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1 **Title**

2 An open-label randomized, controlled trial of the effect of lopinavir/ritonavir,
3 lopinavir/ritonavir plus IFN- β -1a and hydroxychloroquine in hospitalized patients with
4 COVID-19

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149

150 **Key words:** COVID-19; SARS-CoV-2; hydroxychloroquine; interferon beta-1a;
151 lopinavir/ritonavir; randomized controlled trial

152

153 **Running title**

154 Treatments of inpatients with COVID-19

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160

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162 **Abstract**

163 **Objectives**

164 We evaluated the clinical, virological and safety outcomes of lopinavir/ritonavir,
165 lopinavir/ritonavir-interferon (IFN)- β -1a, hydroxychloroquine or remdesivir in comparison to
166 standard of care (control) in COVID-19 inpatients requiring oxygen and/or ventilatory
167 support.

168 **Methods**

169 We conducted a phase 3 multi-centre open-label, randomized 1:1:1:1:1, adaptive, controlled
170 trial (DisCoVeRy), add-on trial to Solidarity (NCT04315948, EudraCT2020-000936-23). The
171 primary outcome was the clinical status at day 15, measured by the WHO 7-point ordinal
172 scale. Secondary outcomes included SARS-CoV-2 quantification in respiratory specimens,
173 pharmacokinetic and safety analyses. We report the results for the lopinavir/ritonavir-
174 containing arms and for the hydroxychloroquine arm, which were stopped prematurely.

175 **Results**

176 The intention-to-treat population included 583 participants (lopinavir/ritonavir, n=145;
177 lopinavir/ritonavir-IFN- β -1a, n=145; hydroxychloroquine, n=145; control, n=148), among
178 whom 418 (71.7%) were male, the median age was 63 years (IQR, 54-71) and 211 (36.2%)
179 had a severe disease. The day-15 clinical status was not improved with investigational
180 treatments: lopinavir/ritonavir *versus* control, adjusted odds ratio (aOR) 0.83, (95%
181 confidence interval [CI] 0.55-1.26, P=0.39); lopinavir/ritonavir-IFN- β -1a *versus* control, aOR
182 0.69 (95%CI 0.45-1.04, P=0.08); hydroxychloroquine *versus* control, aOR 0.93 (95%CI 0.62-
183 1.41, P=0.75). No significant effect of investigational treatment was observed on SARS-CoV-
184 2 clearance . Trough plasma concentrations of lopinavir and ritonavir were higher than those
185 expected, while those of hydroxychloroquine were those expected with the dosing regimen.

186 The occurrence of Serious Adverse Events was significantly higher in participants allocated to
187 the lopinavir/ritonavir-containing arms.

188 **Conclusion**

189 In adults hospitalized for COVID-19, lopinavir/ritonavir, lopinavir/ritonavir-IFN- β -1a and
190 hydroxychloroquine did not improve the clinical status at day 15, nor SARS-CoV-2 clearance
191 in respiratory tract specimens.

192 **Introduction**

193 Worldwide research efforts against SARS-CoV-2 initially focused on repurposed drugs that
194 showed broad-spectrum antiviral activity against coronaviruses [1,2]. Lopinavir/ritonavir
195 [3,4], type I interferon (IFN) [5–7], hydroxychloroquine [8–10], and remdesivir [11] were
196 among the first investigational treatments to be tested on the basis of their *in vitro* activity
197 against SARS-CoV-2.

198 The DisCoVeRy trial is a European randomized controlled trial evaluating the clinical and the
199 virological efficacy, as well as the safety, of lopinavir/ritonavir, lopinavir/ritonavir plus IFN-
200 β -1a, hydroxychloroquine, and remdesivir as compared with standard of care in adults
201 hospitalized for COVID-19 [12]. As an add-on trial to the international Solidarity trial
202 sponsored by the World Health Organization (WHO), it has contributed to data acquisition on
203 in-hospital mortality, need for mechanical ventilation and time to hospital discharge. Interim
204 analyses of these variables concluded to futility, leading to discontinuation of three treatment
205 arms while inclusions continued in the remdesivir arm [13]. The DisCoVeRy trial was
206 designed to further document clinical outcomes, virological kinetics, treatment
207 pharmacokinetics and related safety data. We report here the results for the
208 lopinavir/ritonavir, lopinavir/ritonavir plus IFN- β -1a and hydroxychloroquine arms.

209 **Methods**

210 ***Trial design and oversight***

211 DisCoVeRy is a phase 3 open-label, adaptive, multicenter, randomized, superiority-controlled
212 trial which evaluates the efficacy and safety of repurposed drugs in adults hospitalized for
213 COVID-19. Sponsored by the Institut national de la santé et de la recherche médicale (Inserm,
214 France), the trial was approved by the Ethics Committee (CPP Ile-de-France-III, approval
215 #20.03.06.51744). Written informed consent was obtained from all included participants or
216 their legal representative, when unable to consent. The trial was conducted in accordance with

217 the Declaration of Helsinki and national laws and regulations and declared on the
218 clinictrials.gov registry (NCT 04315948) and on the European Clinical Trials Database
219 (2020-000936-23).

220 ***Study population***

221 Eligible participants were adults (≥ 18 -year-old) hospitalized with a PCR-proven (< 72 hours)
222 SARS-CoV-2 infection and pulmonary rales or crackles with a peripheral oxygen saturation \leq
223 94% or requiring supplemental oxygen. Inclusion and exclusion criteria are presented in the
224 Supplementary Appendix.

225 ***Interventions and randomization***

226 Participants were randomly assigned to treatment arms in a 1:1:1:1 ratio, through computer-
227 generated blocks of various sizes and stratification by administrative region and severity of
228 disease at enrolment (moderate: hospitalized participants not requiring oxygen or receiving
229 low-flow supplemental oxygen; severe: hospitalized participants requiring non-invasive
230 ventilation or high-flow oxygen devices, invasive mechanical ventilation or extracorporeal
231 membrane oxygenation (ECMO)). Randomization was implemented in the electronic Case
232 Report Form to ensure appropriate allocation concealment. Investigational arms were standard
233 of care (SoC, control), SoC plus lopinavir/ritonavir (400 mg lopinavir and 100 mg ritonavir
234 orally twice on day for 14 days [3,14]), SoC plus lopinavir/ritonavir plus IFN- β -1a (44 μ g of
235 subcutaneous IFN- β -1a on days 1, 3, and 6), SoC plus hydroxychloroquine (400 mg orally,
236 twice on day 1 as a loading dose followed by 400 mg once daily for 9 days) [15]. Supportive
237 treatments corticosteroids, anticoagulants or immunomodulatory agents were allowed except
238 antivirals. Enrolment in another investigative trial was not allowed.

239 ***Clinical and laboratory monitoring***

240 Participants were assessed at days 3, 5, 8, 11, 15 ± 2 and 29 ± 3 while hospitalized. If discharge
241 occurred before day 15, face-to-face visits were set up for days 15 ± 2 and 29 ± 3 visits, for

242 efficacy and safety evaluations. Clinical data, concomitant medications, adverse events (AEs)
243 and measurements for safety biological data (blood cell counts, serum creatinine and liver
244 aminotransferases) were collected. Nasopharyngeal swab (NPS) and lower respiratory tract
245 (LRT) specimens were collected for SARS-CoV-2 RNA quantification. For lopinavir and
246 ritonavir, trough plasma concentrations were obtained at days 1 and 3, 12h (\pm 2h) after the last
247 administration and for hydroxychloroquine, at day 1 12h (\pm 2h) and at day 3 24h (\pm 4h) after
248 the last administration.

249 ***Outcomes measures***

250 The primary outcome measure was the clinical status at day 15 as measured on the 7-point
251 ordinal scale of the WHO Master Protocol (v3.0, March 3, 2020): 1. Not hospitalized, no
252 limitation on activities; 2. Not hospitalized, limitation on activities; 3. Hospitalized, not
253 requiring supplemental oxygen; 4. Hospitalized, requiring supplemental oxygen; 5.
254 Hospitalized, on non-invasive ventilation or high flow oxygen devices; 6. Hospitalized, on
255 invasive mechanical ventilation or ECMO; 7. Death.

