Phase 1 trial of dasatinib combined with afatinib for epidermal growth factor receptor- (EGFR-) mutated lung cancer with acquired tyrosine kinase inhibitor (TKI) resistance

Ben C. Creelan1, Jhanelle E. Gray1, Tawee Tanvetyanon1, Alberto A. Chiappori1, Takeshi Yoshida2, Michael J. Schell3, Scott J. Antonia1 and Eric B. Haura1

BACKGROUND: Bypass activation of Src family kinases can confer resistance to EGFR tyrosine kinase inhibitors (TKIs) based on preclinical models. We prospectively assessed the safety and clinical activity of dasatinib and afatinib in combination for patients with resistant EGFR-mutant lung cancer.

METHODS: An open-label, dose-escalation phase 1/2 trial (NCT01999985) with 2-stage expansion was conducted with 25 lung cancer patients. Dose expansion required activating EGFR mutations and progression following prior EGFR TKI.

RESULTS: Patients were 72% Caucasian and received median of 2 prior lines of therapy. Maximum-tolerated dose was 30 mg afatinib with 100 mg dasatinib. New or increased pleural effusions were observed in 56% of patients. No radiologic responses were observed, although several EGFR-mutant TKI-resistant patients (26%) had prolonged stable disease over 6 months. The combination reduced the EGFR mutation and T790M variant allele frequency in cell-free DNA (p < .05). Nonetheless, the threshold for futility was met, based on 6-month progression-free survival. For EGFR TKI-resistant patients, median progression-free survival was 3.7 months (95% confidence interval (CI), 2.3–5.0) and overall survival was 14.7 months (95% CI, 8.5–20.9).

CONCLUSIONS: The combination had a manageable toxicity profile and in vivo T790M modulation, but no objective clinical responses were observed.
hypothesised that this combination would lead to durable disease control in EGFRm NSCLC patients with acquired TKI resistance. Therefore, we conducted a phase 1/2 trial with the primary objective of characterising the safety and clinical activity of dasatinib with afatinib in this population. We also hypothesised that assessment of cell-free DNA for EGFR mutations could serve as an additional readout of drug efficacy.

**MATERIALS AND METHODS**

This was an open-label, single-centre, phase 1 study with a modified 3+3 dose-escalation design, followed by an expansion cohort with a 2-stage design (NCT01999985). The trial was approved by Liberty Institutional Review Board Inc., assurance number IRB00003411. For dose escalation, patients were required to have stage 4 NSCLC with progression after ≥1 standard therapy. For dose expansion, patients were required to have an activating EGFR exon 19 or 21 mutation, with disease progression after first-line epidermal growth factor receptor tyrosine kinase inhibitor resistance. EGFR mutation status assessed by plasma or tissue test: T790M? detected, T790M? unknown, T790M+ wildtype EGFR n=1, T790M– wildtype EGFR n=4, T790M? unknown EGFR n=3.

Of the 31 patients screened, 25 were eligible and treated (Fig. 1). The most common serious AEs, regardless of causality, were pneumonia (24%), diarrhoea (12%), and pleural effusion (12%).

**RESULTS**

Patient characteristics
Of the 31 patients screened, 25 were eligible and treated (Fig. 1). Patients had received a median of 2 (range, 1–5) prior systemic therapies for stage 4 NSCLC and had progressive measurable disease (Supplemental Table S1). Patients with an activating EGFR mutation had previously progressed on a first- or second-generation EGFR TKI. No patients had evidence of small cell transformation, and none had received prior third-generation TKIs, such as osimertinib.

Safety and tolerability
Dose escalation proceeded through 2 dose levels of up to 40 mg afatinib with 100 mg dasatinib. Although no protocol-defined DLT was observed, we decided to deescalate after 3 patients were enrolled at the 40 mg dose level, due to the persistence of grade 2 diarrhoea despite optimal medical management in 2 patients. These two events were therefore recorded as DLTs for monitoring purposes.

Patients received a mean of 3.2 months of continuous oral drug exposure. Adverse events (AEs) for both dose levels in all patients are summarised together (Fig. 2). The most common drug-related AEs were diarrhoea (72%) and rash (64%) (Supplemental Table S2). The most common serious AEs, regardless of causality, were pneumonia (24%), diarrhoea (12%), and pleural effusion (12%).
Although drug-related AEs were generally manageable, 24% of patients eventually required dose reductions or interruptions for the management of AEs.

