The role of androgens on COVID-19 is well established. Proxalutamide is a second-generation, non-androgen receptor, sars-cov-2, covid-19 Keywords: proxalutamide, tmprss2, pandemic, transmembrane protease serine 2, clinical trial, sepsis, antiandrogens, Endocrinology/Diabetes/Metabolism, Internal Medicine, Infectious Disease Categories: Gynecology/Obstetrics, Internal Medicine, Infectious Disease

Introduction Early in 2020, when the pandemics of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its resulting disease, coronavirus disease 2019 (COVID-19), was announced, early reports already indicated that males were an independent risk factor for severe COVID-19 and death, since the overrepresentation of males was not fully justified by the presence of comorbidities, body mass index (BMI), habits or age [1,2]. The observation that males with androgenetic alopecia (AGA) were prevalent in COVID-19 intensive care units (ICUs) allowed different teams of researchers to hypothesize and was further confirmed that male AGA patients presented similar results in all COVID-19 outcomes, and mortality rates, and overall and poor risk stratification hospitalization stay. Results of proxalutamide and placebo groups were also compared between the North and South arms. Analysis was also performed stratified by sex and baseline WHO COVID-19 Classification Score. Proxalutamide increased recovery rate, reduced mortality rate and shortened hospital stay in hospitalized COVID-19 patients. Results were similar between the two different arms, providing further consistency for the efficacy of proxalutamide when used in late-stage COVID-19. However, the observation from these studies reinforce the androgens theory for COVID-19. The biological plausibility for the androgen theory on COVID-19 is corroborated by the fact that SARS-CoV-2 cell entry largely depends on an endogenous transmembrane protease, serine 2 (TMPRSS2), that primes the virus, a critical step for its entrance through the coupling to the angiotensin-converting enzyme 2 (ACE2) receptor [18], and the inhibition of TMPRSS2 would reduce SARS-CoV-2 infectivity. The only known endogenous regulators of the TMPRSS2 expression are the androgens [19]. The virus dependence on TMPRSS2 varies across different variants [20].

Materials and methods Upon randomization, hospitalized COVID-19 patients received either proxalutamide 300 mg daily or placebo for 14 days, in addition to usual care, in a proxalutamide placebo ratio of 1:1. The North arm and 4:1 to the South arm (ratio was modified due to preliminary report of high drug efficacy). Patients that did not require oxygen use (scores 3 and 4) did not present statistically significant improvement in recovery outcomes. The patients that worsened in all-outcome studies presented significant improvements in all outcomes (p < 0.001 for all) on all evaluated outcomes. Proxalutamide was compared to placebo group for 14-day and 28-day recovery discharge alive from the hospital and mortality rates, and overall and poor risk stratification hospitalization stay. Results of proxalutamide and placebo groups were also compared between the North and South arms. Analysis was also performed stratified by sex and baseline WHO COVID-19 Classification Score. Proxalutamide increased recovery rate, reduced mortality rate and shortened hospital stay in hospitalized COVID-19 patients. Results were similar between the two different arms, providing further consistency for the efficacy of proxalutamide when used in late-stage COVID-19. However, the observation from these studies reinforce the androgens theory for COVID-19. The biological plausibility for the androgen theory on COVID-19 is corroborated by the fact that SARS-CoV-2 cell entry largely depends on an endogenous transmembrane protease, serine 2 (TMPRSS2), that primes the virus, a critical step for its entrance through the coupling to the angiotensin-converting enzyme 2 (ACE2) receptor [18], and the inhibition of TMPRSS2 would reduce SARS-CoV-2 infectivity. The only known endogenous regulators of the TMPRSS2 expression are the androgens [19]. The virus dependence on TMPRSS2 varies across different variants [20].

Results A total of 778 subjects were included (645 from the North, 317 from the proxalutamide group and 328 from the placebo group). Recovery rate was 121.5% higher in proxalutamide than placebo group at day 14 (81.1% vs. 66.4%). Recovery rate (95% CI; 77.6-135.3; p < 0.0001), and 91.1% higher at day 28 (98.1% vs. 71.5%; p < 0.0001). All-cause mortality rate was 80% lower in proxalutamide than placebo group at Day 14 (4.0% vs. 37.3%; Risk ratio (RR); 0.20; 95% CI: 0.14-0.29; p < 0.0001), and 78% lower at Day 20 (10.6% vs. 44.2%; RR: 0.22; 95% CI: 0.16-0.30). Post-randomization time-to-discharge was shorter in proxalutamide (median, 9 days; interquartile range (IQR), 3-14) than placebo group (median, 9 days; IQR, 6-14; p < 0.0001). Results were statistically similar between North and South arms for all measured outcomes. Males and women presented similar results in all outcomes. Patients that did not require oxygen use (scores 3 and 4) did not present statistically significant improvement in recovery outcomes. The patients that worsened in all-outcome studies presented significant improvements in all outcomes (p < 0.001 for all) on all evaluated outcomes. Proxalutamide was compared to placebo group for 14-day and 28-day recovery discharge alive from the hospital and mortality rates, and overall and poor risk stratification hospitalization stay. Results of proxalutamide and placebo groups were also compared between the North and South arms. Analysis was also performed stratified by sex and baseline WHO COVID-19 Classification Score. Proxalutamide increased recovery rate, reduced mortality rate and shortened hospital stay in hospitalized COVID-19 patients. Results were similar between the two different arms, providing further consistency for the efficacy of proxalutamide when used in late-stage COVID-19. However, the observation from these studies reinforce the androgens theory for COVID-19. The biological plausibility for the androgen theory on COVID-19 is corroborated by the fact that SARS-CoV-2 cell entry largely depends on an endogenous transmembrane protease, serine 2 (TMPRSS2), that primes the virus, a critical step for its entrance through the coupling to the angiotensin-converting enzyme 2 (ACE2) receptor [18], and the inhibition of TMPRSS2 would reduce SARS-CoV-2 infectivity. The only known endogenous regulators of the TMPRSS2 expression are the androgens [19]. The virus dependence on TMPRSS2 varies across different variants [20].

Conclusion Proxalutamide increased recovery rate, reduced mortality rate and shortened hospital stay in hospitalized COVID-19 patients. Results were similar between the two different arms, providing further consistency for the efficacy of proxalutamide when used in late-stage COVID-19. How to cite this article Cadegiani F A, Zimmerman R A, Forseca D N et al. (December 25, 2021) Final Results of a Randomized, Placebo-Controlled, Two-Arm, Parallel Clinical Trial of Proxalutamide for Hospitalized COVID-19 Patients: A Multiregional, Joint Analysis of the Proxa-Rescue AndroCoV Trial. Cureus 13(12): e20691. DOI: 10.7759/cureus.20691
From all the existing observations and strong, convergent mechanistic exploration, we hypothesized that anti-androgens could be a potential target to protect against COVID-19 [27,28].

Dutasteride, a broad 5α-reductase inhibitor, demonstrated to reduce viral load and accelerate the recovery process in early COVID-19 [14]. Provectamide, a second-generation nonsteroidal androgen receptor antagonist initially developed to be an additional option for ADT in males with castration-resistant prostate cancer, was shown to be more potent than other antiandrogen compounds such as enzalutamide or bicalutamide in inhibiting androgen activity, and presented concurrent activity on ACE-2, as well as anti-inflammatory properties [29].

Provectamide was chosen to reduce hospitalization rate in both males [28] and females [27], and induced significant reduction in inflammatory and thrombotic markers, which was particularly observed in subjects with initial high circulating C-reactive (cCRP) and D-dimer levels [28,29]. The fact that provectamide may not be restricted to androgen activity in COVID-19, but also act as an strong anti-inflammatory, anti-thrombotic, and possibly immunomodulatory agent, allowed the hypothesis that provectamide could also be effective to COVID-19 in later, more severe stages, during hospitalization.

As expected, provectamide demonstrated a reduction of more than 75% in mortality rate and increase of more than 100% in recovery rate at Day 14 in two distinct geographical regions, in a randomized, double-blind, placebo-controlled, multinational clinical trial with two different arms (Northern Brazil and Southern Brazil) [28,29]. Due to the unexpected efficacy of provectamide, further confirmation of the findings was critical. The use of the exact same protocol with the ethical adaptations to a different region could help indicate whether these findings were consistent and increase the statistical power of the analysis while fully respecting ethical issues.

The present study is the joint analysis of the randomized clinical trial (RCT) that tested provectamide on patients hospitalized due to COVID-19 conducted in two different regions, as two different arms, aiming to provide the final results for the Proxe-Rescue AndroCoV Trial and to evaluate whether findings were consistent between arms.

Materials And Methods

Design

This is a joint analysis of the arms of a double-blind, placebo-controlled, two-arm (arm as group population – two groups parallel RCT conducted in two different regions of Brazil – Northern arm and Southern arm) multicenter randomized clinical trial, at 13 hospital settings in nine cities. The North arm included the following hospitals and respective cities: Samad Hospital, Oscar Nobilior Hospital and Hospital Promicrod, in Maués, Hospital Regional José Mendes, in Itacoatiara, Hospital Regional (former Cohen, in Porto Alegre, Hospital de Campanha de Manaus, in Manaus, Hospital Regional Dr. Hamilton Dias, in Manaus, and hospital Regional Dr. Dinell de Silva, in Manaus. All hospitals and cities in the North arm are located in the state of Amazonas (AM), in the Northern region of Brazil. The South arm included the following hospitals and respective cities and states: Hospital Arcanjo São Miguel, in Gramado, State of Rio Grande do Sul (RS), Hospital da Brigada Militar de Porto Alegre (HMPA), in Porto Alegre, State of Rio Grande do Sul (RS), and Hospital Gruppo Chega, in Chapéu, State of Santa Catarina (SC). Both states are located in the Southern region of Brazil.

The protocol of the present study was approved by the National Ethics Committee (Comitê de Ética em Pesquisa da Comissão Nacional de Ética em Pesquisa - Ministério da Saúde (CNPEN-M) - approval number 4.153.035) on January 27th, 2021, and is registered in clinicaltrials.gov as two different numbers: the North arm (NCT04703002) and the South arm (NCT05152458). Dataset is publicly available in the appendices.

Study population

Patients with known COVID-19 (positive test SARS-CoV-2 real-time reverse transcription polymerase chain reaction (RT-PCR) test) with the following criteria: a) SARS-CoV-2 RT-PCR test positive; b) COVID-19 pneumonia by chest X-ray or CT scan; c) fever > 38°C; d) oxygen saturation level < 95%, e) respiratory distress index > 30, and f) intensity of hospitalization, either moderate or severe, as approved by the National Ethics Committee.

Patients were randomized to receive either provectamide (500 mg/day plus usual care or a placebo plus usual care for 14 days). The provectamide/placebo randomization ratio was 1:1 in the North arm and 4:1 in the South arm.”

