14	1.7 (0–6.9)	9	1.5 (0–10.7)	23
AUC Ratio€ 420/774	0.068 (0.004–0.129)	13	0.056 (0–0.109)	11	0.066 (0–0.127)	24	0.102 (0.011–0.209)	14	0.094 (0–0.149)	9	0.096 (0–0.209)	23
Cmax (mg/L)	0.070 (0.010–0.203)	13	0.048 (0–0.119)	11	0.054 (0–0.203)	24	0.100 (0.009–0.558)	14	0.178 (0–0.412)	9	0.153 (0–0.558)	23
Tmax (hrs)	5 (1–24)	13	2 (1–11.4)	11	4 (1–24)	24	2 (0–10)	14	2.5 (1–6)	9	2 (0–10)	23
T1/2 (hrs)	9.6 (7–70.4)	8	12.3 (3.2–40.8)	6	9.7 (3.2–70.4)	14	22.2 (6–71.1)	10	17.7 (3.4–28.8)	6	22.2 (3.4–71.1)	16

* Data are presented as median (range).

** AUC, area under the observed concentration vs. time curve;

£ Cmax, maximum observed concentration;

§ CL/F, apparent clearance/oral bioavailability (F);

† Tmax, time of observed maximum concentration;

‡ T1/2, terminal phase half-life (note half-lives were too long to be determined for some patients);

¥ n, the number of concentration vs. time profiles used for each median value;

€ AUC Ratio, the ratio of AUCs calculated as (AUC OSI-420)/(AUC OSI-774).

ƛ OSI-420 was undetectable in one patient treated on Arm B

Table 5

Pharmacogenetics of Select Genes Relevant for Erlotinib Disposition

		Genotype*						Allele		Freq**
Gene	**SNP ID**	**M/M**	**n**	**M/m**	**n**	**m/m**	**n**	**M**	**m**	**m**
CYP1A1	rs1048943	A/A	25	A/G	1	G/G	0	51	1	0.019
	rs2606345	C/C	20	C/A	5	A/A	1	45	7	0.135
	rs4646421	C/C	10	C/T	9	T/T	1	29	11	0.275
CYP1A2	rs2069526	T/T	20	T/G	2	G/G	2	42	6	0.125
	rs762551	C/C	8	C/A	3	A/A	5	19	13	0.406
	rs2470890	C/C	9	C/T	1	T/T	0	19	1	0.050
CYP3A4	rs2740574	G/G	9	G/A	14	A/A	3	32	20	0.385
	rs35599367	C/C	41	C/T	1	T/T	0	83	1	0.012
CYP3A5	rs776746	A/A	10	A/G	20	G/G	9	40	38	0.487
	rs41303343	-/-	22	-/T	4	T/T	0	48	4	0.077
	T12952C	T/T	22	T/C	3	C/C	0	47	3	0.060
	C31611T	C/C	5	C/T	14	T/T	6	24	26	0.520
ABCB1	rs1128503	C/C	11	C/T	2	T/T	2	24	6	0.200
	rs1045642	C/C	18	C/T	13	T/T	2	49	17	0.258
	rs1564481	C/C	11	C/T	9	T/T	0	31	9	0.225
ABCG2	rs2622605	T/T	18	T/C	7	C/C	1	43	9	0.173
	rs2622624	A/A	11	A/G	7	G/G	1	29	9	0.237
	rs3114018	A/A	8	A/C	10	C/C	2	26	14	0.350

* The number of patients (n) with genotypes for major (M) and minor (m) alleles.

** Freq is the frequency of minor allele occurrence.

Table 6

Population PK parameter estimates for erlotinib and OSI-420

Model Term	Base model (n=26)		Covariate model (LBM, n=26)	
	Estimate (CV %)	Bootstrap median (95% CI)	Estimate (CV %)	Bootstrap median (95% CI)
OBJ	968.48	--	963.26	--
θ_{Ka} (h^{-1})	1.04	--	1.04	--
θ_{CL} (L/h)	6.22 (8.5)	6.24 (4.72, 8.11)	6.4 (9.0)	6.37 (4.99, 8.09)
θ_{V1} (L)	284 (15.7)	275.00 (193.00, 448.05)	283 (37.1) 2	74.00 (195.00, 447.03)
θ_{FM}	1.41 (12.8)	1.22 (0.47, 3.33)	0.88 (26.4)	1.03 (0.23, 3.49)
θ_{CLM} (L/h)	21.5 (8.9)	23.60 (4.76, 47.91)	32.6 (10.4)	27.80 (4.04, 55.60)
θ_{V2} (L)	7.18 (62.8)	7.67 (0.09, 21.91)	11.6 (12.1)	8.99 (0.04, 26.11)
Shared ETA scale factor, θ_{SE}	3.46 (37.0)	3.18 (−148.10, 16.11)	5.96 (56.5)	6.42 (2.29, 577.30)
θ_{LBM}	--	--	1.71 (27.8)	1.75 (0.69, 3.08)
IIV_{CL}	0.06 (80.8)	0.24 (0.005, 0.44)	0.02 (127.1)	0.13 (0.001, 0.35)
IIV_{V1}	0.92 (50.5)	0.90 (0.40, 1.33)	0.95 (50.7)	0.92 (0.43, 1.37)
IOV_{CL}	0.38 (55.5)	0.58 (0.29, 0.89)	0.30 (67.9)	0.51 (0.15, 0.85)
Residual error for erlotinib, ε_1	1.2 (37.2)	1.08 (0.66, 1.47)	1.2 (37.1)	1.07 (0.65, 1.48)
Residual error for OSI-420, ε_2	0.78 (23.3)	0.87 (0.37, 1.06)	0.78 (24.2)	0.87 (0.67, 1.06)

OBJ, objective function value; LBM, lean body mass; IIV, inter-individual variability; IOV, inter-occasion variability; CV%, standard error of estimate expressed as coefficient of variation (%); The shared eta scale factor was estimated as described in the text. IIV, IOV and residual errors are expressed as variances.

Table 7

Tumor Genetics

	Tested	Positive (%)	No. of DC at 3m
EGFR mutations	45	2 (4)	1
[E]Exon 19 indel	**F**	1	1
Exon 21 mutation		0	0
R776L mutation		1	0
EGFR gene amplification	47	17 (36)	3
ALK gene translocation	39	5 (13)	0
ALK gene amplification	39	1	1
KRAS mutation	46	8 (17)	2
G12C (c.32 G>T)		4	0
G12D (c.33 G>A)		1	0
G12V (c.33 G>T)		1	1
5’-UTR (−9C>T, −6 C>T)		2	1
Total		32	7