256 Secondary efficacy outcome measures were the clinical status at day 29 and the time to an
257 improvement of 2 categories as measured on the 7-point ordinal scale or hospital discharge
258 until day 29, the time to National Early Warning Score 2 (NEWS2) \leq 2 or hospital discharge
259 until day 29, the time to hospital discharge until day 29, oxygenation- and ventilator-free days
260 until day 29, 29-day mortality, and the SARS-CoV-2 detection and quantitative normalized
261 viral loads. Trough plasma concentrations of lopinavir, ritonavir and hydroxychloroquine
262 were measured at days 1 and 3. Secondary safety outcomes included the cumulative incidence
263 of any grade 3 or 4 AE, or of any serious adverse event (SAE, according to the DAIDS Table
264 for Grading the Severity of Adult and Paediatric Adverse Events, v2.1, July 2017) and the
265 proportion of patients with a premature suspension or discontinuation for any reason of the
266 investigational treatments.

267 ***Virological methods***

268 Determination of normalized viral load blinded to treatment arm was performed on NPS and
269 LRT specimens by RNA extraction on the EMAG® platform (bioMérieux, Marcy-l'Étoile,
270 France). The SARS-CoV-2 load was measured by quantitative RT-PCR, according to a scale
271 of calibrated in-house plasmid, using the RT-PCR RdRp-IP4 developed by the Institut Pasteur
272 (Paris, France) [16]. The amplification protocol was developed using QuantStudio 5 rtPCR
273 Systems (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The number of cells in
274 sample (quality criteria for NPS and normalization tool for viral load determination) was
275 checked using the CELL Control r-gene® kit (Argene-BioMérieux, Marcy-l'Étoile, France). If
276 cell quantification was below 500 cells/reaction, the quality of the sample was considered too
277 low to be measured. We computed a normalized SARS-CoV-2 load by dividing the viral load
278 by the number of cells. All viral loads strictly below 1 log₁₀ RNA copies/10 000 cells were
279 considered under the limit of detection and were reported as negative.

280 ***Pharmacological methods***

281 Plasma concentrations of lopinavir, ritonavir and hydroxychloroquine were determined using
282 liquid chromatography coupled with tandem mass spectrometry [17,18]. The limits of
283 quantification were 30 ng/mL for lopinavir and ritonavir, and 10 ng/mL for
284 hydroxychloroquine.

285 ***Sample size calculation***

286 The sample size was determined assuming the following scenario under SoC for each item of
287 the ordinal scale at day 15: 1, 42%; 2, 38%; 3, 8%; 4, 7%; 5, 2%; 6, 1%; 7, 2%. At the time of
288 the trial design, there was a significant uncertainty with these assumptions. We powered the
289 study for an odds ratio of 1.5 (an odds ratio higher than 1 indicates superiority of the
290 experimental treatment over the control for each ordinal scale category), with 90% power and
291 using an overall two-sided type I error rate of 0.05. Adjusting for multiplicity of 4 pairwise

292 comparisons with the control arm in a 5-arm setting, the two-sided false positive error rate
293 would be 0.0125. We determined that the inclusion of 620 patients in each treatment arm was
294 required.

295 *Statistical and interim analyses*

296 An independent data safety and monitoring board (DSMB) externally reviewed the trial data
297 periodically. Based on interim analyses (see Supplementary Appendix), enrolment in the
298 hydroxychloroquine arm was prematurely stopped on June 17th, and enrolment in lopinavir-
299 containing arms was stopped on June 29th 2020.

300 For the 7-point ordinal scale, data were analyzed using a proportional odds model, which
301 assumes a common odds ratio between the 7 points of the ordinal scale. All analyses were
302 stratified by severity at randomization, and adjusted effect measures are reported. Full
303 statistical methods are presented in Supplementary Appendix.

304 **Results**

305 *Patient's characteristics at baseline*

306 Between March 22nd and June 29th, 603 participants were randomized across 30 sites in
307 France and 2 in Luxembourg; 583 were evaluable for analysis (Supplementary Figure S1):
308 control arm, n=148; lopinavir/ritonavir arm, n=145; lopinavir/ritonavir plus IFN- β -1a arm,
309 n=145; hydroxychloroquine arm, n=145. Participants' baseline characteristics are presented in
310 Table 1. Participants were mostly male (n=418, 71.7%), median age was 63 years (IQR, 54-
311 71). The median time from symptoms onset to randomization was 9 days (IQR, 7-12). The
312 most frequent underlying conditions were obesity (n=166, 28.7%), chronic cardiac disease
313 (n=151, 26.0%) and diabetes mellitus (n=128, 22.0%). At baseline, severe disease accounted
314 for 211 (36.2%) participants. Concomitant treatments are listed in Supplementary Table 1.

315 *Primary endpoint*

316 The distribution of the 7-point ordinal scale at day 15 is presented in Figure 1 and Table 2.
317 Adjusted OR for clinical improvement (aOR) were not in favor of investigational treatments
318 (*i.e.*, below 1): lopinavir/ritonavir *versus* control, aOR 0.83 (95% confidence interval [CI]
319 0.55-1.26, P=0.39); lopinavir/ritonavir plus IFN- β -1a *vs.* control, aOR 0.69 (95%CI 0.45-1.04,
320 P=0.08); hydroxychloroquine *vs.* control, aOR 0.93 (95%CI 0.62-1.41, P=0.75).

321 ***Secondary endpoints***

322 There was no significant difference between any of the treatment and control arms on the 7-
323 point ordinal scale at day 29 (Figure 1 and Table 2). The time to improvement of 2 categories
324 of the same scale or hospital discharge within day 29 was significantly higher in
325 lopinavir/ritonavir-containing arms than in the control arm: lopinavir/ritonavir *versus* control,
326 HR=0.71 (95%CI 0.54-0.93, P=0.012 and lopinavir/ritonavir plus IFN- β -1a *versus* control,
327 HR=0.70 (95%CI 0.54-0.92, P=0.009). The time to NEWS \leq 2 or hospital discharge within 29
328 days was significantly higher in the lopinavir/ritonavir plus IFN- β -1a arm than in the control
329 arm (HR=0.75, 95%CI 0.56-0.99, P=0.046), as was the time to hospital discharge within day
330 29 (HR=0.72, 95%CI 0.54-0.96, P=0.026). No other significant difference was observed for
331 other secondary outcomes (Table 2 and Supplementary Figures S2-S4).

332 ***Virological endpoints***

333 The slope of the decrease of the viral loads in NPS over time was not significantly affected by
334 any of the investigational treatments (Figure 2). No significant difference in the proportion of
335 participants with detectable viral loads at each sampling time was observed in the NPS nor in
336 the LRT specimens (Supplementary Table S2 and S3).

337 ***Trough concentrations of experimental treatments***

338 At day 3, median trough plasma concentrations of lopinavir were 20 328 ng/mL (IQR,
339 13 033-26 640) and 20 028 ng/mL (15 290-25 718) and of ritonavir were 536 ng/mL (312-1
340 010) and 606 ng/mL (388-1 070) in the lopinavir/ritonavir and in the lopinavir/ritonavir plus

341 IFN- β -1a, respectively (Supplementary Table S4). Median trough plasma concentrations of
342 hydroxychloroquine were 120 ng/mL (65-271).

343 ***Safety***

344 The safety analysis included 579 participants (control, n=148; lopinavir/ritonavir, n=144;
345 lopinavir/ritonavir plus IFN- β -1a, n=144; hydroxychloroquine, n=143). Safety outcomes are
346 presented in Table 3. Among 2399 reported AEs, 477 were graded 3 or 4 in 205 patients and
347 mostly reported in lopinavir/ritonavir-containing arms (Table 3).