The COVID-19 patient severity scale [16] was the main driver to classify and detect outcomes, since it classifies according to the clinical severity level, reveals and distinguishes patients that are discharged alive from the hospital, on oxygen use, higher oxygen need, on mechanical ventilation, and that died, according to the score. The scale was used for counting the Day 0, from Day 1 to Day 14, Day 21 and Day 28 (or until discharge or death). The clinical score is defined as: 1. Not hospitalized, no limitations on activities; 2. Also not hospitalized, but on the limitation on activities; 3. Hospitalized, but not or no longer requiring discharge or death). The clinical score is defined as: 1. Not hospitalized, no limitations on activities; 2. Also not hospitalized, but on the limitation on activities; 3. Hospitalized, but not or no longer requiring discharge or death).
Participants discharged from the hospital alive were actively monitored by local investigators, with post-discharge medical appointments in the same sites where they were hospitalized. Upon discharge from the hospital, patients were instructed to return in case of relapse, worsening, or new symptoms. Hospital readmissions were actively surveilled at all sites and informed to the supervising research team.

Local investigators that recruited and/or followed participants directly were kept blinded until all patients completed the study. The principal investigator was blinded for the North arm until March 10, 2021, and unblinded for the South arm. A daily analysis was performed by the principal investigator, and a weekly analysis by a data safety monitoring board (DSMB) and an interim analysis was performed weekly, unless a period of recruitment was shorter than 14 days (time to receive the first report from the DSMB).

Protocol modification

Due to the high efficacy reported in the first state where the study was conducted, the principal investigator decided to change the active:placebo ratio from 1:1 to 4:1, aiming to reduce the exposure to placebo. As per the protocol approved, we could not continue the study as a single-arm study, since this is not permitted in the country where it was conducted (Brazil). The randomization method was changed from blocks to individualized sets.

Efficacy assessment

The primary outcome to evaluate the efficacy of proxalutamide in patients hospitalized due to COVID-19 was the recovery rate 14 days after randomization, based on scores 1 and 2 at the 8-point COVID-19 ordinal scale, or the proportion of patients discharged from hospital alive before 14 days of treatment.

Secondary outcomes included recovery rate (scores 1 and 2) at Day 28, all-cause mortality rate (score 8) at Day 14 and at Day 28, hospitalization stay (days) and time-to-discharge alive after randomization (days).

The efficacy of the recovery and all-cause mortality rates was measured through the ratio between the rates of the proxalutamide group and the placebo group, and the efficacy of the other two outcomes was determined by the comparison between the groups.

Safety assessment

Treatment-emergent adverse effects (TEAEs) were actively searched. Grades 3 to 5 adverse effects (SAEs) measured included death, mechanical ventilation, shock requiring vasopressors, liver damage, kidney injury and disease progression. Grades 1 and 2 adverse effects (AEs) actively questioned were gastrointestinal symptoms (diarrhea, vomiting, nausea, abdominal pain, and dyspepsia), palpitations and instability. Other symptoms were spontaneously reported.

Presentation of the results

The primary results of the North arm and the South arm comparing the proxalutamide group with the placebo group of each arm were presented elsewhere [30,31]. In this joint analysis, different comparisons were performed.

Proxalutamide versus placebo

Active groups from the South and North arms and placebo groups from the South and North arms were combined to analyze the efficacy of proxalutamide compared to placebo in an overall, multicentric, multinational perspective. Baseline characteristics, outcomes and adverse effects (AEs) were compared between active groups combined and placebo groups combined.

Baseline characteristics were compared for age, sex, comorbidities, time from hospitalization to randomization, and medications used during hospitalization. Outcomes were compared for 14-day and 28-day recovery rate, 14-day and 28-day mortality rate, hospitalization length stay and post-randomization time-to-discharge alive from hospital. Outcomes were also compared between subgroups according to sex and baseline clinical score, except for hospitalization overall and post-randomization length stay.

North versus South

Results in the North arm were compared to the results in the South arm. The analysis was performed by comparing proxalutamide group of the North arm with the proxalutamide group of the South arm, and the placebo-group of the North arm was compared to the placebo group of the South arm. Comparisons were performed for the same baseline characteristics, outcomes and adverse effects compared between combined active and combined placebo groups. Outcomes were compared overall and according to sex.

Absolute differences between active and placebo groups from each arm were calculated, estimating the number of patients that would have been benefited from the intervention if all participants of that arm received proxalutamide. The number was provided as the number of patients expected to be benefited over the overall number of patients of the respective arm. Absolute differences were then compared between the North and the South arm to evaluate whether the level of efficacy was similar between arms or not, for recovery and mortality rates at Day 14 and Day 28, for overall and sex-specific.

We did not compare the outcomes and adverse effects of overall participants between the North and the South arm due to the difference between the proxalutamide placebo-ratio, which would lead to confounding differences. Figure 4 illustrates the comparisons that were performed between different groups and arms throughout this analysis.
FIGURE 1: Illustrative description of the comparisons performed in the present analysis.

**Statistical analysis**

In the North arm, intention-to-treat (ITT) protocol was employed, whereas in the South arm a modified ITT (mITT) was employed. All subjects that remained at least 24 hours after the first dose of proxalutamide or placebo were considered for the mITT. The difference occurred because in the North arm sites were unable to communicate the discontinuation of the drug within less than 24 hours after randomization, which did not allow the employment of the mITT, whereas this communication was instantaneous in the South arm. Indeed, in the North arm, ITT and mITT would not lead to any differences.

To determine the efficacy of proxalutamide compared to usual care, combined proxalutamide groups (i.e., proxalutamide group from the South and North arms together) were compared to combined placebo groups (i.e., placebo group from the South and North arms together). Cox proportional hazard model was employed to calculate the hazard ratio (HR) and 95% confidence interval (95% CI), for 14-Day and 28-Day all-cause mortality rate, and the rate ratio (RR) of the 14-Day and 28-Day recovery rate. The Wilcoxon Rank Sum and Wilcoxon-Mann-Whitney U tests were employed to compare the differences of the ordinal scale scores at Day 14, length of hospitalization stay, and post-randomization time-to-discharge alive from the hospital, and the following baseline characteristics: median age and interval between hospitalization and randomization. Chi-Square Test was employed to compare the other baseline characteristics and TEAEs. XLSTAT 2021.5 (Addinsoft, Paris, France) and Easy Med Stat were used to run the statistical analysis.

To evaluate the level of similarity between the North and South arms, baseline characteristic of each arm were compared using the same statistical tools as used to compare proxalutamide and placebo groups. To assess the consistency between arms, the outcomes of the active and placebo groups from the North arm were compared to the respective groups from the South arm using Cox proportional hazard model and the TEAES were compared using Chi-Square Test.

To assess whether there were differences between the level of efficacy of proxalutamide arms between regions, the estimated number of additional subjects to be benefited from the treatment if all participants were treated with proxalutamide were calculated for all outcomes in the North and in the South arm, and then compared using Cox proportional hazard model.

Similar statistical tools were employed for the comparative analysis of sex- and baseline COVID ordinary score-specific subpopulations for the corresponding outcomes and TEAEs.

**Results**

A total of 778 subjects were included in the study, including 645 from the North arm (317 from the proxalutamide group and 328 from the placebo group), and 133 from the South arm (106 from the proxalutamide group and 27 from the placebo group) of the Proxa-Rescue AndroCoV trial. Figure 2 depicts the CONSORT flowchart. No transmen, transwomen, non-binary, or intersex subjects were included.
Proxalutamide versus placebo

Table 1 describes the baseline characteristics of overall, proxalutamide, and placebo groups resulted from the joint analysis of the North and the South arms. Except for the use of medications, none of the characteristics was statistically significantly different between groups. However, participants from the proxalutamide group were marginally older (p=0.062) and had marginally more type 2 diabetes (p=0.055) than the placebo group. None of the groups presented patients with chronic kidney disease (CKD), since this was criteria for exclusion per the protocol.

| Characteristic                                | Overall N=778 | Proxalutamide N=423 | Placebo N=355 | p-value |
|-----------------------------------------------|---------------|---------------------|---------------|---------|
| Age                                           |               |                     |               |         |
| Median – years (IQR)                          | 51 (41-62)    | 52 (42-62)          | 51 (39-62)    | 0.062   |
| > 55 yr – no. (%)                             | 298 (38.0%)   | 158 (37.8%)         | 137 (38.6%)   | 0.77    |
| Sex – no. (%)                                 |               |                     |               | 0.26    |
| Female                                        | 314 (40.4%)   | 163 (38.5%)         | 151 (42.5%)   |         |
| Male                                          | 464 (59.6%)   | 260 (61.5%)         | 204 (57.5%)   |         |
| Comorbidities                                 |               |                     |               |         |
| Body mass index over 30 kg/m² – no. (%)       | 68 (8.7%)     | 39 (9.2%)           | 29 (8.2%)     | 0.70    |
| Hypertension – no. (%)                        | 215 (27.6%)   | 124 (29.3%)         | 91 (25.6%)    | 0.26    |
| Type 2 diabetes mellitus – no. (%)            | 97 (12.5%)    | 55 (13.0%)          | 42 (11.8%)    | 0.055   |
| Chronic obstructive pulmonary disorder – no. (%) | 15 (2.3%)   | 9 (2.1%)            | 6 (1.7%)      | 0.81    |
| Chronic kidney disease – no. (%)              | 0             | 0                   | 0             | n/a     |
| Coexisting conditions – no. (%)               |               |                     |               |         |
| 0                                             | 597 (65.2%)   | 267 (63.1%)         | 330 (65.3%)   | 0.19    |
| 1                                             | 157 (17.0%)   | 92 (21.7%)          | 65 (18.3%)    | 0.26    |
| 2+                                            | 114 (14.8%)   | 64 (15.1%)          | 50 (14.1%)    | 0.65    |
| Median time from hospitalization to randomization (IQR) – days | 2 (1-4)     | 2 (1-4)             | 2 (1-4)       | 0.16    |
| Score on the Coronavirus Disease 2019 ordinal scale – no. (%) |               |                     |               |         |
| 3: Hospitalized, not requiring supplemental oxygen – no longer requires ongoing medical care | 2 (0.3%)     | 0                   | 2 (0.6%)    | 0.35    |
| 4: Hospitalized, not requiring supplemental oxygen, requiring ongoing medical care (COVID-19 related or otherwise) | 24 (3.1%)    | 12 (2.8%)           | 12 (3.3%)    | 0.65    |
| 5: Hospitalized, requiring supplemental oxygen | 232 (31.4%)  | 136 (32.2%)         | 116 (32.7%)   | 0.83    |
| 6: Hospitalized, receiving non-invasive ventilation or high flow oxygen devices | 900 (44.8%) | 375 (65.0%)         | 225 (63.4%)  | 0.64    |

