

Ziritaxestat, a Novel Autotaxin Inhibitor, and Lung Function in Idiopathic Pulmonary Fibrosis The ISABELA 1 and 2 Randomized Clinical Trials

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1 **1. Title page**

2 **Effect of Ziritaxestat, a Novel Autotaxin Inhibitor, vs Placebo on**
3 **Lung Function in Idiopathic Pulmonary Fibrosis: The ISABELA 1**
4 **and 2 Randomized Clinical Trials**

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54 **Key Points**

55 **Question:** Does the autotaxin inhibitor ziritaxestat improve outcomes, compared with
56 placebo, in patients with idiopathic pulmonary fibrosis (IPF) who continue to receive standard
57 care (with pirfenidone, nintedanib or neither)?

58 **Findings:** In two clinical trials, ISABELA 1 and 2, no reduction in the 52-week rate of decline
59 in forced vital capacity (a measure of lung function) was seen in the ziritaxestat groups
60 versus placebo, and combined data from both studies showed all-cause mortality rates were
61 numerically higher for ziritaxestat than placebo. Therefore, the trials were stopped early.

62 **Meaning:** The autotaxin inhibitor ziritaxestat was ineffective as a treatment for IPF.

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66 **2. Abstract**

67 **Importance:** There is a major unmet need for effective, well-tolerated treatments for
68 idiopathic pulmonary fibrosis (IPF).

69 **Objective:** To assess the efficacy and safety of the autotaxin inhibitor ziritaxestat in patients
70 with IPF.

71 **Design, Setting and Participants:** ISABELA 1 and 2 were two identically designed,
72 randomized, double-blind, placebo-controlled phase 3, global studies, conducted in Europe,
73 the Middle East and Africa, Latin America, North America and Asia-Pacific. A total of 1306
74 patients with IPF were randomized: 525 patients at 106 sites in ISABELA 1 and 781 patients
75 at 121 sites in ISABELA 2. In ISABELA 1 and 2, respectively, enrollment began on 28
76 November, 2018 and 05 November, 2018 and follow-up was completed early due to study
77 termination on 12 April, 2021 and 30 March 2021.

78 **Interventions:** Patients were randomized 1:1:1 to receive oral ziritaxestat 600 mg,
79 ziritaxestat 200 mg or placebo once daily, on top of local standard of care (pirfenidone,
80 nintedanib or neither) for at least 52 weeks.

81 **Main Outcomes and Measures:** The primary endpoint was annual rate of decline of forced
82 vital capacity (FVC). Key secondary endpoints were disease progression, time to first
83 respiratory-related hospitalization and change from baseline in St George's Respiratory
84 Questionnaire total score (which ranged from 0 to 100, with higher scores indicating poorer
85 health-related quality of life). Other secondary endpoints included time to respiratory-related
86 mortality, time to all-cause mortality or respiratory-related hospitalization, time to first acute
87 IPF exacerbation, change from baseline in FVC, 6-minute walk test distance and safety and
88 tolerability.

89 **Results:** At the time of study termination, 525 and 781 patients were randomized, in
90 ISABELA 1 and 2 respectively (mean [SD] age: 70.0 [7.2] and 69.8 [7.1] years; male: 82.4%
91 and 81.2%, respectively). The trials were terminated early after an independent data

Commented [OGT1]: "Time to exacerbation" is not included here, but it is included in the "Other secondary outcomes" section of the text.

92 monitoring committee concluded that the benefit–risk profile of ziritaxestat no longer
93 supported their continuation. Ziritaxestat did not improve the annual rate of FVC decline
94 versus placebo in either study: in ISABELA 1 the least-squares mean (95% CI) annual rate
95 of FVC decline was –124.6 mL (–178.0, –71.2) with ziritaxestat 600 mg vs –147.3 mL
96 (–199.8, –94.7) with placebo (difference [95% CI]: 22.7 [–52.3, 97.6]), and –173.9 mL
97 (–225.7, –122.2) with ziritaxestat 200 mg (difference vs placebo [95% CI]: –26.7 [–100.5,
98 47.1]). In ISABELA 2 the least-squares mean (95% CI) annual rate of FVC decline was–
99 173.8 mL (–209.2, –138.4) with ziritaxestat 600 mg vs –176.6 mL (–211.4, –141.8) with
100 placebo (difference [95% CI] 2.8 [–46.9, 52.4]) and –174.9 mL (–209.5, –140.2) with
101 ziritaxestat 200 mg (difference vs placebo [95% CI] 1.7 [–47.4, 50.8]). There was no benefit
102 with ziritaxestat versus placebo for key secondary outcomes. In ISABELA 1, all-cause
103 mortality was 8.0% and 4.6% with ziritaxestat 600 mg and 200 mg, respectively, and 6.3%
104 with placebo (hazard ratio [95% CI] vs placebo: 1.4 [0.6, 3.1] and 0.7 [0.3, 1.8]); in ISABELA
105 2, it was 9.3% and 8.5% with ziritaxestat 600 mg and 200 mg, respectively, and 4.7% with
106 placebo (hazard ratio [95% CI] vs placebo: 2.1 [1.1, 4.3] and 1.9 [0.9, 3.8]).

107 **Conclusions and Relevance:** Ziritaxestat did not improve clinical outcomes compared with
108 placebo in patients with IPF receiving standard of care treatment with pirfenidone,
109 nintedanib, or neither.

110 **Trial Registration:** ClinicalTrials.gov Identifiers: [NCT03711162](https://clinicaltrials.gov/ct2/show/study/NCT03711162); [NCT03733444](https://clinicaltrials.gov/ct2/show/study/NCT03733444).

111

112 **3. Introduction**

113 Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease associated with progressive and
114 irreversible fibrosis, dyspnea, lung function decline and loss of quality of life.^{1,2} Median
115 survival without treatment is approximately 3 years, with respiratory failure being the most
116 frequent cause of death.³ While treatment with pirfenidone or nintedanib slows disease
117 progression, patients continue to suffer loss of lung function and premature death.⁴
118 Furthermore, in a substantial proportion of patients, pirfenidone and nintedanib are
119 associated with adverse effects, which may lead to treatment discontinuation.⁵ Thus, there
120 remains a major unmet need for more effective, better tolerated IPF treatments.