348 A total of 608 SAEs were reported in 274 participants; 149 (24.5%) were related to the
349 investigational drug according to investigator's judgment (lopinavir/ritonavir arm, n=37;
350 lopinavir/ritonavir plus IFN- β -1a arm, n=71; hydroxychloroquine arm, n=41). A significantly
351 higher number of patients experienced at least one SAE in the lopinavir/ritonavir-containing
352 arms than in the control arm (Table 3). The most frequently reported SAEs were acute
353 respiratory failure (n=65, 11%), acute kidney injury (n=50, 8.2%), acute respiratory distress
354 syndrome (n=47, 8%), arrhythmia (n=41, 7%), pulmonary embolism (n=27, 5%), and sepsis
355 including those related to super-infections (n=21, 4%). Thirteen percent (n=76) of participants
356 developed at least one kidney-related SAE. Among them, 12 had acute renal failure upon
357 admission, and 66 were critically-ill ventilated patients with acute kidney injury. Among 57
358 fatal SAEs, 23 had a pulmonary origin, and 34 had a non-pulmonary origin. Four non-
359 pulmonary-related deaths were linked to investigational treatments by investigators
360 (lopinavir/ritonavir arm, n=1; lopinavir/ritonavir plus IFN- β -1a arm, n=3).

361 **Discussion**

362 We report here the results of the DisCoVeRy clinical trial, evaluating lopinavir/ritonavir with
363 or without IFN- β -1a, or hydroxychloroquine in comparison with control for the treatment of
364 inpatients with COVID-19. Participants had mostly moderate disease (63.4%) covering a
365 large spectrum of clinical presentations. Inclusions were prematurely stopped for futility, so

366 that the number of included patients is lower than the estimated sample size. Consistently with
367 Solidarity results, investigational treatments failed to improve the clinical course of COVID-
368 19. No effect on SARS-CoV-2 clearance was observed, using a reproducible normalized
369 method. Furthermore, significantly more SAEs were reported in the lopinavir/ritonavir-
370 containing arms than in the control arm.

371 Two randomized trials conducted in hospitalized COVID-19 patients found no benefit of
372 lopinavir/ritonavir in terms of 28-day mortality or of progression to mechanical ventilation or
373 death [9,19]. No added benefit was observed using IFN- β -1a, as the median time to
374 randomization of 9 days may have been too long to allow an immune-mediated boosting
375 effect on viral clearance. We observed plasma overexposure of lopinavir relative to target
376 concentrations obtained in HIV-infected patients, possibly responsible for the higher rate of
377 SAEs and more acute kidney injury than controls. The SARS-CoV-2-induced inflammatory
378 burden may have reduced Cytochrome P450 activity and modified plasma α -1-acid
379 glycoprotein levels, an acute phase protein which binds protease inhibitors [20,21]. Reported
380 *in-vitro* half maximal inhibitory concentration (EC50) for SARS-CoV2 is 16,400 ng/mL [22]
381 (while the EC50 for HIV is 70 ng/mL [23]), an over 200-fold difference, suggesting that
382 significantly higher concentrations of lopinavir are needed to enhance SARS-CoV-2
383 clearance. A recent physiologically-based pharmacokinetic model suggested that standard
384 regimens of lopinavir/ritonavir are not sufficient to achieve efficacy through unbound lung
385 concentrations [24]. In our study, trough lopinavir plasma concentrations at day 3 were more
386 than 2-fold higher than expected with the standard dose [25], but were below the EC50 of
387 SARS-CoV2 in 25% of participants.

388 Several larger-scale randomized controlled trials conducted in hospitalized COVID-19
389 patients failed to demonstrate the clinical efficacy of hydroxychloroquine [26,27] . Our results
390 are in line with these conclusions. We report that hydroxychloroquine does not accelerate

391 SARS-CoV-2 clearance, consistently with preclinical data [28]. Based on *in vitro* EC50
392 against SARS-CoV-2 (242 ng/mL), the target plasma concentration was reached in only 25%
393 of participants at day 3, and optimal intrapulmonary exposure might have been only achieved
394 at day 10 [10,15]. It could be argued that the dosing regimen administered in the DisCoVeRy
395 trial was insufficient to rapidly reach target concentrations. However, Solidarity and Recovery
396 trials, which both used a doubled hydroxychloroquine dosing regimen, did not bring evidence
397 of clinical benefit either [13,27].

398 The trial has limitations: the complexity of blinding treatments with different routes of
399 administration and the need to initiate the trial very rapidly led to choose an open-labelled
400 design. The trial did not target patients at the early phase of the disease nor include arms
401 testing anti-inflammatory agents that could be used as part of the SoC in any arm. In addition,
402 the trial was performed in the early phase of the COVID-19 pandemics and the SoC
403 underwent substantial changes over time, adapting to knowledge acquisition, especially
404 regarding the use of corticosteroids in COVID-19.

405 **Conclusion**

406 In patients admitted to hospital with COVID-19, lopinavir/ritonavir, lopinavir/ritonavir plus
407 IFN- β -1a and hydroxychloroquine were not associated with clinical improvement at day 15
408 and day 29, nor reduction in viral shedding, and generated significantly more SAEs in
409 lopinavir/ritonavir-containing arms. These findings do not support the use of these
410 investigational treatments for patients hospitalized with COVID-19.

411 **Authors contribution**

412 Writing – Original Draft: FA, CB; Writing – Review & Editing: NPS, JP, MBD, DB, ADi,
413 MH, MPL, GP, DC, YY, FM; Conceptualization: FA, NPS, JP, MBD, GP, BL, DC, YY, FM;
414 Investigation: FA, NPS, JP, MBD, Adi, NM, FXL, FR, FG, AK, SJ, JR, SN, FD, RCJ, KB,
415 JCN, VT, AC, CDu, JC, SL, JM, RG, BM, EF, VP, SG, OL, KL, JPL, AM, GMB, LB, ÉBN,
416 AGB, OE, LP, FW, JCR, JR, TS, MH, CA, MPL, GP; Methodology: FA, NPS, JP, MBD,
417 DC, CB, FM; Data curation: ADi, ADe, NM, ADu, TA; Formal Analysis: DB, ADu, DC, CB,
418 FM
419 Project Administration: FA, CD, FM; Funding Acquisition: FA, CDe, JS, DC, YY, FM.

420

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427

428 **Declaration of interests**

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560

1 **Figures Legends**

2 **Figure 1. Clinical status, as measured by the 7-point ordinal scale, at day 15 and day 29**
3 **of patients from the intention-to-treat population of the DisCoVeRy trial, according to**
4 **treatment arm and disease severity at baseline.**

5 Reported numbers refer to the proportion of patients with the corresponding level in each group.
6 L/r, Lopinavir/ritonavir; L/r + IFN, Lopinavir/ritonavir + interferon β -1a; HCQ,
7 Hydroxychloroquine.

8

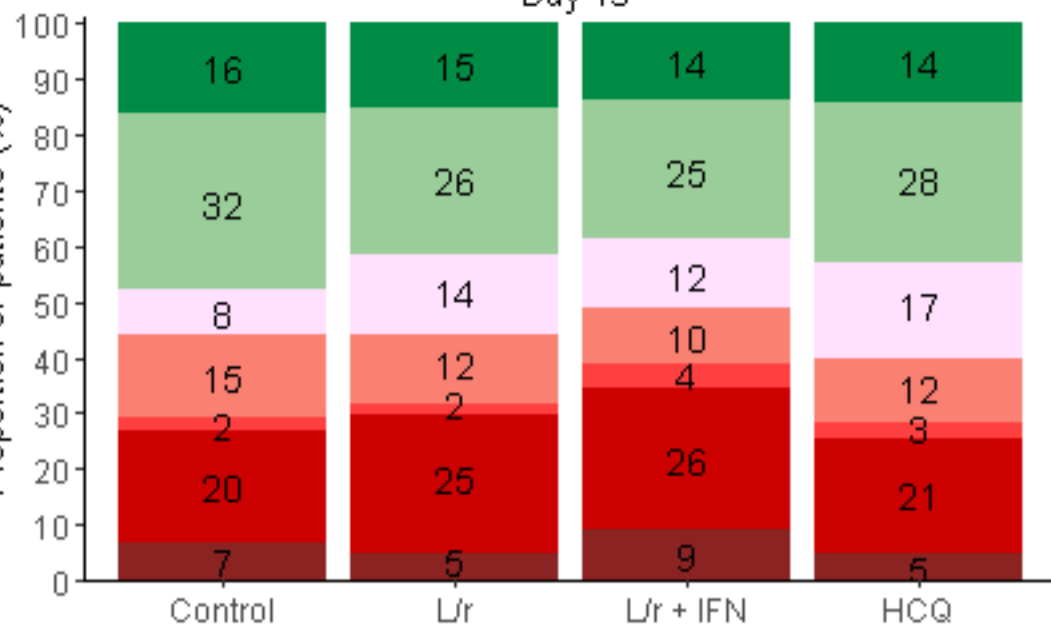
9 **Figure 2. Evolution of the normalized SARS-CoV-2 viral load in nasopharyngeal swabs**
10 **between baseline and day 15 in the intention-to-treat population of the DisCoVeRy trial:**
11 **means (95% CI) of the log viral loads (panel A), mean changes from baseline (95% CI) of**
12 **the log viral loads (panel B).**

13 L/r, Lopinavir/ritonavir (blue line); L/r + IFN, Lopinavir/ritonavir + interferon β -1a (yellow
14 line); HCQ, Hydroxychloroquine (red line); control (black line).

