Proxalutamide (GT0918) Improves Lung Injury in Hospitalized COVID-19 Patients – an Analysis of the Radiological Findings of the Proxa-Rescue AndroCoV Trial

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Abstract

Introduction: Antiandrogen are good candidates against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) due to the inhibition of its entry into host cells by the suppression of TMPRSS2, an enzyme that primes the SARS-CoV-2 spike (S) protein and is key for its cell entry. Proxalutamide is a second-generation nonsteroidal anti-androgen (NSAA) with strong activities on androgen receptor (AR) antagonism, suppression of AR nuclear expression, and downregulation of the membrane-attached angiotensin converting enzyme-2 (ACE2). The efficacy of proxalutamide was previously demonstrated for early COVID-19 patients, and has now demonstrated efficacy to reduce deaths in hospitalized COVID-19 patient in a double-blind, placebo-controlled randomized clinical trial (RCT). Whether radiological changes would follow the improvement in clinical outcomes with proxalutamide is not established. The present *post-hoc* analysis aims to evaluate whether proxalutamide improves lung injury observed through chest computed tomography (CT) scans, in addition to the clinical improvement, thus providing further objective evidence of the drug response in COVID-19.

Methods: This is a *post-hoc* analysis of the radiological findings of a double-blinded, placebo-controlled, prospective, two-arm RCT (The Proxa-Rescue AndroCoV Trial) with all enrolled patients from the three participating institutions of the city of Manaus, Amazonas, Brazil, that had at least two chest CT scans during hospitalization. The quantification of lung parenchyma involvement was performed by independent board-certified radiologists with expertise in analysis of COVID-19 images, that were blind to the assigned intervention in the RCT. A first chest CT scan was performed upon randomization and a second CT scan was performed approximately five days later, whenever patient transportation was feasible.

Results: Of the 395 patients initially evaluated, 72 and 179 patients from the proxalutamide and placebo arms, respectively, were included (n=251). Baseline and clinical characteristics, interval between first and second chest CT scans, and percentage of lung parenchyma affected in the baseline chest CT scan were similar between groups. In the second chest CT scan, the percentage of lungs affected (Median – IQR) was 35.0% (25.0-57.5%) in the proxalutamide group versus 67.5% (50.0-80.0%) in the placebo group ($p < 0.001$). The absolute and relative change between the second and first chest CT scans (Median – IQR) were -15.0 percent points (p.p.) (-30.0 – 0.0p.p.)

and -25.0% (-50.0 – 0.0%) in the proxalutamide group, respectively, and +15.0p.p. (0.0 - +30.0p.p.) and +32.7% (0.0 - +80.0%) in the placebo group, respectively ($p < 0.001$ for both absolute and relative changes).
Conclusion: Proxalutamide improves lung opacities in hospitalized COVID-19 patients when compared to placebo. (NCT04728802)

Keywords: COVID-19, SARS-CoV-2, proxalutamide, antiandrogen, non-steroidal antiandrogen (NSAA), lung injury.

Introduction

Antiandrogen drugs are good candidates against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) due to the inhibition of its entry into cells.[1] The blockade of viral cell entry would occur indirectly by the mitigation of the structural modification of the SARS-CoV-2 spike (S) protein that is required for infection. This inhibition occurs though the reduction of the expression of the endogenous transmembrane protease, serine 2 (TMPRSS2). The enzyme is responsible for priming the viral S protein, allowing its proper coupling to ACE-2 receptor and consequent cell entry.[2] The suppression of the TMPRSS2 expression occurs through the inhibition of the TMPRSS2 promoter, that includes a 15 base pair androgen response element.[3] Since androgens are the only known endogenous regulators of TMPRSS2, antiandrogens play a key role in the inhibition of TMPRSS2 expression. Pre-clinical studies have shown that nonsteroidal antiandrogens down regulate TMPRSS2[4] and inhibit viral replication in human cell culture.[5,6] However, other mechanisms to explain the potential interplay between antiandrogens and SARS-CoV-2, such as *downregulation* of ACE-2 receptors, may play an additional role, and should be further elucidated.

