Lorlatinib in advanced *ROS1*-positive non-small-cell lung cancer: a multicentre, open-label, single-arm, phase 1–2 trial

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Summary

Background Lorlatinib is a potent, brain-penetrant, third-generation tyrosine kinase inhibitor (TKI) that targets ALK and ROS1 with preclinical activity against most known resistance mutations in *ALK* and *ROS1*. We investigated the antitumour activity and safety of lorlatinib in advanced, *ROS1*-positive non-small-cell lung cancer (NSCLC).

Methods In this open-label, single-arm, phase 1–2 trial, we enrolled patients (aged ≥18 years) with histologically or cytologically confirmed advanced *ROS1*-positive NSCLC, with or without CNS metastases, with an Eastern Cooperative Oncology Group performance status of 2 or less (≤1 for phase 1 only) from 28 hospitals in 12 countries worldwide. Lorlatinib 100 mg once daily (escalating doses of 10 mg once daily to 100 mg twice daily in phase 1 only) was given orally in continuous 21-day cycles until investigator-determined disease progression, unacceptable toxicity, withdrawal of consent, or death. The primary endpoint was overall and intracranial tumour response, assessed by independent central review. Activity endpoints were assessed in patients who received at least one dose of lorlatinib. This study is ongoing and is registered with ClinicalTrials.gov, NCT01970865.

Findings Between Jan 22, 2014, and Oct 2, 2016, we assessed 364 patients, of whom 69 with *ROS1*-positive NSCLC were enrolled. 21 (30%) of 69 patients were TKI-naive, 40 (58%) had previously received crizotinib as their only TKI, and eight (12%) had previously received one non-crizotinib ROS1 TKI or two or more ROS1 TKIs. The estimated median duration of follow-up for response was 21·1 months (IQR 15·2–30·3). 13 (62%; 95% CI 38–82) of 21 TKI-naive patients and 14 (35%; 21–52) of 40 patients previously treated with crizotinib as their only TKI had an objective response. Intracranial responses were achieved in seven (64%; 95% CI 31–89) of 11 TKI-naive patients and 12 (50%; 29–71) of 24 previous crizotinib-only patients. The most common grade 3–4 treatment-related adverse events were hypertriglyceridaemia (13 [19%] of 69 patients) and hypercholesterolaemia (ten [14%]). Serious treatment-related adverse events occurred in five (7%) of 69 patients. No treatment-related deaths were reported.

Interpretation Lorlatinib showed clinical activity in patients with advanced *ROS1*-positive NSCLC, including those with CNS metastases and those previously treated with crizotinib. Because crizotinib-refractory patients have few treatment options, lorlatinib could represent an important next-line targeted agent.

Funding Pfizer.

Introduction

Chromosomal rearrangements of *ROS1,* a gene that encodes for the receptor tyrosine kinase ROS1, can lead to the formation of oncogenic fusion proteins with constitutive kinase activity.[1] *ROS1* rearrangements are identified in approximately 1–2% of patients with non-small-cell lung cancer (NSCLC) and define a distinct molecular subset of NSCLC with sensitivity to ROS1 inhibition.[2–5]

Because of amino acid similarities between the kinase domains of ROS1 and ALK, some ALK tyrosine kinase inhibitors (TKIs) are also active against ROS1.[6–10] For example, crizotinib, a multitargeted inhibitor of the tyrosine kinases ALK, ROS1, and MET, is approved in many countries for the treatment of patients with advanced *ROS1*-positive NSCLC. The second-generation ALK inhibitor ceritinib has also shown antitumour activity in a cohort of crizotinib-naive Korean patients with *ROS1*-positive NSCLC.[6] The majority of ROS1-positive patients initially respond to crizotinib (or ceritinib), but most patients eventually relapse because of resistance. Across retrospective and prospective studies of crizotinib, median progression-free survival ranged from 6 months to 19 months.[7,8,11,12]

Resistance to crizotinib can be mediated by secondary mutations within the *ROS1* kinase domain (ie, on-target resistance), or by activation of alternative signalling pathways (ie, off-target or ROS1-independent resistance). Overall, the most common cause of crizotinib resistance is the solvent-front mutation Gly2032Arg, which sterically impedes drug binding.[13] Several other resistance mutations within *ROS1* have been reported at lower frequency.[13–15] Although acquired resistance is a major cause of crizotinib failures, relapses are also common in the CNS.[16,17] Given the poor blood–brain barrier penetration of crizotinib, CNS progression probably

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Research in context

Evidence before this study

The multitargeted tyrosine kinase inhibitor (TKI) crizotinib, which targets ALK, ROS1, and MET, is approved in many countries for the treatment of patients with advanced ROS1-positive non-small-cell lung cancer (NSCLC). Although most patients with ROS1-positive NSCLC initially derive clinical benefit from crizotinib, most patients will eventually relapse because of acquired resistance or CNS progression. We used PubMed to search the biomedical literature for studies on crizotinib-resistant ROS1-positive NSCLC using the terms "ROS1", "lung cancer", and "clinical trial". No date or language restrictions were applied. The search identified two targeted therapies—ceritinib and entrectinib—with clinical activity in crizotinib-naive patients but no clinical activity in crizotinib-resistant disease, and a case report describing the activity of a multitargeted agent, cabozantinib, in one patient with acquired resistance to crizotinib. Several other ROS1-targeted agents are currently in clinical trials; however, treatment options for relapsing patients are generally limited and include cytotoxic chemotherapy regimens or radiation. Lorlatinib is a potent, oral, selective, third-generation macrocyclic TKI of ALK and ROS1 that was designed to penetrate the blood–brain barrier. Preclinical studies have also shown that lorlatinib retains variable potency against known *ROS1* resistance mutations. In the phase 1 portion of this ongoing phase 1–2 study, lorlatinib showed preliminary antitumour activity in patients with ROS1-positive NSCLC, most of whom had previously received crizotinib.

Added value of this study

This study shows that lorlatinib has clinical activity in patients with advanced ROS1-positive NSCLC who are ROS1 TKI-naive or who have previously received crizotinib. Marked intracranial activity was also observed in both TKI-naive and crizotinib-pretreated patients. In addition, lorlatinib was generally well tolerated, with no new safety signals reported.