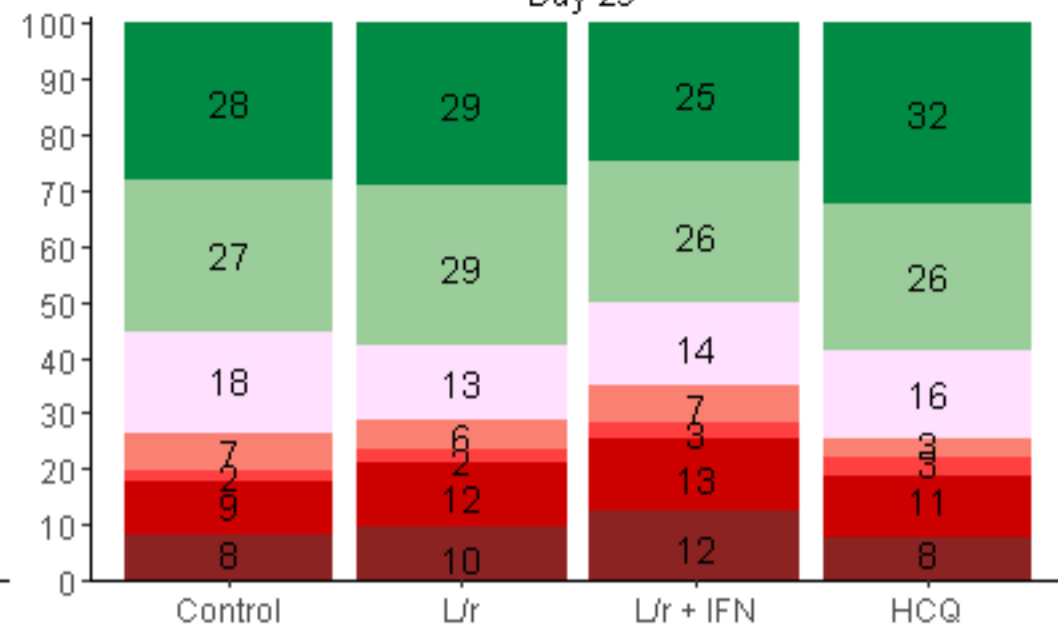
15 LSMD, least-square mean difference; 95%CI, 95% confidence interval.