Proxalutamide is a second-generation nonsteroidal anti-androgen (NSAA) that is more potent than other NSAAs, such as enzalutamide or bicalutamide.[7] In addition to the competitive antagonism in the androgen receptor (AR), NSAAs also prevent androgen receptor nuclear translocation and binding to DNA.[8]

The efficacy of proxalutamide was previously demonstrated for SARS-CoV-2 positive men in an outpatient setting.[9,10] The Proxa-Rescue AndroCoV Trial has now demonstrated the efficacy of proxalutamide for hospitalized COVID-19 men and women patients regarding clinical recovery speed (128% increase in recovery speed) and reduction of mortality rate (77.7% reduction in the 28-day mortality rate), though a double-blinded, placebo-controlled, multicenter randomized clinical trial, when compared to patients under usual care.[11]

Radiological improvement of COVID-19 tends to occur later in the recovery process, with easing of lungs appearance on chest computed tomography (CT) scan usually observed only after seven to 14 days [12] . Due to the dramatic improvement observed in part of the patients of the proxalutamide arm, we hypothesized that an early improvement in the radiological aspect would also occur, which would reinforce the efficacy of proxalutamide for hospitalized COVID-19 patients.

The objective of the present analysis is to compare the radiological findings of hospitalized COVID-19 patients included in the RCT between the proxalutamide and placebo arms, to reinforce the efficacy of proxalutamide observed clinically through increase in recovery speed and reduction of mortality rate.

Methods

Trial Design, Setting and Locations

Study design, criteria for eligibility, randomization, procedures, outcomes are described elsewhere.[11]

This is a *post-hoc* analysis of the radiological findings of a double-blinded, placebo-controlled, prospective, two-arm randomized clinical trial (RCT), that encompassed the three institutions of the city of Manaus, Amazonas, Brazil, that participated in the study. The other five centers included in the RCT were not included due to the lack of

available CTs for regular evaluation of COVID-19. The study was conducted between February 1 and April 15, 2021, including enrollment and follow-up.

The RCT was approved by Brazilian National Ethics Committee of the Ministry of Health, under the approval number 4.513.425 of the process number (CAAE) 41909121.0.0000.5553 (original name of the Ethics Committee: Comitê de Ética em Pesquisa (CEP) do the Comitê Nacional de Ética em Pesquisa (CONEP) do Ministério da Saúde - CEP/CONEP/MS). All data used for the present *post-hoc* analysis was entirely covered by the approval obtained with the Brazilian National Ethics Committee of the Ministry of Health (MS) (approval number 4.513.425). The RCT was registered in clinicaltrials.gov (NCT04728802).

Eligibility criteria

In short, for inclusion, men and women above 18 years old hospitalized due to COVID-19 confirmed with a positive real-time reverse transcription polymerase chain reaction (rtPCR) test for SARS-CoV-2 (Cobas SARS-CoV-2 rtPCR kit test protocol, Roche, USA) were considered.

Exclusion criteria included mechanical ventilation at the time of randomization, known congestive heart failure class III or IV (New York Heart Association), immunosuppression, alanine transferase (ALT) above five times ULN (> 250 U/L), creatinine above 2.5 mg/ml or a calculated eGFR below 30 ml/min, current use of antiandrogen medications, planning to attempt to have kids within 90 days after the intervention, and women that were pregnant or breastfeeding.

For the present *post-hoc* analysis of the radiological findings, all patients that participated in the Proxa-Rescue AndroCoV Trial[11] from the three hospitals located in the city of Manaus, Amazonas, Brazil, were included. There were no selection criteria among patients enrolled in the RCT from these hospitals for the initial assessment. Patients with at least two chest CT scans during hospitalization were included in the present analysis, since at least two scans were needed for comparison purposes. All potential limitations of a subgroup *post-hoc* analysis of a RCT described by Pocock *et al* were addressed.[13]

Procedures

Patients were randomized to receive either proxalutamide 300 mg/day plus usual care or a placebo plus usual care for 14 days in a 1:1 ratio. If patients were discharged before 14 days, they were instructed to continue treatment. Therapy compliance was monitored daily for both inpatients and patients that were discharged until day 14, and then in days 21 and 28 if discharged before, or daily if still hospitalized.

The COVID-19 8-point ordinal scale was used as the parameter for monitoring. The ordinary clinical scale is defined as: 8. Death; 7. Hospitalized, on invasive mechanical ventilation; 6. Hospitalized, on non-invasive ventilation or high flow oxygen devices; 5. Hospitalized, requiring supplemental oxygen; 4. Hospitalized, not requiring supplemental oxygen- requiring ongoing medical care (COVID-19 related or otherwise); 3. Hospitalized, not requiring supplemental oxygen - no longer requires ongoing medical care; 2. Not hospitalized, limitation on activities; and 1. Not hospitalized, no limitations on activities.[11]

Baseline characteristics, previous medical history, comorbidities and concomitant medications were recorded. Usual care for hospitalized COVID-19 patients as per the hospitals protocol included enoxaparin, colchicine, methylprednisolone or dexamethasone, and antibiotic therapy as required. The usual care was not changed for the RCT.

