Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Shaw AT, Ou S-HI, Bang Y-J, et al. Crizotinib in *ROS1*-rearranged non–small-cell lung cancer. N Engl J Med 2014;371:1963-71. DOI: 10.1056/NEJMoa1406766

SUPPLEMENTARY APPENDIX

Supplement to: Shaw et al. Crizotinib in ROS1-Rearranged Non-Small Cell Lung Cancer

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RECIST version 1.0 Tumor Assessment Criteria

At baseline, tumor lesions will be categorized as measurable or non-measurable (defined below).

All baseline evaluations should be performed as close as possible to the first day of study treatment and never more than 4 weeks before starting therapy.

Measurable Lesions

- Lesions that can be accurately measured in at least 1 dimension (longest diameter to be recorded) as ≥2.0 cm with conventional techniques or ≥1.0 cm with spiral CT scan.

- A tumor lesion that is situated in a previously irradiated area is eligible for measurable disease provided: 1) there has been documented disease progression in this site; 2) the criteria for measurability as outlined above are met; 3) this is not the only site of measurable disease.

- All measurements should be determined using a ruler, calipers or digital technology, and recorded on the CRF in metric notation.

Nonmeasurable Lesions

All other lesions, including small lesions (longest diameter <2.0 cm with conventional techniques or <1.0 cm with spiral CT) and truly nonmeasurable lesions. Truly nonmeasurable lesions include bone lesions, leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitis cutis or pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions.

Documentation of Target and Nontarget Lesions

All measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and measured and recorded at baseline. Target lesions (measurable) should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically. A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

All other lesions (or sites of disease) should be identified as nontarget lesions and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as present or absent.

Techniques for Assessing Measurable Disease

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at screening and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical (physical) examination when both methods have been used to assess the antitumor effect of a treatment.

Accepted methods of tumor assessment include:

Clinical examination: clinically detected lesions will only be considered measurable when they are superficial (eg, skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended.

Chest x-ray: lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

CT and MRI: CT and MRI are the best currently available and most reproducible methods of measuring target lesions selected for response assessment. Conventional CT and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm.

Ultrasound: should not be used to measure tumor lesions for objective response evaluation. It is however a possible alternative to clinical measurements of superficial palpable nodes, subcutaneous lesions and non-small cell lung nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy and Laparoscopy: The utilization of these techniques for objective tumor evaluation has not yet been fully or widely validated. Utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete histopathologic response when biopsy specimens are obtained.

Tumor markers: tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered a complete clinical response.

Cytology and histology: the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

Response Criteria

The following RECIST criteria will be the primary method utilized in this study for the assessment and reporting of tumor response data.

Complete Response (CR): Disappearance of all target and nontarget lesions, normalization of tumor marker levels, and no appearance of new lesions indicates complete response. Each of these must be documented on 2 occasions separated by at least 4 weeks.

Partial Response (PR): At least a 30% decrease in the sum of the LDs of target lesions (taking as reference the baseline sum), without progression of nontarget lesions and no appearance of new lesions indicates partial response. Each of these must be documented on 2 occasions separated by at least 4 weeks.

Stable Disease (SD): Neither PR or PD criteria are met.

Progressive Disease (PD): ≥20% increase in the sum of the LD of target lesions taking as references the smallest sum LD recorded since the treatment started, unequivocal progression of existing nontarget lesions, or the appearance of 1 or more new lesions.
The occurrence of a pleural effusion or ascites is also considered PD if substantiated by cytologic investigation and not previously documented. Pathologic fracture or collapse of bone is not necessarily evidence of disease progression; however, new bone lesions not previously documented are considered PD.

In cases where procedures used to assess tumor size suggest tumor necrosis or intratumor bleeding coincident with an increase in size, a PET scan or ultrasound should be considered because it is important to be sure that increasing lesions are due to increased tumor growth and not necrosis or bleeding.