16

All patients
Day 15

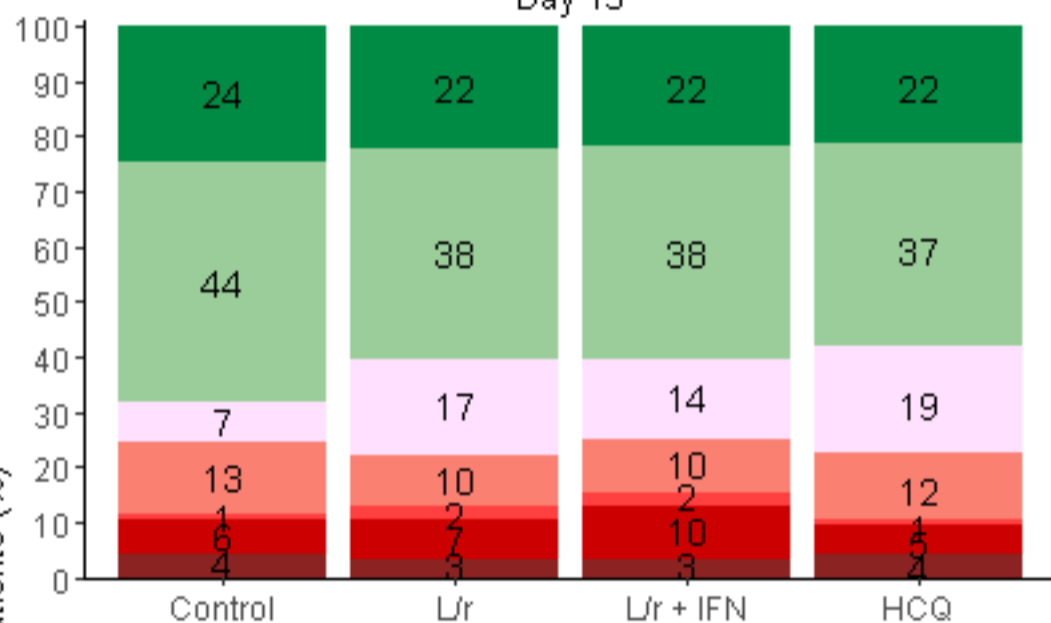


All patients
Day 29



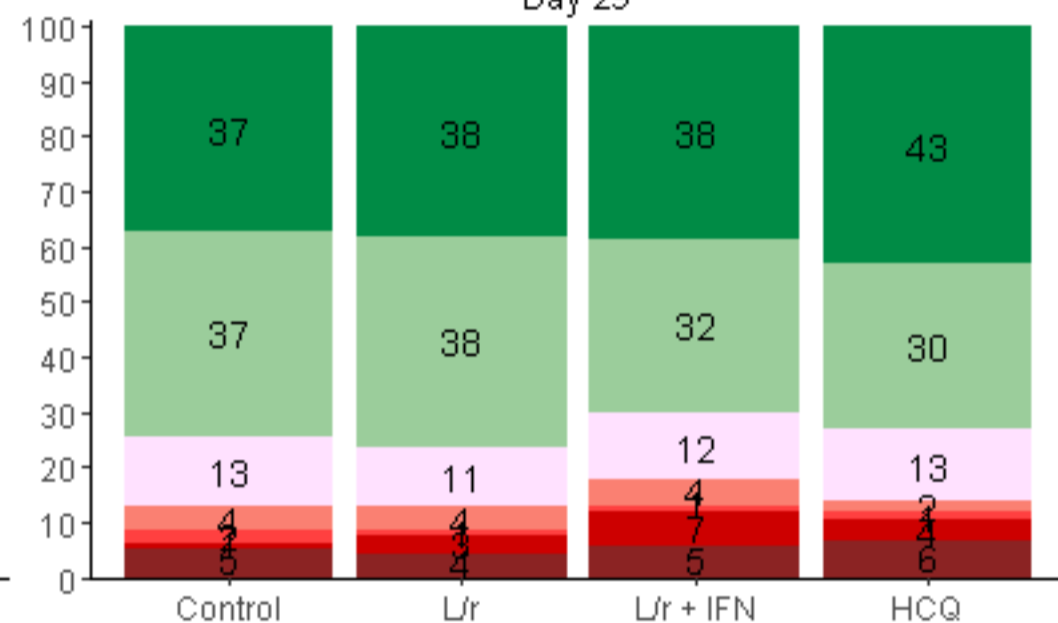
Moderate disease

Day 15



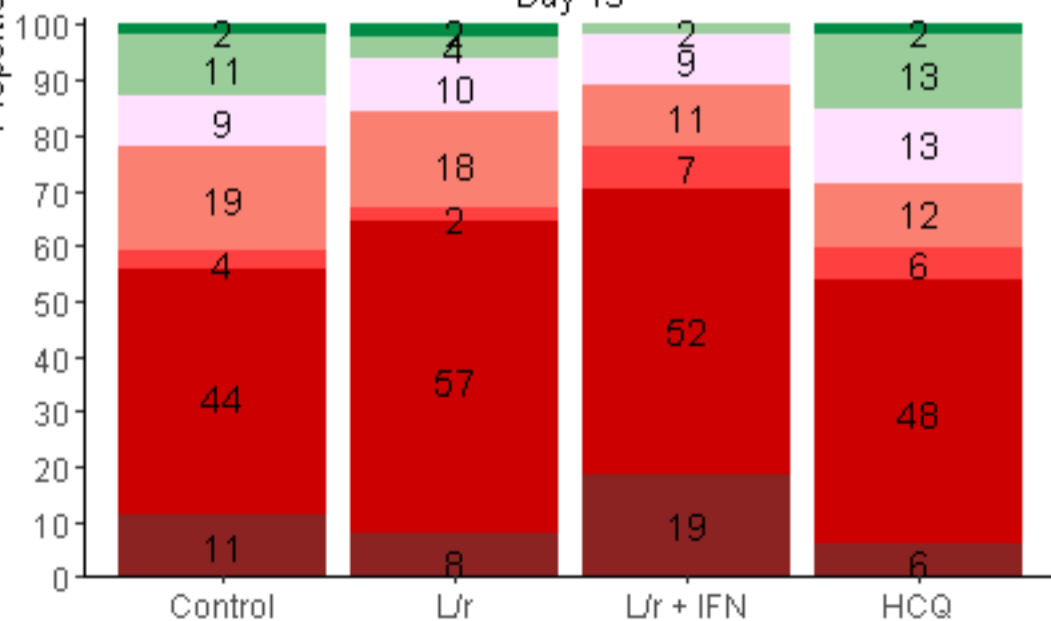
Moderate disease

Day 29



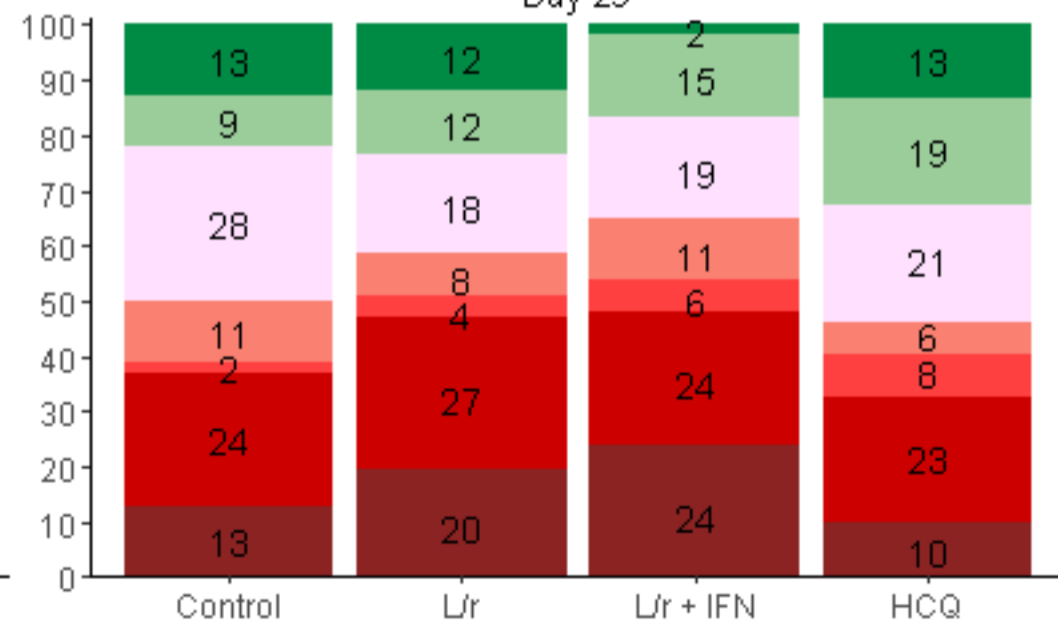
Severe disease

Day 15



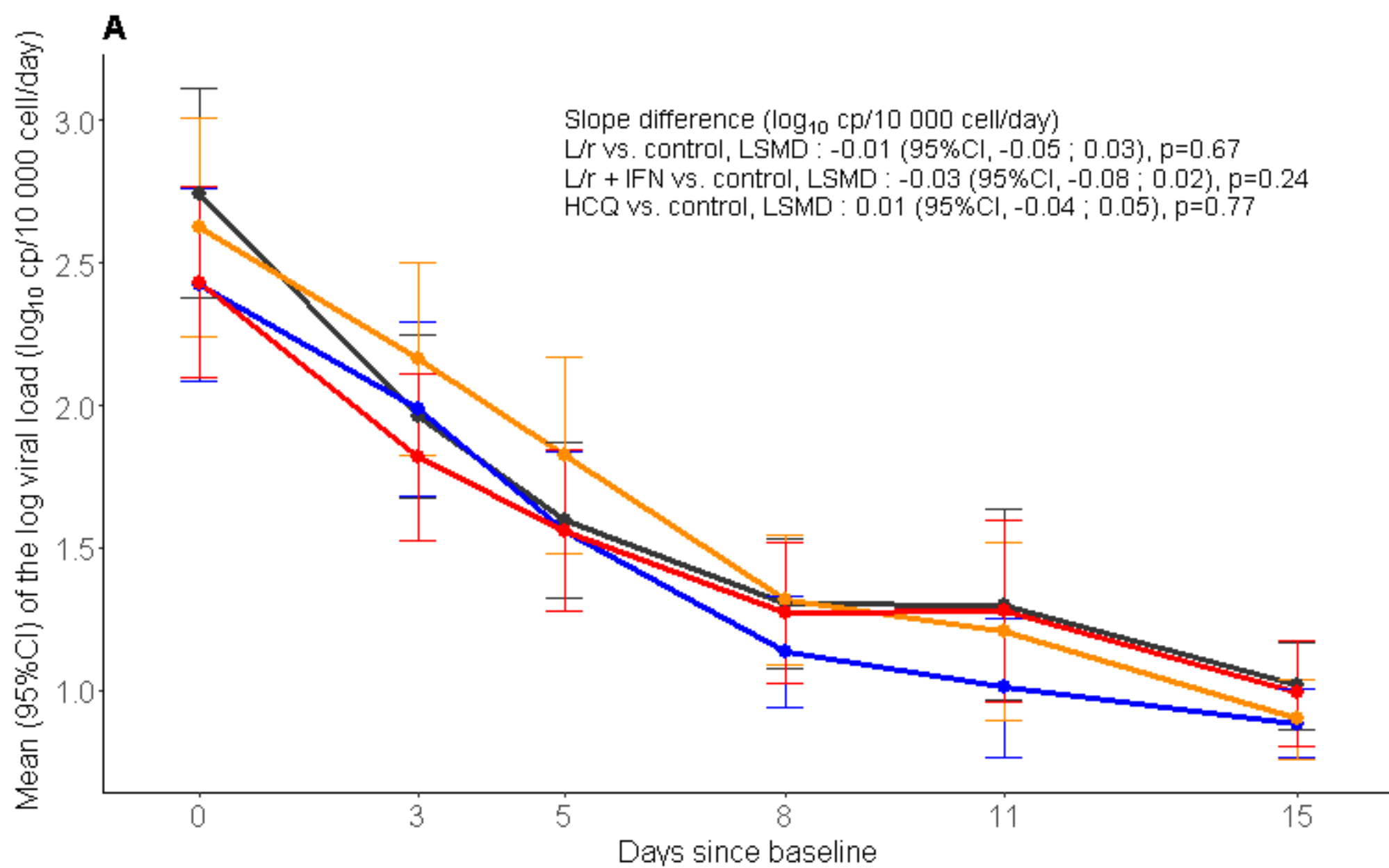
Severe disease

Day 29



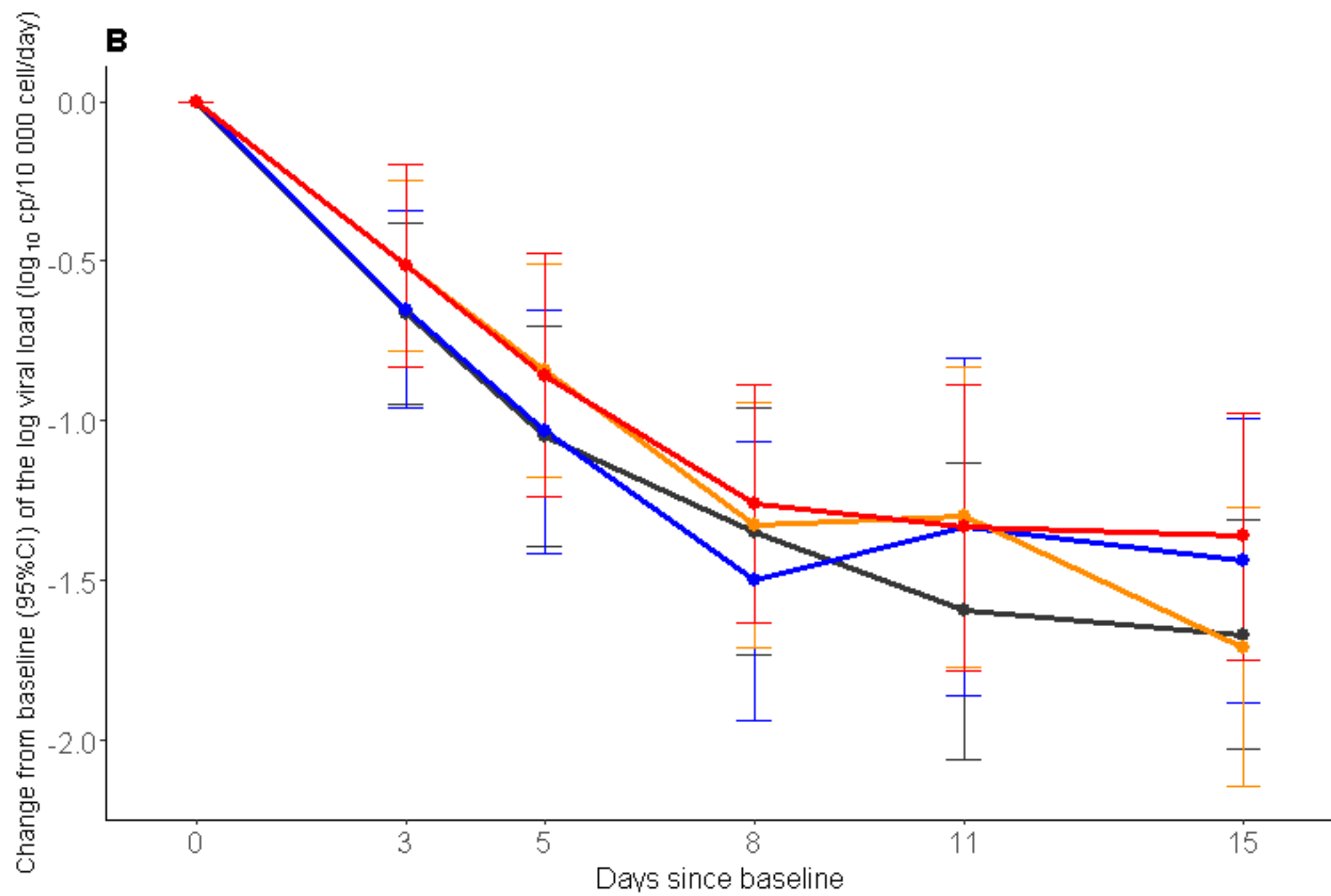
7-point
Ordinal Scale

- 1. Not hospitalized, no limitations on activities
- 2. Not hospitalized, limitations on activities
- 3. Hospitalized, not requiring supplemental oxygen
- 4. Hospitalized, requiring supplemental oxygen
- 5. Hospitalized, on non-invasive ventilation or high flow oxygen devices
- 6. Hospitalized, on invasive mechanical ventilation or ECMO
- 7. Death



Number of viral loads

Control	87	105	95	82	57	101
L/r	88	99	92	82	56	96
L/r + IFN	79	104	89	82	54	85
HCQ	95	97	88	79	62	95



Number of viral loads

Control	87	73	66	59	39	68
L/r	88	68	64	56	40	64
L/r + IFN	79	68	56	54	38	57
HCQ	95	71	67	59	47	72

Table 1. Baseline characteristics of patients included in the intention to treat population of the present analysis of DisCoVeRy trial.

NPS, Nasopharyngeal swabs; LRT, Lower respiratory tract.

* denotes variables with missing data. Data on chronic cardiac disease, chronic pulmonary disease, mild liver disease, chronic neurological disorder, active cancer and diabetes mellitus were missing for 2 patients; data on chronic kidney disease were missing for 3 patients; data on auto-inflammatory disease were missing for 1 patient; data on obesity were missing for 5 patients; data on smoking status were missing for 30 patients; data on the time from symptoms onset to randomization were missing for 8 patients; data on BMI were missing for 83 patients; data on randomization site were missing for 1 patient; data on viral load from NPS were missing for 234 patients; data on viral load from LRT specimens were missing for 527 patients; data for lymphocyte count were missing for 90 patients; data for neutrophil count were missing for 136 patients; data on creatinine were missing for 15 patients; data on AST/SGOT were missing for 56 patients; data on ALT/SGPT were missing for 51 patients; data on CRP were missing for 137 patients; data on D-Dimers were missing for 299 patients; data on PCT were missing for 356 patients; data on ferritin were missing for 421 patients.