Before the onset of the RCT, a random sequence using 4, 6 and 8 block sizes and a list length for 662 treatments was created thought a randomization software. [14] The randomization sequence and allocation concealment were performed remotely and was not stratified by institution. Pre-packing of tablets of either active or placebo group was manufactured to have identical physical characteristics, and was manufactured and transported by Kintor Pharmaceuticals Ltd. Suzhou, China.

Protocol for the exploratory analysis of the chest CT scans

As per the protocol of the three hospitals located in the city of Manaus, Amazonas, Brazil, patients hospitalized due to COVID-19 had chest CT-scans approximately every five days, or whenever it was feasible to transport patient to the CT-scan room. A first chest CT scan was performed upon randomization and a second CT scan was performed approximately five days later, whenever patient health condition permitted the transportation. Patients in ICU or clinically unstable were not eligible for the five-day interval CT-scan follow-up.

The analysis of the chest CT scan was performed by board-certified radiologists with previous clinical expertise in COVID-19, that quantified the percentage of lung parenchyma involved in COVID-19, based on the classifications proposed by Xie et al, Zhao et al, Pan et al, Li et al, Chung et al, and Yuan et al,[15-20] following the standardization proposed by Martinez Chamorro et al.[12] The three hospitals unified and standardized the methods for the quantification of lung affected in order to avoid inter-operator differences. All chest CT scans were performed in CT SOMATOM model with 64-slice data acquisition (Simens Healthineers, Siemens, Germany).

Bilateral reticular patterns, peripheral bilateral ground-glass opacities, and patchy or confluent multifocal consolidation were considered as findings consistent with COVID-19 pneumonia. Central consolidation and unilateral ground-glass opacities were considered as indeterminate for COVID-19. Pneumothorax, pneumomediastinum, pleural effusion, lobar consolidation, military patterns or cavitation were not considered as part of COVID-19 pneumonia, although a series of case reports have described the first two characteristics. Long term fibrotic changes, such as honeycombing or traction bronchiectasis, could be consequences of COVID-19, but were not considered as part of the quantification of lungs affected. In short, only CO-RADS 6 were included in this analysis. All patients had diagnosis of COVID-19 through positive rtPCR-SARS-CoV-2 test.[21]

The analyses were performed in a complete independent manner. Radiologists were not informed whether patients were or were not participating in the RCT, as well as in which arm they were designated.

For quantification purposes, whenever an interval of percentage was provided instead of an exact percentage, this was replaced by an exact value, as following: <5% = 2%; <10% = 5%: <25% = 10%; 10-25% = 20%; <30% = 15%; <50% = 30%; 25-50% = 40%; >30% = 50%: 50-60% = 60%; >50% = 70%; 50-75% = 65%; >75% = 90%; >80% = 90%; and >90% = 95%.

For the present exploratory analysis, all COVID-19 hospitalized patients enrolled in the RCT from the three hospitals of the city of Manaus, Amazonas, were initially considered. Among these patients, all those with at least two chest CT scans during hospitalization were included for the analysis.

Endpoints

The differences in the quantification of lung parenchyma affected by COVID-19 chest, seen through chest CT scan results, between the baseline (first) and second exam, in terms of: 1. absolute changes (in points percent – p.p.; *eg.*. If the first CT scan showed 50% of lungs affected and the second CT scan showed 25% of lungs affected, a 25p.p. reduction – -25p.p – was observed); and 2. relative changes (percentage of change.; *eg.*. If the first CT scan showed 50% of lungs affected and the second CT scan showed 25% of lungs affected, a 50%. reduction – -50% – was observed, compared to the first CT scan), were compared between proxalutamide and placebo arms.
Baseline characteristics and clinical outcomes are described to evaluate whether the dimension of the drug efficacy of the RCT is represented in this *post-hoc* analysis.

Statistical Analysis

An original intention-to-treat (ITT) protocol (unmodified) was used for data analysis. Analysis was not stratified by sex since both men and women presented similar clinical responses to proxalutamide in the RCT compared to placebo.

