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Biomarkers of extracellular matrix turnover in patients with idiopathic pulmonary fibrosis given nintedanib (INMARK study): a randomised, placebo-controlled study

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SUMMARY

Background A hallmark of idiopathic pulmonary fibrosis is the excess accumulation of extracellular matrix in the lungs. Degradation of extracellular matrix generates free-circulating protein fragments called neoepitopes. The aim of the INMARK trial was to investigate changes in neoepitopes as predictors of disease progression in patients with idiopathic pulmonary fibrosis and the effect of nintedanib on these biomarkers.

Methods In this randomised, double-blind, placebo-controlled trial, patients with a diagnosis of idiopathic pulmonary fibrosis within the past 3 years and forced vital capacity (FVC) of 80% predicted or higher were eligible to participate. Patients were recruited from hospitals, private practices, clinical research units, and academic medical centres. Patients were randomly assigned (1:2) with the use of a pseudo-random number generator to receive oral nintedanib 150 mg twice a day or placebo for 12 weeks in a double-blind fashion, followed by openlabel nintedanib for 40 weeks. The primary endpoint was the rate of change in C-reactive protein (CRP) degraded by matrix metalloproteinases 1 and 8 (CRPM) from baseline to week 12 in the intention-to-treat population. The trial has been completed and is registered with ClinicalTrials.gov, number NCT02788474, and with the European Clinical Trials Database, number 2015-003148-38.

Findings Between June 27, 2016, and May 15, 2017, 347 patients were randomly assigned to the nintedanib group (n=116) or to the placebo group (n=231). One patient from the placebo group was not treated owing to a randomisation error. At baseline, mean FVC was 97·5% (SD 13·5) predicted. In the double-blind period, 116 patients received nintedanib and 230 patients received placebo. The rate of change in CRPM from baseline to week 12 was -2·57 × 10^{-3} ng/mL/month in the nintedanib group and -1·90×10^{-3} ng/mL/month in the placebo group (between-group difference -0·66 × 10^{-3} ng/mL/month [95% CI -6·21 × 10^{-3} to 4·88 × 10^{-3}]; p=0·8146). The adjusted rate of change in FVC over 12 weeks was 5·9 mL in the nintedanib group and -70·2 mL in the placebo group (difference 76·1 mL/12 weeks [31·7 to 120·4]). In

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patients who received placebo for 12 weeks followed by open-label nintedanib, rising concentrations of CRPM over 12 weeks were associated with disease progression (absolute decline in FVC ≥10% predicted or death) over 52 weeks. In the double-blind period, serious adverse events were reported in eight (7%) patients given nintedanib and 18 (8%) patients given placebo. Grade 3 diarrhoea was reported in two (2%) patients in the nintedanib group and two (1%) patients in the placebo group. No patients had grade 4 diarrhoea.

Interpretation In patients with idiopathic pulmonary fibrosis and preserved lung function, treatment with nintedanib versus placebo for 12 weeks did not affect the rate of change in CRPM but was associated with a reduced rate of decline in FVC. These results suggest that change in CRPM is not a marker of response to nintedanib in patients with idiopathic pulmonary fibrosis.

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Introduction

Idiopathic pulmonary fibrosis is a progressive, fibrosing, interstitial lung disease characterised by decline in lung function and worsening dyspnoea.[1] It carries a poor prognosis, with a median post-diagnosis survival in untreated patients of approximately 3 years.[2] The rate at which idiopathic pulmonary fibrosis progresses varies among individuals and cannot accurately be predicted based on the available measures.[3,4] Thus, biomarkers need to be identified to predict disease progression early in the course of the disease, guide treatment decisions, and provide a measure of treatment response in individual patients with idiopathic pulmonary fibrosis.

In idiopathic pulmonary fibrosis, fibrosis is thought to arise as a result of repeated epithelial injury, coupled with aberrant wound healing and abnormal epithelial- mesenchymal interactions.[5-8] Fibroblasts migrate to the site of injury and differentiate into myofibroblasts, which secrete excessive amounts of extracellular matrix.[9]

RESEARCH IN CONTEXT

Evidence before this study

Degradation of the extracellular matrix by metalloproteinases generates free-circulating protein fragments known as neoepitopes. Data from the prospective observational PROFILE study showed that in patients with idiopathic pulmonary Abrosis$_1$ a higher rate of increase in concentrations of certain neoepitopes over 3 months was associated with worse survival, with CRPM showing the strongest association. We searched PubMed for all English-language papers published between Jan 1, 1990, and March 1, 2019, using the search terms "nintedanib" and "neoepitope" and no papers were found. To our knowledge, this is the first trial to investigate the predictive value of neoepitopes for disease progression in patients with idiopathic pulmonary fibrosis receiving antifibrotic therapy.

Added value of this study

The results of the INMARK trial showed that in patients with idiopathic pulmonary fibrosis and preserved lung volume, treatment with nintedanib versus placebo for 12 weeks did not affect the rate of change in biomarkers indicative of extracellular matrix turnover, including CRPM. However, in patients who received placebo for 12 weeks and then open-label nintedanib for 40 weeks, rising concentrations of CRPM over 12 weeks were associated with disease progression (defined as an absolute decline in FVC of 10% or more predicted or death) over 52 weeks. The rate of decline in FVC was lower in patients given nintedanib versus placebo over 12 weeks.