121 Pulmonary fibrosis in IPF is believed to develop when aberrant responses to lung injury,
122 including epithelial apoptosis and fibroblast recruitment, occur.⁶⁻⁸ Lysophosphatidic acid
123 (LPA) is thought to be at least partially responsible for mediating such responses.^{6,7}
124 Autotaxin, an enzyme involved in the production of LPA,⁹ is upregulated in patients with IPF
125 and is therefore a potential target for novel IPF therapies.^{6,10} Ziritaxestat (GLPG1690) is a
126 small-molecule, selective autotaxin inhibitor,¹¹⁻¹⁵ which showed promising results in a phase
127 2a study conducted in 23 patients with IPF.¹⁶ Ziritaxestat was well tolerated and those
128 treated with ziritaxestat demonstrated a smaller mean change from baseline in forced vital
129 capacity (FVC) at Week 12 versus placebo. Further, ziritaxestat reduced the concentration of
130 plasma LPA, with a maximum reduction from baseline of approximately 90%, confirming
131 target engagement.¹⁶

132 To further evaluate the efficacy and safety of ziritaxestat for the treatment of IPF, ISABELA 1
133 and 2 (NCT03711162; NCT03733444), two identically designed phase 3 IPF studies were
134 subsequently conducted.

135

136 **4. Methods**

137 **4.1. Study design and eligibility criteria**

138 ISABELA 1 and 2 were randomized, double-blind, placebo-controlled, global studies (eFigure
139 1; see Supplement for protocol and statistical analysis plan).¹⁷ Patients were recruited from
140 pulmonary clinics; patients were recruited from 106 sites in 14 countries in ISABELA 1 and
141 from 121 sites in 15 countries in ISABELA 2. Eligible men or women were aged ≥ 40 years
142 and had been diagnosed with IPF within the previous 5 years. IPF diagnosis was confirmed
143 through central review of a high-resolution computed tomography scan of the chest
144 performed within the 12 months prior to screening and lung biopsy (if available). At the time
145 of enrollment, patients were receiving treatment with local standard of care (SoC; a stable
146 dose of pirfenidone or nintedanib for at least 2 months prior to screening, or neither therapy).
147 Eligible patients had to be able to walk ≥ 150 m in the 6-minute walk test at screening visit 1.
148 At visit 2, resting arterial oxygen saturation (SpO_2) had to be $\geq 88\%$ with a maximum of 6 L
149 O_2 /min for the oxygen titration test; during the walk, SpO_2 had to be $\geq 83\%$ with 6 L O_2 /min or
150 $\geq 88\%$ with 0, 2, or 4 L O_2 /min. Additional inclusion criteria included FVC $\geq 45\%$ predicted of
151 normal, forced expiratory volume in 1 second (FEV_1)/FVC ≥ 0.7 , and diffusing capacity of the
152 lung for carbon monoxide (DLCO) corrected for hemoglobin $\geq 30\%$ predicted of normal.
153 Exclusion criteria included immunosuppressive conditions (e.g. HIV, congenital, acquired or
154 medication induced); hepatitis B or C positive serology; malignancy within the past 5 years
155 (except for carcinoma in situ of the uterine cervix, basal carcinoma of the skin that shows no
156 evidence of recurrence following treatment, prostate cancer that has been medically
157 managed with active surveillance or watchful waiting, squamous cell carcinoma of the skin if
158 fully resected, and Ductal Carcinoma in situ); certain medications (e.g., strong inducers or
159 inhibitors of CYP3A4, and potent inducers or inhibitors of P-gp); acute IPF exacerbation
160 within 6 months; lower respiratory tract infection requiring antibiotics within 4 weeks; severe
161 pulmonary hypertension; lung volume reduction surgery or lung transplant; interstitial lung
162 disease with known primary diseases (such as sarcoidosis or amyloidosis), exposures (such

163 as radiation) or drugs (such as amiodarone); unstable cardiovascular, pulmonary (other than
164 IPF), or other disease within 6 months; creatine clearance <30 mL/min; hemoglobin level
165 <10 g/dL; alcohol or substance abuse; pregnancy; abnormal liver function test. Patients
166 attended two screening visits; at Visit 1 assessments included inclusion and exclusion
167 criteria, demographics, medical history, alcohol consumption and smoking habits, St
168 George's Respiratory Questionnaire (SGRQ), electrocardiogram, SpO₂ test, spirometry,
169 DLCO, adverse events, prior and concomitant medication, physical examination and vital
170 signs, blood sampling, and the six-minute walk test. Assessments at the second screening
171 visit were spirometry, SpO₂ test, oxygen titration test for the six-minute walk test, adverse
172 events and concomitant medication. Patients were randomized at Visit 3. Following
173 randomization patients were assessed at Weeks 2, 4, 8, 12, 18, 26, 34, 42 and 52.
174 Spirometry assessments were performed at each of these visits and then every 12 weeks;
175 blood samples for pharmacokinetic/pharmacodynamic assessments were collected at Week
176 12, 26, 34 and 52 and then every 24 weeks. If patients could not attend study sites due to
177 the COVID-19 pandemic, study visits were conducted virtually or via phone calls.

178 In both studies, patients were randomized 1:1:1 to receive oral ziritaxestat 600 mg,
179 ziritaxestat 200 mg or matching placebo once daily on top of local SoC until the last patient
180 completed 52 weeks, to allow the collection of long-term efficacy and safety data. Initiation of
181 pirfenidone or nintedanib or switching SoC during the studies was allowed. Treatment was
182 allocated to each patient using a centralized electronic system (interactive web-based
183 response system) with permuted blocks. Both patients and study personnel were blinded to
184 treatment allocation. Randomization was stratified by local SoC for IPF (nintedanib or
185 pirfenidone) or neither, and recruitment could be restricted in one or more strata, while the
186 other strata continue to recruit to maintain a balanced proportion of patients between the
187 three strata. Clinical study personnel monitored the amount of study drug dispensed to a
188 patient and the amount returned to assess treatment compliance. For doses taken at home,
189 the intake was reported using patient diary cards. The study drug was to be discontinued if

190 aspartate transaminase or alanine transaminase levels exceeded 8 times the upper limit of
191 normal or were ≥ 3 times the upper limit of normal with signs of severe liver damage.