Implications of all the available evidence

Patients relapsing on crizotinib currently have few treatment options. Lorlatinib might represent an important next-line targeted agent in advanced ROS1-positive NSCLC, including for patients with CNS metastases.

reflects pharmacokinetic failure rather than resistance to crizotinib. For patients who relapse systemically, in the CNS, or both, several ROS1-targeted drugs are being evaluated in clinical trials, but standard treatment options are limited and include cytotoxic chemotherapy (eg, platinum and pemetrexed-based combinations) or radiotherapy.[18,19]

Lorlatinib is a potent, oral, selective, third-generation macrocyclic TKI that targets ALK and ROS1. With use of structure-based design, lorlatinib was specifically developed to penetrate the blood–brain barrier through reduction of P-glycoprotein-1-mediated efflux.[20] Preclinical studies have shown that lorlatinib potently inhibits ROS1 and retains variable potency against different *ROS1* resistance mutations.[21–23] In the phase 1 portion of an ongoing phase 1–2 study, lorlatinib showed preliminary antitumour activity in patients with *ROS1*-positive NSCLC, most of whom had previously received crizotinib.[24] Here, we present antitumour activity and safety results, as well as a planned molecular analysis, from this phase 1–2 study of lorlatinib in patients with advanced *ROS1*-positive NSCLC.

Methods

Study design and participants

Patients with advanced *ROS1*-positive NSCLC were treated with lorlatinib in an ongoing, open-label, single-arm, phase 1–2 study done in 28 hospitals in 12 countries worldwide (appendix p 3), for which the full method has been published previously.[24,25] *ROS1*-positive patients from two substudies, a Japanese lead-in cohort (Japan-LIC) and a drug–drug interaction and Holter monitoring (DDI) study, were also included (appendix p 2).

See Online for appendix

Eligible patients (aged ≥18 years) had histologically or cytologically confirmed metastatic NSCLC with a *ROS1* rearrangement and an Eastern Cooperative Oncology Group performance status of 2 or less (≤1 for phase 1 only). ROS1 positivity was established by fluorescence in situ hybridisation, RT-PCR, or next-generation sequencing via a local laboratory developed test. Asymptomatic treated or untreated CNS metastases were permitted. Patients were required to have at least one measurable target extracranial (or intracranial for DDI only) lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Inclusion criteria allowed both treatment-naive patients in the advanced setting and patients with disease progression after at least one previous ROS1 inhibitor therapy (phase 1) or any number of previous therapies (ie, chemotherapy or ROS1 inhibitor therapies; phase 2, Japan-LIC, and DDI). Patients with leptomeningeal disease or carcinomatous meningitis were eligible in the phase 2 study if the leptomeningeal disease or carcinomatous meningitis was visualised on MRI or if baseline CSF cytology was positive. Patients were required to have adequate function of the bone marrow (evaluated by laboratory tests of absolute neutrophil count, platelets, and haemoglobin), and adequate pancreatic (evaluated by tests of amylase and lipase), renal (evaluated by a test of creatinine), and liver (evaluated by tests of total bilirubin, aspartate aminotransferase, and alanine aminotransferase) function.

Exclusion criteria included previous treatment with immunotherapy, spinal cord compression; active and clinically significant infection; clinically significant cardiovascular disease; predisposing characteristics for

acute pancreatitis; history of extensive, disseminated, bilateral, or grade 3–4 interstitial fibrosis or interstitial lung disease; severe, acute, or chronic psychiatric conditions; acute malignant disease (other than NSCLC, non-melanoma skin cancer, in-situ cervical cancer, papillary thyroid cancer, ductal carcinoma in situ of the breast, or localised and presumed cured prostate cancer) within the past 3 years; active inflammatory gastrointestinal disease, chronic diarrhoea, symptomatic diverticular disease, previous gastric resection, or gastric band; or abnormal left ventricular ejection fraction (LVEF). The full list of inclusion and exclusion criteria is provided in the protocol.

All patients provided written, informed consent before participation. The institutional review board or independent ethics committee at each participating centre approved the protocol, which complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws. The protocol is included in the appendix (pp 20–219).

Procedures

Lorlatinib was administered orally in continuous 21-day cycles at escalating doses (10 mg once daily to 100 mg twice daily) in phase 1, and at a starting dose of 100 mg once daily in phase 2, Japan-LIC, and DDI. Treatment continued until investigator-determined disease progression, unacceptable toxicity, withdrawal of consent, or death. Treatment could be continued after progression if the patient was still experiencing clinical benefit, at the discretion of the investigator.

Temporary treatment discontinuations and dose reductions were permitted to manage toxic effects at the discretion of the investigator. Patients who required more than three dose reductions were discontinued from treatment.

All patients underwent baseline tumour imaging by CT and brain imaging by MRI. CT (chest, abdomen, and pelvis) and MRI scans were done every 6 weeks for the first 18 months in phase 1 and Japan-LIC, and for the first 30 months in phase 2 and DDI; scans were done every 12 weeks thereafter until disease progression or the start of a new anticancer treatment. Response was assessed according to modified RECIST version 1.1, which allowed for five or fewer CNS target lesions, as assessed by independent central radiology review.

In all four substudies (phase 1, phase 2, Japan-LIC, and DDI), haematological and lipid assessments were done at screening, day 1 of cycle 1, day 15 of cycle 1, day 1 of each subsequent cycle, and at the end of treatment. Coagulation and urinalysis assessments were done at screening, day 1 of cycle 1, and at the end of treatment. Pregnancy tests were done at screening, day –7 (lead-in pharmacokinetic analysis), day 1 of cycle 1, day 1 of each subsequent cycle, and at the end of treatment. LVEF assessments (echocardiogram or multigated acquisition scans) were done at screening, before dose at day 1 of cycle 2, before dose at day 1 of cycle 3, before dose at day 1 of cycle 5, every two cycles thereafter until approximately 18 months after day 1, and then every four cycles on day 1 (or within 2 days before and after) thereafter.

Adverse events were assessed in all patients at baseline, every week for the first cycle, and every 3 weeks thereafter until at least 28 days after the final dose of lorlatinib and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03.