Implications of all the available evidence

Measuring circulating concentrations of neoepitopes over 12 weeks might have value in the prediction of disease progression in patients with idiopathic pulmonary fibrosis, but not in determining response to nintedanib. Change in FVC is a better marker of response to nintedanib than changes in blood biomarkers and shows a significant difference versus placebo after 12 weeks of treatment.

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The extracellular matrix is degraded by matrix metalloproteinases (MMPs), resulting in extracellular matrix turnover. When extracellular matrix or other proteins are degraded by MMPs, unique free-circulating protein fragments called neoepitopes are generated.[10,11] In the prospective, observational PROFILE study, serum concentrations of neoepitopes and other blood biomarkers were investigated as predictors of disease progression in patients with idiopathic pulmonary fibrosis who were naive to antifibrotic treatments.[12,13] Increased concentrations of six of 11 of the measured neoepitopes (biglycan degraded by MMP-2/9 [BGM], collagen 1 degraded by MMP-2/9/13 [C1M], collagen 3 degraded by ADAMTS-1/4/8 [C3A], collagen 3 degraded by MMP-9 [C3M], collagen 6 degraded by MMP-2/9 [C6M], and C-reactive protein (CRP) degraded by MMP- 1/8 [CRPM]) over 6 months were associated with disease progression, defined as an absolute decline in forced vital capacity (FVC) of 10% predicted or more or death at month 12. A higher rate of increase in six neoepitopes (BGM, C1M, C3M, collagen 5 degraded by MMP-2/9 [C5M], C6M, and CRPM) over 3 months was associated with worse survival. Furthermore, mortality was significantly higher in patients with increasing concentrations of C1M, C5M, C6M, and CRPM over 3 months than in those with stable or decreasing concentrations, with the strongest association shown with CRPM.[12]

Nintedanib is an approved treatment for idiopathic pulmonary fibrosis and slows disease progression by reducing the rate of decline in FVC.[14,15] Non-clinical studies have shown that nintedanib has antifibrotic effects that include effects on extracellular matrix deposition.[16-19] Currently, no biomarkers predict or indicate response to nintedanib in patients with idiopathic pulmonary fibrosis.

We did the INMARK trial to confirm the association between changes in neoepitopes and disease progression in individuals with idiopathic pulmonary fibrosis and to investigate whether treatment with nintedanib resulted in changes in these biomarkers.

Methods

STUDY DESIGN

The INMARK trial comprised a randomised, doubleblind, placebo-controlled period followed by an openlabel period in which all patients received nintedanib (appendix p 9). The trial design has been described.[20] Patients were enrolled at 78 sites in 13 countries (Australia, Belgium, Czech Republic, Finland, France, Germany, Hungary, Japan, Poland, South Korea, Spain, UK, USA). Institutional review board or independent ethics committee approval was obtained at each site before study initiation. The INMARK trial was done in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, and in accordance with the International Conference on Harmonisation Harmonised Tripartite Guideline for Good Clinical Practice.

PARTICIPANTS

Eligible patients were aged 40 years or older, with a diagnosis of idiopathic pulmonary fibrosis according to the 2011 American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, Latin American Thoracic Association (ATS/ERS/JRS/ALAT) guidelines[21] within 3 years, a high-resolution chest CT scan (performed within 18 months of screening) and surgical lung biopsy sample pattern (if available) consistent with a diagnosis of idiopathic

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pulmonary fibrosis, assessed by central review, and FVC of 80% predicted or higher. Eligibility was restricted to patients with FVC of 80% predicted or higher given the ethical considerations around doing a trial with a placebo- controlled period in patients with idiopathic pulmonary fibrosis. Patients with alanine aminotransferase (ALT), aspartate aminotransferase (AST), or total bilirubin concentrations of more than 1·5 × upper limit of normal (ULN) at screening (or any combination of these three), forced expiratory volume in 1 s:FVC ratio of less than 0·70, myocardial infarction within 6 months or unstable angina within 1 month of screening, bleeding risk, or a history of a thrombotic event within 12 months of screening were not eligible. All patients provided written informed consent before entering the trial.

RANDOMISATION AND MASKING

Following a screening period, patients were randomly assigned 1:2 to receive oral nintedanib 150 mg twice a day or placebo (appendix p 9). A 1:2 randomisation ratio was used to increase the power to assess the association between changes in serum biomarkers over 12 weeks and disease progression in placebo-treated patients. The study sponsor allocated patients via an interactive voice and web-based response system, using a pseudo-random number generator in block sizes of 6. Nintedanib and placebo were provided by the sponsor as capsules with identical appearance. Patients, investigators, and other personnel involved in the trial conduct and analysis were masked to treatment assignment until after database lock. The success of masking was not evaluated.

PROCEDURES

Nintedanib (Boehringer Ingelheim, Biberach, Germany) was supplied as soft gelatine capsules to be taken orally. Patients were to take nintedanib 150 mg twice a day or placebo double-blind for 12 weeks, followed by openlabel nintedanib for 40 weeks (appendix p 9). Treatment interruptions and dose reductions to nintedanib 100 mg twice a day were recommended to manage adverse events. After resolution of the adverse event, nintedanib could be reintroduced or re-escalated to 150 mg twice a day at the discretion of the investigator, or both.