192 The studies were conducted in accordance with the Declaration of Helsinki and local ethical
193 and legal requirements. Study protocols were approved by the independent ethics
194 committee/institutional review board for each site or country, as applicable. All patients
195 provided written informed consent. Enrollment began in November 2018; the studies were
196 terminated early in February 2021.

197 **4.2. Endpoints**

198 The primary endpoint was annual rate of FVC decline up to Week 52. Key secondary
199 endpoints were: disease progression (a composite endpoint of first occurrence of $\geq 10\%$
200 absolute decline in percent predicted FVC or all-cause mortality at Week 52); time to first
201 respiratory-related hospitalization until the end of the study; and change from baseline in
202 SGRQ total score at Week 52. Other secondary endpoints included time to respiratory-
203 related mortality, time to all-cause mortality or respiratory-related hospitalization, time to first
204 acute IPF exacerbation, change from baseline in FVC, 6-minute walk test distance and
205 safety and tolerability over time until the end of the study (**eMethods**).

206 Data were reviewed by an independent data monitoring committee (IDMC) and Clinical
207 Endpoint Adjudication Committee (CEAC). The IDMC assessed potential safety risks and
208 examined unblinded FVC data to assess the effect of treatment on lung function so a
209 recommendation could be made to the study sponsor regarding study continuation. The
210 IDMC regularly reviewed the data (every 3 to 4 months); and in addition, an interim futility
211 analysis was planned once at least 25% of patients from the two studies combined had
212 completed 52 weeks of treatment. However, the studies were terminated after the 6th regular
213 data review, before the interim futility analysis occurred. The CEAC adjudicated major
214 events, including exacerbations, hospitalizations and deaths, based on blinded data.

215 **4.3. Pharmacokinetic and pharmacodynamic assessments**

216 Pharmacokinetic assessments included the plasma concentration of ziritaxestat, pirfenidone
217 and nintedanib. Target engagement (autotaxin inhibition) was determined by LPA C18:2
218 plasma concentration. Modelling was performed to determine exposure–response
219 relationships (**eMethods**).¹⁸

220 **4.4. Statistical analyses**

221 The full analysis set was used for primary and safety analyses and comprised all
222 randomized patients who received at least one dose of study drug. Assuming ziritaxestat
223 600 mg had an effect on FVC of ≥ 80 mL versus placebo, a sample size of 250 per group
224 would provide 80% power to show a significant effect. Therefore, each study was expected
225 to enroll 750 patients (250 patients in the ziritaxestat 600 mg, ziritaxestat 200 mg and
226 placebo groups).

227 For the primary endpoint, a random coefficient regression model (linear slope model¹⁹) was
228 used, which included sex, age, height and stratification factor as covariates and a random
229 slope and intercept. A protocol-defined sensitivity analysis was performed to assess annual
230 rate of FVC decline up to the end of the trials using all study data and a post hoc sensitivity
231 analysis was performed to assess FVC decline up to the end of the trials using all study data
232 according to treatment strata.

233 Logistic regression analysis was used to assess the proportion of patients with disease
234 progression at Week 52. Time-to-event data are presented as Kaplan–Meier estimates. A
235 Cox proportional hazards model was used to estimate and test hazard ratios (HRs) for each
236 dose of ziritaxestat versus placebo. A mixed-effect model was applied to the SGRQ total
237 score (**eMethods**). To account for multiple testing due to two doses of ziritaxestat being
238 compared with placebo, a Bonferroni approach was used with higher priority given to the
239 high-dose group. The primary endpoint was tested at the alpha 4% and 1% level when
240 comparing ziritaxestat 600 mg and 200 mg, respectively, with placebo. The rate of decline

241 model, which assumes a linear trend over time, was applied to all available data. Therefore,
242 imputation of missing data was not performed.

243

244 **5. Results**

245 **5.1. Study termination**

246 The studies were terminated early, following a planned review of pooled unblinded data by
247 the IDMC. The IDMC concluded that there were safety concerns regarding increased
248 mortality in the ziritaxestat 600 mg group, and a lack of efficacy in all treatment arms and
249 therefore the benefit–risk profile of ziritaxestat no longer supported the continuation of the
250 studies. At that time, 1116 and 1431 patients had been screened in ISABELA 1 and 2,
251 respectively.

252 **5.2. Patient disposition and baseline characteristics**

253 In ISABELA 1 and 2, 525 and 781 patients were randomized and 523 and 777 received at
254 least one dose of their assigned study drug, respectively (**Figure 1**). After the premature
255 termination of the trials all patients discontinued study drug (**Figure 1**). At the time of study
256 termination, enrollment was ongoing in ISABELA 1 and had completed in ISABELA 2.

257 The median duration between HRCT scan and study enrollment was 51 days overall in
258 ISABELA 1 (41, 58 and 60 days in the ziritaxestat 600 mg, ziritaxestat 200 mg and placebo
259 groups, respectively) and 50 days overall in ISABELA 2 (64, 50 and 43 days in the
260 ziritaxestat 600 mg, ziritaxestat 200 mg and placebo groups, respectively). In the ziritaxestat
261 600 mg, ziritaxestat 200 mg and placebo groups, 20, 27 and 19 lung biopsies were
262 reviewed, respectively, in ISABELA 1, as were 28, 33 and 28, respectively, in ISABELA 2. In
263 each study, baseline demographics and disease characteristics were similar in the
264 ziritaxestat 600 mg, 200 mg and placebo groups (**Table 1**). There was a greater proportion
265 of Asian patients in ISABELA 2 than ISABELA 1 (28.1% vs 5.5%) owing to the geographical

266 location of sites. Mean baseline FVC and percent predicted FVC were slightly higher in
267 ISABELA 1 than ISABELA 2 (2921.1 mL vs 2764.7 mL and 79.3% vs 77.3%, respectively); a
268 higher proportion of patients in ISABELA 2 were former smokers (71.4% vs 61.6%) (**Table**
269 **1**).