New or increased pleural effusions occurred in 72% of patients on reimaging computed tomography (CT) scans (Supplemental Table S3). In many instances, it was difficult to ascertain whether effusions were attributable to dasatinib or lung cancer, since enlargement of pre-existing malignant effusions could be attributable to either cause. Symptomatic pleural effusions were managed with thoracentesis, corticosteroid tapers, or dose reductions of dasatinib to 50 mg daily, if recrudescence occurred.

No QTc prolongations on serial electrocardiograms were detected during the trial (Supplemental Fig. 1A). No decrease in mean left ventricular ejection fraction below normal levels (<50%) was detected (Supplemental Fig. 1B).

Pharmacodynamics
For patients with activating EGFRm with acquired TKI resistance, an overall decrease in EGFRm-variant allele frequency was observed during the course of treatment (Fig. 3a). The interpretation was limited by a high degree of deviation in variant allele frequencies between patients. Likewise, a decrease in T790M allele frequency was observed among patients with baseline detectable T790M (Fig. 3b). Samples for subsequent time points were not collected.

Treatment outcomes and overall survival
No radiologic responses were observed, although several EGFRm TKI-resistant patients had stable disease as best response (Fig. 4). One patient with acquired T790M had stable disease lasting more than 12 months. The trial met the interim stopping threshold for futility based upon 6-month progression-free survival. For all EGFR TKI-resistant patients, median progression-free survival was 3.7 months (95% confidence interval (CI), 2.3–5.0). Median overall survival was 14.7 months (95% CI, 8.5–20.9) at a median follow-up of 25 months. EGFRm patients lacking detectable T790M at entry had worse overall survival than those with detectable T790M at entry, with a hazard ratio of 4.0 (95% CI, 1.3–13.1), p = .02. This may be partly attributable to subsequent osimertinib as the next
line of therapy for patients who were T790M positive at study entry, as shown in Fig. 5. All deaths were attributable to progressive cancer. No ostensible clinical benefit was detected in the EGFR wild-type population, with a median progression-free survival of 1.8 months (95% CI, 0.7–3.0) and median overall survival of 5.1 months (95% CI, 2.4–7.8).

**DISCUSSION**

In this phase 1 trial, it was feasible to combine dasatinib with afatinib in advanced NSCLC at biologically active doses. Nonetheless, pleural effusion remained a prominent adverse effect, with most patients who had been effusion free at study entry exhibiting pleural effusions on their reimaging CT scans. The inclusion of patient with mild pleural effusions may have confounded the interpretation of this adverse effect. Nevertheless, our goal was to enrol representative patients, since malignant pleural effusions occur in almost 40% of metastatic NSCLC. Moreover, the clinical activity of the combination was limited and did not justify further investigation, especially since third-generation EGFR TKIs such as osimertinib have become available and demonstrate significant responses in a similar patient population.

This combination was observed to modestly decrease the fraction of plasma-mutant EGFR and T790M alleles at week 4. In contrast, complete clearance of plasma T790M occurred at week 6 among most patients on the AURA osimertinib trial and was associated with durable response. Although dasatinib has modest in vitro binding affinity to mutant EGFR kinases, T790M appears to cause steric hindrance with the chloro-methyl-phenyl ring of dasatinib. Nonetheless, dasatinib is hypothesised to suppress bypass tracks through SRC/AKT, rather than direct inhibition of the T790M EGFR kinase.