Patients were to attend clinic visits at screening, randomisation, at weeks 4, 8, 12, 16, 20, 24, 36, and 52, and a follow-up visit 4 weeks later. Patients who prematurely discontinued trial drug were asked to attend all visits as planned. Blood samples for biomarker analysis were collected at baseline and at each visit up to week 52. Collection was standardised across centres. Blood was collected with anticoagulant-free, gelcontaining serum separation tubes (Becton Dickenson, Oxford, UK). Samples were allowed to clot at room temperature for approximately 60 min. Serum was separated by centrifugation and aliquoted before freezing. Samples were shipped to a central laboratory for storage, and later shipped in batches to the sponsor or a contractor of the sponsor for analysis. Serum concentrations of CRPM, C1M, and C3M, were measured with the use of an ELISA method as previously described.[22] All samples for CRPM and most samples for C3M and C1M taken during the randomised treatment period were analysed in the same batch. In-clinic spirometry was done at each visit, in accordance with ATS/ERS guidelines.[23] Spirometry devices were supplied to all participating centres and the results were centrally reviewed.

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OUTCOMES

The primary endpoint was the rate of change (slope) in CRPM from baseline to week 12 (expressed in ng/mL/month). The key secondary endpoint was the proportion of patients with disease progression, defined as an absolute decline in FVC of 10% predicted or more or death, over 52 weeks. Other secondary endpoints were the rates of change in serum C1M and C3M concentrations from baseline to week 12. Other prespecified endpoints included the rate of decline in FVC (mL/year), absolute changes from baseline in FVC (in mL and % predicted) at week 52, time to decline in FVC of 5% predicted or more and 10% predicted or more over 52 weeks, time to decline in FVC of 5% predicted or more or death, time to decline in FVC of 10% predicted or more or death over 52 weeks, and time to first investigator-reported acute exacerbation over 52 weeks. Acute exacerbations were defined as in the INPULSIS trials[15] and were not adjudicated. The rate of change in serum CRP from baseline to week 12 was assessed as a prespecified exploratory endpoint.

Safety was assessed via the recording of adverse events with onset after the first dose and up to 28 days after the last dose of study drug and measurement of laboratory parameters. Adverse events were coded with the use of the Medical Dictionary for Regulatory Activities version 21.0. Patients met criteria for Hy's Law if they had ALT or AST concentrations of 3 × ULN or higher and bilirubin concentrations of 2 × ULN or higher and no reason was found to explain the combination of increased hepatic transaminases and bilirubin concentrations.

STATISTICAL ANALYSIS

Based on two-sided tests (α=0·05), we calculated that a sample size of 300 would provide 90% power to detect a relative difference between groups of 20%, assuming a SD of 50% on the primary endpoint, based on a 1:2 randomisation between nintedanib and placebo. Based on data from the INPULSIS trials,[15] we expected that 40% of patients in the placebo group would have disease progression over 52 weeks. We calculated a sample size of 233 patients in the placebo group would provide 88% power to detect a difference in the proportion of patients with disease progression over 52 weeks of 20%. Therefore, we planned a sample size of 350 patients (233 randomly assigned to placebo, 117 to nintedanib).

In patients who received one or more doses of trial medication, we analysed the rate of change in serum CRPM concentration from baseline to week 12 using a random coefficient regression model (with random slopes and intercepts), including treatment-by-time, sex, age, and height as covariates. Missing data were not imputed. We analysed rates of change in C3M, C1M, and CRP concentrations using the same model. For the analysis of the rate of change in biomarkers from baseline to week 12, we included baseline in the response variable, together with the timepoints at week 4, 8, and 12, rather than as an adjusting covariate, to increase the amount of data for the estimation of each endpoint. Biomarker data were not normally distributed; CRPM, C3M, and CRP data required log10 transformation and C1M data required negative reciprocal root transformation before analysis. We report on analyses related to changes in CRPM concentrations and disease progression over 52 weeks (appendix p 2).

We analysed the rate of change in FVC over 12 weeks using a random coefficient regression, including treatment-by-time, sex, age, and height as covariates. We analysed the annual rate of change in FVC, assessed over 52 weeks, using a random coefficient piecewise regression with treatment-by-time, sex, age, and height as covariates and piecewise knot at week 12. We used