270 Upon enrollment in ISABELA 1, 39.8% and 34.6% of patients were taking pirfenidone and
271 nintedanib, respectively, while 25.6% received neither. In ISABELA 2, 32.4% and 34.9% of
272 patients were taking pirfenidone and nintedanib, respectively, while 32.7% received neither
273 (**Table 1**). The mean dose of pirfenidone at enrollment was 2216 mg/day in ISABELA 1
274 (2184, 2165 and 2300 mg/day in the ziritaxestat 600 mg, ziritaxestat 200 mg and placebo
275 groups, respectively) and 2083 mg/day in ISABELA 2 (2020, 2031 and 2201 mg/day in the
276 ziritaxestat 600 mg, ziritaxestat 200 mg and placebo groups, respectively). The mean dose
277 of nintedanib at enrollment was 283 mg/day in ISABELA 1 (293, 278 and 278 mg/day in the
278 ziritaxestat 600 mg, ziritaxestat 200 mg and placebo groups, respectively) and 267 mg/day
279 in ISABELA 2 (262, 270 and 270 mg/day in the ziritaxestat 600 mg, ziritaxestat 200 mg and
280 placebo groups, respectively). Of patients taking neither pirfenidone nor nintedanib at the
281 time of enrollment, 79.1% and 68.1% in ISABELA 1 and 2, respectively, had never been
282 treated with either therapy. Mean IPF duration was shorter in the group taking neither
283 pirfenidone nor nintedanib (1.8 years in both ISABELA 1 and 2) than in the pirfenidone (2.4
284 years and 2.6 years in ISABELA 1 and 2, respectively) or nintedanib strata (2.4 years in both
285 ISABELA 1 and 2) (**eTable 1**). Percent predicted FVC was slightly lower in patients who
286 received either pirfenidone or nintedanib (75.0–77.4%) than in those who received neither
287 (85.6% in ISABELA 1; 79.9% in ISABELA 2) (**eTable 1**).

288 **5.3. Treatment duration and exposure**

289 Treatment exposures were longer in ISABELA 2 than in ISABELA 1 (**eTable 2**), reflecting
290 that the patients were followed up for longer on average. Treatment compliance was high in
291 all treatment groups (median was 100% in each treatment arm in both ISABELA 1 and 2;

292 **eTable 2).** The dose of nintedanib was reduced or interrupted in a greater proportion of
293 patients in the ziritaxestat 600 mg group than ziritaxestat 200 mg or placebo groups (**Table**
294 **2**); in the ziritaxestat 600 mg group, the proportion of patients who underwent dose
295 reductions or interruptions of SoC was greater in the nintedanib stratum than pirfenidone
296 stratum (**Table 2**). Diarrhea was the most common TEAE leading to nintedanib dose
297 reductions (ISABELA 1: 6.7%, 4.9% and 3.3% in the ziritaxestat 600 mg, ziritaxestat 200 mg
298 and placebo groups, respectively; ISABELA 2: 22.5%, 6.6% and 8.8% in each group,
299 respectively) or interruptions (ISABELA 1: 10.0%, 8.2% and 6.7% in the ziritaxestat 600 mg,
300 ziritaxestat 200 mg and placebo groups, respectively; ISABELA 2: 22.5%, 13.2% and 8.8%
301 in each group, respectively). Mean daily doses of pirfenidone and nintedanib are given in
302 **eTable 3.** During ISABELA 1 and 2, respectively, 5.0% and 5.9% of patients switched from
303 nintedanib to pirfenidone, while 1.9% and 1.6% switched from pirfenidone to nintedanib. In
304 ISABELA 1 and 2, respectively, 11.9% and 9.1% of patients who were assigned ziritaxestat
305 alone (i.e. not on top of pirfenidone or nintedanib) initiated SoC during the studies (**eTable**
306 **4**).

307 **5.4. Primary endpoint: annual rate of FVC decline**

308 Ziritaxestat did not improve annual rate of FVC decline versus placebo (**Table 3**). In
309 ISABELA 1, the least-squares (LS) mean (standard error [SE]) annual rate of FVC decline
310 up to Week 52 was -124.6 (27.2) mL with ziritaxestat 600 mg, -173.9 (26.3) mL with
311 ziritaxestat 200 mg and -147.3 (26.7) mL with placebo. Corresponding data for ISABELA 2
312 were -173.8 (18.0) mL with ziritaxestat 600 mg, -174.9 (17.7) mL with ziritaxestat 200 mg
313 and -176.6 (17.7) mL with placebo. Pooled data from both studies are shown in **Table 3**.
314 Similar results were observed in the sensitivity analysis, which included all data until the end
315 of the studies; when analyzed by stratum, annual rate of FVC decline was greatest in the
316 pirfenidone group (**Table 3**). In each trial, the treatment-stratum-time interaction p-value
317 was <0.001 indicating a significant interaction. For the primary endpoint, 19 patients (1%)
318 from the full analysis set (N=1281) were excluded from the linear slope model analysis due

319 to missing post-baseline FVC data. At Week 52, FVC data were missing for 92 patients
320 (17%).

321 **5.5. Key secondary endpoints**

322 At Week 52, disease progression was similar across treatment arms, both when data from
323 ISABELA 1 and 2 were pooled and assessed separately (**eFigure 2**).

324 For time to first respiratory-related hospitalization outcomes were worse in the ziritaxestat
325 arm versus placebo in both ISABELA 1 and 2 (**eFigure 3**); reasons for hospitalization are
326 shown in **eTable 5**. Change from baseline in SGRQ total score at Week 52 was similar
327 across treatment groups in each study (**eTable 6**).

328 **5.6. Other secondary endpoints**

329 For the following endpoints, worse outcomes were observed with ziritaxestat versus placebo:
330 time to respiratory-related mortality, time to all-cause mortality or respiratory-related
331 hospitalization, or time to first acute IPF exacerbation (**eFigures 4–6**).

332 Pooled study data showed observed mean change from baseline in FVC was similar across
333 treatment arms at Week 52 (**eFigure 7**). Changes from baseline in FVC per stratum are
334 shown in **eFigures 8 and 9**.

335 The 6-minute walk test distance decreased from baseline to Week 52 in all treatment arms in
336 ISABELA 1 and 2 (**eTable 6**).