Determination of Best Overall Response:

The best overall response is the best response recorded from the start of treatment until disease progression/recurrence. For PD, taking as reference the smallest measurements recorded since treatment started. For CR and PR the best response assignment will depend on the achievement of both measurement and confirmation (at the minimum of 28 days) criteria. Stable disease rate will be defined as the percentage of patients with stable disease based on the total number of patients evaluable for response.

Determination of best overall response is summarized in the Table on the following page:

Determination of Best Overall Response			
Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR[a]	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR[b]	Non-PD	No	PR
SD[c]	Non-PD	No	SD
PD[d]	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

[a] Complete response.
[b] Partial response.
[c] Stable disease.
[d] Progressive disease.

Reference: Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205-216.

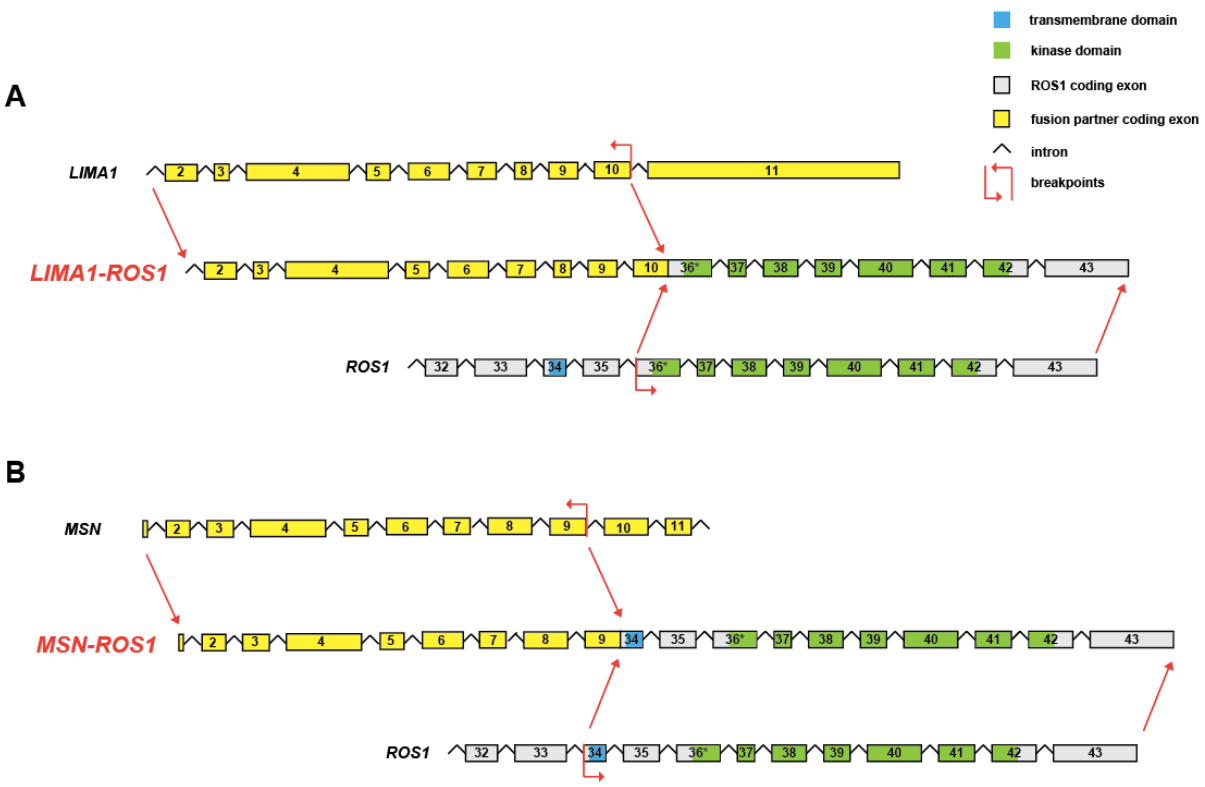

Figure S1. Genomic organization of two novel ROS1 fusions in NSCLC.
Shown is a schematic of the genomic structures of the LIMA1-ROS1 and MSN-ROS1 fusions. Both fusions include the entire tyrosine kinase domain of ROS1. Exons encoding the kinase domain are shaded in green and were mapped using MapBack. The numbering of exons corresponds to coding, translated exons. Exons, but not introns, are drawn to scale. Breakpoints are represented by red right angle arrows.