TABLE 1: Baseline characteristics of the overall, proxalutamide and placebo groups of the North and South arms combined.
| Day 14 Scores – median (IQR) | 2 (1-7) | 1 (1-2) | 6 (2-4) | p = 0.0001 |
|-----------------------------|---------|---------|---------|-------------|
| Recovery rate over 14 days, no. (%) | 473 (80.8%) | 343 (81.1%) | 120 (26.6%) | 2.27 (1.92 – 2.65) NNT = 2.2 | p < 0.0001 |
| Females | 185 (59.9%) | 132 (81.0%) | 53 (28.1%) | 2.01 (1.83 – 2.20) p=0.0001 | NNT=2.2 |
| Males | 288 (82.1%) | 211 (81.2%) | 77 (37.7%) | 2.15 (1.79 – 2.59) p=0.0001 | NNT=2.3 |
| Baseline Score 3 and 4 | 22 (94.6%) | 10 (93.3%) | 12 (85.7%) | 0.97 (0.70 – 1.35) p=0.80 | NNT=n/a |
| Baseline Score 5 | 183 (72.3%) | 123 (80.8%) | 60 (51.7%) | 1.73 (1.44 – 2.09) p=0.0001 | NNT=2.6 |
| Baseline Score 6 | 268 (53.7%) | 210 (76.6%) | 58 (25.8%) | 2.07 (2.36 – 3.70) p=0.0001 | NNT=2.2 |
| Recovery rate over 28 days, no. (%) | 333 (68.5%) | 364 (86.1%) | 169 (47.6%) | 1.81 (1.61 – 2.03) NNT = 2.6 | p < 0.0001 |
| Females | 215 (68.5%) | 142 (87.1%) | 73 (48.3%) | 1.80 (1.51 – 2.12) p=0.0001 | NNT=2.6 |
| Males | 318 (68.5%) | 222 (88.4%) | 56 (47.1%) | 1.81 (1.66 – 2.01) p=0.0001 | NNT=2.6 |
| Baseline Score 3 and 4 | 23 (96.5%) | 11 (91.7%) | 12 (85.7%) | 1.07 (0.81 – 1.41) p=0.63 | NNT=16.8 |
| Baseline Score 5 | 203 (80.2%) | 128 (80.4%) | 75 (54.7%) | 1.45 (1.25 – 1.67) p=0.0001 | NNT=3.5 |
| Baseline Score 6 | 307 (61.5%) | 225 (62.1%) | 82 (25.8%) | 2.25 (1.88 – 2.70) p=0.0001 | NNT=2.6 |
| All-cause mortality rate over 14 days, no. (%) | 172 (22.1%) | 34 (0.0%) | 138 (28.9%) | 0.21 (0.15 – 0.30) NNT = 3.2 | p < 0.0001 |
| Females | 66 (21.0%) | 11 (0.7%) | 55 (28.4%) | 0.18 (0.10 – 0.34) p=0.0001 | NNT=3.4 |
| Males | 106 (22.8%) | 23 (0.8%) | 83 (40.7%) | 0.21 (0.14 – 0.33) p=0.0001 | NNT=3.1 |
| Baseline Score 3 and 4 | 1 (3.8%) | 0 (0.0%) | 1 (7.1%) | 0.26 (0.12 – 0.50) p=0.0001 | NNT=16.3 |
| Baseline Score 5 | 35 (15.0%) | 5 (2.8%) | 30 (28.4%) | 0.13 (0.03 – 0.32) p=0.0001 | NNT=4.0 |
| Baseline Score 6 | 133 (26.7%) | 29 (10.6%) | 104 (46.2%) | 0.23 (0.16 – 0.33) p=0.0001 | NNT=2.5 |
| All-cause mortality rate over 28 days, no. (%) | 216 (27.8%) | 45 (10.6%) | 171 (48.2%) | 0.22 (0.16 – 0.30) NNT = 2.7 | p < 0.0001 |
| Females | 87 (27.7%) | 14 (8.6%) | 73 (48.3%) | 0.18 (0.10 – 0.30) p=0.0001 | NNT=2.5 |
| Males | 129 (27.8%) | 31 (11.9%) | 98 (48.0%) | 0.25 (0.17 – 0.37) p=0.0001 | NNT=2.7 |
| Baseline Score 3 and 4 | 2 (7.7%) | 0 (0.0%) | 2 (14.3%) | 0.23 (0.01 – 0.48) p=0.0001 | NNT=7.8 |
| Baseline Score 5 | 45 (7.9%) | 6 (4.4%) | 38 (22.5%) | 0.13 (0.06 – 0.30) p=0.0001 |
Recovery rate over 14 days after randomization, as the primary outcome of this study, was 127.6% higher in the proxalutamide group (81.1%) than in the placebo group (36.6%) [Recovery ratio (RecR) 2.21; 95% confidence interval (95% CI), 1.92-2.56]. At Day 28, recovery rate was 81.9% higher in the proxalutamide group (86.1%) than in the placebo group (47.6%) (Rec, 1.81; 95% CI, 1.61-2.03). Median clinical score at Day 14 after randomization was significantly lower in the proxalutamide group [1; interquartile range (IQR), 1-2] than in the placebo group (6; IQR, 2-8) (p < 0.0001).

All-cause mortality rate at Day 14 was 879% lower in the proxalutamide group (8.0%) compared to the placebo group (38.9%) [Risk ratio (RR), 0.21; 95% CI, 0.15-0.30]. At Day 28, all-cause mortality rate was 78% lower in the proxalutamide group (10.6%) than in the placebo group (48.2%) (RR, 0.22; 95% CI 0.16-0.30).

Hospitalization stay was shorter in the proxalutamide group (median, 8 days; IQR, 6-13) than in the placebo group (median, 12 days; IQR, 8-18) (p<0.0001). Conversely, post-randomization time-to-discharge alive from the hospital was shorter in the proxalutamide group (median, 5 days; IQR, 3-8) than in the placebo group (median, 9 days; IQR, 6-14) (p<0.0001).

Figure 4 illustrates the proportion of patients discharged alive in males (A) and in females (B), and Kaplan-Meier survival estimate of males (C) and females (D), when evaluated according to sex, recovery rate at Day 14 between males and females (15% and 151% higher recovery rate in the proxalutamide group, respectively), as well as at Day 28 (15% and 82%, respectively) (p<0.0001 between proxalutamide and placebo groups for all). All-cause mortality rates in males were similar to females at Day 14 (75% and 82% improvement, respectively) and at Day 28 (75% and 82% improvement, respectively) (p<0.0001 between proxalutamide and placebo groups for all).
FIGURE 4: Proxalutamide group compared to placebo group stratified by sex.

A. Proportion of patients discharged alive from the hospital in males; B. Proportion of patients discharged alive from the hospital in females; C. Kaplan-Meier survival probability in males; D. Kaplan-Meier survival probability in females.

Figure 5 shows the proportion of patients discharged from hospital alive (A, B and C) and the Kaplan-Meier survival estimate (D, E and F) according to the WHO COVID-19 Ordinary Clinical scores 3 and 4, score 5, and score 6, respectively. According to baseline clinical score, recovery rates at Days 14 and 28 were similar between proxalutamide and placebo groups at scores 3 and 4 (80.3% vs 84.7% at Day 14, p=0.87; 85.7% vs 88.7% at Day 28, p=0.63, respectively), while significantly higher in score 5 (95.4% vs 64.7% at Day 28, p<0.0001 for both) and in score 6 (76.6% vs 44.7% at Day 14 vs 14.2% vs 57.0% at Day 28, p=0.0001 for both).

FIGURE 5: Proxalutamide group compared to placebo group stratified by baseline WHO COVID-19 ordinary score.

A. Proportion of patients discharged alive from the hospital in subjects with baseline scores 3 and 4; B. Proportion of patients discharged alive from the hospital in subjects with baseline score 5; C. Proportion of patients discharged alive from the hospital in subjects with baseline score 6; D. Kaplan-Meier survival probability in subjects with baseline scores 3 and 4; E. Kaplan-Meier survival probability in subjects with baseline score 5; F. Kaplan-Meier survival probability in subjects with baseline score 6.

Table 3 describes the number and percentage of subjects that required the use of concurrent medications during the period of the study. All antibiotics, including cephalosporins (ceftriaxone, cefepime, cefuroxime), macrolides (azithromycin, clarithromycin), vancomycin, meropenem, and piperacillin/tazobactam, were used in a larger percentage of participants from the placebo group than from the proxalutamide group (p<0.0001 for all). Colchicine was also used in a larger percentage of patients from the placebo group (p<0.0001).
Concomitant medications – no. (%)

|                          | Overall N=778 | Proxalutamide N=423 | Placebo N=355 | p-value   |
|--------------------------|--------------|---------------------|---------------|-----------|
| Cephalosporins (Cefuroxime, Ceftriaxone, Cefepime) | 659 (84.7%) | 362 (85.8%) | 297 (84.8%) | <0.0001 |
| Colchicine               | 578 (74.0%)  | 255 (60.3%) | 323 (90.4%) | <0.0001 |
| Macrolides (azithromycin, clarithromycin) | 694 (89.2%) | 398 (94.2%) | 336 (95.2%) | <0.0001 |
| Glucocorticosteroids (dexamethasone, methylprednisolone) | 778 (100.0%) | 423 (100.0%) | 355 (100.0%) | 1.00     |
| Enoxaparin               | 787 (98.6%)  | 420 (99.3%) | 355 (100.0%) | 0.003    |
| Colchicine               | 576 (74.0%)  | 255 (60.3%) | 321 (90.4%) | <0.0001 |
| Glucocorticosteroids     | 778 (100.0%) | 423 (100.0%) | 355 (100.0%) | 1.00     |
| Enoxaparin               | 787 (98.6%)  | 420 (99.3%) | 355 (100.0%) | 0.003    |
| Meropenem                | 51/636 (8.0%) | 3/307 (1.0%) | 48/329 (14.6%) | <0.0001 |
| Piperacillin/Tazobactam  | 148/636 (23.3%) | 24/307 (7.8%) | 124/329 (37.7%) | <0.0001 |