Molecular profiling of tissue (archival or de-novo) and blood was done to identify potential biomarkers of response and resistance to lorlatinib. Peripheral blood samples were collected at screening, at the beginning of cycle 3 (phase 2, Japan LIC, and DDI only), and at the end-of-treatment visit for plasma circulating free DNA (cfDNA) analysis of *ROS1* kinase domain mutations or gene rearrangements with a next-generation sequencing panel (Guardant360, panel version 2.10, bioinformatics pipeline version 3.7; Guardant Health, Redwood City, CA, USA). All patients were required to have archival or de-novo tissue samples available and collected before enrolment; archival tissue could have been collected at any time after diagnosis. In phase 1 and Japan-LIC, de-novo biopsy was required if archival tumour tissue was not available. In phase 2 and DDI, a de-novo biopsy was mandatory unless it was considered a safety risk to the patient. Tumour tissue was analysed with a *ROS1* kinase domain mutation-focused next-generation sequencing panel (ROS1 exons 36–42; MolecularMD, Portland, OR, USA).

Outcomes

The primary endpoint was objective tumour response and intracranial tumour response (defined as a confirmed complete response or partial response as the best overall or intracranial response) according to modified RECIST version 1.1, as assessed by independent central radiology review. Key secondary endpoints included: duration of response (time from the first documentation of objective response to the first documentation of progression or death from any cause); intracranial duration of response; time to first tumour response (time from first dose to first documentation of objective tumour response); time to intracranial progression (time from first dose to first documentation of intracranial disease progression); progression-free survival (time from first dose to first documentation of objective disease progression or to death on study due to any cause); probability of first event being a CNS progression, non-CNS progression, or death; and safety and tolerability. Selected molecular profiling of cfDNA and tumour tissue was also a prespecified secondary endpoint. Secondary endpoints not reported here were CSF concentration of lorlatinib, corrected QT interval, LVEF, disease control, overall survival, patient-reported outcomes, response to previous

systemic therapies, pharmacokinetics, vital signs, and mood, cognitive function, and suicide ideation and behaviour (phase 2 only); these endpoints will be reported elsewhere.

Statistical analysis

The analyses presented are based on the entire population of *ROS1*-positive patients who entered the study in its various parts, with different previous treatments and at different doses. As such, overall sample size was not predefined, nor was it based on any predefined statistical hypothesis.

Analyses of antitumour activity and safety in this report were based on all patients who received at least one dose of lorlatinib. Molecular analyses were prespecified and based on all patients with documented *ROS1* rearrangements who had received at least one dose of lorlatinib, with at least one molecular biomarker (eg, markers of resonse and resistance to lorlatinib, including *ROS1* kinase mutations) assayed from a tumour or cfDNA sample. Antitumour activity and molecular analyses were summarised for all patients as well as patients with CNS metastases by previous line of treatment: ROS1 TKI-naive, previous crizotinib, or other (defined as patients who had previously received one non-crizotinib ROS1 TKI or at least two ROS1 TKIs), with or without any number of chemotherapy regimens. Patients who had previously received either one non-crizotinib ROS1 TKI or at least two ROS1 TKIs were analysed separately from those who had previously received crizotinib only, because mechanisms of resistance differ depending on previous TKI exposure. Analyses according to previous treatment were not prespecified in the protocol.

We calculated the corresponding 95% CIs for overall response using the exact method based on the binomial distribution. Waterfall plots were generated for best target lesion tumour size percentage change from baseline for all patients and patients with *ROS1* gene alterations at screening, including the Gly2032Arg mutation. We analysed time-to-event endpoints, such as duration of response, progression-free survival, and time to intracranial progression, using Kaplan-Meier estimates of median time and associated two-sided 95% CIs on the basis of the Brookmeyer and Crowley method. We analysed duration of follow-up for objective response using the reverse Kaplan-Meier method. Time to first tumour response was summarised with descriptive statistics.

Patients were analysed for progressive disease events, categorised as having either CNS or non-CNS progression. Any new CNS lesions or progression of pre-existing CNS lesions versus baseline was considered a CNS progression, and any new lesion or progression of pre-existing lesions in areas outside the CNS was considered non-CNS progression. We estimated the probability of a first event being a CNS progression, non-CNS progression, or death by cumulative incidences using a competing risks approach in patients with or without baseline CNS metastases. If both CNS and non-CNS progression were presented simultaneously as the first progressive event, patients were considered to have CNS progression as their first event. The data cutoff date was Feb 2, 2018. All analyses were done with SAS (version 9.4). This study is ongoing and is registered with ClinicalTrials.gov, NCT01970865.

	Participants (n=69)
Age, years	54·0 (44·0–61·0)
Sex	
Male	30 (43%)
Female	39 (57%)
Race	
White	36 (52%)
Asian	22 (32%)
Black	2 (3%)
Other	4 (6%)
Not specified*	5 (7%)
ECOG performance status	
0	27 (39%)
1	40 (58%)
2	2 (3%)
Brain metastases present at baseline†	39 (57%)
Previous brain-directed radiotherapy, n/N (%)	19/39 (49%)
Number of previous chemotherapy regimens	
0	15 (22%)
1	19 (28%)
2	19 (28%)
3	13 (19%)
≥4	3 (4%)
Number of previous ROS1 TKIs	
0	21 (30%)
1	41 (59%)‡
2	3 (4%)§
3	4 (6%)§

Data are median (IQR) or n (%). ECOG=Eastern Cooperative Oncology Group. TKI=tyrosine kinase inhibitor. *In France, information about race was not collected as directed by local regulations. †By independent central review. ‡40 of 41 patients received crizotinib; one patient received entrectinib. §In seven patients who had received two or three previous TKIs, TKIs included cabozantinib, ceritinib, crizotinib, and DS-6051b.

Table 1: **Baseline characteristics**

Role of the funding source

This study was designed by the sponsor and study investigators. Data were collected by investigators and analysed by the sponsor. All authors, including those employed by the sponsor of the study, contributed to the interpretation of the data and the development, writing, and approval of the manuscript. All authors had full access to the raw data. The first author wrote the first draft of the manuscript in conjunction with the sponsor and had final responsibility for the decision to submit for publication.