337 **5.7. All-cause mortality**

338 Mortality rate (until end of study) was numerically higher with both doses of ziritaxestat
339 versus placebo in ISABELA 2 and with ziritaxestat 200 mg versus placebo in ISABELA 1
340 (**Table 4**). Pooled data showed all-cause mortality was 8.9% and 7.0% with ziritaxestat 600
341 mg and 200 mg, respectively, versus 5.5% with placebo (HR [95% confidence interval]; 1.8
342 [1.1, 3.0] and 1.3 [0.8, 2.3], respectively; **Table 4**).

343 Respiratory-related deaths were the primary cause of mortality (adjudicated events that took
344 place during the trials), occurring in 3.6%, 3.5% and 1.8% of patients in the ziritaxestat 600
345 mg, 200 mg and placebo groups, respectively, in ISABELA 1, and in 5.8%, 4.2% and 1.6%
346 of patients in each group, respectively, in ISABELA 2 (**eTable 7**).

347 **5.8. Treatment-emergent adverse events**

348 In ISABELA 1, 78.7%, 84.6% and 84.5% of patients in the ziritaxestat 600 mg, 200 mg and
349 placebo groups, respectively, had treatment-emergent adverse events (TEAEs); the
350 corresponding data for ISABELA 2 were 81.1%, 85.8% and 75.6% (**Table 2**). Serious TEAEs
351 occurred in 21.8%, 21.7% and 20.7% of patients in the ziritaxestat 600 mg, 200 mg and
352 placebo groups, respectively, in ISABELA 1, and in 24.7%, 24.2% and 16.3% of these
353 groups in ISABELA 2 (**Table 2**). TEAE data according to stratum are shown in **eTable 8** and
354 TEAEs with a $\geq 5\%$ incidence in at least one treatment group are listed in **eTable 9**; the most
355 common TEAEs were gastrointestinal disorders.

356 TEAEs leading to death occurred in 4.6%, 3.4% and 4.6% of patients in the ziritaxestat 600
357 mg, 200 mg and placebo groups, respectively, in ISABELA 1, and 8.5%, 7.7% and 3.9% of
358 these groups in ISABELA 2 (**Table 2**). IPF was the most common TEAE leading to death in
359 the ziritaxestat 600 mg group in both ISABELA 1 and 2 (n=4 and n=5) while coronavirus
360 disease 2019 (COVID-19) pneumonia and COVID-19 were the most common TEAEs
361 leading to death in the ziritaxestat 200 mg group in ISABELA 1 and 2, respectively (n=2 and
362 n=4) (**eTable 10**). The causes of death as provided by the investigator are listed in **eTable**
363 **11**. Overall, COVID-19–related TEAEs occurred in 3.8% of patients (**eTable 12**). Adjudicated
364 deaths due to COVID-19 occurred in 0%, 1.8% and 0.6% of patients in the ziritaxestat 600
365 mg, ziritaxestat 200 mg and placebo groups, respectively, in ISABELA 1, and 0.8%, 1.9%
366 and 0% of these groups, respectively, in ISABELA 2. The impact of COVID-19 on study
367 visits is provided in **eTable 13**.

368 **5.9. Pharmacokinetics and target engagement**

369 Pharmacokinetic parameters of ziritaxestat are shown in **eTable 14**. A mixture model, which
370 accounted for two responder sub-types, showed that in over 70% of the patients on active
371 therapy, LPA level decreased from baseline (**eFigure 10**). In the remaining, smaller cohort,
372 there was an increase in LPA level post-treatment, not attributable to any difference in
373 exposure to ziritaxestat.

374 **6. Discussion**

375 In the ISABELA studies, ziritaxestat did not lead to a reduction in the annual rate of FVC
376 decline versus placebo; thus, the primary endpoint was not met. Ziritaxestat also failed to
377 show benefit in secondary efficacy endpoints, such as time to first respiratory-related
378 hospitalization, respiratory-related mortality, all-cause mortality or respiratory-related
379 hospitalization, or acute IPF exacerbation, SGRQ total score or 6-minute walk test distance.
380 All-cause mortality data showed a higher proportion of deaths with ziritaxestat 600 mg than
381 placebo in ISABELA 1, and with each ziritaxestat dose than placebo in ISABELA 2.

382 Ziritaxestat did not reduce FVC decline compared with placebo, unlike in the prior phase 2a
383 study,¹⁶ although the latter included a limited number of patients. As in the phase 2a study,
384 decreases in LPA were observed following ziritaxestat dosing; therefore, lack of target
385 engagement is not considered the reason for the absence of effect on clinical endpoints. It is
386 unknown why the positive results of the prior phase 2a study were not replicated in the
387 ISBAELA studies, but the limitations associated with early phase trials such as small sample
388 sizes, short duration and limited use of SoC therapies may be contributing factors. Lung
389 function in patients on SoC was expected to decline at a slower rate than in untreated
390 patients; however, this was not the case in the ISABELA studies. The extent of FVC decline
391 in the ISABELA trials differed between strata and was greatest in patients taking pirfenidone.
392 While suboptimal pirfenidone dosing was observed in some patients, it was not overly
393 frequent, and is not thought to explain the apparent worsening of lung function. In addition,
394 phase 1 data show ziritaxestat does not affect pirfenidone concentration. Therefore, the
395 reason for this finding remains unclear.

396 Unlike for pirfenidone, phase 1 studies show ziritaxestat increases plasma levels of
397 nintedanib. An increase in nintedanib levels could have led to the higher proportion of dose
398 reductions, interruptions and non-diarrheal TEAEs observed with nintedanib versus
399 pirfenidone in the ziritaxestat 600 mg group of the ISABELA trials. However, the dose of
400 nintedanib administered has been shown to predict risk of diarrhea (which was the most
401 frequent TEAE in the ziritaxestat 600 mg group), better than plasma exposure²⁰. In the prior
402 phase 2a study, ziritaxestat was administered as monotherapy and treatment with nintedanib
403 was prohibited reducing the probability of dose reductions or treatment interruptions due to
404 drug–drug interactions.

