

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

Supplementary Data

Tumor Genetics Analysis

DNA extraction. Separate tumor biopsy specimens were obtained from each patient and either immediately frozen in liquid nitrogen or formalin fixed and paraffin embedded (FFPE) using standard procedures. Frozen samples were thawed and grossly dissected by a surgical pathologist to enrich the tumor sample. FFPE tissue sections were deparaffinized with xylene followed by overnight proteinase K digestion at 55 °C. The genomic DNA was purified from the digested tissue according to manufacturer's protocol (QIAamp DNA Micro Kit, Qiagen, Valencia, CA).

Detection of somatic mutations present in exon 19, 20 and 21 of EGFR was performed as described previously with modification. Briefly, 10 ng genomic DNA were amplified using forward and reverse primers flanking each exons with the Hotstar Taq-Master Mix (QIAGEN) (primer sequences were provided if specifically requested). PCR conditions included an initial 95°C step for 10 minutes and followed by 34 cycles of 94 °C 30 seconds, 58 °C 45 seconds, 72 °C 90 seconds and finally 72 °C for 10 minutes in GeneAmp PCR system 9700 (Applied Biosystems, ABI). The polymerase chain reaction (PCR) Sequencing of amplified DNA was accomplished with a Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) on 3130xl Genetic Analyzer (ABI) following the manufacturer's protocols. Forward and reverse sequences were aligned with the EGFR wild-type sequence (NCBI Accession Number NM_005228.3), and chromatograms were manually evaluated. High quality data confirming mutations in both the forward and reverse directions are reported in the manuscript.

Detection of somatic mutations present in exon 1 of KRAS was performed using a two-step PCR procedure. Briefly, the forward 5'-TACTGGTGGAGTATTTGATAGTG-3' and reverse 5'-CTGTATCAAAGAATGGTCCTG-3' primers were used in the first round of PCR to amplify 10 ng of DNA in a 25 µL PCR reaction. A second pair of primers was used to amplify 1 µL of the PCR products obtained in the first round using the forward 5'- TGTAAACGGCCAGTTAGTGTATTAACCTTATGTG-3' and reverse 5'-CAGGAAACAGCTATGACCACCTCTATTGTTGGATCATATTCG-3' primers in which M13, a bacteriophage sequence was introduced to facilitate DNA sequencing. The PCR products were subjected to bidirectional DNA sequencing use M13 sequencing primers as described above.

EGFR amplification and ALK gene translocation. EGFR amplification and ALK gene translocation were evaluated using fluorescence in situ hybridization (FISH) with methods described previously with modification³⁻⁵. Deparaffinized slides were processed and hybridized with the commercialized probe kits from Abbott for EGFR (Vysis®LSI® EGFR SpectrumOrange/CEP®7 SpectrumGreen Dual Probe Set) and

ALK (LSI ALK Dual Color, Break Apart Rearrangement Probe) according to validated clinically standard FISH procedures. The FISH slides were scanned using a semi-automated FISH analyzing system, BioView, and manually by a board certified Pathologist (Zhao) using a fluorescence microscope (Nikon BX). The copy number variances of EGFR were classified as A) amplification (EGFR/CEP7>2.1 or >15 copies of EGFR/nuclei); B) positive (4-14 copies of EGFR/nucleus >20% of the tumor cells); and C) negative (EGFR/CEP7<2.1 or <4 copies of EGFR/nucleus). Translocation of the ALK gene was determined by the breakapart ratio of ALK probe in at least >15/100 tumor nuclei.

REFERENCES

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FIGURE LEGENDS

Figure S1. CONSORT Diagram. CONSORT diagram for 58 African Americans with advanced non-small cell lung cancer who were enrolled on study⁶. Fifty-five (55) patients received erlotinib, and 52 patients were evaluable for the primary outcome, disease control rate at 3 months.

Figure S2. Randomization scheme. Patients in Arm A received 150 mg daily unless dose reduction was required for toxicity. Patients in Arm B received an initial dose of 150, 175 or 200 mg daily based on body weights of <80 kg, 80-90 kg, and > 90 kg, respectively. After cycle 1, Arm B patients who did not experience skin rash were dose-escalated weekly until skin rash was observed or until they reached the maximum 200 mg daily dose.