Results

Between Jan 22, 2014, and Oct 2, 2016, 364 patients were initially assessed, of whom 69 patients with *ROS1*-positive NSCLC were enrolled and included in the antitumour activity and safety analyses. Of these 69 patients, 12 patients were from the phase 1 portion, 47 were from the phase 2 portion, one was from the Japan-LIC, and nine were from the DDI (appendix pp 4, 11). Baseline clinical characteristics are shown in table 1. Among the 69 patients, 21 (30%) were TKI-naive, 40 (58%) had previously received crizotinib as their only TKI, and eight (12%) had previously received one non-crizotinib ROS1 TKI or two or more ROS1 TKIs. 54 (78%) had received at least one previous chemotherapy regimen. CNS metastases were present at baseline in 39 (57%) of 69 patients; 19 (49%) of whom had received previous brain-directed radiotherapy. The estimated median duration of follow-up for response was 21·1 months (IQR 15·2–30·3). All patients were treated with lorlatinib at 100 mg per day, except for ten phase 1 patients who received doses ranging from 10 mg per day to 100 mg twice daily. Of the ten patients, five (50%) received lorlatinib at doses of less than 100 mg per day.

Among the 69 patients with *ROS1*-positive NSCLC, 28 (41% [95% CI 29–53]) had an objective response. Of the 21 TKI-naive patients, 13 (62% [38–82]) had an objective response, with two patients (10%) achieving a complete response and 11 (52%) achieving a partial response (table 2, figure 1A). At the data cutoff, six (46%) of 13 responses were ongoing and median duration of response was 25·3 months (95% CI 7·5–31·9). Median time to first tumour response was 1·4 months (IQR 1·4–1·4). Objective responses were achieved in five (45% [95% CI 17–77]) of 11 patients with baseline CNS metastases and in eight (80% [44–98]) of ten patients without baseline CNS metastases.

Of the 40 patients who had received crizotinib as their only previous ROS1 TKI, objective responses were observed in 14 patients (35% [95% CI 21–52]), including two patients (5%) with complete response and 12 patients (30%) with partial response (table 2, figure 1B). Eight (57%) of 14 responses were ongoing at time of analysis, and the median duration of response was 13·8 months (95% CI 9·7–not reached). Median time to first tumour response was 2·1 months (IQR 1·4–2·8). Responses were observed in six (25% [95% CI 10–47]) of 24 patients with baseline CNS metastases and eight (50% [25–75]) of 16 patients without baseline CNS metastases.

Lorlatinib was active in the one *ROS1*-positive patient with leptomeningeal disease at baseline. This patient achieved an overall partial response, intracranial complete response, and had not progressed at the time of analysis.

Extracranial responses were consistent with overall responses observed in both TKI-naive patients and in patients who had previously received only crizotinib (table 2, appendix p 12).

At the time of data cutoff, 13 (62%) of 21 TKI-naive patients and 23 (58%) of 40 crizotinib-treated patients had experienced a progression-free survival event. Median progression-free survival was 21·0 months (95% CI 4·2–31·9) in TKI-naive patients and 8·5 months (4·7–15·2) in crizotinib-treated patients (figure 1C).

Eight patients had received one previous non-crizotinib ROS1 TKI or two or more ROS1 TKIs; treatment history and antitumour activity of lorlatinib in these patients are summarised in the appendix (pp 5, 13).

Baseline CNS metastases (measurable, non-measurable, or both) were present in 11 (52%) of the 21 TKI-naive patients and intracranial responses were observed in seven (64% [95% CI 31–89]) of these patients (table 2, appendix p 14). Six TKI-naive patients had measurable baseline CNS metastases and four (67% [95% CI 22–96])

	TKI-naive	Previous crizotinib only
Overall		
Number of patients	21	40
Best overall response		
Complete response	2 (10%)	2 (5%)
Partial response	11 (52%)	12 (30%)
Stable disease	6 (29%)	16 (40%)
Objective progression	2 (10%)	4 (10%)
Indeterminate*	0	6 (15%)
Patients with confirmed objective response	13 (62%)	14 (35%)
95% CI†	38–82	21–52
Time to first tumour response, months, median (IQR)	1·4 (1·4–1·4)	2·1 (1·4–2·8)
Duration of response, months, median (95% CI‡)	25·3 (7·5–31·9)	13·8 (9·7–NR)
Extracranial		
Number of patients	21	40
Best overall extracranial response		
Complete response	2 (10%)	2 (5%)
Partial response	11 (52%)	12 (30%)
Stable disease	6 (29%)	17 (43%)
Objective progression	2 (10%)	3 (8%)
Indeterminate*	0	6 (15%)
Patients with confirmed extracranial objective response	13 (62%)	14 (35%)
95% CI†	38–82	21–52
Intracranial		
Number of patients with baseline CNS metastases§	11	24
Best overall intracranial response		
Complete response	5 (45%)	9 (38%)
Partial response	2 (18%)	3 (13%)
Stable disease	2 (18%)	6 (25%)
Objective progression	2 (18%)	2 (8%)
Indeterminate*	0	4 (17%)
Patients with confirmed intracranial objective response	7 (64%)	12 (50%)
95% CI†	31–89	29–71
Duration of intracranial response, months, median (95% CI‡)	NR (5·7–NR)	NR (11·0–NR)

Data are n (%) unless otherwise specified. NR=not reached. TKI=tyrosine kinase inhibitor. *Patients defined as indeterminate if (1) only baseline assessment available; (2) tumour assessments incomplete; or (3) first response assessment of stable disease at an interval less than 6 weeks from treatment start and no subsequent disease evaluation. †Using exact method based on binomial distribution. ‡Using Brookmeyer and Crowley method. §CNS metastases were measurable or non-measurable.