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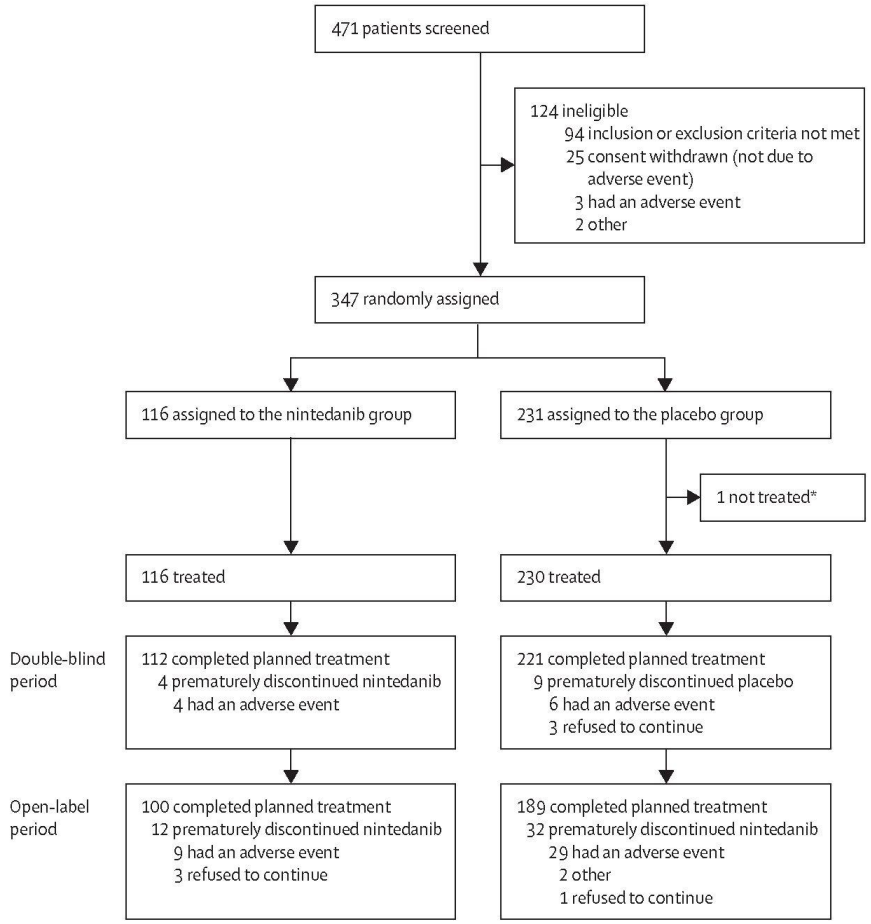
the Kenward-Roger approximation to estimate denominators degrees of freedom in all the random coefficient regression models. We compared the time to an absolute decline in FVC of 5% predicted or more or 10% predicted or more between treatment groups using a Cox regression model with terms for treatment, sex, age, and height. All the analyses presented in this manuscript were prespecified. Analyses were performed using SAS version 9.4. Data on absolute changes from baseline in FVC (in mL and % predicted), acute exacerbations, and safety are presented descriptively.

The INMARK trial was registered with ClinicalTrials. gov (NCT02788474) and the European Clinical Trials Database (EudraCT 2015-003148-38).

ROLE OF THE FUNDING SOURCE

The sponsor participated in the study design, data collection, statistical analyses, data interpretation, and the writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Figure 1: Trial profile



*Owing to a randomisation error.

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Results

Between June 27, 2016, and May 15, 2017, 471 patients were screened, of whom 124 were ineligible and 347 were randomly assigned. Reasons for exclusion are shown in figure 1. One patient assigned to placebo did not receive treatment owing to a randomisation error. A total of 346 patients (116 in the nintedanib group, 230 in the placebo group) were treated in the double-blind period and assessed for the primary endpoint (figure 1).

Most patients were men (n=262; 76%), white (n=214; 62%) and ex-smokers (n=238; 69%). At baseline, mean FVC was 97·5% (SD 13·5) predicted, and diffusing capacity of the lung for carbon monoxide (corrected for haemoglobin) was 64·0% (19·8) predicted (table 1). Median exposure to nintedanib or placebo in the randomly assigned double-blind period was 12 weeks (appendix p 3). Median exposure to study medication over the whole treatment period was 52 weeks (appendix p 3). During the double-blind treatment period, 14 (12%) patients in the nintedanib group and ten (4%) patients in the placebo group had one or more dose reductions, and ten (9%) patients in the nintedanib group and ten (4%) patients in the placebo group had one or more treatment interruptions.

Information on rate of change in CRPM was not available for one patient in the placebo group. Assay performance characteristics are summarised in the appendix (p 4).

We found no significant difference between nintedanib and placebo in the adjusted rate of change in serum CRPM concentrations from baseline to week 12 (between- group difference -0·66 × 10^{-3} ng/mL/month [95% CI -6·21 × 10^{-3} to 4·88 × 10^{-3}]; p=0·8146; figure 2). Adjusted mean serum CRPM concentrations over 52 weeks (corrected for batch effects) are presented in the appendix (p 10). We found no significant differences between the nintedanib and placebo groups in the adjusted rates of change in serum C1M or C3M concentrations from baseline to week 12 (table 2). The adjusted rate of change in serum CRP concentration from baseline to week 12 was significantly higher in the nintedanib group than in the placebo group (table 2). The median absolute change from baseline in CRP concentration at week 52 was 0·48 mg/L (range -17·5 to 63·9) in the nintedanib group and 0·24 mg/L (-98·7 to 104·8) in patients treated with placebo for 12 weeks, followed by nintedanib for 40 weeks. Adjusted mean serum CRP concentrations over 52 weeks are presented in the appendix (p 11). The number of patients who provided CRP concentrations, and the median, minimum, and maximum values, at each visit are shown in the appendix (p 5).

