APPLIED BIOLOGY

CLINICAL STUDY PROTOCOL

Anti-Androgen Treatment for COVID-19 (Proxalutamide - GT0918)

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Clinical Study: Anti-Androgen Treatment for COVID-19

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Clinical Study: Anti-Androgen Treatment for COVID-19

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Name	**Signature**	**Date**
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Andy Goren, MD		

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List of Abbreviations

CFR	Code of Federal Regulations
CRF	Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IRB	Institutional Review Board
EC	Ethics Committee
HCP	Healthcare Professional
CDC	US Center for Disease Control
SAE	Serious Adverse Event
AE	Adverse Event
ARDS	Acute Respiratory Distress Syndrome
UP	Unanticipated Problem

1. Background

1. Overview

During the continuing SARS-CoV-2 (COVID-19) pandemic, several studies have reported a significant difference in the rate of severe cases between adult females and adult males (42% vs 58%).[1] Among children under the age of 14, the rate of severe cases was reported to be extremely low.[1] To explain this difference, several theories have been proposed including cigarette smoking and lifestyle habits. However, no theory fits both the gender difference in severe cases as well as reduced risk in pre-pubescent children. Our past research on male androgenetic alopecia (AGA) has led us to investigate an association between androgens and COVID-19 pathogenesis.[2] In normal subjects, androgen expression demonstrates significant variation between men and women as well as between adults and pre-pubescent children.

SARS-CoV-2 primarily infects type II pneumocytes in the human lung. SARS-CoV-2 enters pneumocytes, by anchoring to the ACE2 cell surface receptor. Prior to receptor binding, viral spike proteins undergo proteolytic priming by the transmembrane protease, serine 2 (TMPRSS2).[3–5] TMPRSS2 inhibition or knock down reduces ability of SARS-CoV-1 (a related virus to SARS-CoV-2) to infect cells in vitro.[6] Additionally, TMPRSS2 also facilitates entry of influenza A and influenza B into primary human airway cells and type II pneumocytes.[7]

The human TMPRSS2 gene has a 15 bp androgen response element and in humans, androgens are the only known transcription promoters for the TMPRSS2 gene.[8-10] In a study of androgen-stimulated prostate cancer cells (LNCaP), TMPRSS2 mRNA expression increase was mediated by the androgen receptor.[10] Further, the ACE2 receptor, also critical for SARS-CoV-2 viral infectivity, is affected by male sex hormones with higher activity found in males.[11]

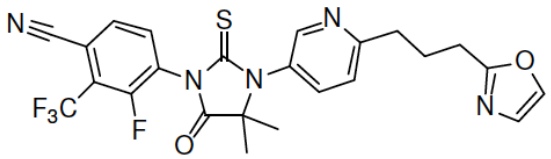
Previously, we have reported the results from two retrospective cohort analysis demonstrating the protective effect of 5-alpha-reductase inhibitors (5ARi) for men with COVID-19.[17] In a study of 77 men hospitalized with COVID-19 we found among men taking 5ARis, 8% were admitted to the ICU compared to 58% of men not taking 5ARis ($P = 0.0015$). In the cohort, 5ARis were associated with reduced risk for ICU admissions RR 0.14 (95% CI: 0.02–0.94).[17] Similarly, we have demonstrated that the frequency of COVID-19 symptoms was drastically reduced for men using 5ARis in an outpatient setting. A statistically significant ($p<0.05$) reduction in the frequency of 20 of the 29 clinical symptoms was observed in AGA men using 5ARis compared to AGA men not using 5ARis. For example, 38% and 2% of men presented with low-grade fever, 60% and 6% with dry cough, and 88% and 15% reported anosmia in the non-5ARi and 5ARi groups, respectively.[18]

One limitation of 5ARis is the time course required to achieve systemic DHT reductions. As such, we explored the use of a novel second generation androgen receptor antagonist Proxalutamide as a means for rapid reduction in AR activity. Proxalutamide (GT0918) demonstrates a dual mechanism of action. It is highly effective in inhibiting AR as well as

exhibiting pharmacological effects of inducing the down-regulation of AR expression; the mechanism that is not present in bicalutamide and enzalutamide. Because of the dual mechanism of action, it is expected to be a more effective and less toxic second-generation anti-androgen drug therapy. Clinical evidence has demonstrated that Proxalutamide lowers AR expression and activity. Additionally, it has been reported that Proxalutamide lowers the expression of ACE2. Both would be beneficial for preventing SARS-CoV-2 entry into lung cells.

1.1. Investigational Drug

Proxalutamide (200 mg) q.d.