Table 2: **Antitumour activity by independent central review**

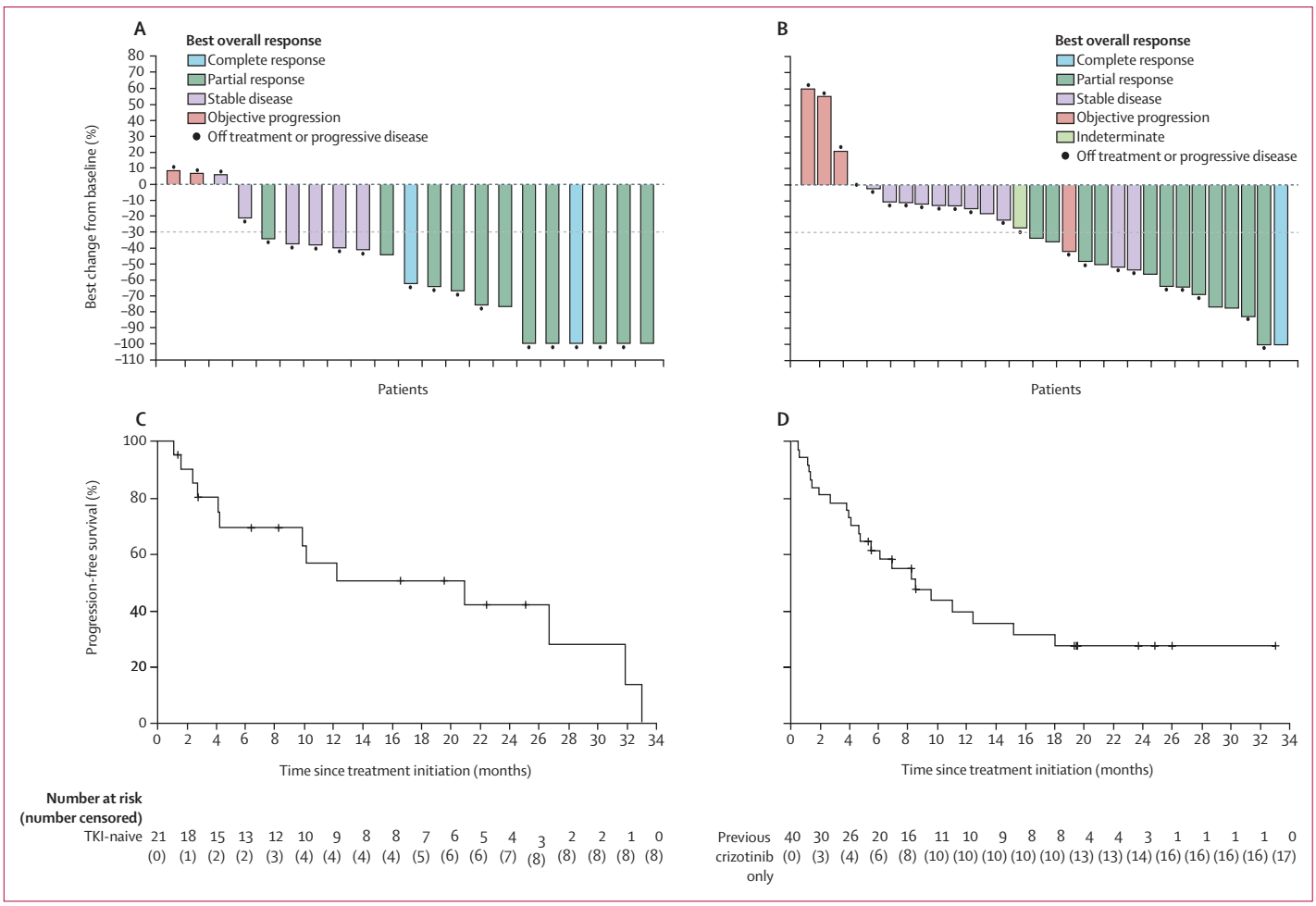

Figure 1: **Antitumour activity of lorlatinib in patients with ROS1-positive non-small-cell lung cancer**
(A) TKI-naive patients. (B) Patients who had previously received crizotinib only. In (A) and (B), patients with at least one on-study target lesion assessment as per independent central review were included. If any radiological procedure was different and not interchangeable from the procedure at screening, the percentage change from baseline could not be calculated and is not displayed. The dashed line shows a 30% reduction in target lesions, which is the threshold for partial response. (C) Progression-free survival among TKI-naive patients. (D) Progression-free survival among patients who had previously received crizotinib only. Vertical lines on the curves indicate censoring of data. TKI=tyrosine kinase inhibitor.

of these patients achieved intracranial responses. Median intracranial duration of response was not reached (95% CI 5·7–not reached), with only three (43%) of seven patients having progression events; intracranial response durations ranged from 1·4 months to 34·7 months (appendix p 6). Among all TKI-naive patients (n=21), median time to intracranial progression could not be estimated at the time of data cutoff.

Of the 24 crizotinib-treated patients with baseline CNS metastases (measurable, non-measurable, or both), 12 (50% [95% CI 29–71]) achieved intracranial responses (table 2, appendix p 14). Of the ten crizotinib-treated patients with measurable baseline CNS metastases, five (50% [95% CI 19–81]) had an intracranial response. Median intracranial duration of response was not reached (95% CI 11·0–not reached), with only three (25%) of 12 patients having progression events; intracranial response durations ranged from 1·4 months to 20·7 months (appendix p 6). Among all crizotinib-treated patients (n=40), median time to intracranial progression could not be estimated at the time of data cutoff.

The cumulative incidences of CNS progression, non-CNS progression, and death in patients with *ROS1*-positive NSCLC treated with lorlatinib are shown in the appendix (pp 15, 16). In TKI-naive patients with baseline CNS metastases, the cumulative incidence of CNS progression at 24 months was 0·29 (95% CI 0·07–0·56) and the cumulative incidence of non-CNS progression at 24 months was 0·20 (0·03–0·47). In crizotinib-treated patients with baseline CNS metastases, the cumulative incidence of CNS progression at 24 months was 0·19 (0·06–0·38) and the cumulative incidence of non-CNS progression at 24 months was 0·59 (0·35–0·77; appendix p 7).