Figure S3. Comparison of Cycle 1 AUCs for erlotinib and OSI-420 relative to smoking status. Box and whisker plots showing smoking status vs. (A) erlotinib (OSI-774) and (B) OSI-420 AUC during cycle 1. Mann-Whitney-Wilcoxon test for medians yields p-values 0.2671 and 0.5719, respectively for erlotinib and OSI-420 AUCs compared to smoking status.

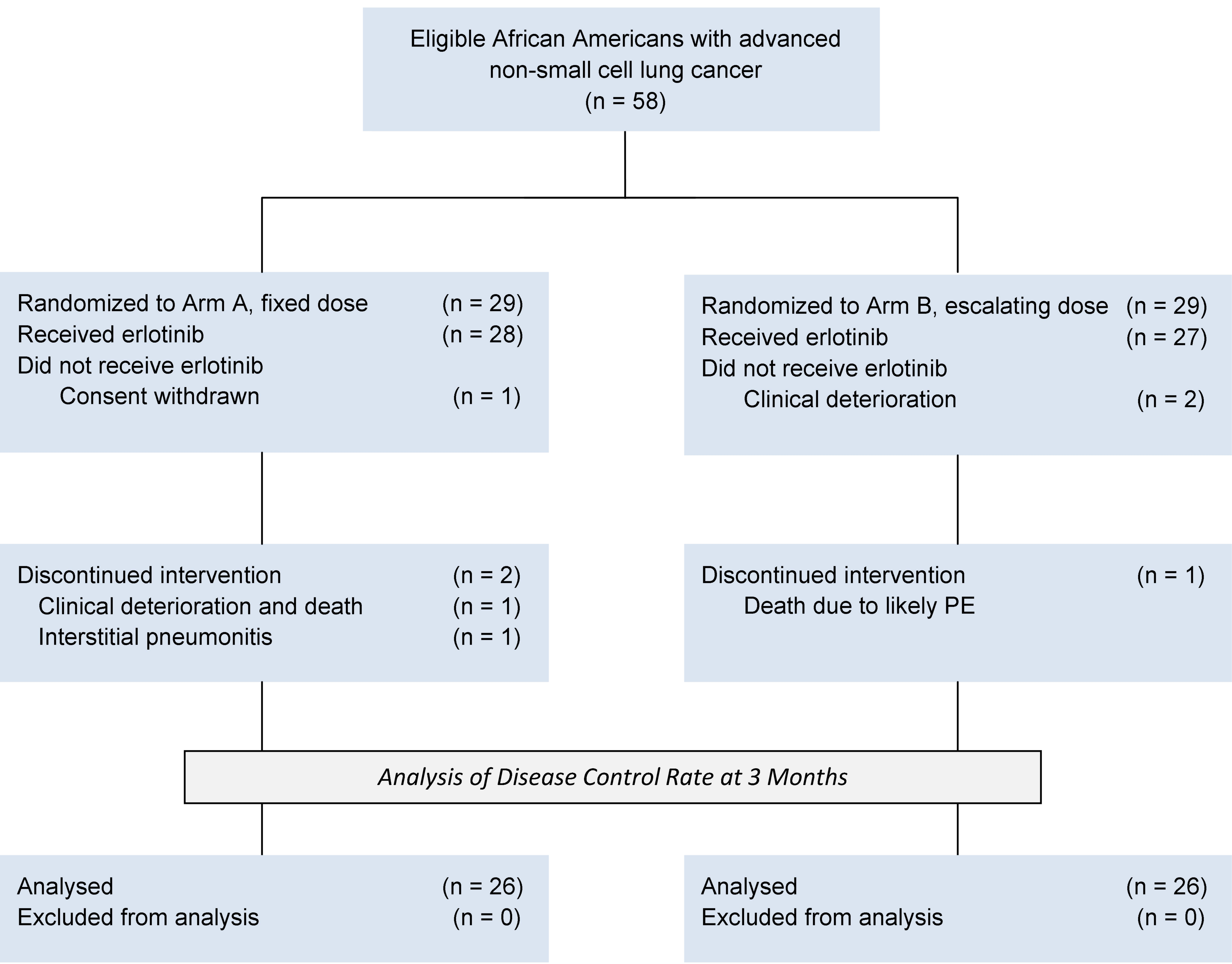
Figure S4. Pharmacokinetics and Impact of Smoking for Erlotinib (OSI-774) and OSI-420. Semi-log concentration vs. time plots displaying the mean +/- SD of erlotinib (panels A and B) and OSI-420 (panels C and D). Each compound was simultaneously measured with a validated LC/MS/MS assay in patient plasma samples up to 24 hours after the first dose in cycle 1 (panels A and C) and cycle 2 (panels B and D).