**TABLE 3: Medications used during the period of the study.**

Table 3 describes the treatment-emergent adverse effects (TEAEs). Proxalutamide group has a significantly lower percentage of subjects with at least one TEAE (p<0.0001), Grade 5 adverse effect (AE) (death) at Days 14 and 28 (p<0.0001 for both), and Grades 3 and 4 AEs, including shock requiring vasopressors (p<0.0001), mechanical ventilation at Day 14 (p<0.0001), renal failure (p=0.0008) and liver injury (p=0.0001), while marginally lower in both males and females, except for liver damage in males (p=0.094) and mechanical ventilation at Day 14 (p=0.07 for males and p=0.14 for females). Among Grades 1 and 2 AEs, diarrhea was significantly more present in the proxalutamide group than in the placebo group (p<0.0001 for overall, p=0.0009 for females and p=0.0001 for males), while irritability and spontaneous erection were marginally higher among subjects in the proxalutamide group (p=0.056 and p=0.091, respectively).
Characteristic | Overall N=778 (females = 314) (males = 464) | Proxalutamide N=423 (females = 163) (males = 260) | Placebo N=355 (females = 151) (males = 204) | p-value |
--- | --- | --- | --- | --- |
Number of subjects with 1 or more TEAE | 387 (49.7%) | 147 (34.7%) | 240 (67.6%) | <0.0001 |
Grade 5 – n (%) | | | | |
Death, Day 14 | 172 (22.1%) | 34 (8.0%) | 138 (38.9%) | <0.0001 |
Females | 66 (21.0%) | 11 (6.7%) | 55 (36.4%) | <0.0001 |
Males | 107 (22.8%) | 23 (8.8%) | 83 (40.7%) | <0.0001 |
Death, Day 28 | 216 (27.8%) | 45 (9.3%) | 171 (46.2%) | <0.0001 |
Females | 87 (27.7%) | 10 (6.1%) | 73 (48.3%) | <0.0001 |
Males | 129 (27.8%) | 35 (11.5%) | 98 (48.0%) | <0.0001 |
Grade 4 or 3 – n (%) | | | | |
Shock, requiring vasopressors | 227 (29.2%) | 49 (11.6%) | 178 (50.1%) | <0.0001 |
Mechanical ventilation, Day 14 | 47 (6.0%) | 7 (1.6%) | 40 (11.3%) | <0.0001 |
Females | 23 (7.3%) | 3 (1.8%) | 20 (13.2%) | 0.001 |
Males | 24 (5.2%) | 4 (1.5%) | 20 (9.8%) | 0.0006 |
Disease progression | 235 (30.2%) | 53 (12.5%) | 182 (51.3%) | <0.0001 |
Females | 93 (29.6%) | 15 (9.2%) | 78 (51.7%) | <0.0001 |
Males | 142 (30.8%) | 38 (14.6%) | 104 (51.0%) | <0.0001 |
Renal failure (creatinine increase > 100%) | 29 (3.7%) | 6 (1.4%) | 23 (6.5%) | 0.002 |
Females | 12 (3.8%) | 1 (0.6%) | 11 (7.3%) | 0.002 |
Males | 17 (3.7%) | 5 (1.9%) | 12 (5.9%) | 0.043 |
Liver damage (ALT > 250 U/L or >100% increase) | 30 (3.9%) | 8 (1.9%) | 22 (6.2%) | 0.002 |
Females | 11 (3.5%) | 1 (0.6%) | 10 (6.6%) | 0.004 |
Males | 19 (4.1%) | 7 (2.7%) | 12 (5.9%) | 0.10 |
Grades 2 or 1 – n (%) | | | | |
Diarhea | 89 (11.4%) | 77 (18.2%) | 12 (3.4%) | <0.0001 |
Females | 36 (11.5%) | 26 (17.8%) | 7 (4.6%) | 0.0033 |
Males | 53 (11.4%) | 50 (10.5%) | 5 (2.3%) | <0.0001 |
Abdominal pain | 7 (0.9%) | 5 (1.2%) | 2 (0.6%) | 0.46 |
Females | 1 (0.3%) | 1 (0.6%) | 0 (0.0%) | 1.00 |
Males | 6 (1.3%) | 4 (1.5%) | 2 (1.0%) | 0.70 |
Irritability | 9 (1.2%) | 9 (2.1%) | 0 (0.0%) | 0.005 |
Females | 1 (0.3%) | 1 (0.6%) | 0 (0.0%) | 1.00 |
Males | 8 (1.7%) | 8 (2.1%) | 0 (0.0%) | 0.01 |
Spontaneous erection (males) | 7 (1.5%) | 7 (1.7%) | 0 (0.0%) | 0.02 |
Vomiting, dyspepsia, or palpitations | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | n/a |

**TABLE 4: Adverse effects in overall and sex-stratified populations.**

TEAE = Treatment emergent adverse effect; n/a = non-applicable; ALT = Alanine transferase

**North versus South**

Table 5 compared the baseline characteristics of the North arm of the Proxa-Rescue Trial and the South arm of the same trial. Participants of the South arm were older (median age, 55 y/o vs 50 y/o in the North arm, p=0.0004; 50.4% above 55 y/o versus 36.9% in the North arm, p=0.003), had more males (73.7% vs 56.7% in the North arm, p=0.001), fewer previously healthy (no comorbidities) subjects (55.6% vs 66.8% in the North arm, p=0.02), lower time from hospitalization to randomization (p<0.0001), and lower percentage of participants in severe state (Score 6) at baseline (35.4% vs 46.3% in the North arm, p=0.01).
**TABLE 5: Baseline characteristics in the North arm versus the South arm.**

| Characteristic | South N=132 | North N=645 | p-value |
|---------------|-------------|-------------|---------|
| **Age** | | | |
| Median – years (IQR) | 55 (46-63) | 50 (40-61) | <0.0001 |
| > 55 yr – no. (%) | 87 (65.4%) | 238 (36.9%) | 0.003 |
| **Sex – no. (%)** | | | |
| Male | 98 (73.7%) | 365 (56.7%) | 0.001 |
| Female | 35 (26.3%) | 279 (43.3%) | | |
| **Comorbidities** | | | |
| Body mass index over 30 kg/m² – no. (%) | 15 (11.3%) | 53 (8.2%) | 0.24 |
| Hypertension – no. (%) | 40 (30.1%) | 175 (27.1%) | 0.52 |
| Type 2 diabetes mellitus – no. (%) | 18 (13.8%) | 79 (12.2%) | 0.27 |
| Chronic obstructive pulmonary disorder – no. (%) | 2 (1.5%) | 16 (2.5%) | 0.73 |
| Chronic kidney disease – no. (%) | 0 (0.0%) | 0 (0.0%) | n/a |
| **Coexisting conditions – no. (%)** | | | |
| 0 | 74 (55.6%) | 431 (66.8%) | 0.026 |
| 1 | 34 (25.6%) | 124 (19.2%) | 0.091 |
| 2+ | 25 (18.8%) | 90 (14.0%) | 0.15 |
| Median time from hospitalization to randomization (IQR) – days | 1.0 (1.0-3.0) | 2.0 (1.0-4.0) | <0.0001 |
| **Score on the Coronavirus Disease 2019 ordinal scale – no. (%)** | | | |
| 4. Hospitalized, not requiring supplemental oxygen, requiring ongoing medical care (COVID-19 related or otherwise) | 6 (4.5%) | 20 (3.1%) | 0.41 |
| 5. Hospitalized, requiring supplemental oxygen | 56 (42.1%) | 196 (30.4%) | 0.006 |
| 6. Hospitalized, receiving non-invasive ventilation or high flow oxygen devices | 71 (53.4%) | 429 (66.5%) | 0.01 |
| **Concomitant medications – no. (%)** | | | |
| Cephalosporins (Cefuroxime, Ceftriaxone, Cefepime) | 118 (88.7%) | 638 (98.9%) | 0.0005 |
| Colchicine | 69 (51.9%) | 407 (63.1%) | 0.027 |
| Macrolides (azithromycin, clarithromycin) | 61 (46.9%) | 631 (97.8%) | <0.0001 |
| Glucocorticosteroids (dexamethasone, methylprednisolone) | 133 (100.0%) | 645 (100.0%) | 1.00 |
| Enoxaparin | 122 (91.7%) | 645 (100.0%) | 0.0009 |
| Omeprazole | 133 (100.0%) | 645 (100.0%) | 1.00 |

Participants in the South arm used less antibiotics, including cephalosporins (88.7% vs 98.9% in the North arm, p=0.0005) and macrolides (46.9% vs 97.8% in the North arm, p<0.0001). Patients in the South arm also used less enoxaparin (91.7% vs 100% in the North arm, p=0.0005) and colchicine (51.9% vs 63.1% in the North arm, p=0.02). All patients from both arms used glucocorticoids and omeprazole.