Cox proportional hazards model was used to calculate hazard ratio (HR) for all-cause 14-day and 28-day mortality and their 95% confidence interval (CI), to measure the effects of proxalutamide versus placebo. Non-parametric Kruskal-Wallis Test was employed to measure the effects of proxalutamide *versus* placebo for radiological

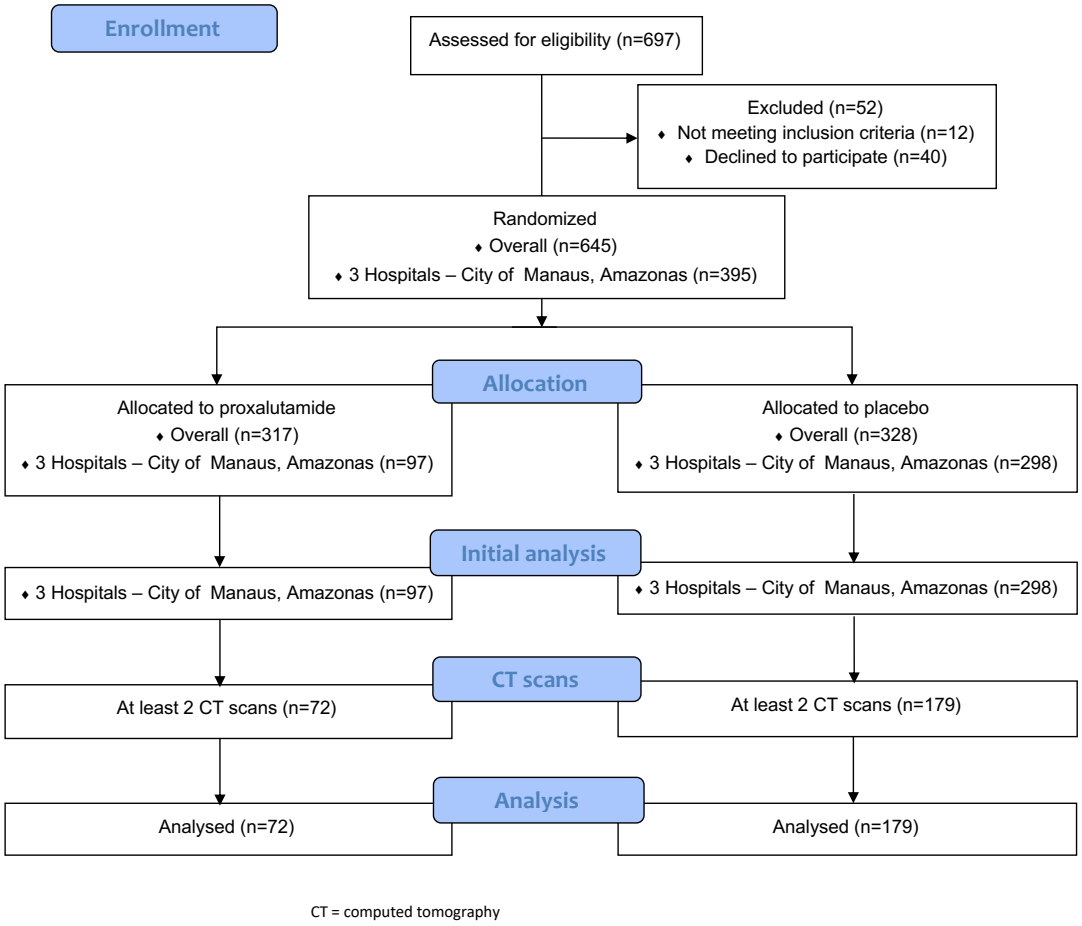
endpoints, and disclosed as *p-values*. All statistical tests were performed using IBM-SPSS statistics version 25.0 software (IBM, USA).

Results

The flowchart depicting the subject selection in the proposed protocol is shown in Figure 1. A total of 395 patients were initially evaluated, including 97 patients in the active arm and 298 patients in the placebo arm, which corresponds to all patients enrolled in the three hospitals from Manaus, Amazonas, Brazil. Imbalances between sites in terms of active and placebo proportions are explained elsewhere.[11] Of these, 72 patients from the proxalutamide arm and 179 patients from the placebo arm had at least two chest CT scans, and were included in the present analysis.

Figure 1. Protocol flowchart.

Figure 1. Procotol flowchart –
Analysis of the chest CT scans in the Proxa-Rescue AndroCoV Trial.



Baseline characteristics, including age, proportion between males and females, and presence of comorbidities, and additional parameters such as median time since hospitalization, distribution of the score in the COVID-19 ordinary scale, and use of concomitant medications were similar between proxalutamide and placebo group (Table 1).