	TKI-naive*	Any previous ROS1 TKI†	
		No mutations	≥1 mutation
Circulating free DNA			
Number of patients with analysable samples	17	33	6
Best overall response			
Complete response	2 (12%)	1 (3%)	0
Partial response	8 (47%)	8 (24%)	0
Stable disease	5 (29%)	14 (42%)	5 (83%)
Objective progression	2 (12%)	4 (12%)	1 (17%)
Indeterminate‡	0	6 (18%)	0
Responders	10 (59%)	9 (27%)	0
Tumour tissue (de novo)			
Number of patients with analysable samples	7	11	5
Best overall response			
Complete response	1 (14%)	0	0
Partial response	4 (57%)	1 (9%)	2 (40%)
Stable disease	2 (29%)	6 (55%)	3 (60%)
Objective progression	0	2 (18%)	0
Indeterminate‡	0	2 (18%)	0
Responders	5 (71%)	1 (9%)	2 (40%)

Data are n (%) unless otherwise specified. TKI=tyrosine kinase inhibitor. *All TKI-naive patients had no mutations. †Includes patients treated with crizotinib only, patients previously treated with one previous non-crizotinib ROS1 TKI, and patients treated with two or more ROS1 TKIs. ‡Patients defined as indeterminate if (1) only baseline assessment available; (2) tumour assessments incomplete; or (3) first response assessment of stable disease at an interval less than 6 weeks from treatment start and no subsequent disease evaluation.

Table 3: **Activity by presence or absence of *ROS1* mutations in circulating free DNA or tumour tissue (de-novo)**

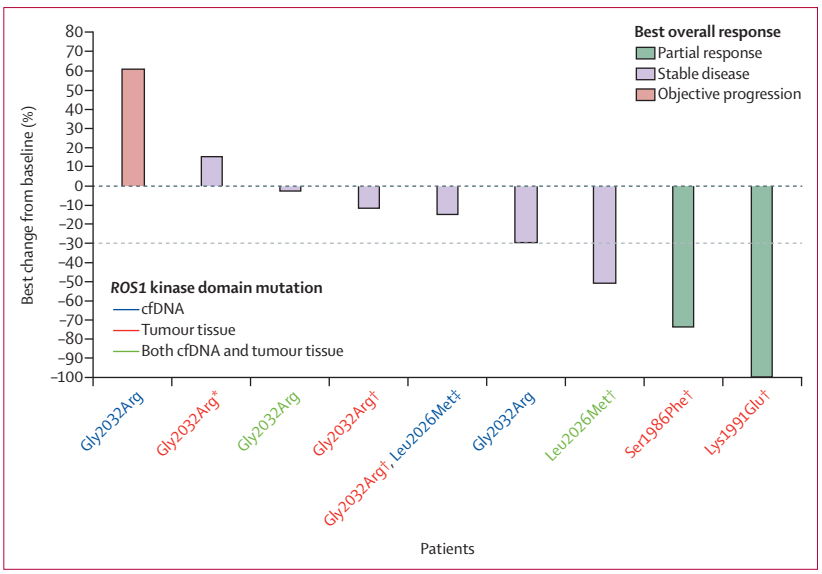

Figure 2: **Best percentage change in tumour size from baseline in patients with at least one *ROS1* kinase domain mutation in cfDNA or tumour tissue (archival or de-novo)**
All patients had received prior crizotinib. The dashed line shows a 30% reduction in target lesions, which is the threshold for partial response. cfDNA=circulating free DNA. *Patient previously received crizotinib and DS6051B. †*ROS1* mutation found in de-novo tumour sample. §Patient previously received crizotinib and ceritinib, and also had the silent Ile2025Ile mutation in cfDNA.

68 patients with *ROS1*-positive NSCLC had plasma samples, tumour tissue (archival or de-novo) samples, or both available for analysis, including 21 patients who were TKI-naive, 39 who had previous crizotinib only, and eight with one previous non-crizotinib ROS1 TKI or two or more ROS1 TKIs (appendix p 17). Of the 60 patients with plasma samples, six (10%) patients had at least one *ROS1* mutation, including four patients who also had a *ROS1* gene rearrangement detectable in cfDNA, 19 (32%) had only a *ROS1* gene rearrangement in cfDNA, 19 (32%) had no *ROS1* alteration (gene rearrangement or mutation) detected, 12 (20%) had no cfDNA detected, and four (7%) had non-analysable samples. 53 patients had archival or de-novo (29 [55%] de-novo) tumour tissue available, of whom seven patients (13%) had at least one *ROS1* mutation detected, 37 (70%) had no detectable *ROS1* mutations, and nine (17%) had non-analysable samples (appendix p 8).

None of the patients who were TKI-naive had detectable *ROS1* mutations in cfDNA or tumour tissue. Among the 41 patients who had previously received any ROS1 TKI and had plasma samples available, six (15%) had detectable *ROS1* mutations in cfDNA, including Gly2032Arg in four patients, Leu2026Met in one patient, and Leu2026Met and the silent Ile2025Ile mutation in one patient. Among the 21 patients who had previously received any ROS1 TKI and had a de-novo tumour specimen, five (24%) had *ROS1* mutations detected in tumour tissue. Gly2032Arg was the most common *ROS1* mutation detected in two patients, followed by Leu2026Met, Ser1986Phe, and Lys1991Glu, each detected in one patient (appendix p 18).

For those patients who had previously received any ROS1 TKI, we evaluated responses according to presence or absence of detectable *ROS1* mutations. On the basis of plasma genotyping, responses were observed in nine (27%) of 33 patients without a *ROS1* mutation detected and in none of six patients with a *ROS1* mutation. On the basis of tissue genotyping of de-novo specimens, responses were observed in one (9%) of 11 patients without a *ROS1* mutation detected and two (40%) of five patients with a *ROS1* mutation (table 3).