** moderate disease: hospitalized participants receiving low-flow supplemental oxygen or not requiring oxygen; severe disease: hospitalized participants requiring non-invasive ventilation or high-flow oxygen devices, invasive mechanical ventilation or ECMO

	Overall (N=583)	Control (N=148)	Lopinavir/ritonavir (L/r) (N=145)	Lopinavir/Ritonavir + Interferon β-1a (L/r + IFN) (N=145)	Hydroxychloroquine (HCQ) (N=145)
Median age — yr [IQR]	63 [54-71]	62 [52-71]	63 [55-71]	64 [53-71]	65 [55-71]
Male sex — no. (%)	418 (71.7%)	105 (70.9%)	106 (73.1%)	103 (71.0%)	104 (71.7%)
Coexisting condition* — no. (%)					
- Chronic cardiac disease	151 (26.0%)	39 (26.4%)	35 (24.1%)	36 (25.2%)	41 (28.3%)
- Chronic pulmonary disease	88 (15.1%)	31 (20.9%)	19 (13.1%)	19 (13.3%)	19 (13.1%)
- Chronic kidney disease (stage 1 to 3)	24 (4.1%)	7 (4.7%)	2 (1.4%)	5 (3.5%)	10 (6.9%)
- Mild liver disease	13 (2.2%)	6 (4.1%)	3 (2.1%)	0 (0.0%)	4 (2.8%)

	Overall (N=583)	Control (N=148)	Lopinavir/ritonavir (L/r) (N=145)	Lopinavir/Ritonavir + Interferon β-1a (L/r + IFN) (N=145)	Hydroxychloroquine (HCQ) (N=145)
- Chronic neurological disorder (including dementia)	23 (4.0%)	6 (4.1%)	5 (3.4%)	4 (2.8%)	8 (5.5%)
- Active cancer	35 (6.0%)	10 (6.8%)	8 (5.5%)	6 (4.1%)	11 (7.6%)
- Auto-inflammatory disease	26 (4.5%)	8 (5.4%)	4 (2.8%)	9 (6.3%)	5 (3.4%)
- Obesity	166 (28.7%)	46 (31.3%)	36 (24.8%)	41 (28.7%)	43 (30.1%)
- Diabetes mellitus	128 (22.0%)	35 (23.6%)	35 (24.1%)	27 (18.9%)	31 (21.4%)
- Current smoker	18 (3.3%)	5 (3.5%)	4 (2.9%)	5 (3.6%)	4 (3.0%)
Median time from symptom onset to randomization* — days [IQR]	9.0 [7.0-12.0]	10.0 [7.0-12.0]	10.0 [7.0-13.0]	10.0 [7.0-12.0]	8.0 [7.0-11.0]
Baseline severity of COVID-19** — no. (%)					
- Moderate	372 (63.8%)	94 (63.5%)	94 (64.8%)	91 (62.8%)	93 (64.1%)
- Severe	211 (36.2%)	54 (36.5%)	51 (35.2%)	54 (37.2%)	52 (35.9%)
Randomization site* — no. (%)					
- ICU	254 (43.6%)	64 (43.2%)	65 (44.8%)	65 (45.1%)	60 (41.4%)
- Conventional unit (e.g. : infectious disease unit, internal medicine, pneumology)	328 (56.4%)	84 (56.8%)	80 (55.2%)	79 (54.9%)	85 (58.6%)
7-point ordinal scale at baseline — no. (%)					
- 3. Hospitalized, not requiring supplemental oxygen	27 (4.6%)	8 (5.4%)	4 (2.8%)	9 (6.2%)	6 (4.1%)
- 4. Hospitalized, requiring supplemental oxygen	341 (58.5%)	84 (56.8%)	88 (60.7%)	84 (57.9%)	85 (58.6%)
- 5. Hospitalized, on non-invasive ventilation or high flow oxygen devices	63 (10.8%)	21 (14.2%)	15 (10.3%)	13 (9.0%)	14 (9.7%)
- 6. Hospitalized, on invasive mechanical ventilation or ECMO	152 (26.1%)	35 (23.6%)	38 (26.2%)	39 (26.9%)	40 (27.6%)