Despite promising preclinical activity, dasatinib is characterised by a short terminal half-life in plasma, which may constrain its inhibitory activity of SFKs in human tissue. To this end, the reported tolerability and efficacy of dasatinib as a single agent across multiple solid tumours has been unsatisfactory. Two phase 1/2 NSCLC trials of single-agent dasatinib at higher doses were closed due to a high frequency of AEs and a paucity of
durable responses. Likewise, additional trials of dasatinib with erlotinib reported low or absent responses in patients with acquired EGFR TKI resistance. Finally, a phase 2 trial of dasatinib for inactivating BRAF mutations was closed due to a lack of efficacy amongst the study population. Therefore, a trial testing osimertinib with dasatinib in EGFRm NSCLC based upon baseline plasma or tumour CRIPTO-1 expression is ongoing. Additionally, YAP amplification has been proposed to confer resistance in up to 4% of EGFR TKI-resistant patients, which may implicate SFK as important in mediating resistance. Therefore, there may yet be a further role for SFK inhibitors in these narrowly defined molecular subgroups. In our study, tumour biopsies were not performed, and thus we could not detect phospho-Src, YES amplification, or CRIPTO-1 alterations within the tumour. Future studies of SFK in combination with EGFR targeting agents should focus on subsets of patients with likely mechanisms of resistance driven by SFK.

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**AUTHOR CONTRIBUTIONS**

The authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE). B.C.C. was the principal investigator who helped to design, conducted the trial, analysis of results, and wrote the primary manuscript. J.E.G. helped by reviewing trial design, contributing patients, and review of the manuscript. T.T. helped by contributing patients and manuscript review. A.A.C. helped by discussing trial design, contributing patients, and review of the manuscript. T.Y. helped by writing the protocol rationale, and review of the manuscript. M.J.S. provided statistical justification for the study design, analysis, and review of the manuscript. S.J.A. helped by contributing patients and review of the manuscript. C.B. provided the study rationale, supervised the study design, contributed patients, and contributed to the analysis and manuscript review.

**ADDITIONAL INFORMATION**

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**Ethics approval and consent to participate:** The study was institutional review board approved, Federal wide assurance number IRB00003411, Liberty Institutional Review Board, Inc. All participants provided written informed consent. The study was performed in accordance with the Declaration of Helsinki.

**Data availability:** Raw data and materials generated during the study are available upon request to the author study team, thoraccrc@moffitt.org.