405 As stated, the IDMC's recommendation to terminate the studies was based on both lack of
406 efficacy and a perceived increased mortality risk. Pooled data from ISABELA 1 and 2
407 showed an all-cause mortality rate of 8.9%, 7.0% and 5.5% with ziritaxestat 600 mg,
408 ziritaxestat 200 mg and placebo, respectively, over a study duration of upwards of 100
409 weeks. A greater proportion of patient deaths occurred with ziritaxestat 600 mg than placebo
410 in ISABELA 1, and with both ziritaxestat doses versus placebo in ISABELA 2. At the time of
411 study termination, the number of patients enrolled and the number of patient-years of study
412 drug exposure were greater in ISABELA 2 than ISABELA 1. Compared with ISABELA 1,
413 there was a greater proportion of Asian patients in ISABELA 2. Whether this contributed to
414 the greater proportion of deaths (including COVID-19–related deaths) in ISABELA 2 requires
415 greater understanding of regional/racial differences in patients with IPF.

416 The COVID-19 pandemic, which arose after the studies were initiated, had some impact on
417 study conduct and resulted in many clinic-based visits being missed by patients. Study
418 safety was ensured by permitting telephone visits in place of scheduled clinic visits and
419 performance of blood safety assessments in local laboratories. Fortunately, the proportion of
420 COVID-19–related deaths was low, although where these occurred, they disproportionately
421 affected the ziritaxestat treatment arms. A limited proportion of patients had missing
422 spirometry data (1% had no post-baseline FVC data), which arguably did not affect

423 analyses, and in addition, a mixed model for repeated measures was used, which mitigates
424 the impact of some missing data.

425 Despite the regulatory requirement, testing new IPF medications on top of SoC is
426 challenging as lung function in patients on currently available therapy is likely to decline at a
427 slower rate than in untreated patients; however, as noted, this was not the case in the
428 ISABELA trials. Furthermore, variability among individual patients may be amplified when
429 testing a new therapy on top of local SoC, which can make data interpretation challenging.
430 While the design of the ISABELA studies is not considered to have contributed to the
431 negative findings, possible considerations for future IPF studies, which may increase the
432 likelihood of identifying treatment effects, include the use of adaptive designs employing a
433 Bayesian approach and biomarker-based enrichment strategies using prognostic biomarkers
434 of early or more rapid disease progression.²¹ Knowledge of patients' prior change in lung
435 function would also allow better understanding of the rate of decline following the
436 introduction of therapy. While the ISABELA studies failed, they demonstrate the potential
437 value of observing data for ≥ 52 weeks and provide information regarding the utility of
438 different clinical endpoints. In addition, information collected during studies, such as the
439 faster than anticipated decline in FVC in the SoC strata, adverse events associated with
440 SoC, and treatment patterns (e.g., the proportion of patients switching or initiating SoC
441 during the trials), may help inform the design of future IPF studies.

442 Further investigation is needed to determine why the ISABELA trials failed. This may be
443 determined by ongoing studies of other autotaxin inhibitors with different pharmacological
444 characteristics to those of ziritaxestat (such as BBT-877²²) or LPA receptor antagonists
445 (such as BMS-986278²³). Of note, the LPA receptor antagonist BMS-986020 was
446 discontinued due to hepatobiliary toxicity; however, this was found to be unrelated to LPA
447 antagonism^{24,25} and, indeed, no such safety issues were identified in the ISABELA trials.

448 **7. Limitations**

449 The early termination of the studies is considered a possible limitation as it may have
450 reduced our ability to adequately interpret the effect of treatment on the primary endpoint.
451 Enrollment in ISABELA 1 was not complete so fewer patients entered the study than
452 planned and outcomes beyond Week 52 were not captured for all patients. Another limitation
453 is the missing data or visits due to the COVID-19 pandemic. The pandemic may also have
454 influenced trial participation, and therefore the patient population may not reflect those in
455 former IPF trials. In addition, the studies were not powered to assess true differences
456 between strata (nintedanib vs pirfenidone vs no SoC).

457 8. Conclusion

458 The ISABELA studies did not demonstrate a benefit in their primary endpoint of annual rate
459 of FVC decline, or in key secondary endpoints, compared with SoC in patients with IPF.

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461 Author contributions

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10. Tables

Table 1. Baseline demographics, disease characteristics and stratum

	ISABELA 1			ISABELA 2		
	Ziritaxestat 600 mg (n=174)	Ziritaxestat 200 mg (n=175)	Placebo (n=174)	Ziritaxestat 600 mg (n=259)	Ziritaxestat 200 mg (n=260)	Placebo (n=258)
Age, years	69.4 (7.2)	70.0 (6.7)	70.6 (7.7)	69.2 (7.2)	69.7 (7.3)	70.6 (6.6)
Male, n (%)	142 (81.6)	143 (81.7)	146 (83.9)	209 (80.7)	213 (81.9)	209 (81.0)
Female, n (%)	32 (18.4)	32 (18.3)	28 (16.1)	50 (19.3)	47 (18.1)	49 (19.0)
Race, n (%)						
American Indian or Alaska native	10 (5.7)	6 (3.4)	6 (3.4)	2/251 (0.8)	1/252 (0.4)	2/252 (0.8)
Asian	11 (6.3)	9 (5.1)	9 (5.2)	72/251 (28.7)	72/252 (28.6)	68/252 (27.0)
Black or African American	1 (0.6)	0	0	2/251 (0.8)	0/252 (0.0)	1/252 (0.4)
Multiple	0	0	0	0/251 (0.0)	1/252 (0.4)	2/252 (0.8)
Native Hawaiian or Other Pacific Islander	0	0	0	1/251 (0.4)	0/252 (0.0)	1/252 (0.4)
White	152 (87.4)	160 (91.4)	159 (91.4)	174 (69.3)	178/252 (70.6)	178/252 (70.6)
Geographical region, n (%)						
Asia-Pacific	24 (13.8)	29 (16.6)	22 (12.6)	67 (25.9)	76 (29.2)	71 (27.5)
EMEA	73 (42.0)	70 (40.0)	77 (44.3)	100 (38.6)	90 (34.6)	86 (33.3)
Latin America	35 (20.1)	21 (12.0)	22 (12.6)	30 (11.6)	33 (12.7)	27 (10.5)
Northern America	42 (24.1)	55 (31.4)	53 (30.5)	62 (23.9)	61 (23.5)	74 (28.7)
Former smoker, n (%)	99 (56.9)	110 (62.9)	113 (64.9)	177 (68.3)	195 (75.0)	183 (70.9)
Current smoker, n (%)	5 (2.9)	2 (1.1)	0	5 (1.9)	2 (0.8)	5 (1.9)
BMI, kg/m ²	27.6 (4.0)	28.3 (3.9)	27.9 (3.8)	26.7 (3.9)	27.3 (3.7)	27.1 (4.3)
Oxygen saturation at rest, %	95.8 (2.1)	95.8 (2.0)	95.7 (1.9)	95.6 (2.2)	95.5 (2.0)	95.7 (2.1)