The adjusted rate of change in FVC over 12 weeks was 5·9 mL/12 weeks (SE 18·5) in the nintedanib group and-70·2 mL/12 weeks (13·1) in the placebo group (difference 76 ·1 mL/12 weeks [95% CI 31·7 to 120·4]; p=0·0008; figure 3). The adjusted rate of change in FVC over 52 weeks was -88·8 mL/year (SE 23·9) in the nintedanib group and -104·1 mL/year (17·0) in patients treated with placebo for 12 weeks, followed by nintedanib for 40 weeks (difference 15·3 mL/year [95% CI -42·5 to 73·0]; p=0·60). Over 52 weeks, disease progression was observed in 29 (25%) patients in the nintedanib group and 70 (30%) patients treated with placebo for 12 weeks, followed by nintedanib for 40 weeks (odds ratio [OR] 0·77 [95% CI 0·46 to 1·27]; p=0·3116; figure 4). Over 52 weeks, 29 (25%) patients in the nintedanib group and 67 (29%) patients treated with placebo for 12 weeks, followed by nintedanib for 40 weeks, had an absolute decline in FVC of 10% or more predicted (hazard ratio [HR] 0·87 [95% CI 0·56 to 1·35]; p=0·44); 65 (56%) and 132 (57%) patients had an absolute decline in FVC of 5% or more predicted (HR 0·92 [0·69 to 1·25]; p=0·48). Mean absolute changes from baseline in FVC (mL

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and % predicted) over 52 weeks are presented in the appendix (pp 12-13). Time to absolute decline in FVC of 10% predicted or more or death and time to absolute decline in FVC of 5% predicted or more or death are presented in the appendix (pp 14-15). In total, eight patients had an acute exacerbation over the 52-week study period. One patient in the nintedanib group had an acute exacerbation during the double-blind period and seven patients who initially received placebo had an acute exacerbation during the nintedanib open-label period.

Among patients who received placebo for 12 weeks, 32 (36%) of 89 patients with rising serum CRPM concentrations over 12 weeks versus 37 (26%) of 140 patients with stable or decreasing serum CRPM concentrations over 12 weeks had disease progression over 52 weeks (appendix p 6). Baseline characteristics were similar in the subgroups of patients with rising and stable or decreasing CRPM concentrations (appendix p 7). Although rising versus stable or decreasing serum CRPM concentration over 12 weeks was significantly associated with disease progression over 52 weeks in the placebo group (OR 1·87 [95% CI 1·02 to 3·44]; p=0·04), no significant association was found in the placebo group between rate of change in serum CRPM concentration over 12 weeks and disease progression over 52 weeks (p=0·21). Furthermore, treatment with nintedanib did not have a significant effect on the association between the rate of change in serum CRPM concentration over 12 weeks and disease progression over 52 weeks (interaction estimate -45·57 [95% CI -109·55 to 16·37]; p=0·15) or on the association between rising versus stable or decreasing CRPM concentrations over 12 weeks and disease progression over 52 weeks (interaction estimate -0·80 [-1·90 to 0·26]; p=0·14; appendix p 6). Adjustment for the rate of change in serum CRPM concentration over 12 weeks did not influence the effect of nintedanib versus placebo on disease progression over 52 weeks (OR 0·77 [95% CI 0·46 to 1·27]; p=0·32).

Adverse events reported in the nintedanib and placebo groups in the randomised double-blind period are shown in table 3. Diarrhoea was the most frequent adverse event in patients in the nintedanib group (54 [47%] *vs* 42 [18%] patients in the placebo group; table 3). Grade 3 diarrhoea adverse events were reported in two (2%) patients in the nintedanib group and two (1%) patients in the placebo group in the double-blind period. No grade 4 diarrhoea adverse events were recorded. No patients had ALT or AST (or both) concentrations of 3 × ULN or higher and bilirubin concentration of 2 × ULN or higher in the randomised double-blind period (appendix p 8). One patient who initially received placebo fulfilled criteria for Hy's Law during the nintedanib open-label period; following treatment discontinuation, ALT and AST concentrations returned to normal.

Table 1: Baseline characteristics of patients in the INMARK trial

Data are mean (SD) or n (%). DLco=diffusing capacity of the lung for carbon monoxide. CRPM=C-reactive protein degraded by matrix metalloproteinases 1 and 8. CRP=C-reactive protein. C1M=collagen 1 degraded by matrix metalloproteinases 2, 9, and 13. C3M=collagen 3 degraded by matrix metalloproteinase 9. FVC=forced vital capacity. FEV_1=forced expiratory volume in 1 s. *Data on race were not collected in France due to local regulation. †orrected for haemoglobin. ‡n=111 for nintedanib and n=221 for placebo. §n=228 for placebo. ¶n=109 for nintedanib and n=221 for placebo. ||n=227 for placebo.