Of the two responders with *ROS1* mutations detected in de-novo tumour tissue, both had partial responses; one patient had a Lys1991Glu mutation (duration of response 11·1 months) and one patient had a Ser1986Phe mutation (duration of response 23·3 months and ongoing at data cutoff; figure 2). Of six patients with Gly2032Arg detected in cfDNA or de-novo tumour tissue, five had stable disease (with durations of stable disease from 2·9 months to 9·6 months) and one had progressive disease as best response. One patient who had previously received crizotinib had a Leu2026Met mutation, on the basis of both cfDNA and de-novo tumour tissue profiling, and they had stable disease lasting 2·7 months. Besides *ROS1* rearrangements or mutations, other genetic

	Grade 1–2	Grade 3	Grade 4
Any treatment-related adverse event	32 (46%)	30 (43%)	4 (6%)
Hypercholesterolaemia*	45 (65%)	8 (12%)	2 (3%)
Hypertriglyceridaemia*	29 (42%)	13 (19%)	0
Oedema*	27 (39%)	1 (1%)	0
Peripheral neuropathy*	24 (35%)	1 (1%)	0
Cognitive effects*	18 (26%)	1 (1%)	0
Weight increased	11 (16%)	5 (7%)	0
Mood effects*	11 (16%)	0	0
Fatigue*	7 (10%)	1 (1%)	0
ALT increased	7 (10%)	1 (1%)	0
AST increased	7 (10%)	0	1 (1%)
Dizziness	6 (9%)	2 (3%)	0
Lipase increased	4 (6%)	4 (6%)	0
Amylase increased	6 (9%)	1 (1%)	0
Arthralgia	7 (10%)	0	0
Constipation	7 (10%)	0	0
Nausea	7 (10%)	0	0
Thrombocytopenia	6 (9%)	0	1 (1%)
Hypophosphataemia	2 (3%)	4 (6%)	0
Headache	3 (4%)	1 (1%)	0
Dyspnoea	2 (3%)	1 (1%)	0
Localised oedema	2 (3%)	1 (1%)	0
Presyncope	1 (1%)	1 (1%)	0
Diabetes mellitus	0	1 (1%)	0
GGT increased	0	0	1 (1%)
Hyponatraemia	0	1 (1%)	0
Mental status changes	0	1 (1%)	0
Night sweats	0	1 (1%)	0

Data are n (%). Table includes treatment-related adverse events that were reported in at least 10% of patients and all grade 3–4 treatment-related adverse events. No deaths due to treatment-related adverse events were reported. ALT=alanine aminotransferase. AST=aspartate aminotransferase. GGT=gamma-glutamyltransferase. *Cluster term comprising adverse events that represent similar clinical symptoms or syndromes.

Table 4: **Treatment-related adverse events in ROS1-positive patients (n=69)**

alterations, such as *TP53* mutations, were identified in the plasma of crizotinib-naive and crizotinib-resistant patients before lorlatinib treatment. However, there was no apparent association between lorlatinib response and specific co-alterations (appendix p 19).

Of 69 patients treated with lorlatinib, 66 (96%) had at least one treatment-related adverse event (table 4). The most common grade 3–4 treatment-related adverse events were hypertriglyceridaemia (13 [19%] patients) and hypercholesterolaemia (ten [14%]). No deaths due to adverse events were observed. Serious treatment-related adverse events occurred in five (7%) of 69 patients (appendix p 9). Temporary discontinuations associated with treatment-related adverse events were reported in 25 (36%) of 69 patients and dose reductions associated with treatment-related adverse events were reported in 17 (25%). The most common causes of temporary discontinuations were treatment-related hypertriglyceridaemia (six [9%] patients), oedema (five [7%]), peripheral neuropathy (four [6%]), and hypercholesterolaemia (three [4%]). The most common causes of dose reductions were oedema (four [6%]), peripheral neuropathy (three [4%]), hypercholesterolaemia (three [4%]), and hypertriglyceridaemia (one [1%]). One (1%) of 69 patients permanently discontinued lorlatinib because of treatment-related grade 2 elevated aminotransferases. No treatment-related deaths were reported; a list of all causes of death is in the appendix (p 10).

Discussion

In this multicentre, open-label, single-arm, phase 1–2 study, we examined the antitumour activity and safety of the third-generation TKI lorlatinib, which inhibits ALK and ROS1, in patients with advanced *ROS1*-positive NSCLC. Among TKI-naive patients, lorlatinib showed potent antitumour activity, with 62% of patients achieving a response and median progression-free survival of 21·0 months. Clinical activity was also observed among patients who were previously treated with crizotinib. Of note, lorlatinib showed marked intracranial activity, with an intracranial response in at least 50% of patients in both the TKI-naive and crizotinib-pretreated groups. In addition, lorlatinib was generally well tolerated, with asymptomatic laboratory abnormalities comprising the majority of grade 3–4 adverse events and only 1% of patients discontinuing from lorlatinib because of a treatment-related adverse event. No new safety signals were reported.

Currently, the first-generation multitargeted TKI crizotinib is the only approved treatment for patients with advanced *ROS1*-positive NSCLC. In an update to the registrational phase 1 PROFILE 1001 study, now with a median follow-up exceeding 5 years, treatment with crizotinib resulted in 72% of patients having an objective response and a median progression-free survival of 19·3 months,[26] similar to the activity of lorlatinib in TKI-naive patients. For both crizotinib and lorlatinib, durations of response were similarly long, with a median duration of response of 24·7 months for crizotinib and 25·3 months for lorlatinib. The second-generation ALK inhibitor ceritinib has also been studied in advanced *ROS1*-positive NSCLC. Among 30 Korean patients with crizotinib-naive disease, ceritinib induced responses in 67% of patients; median progression-free survival was 19·3 months and median duration of response was 21·0 months.[6] Similarly, in a pooled analysis of 53 *ROS1*-positive patients from three separate trials who were treated with the ROS1 and TRK inhibitor entrectinib, 77% of patients had responses, median progression-free survival was 19·0 months, and median duration of response was 24·6 months.[10] Although cross-trial comparisons are inherently limited, lorlatinib appears to have at least comparable clinical activity with other ROS1 inhibitors in the TKI-naive setting.