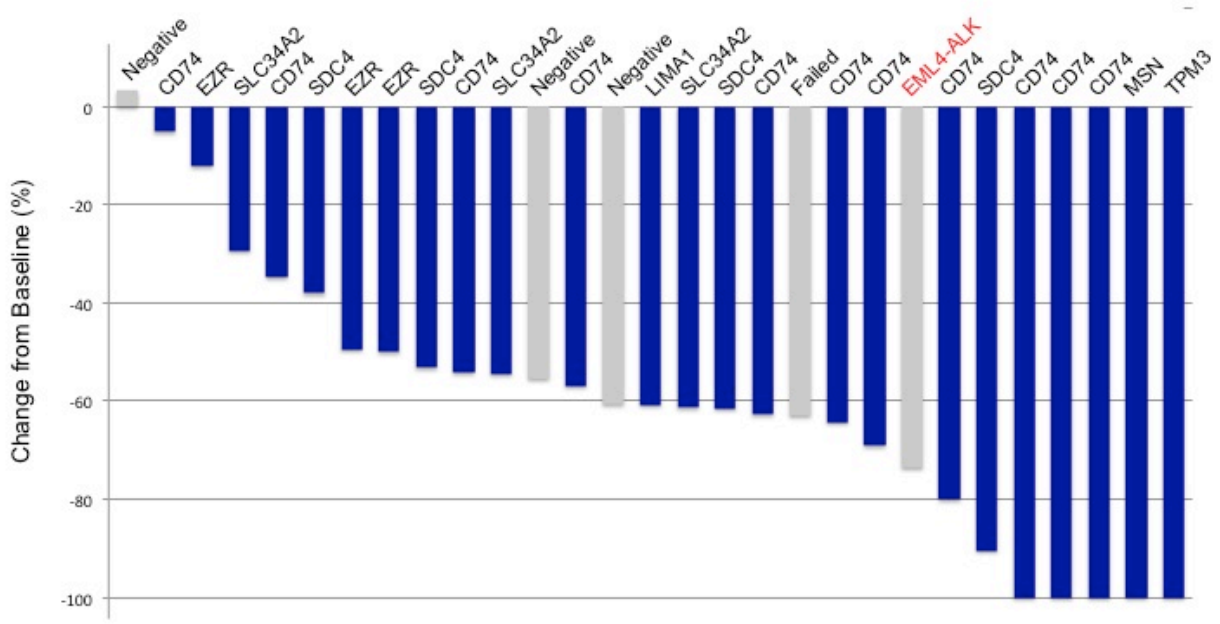

Figure S2. Best response to crizotinib and correlation with ROS1 fusion partners.
This waterfall plot includes all patients who had sufficient tumor tissue for additional characterization of the ROS1 fusion partner. For each patient, the fusion partner (if one was identified) is shown above the bar corresponding to the patient's best tumor response. Those cases where the NGS assay failed to detect a ROS1 fusion partner are also included and depicted by gray bars. One patient with an EZR-ROS1 fusion was not evaluable due to early death and hence is not shown here.