**REFERENCES**

1. Stabile, L. P., He, G., Lui, V. Y. W., Thomas, S. M., Henry, C. & Gubish, C. T. et al. c-Src activation mediates erlotinib resistance in head and neck cancer by stimulating c-Met. *Clin. Cancer Res.* 19, 380–392 (2013).
2. Yoshida, T., Zhang, G., Smith, M. A., Lopez, A. S., Bai, V. & Li, J. et al. Tyrosine phosphoproteomics identifies both codrivers and cotargeting strategies for T790M-related EGFR-TKI resistance in non-small cell lung cancer. *Clin. Cancer Res.* 20, 4059–4074 (2014).
3. Wotanabé, S., Yoshida, T., Kawakami, H., Takegawa, N., Tanizaki, J. & Hayashi, H. et al. T790M-selective EGFR-TKI combined with dasatinib as an optimal strategy for overcoming EGFR-TKI resistance in T790M-positive non-small cell lung cancer. *Mol. Cancer Ther.* 16, 2563–2571 (2017).
4. Haura, E. B., Tanvetyanon, T., Chiappori, A., Williams, C., Simon, G. & Antonia, S. et al. Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non–small-cell lung cancer. *J. Clin. Oncol.* 28, 1387 (2010).
5. Sos, M. L., Koker, M., Weir, B. A., Heynck, S., Rabinovisky, R. & Zander, T. et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. *Clin. Cancer Res.* 69, 3256–3261 (2009).
6. Kanda, R., Kawahara, A., Watarai, K., Murakami, Y., Sonoda, K. & Maeda, M. et al. Erlotinib resistance in lung cancer cells mediated by integrin β1/Src/Akt-driven Erk signaling. *Cancer Res.* 73, 6243–6253 (2013).
7. Lee, T.-F., Tseng, Y.-C., Nguyen, P. A., Li, Y.-C., Ho, C.-C. & Wu, C.-W. Enhanced YAP expression leads to EGFR TKI resistance in lung adenocarcinomas. *Sci. Rep.* 8, 271 (2018).
8. Park, K.-S., Raffeld, M., Moon, Y. W., Xi, L., Bianco, C. & Pham, T. et al. CRIPTO1 expression in EGFR-mutant NSCLC elicits intrinsic EGFR-inhibitor resistance. *J. Clin. Investig.* 124, 3003–3015 (2014).
9. Zhang, H., Zhang, B., Gao, L., Zhang, L., Zhu, K. & Cheng, R. et al. Clinical significance of cripto-1 expression in lung adenocarcinomas. *Oncotarget* 8, 79087 (2017).
10. Fan, P.-D., Narzisi, G., Jayaparakash, A., Venturini, E., Robine, N. & Smibert, P. et al. YES1 amplification as a mechanism of acquired resistance (AR) to EGFR tyrosine kinase inhibitors (TKIs) identified by a transposon mutagenesis screen and clinical genomic testing. *Am. Soc. Clin. Oncol.* 35(Suppl.), 9043 (2017).
11. Fan, P.-D., Narzisi, G., Jayaparakash, A. D., Venturini, E., Robine, N. & Smibert, P. et al. YES1 amplification is a mechanism of acquired resistance to EGFR inhibitors identified by transposon mutagenesis and clinical genomics. *Proc. Natl. Acad. Sci. USA* 115, E6030–E6038 (2018).
12. Ichihara, E., Westover, D., Meador, C. B., Yan, Y., Bauer, J. A. & Lu, P. et al. SFK/FAK signaling attenuates osimertinib efficacy in both drug-sensitive and drug-resistant models of EGFR-mutant lung cancer. *Cancer Res.* 77, 2990–3000 (2017).
13. Gold, K. A., Lee, J. J., Hanun, N., Tang, X., Price, J. & Kawedia, J. D. et al. A phase I/II study combining erlotinib and dasatinib for non-small cell lung cancer. *Oncology* 19, 1040 (2014).
14. Johnson, M. L., Riel, G. J., Rizvi, N. A., Azzoli, C. G., Kris, M. G. & Sima, C. S. et al. Phase II trial of dasatinib for patients with acquired resistance to treatment with the epidermal growth factor receptor tyrosine kinase inhibitors erlotinib or gefitinib. *J. Thorac. Oncol.* 6, 1128–1131 (2011).
15. Porcel, J. M., Gasol, A., Bielsa, S., Civit, C., Light, R. W. & Salud, A. Clinical features and outcome on osimertinib in the AURA trial. *J. Thorac. Oncol.* 11, 1728–1735 (2016).
16. Thress, K. S., Markovets, A., Barrett, J. C., Chmiielecki, J., Goldberg, S. B. & Shepherd, F. A. et al. Complete clearance of plasma EGFR mutations as a predictor of outcome on osimertinib in the AURA trial. *Am. Soc. Clin. Oncol.* 35(Suppl.), 9018 (2017).
17. Sos, M. L., Michel, K., Zander, T., Weiss, J., Frommolt, P. & Peifer, M. et al. Predicting drug susceptibility of non-small cell lung cancers based on genetic lesions. *J. Clin. Investig.* 119, 1727–1740 (2009).
18. Christopher, L. J., Cui, D., Wu, C., Luo, R., Manning, J. A. & Bonacorsi, S. J. et al. Metabolism and disposition of dasatinib after oral administration to humans. *Drug Metab. Dispos.* 36, 1357–1364 (2008).
19. Brunner, A. M., Costa, D. B., Heist, R. S., Garcia, E., Lindeman, N. I. & Sholl, L. M. et al. Treatment-related toxicities in a phase II trial of dasatinib in patients with squamous cell carcinoma of the lung. *J. Thorac. Oncol.* 8, 1434–1437 (2013).

**Phase 1 trial of dasatinib combined with afatinib for epidermal growth**

BC Creelan et al.

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Phase II study of dasatinib in patients with advanced non–small-cell lung cancer. J. Clin. Oncol. 28, 4609 (2010).

21. Trial of dasatinib in patients with advanced cancers harboring DDR2 mutation or inactivating B-RAF mutation (ed. Medicine UNLo). ClinicalTrialsgov Identifier: NCT01514864. ClinicalTrials.gov (Bristol-Myers Squibb, 2015) https://clinicaltrials.gov/ct2/show/NCT01514864.

22. Kim, C., Liu, S., Subramaniam, D. & Giaccone, G. P1.01-47 Phase I/II trial of dasatinib and osimertinib in patients with advanced EGFR-mutant non-small cell lung cancer (NSCLC). J. Thorac. Oncol. 13, S479 (2018).