Table 6 compares results between proxalutamide groups and between placebo groups from the South and the North arm. Figure 6 and Figure 7 illustrate, respectively, recovery rates and mortality rates in the North arm. Recovery rate at Days 14 and 28, and mortality rate at Days 14 and 28 were statistically similar between placebo groups and between proxalutamide groups. Hospitalization stay was slightly longer in the active group in the North compared to the South (p=0.05) while was lower in the placebo group in the North compared to the South (p=0.045). Post-randomization length of hospital stay was significantly shorter in the placebo group of the North arm (median, 10 days; IQR, 9-15) than the placebo group of the South arm (median, 12 days; IQR, 9-15) (p=0.005).
| Characteristic | South Active N=106 Male (n=50) Female (n=56) (sc. 3/4 = 5) (sc. 5 = 50) (sc. 6 = 21) | North Active N=157 Male (n=82) Female (n=75) (sc. 3/4 = 5) (sc. 5 = 52) (sc. 6 = 216) | South Placebo N=27 Male (n=13) Female (n=14) (sc. 3/4 = 3) (sc. 5 = 14) (sc. 6 = 13) | North Placebo N=128 Male (n=101) Female (n=27) (sc. 3/4 = 13) (sc. 5 = 100) (sc. 6 = 212) | Active between regions (P-value) | Placebo between regions (P-value) |
|---------------|------------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------------------|-----------------------------|-----------------------------|
| Day 14 WHO COVID-19 Clinical Score – median (IQR)                     | 1 (1-2)                                                          | 1 (1-2)                                                          | 1 (1-2)                                                          | 4 (2-8)                                                          | 7 (2-8)                                                          | 0.81 (0.27) |
| Day 18 WHO COVID-19 Clinical Score – median (IQR)                     | 1 (1-1)                                                          | 1 (1-1)                                                          | 1 (1-1)                                                          | 2 (1-4)                                                          | 7 (2-8)                                                          | 0.00 (0.33) |
| Recovery rate over 14 days, no. (%))                                  | 85 (80.2)                                                       | 258 (81.4)                                                      | 13 (46.1)                                                        | 117 (39.7)                                                      | 0.79 (0.16) |
| Females                                                               | 24 (80.0)                                                       | 158 (82.0)                                                      | 1 (20.0)                                                        | 81 (34.9)                                                        | 0.81 (0.34) |
| Males                                                                 | 61 (82.9)                                                       | 149 (81.0)                                                      | 9 (40.9)                                                        | 68 (32.3)                                                        | 0.71 (0.86) |
| Baseline Score 3 and 4                                                | 3 (80.0)                                                        | 7 (100.0)                                                       | 1 (100)                                                          | 11 (94.8)                                                        | 0.16 (0.16) |
| Baseline Score 5                                                     | 41 (95.3)                                                       | 82 (88.2)                                                       | 9 (59.2)                                                        | 51 (48.5)                                                        | 0.12 (0.11) |
| Baseline Score 8                                                     | 41 (70.1)                                                       | 168 (77.8)                                                      | 3 (23.1)                                                        | 55 (25.5)                                                        | 0.27 (0.52) |
| Recovery rate over 28 days, no. (%))                                  | 93 (87.7)                                                       | 271 (85.5)                                                      | 14 (51.6)                                                        | 155 (47.3)                                                      | 0.55 (0.03) |
| Females                                                               | 27 (90.0)                                                       | 117 (88.0)                                                      | 2 (40.0)                                                        | 71 (34.9)                                                        | 0.74 (0.72) |
| Males                                                                 | 66 (89.5)                                                       | 154 (83.7)                                                      | 9 (40.9)                                                        | 68 (32.3)                                                        | 0.19 (0.74) |
| Baseline Score 3 and 4                                                | 4 (80.0)                                                        | 7 (100.0)                                                       | 1 (100)                                                          | 11 (94.8)                                                        | 0.32 (0.16) |
| Baseline Score 5                                                     | 43 (100.0)                                                      | 86 (92.5)                                                       | 9 (59.2)                                                        | 66 (64.1)                                                        | 0.008 (0.39) |
| Baseline Score 8                                                     | 47 (91.0)                                                       | 178 (82.0)                                                      | 4 (20.0)                                                        | 76 (56.5)                                                        | 0.61 (0.67) |
| All-cause mortality rate over 14 days, no. (%))                       | 7 (6.6)                                                         | 27 (9.5)                                                        | 8 (28.6)                                                        | 130 (28.6)                                                       | 0.68 (0.34) |
| Females                                                               | 0 (0.0)                                                         | 11 (3.3)                                                        | 2 (40.0)                                                        | 53 (38.3)                                                        | 0.22 (1.00) |
| Males                                                                 | 7 (9.2)                                                         | 16 (6.7)                                                        | 6 (27.3)                                                        | 77 (42.3)                                                        | 1.00 (0.52) |
| Baseline Score 3 and 4                                                | 0 (0.0)                                                         | 0 (0.0)                                                         | 0 (0.0)                                                         | 1 (77.8)                                                         | 1.00 (1.00) |
| Baseline Score 5                                                     | 1 (2.3)                                                         | 4 (4.3)                                                         | 2 (15.4)                                                        | 31 (30.1)                                                        | 1.00 (0.34) |
| Baseline Score 8                                                     | 8 (10.3)                                                        | 23 (10.6)                                                       | 6 (48.2)                                                        | 58 (46.2)                                                        | 1.00 (1.00) |
| All-cause mortality rate over 28 days, no. (%))                       | 10 (9.4)                                                        | 25 (11.0)                                                       | 9 (33.3)                                                        | 162 (48.4)                                                       | 0.72 (0.11) |
| Females                                                               | 2 (6.7)                                                         | 12 (0.9)                                                        | 2 (40.0)                                                        | 71 (48.6)                                                        | 1.00 (1.00) |
| Males                                                                 | 8 (10.5)                                                        | 23 (12.6)                                                       | 7 (31.8)                                                        | 91 (30.0)                                                        | 0.83 (0.12) |
| Baseline Score 3 and 4                                                | 0 (0.0)                                                         | 0 (0.0)                                                         | 0 (0.0)                                                         | 2 (18.4)                                                         | 1.00 (1.00) |
| Baseline Score 5                                                     | 1 (2.3)                                                         | 5 (5.4)                                                         | 2 (15.4)                                                        | 37 (25.9)                                                        | 0.66 (0.21) |
| Baseline Score 8                                                     | 9 (15.5)                                                        | 30 (13.8)                                                       | 7 (53.8)                                                        | 123 (58.0)                                                       | 0.63 (0.78) |
| Median hospitalization days (GFR)                                    | 8 (5-10)                                                        | 8 (6-13)                                                        | 14 (12-18)                                                      | 12 (8-18)                                                        | 0.07 (0.12) |
| Post-randomization to alive hospital discharge, median days (GFR)     | 5 (3-8)                                                         | 5 (3-9)                                                         | 12 (9-16)                                                      | 10 (6-14)                                                        | 1.00 (0.025) |

**TABLE 6: Outcomes in active and placebo groups in the North compared to the South arm.**

IQR = Interquartile range
Table 7 describes the differences of each outcome between the active and placebo groups, translated as estimated number of patients that would have changed the outcome if all participants were from the same group, in the South and in the North arm. The difference in the recovery rate at Day 14 between proxalutamide and placebo arms was significantly higher in the North than in the South arm (p=0.009), while was similar at Day 28 (p=0.66). However, the difference in recovery rate at Day 28 in males was higher in the North than in the South arm (p=0.039). The difference in all-cause mortality between proxalutamide and placebo groups at Day 14 was marginally higher in the North than in the South arm (p=0.073) and significantly higher at Day 28 (p=0.004). Among females, there were no differences between the North and South arm (p=0.12 at Day 14 and p=0.34 at Day 28), whereas males had significantly more benefit of survival in the North arm compared to the South arm at both Days 14 (p=0.056) and 28 (p=0.006).
| Difference (expected difference in terms of no. of patients / overall no. of patients) | Difference between Active and Placebo Arms (South) | Difference between Active and Placebo Arms (North) | South Risk ratio (95% CI) | North Risk ratio (95% CI) | Difference between responses in the North vs South (P-value) |
|---|---|---|---|---|---|
| Recovery rate over 14 days, no. (%) | 42.61/133 | 294.83/645 | 1.67 (1.11 – 2.46) | 2.28 (1.90 – 2.69) | 0.009 |
| Females | 21.00/35 | 131.13/279 | 4.93 (3.09 – 8.1) | 2.36 (1.60 – 3.47) | 0.11 |
| Males | 41.14/98 | 383.67/666 | 2.21 (1.21 – 3.87) | 1.62 – 3.74 | 0.00 |
| Baseline Score 3 and 4 | 2.46/8 | 1.23/30 | 0.66 (0.23 – 1.83) | 1.32 (0.32 – 5.05) | 0.60 |
| Males | 2.46/8 | 1.23/30 | 0.66 (0.23 – 1.83) | 1.32 (0.32 – 5.05) | 0.60 |
| Baseline Score 5 | 14.62/56 | 73.52/197 | 1.41 (1.06 – 1.86) | 1.19 (0.96 – 1.47) | 0.18 |
| Females | 33.88/71 | 223.64/429 | 3.01 (1.70 – 5.24) | 3.19 (2.37 – 4.31) | 0.50 |
| Baseline Score 6 | 47.73/133 | 246.58/645 | 1.69 (1.17 – 2.45) | 1.60 – 2.05 | 0.06 |
| Females | 26.31/68 | 137.43/366 | 1.16 (1.02 – 1.24) | 1.03 – 1.15 | 0.03 |
| Males | 2.29/6 | 3.18/20 | 1.25 (0.52 – 2.24) | 0.90 – 1.67 | 0.82 |
| Baseline Score 5 | 15.02/56 | 53.96/197 | 1.44 (1.01 – 2.03) | 1.23 (0.92 – 1.69) | 0.50 |
| Females | 35.69/71 | 195.25/429 | 2.34 (1.86 – 2.91) | 2.32 (1.80 – 2.96) | 0.40 |
| All-cause mortality rate over 14 days, no. (%) | 30.52/133 | 195.59/645 | 0.22 (0.09 – 0.50) | 0.14 (0.07 – 0.27) | 0.073 |
| Females | 14.00/35 | 78.20/279 | 0.84 (0.40 – 1.49) | 0.44 (0.22 – 0.80) | 0.12 |
| Males | 7.75/35 | 123.03/466 | 0.19 (0.13 – 0.29) | 0.19 (0.13 – 0.32) | 0.005 |
| Baseline Score 3 and 4 | 0.00/8 | 1.54/20 | 0.33 (0.17 – 0.70) | 0.35 (0.20 – 0.63) | 0.38 |
| Baseline Score 5 | 7.31/56 | 50.92/197 | 0.15 (0.02 – 0.97) | 0.08 (0.05 – 0.11) | 0.36 |
| Females | 25.42/71 | 152.65/429 | 0.22 (0.08 – 0.57) | 0.15 (0.05 – 0.34) | 0.34 |
| All-cause mortality rate over 28 days, no. (%) | 31.79/133 | 247.03/645 | 0.28 (0.13 – 0.67) | 0.22 (0.18 – 0.31) | 0.004 |
| Females | 11.67/35 | 110.51/279 | 0.17 (0.03 – 0.33) | 0.18 (0.11 – 0.33) | 0.54 |
| Males | 20.89/56 | 137.23/366 | 0.33 (0.13 – 0.81) | 0.25 (0.17 – 0.38) | 0.005 |
| Baseline Score 3 and 4 | 0.00/8 | 3.08/20 | 0.33 (0.17 – 0.70) | 0.30 (0.12 – 0.80) | 0.96 |
| Baseline Score 5 | 7.31/56 | 48.82/197 | 0.15 (0.02 – 0.71) | 0.15 (0.06 – 0.38) | 0.13 |
| Females | 27.21/71 | 188.88/429 | 0.28 (0.13 – 0.62) | 0.24 (0.17 – 0.34) | 0.71 |

**TABLE 7: Differences between active and placebo groups in the South arm and in the North arm.**

As shown in Table 7, there were no significant differences between proxalutamide groups and between placebo-group in 34% (grade 5 in 34%), except for a marginally increased chance of disease progression in females of the placebo group from the South arm (0.0%) compared to the North arm (0.1%) (p = 0.051). Diarrhea was more commonly present among patients of the proxalutamide group from the South arm (24.7%) than in the North arm (16.1%) (p = 0.049). Irritability and spontaneous erection were more commonly...
observed among overall and males of the proxalutamide group from the South arm (4.7% and 6.6%, respectively) than from the North arm (1.2% and 1.4%, respectively) (p=0.046 and p=0.032, respectively).