Of the patients included in the present exploratory analysis, the 14-day mortality was 3.1% in the proxalutamide group and 38.9% in the placebo group, with a mortality risk ratio (RR) of 0.08 (0.03-0.14). The 28-day mortality was 6.2% in the proxalutamide group and 48.7% in the placebo group, with a mortality RR of 0.13 (0.06-0.28). The

median hospitalization length stay was 8.0 days in the proxalutamide group and 12.0 days in the placebo group ($p < 0.0001$) (Table 1).

No drug-related severe adverse effects (SAEs) were reported (Table 1).

Table 1. Baseline clinical characteristics, outcomes, and adverse effects.

INTENTION-TO-TREAT IN THE THREE INTITUTIONS LOCATED IN THE CITY OF MANAUS, AMAZONAS, BRAZIL				
Characteristic	**Overall** N= 395	**Proxalutamide** N=97	**Placebo** N=298	p
Age				
Median – years (IQR)	47.5 (38-59)	46.5 (39-59)	47.5 (37-60)	*n/s*
> 55 yr – no. (%)	254 (36.5%)	34 (35.1%)	110 (36.9%)	*n/s*
Sex – no. (%)				
Female	168 (42.6%)	37 (38.2%)	131 (44.0%)	*.527*
Male	227 (57.4%)	60 (61.8%)	167 (56.0%)	
BMI > 30 – no. (%)	35 (8.9%)	s12 (12.4%)	23 (7.7%)	*n/s*
Hypertension – no. (%)	99 (25.1%)	30 (30.9%)	69 (23.2%)	*n/s*
Type 2 diabetes mellitus – no. (%)	35 (8.9%)	13(13.4%)	32 (10.7%)	*n/s*
COPD – no. (%)	12 (3.3%)	5 (5.2%)	7 (2.3%)	*n/s*
Chronic kidney disease – no. (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	*n/s*
Median time from hospitalization to randomization (IQR) – days	2.0 (1.0-4.0)	2.0 (1.0-3.0)	2.0 (1.0-4.0)	*n/s*
Score on the COVID-19 ordinal scale– no. (%)				
3. Hospitalized, not requiring supplemental oxygen - no longer requires ongoing medical care	2 (0.5%)	0 (0.0%)	2 (0.7%)	*n/s*
4. Hospitalized, not requiring supplemental oxygen, requiring ongoing medical care (COVID-19 related or otherwise)	13 (3.3%)	3 (3.1%)	10 (3.4%)	*n/s*
5. Hospitalized, requiring supplemental oxygen	117 (29.6%)	22 (22.7%)	95 (31.9%)	*n/s*
6. Hospitalized, receiving non-invasive ventilation or high flow oxygen devices	263 (66.6%)	72 (74.2%)	191 (64.1%)	*n/s*
Concomitant medications – no. (%)				
Ceftriaxone	638 (98.9%)	95 (97.9%)	295 (99.0%)	*n/s*
Colchicine	407 (63.1%)	97 (100%)	298 (100%)	*n/s*
Enoxaparin	645 (100%)	97 (100%)	298 (100%)	*n/s*
Macrolides (azithromycin, clarithromycin)	631 (97.8%)	89 (91.7%)	296 (99.3%)	*n/s*
Glucocorticosteroids (dexamethasone, methylprednisolone)	645 (100%)	97 (100%)	298 (100%)	*n/s*
Omeprazole	645 (100%)	97 (100%)	298 (100%)	*n/s*
Score on the COVID-19 ordinal scale at Day 14– Median (IQR)	5.0 (1-7)	1.0 (1-2)	6.5 (2-8)	< 0.001
Score on the COVID-19 ordinal scale at Day 28– Median (IQR)	4.0 (1-8)	1.0 (1-1)	5.5 (2-8)	< 0.001
Mortality at 14 days	119 (30.1%)	3 (3.1%)	116 (38.9%)	< 0.001
Mortality at 28 days	151 (38.2%)	6 (6.2%)	145 (48.7%)	< 0.001
Median hospitalization days (IQR)	11.0 (7.0-17.0)	8.0 (6.0-12.0)	12.0 (8.0-18.0)	< 0.001
Median hospitalization days after randomization (IQR)	8.0 (5.5-12.0)	6.0 (4.0-8.0)	9.0 (6.0-14.0)	< 0.001
Grade 5 – n (%)				
Death, Day 14	119 (30.1%)	3 (3.1%)	116 (38.9%)	< 0.001