	Nintedanib group (n=116)	Placebo group (n=230)
Age, years	70·5 (7·7)	70·2 (7·2)
Sex		
Men	93 (80%)	169 (73%)
Women	23 (20%)	61 (27%)
Weight, kg	79·1 (16·5)	76·8 (15·7)

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Body-massindex, kg/m²	27·7 (4·3)	27·2 (4·1)
Race		
White	70 (60%)	144 (63%)
Asian	35 (30%)	68 (30%)
Missing*	11 (9%)	18 (8%)
Time since diagnosis of idiopathic pulmonary fibrosis, years	0·8 (0·8)	0·9 (1·0)
Smoking status		
Never	31 (27%)	63 (27%)
Former	80 (69%)	158 (69%)
Current	5 (4%)	9 (4%)
FVC, mL	3270 (848)	3227 (795)
FVC, % predicted	96·6 (15·2)	98·0 (12·6)
FEV_1:FVC ratio, %	79·3 (7·0)	79·6 (6·0)
DLco, % predicted†‡	60·9 (16·6)	65·5 (21·2)
CRPM, ng/mL§	12·0 (6·9)	12·2 (10·2)
CRP, mg/L¶	3·6 (6·2)	3·7 (8·2)
C1M, ng/mL||	34·3 (29·0)	32·7 (20·3)
C3M, ng/mL§	12·1 (3·1)	12·7 (4·0)

Figure 2: **Rate of change in serum CRPM concentration from baseline to week 12**
Data based on log10 transformed values, presented with SE. CRPM=C-reactive protein degraded by matrix metalloproteinases 1 and 8.

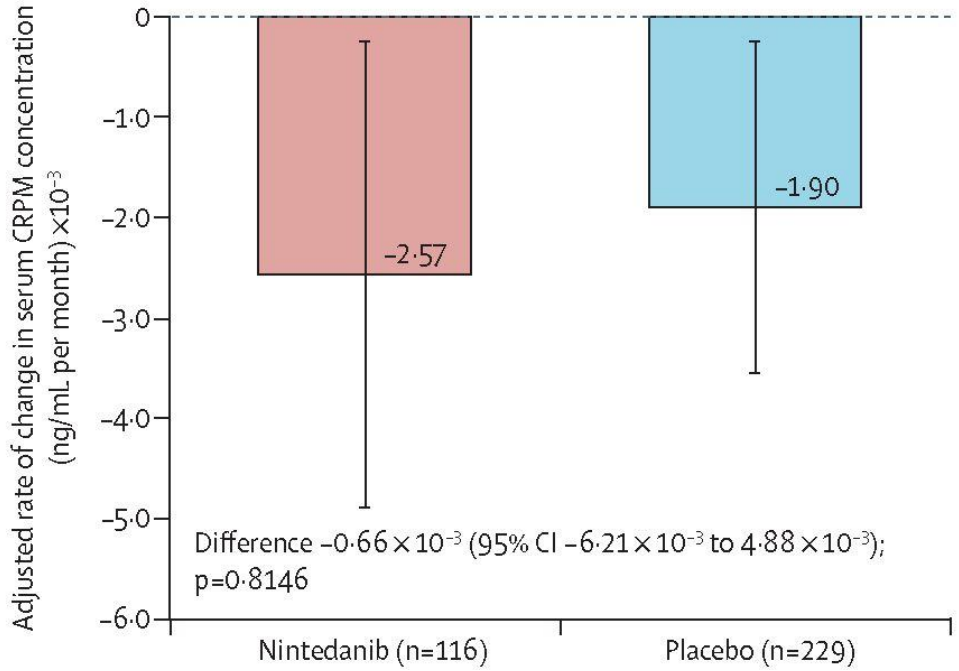

Table2: **Rates of change in C1M, C3M, and CRP from baseline to week 12**
Data are adjusted rates (SE), unless otherwise specified. Rates of change in C3M and CRP were based on log10 transformed values; rates of change in C1M were based on negative reciprocal root transformed values. CRP=C-reactive protein. C1M=collagen 1 degraded by matrix metalloproteinases 2, 9, and 13. C3M=collagen 3 degraded by matrix metalloproteinase 9. *n=227.

	Nintedanib group (n=116)	Placebo group (n=229)	Difference (95% CI)	p
Adjusted rate of change in serum C1M from baseline to week 12 (× 10^{-3} ng/mL/month)	1·62 (1·72)	0·41 (1·27)	1·21 (-2·73 to 5·15)	
Adjusted rate of change in serum C3M from	-3·98 (2·19)	-0·91 (1·58)	-3·07	

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baseline to week 12 (× 10^{-3} ng/mL/month)			(-8·23 to 2·09)	
Adjusted rate of change in serum CRP from baseline to week 12 (× 10^{-3} mg/L/month)	40·60 (13·04)	-6·26 (9·55)*	46·85 (16·74 to 76·97)	0·

Figure 3: **Rate of change in FVC over 12 weeks**
Data presented with SE. FVC=forced vital capacity.