Table S1. Pharmacokinetic Parameter Estimates for Cycle 1 Dose Levels 150 mg and 175 mg.

Parameter* (units)	Cycle 1					Cycle 2						
	150 mg	n*	175 mg	n	Total	n	150 mg	n	175 mg	n	Total	n
Erlotinib (OSI-774)												
AUC ₀₋₂₄ ** (mg/L*hr)	12.4 (3.4-35.8)	20	13.5 (10.2-31.6)	5	12.6 (3.4-35.8)	25	24.0 (1.4-71.5)	21	21.7 (6.6-47.2)	3	22.9 (1.4-71.5)	24
Cmax [£] (mg/L)	0.90 (0.16-2.1)	20	0.79 (0.40-1.9)	5	0.88 (0.16-2.1)	25	1.7 (0.28-3.2)	21	1.6 (1.5-2.3)	3	1.6 (0.28-3.2)	24
CL/F [§] (L/hr)	12.1 (4.2-44.5)	20	12.9 (5.5-17.1)	5	12.3 (4.2-44.5)	25	6.2 (2.1-110)	21	8.1 (3.7-26.4)	3	6.6 (2.1-110)	24
Tmax [†] (hrs)	5 (1-24)	20	2 (1-2.3)	5	4 (1-24)	25	2 (1-10)	21	1 (0-10)	3	2 (0-10)	24
T1/2 [‡] (hrs)	11.2 (2.1-116)	17	26.0 (12.0-30.0)	3	11.6 (2.1-116)	20	25.9 (1.7-68.5)	19	12.9 (3.2-22.5)	2	22.5 (1.7-68.5)	21
OSI-420^λ												
AUC ₀₋₂₄ (mg/L*hr)	0.902 (0.0-4.5)	19	0.71 (0.61-1.1)	5	0.87 (0-4.5)	24	1.5 (0.0-10.7)	20	1.8 (0.9-4.7)	3	1.5 (0-10.7)	23
AUC Ratio [€] 420/774	0.067 (0.0-0.129)	19	0.060 (-0.21.084)	5	0.066 (0-0.127)	24	0.096 (0.0-0.209)	20	0.083 (0-0.139)	3	0.096 (0-0.209)	23
Cmax (mg/L)	0.054 (0.0-0.203)	19	0.061 (0.01-112)	5	0.054 (0-0.203)	24	0.127 (0.0-0.558)	20	0.268 (-157.412)	3	0.153 (0-0.558)	23
Tmax (hrs)	5 (1-24)	19	2 (2-2.6)	5	4 (1-24)	24	2 (0-10)	20	5 (1-6)	3	2 (0-10)	23
T1/2 (hrs)	9.6 (7-70.4)	10	18.4 (3.2-40.8)	4	9.7 (3.2-70.4)	14	22.4 (4.8-71.1)	13	12.1 (3.4-28.8)	3	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); ^λn, 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). * OSI-420 was undetectable in one patient treated at 150 mg). Note, data is categorized by cycle 1 dose level (i.e. 150 mg or 175 mg in cycle 1) and does not reflect the dose level patients may have received in cycle 2.