| Adverse effect | South Active (N=106) Male (n=70) Female (n=36) | North Active (N=217) Male (n=134) Female (n=83) | South Placebo (N=27 Male (n=12) Female (n=15)) | North Placebo (N=128 Male (n=62) Female (n=66)) | South between active versus placebo (p-value) | North between active versus placebo (p-value) | Active between regions (p-value) | Placebo between regions (p-value) |
|----------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Number of subjects with 1 or more TEAE | 38 (26.5%) 109 (34.4%) 15 (55.0%) 225 (88.6%) | 0.042 <0.001 0.78 0.23 |
| Grades 5 – 1 (%) |  
| Death, Day 14 | 7 (8.0%) 27 (8.5%) 8 (29.0%) 130 (38.6%) | 0.001 <0.001 0.68 0.34 |
| Females | 7 (7.9%) 11 (8.3%) 6 (27.3%) 53 (36.3%) | 0.03 <0.001 0.22 1.00 |
| Males | 0 (0.0%) 16 (8.7%) 2 (40.0%) 77 (42.3%) | 0.02 <0.001 1.00 0.52 |
| Death, Day 28 | 10 (9.4%) 35 (11.0%) 9 (33.3%) 162 (46.4%) | 0.002 <0.001 0.72 0.11 |
| Females | 8 (10.5%) 12 (9.0%) 7 (31.0%) 71 (46.5%) | 0.016 <0.001 1.00 1.00 |
| Males | 2 (8.7%) 23 (12.0%) 2 (40.0%) 91 (50.0%) | 0.041 <0.001 0.83 0.12 |
| Grades 4 or 3 (%) |  
| Shock, requiring vasoressors | 12 (11.3%) 37 (11.7%) 10 (37.0%) 168 (51.2%) | 0.001 <0.001 0.92 0.21 |
| Mechanical ventilation, Day 14 | 3 (2.0%) 4 (1.3%) 4 (14.0%) 37 (11.3%) | 0.024 0.038 0.29 0.58 |
| Females | 2 (8.7%) 1 (0.8%) 1 (20.0%) 20 (13.7%) | 0.42 0.089 0.071 0.83 |
| Males | 1 (1.3%) 3 (1.6%) 3 (13.6%) 17 (9.9%) | 0.036 0.26 0.85 0.52 |
| Mechanical ventilation, Day 28 | 0 (0.0%) 5 (1.6%) 2 (7.4%) 41 (12.5%) | 0.056 <0.001 0.37 0.71 |
| Females | 0 (0.0%) 1 (0.8%) 0 22 (15.1%) n/a <0.001 0.82 0.66 |
| Males | 0 (0.0%) 4 (2.2%) 2 (9.1%) 19 (10.4%) | 0.056 0.001 0.37 0.85 |
| Dose increase | 2 (1.6%) 36 (11.4%) 14 (51.9%) 168 (51.2%) | 0.0001 <0.001 0.20 0.95 |
| Females | 3 (10.0%) 12 (9.0%) 4 (80.0%) 74 (50.7%) | 0.0009 <0.001 0.87 0.055 |
| Males | 14 (18.4%) 24 (13.0%) 10 (45.5%) 94 (51.6%) | 0.007 <0.001 0.26 0.60 |
| Renal failure (creatinine increase > 100%) | 1 (0.9%) 5 (1.6%) 2 (7.4%) 21 (6.4%) | 0.087 0.29 1.00 0.69 |
| Females | 0 (0.0%) 1 (0.8%) 0 (0.0%) 11 (7.5%) n/a 0.33 1.00 1.00 |
| Males | 1 (1.3%) 4 (2.2%) 2 (9.1%) 10 (5.5%) | 0.11 0.58 1.00 0.62 |
| Liver damage (ALT > 250 IU or 100% increase) | 4 (3.0%) 4 (1.3%) 3 (11.1%) 19 (5.8%) | 0.14 0.32 0.11 0.23 |
| Females | 1 (3.3%) 0 (0.0%) 0 (0.0%) 10 (8.8%) | 0.73 0.37 0.18 1.00 |
| Males | 3 (3.9%) 4 (2.2%) 3 (13.6%) 9 (4.9%) | 0.11 0.85 0.42 0.20 |
| Grades 2 or 1 (%) |  
| Discrepancy | 28 (24.3%) 51 (16.1%) 1 (3.7%) 11 (3.4%) | 0.058 0.005 0.099 1.00 |
| Females | 7 (23.3%) 22 (16.5%) 0 (0.0%) 7 (4.8%) | 0.44 0.081 0.43 1.00 |
| Males | 19 (25.5%) 26 (15.8%) 1 (4.5%) 4 (2.2%) | 0.087 0.025 0.11 0.44 |
| Abdominal pain | 2 (1.9%) 3 (0.9%) 1 (3.7%) 1 (0.3%) | 0.58 0.89 0.60 0.15 |
| Females | 0 (0.0%) 1 (0.8%) 0 (0.0%) 0 (0.0%) n/a 0.91 1.00 n/a |
| Males | 2 (2.0%) 2 (1.1%) 1 (4.5%) 1 (0.5%) | 0.05 0.93 0.58 0.20 |
| Insomnia | 5 (4.7%) 4 (1.3%) 0 (0.0%) 0 (0.0%) | 0.47 0.78 0.048 n/a |
| Females | 0 (0.0%) 1 (0.8%) 0 (0.0%) 0 (0.0%) n/a 0.91 1.00 n/a |
| Males | 5 (6.6%) 3 (1.6%) 0 (0.0%) 0 (0.0%) | 0.41 0.79 0.049 n/a |
| Somnolence (Males) | 3 (3.3%) 4 (2.2%) 0 (0.0%) 0 (0.0%) | 0.62 0.73 0.42 n/a |
| Vomiting, dyspepsia, or nausea | 0 (0.0%) 1 (0.0%) 0 (0.0%) 1 (0.0%) n/a n/a n/a n/a |

**TABLE 8: Adverse effects in proxalutamide and placebo groups in the South arm and in the North arm.**

TEAE = Treatment emergent adverse effect; n/a = non-applicable; ALT = Alanine transferase
Discussion

This final, joint analysis of the Proxa-Rescue Trial including both South and North arms with proxalutamide for hospitalized COVID-19 patients demonstrated that proxalutamide is effective for COVID-19 in later stages of the disease and that benefits of proxalutamide for this population were consistent across different regions with distinct demographic characteristics.

In the South as in the North: successful reproduction of the findings from the Proxa-Rescue AndroCoV trial

The fact that this RCT demonstrated a large efficacy of proxalutamide in patients hospitalized due to COVID-19 in Northern Brazil, we had to address the question of whether these findings would be reproducible in a population with distinct characteristics in a different region.

The important improvements in clinical outcomes [35], including reductions in mortality rate and hospital length stay, and increase in recovery speed rate, observed in the first study on hospitalized patients, were replicated in the Proxa-South Rescue Trial [35].

The recovery rate ratio was similar in Day 14, of 2.28 (95% CI: 1.95 - 2.64) in the North and 2.22 (95% CI: 1.54 - 3.15) in the South. At Day 28, the recovery rate ratio was slightly lower in the South (1.31; 95% CI: 1.10 - 1.58) than in the North (2.01; 95% CI: 1.60 - 2.50). The speed of recovery rate at Day 14, the primary objective of these RCTs, showed that proxalutamide led to an increase of approximately 120% to 175% in speed recovery, quite similar between groups.

The all-cause mortality rate ratio between the proxalutamide arm and the placebo arm at Day 14 was similar between studies: 0.22 (95% CI: 0.19 - 0.56) in the South arm and 0.21 (95% CI: 0.14 - 0.32) in the North arm. At Day 28, all-cause mortality rate ratio was slightly higher in the placebo arm (0.39; 95% CI: 0.31 - 0.53) than in the North arm (0.22; 95% CI: 0.14 - 0.31). The reduction of all-cause mortality at both Days 14 and 28 was between 78% and 73%, almost identical between the studies in the North and in the South.

Overall and post-randomization hospital stay until discharge in the placebo group was shorter in the North arm than the North arm: Overall hospital stay: median, 12 days; 95%CI, 9 - 16; post-randomization hospital stay: median: 10 days; 95%CI, 6 - 14) than in the South arm (Overall hospital stay: median, 14 days; 95% CI: 11 - 18; post-randomization hospital stay: median: 12 days; 95% CI: 8 - 10) (p = 0.012 and 0.025, respectively). This is possibly resulted from the increased need to discharge patients from the North arm, due to the collapsed situation in hospitals, which were worse in Northern than Southern Brazil.

The sole non-severe TEA more commonly present among patients from the placebo group was diabetes, which corresponds to the same sole TEA reported in the study conducted in the North.

In the North arm, patients were younger, had fewer males and had higher proportion of subjects without comorbidities than in the South arm. This is in accordance with the regional demographic differences between regions in Brazil [35]. In the case of higher prevalence of male sex in the South arm, approximately 46% of the participants were originally from a military hospital, where the vast majority of patients are males. Conversely, there were approximately 28% more patients in severe state (score ≥ 6) in the North arm than in the South arm (p = 0.001). While patients in Southern Brazil had a higher risk of dying from COVID-19, patients in Northern Brazil presented more severe COVID-19 states, possibly due to the collapse in the overall health system in the state of Amazonas during the trial, which hampered non-severe COVID-19 patients from being hospitalized, even fulfilling criteria for hospitalizations.

The higher percentage of participants that used antibiotics in the North arm compared to the South arm may reflect the higher proportion of patients in the placebo group in this arm (1:1) than in the South arm (4:1), and the higher percentage of severe patients in the North arm compared to the South.

Recovery and mortality rates presented better results in the North than in the South arm: recovery rate 128% versus 67% Higher at Day 14 (p=0.0199) and 81% versus 51% at Day 28 (non-significantly: p=0.56), and all-cause mortality rate 65% versus 78% lower at Day 14 (marginally significant: p=0.075) and 78% versus 96% at Day 28 (p=0.004). The difference in the size of efficacy, which shows a more prominent response with proxalutamide in the North arm, can be fully explained by the higher proportion of patients in score ≥ 6 in the North (approximately two thirds) than in the South arm (approximately half of participants), since stratified by baseline score, all differences vanish.

As mentioned, compared to Northern Brazil, hospitals in Southern Brazil were slightly less, although overwhelmsingly occupied. As a result, criteria for hospitalization were adapted to encompass patients that needed medical assistance even in both regions, but patients at a higher severity were to be hospitalized in the North than in the South, which explains the differences in the proportion of patients in score ≥ 6.

Differences in mortality rate in the placebo group between Northern and Southern Brazil were not significant and reflected regional differences observed in Brazil [30,33]. In both studies, the all-cause mortality rate in the placebo group was smaller or similar to the COVID-19 in-hospital mortality rate in the respective regions [34] - of approximately 50% in the North arm and 53% in the South arm [35].

Overall, results were exceptionally similar between the populations in the North and in the South, despite the slight demographic differences, indicating a high consistency of the findings when validated externally within the study. Of note, in both cases, P1 (gamma) variant was the cause of COVID-19 in virtually all patients, and standard of care was in general similar between all hospitals.

The fact that comparisons between active groups and between placebo groups in all major outcomes showed similar responses between regions reinforces the efficacy of proxalutamide in terms of recovery and mortality rates.