Death, Day 28	151 (38.2%)	6 (6.2%)	145 (48.7%)	< 0.001
Grades 4 or 3 – n (%)				
Mechanical ventilation, Day 14	36 (9.1%)	3 (3.1%)	33 (11.1%)	< 0.001
Mechanical ventilation, Day 28	3 (0.8%)	1 (1.0%)	2 (0.7%)	n/s
Renal failure (creatinine increase > 100%)	22 (5.6%)	3 (3.1%)	19 (6.4%)	0.07
Liver damage (ALT > 250 U/L or >100% increase)	20 (5.1%)	4 (4.1%)	16 (5.4%)	n/s
Grades 2 or 1 – n (%)				
Diarrhea	32 (8.1%)	22 (22.7%)	10 (3.4%)	0.005
Abdominal pain	3 (0.8%)	2 (2.1%)	1 (0.3%)	n/s
Irritability	4 (1.0%)	4 (4.1%)	0 (0.0%)	n/s
Spontaneous erection	4 (1.0%)	4 (4.1%)	0 (0.0%)	n/s
Vomiting, dyspepsia, or palpitations	0 (0.0%)	0 (0.0%)	0 (0.0%)	-

CI = Confidence interval; IQR = Interquartile range; BMI = body mass index; COPD: chronic obstructive pulmonary disorder, COVID-19: coronavirus disease 2019.

The number of patients with at least two chest CT scans during hospitalization due to COVID-19 was 72 (74.2%) in the proxalutamide group and 179 (60.1%) in the placebo group ($p < 0.001$). The median interval between two chest CT scans was 5.0 days for both groups (p = n/s).

Radiological findings are described in Table 2. Figure 2 illustrates the percentage of lung parenchyma affected in the baseline and on-treatment chest CT scans (A) and the variation of percentage of lungs affected between baseline and on-treatment chest CT scans (B). The percentage of lung parenchyma involvement due to COVID-19 in the baseline chest CT scan (Median – Interquartile range (IQR)) was 60.0% in both proxalutamide and placebo groups, when all patients with a baseline chest CT scan were considered. When only those with at least two chest CT scans were considered, the percentage of lung affected (Median – IQR) was 60.0% (45.0-70.0%) in the proxalutamide group and 50.0% (30.0-70.0%) in the placebo group. In both cases, baseline chest CT scan was statistically similar between groups.

The percentage of lungs with COVID-19 opacities in the second chest CT scan (Median – IQR) was 35.0% (25.0-57.5%) in the proxalutamide group and 67.5% (50.0-80.0%) in the placebo group ($p < 0.001$).

The absolute change between the second and first chest CT scans (Median – IQR) was -15.0 percent points (p.p.) (-30.0 – 0.0p.p.) in the proxalutamide group, and +15.0p.p. (0.0 - +30.0p.p.) in the placebo group ($p < 0.001$).

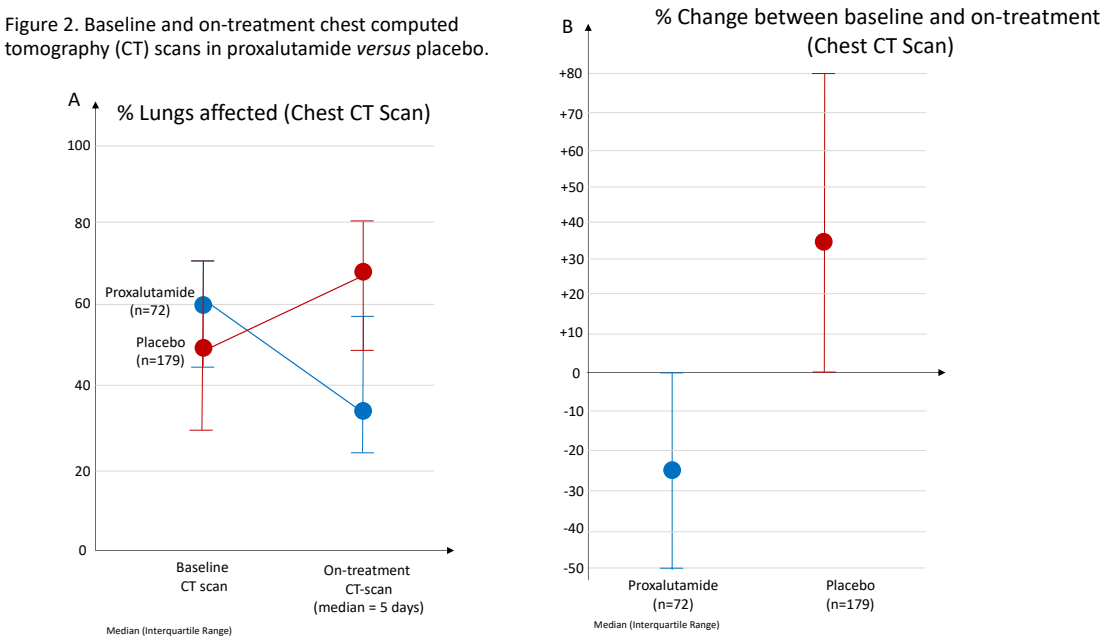
The relative change in terms of percentage between the second and first chest CT scans (Median – IQR) was -25.0% (-50.0 – 0.0%) in the proxalutamide group and +32.7% (0.0 - +80.0%) in the placebo group ($p < 0.001$).