Receiving oxygen at rest, n (%)	11 (6.3)	7 (4.0)	9 (5.2)	16 (6.2)	10 (3.8)	13 (5.0)
Duration of IPF, years	2.3 (1.4)	2.3 (1.4) [174]	2.3 (1.4)	2.2 (1.5)	2.3 (1.5)	2.2 (1.5) [257]
Duration of IPF, years, median (min, max) [n]	2.1 (0.1, 5.8)	2.1 (0.1, 5.5) [174]	2.0 (0.1, 6.6)	2.0 (0.1, 11.8)	2.1 (0.1, 6.3)	1.9 (0.1, 5.8) [257]
FVC, mL	2947.0 (820.8)	2873.2 (815.8)	2943.3 (738.7)	2775.7 (823.2)	2768.6 (701.9)	2749.5 (785.1) [256]
Percent predicted FVC, ^a %	80.4 (17.5)	77.9 (18.1)	79.7 (15.9)	76.5 (16.8)	77.7 (15.5)	77.6 (17.2) [256]
FEV ₁ /FVC ratio, %	82.0 (5.9)	82.4 (5.6)	82.3 (4.7)	82.9 (5.8)	82.8 (5.7)	82.7 (6.0) [256]
SGRQ total score, ^b %	34.1 (18.5) [173]	35.9 (19.1)	35.2 (17.9) [173]	36.9 (20.2) [258]	36.6 (20.3) [258]	34.9 (19.2) [257]
6MWT distance, ^c m	416.3 (101.6) [173]	403.7 (105.1)	400.8 (119.1)	408.3 (110.9)	395.7 (133.9) [257]	400.4 (105.4) [257]
Percent predicted DLCO corrected for hemoglobin, %	56.4 (18.9)	54.0 (16.3)	53.1 (16.3)	54.3 (19.6) [258]	53.1 (17.5) [259]	57.1 (21.7) [257]
Concomitant use of proton pump inhibitors	91 (52.3)	90 (51.4)	86 (49.4)	133 (51.4)	131 (50.4)	139 (53.9)
Stratum, n (%)						
Pirfenidone ^d	69 (39.7)	70 (40.0)	69 (39.7)	84 (32.4)	85 (32.7)	83 (32.2)
Nintedanib ^d	60 (34.5)	61 (34.9)	60 (34.5)	89 (34.4)	91 (35.0)	91 (35.3)
Neither	45 (25.9)	44 (25.1)	45 (25.9)	86 (33.2)	84 (32.3)	84 (32.6)

^aPredicted FVC calculated using the 2012 Global Lung Function Initiative Equations.

^bSGRQ is a 50-item health-related quality of life patient questionnaire with domains covering respiratory symptoms, activity levels, and psychosocial impact. Scores range from 0-100, with a higher score indicating poorer health-related quality of life.

^cAverage 6MWT distance in healthy subjects aged 55–75 years is 659 metres.

^dSee **eTable 3** for doses of standard of care medication²⁶.

Values are mean (SD) [n] unless otherwise stated. Full analysis set.

6MWT, 6-minute walk test; BMI, body mass index; DLCO, diffusing capacity of the lung for carbon monoxide; FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity; IPF, idiopathic pulmonary fibrosis; SD, standard deviation; SGRQ, St George Respiratory Questionnaire.

Table 2. Treatment-emergent adverse events and dose reductions/interruptions/discontinuations

Patients, n (%)	ISABELA 1			ISABELA 2		
	Ziritaxestat 600 mg (n=174)	Ziritaxestat 200 mg (n=174)	Placebo (n=174)	Ziritaxestat 600 mg (n=259)	Ziritaxestat 200 mg (n=260)	Placebo (n=258)
TEAE	137 (78.7)	148 (84.6)	147 (84.5)	210 (81.1)	223 (85.8)	195 (75.6)
Serious TEAE	38 (21.8)	38 (21.7)	36 (20.7)	64 (24.7)	63 (24.2)	42 (16.3)
Death	8 (4.6)	6 (3.4)	8 (4.6)	22 (8.5)	20 (7.7)	10 (3.9)
Worst TEAE severity						
Death	9 (5.2)	6 (3.4)	8 (4.6)	22 (8.5)	20 (7.7)	10 (3.9)
Life-threatening	3 (1.7)	5 (2.9)	4 (2.3)	5 (1.9)	1 (0.4)	4 (1.6)
Severe	29 (16.7)	35 (20.0)	26 (14.9)	38 (14.7)	44 (16.9)	33 (12.8)
Moderate	82 (47.1)	69 (39.4)	80 (46.0)	111 (42.9)	108 (41.5)	108 (41.9)
Mild	14 (8.0)	33 (18.9)	29 (16.7)	34 (13.1)	50 (19.2)	40 (15.5)
Treatment-related TEAE to study drug	60 (34.5)	53 (30.3)	53 (30.5)	96 (37.1)	80 (30.8)	70 (27.1)
Treatment-related TEAE to pirfenidone	22 (12.6)	28 (16.0)	26 (14.9)	19 (7.3)	34 (13.1)	30 (11.6)
Treatment-related TEAE to nintedanib	50 (28.7)	39 (22.3)	26 (14.9)	80 (30.9)	54 (20.8)	42 (16.3)
Dose reductions/interruptions/discontinuations						
Study drug:						
Permanently stopped	18 (10.3)	10 (5.7)	13 (7.5)	29 (11.2)	27 (10.4)	18 (7.0)
Interrupted	30 (17.2)	29 (16.6)	23 (13.2)	57 (22.0)	37 (14.2)	33 (12.8)
Reduced	9 (5.2)	6 (3.4)	3 (1.7)	30 (11.6)	12 (4.6)	9 (3.5)
Pirfenidone:						
Permanently stopped	3 (1.7)	2 (1.1)	4 (2.3)	8 (3.1)	3 (1.2)	8 (3.1)
Interrupted	4 (2.3)	4 (2.3)	5 (2.9)	8 (3.1)	9 (3.5)	5 (1.9)
Reduced	1 (0.6)	3 (1.7)	3 (1.7)	5 (1.9)	6 (2.3)	2 (0.8)
Nintedanib:						
Permanently stopped	4 (2.3)	6 (3.4)	0	10 (3.9)	3 (1.2)	2 (0.8)
Interrupted	13 (7.5)	10 (5.7)	10 (5.7)	25 (9.7)	19 (7.3)	14 (5.4)
Reduced	7 (4.0)	3 (1.7)	6 (3.4)	24 (9.3)	8 (3.1)	10 (3.9)