Eligible African Americans with advanced non-small cell lung cancer (n = 58)

Randomized to Arm A, fixed dose (n = 29)
 Received erlotinib (n = 28)
 Did not receive erlotinib (n = 1)
 Consent withdrawn (n = 1)

Randomized to Arm B, escalating dose (n = 29)
 Received erlotinib (n = 27)
 Did not receive erlotinib (n = 2)
 Clinical deterioration (n = 2)

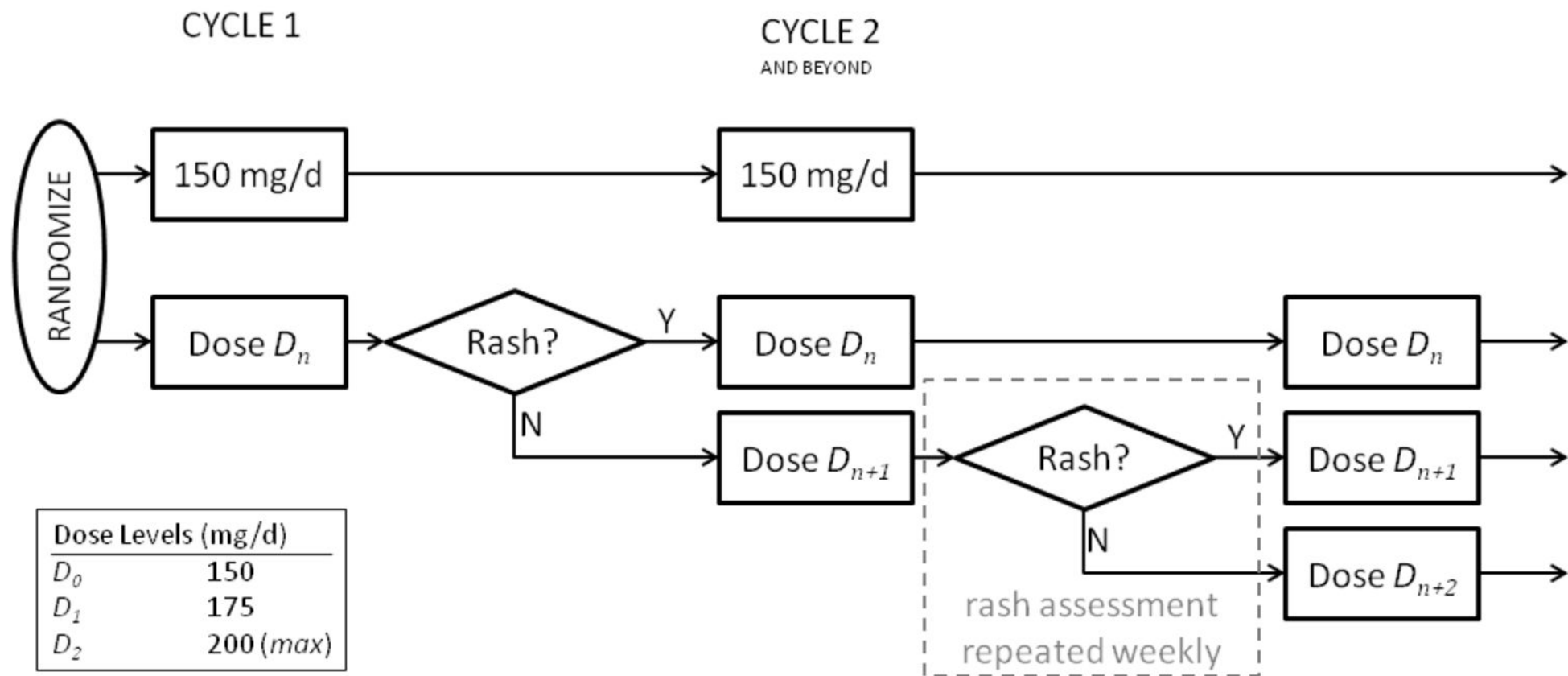
Discontinued intervention (n = 2)
 Clinical deterioration and death (n = 1)
 Interstitial pneumonitis (n = 1)

Discontinued intervention (n = 1)
 Death due to likely PE (n = 1)