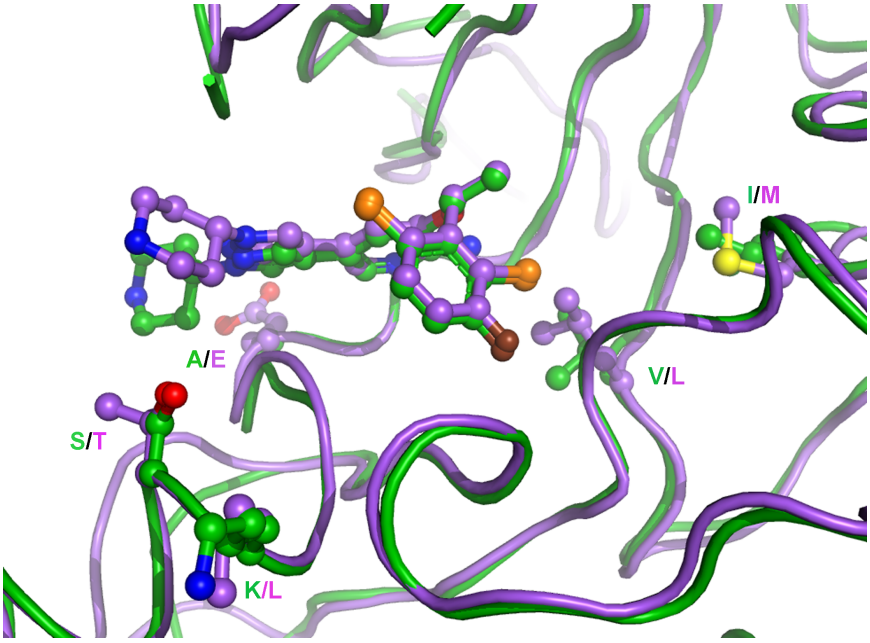

Figure S3. Structural similarity between ROS1 and ALK crizotinib binding sites.
Shown is the superposition of crystal structures of crizotinib complexes with ROS1 (purple, wwPDB entry 3ZBF) and ALK (green, wwPDB entry 2XP2) (refs Awad et al and Huang et al). The overall protein conformations and interactions with crizotinib are highly similar. Nearly all of the residues in the binding site are identical and for clarity only the side chains for the ones that differ have been depicted. Of the sequence differences only the S/T and V/L positions make contact with crizotinib.