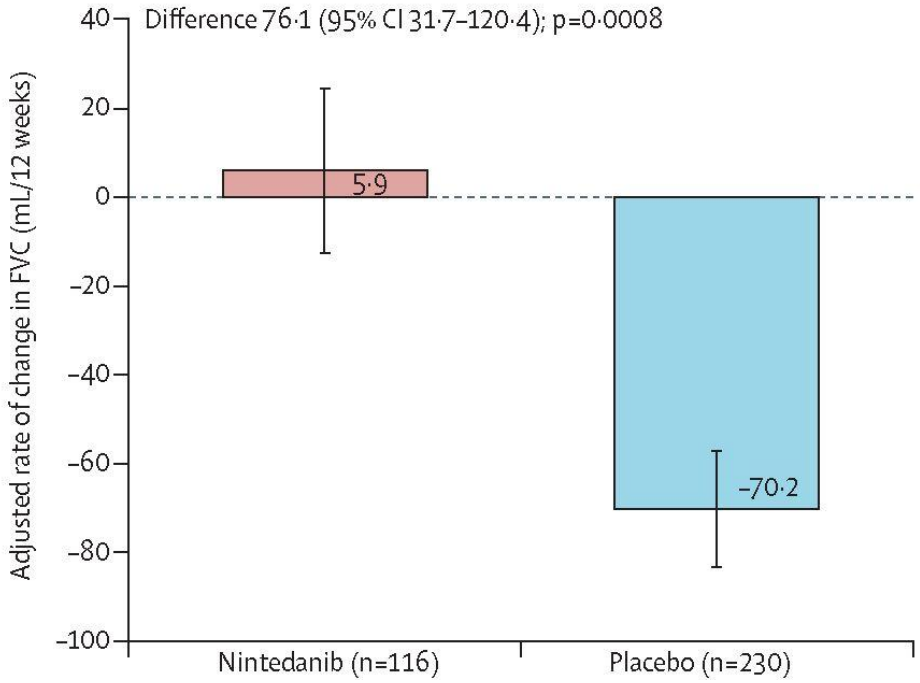

Figure4: **Proportion of patients with disease progression (absolute decline in FVC of 10% or more predicted or death) over 52 weeks**

FVC=forced vital capacity. *Patients received placebo (blinded) for 12 weeks followed by nintedanib (open label) for 40 weeks.

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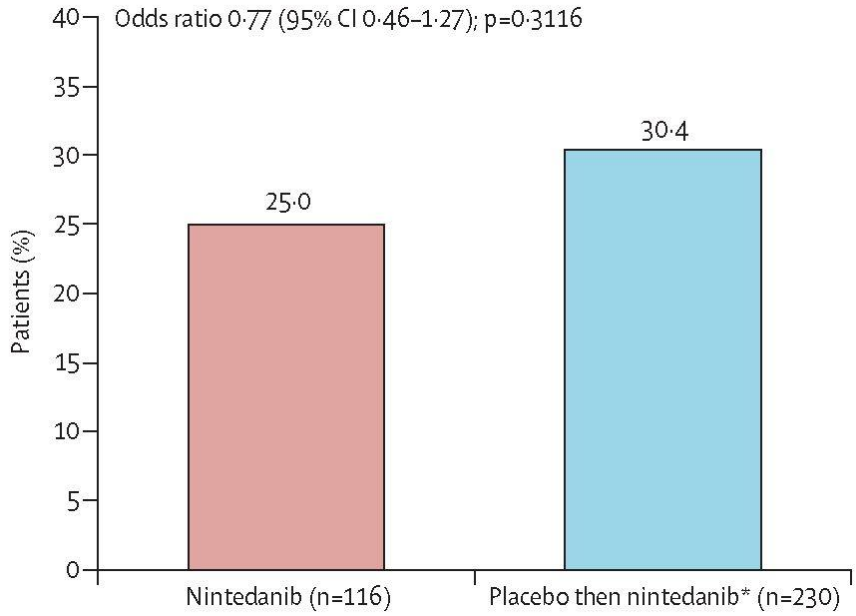

Discussion

To our knowledge, the INMARK trial is the first study to investigate the predictive value of serum biomarkers for disease progression in patients with idiopathic pulmonary fibrosis taking antifibrotic therapy. Treatment with nintedanib versus placebo for 12 weeks did not affect the rate of change in CRPM concentration, a marker of extracellular matrix turnover. Similarly, over 12 weeks there were no significant differences between nintedanib and placebo in the rates of change in C1M or C3M concentrations. Over 12 weeks, there was a significant difference in the rate of FVC decline in individuals in the nintedanib group compared with the placebo group (5·9 *vs* -70·2 mL/12 weeks), suggesting that change in FVC, an established measure of disease progression in patients with idiopathic pulmonary fibrosis,[24] is a better biomarker of response to nintedanib than change in CRPM concentration. A significant difference in FVC between the nintedanib and placebo groups was also observed at week 12 in post-hoc analyses of the INPULSIS trials.[25] These findings support the feasibility of assessing the efficacy of antifibrotic drugs based on change in FVC in studies of much shorter duration than have traditionally been done, with potentially important implications for accelerating drug development. Interestingly we observed a small increase in mean FVC over 12 weeks in patients in the nintedanib group. A post-hoc analysis of data from the INPULSIS trials showed that based on analyses of the annual rate of decline in FVC (mL/year), 24·8% of patients in the nintedanib and 9·0% of patients in the placebo group had an improvement or no decline in FVC over 52 weeks.[26] In these patients, median improvements in FVC at week 52 were 76·5 mL and 57·5 mL in the nintedanib and placebo group, respectively. The mechanisms by which nintedanib might lead to an improvement in FVC are not understood, but it has been hypothesised that its effects on lung fibrosis, vascular proliferation, or restoration of alveolar structures might contribute to an improvement in lung function.[26]

Similar rates of FVC decline over 52 weeks were observed in patients in the nintedanib group as in those who received placebo for 12 weeks and then nintedanib for 40 weeks, but the 12-week delay in initiation of therapy did not appear to be fully compensated for over the 52-week