Full analysis set. Events listed in table occurred during the trial, on or after the start of study drug intake and within 30 days after the last dose of study drug until the end of the study.

TEAE, treatment-emergent adverse event.

Table 3. Annual rate of FVC decline up to Week 52 and until end of study

	Ziritaxestat 600 mg	Ziritaxestat 200 mg	Placebo	Difference between ziritaxestat 600 mg and placebo ^a	Difference between ziritaxestat 200 mg and placebo ^a
Annual rate of FVC decline up to Week 52					
ISABELA 1 LS mean (95% CI) [n]	-124.6 (-178.0, -71.2) [174]	-173.9 (-225.7, -122.2) [175]	-147.3 (-199.8, -94.7) [174]	22.7 (-52.3, 97.6)	-26.7 (-100.5, 47.1)
ISABELA 2 LS mean (95% CI) [n]	-173.8 (-209.2, -138.4) [259]	-174.9 (-209.5, -140.2) [260]	-176.6 (-211.4, -141.8) [258]	2.8 (-46.9, 52.4)	1.7 (-47.4, 50.8)
ISABELA 1 and 2 (pooled) LS mean (95% CI) [n]	-156.5 (-186.2, -126.7) [428]	-173.7 (-202.7, -144.7) [431]	-165.7 (-194.9, -136.4) [422]	9.2 (-32.5, 50.9)	-8.0 (-49.2, 33.1)
Annual rate of FVC decline until end of study (ISABELA 1 and 2 pooled)					
All strata LS mean (95% CI) [n]	-161.0 (-187.4, -134.5) [428]	-174.4 (-200.0, -148.7) [431]	-169.0 (-195.0, -143.0) [422]	8.0 (-29.0, 45.1)	-5.4 (-41.9, 31.1)
Pirfenidone LS mean (95% CI) [n]	-194.3 (-238.2, -150.3) [151]	-217.9 (-259.5, -176.3) [154]	-189.0 (-231.6, -146.3) [149]	-5.3 (-66.6, 56.0)	-29.0 (-88.6, 30.6)
Nintedanib LS mean (95% CI) [n]	-132.4 (-176.2, -88.6) [146]	-132.2 (-175.2, -89.2) [151]	-163.1 (-205.7, -120.5) [147]	30.7 (-30.4, 91.9)	30.9 (-29.7, 91.5)
Neither LS mean (95% CI) [n]	-155.1 (-204.8, -105.3) [131]	-168.4 (-217.5, -119.3) [126]	-149.1 (-199.5, -98.6) [126]	-6.0 (-76.9, 64.9)	-19.3 (-89.7, 51.1)

^aPositive difference in values indicates that patients receiving ziritaxestat deteriorate less than those receiving placebo.

Full analysis set.

CI, confidence interval; FVC, forced vital capacity; LS, least squares; SE, standard error.

Table 4. Risk of all-cause mortality with ziritaxestat versus placebo (until the end of the studies)

	Ziritaxestat 600 mg (N=428)		Ziritaxestat 200 mg (N=431)		Placebo (N=422)		Ziritaxestat 600 mg versus placebo		Ziritaxestat 200 mg versus placebo	
	Events n (%)	Incidence rate per 100 PYE	Events n (%)	Incidence rate per 100 PYE	Events n (%)	Incidence rate per 100 PYE	Difference in incidence rate (CI)	HR (CI)	Difference in incidence rate (CI)	HR (CI)
ISABELA 1	14 (8.0)	8.1	8 (4.6)	4.3	11 (6.3)	6.3	1.8 (-4.3, 8.0)	1.4 (0.6, 3.1)	-1.9 (-7.5, 3.6)	0.7 (0.3, 1.8)
ISABELA 2	24 (9.3)	8.9	22 (8.5)	8.1	12 (4.7)	4.4	4.5 (0.0, 9.1)	2.1 (1.1, 4.3)	3.7 (-0.7, 8.1)	1.9 (0.9, 3.8)
ISABELA 1 and 2 pooled	38 (8.9)	8.6	30 (7.0)	6.6	23 (5.5)	5.1	3.5 (-0.1, 7.1)	1.8 (1.1, 3.0)	1.5 (-1.9, 4.8)	1.3 (0.8, 2.3)

Full-analysis set.

CI, confidence interval; HR, hazard ratio; IR, incidence rate; PYE, patient years of exposure.

11. Figure legends

Figure 1. Patient disposition in ISABELA 1 (a) and ISABELA 2 (b)

At the time of study termination, enrollment was ongoing in ISABELA 1 and had completed in ISABELA 2. The discrepancy between the number of deaths reported here for patient disposition and in the results is because of incomplete data cleaning due to the early termination of the studies; the total number of deaths was ziritaxestat 600 mg: 14, ziritaxestat 200 mg: 8, placebo: 11 in ISABELA 1 and ziritaxestat 600 mg: 24, ziritaxestat 200 mg: 22, placebo: 12 in ISABELA 2.

* FVC \geq 45% predicted of normal, FEV₁/FVC \geq 0.7 and DLCO corrected for hemoglobin \geq 30% predicted of normal.

DLCO, diffusing capacity for carbon monoxide; FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity; HRCT, high-resolution computed tomography