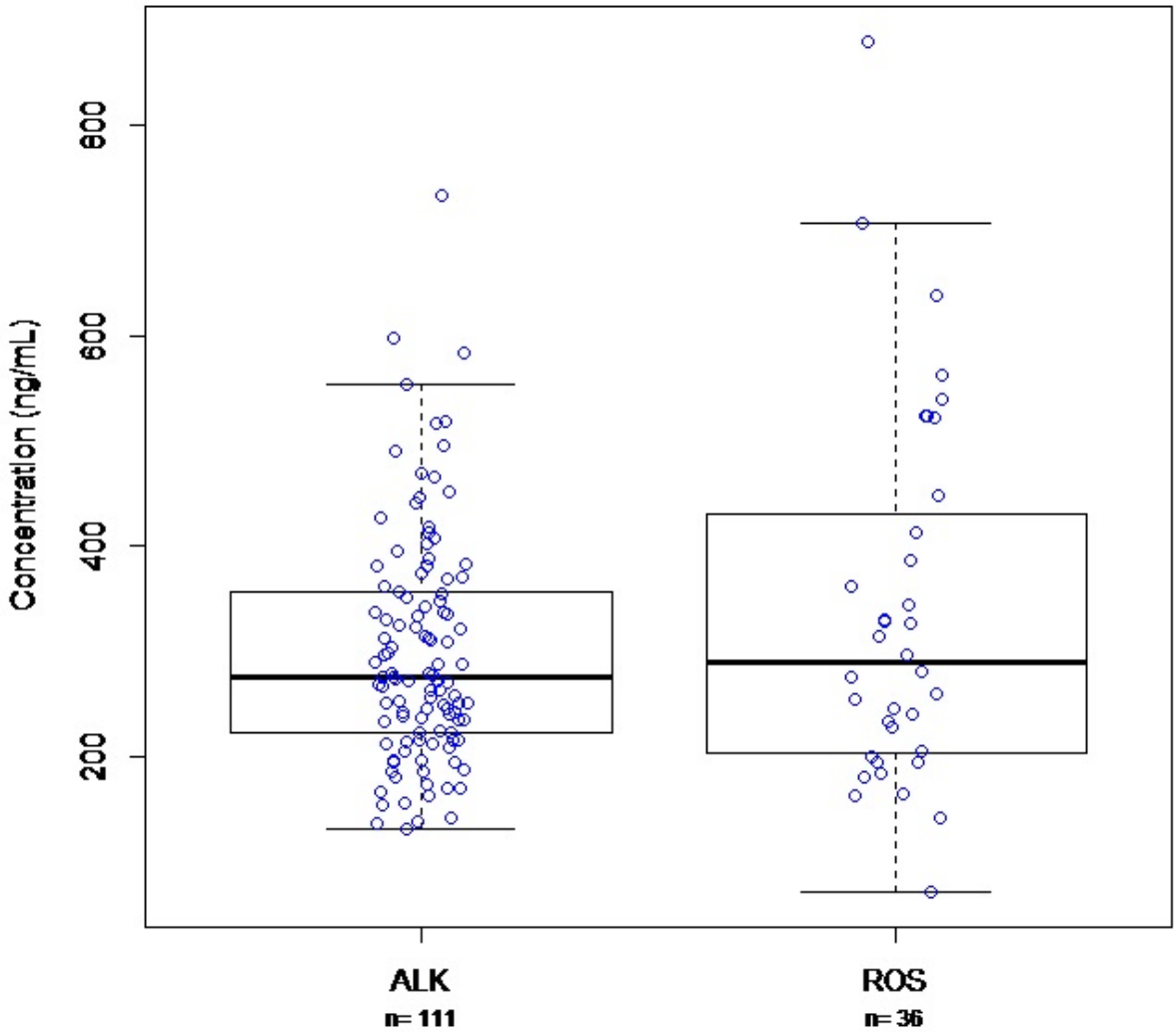

Figure S4. Pharmacokinetic profile of crizotinib.

This figure shows a comparison of the steady state trough concentrations ($C_{trough, ss}$) of crizotinib in ROS1 (right) versus ALK (left) patients in the phase 1 study. Blood samples for pharmacokinetic assessment were collected on days 1 and 15 of cycles 1 and 2 and on day 1 of cycles 3, 4 and/or 5, as specified in the protocol. The arithmetic mean $C_{trough, ss}$ of crizotinib in each patient was calculated using all evaluable pre-dose plasma concentrations from cycle 1 day 15 through cycle 5 day 1, wherever available.

Table S1. Summary of Prior Anticancer Therapies.

	ROS1 Cohort (N=50)
Any prior therapy	43 (86)
Platinum agent (cisplatin or carboplatin)	40 (80)
Pemetrexed	36 (72)
Taxane (paclitaxel or docetaxel)	20 (40)
Bevacizumab	16 (32)
EGFR inhibitor (erlotinib or gefitinib)	16 (32)
Gemcitabine	11 (22)
Vinorelbine	3 (6)

Table S2. Summary of Confirmed Responses.*

	ROS1 Cohort (N=50)
Type of response – no. (%)	
Complete response	3 (6)
Partial response	33 (66)
Stable disease	9 (18)
Progressive disease	3 (6)
Early death†	2 (4)
Objective response rate (%)	72
95% CI	58 - 84
Time to first response (wks)‡	
Median	7.9
Range	4.3 – 32.0
Duration of response (mos)§	
Median	17.6
95% CI	14.5 – NR

95% CI, 95% confidence interval (estimated using the exact binomial method based on the F distribution)

* Tumor responses were assessed by investigators using Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0.

† Two patients died within 6 weeks from first dose.

‡ Time to response was calculated from the date of first dose of study drug to the date of first documentation of partial or complete response.

§ Duration of response was calculated from the date of first documentation of partial or complete response to the date of RECIST-defined progression or death. Median response duration was estimated using the Kaplan-Meier method.