	Overall (N=583)	Control (N=148)	Lopinavir/ritonavir (L/r) (N=145)	Lopinavir/Ritonavir + Interferon β-1a (L/r + IFN) (N=145)	Hydroxychloroquine (HCQ) (N=145)
Median NEWS-2 at baseline*, median [IQR]	9.0 [7.0-12.0]	9.0 [7.0-12.0]	9.0 [7.0-11.0]	10.0 [7.0-12.0]	9.0 [6.0-11.0]
Median viral load at baseline, median [IQR]					
- on NPS (log10 copies/10 000 cells)	2.4 [0.7-3.7] (n=349)	2.5 [1.1-3.9] (n=87)	2.4 [0.7-3.6] (n=88)	2.5 [0.7-3.8] (n=79)	2.0 [0.7-3.4] (n=95)
- on LRT specimens (log10 copies/10 000 cells)	4.1 [2.8-4.9] (n=56)	3.6 [2.4-4.5] (n=14)	4.4 [3.2-4.8] (n=14)	3.5 [1.0-4.8] (n=10)	4.3 [3.3-5.5] (n=18)
Biological data at baseline*, median [IQR]					
- Minimal lymphocytes count (G/L)	0.9 [0.6-1.2]	0.9 [0.6-1.4]	0.8 [0.6-1.2]	0.9 [0.7-1.3]	0.9 [0.6-1.1]
- Maximal neutrophils count (G/L)	5.8 [4.0-7.9]	5.7 [4.1-7.8]	6.3 [4.3-8.0]	5.7 [3.9-8.3]	5.6 [3.8-7.8]
- Maximal plasma creatinine (μmol/L)	74.0 [62.0-91.0]	72.5 [60.0-88.0]	73.5 [62.0-88.0]	77.0 [65.0-91.0]	74.0 [62.0-93.0]
- Maximal SGOT (U/L)	49.0 [35.0-72.0]	53.0 [38.0-74.0]	47.0 [34.0-64.0]	47.0 [35.0-70.0]	53.5 [34.0-81.0]
- Maximal SGPT (U/L)	37.0 [25.0-63.0]	41.0 [25.0-62.0]	34.0 [22.5-60.5]	37.0 [24.0-59.0]	41.5 [26.0-67.0]
- Maximal plasma C-reactive protein (mg/L)	119.5 [72.0- 185.0]	132.0 [86.0- 191.0]	124.0 [75.0-188.0]	105.0 [57.0-164.0]	118.0 [72.0-188.0]
- Maximal plasma D-dimers (μg/L)	1080.0 [649.0- 1860.0]	1170.0 [689.0- 2000.0]	1060.0 [626.0-1987.0]	956.0 [560.0-1673.0]	1140.0 [654.0-1820.0]
- Maximal procalcitonin (ng/mL)	0.2 [0.1-0.9]	0.3 [0.1-1.1]	0.2 [0.1-0.6]	0.3 [0.1-0.9]	0.3 [0.1-0.9]
- Maximal ferritin (mg/L)	480.5 [2.0- 1344.0]	98.0 [2.0- 1041.0]	608.0 [2.0-1288.0]	761.0 [3.0-1344.0]	377.0 [2.0-1610.0]

Table 2. Primary and secondary outcomes for patients included in the present analysis DisCoVeRy trial, according to disease severity at baseline.

Analyses were stratified on the disease severity at baseline (moderate: 7-point ordinal scale 3 or 4; severe: 7-point ordinal scale 5 or 6), and adjusted effect measures are reported in the table. NP, Nasopharyngeal; LRT, Lower respiratory tract; OR, Odds-ratio; HR, Hazard ratio; LSMD, least-square mean difference.

	Overall (N=583)		Control (N=148)		Lopinavir/ritonavir (L/r) (N=145)		Lopinavir/ritonavir + interferon β-1a (L/r + IFN) (N=145)		Hydroxychloroquine (HCQ) (N=145)		L/r vs. control Effect measure (95% CI)	L/r + IFN vs. control Effect measure (95% CI)	HCQ vs. control Effect measure (95% CI)
	Moderate (N=372)	Severe (N=211)	Moderate (N=94)	Severe (N=54)	Moderate (N=94)	Severe (N=51)	Moderate (N=91)	Severe (N=54)	Moderate (N=93)	Severe (N=52)			
7-point ordinal scale at day 15, n (%)													
1. Not hospitalized, no limitations on activities	84 (22.6%)	3 (1.4%)	23 (24.5%)	1 (1.9%)	21 (22.3%)	1 (2.0%)	20 (22.0%)	0 (0.0%)	20 (21.5%)	1 (1.9%)	OR=0.83 (0.55 to 1.26) [P=0.39]	OR=0.69 (0.45 to 1.04) [P=0.08]	OR=0.93 (0.62 to 1.41) [P=0.75]
2. Not hospitalized, limitation on activities	146 (39.2%)	16 (7.6%)	41 (43.6%)	6 (11.1%)	36 (38.3%)	2 (3.9%)	35 (38.5%)	1 (1.9%)	34 (36.6%)	7 (13.5%)			
3. Hospitalized, not requiring supplemental oxygen	54 (14.5%)	22 (10.4%)	7 (7.4%)	5 (9.3%)	16 (17.0%)	5 (9.8%)	13 (14.3%)	5 (9.3%)	18 (19.4%)	7 (13.5%)			
4. Hospitalized, requiring supplemental oxygen	41 (11.0%)	31 (14.7%)	12 (12.8%)	10 (18.5%)	9 (9.6%)	9 (17.6%)	9 (9.9%)	6 (11.1%)	11 (11.8%)	6 (11.5%)			
5. Hospitalized, on non-invasive ventilation or high flow oxygen devices	6 (1.6%)	10 (4.7%)	1 (1.1%)	2 (3.7%)	2 (2.1%)	1 (2.0%)	2 (2.2%)	4 (7.4%)	1 (1.1%)	3 (5.8%)			
6. Hospitalized, on invasive mechanical ventilation or ECMO	27 (7.3%)	106 (50.2%)	6 (6.4%)	24 (44.4%)	7 (7.4%)	29 (56.9%)	9 (9.9%)	28 (51.9%)	5 (5.4%)	25 (48.1%)			
7. Death	14 (3.8%)	23 (10.9%)	4 (4.3%)	6 (11.1%)	3 (3.2%)	4 (7.8%)	3 (3.3%)	10 (18.5%)	4 (4.3%)	3 (5.8%)			
7-point ordinal scale at day 29, n (%)													
1. Not hospitalized, no limitations on activities	146 (39.2%)	21 (10.0%)	35 (37.2%)	7 (13.0%)	36 (38.3%)	6 (11.8%)	35 (38.5%)	1 (1.9%)	40 (43.0%)	7 (13.5%)	OR=0.93 (0.62 to 1.41) [P=0.74]	OR=0.76 (0.50 to 1.15) [P=0.19]	OR=1.16 (0.77 to 1.75) [P=0.49]
2. Not hospitalized, limitation on activities	128 (34.4%)	29 (13.7%)	35 (37.2%)	5 (9.3%)	36 (38.3%)	6 (11.8%)	29 (31.9%)	8 (14.8%)	28 (30.1%)	10 (19.2%)			
3. Hospitalized, not requiring supplemental oxygen	45 (12.1%)	45 (21.3%)	12 (12.8%)	15 (27.8%)	10 (10.6%)	9 (17.6%)	11 (12.1%)	10 (18.5%)	12 (12.9%)	11 (21.2%)			
4. Hospitalized, requiring supplemental oxygen	14 (3.8%)	19 (9.0%)	4 (4.3%)	6 (11.1%)	4 (4.3%)	4 (7.8%)	4 (4.4%)	6 (11.1%)	2 (2.2%)	3 (5.8%)			