Chemical Name	4-[4,4-dimethyl-3-[6-[3-(2-oxazolyl)propyl]-3-pyridinyl]-5-oxo-2-thioxo-1-imidazolidinyl]-3-fluoro-2-(trifluoromethyl)-benzonitrile
CAS Registry No.	1398046-21-3
Company Code	GT0918
Chemical Structure	
Molecular Formula	$C_{24}H_{19}F_4N_5O_2S$
Relative Molecular Mass	517.50

Proxalutamide (GT0918) demonstrates a dual mechanism of action. It is highly effective in inhibiting AR as well as exhibiting pharmacological effects of inducing the down-regulation of AR expression; the mechanism that is not present in bicalutamide and enzalutamide. Because of the dual mechanism of action, it is expected to be a more effective and less toxic second-generation anti-androgen drug therapy. Clinical evidence has demonstrated that Proxalutamide lowers AR expression and activity. Additionally, it has been reported that Proxalutamide lowers the expression of ACE2. Both would be beneficial for preventing SARS-CoV-2 entry into lung cells.

The Proxalutamide tablets (100mg per tablet) will be manufactured by:

TOT BIOPHARM International Company Limited. No. 120 changyang Street, Suzhou Industrial Park, Suzhou City, Jiangsu Province, China

1.1. Pre-Clinical and Prior Clinical Data

1.1.1. Prior Pre-Clinical and Clinical Safety Data

No prior pre-clinical safety data exists as to the use of Proxalutamide for the treatment of COVID-19; however, pre-clinical studies have been conducted in support of the US FDA IND approval of Phase-1 human trials of Proxalutamide in castration resistant prostate cancer. Selected pre-clinical animal studies conducted with Proxalutamide are provided below.

Tissue Distribution and Excretion of GT0918 in Rats

The tissue distribution of GT9018 in male SD rats was determined at 0.5, 3 and 12 hours following an oral single administration (20 mg/kg). Proxalutamide was extensively distributed to most of the tissues/organs with high concentrations found in fat tissue and liver, followed by stomach, pancreas and intestine in rats. Proxalutamide distribution was also observed in bone marrow in rats. Peak Proxalutamide concentrations were achieved in most tissues at 3 hours following the administration and a certain degree of elimination was observed in these tissues at 12 hours following the administration.

The excretion profiles (bile, urine and feces) following a single oral dose of Proxalutamide (20 mg/kg) to 6 male SD rats was studied. The data indicated that following a single oral administration of Proxalutamide to rats, the cumulative fractional excretion of Proxalutamide in bile within 36 hours was 0.010 ± 0.006%, the cumulative fractional excretion of Proxalutamide in urine within 72 hours was 0.014 ± 0.009% and the cumulative fractional excretion of Proxalutamide in feces within 72 hours was 10.785 ± 4.547%. Proxalutamide was mainly excreted in the feces (about 10%) and in trace amounts through bile and urine (0 to 0.1%) in rats.

Pharmacokinetic Profiles of Proxalutamide in SD Rats

Pharmacokinetic properties of Proxalutamide were studied in SD rats following single oral dosing at 10, 20, 40 and 80 mg/kg, single IV dosing at 5 mg/kg and repeated PO dosing at 20 mg/kg daily for 8 consecutive days. After single oral dosing, Proxalutamide reached maximal plasma concentrations in 3 to 5 hours and then decreased with the elimination half-life 2.0 to 2.5 hours. Both AUC and Cmax were proportional to dose. The absolute bioavailability was 74 - 100%. After repeated dosing for 8 days, AUC and Cmax increased about 50% comparing to single dose at the same level, indicating some drug accumulation after repeated dosing.

Pharmacokinetic Profiles of Proxalutamide in Beagle Dogs

Pharmacokinetic properties of Proxalutamide were studied in Beagle dogs following single oral dosing at 2, 5 and 10 mg/kg under fasting conditions, single IV dosing at 1 mg/kg, a single oral dosing at 20 mg/kg (with two different API processes) under fed conditions, and repeated PO dosing at 20 mg/kg daily for 7 consecutive days. After single

oral dosing, Proxalutamide reached maximal plasma concentrations in 2 to 2.5 hours and then decreased with the elimination half-life 9.5 to 11.8 hours. Both AUC and Cmax were proportional to dose. The absolute bioavailability was 36.5 – 52.5%. Under fed conditions, Cmax markedly decreased about 60% with Tmax increase from 3 hours to 8.3 hours but slightly increased in AUC compared to those respective PK parameters under fasting conditions at the same dose. After repeated dosing for 8 days, AUC and Cmax increased about 70% and 150%, respectively comparing to single dose at the same level, indicating drug accumulation after repeated dosing.