Overall results

When analyzed for overall participants from the placebo group were marginally older than participants from the placebo group because, as mentioned, in the South, where the randomization ratio was 1:1, patients were older than patients in the North. Conversely, older patients tend to present more comorbidities, and T2DM was marginally more present in the placebo group. This is a conservative bias that may underestimate the efficacy of proxalutamide.

Disease progression occurred approximately 4.1 times more frequently in the placebo than the placebo group, while progression to shock requiring vasopressor was 4.6 times more frequent in the placebo group (p<0.001 for both). Accordingly, the proportion of patients in the placebo group with renal injury and liver damage was 4.5 and 7.2 times higher than patients in the placebo group (p<0.008 and p=0.003, respectively). The remarkable reduction in disease progression and liver and kidney injuries reinforces the efficacy of proxalutamide to interrupt the COVID-19 disease course during the second and third phases of the disease. The resulted reduction in mortality rate likely reflects not only the improvement of COVID-19 per se, but also the numerous secondary complications causally the progression of the disease.

Reduction in hospitalization stay was 55% (p≤0.001) with proxalutamide, particularly when proxalutamide is initiated earlier in hospitalization, since post-randomization time to discharge alive from the hospital was almost 55% lower in the placebo group (p≤0.001). This is particularly important during outbreaks and collapses of hospitals, when earlier discharge may allow undertaken patients to be hospitalized.

The larger proportion of patients from the placebo arm that used different classes of antibiotics reflect the higher proportion of patients in the placebo group in both arms (1:1) than in the South arm (4:1), and the higher percentage of severe patients in the North arm compared to the South.
severe COVID-19. Proxalutamide also demonstrated theoretical reduction of the COVID-19-triggered endothelial dysfunction, observed through substantial decrease in D-dimer levels. To which extent anti-inflammatory, anti-thrombotic and immunomodulatory effects of proxalutamide were directly mediated or indirectly induced by reduction in viral load is not clarified. The prevailing SARS-CoV-2 variant during the course of the trials was the P.1 (gamma), which, unlike other variants, seemed to present persistent viral activity after seven to 14 days of the disease, as observed through the persistent, unexplained and refractory increase of inflammatory and thrombotic markers during hospitalization [36-39]. Figure 8 summarizes the critical mechanisms in late-stage COVID-19 that lead to death, and the proposed mechanisms of protection conferred by proxalutamide.

The unexpected spontaneous prolonged erection among males in the proxalutamide group may have occurred due to a short-term rise in testosterone levels, as seen in our previous study in male outpatients [29]. In females as in males: an unexpected balance in the results

While it would be expected that males would present a more substantial improvement in clinical outcomes due to the higher circulating testosterone and dihydrotestosterone (DHT) levels compared to women, reduction in overall mortality rate and increase in recovery rate with proxalutamide were unexpectedly observed both in males and females. Responses not only occurred in a non-gender specific manner but were more marked in women in both recovery rate ratio at Day 14 (4.0 vs 2.5 in men) and at Day 28 (2.2 vs 1.4 in men) and mortality risk ratio at Day 14 (90% reduction vs 66% reduction in men) and at Day 28 (85% reduction vs 67% reduction in men). This finding is consistent with the findings in the North arm, where both males and females responded similarly to proxalutamide, when compared to the placebo arm.

The unexpected response in women may be justified by the fact that although females have lower androgen circulating levels, they tend to be more sensitive to androgens due to potential higher sensitivity of the androgen receptor (AR) and inherently upregulation of the AR, leading to increased density of AR in the cytoplasm. The hyperresponsiveness to androgens in a manner that slightly increased testosterone levels may be sufficient to become an independent risk factor for severe COVID-19 [40]. Consequently, antagonism of AR or blockage of more active androgen hormones may lead to more prominent responses.

Some of the differences between females of the placebo groups can be justified by the small number of participants in the group from the South arm. This peculiarity should be considered in comparisons that encompass female placebo groups.

The level of COVID-19 severity as a predictor of clinical response to proxalutamide

The improvement of recovery rate at Days 14 and 28 was higher in score 6 (196% and 114%, respectively) than score 5 (75% and 46%, respectively) or scores 3 and 4 (-3% and 7%, respectively). However, reduction in mortality rate at Days 14 and 28 was slightly higher in moderate score 5 (patients 87% for both) than score 6 (77% and 75%, respectively) or score 4 (62% and 77%, respectively).

The importance of the analysis according to the baseline clinical score is to predict subjects that should present better responses. From this analysis, apparently the patients that are more benefited from proxalutamide are those that required oxygen supplement but still not needing higher oxygen flow or additional non-invasive respiratory devices, i.e., in score 5, and in any case when oxygen is needed (scores 5 and 6) compared to cases when oxygen is not needed (scores 3 and 4).

The P.1 (Gamma) SARS-CoV-2 variant and its implications in the efficacy of proxalutamide

The P.1 (Gamma) variant of SARS-CoV-2 is a variant of concern (VOC) that, although not officially recognized, is a strong candidate to be considered the first variant of high consequence (VOHC) [36], since it has been demonstrated to lead to more severe clinical disease, increased hospitalization and increased lethality, with an up to 4-time higher risk of in-hospital death compared to the B.1.617.2/AY lineages (Delta) variant and to present increased transmissibility and reduction in the efficacy and response of the therapies against COVID-19 [37,38]. Since vaccination rates were virtually null by the time of the peak of P.1 variant, the efficacy of the vaccines to prevent infection or attenuate clinical disease for the P.1 variant is unclear.

To our knowledge, proxalutamide was the first drug tested in hospitalized patients due to COVID-19 in the P.1 (Gamma) variant, demonstrated not by the fact that the P.1 variant was present in more than 90% of tested subjects in the regions where the RCTs were conducted during the period of the studies, but...
proxalutamide was tested in the Proxa-Rescue AndroCoV trial, whether proxalutamide will be as effective for SARS-CoV-2 variants, which is particularly overwhelming in the case of the P.1 (Gamma) variant, for which proxalutamide may have been more effective for hospitalized COVID-19 patients due to the P.1 (gamma) variant, than what it would be for other variants. The key difference is that in the P.1 (Gamma) variant viral activity was enhanced, which despite the use of potent anticoagulants, both of which indicating direct viral activity persisting throughout all COVID-19 stages. The same occurred to other drugs as being a potential critical aspect for the efficacy of the treatment against COVID-19.

Scientific aspects of studies on COVID-19

Some noteworthy particularizations of the research on COVID-19 must be considered for the advance of the quality of the research on COVID-19. First, the importance of employing all-cause mortality rate, not "COVID-19-related" mortality rate, as a final outcome. The importance of this difference relies on the fact that mortality in COVID-19 is largely represented by indirect effects of COVID-19. In addition, whether deaths occurred due to COVID-19 or to a secondary complication is less critical than whether deaths occurred or not.

Second, the crisis of reproducibility in science, largely discussed in the scientific community, is particularly present in COVID-19, since numerous in the SARS-CoV-2 lead to changes in the efficacy of direct or indirect antiviral agents, as well as drugs that target the host cell. Changes in the efficacy of direct antivirals are easy to comprehend since these drugs depend on specific portions of the virus to act. Conversely, mutations may also lead to changes in the level of dependency of SARS-CoV-2 on each of the steps for its cell entry, which may affect the ability of drugs that hamper one or more steps of the process of SARS-CoV-2 cell entry to prevent its successful entry. For instance, certain SARS-CoV-2 variants have a stronger dependency on the priming process that is enabled by TMPRSS2, while others tend to have their direct cell entry not mediated by ACE2 binding [36,41-43].

Due to the meaningful changes in the response to drugs according to the variant, we claim that studies on COVID-19 to test the efficacy of drugs should be considered in variant-dependent, i.e., the observed efficacy of a certain molecule for COVID-19 should be extrapolated for other variants with a cautious and lower degree of certainty, in a similar manner how vaccines for COVID-19 are analyzed.

Third, in COVID-19 studies, self-identified gender, rather than biological sex, should be considered to classify a participant of a research. In case the person is under hormonal therapy to adjust the self-identified gender, the adjustment for the self-identified gender when under hormonal treatment is based on the fact that COVID-19 has androgen- and sex-specific risks, as observed in higher risk in males than in females [22,44-46]. Higher risk in women with hypogonadotropism than women without hypogonadotropism is observed in COVID-19 [30,31], higher risk when on androgen therapy [7], lower risk when in anti-androgen therapy [12-17], lower in estrogen or progesterone therapy [20-24,26-44], and, in the case of transgender females-to-males (FTM), that are self-identified as males while biologically females, also termed as trans men, have higher risk than males-to-females (MTF), that are self-identified as females while biologically males, also termed as trans women [45]. This means that cis and transmen are counted together, and in a different risk profile than cis and transwomen, showing that self-identified gender, rather than biological sex, should be considered to classify within trials on COVID-19.

Forth, interference of politics in science and research on COVID-19 has negatively influenced the inherent impartiality required to evaluate and judge studies and manuscripts. In this matter, competing interests may exert indirect pressure for the acceptance or rejection of specific types of research, according to the results. Although this is speculative, inequalities in the level of review process in several scientific journals have become overwhelming. Due to the unclear interests that have increasingly influenced the scientific methodology and publication, we encourage all scientific community to be fully transparent by declaring all direct and indirect conflicts of interests, not only restricted to the current conflicts, but also extended to the previous conflicts and potential future conflicts, that could influence, at any steps, the results and analysis for both positive and negative responses, as well as decisions and evaluations when acting as reviewers or editors. We also claim that conflicts and competing interests to be more emphasized and visible within the manuscripts and publications.

The Proxa AndroCoV and Proxa-Rescue AndroCoV trials and the currently ongoing Phase 3 trials for COVID-19

In common, all RCTs with proxalutamide for COVID-19, including the RCT in outpatient settings with males, and in hospitalized patients for both males and females, were conducted as a sort of "add-on" therapy, since the standard of care in both outpatients and hospitalized patients, and in both Latin American and Southern Brazil in hospitalized patients, included drugs with efficacy demonstrated to be at least marginally effective, if not effective, including nitazoxanide, ivermectin, antibiotics, enoxaparin, glucocorticoids and other drug classes [48-51].

COVID-19 results from a complex pathophysiology caused by the SARS-CoV-2. Like other viruses, SARS-CoV-2 has multiple sites of action in order to cause the resulting disease, COVID-19. Consequently, once the pathophysiology is complicated, it should not be expected that monotherapy would lead to important improvements in clinical outcomes, unless a drug has multiple actions or is used in a narrow window of opportunity, in the case of direct antivirals. And, in the last case, mutations in the virus may likely result in changes in the efficacy of direct antivirals.