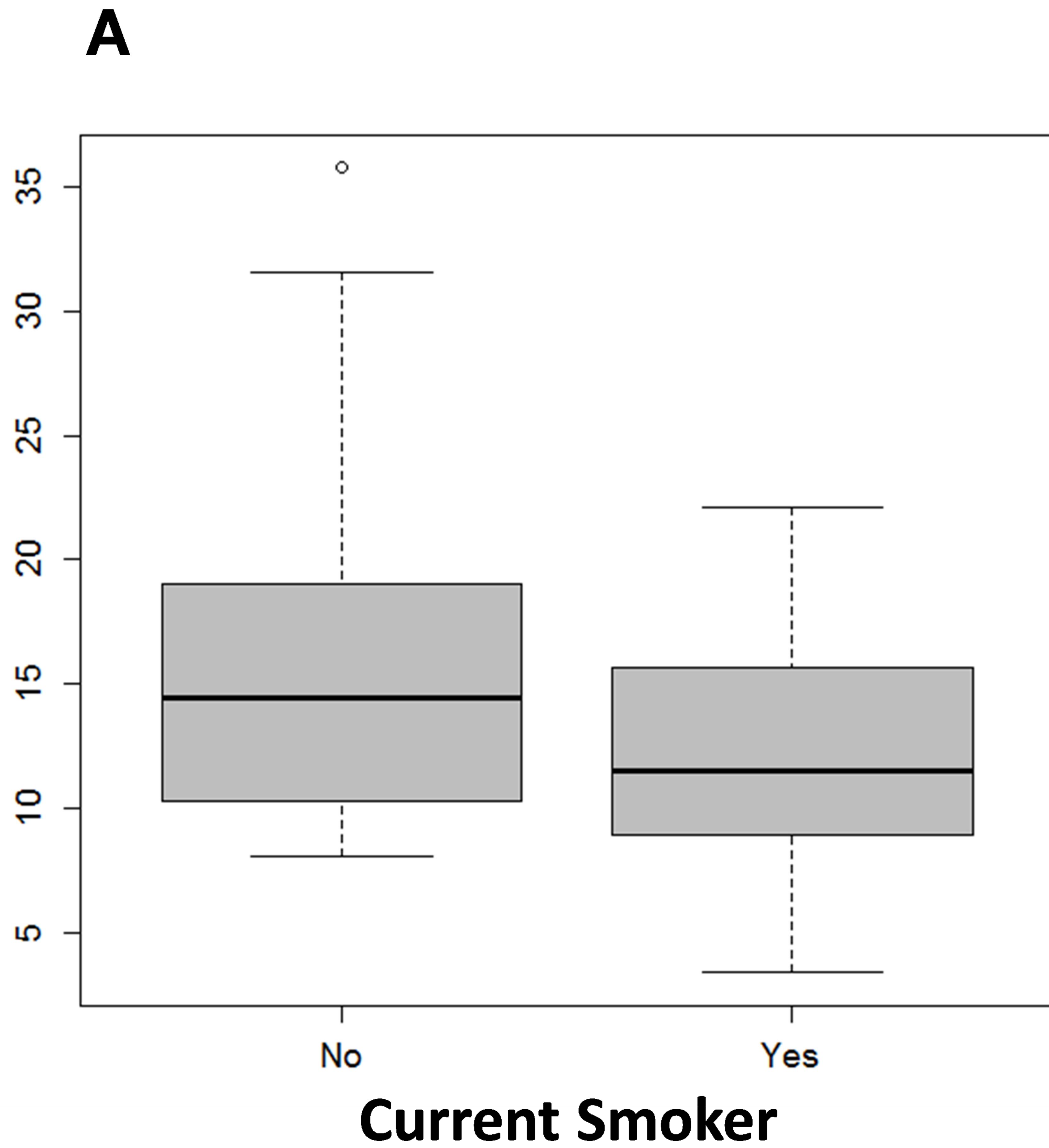
Analysis of Disease Control Rate at 3 Months

Analysed (n = 26)
 Excluded from analysis (n = 0)

Analysed (n = 26)
 Excluded from analysis (n = 0)



Erlotinib AUC (hr x ug/mL)



OSI-420 AUC (hr x ug/mL)