To our knowledge, lorlatinib is one of the first next-generation ROS1 inhibitors to report clinical activity in patients previously treated with crizotinib. Of the 69 *ROS1*-positive patients in this study, 40 had previously received crizotinib. Activity of lorlatinib was notably lower in crizotinib-pretreated patients than in TKI-naive patients, with 35% of patients achieving an objective response and median progression-free survival of 8·5 months. However, responses were durable, with a median duration of response of 13·8 months in crizotinib-pretreated patients. In addition, to eliminate the potential confounding effect of the intracranial activity of lorlatinib on response assessments, we showed that the proportion of patients with extracranial response was similar to the proportion with overall responses. This finding suggests that for about a third of patients, lorlatinib can overcome resistance to crizotinib. Of note, neither ceritinib nor entrectinib appear to be clinically active in crizotinib-resistant *ROS1*-positive NSCLC.[6,27] In a phase 1 study, repotrectinib, a rationally designed inhibitor of ROS1, TRK, and ALK, has shown preliminary antitumour activity, with response in 39% of 18 TKI-pretreated patients with *ROS1*-positive NSCLC; however, larger numbers and longer follow-up are required.[28] Thus, for patients currently relapsing on crizotinib, lorlatinib could represent an important therapeutic option and a potential alternative to cytotoxic chemotherapy.

In both TKI-naive and crizotinib-treated patients, lorlatinib showed robust intracranial activity, consistent with preclinical studies showing that lorlatinib is highly CNS-penetrant.[20,22,29] Brain metastases are found in 19–36% of *ROS1*-positive patients at diagnosis and are common at the time of relapse on crizotinib.[13,16,17] In this study, among patients with baseline measurable or non-measurable CNS disease treated with lorlatinib, the intracranial responses were achieved in 64% of TKI-naive patients and 50% of crizotinib-pretreated patients. Intracranial responses were durable, with median durations of intracranial response not reached at the time of data cutoff. Among patients with baseline CNS metastases, the cumulative incidence of CNS progression at 24 months was low for TKI-naive and crizotinib-pretreated patients. For TKI-naive patients with baseline CNS metastases, the cumulative incidences of extracranial and intracranial progression were similar for TKI-naive patients and crizotinib-pretreated patients at 24 months. By contrast, for patients with baseline CNS metastases and previous crizotinib treatment, the cumulative incidence of extracranial progression was higher than that of intracranial progression at 24 months, although this was not formally compared. These findings suggest an important role for lorlatinib in effectively treating and controlling CNS metastases in patients with *ROS1*-positive NSCLC, irrespective of previous crizotinib exposure.

One of the planned secondary objectives of this study was to evaluate potential biomarkers of response and resistance to lorlatinib. To accomplish this objective, we examined cfDNA and tumour tissue from *ROS1*-positive patients before initiating lorlatinib. As expected, no TKI-naive patients were found to have *ROS1* resistance mutations by plasma or tissue genotyping. By contrast, among patients who had received any previous ROS1 inhibitor, 15% had detectable *ROS1* mutations by plasma genotyping, and 24% of those with de-novo tumour biopsies had *ROS1* mutations by tissue genotyping. Two patients with *ROS1* mutations (Lys1991Glu and Ser1986Phe) achieved durable partial responses, suggesting potent activity of lorlatinib against these two *ROS1* mutants. However, for the most common *ROS1* resistance mutation, Gly2032Arg, no patients achieved an objective response, although some tumour shrinkage was observed in four of six patients. These findings suggest that, in contrast to ALK-positive NSCLC in which lorlatinib retains potency against all clinically identified *ALK* resistance mutations, including the analogous solvent front Gly1202Arg mutation,[30] in *ROS1*-positive NSCLC, lorlatinib loses potency in the presence of ROS1 Gly2032Arg. Because lorlatinib has modest activity against this common mutation, *ROS1* mutations as a class are unlikely to serve as a reliable biomarker of lorlatinib response in crizotinib-resistant, *ROS1*-positive NSCLC. Of note, the frequency of *ROS1* mutations detected in the plasma of resistant patients in this study was lower than previously reported (15% *vs* 33%).[31] This difference could be in part a result of the limitations of plasma genotyping and the small patient numbers included in both studies.

This study has several important limitations. Although this was one of the largest prospective studies of *ROS1*-positive NSCLC, the number of patients was still small, particularly in some subgroups defined by clinical or molecular characteristics. *ROS1* rearrangements are found in only 1–2% of patients with NSCLC, so recruiting large numbers of *ROS1*-positive patients can be challenging. Another limitation is that in the planned molecular analysis, approximately half of the tissue specimens from patients previously treated with ROS1 TKIs were archival rather than de-novo specimens. Archival specimens were primarily obtained at diagnosis, before initial TKI therapy, and would therefore probably not have had resistance alterations that would affect the subsequent response to lorlatinib. The inclusion of archival specimens might explain the lower than expected prevalence of secondary *ROS1* resistance mutations in previously treated patients based on tissue genotyping.[13] Plasma genotyping might also have underestimated the prevalence of *ROS1* resistance mutations,[31] because plasma assays have known limitations in terms of sensitivity, particularly in patients with low tumour burden or intrathoracic-only disease.[32,33] The small number of patients with *ROS1* mutations after previous treatment with a ROS1 TKI prevented analysis of the associations of specific mutations with clinical

response to lorlatinib. In addition, ROS1 fusion partners and breakpoints were not characterised in this study. Because numerous ROS1 fusions have now been described,[15] the small numbers of patients in this study would also have prevented analysis of the association between specific *ROS1* rearrangements and clinical outcome. Larger studies are needed to determine whether *ROS1* mutations, the specific type of *ROS1* rearrangement, or other genetic alterations could serve as predictors of response or resistance to lorlatinib.

In summary, in this phase 1–2 study, lorlatinib showed clinical activity in patients with advanced *ROS1*-positive NSCLC. Clinical activity was observed in both TKI-naive and crizotinib-pretreated patients, and in patients with and without brain metastases. Because patients relapsing on crizotinib have few treatment options, lorlatinib could represent an important next-line targeted agent, including for patients with CNS metastases.

Contributors

Data were collected by the investigators and analysed by the sponsor. All authors, including those employed by the funder of the study, contributed to interpretation of the data, and all authors contributed to the development and approval of the manuscript. All authors had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit the publication.

Declaration of interests

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Data sharing

Upon request, and subject to certain criteria, conditions, and exceptions (see https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual deidentified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines, and medical devices (1) for indications that have been approved in the USA and/or EU or (2) in programmes that have been terminated (ie, development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The deidentified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

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