Consequently, the combination of drugs that act in different sites may lead to a synergistic effect against the SARS-CoV-2, enhancing the efficacy, when compared to each drug alone. The same occurred to other studies, including the "drug cocktail" for the RIV [12,52], hepatitis B [18], and hepatitis C [53]. Indeed, the typical poor responses to viruses observed throughout history may be a consequence of the lack of optimized treatment regimens.

Whether proxalutamide started synergistic effects in the existing treatments drugs - i.e., proxalutamide was not only effective, but also enhanced the efficacy of the other drugs administered - or if proxalutamide worked alone is uncertain, since not only proxalutamide, but also no other antiandrogens have been tested in COVID-19.

Unlike the AndroCoV trials, the phase 3 trials currently ongoing (NCT04906772, NCT04767169 and NCT04844552) are testing proxalutamide in different populations without the rationale of the combination of different drugs as being a potential critical aspect for the efficacy of the treatment against COVID-19.

A second major difference between the AndroCoV Trials, specially the Proxa-Rescue AndroCoV Trial, is that proxalutamide may have been more effective for hospitalized COVID-19 patients due to the P.1 (gamma) variant, than what it would be for other variants. The key difference is that in the P.1 (Gamma) variant viral replication may persist during the second and third stages of the disease [36-39], demonstrated by lymphopenia and unexpected increase in CRP levels despite the use of high-dose glucocorticoid treatment regimens, not explained by bacterial or fungal infections, and also demonstrated by a persistent increase of 23.3% in mortality rate despite the use of potent anticoagulants, both of which indicating direct viral activity persisting throughout all COVID-19 stages [29,31].

Because of the marked differences in the pathophysiology, pathogenicity, and clinical course between SARS-CoV-2 variants, which is particularly overwhelming in the case of the P.1 (Gamma) variant, initial trials proxalutamide was tested in the Proxa-Rescue AndroCoV trial, whether proxalutamide will be as effective...
for COVID-19 caused other variants of the SARS-CoV-2 to emerge. In addition, the currently ongoing trials are not precise replications of the design of the AndroCoV Trials, in the essential aspect of the accompanying drug therapy. Due to the lack of similarity between the AndroCoV and the currently ongoing trials and the differences in the SARS-CoV-2 variants in which proxalutamide is being tested, distinct results in terms of drug efficacy are not unexpected.

Specific aspects of studies on anti-androgens for COVID-19

The role of androgens on COVID-19 is well-established from multiple epidemiological observations [1-17,23], clinical trials [18-30], and strong and definitive biological plausibility [31-34]. Even ADT for castration-resistant prostate cancer, which has been found to independently increase the risk of COVID-19 mortality in case of any secondary illness, when compared to men not under ADT [35], demonstrated not only to lower the mortality of the disease when the case is COVID-19, but also to provide relative protection from severe COVID-19 and death [16,17]. The harmful effects of androgen and protective roles of anti-androgens for COVID-19 have been questioned since low testosterone has been identified as a predictor of poor prognosis among males hospitalized due to COVID-19 [17]. However, the causality relationship between testosterone and disease severity may have been confounded, since in severe states, irrespective of the underlying disease, testosterone is dramatically reduced through multiple hypocalcemic mechanisms, since the intense inflammatory response blunts gonadotropin releasing hormone (GnRH) pulses, until the need of an anti-androgen, pre-catastrophic state, prevailing the corticotropic over the gonadotropic axis, when under severe, acute conditions. As a result, low testosterone is more likely a marker of severity, rather than a causation [18].

Differences between first and second-generation NSAAs, or even between second-generation NSAAs are clinically demonstrated through the fact that failure of treatment of castration-resistant prostate cancer with an NSA is overcome with another NSA [19-21].

Proxalutamide has unique characteristics when compared to other second-generation NSAAs. It is likely the most potent, between five to ten times more potent than other NSAAs, has the ability to suppress the generic expression of the AR gene, has important concurrent actions on the regulation of angiogenesis-co-stimulating enzyme-1 (ACRE1) expression, which is the gate and key regulator of SARS-CoV-2 entry in cells, and exerts important anti-inflammatory effects with dramatic blockage of tumor necrosis factor-alpha (TNF-alpha) and interleukin 1-beta (IL-1beta) [22,23].

Possibly, a beneficial proportion between the different levels of potency of proxalutamide when acting as an anti-androgen, ACE-2 regulator, and an anti-inflammatory may justify its efficacy for COVID-19 throughout different stages of the disease. For instance, a strong anti-androgen without concurrent regulation of the inflammatory response and ACE-2 expression may cause more harm than good at specific stages of COVID-19. In addition, the minimum concentration to be effective on a regulator of the inflammatory response and ACE-2 regulator may be distinct from those used for castration-resistant prostate cancer. Finally, because of the specifications of each NSA, we consider responses to a certain NSA for COVID-19 to be considered as a partial drug-class effect. We encourage that conclusions regarding anti-androgens for COVID-19 should not be extrapolated for other NSAAs, in particular when results are negative, due to the numerous factors that may influence the efficacy of NSA for COVID-19. Finally, researchers should avoid recommending against studies of an entire drug class in the case of anti-androgens, based on the lack of positive results of a single molecule, due to the multiple specificities and peculiarities of each anti-androgen, as described above.

Another critical aspect that must be emphasized when testing anti-androgens for COVID-19 is the treatment duration. In the case of proxalutamide, the risk of COVID-19 relapse and worsening with early interruption of proxalutamide was first identified when used for early COVID-19 in outpatient settings [25-29]. Initially, the protocol was a three-day therapy of 200mg proxalutamide per day. However, two weeks after the beginning of the RCT on outpatients, the unblinded principal investigator noticed, exclusively in participants of the proxalutamide group, a remarkably faster recovery process in the first three days followed by sudden relapse and progression of the disease after discontinuing proxalutamide. This led us to increase the treatment duration from three to seven days, since its safety profile was well-established for chronic use, which was approved by the National Ethics Committee. After changing to seven days of therapy, relapses were no longer reported.

Conversely, in the Pros-Racea AndroCoV trial, we detected a very high mortality rate (approximately 80% mortality rate among participants that did not comply with the 14-day treatment, significantly higher than the placebo arm) [32], while treatment completers had a mortality rate lower than 15%. (Oppositely, compliance in the placebo group did not determine the survival rate, of approximately 50%, whether participants complied or not. Figure 9 demonstrates the differences between proxalutamide and placebo groups depending on the compliance to treatment.)

The sudden loss of antiandrogen and anti-inflammatory protection may explain the dramatic relapse and progression of COVID-19. It is critical that the treatment duration of anti-androgens for COVID-19 should be strictly followed, and we highly recommend against short-duration treatments for COVID-19, of less than seven days for early, non-hospitalized COVID-19, and less than 14 days for hospitalized COVID-19.

Differences between placebo and treatment groups depending on the compliance to treatment.

![Proportion of patients discharged alive from the hospital according to compliance to treatment in proxalutamide (A) and in placebo (B) group.](image)

Limitations

The Pros-Racea AndroCoV RCT was conducted in moderate-to-severe COVID-19 patients infected by the P.1 (Gamma) variant, when hospitals were collapsed in both Northern and Southern Brazil during the period of the study. Despite the demonstrated efficacy that was externally validated within the same study, a) it is unclear that this can be reproduced under normal circumstances and in less pathogenic SARS-CoV-2 variants. The small number of subjects in the placebo group of the South arm may also have limited statistical analyses. For these reasons, we consider it indispensable that further RCTs in distinct environments should be conducted to confirm the present findings, with expected differences in terms of the level of efficacy.

Conclusions

Proxalutamide lowered the progression of the disease in patients at low and more severe states of COVID-19, leading to a remarkable reduction in mortality rate, particularly in patients that required oxygen (scores 5 and 6 of the WHO COVID-19 Ordinary Score). The level of efficacy was similar between the North and
South arm for different outcomes, which reinforces the consistency of the findings. Whether the level of efficacy of gender-stabilization for hystertoped COVID-19 patients will remain in other SAR-CoV-2 variants is unknown. To our knowledge, this is the only study amongst studies that demonstrated an inter-region external validation within the same ICT.

Appendices
TABLE 9: Study dataset.

### Additional Information

#### Disclosures

**Human subjects:** Consent was obtained or waived by all participants in this study. National Ethics Committee (CEP CONBRA) issued approval 4.515.423. The present study was approved by the National Ethics Committee (CEP CONBRA), unrestricted to a specific site, once the research protocol was followed as approved and the study conducted until September 5, 2021, when the approval number to continue the study. In case the study was ongoing, it was revised (the study ended on April 16, 2021). Approval number 4.515.423; process number (CAAE) 44909121.0000.5553. This trial is registered in clinical trials.gov under two different numbers, one for each site, (North site, NCT04720882; South site, NCT05134610). **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissues. **Conflicts of interest:** In compliance with the IEEE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** Kletter Pharmaceuticals, Ltd., the manufacturer of pseudomonadi, provided the drug for the trial, and is conducting phase 3 trials for COVID-19. In case efficacy of pseudomonadi for COVID-19 is confirmed, Kletter Pharmaceuticals, Ltd., plans to market pseudomonadi for COVID-19. AG and FA were consultants for Applied Biology, Inc. BE has served in the past as a clinical director for Applied Biology, Inc. CDP has served as an advisor to Applied Biology, Inc. The other authors have no conflict of interest to declare.

#### Acknowledgements

We acknowledge the teams of the participating hospitals, including healthcare providers and administrative staff for being of essential help during the trial, and the supporting staff including Vincent van Dommelen, MD, Fernando Soffito Jr, MD, Ione Maruri, MD and Marcelo Fourn, MD, Fatima-Nadine Mira, MD, MPH, Brenda Gomes de Almeida, MD, Emídio-Olivera Gorréns, José Eniqrte Miranda Neblina, Raquel Neres Nicalus, Luana Fernanda Mendes-Nicolau, Rafael Xavier Cunha, Maria Fernanda Rodrigues Barreto, Patricia Sousa Silva, Gabriel de Sousa Ferreira, MD, Flavio Benaz-Paiva da Costa Almeida, MD, Angelo Miguens Ribeiro, MD, Felipe Oliveira de Almeida, MD, Antonio Antônio de Sousa Silva, MD, Susana Souza do Rosário, MD, Alexandra Hen, DPhD, Marina Li, MD, Claudia Elizabeth Thompson, PhD and Gerard J. Nau, MD, PhD. We also acknowledge Alexandre Marinho Tenzi, MD, Lucio Carlos Couto, MD, Noberto LC Mattos, MD, Ricardo G. de Costa, MD, Lucio Carlos A. Almendra, MD and John McCoy, PhD, who were not included as authors despite fulfilling authorship criteria, as requested.

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