	Overall (N=583)		Control (N=148)		Lopinavir/ritonavir (L/r) (N=145)		Lopinavir/ritonavir + interferon β-1a (L/r + IFN) (N=145)		Hydroxychloroquine (HCQ) (N=145)		L/r vs. control Effect measure (95% CI)	L/r + IFN vs. control Effect measure (95% CI)	HCQ vs. control Effect measure (95% CI)
	Moderate (N=372)	Severe (N=211)	Moderate (N=94)	Severe (N=54)	Moderate (N=94)	Severe (N=51)	Moderate (N=91)	Severe (N=54)	Moderate (N=93)	Severe (N=52)			
5. Hospitalized, on non-invasive ventilation or high flow oxygen devices	5 (1.3%)	10 (4.7%)	2 (2.1%)	1 (1.9%)	1 (1.1%)	2 (3.9%)	1 (1.1%)	3 (5.6%)	1 (1.1%)	4 (7.7%)			
6. Hospitalized, on invasive mechanical ventilation or ECMO	14 (3.8%)	52 (24.6%)	1 (1.1%)	13 (24.1%)	3 (3.2%)	14 (27.5%)	6 (6.6%)	13 (24.1%)	4 (4.3%)	12 (23.1%)			
7. Death	20 (5.4%)	35 (16.6%)	5 (5.3%)	7 (13.0%)	4 (4.3%)	10 (19.6%)	5 (5.5%)	13 (24.1%)	6 (6.5%)	5 (9.6%)			
Time to improvement of 2 categories of the 7-point ordinal scale or hospital discharge within day 29 (days), median [IQR]	10 [7-16]	19 [14-29]	9 [6-14]	19 [10-29]	11 [7-17]	27 [14-29]	10 [7-19]	22 [15-29]	10 [7-17]	18 [13-29]	HR=0.71 (0.54 to 0.93) [P=0.012]	HR=0.70 (0.54 to 0.92) [P=0.009]	HR=0.79 (0.61 to 1.03) [P=0.08]
Time to National Early Warning Score ≤2 or hospital discharge within 29 days (days), median [IQR]	9 [5-16]	29 [17-29]	8 [5-14]	26 [15-29]	9 [6-16]	29 [22-29]	9 [6-18]	29 [19-29]	9 [5-15]	29 [16-29]	HR=0.83 (0.63 to 1.09) [P=0.17]	HR=0.75 (0.56 to 0.99) [P=0.046]	HR=0.90 (0.68 to 1.18) [P=0.45]
Time to hospital discharge within 29 days (days), median [IQR]	10 [7-20]	29 [19-29]	9 [6-16]	29 [19-29]	12 [8-21]	29 [24-29]	11 [8-26]	29 [28-29]	11 [7-20]	29 [16-29]	HR=0.77 (0.58 to 1.02) [P=0.07]	HR=0.72 (0.54 to 0.96) [P=0.026]	HR=0.83 (0.62 to 1.10) [P=0.20]
Oxygenation-free days until day 29 (days), median [IQR]	22 [15-25]	0 [0-13]	22 [15-25]	4 [0-14]	22 [15-25]	0 [0-12]	22 [13-25]	0 [0-6]	22 [16-25]	3 [0-15]	LSMD=-0.86 (-2.80 to 1.08) [P=0.39]	LSMD=-1.68 (-3.66 to 0.29) [P=0.10]	LSMD=0.17 (-1.84 to 2.17) [P=0.87]
Ventilator-free days until day 29 (days), median [IQR]	29 [29-29]	11 [0-20]	29 [29-29]	14 [0-22]	29 [29-29]	3 [0-19]	29 [29-29]	4 [0-16]	29 [29-29]	14 [1-22]	LSMD=-0.98 (-2.96 to 1.00) [P=0.33]	LSMD=-2.01 (-4.03 to 0.00) [P=0.05]	LSMD=0.09 (-1.93 to 2.10) [P=0.93]
Death within 28 days, no. (%)	19 (5.1%)	35 (16.6%)	5 (5.3%)	7 (13.0%)	4 (4.3%)	10 (19.6%)	4 (4.4%)	13 (24.1%)	6 (6.5%)	5 (9.6%)	OR=1.24 (0.55 to 2.82) [P=0.60]	OR=1.51 (0.69 to 3.34) [P=0.30]	OR=0.93 (0.40 to 2.20) [P=0.88]

Table 3. Summary of adverse events according treatment group in the modified intention to treat population.

In the “Overall” column, numbers refer to number of events and number of patients. In other columns, number refer to number of patients (%). Some patients had more than a single SAE. Analyses were performed on the modified Intention-to-treat population. SAE, Serious Adverse Event. P-value refer to Fisher exact test.

* According to the investigator’ judgement. Among participants with the occurrence of the SAE related to the experimental treatment, 14 (51.9%) in the lopinavir/ritonavir arm, 32 (71.1%) in the lopinavir/ritonavir plus INF-β-1a arm and 12 (48.0%) in the hydroxychloroquine arm discontinued the experimental treatment.

** Including renal failure in 30 patients, hepatic disorders in 18 patients and electrocardiogram abnormalities in 8 patients. IFN treatment was completed in all patients from the Lopinavir/ritonavir + Interferon β-1a arm.

*** Excluding acute renal failures defined based on the RIFLE classification.

	Overall (N=579)	Control (N=148)	Lopinavir/ritonavir (L/r) (N=144)	Lopinavir/ritonavir + Interferon β-1a (L/r + IFN) (N=144)	Hydroxychloroquine (HCQ) (N=143)	L/r vs. control P-value	L/r + IFN vs. control P-value	HCQ vs. control P-value
	no. events / no. patients	no. patients (%)	no. patients (%)	no. patients (%)	no. patients (%)			
Any adverse events	2399 / 450	105 (70.9%)	119 (82.6%)	117 (81.3%)	109 (76.2%)	0.02	0.04	0.35
Any grade 3 or 4 adverse events	477 / 205	48 (32.4%)	56 (38.9%)	58 (40.3%)	43 (30.1%)	0.27	0.18	0.71
Any serious adverse events	608 / 274	57 (38.5%)	76 (52.8%)	78 (54.2%)	63 (44.1%)	0.02	0.01	0.34
Any serious adverse event related to the experimental treatment*	-	-	27 (18.8%)	45 (31.3%)	25 (17.5%)	-	-	-
Death related to the experimental treatment*	-	-	1 (0.1%)	3 (2.1%)	0 (0%)	-	-	-
Premature suspension or discontinuation of the experimental treatment**	77 (13.3%)	-	17 (11.8%)	43 (29.9%)	17 (11.9%)	-	-	-
Most relevant SAEs								

- Acute respiratory failure	65 / 65	18 (12%)	19 (13%)	17 (12%)	11 (8%)	
- Acute Respiratory Distress syndrome	47 / 46	16 (11%)	7 (5%)	10 (7%)	13 (9%)	
- Acute kidney injury***	50 / 50	9 (6%)	16 (11%)	11 (8%)	14 (10%)	
- Acute renal failure based on the RIFLE classification	17 / 17	3 (2%)	3 (2%)	8 (6%)	3 (2%)	
- Arrhythmia	41 / 35	3 (2%)	8 (6%)	12 (8%)	12 (8%)	
- Pulmonary embolism	27 / 27	6 (4%)	10 (7%)	5 (3%)	6 (4%)	
- Transaminases increased	25 / 25	2 (1%)	5 (3%)	12 (8%)	6 (4%)	
- Sepsis	21 / 21	2 (1%)	6 (4%)	7 (5%)	6 (4%)	
- Cholestasis	6 / 6	0 (0%)	2 (1%)	3 (2%)	1 (1%)	