Table 2. Radiological outcomes.

Parameter	**Overall** N=395	**Proxalutamide** N=97 (n=72*)	**Placebo** N=298 (n=179*)	**p**
Number of subjects with at least 1 Chest CT scan – n (%)	357 (90.4%)	89 (91.8%)	268 (89.9%)	n/s
Number of subjects with at least 2 Chest CT scans – n (%)	251 (63.5%)	72 (74.2%)	179 (60.1%)	< 0.001
Number of subjects with unknown percentage of affected lungs in first chest CT scan – n (%)	4 (1.0%)	0 (0.0%)	4 (1.3%)	n/s
Number of subjects with unknown percentage of affected lungs in second chest CT scan – n (%)	4 (1.0%)	1 (1.0%)	3 (1.0%)	n/s
% of affected lungs in the first chest CT-scan, including those without a second exam - Median (IQR)*	60.0 (40.0-70.0)	60.0 (40.0-70.0)	60.0 (40.0-70.0)	n/s
% of affected lungs, excluding those without a second exam - Median (IQR)**	55.0 (40.0-70.0)	60.0 (45.0-70.0)	50.0 (30.0-70.0)	n/s
Interval (days) between first and second chest CT scan - Median (IQR)	5.0 (4.0-7.0)	5.0 (4.0-6.0)	5.0 (4.0-7.0)	n/s
% of affected lungs in the second chest CT-scan - Median (IQR)	55.0 (40.0-70.0)	35.0 (25.0-57.5)	67.5 (50.0-80.0)	< 0.001
Absolute change (%) between first and second chest CT scan - Median (IQR)	0.0 (-10.0 - +20.0)	-15.0 (-30.0 – 0.0)	+15.0 (0.0 - +30.0)	< 0.001
Relative change in terms of percentage between first and second chest CT scan - Median (IQR)	0.0% (-30.0 - +20.0%)	-25.0% (-50.0 - 0.0%)	+32.7% (0.0 - +80.0%)	< 0.001

CT = computed tomograph; IQR = interquartile range
* Included for analysis; **In the day of randomization;

Figure 2. CT scans.



Discussion

The present analysis reinforces the efficacy of proxalutamide for hospitalized patients with COVID-19. The radiological improvement provides an independent and objective evaluation of the efficacy of the drug and predicts better clinical outcomes.[12,21] Unlike reports of clinical improvement, radiological findings analyzed by independent radiologists are not influenced by placebo effect, even though objective parameters such as the 8-point WHO COVID ordinary scale is would also hardly be influenced by this effect, except scores 1 to 4. Hence, an open label study would probably be enough to imply causality, without major interferences of the results from lack of blinding. However, our exploratory analysis derived from a double-blind, placebo-controlled, two-arm RCT. Importantly, radiologists that analyzed the images were blind to the intervention.

We analyzed the results utilizing the most appropriate type of analysis, the unmodified ITT, a more conservative analysis that tend to underestimate drug efficacy,[22] in the primary analysis of the RCT. Utilizing ITT population, we were able to find a 87% reduction in the 28-day all-cause mortality in the city of Manaus. In an on-treatment

(OT) analysis, the magnitude of the results tended to be even higher (92% reduction in 28-day mortality rate).[11]. The conservative nature of the ITT analysis and the higher efficacy on treatment completers, compared to non-treatment completers, showing a "dose response-like" behavior, are also suggestive of the efficacy of proxalutamide for hospitalized COVID-19 patients, in addition to the primary findings.

We avoided selection of patients. Instead, we included all participating subjects from the three hospitals. This regional subgroup analysis maintained a balance between proxalutamide and placebo groups in terms of demographic and baseline characteristics. Samples were precisely representative of the groups in the overall analysis. Typical limitations of a *post-hoc* analysis of a RCT are mostly absent in the present case.[13]

The number of patients without at least two chest TC scans was significantly higher in the placebo group than in the proxalutamide group, possibly because the number of patients that needed ICU was significantly higher in the placebo group (Table 2). Due to the absence of a mobile CT scan, the performance of a CT scan became unfeasible when patients needed ICU, in particular when they were under mechanical ventilation (Table 2).