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period. In clinical practice, the unpredictable nature of idiopathic pulmonary fibrosis and the fact that loss of lung function is irreversible and predictive of mortality argue that prompt treatment of idiopathic pulmonary fibrosis might be the best course of action for individual patients.[2,27,28] It is noteworthy that despite having very well-preserved lung volume at baseline (mean FVC 97·5% predicted), almost a third of the patients in this trial had an FVC decline of 10% predicted or more or died over 52 weeks of follow-up. The rate of decline in FVC over 52 weeks in patients in the nintedanib group was consistent with that observed in other trials.[14,15,29] However, the proportion of patients with disease progression and the frequencies of acute exacerbations and deaths were lower in this trial in patients with preserved lung volume than in other trials.[14,15,29] The adverse event profile of nintedanib was consistent with that observed in previous studies,[14,15,29] with diarrhoea being the most frequent adverse event.

In patients with idiopathic pulmonary fibrosis and preserved FVC who received placebo for 12 weeks and then open-label nintedanib, rising concentrations of the neoepitope CRPM over 12 weeks were associated with disease progression over 52 weeks. This observation is consistent with the findings of the PROFILE study,[12] which was done in patients who had greater impairment in FVC but who were not taking antifibrotic therapy. Over 12 weeks of treatment, there was a slight increase in CRP concentration in the nintedanib group, compared with stable concentrations of CRP in the placebo group. The mechanisms underlying the increase in CRP concentrations in individuals given nintedanib remain speculative. Liver enzyme elevations were rare in patients given nintedanib in this study, suggesting that the increase in CRP concentration did not reflect clinically relevant toxicity in the liver. CRP is a non-specific marker of a wide range of acute and chronic inflammatory conditions including infections, inflammatory diseases and malignancies, and tissue injury.[20] Little is known about the behaviour and relevance of CRP concentrations in patients with idiopathic pulmonary fibrosis; however, the CRP concentrations observed in our study appear to be low compared with those observed in patients with idiopathic pulmonary fibrosis identified in a populationbased study.[21] CRPM is detected following degradation of CRP by MMPs 1 and 8,[22] therefore an increase in the substrate available for MMPs could potentially have influenced the change in CRPM observed in the nintedanib group in our trial.

Limitations of our analyses include the use of a selected patient population with well-preserved lung volume, in whom the pathobiology of extracellular matrix turnover might be different to that in patients with more advanced disease. There were also batch differences for CRPM and C1M that affected values after week 12 (but which did not affect analyses of the primary and key secondary endpoints). The power calculation for the key secondary endpoint was based on the assumption that 40% of patients who initially received placebo would have disease progression over 52 weeks. However, in this trial only 30% of patients randomly assigned to placebo had disease progression over 52 weeks, which might have reduced the power to show a difference between the nintedanib and placebo groups on this endpoint. It should also be noted that disease progression over 52 weeks in the placebo group was analysed in patients who received placebo for 12 weeks followed by nintedanib for 40 weeks.

In conclusion, in patients with idiopathic pulmonary fibrosis and preserved lung volume, treatment with nintedanib for 12 weeks did not affect the rate of change in biomarkers indicative of extracellular matrix turnover compared with placebo. However, a difference in change in FVC was evident between the nintedanib and placebo groups at week 12. Further analyses of biomarkers reflective of epithelial damage, inflammation, and extracellular matrix turnover are ongoing.

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Table 3: **Adverse events in the randomised double-blind period**

Data are n (%) of patients with one or more such event. ALT=alanine aminotransferase. AST=aspartate aminotransferase. *Adverse events reported in more than 5% of patients in either treatment group are shown. †An adverse event that was incapacitating or caused inability to work or to perform usual activities. ‡An adverse event that resulted in death, was life-threatening, required admission to hospital or prolongation of hospitalisation, resulted in persistent or substantial disability or incapacity, was a congenital anomaly or birth defect, or was deemed serious for any other reason. §Three (3%) patients in the nintedanib group and six (3%) patients in the placebo and nintedanib group had fatal adverse events over the whole trial.

	Nintedanib group (n=116)	Placebo group (n=230)
Adverse event(s)	94 (81%)	148 (64%)
Most frequent adverse events*		
Diarrhoea	54 (47%)	42 (18%)
Nausea	17 (15%)	15 (7%)
Decreased appetite	12 (10%)	10 (4%)
Nasopharyngitis	10 (9%)	19 (8%)
Vomiting	9 (8%)	7 (3%)
Headache	7 (6%)	7 (3%)
ALT concentration increased	7 (6%)	2 (1%)
Abdominal pain	6 (5%)	8 (3%)
AST concentration increased	6 (5%)	4 (2%)
Constipation	6 (5%)	3 (1%)
Weight decreased	6 (5%)	2 (1%)
Cough	5 (4%)	16 (7%)
Severe adverse event(s)↑	5 (4%)	7 (3%)
Serious adverse event(s)Φ	8 (7%)	18 (8%)
Required admission to hospital	5 (4%)	13 (6%)
Fatal adverse events§	0	0

Contributors

All authors were involved in the interpretation of the data and in the writing and critical review of the manuscript.

Declaration of interests

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Data sharing

Information on data sharing is provided in the appendix (p 16).

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