The present analysis possibly underestimates the efficacy of proxalutamide for hospitalized patients with COVID-19, for two reasons: 1. Patients that had better responses to proxalutamide did not undergo a second CT scan, since they were discharged before five days, when a second chest CT scan would be performed, as per the hospitals protocol. Consequently, the best responders to to proxalutamide were probably selectively removed from analysis: and 2. Patients in the placebo group that had worse progression of the COVID-19 were not able to undergo a second CT scan because most of them needed ICU before five days, which precluded them from a second CT scan. In this way, patients that had better responses to usual care were probably selectively were included in the analysis. In short, this is an analysis that compared the group that responded relatively worse to proxalutamide, and therefore remained in the hospital for a longer period of time, with the group that presented a better COVID-19 disease course and did not require ICU. This hypothesis is reinforced by the fact that the median percentage of lung affected was 60.0% when all patients from the placebo group were included, and 50.0% when only those patients with at least

two chest CT scans were included, while this difference did not occur in the proxalutamide group. This means that patients that were worse tended to be excluded from the placebo group. A significant improvement even under this conservative bias reinforces the potential efficacy of the drug.

The imbalance between actives and placebos in present analysis is a result of a conservative bias of the RCT: more actives were randomly designated to institutions with fewer resources in rural areas, while more placebos were randomly designated to hospital with better infra-structure. Since in-hospital mortality of COVID-19 is highly variable and largely depends on the hospital resources[23], the use of more actives in institutions with fewer resources avoided overestimation of the drug efficacy. The analysis was conducted as Intention-To-Treat (ITT), *i.e.,* considering patients that dropped out the study, which is another conservative bias, since the efficacy of proxalutamide in hospitalized patients largely depended on a regular and uninterrupted 14-day treatment regimen. In fact, early discontinuation of the drug is highly discouraged.

The radiologists that analyzed the chest CTs were experienced with quantifying the percentage of lungs compromised by COVID-19 as the institutions for which they worked has managed more than 20,000 cases of COVID-19,[24] among which the vast majority underwent chest CT scans. In addition, the correlation between the quantification of COVID-19 lungs opacities by a board-certified radiologist experienced with COVID-19 chest CT-scans and artificial intelligence (AI) is strong in the majority of the cases.[25-27] All these aspects reduce the possibility of operational-bias of the study.

The finding of radiological improvement was unexpected since the interval between the baseline and the second chest CT scan was relatively short (median of five days in both groups). We would expect that radiological changes would occur in the long-, not short-run, as per the capacity of the disease resolution, even under effective therapies.[12,20] We hypothesize that this could be particularly true in our patient population, virtually solely infected by the Variant of Concern (VOC) P.1, arguably one of the most pathogenic SARS-CoV-2 variants described to date[28].

In addition to the strong antiandrogen activity and to the ACE-2 antagonism, further analyses demonstrated that proxalutamide may present direct protective actions in the lungs and vessels,[7-8] as well as anti-inflammatory effects, such as mitigation of tumor necrosis factor alpha (TNF-alpha) and nuclear factor kappa beta (NF-kB).[8] This may explain the dramatic clinical and radiological improvements observed with proxalutamide.

Limitations of the present analysis include the fact that radiological findings are not the primary outcomes, with a subgroup of patients enrolled in three of the eight institutions that participated in the RCT. with the inherent limitations of a post-hoc analysis of a subgroup of patients of the RCT, despite the full representation of the group in the subgroup analysis and balanced characteristics between proxalutamide and placebo groups. [11] The present findings should be strengthened by further external analysis, in particular including an analysis using AI.

To our knowledge, this is the first RCT that demonstrated radiological improvement in response to a drug intervention in COVID-19. These findings reinforce the efficacy of proxalutamide in hospitalized COVID-19 patients.

In conclusion, proxalutamide plus usual hospitalized care demonstrated to improve hospitalized COVID-19 patients radiologically, when compared to placebo plus usual hospitalized care.

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FIGURE LEGEND.

Figure 1. Procotol flowchart – Analysis of the chest CT scans in the Proxa-Rescue AndroCoV Trial.

Figure 2. Baseline and on-treatment chest computed tomography (CT) scans in proxalutamide *versus* placebo.