1.1.2 Prior Clinical Safety Data

Study 1: Proxalutamide Treatment for Non-Hospitalized COVID-19 Male Patients (NCT04446429)

A double-blinded, randomized, prospective, investigational study of Proxalutamide Treatment for Non-Hospitalized COVID-19 Male Patients (NCT04446429) was completed in December 2020. The length of the study was 30 days. Proxalutamide was administered 200mg q.d. for 14 days. Men enrolled in the study were 50 years and older. Two hundred and sixty two men completed the study. 134 men were assigned to the Proxalutamide group and 128 men were assigned to the control group. Thirty five subjects were hospitalized in the control group compared to zero in the Proxalutamide group. The proportion of COVID-19 patients hospitalized was significantly different between the Proxalutamide and control arms; $\chi 2\ (1) = 42.051$, $p<.0001$. The difference in proportions was 27.30% with a 95%CI: [19.79%, 35.59%]. No subject receiving Proxalutamide died compared to 2 (1.56%) in the control group. There were no treatment related adverse event reported during the course of the study.

Study 2: Phase 1 study in 16 males with prostate cancer.

The data from the study was published in a peer review journal. The identifier GT0918 was used to denote Proxalutamide. The abstract from the publication is below:

Abstract Purpose: We conducted preclinical experiments and phase I clinical trial to investigate the safety, pharmacokinetics (PK) and antitumour effects of GT0918 in castration- resistant prostate cancer (CRPC).
Experimental design: An androgen receptor (AR) competitive binding assay was performed, followed by evaluation of GT0918 on AR protein expression. The efficacy of GT0918 was investigated in a castration-resistant xenograft model. A phase I dose-escalation study of GT0918 in CRPC was also carried out to evaluate its safety, PK and antitumour efficacy. Results: GT0918 was demonstrated to inhibit the binding of androgen to AR more potently than MDV3100, and to effectively reduce the AR protein level. GT0918 inhibited the tran- scriptional activity of wild-type AR and AR with clinically relevant ligand-binding domain mutations. Furthermore, GT0918 significantly inhibited the growth of prostate cancer. A total of 16 patients was treated with GT0918 at five dose levels. Among

these 16 patients, 10 and 2 patients, respectively, completed a three-cycle and six-cycle treatment, in which MTD was not reached. All the treatment-related adverse events were grade I, including hypercholesterolemia, hypertriglyceridemia, fatigue and anemia. PK parameters showed that drug exposure increased with dose proportionally from 50 to 300 mg and a saturation was observed between 300mg and 400 mg.

The most significant adverse events from the study are summarized in the following table:

Table 2
Most common adverse events of GT0918 by dose (multiple-dose period, ≥2 cycles).

Adverse event	50 mg (n = 2)		100 mg (n = 4)		200 mg (n = 3)		300 mg (n = 3)		400 mg (n = 4)		Total (n = 16)	
	Drug-related	Drug-irrelative	Drug-related	Drug-irrelative	Drug-related	Drug-irrelative	Drug-related	Drug-irrelative	Drug-related	Drug-irrelative	Drug-related	Drug-irrelative
Blood and lymphatic diseases												
Anaemia	1	0	0	1	0	1	0	0	0	0	1	2
Heart diseases												
Coronary artery disease	0	1	0	0	0	0	0	0	0	0	0	1
Visual diseases												
Eye discomfort	0	0	0	1	0	0	0	0	0	0	0	1
Gastrointestinal diseases												
Constipation	0	0	0	0	0	0	1	0	0	0	1	0
Systemic diseases												
Chest pain	0	0	0	0	0	0	0	1	0	0	0	1
Peripheral oedema	0	0	0	0	0	0	0	2	0	1	0	3
Abnormal examination results												
Prolonged coagulation indicators	0	0	0	0	0	1	0	0	0	0	0	1
QT interval extension	0	0	0	0	0	1	0	0	0	0	0	1
LDH increased	0	0	0	0	0	0	0	1	0	0	0	1
ALP increased	0	0	0	0	0	0	0	1	0	0	0	1
Metabolic and nutritional diseases												
Hypercholesterolemia	0	0	0	0	0	0	2	0	1	0	3	0
Malnutrition	0	0	0	0	0	1	0	0	0	0	0	1
Musculoskeletal and connective tissue diseases												
Arthralgia	0	0	0	0	0	1	0	0	0	0	0	1
Neurological diseases												
Dizziness	0	0	0	0	0	0	0	0	0	1	0	1
Urinary diseases												
Haematuria	0	1	0	0	0	0	0	0	0	0	0	1
Vascular diseases												
Aortic aneurysm	0	0	0	0	0	1	0	0	0	0	0	1
Hot flushes	0	0	0	0	0	0	1	0	0	0	1	0

LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

Study 3: Phase 1 study conducted in 40 males with prostate cancer.

A summary of the study is provided below:

Demographics: All 40 patients in the Safety Analysis Set were male with the mean age at 70.1 years. Three (7.5%) patients were Hispanic or Latino and 37 (92.5%) were not Hispanic or Latino. Thirty-five (87.5%) patients were white, 4 (10%) were black or African American and 1 (2.5%) was Asian. Of the 40 patients with mCRPC tumors, all patients (100%) had received at least two lines of hormone therapies, with 11 (27.5%) having 3 lines and 20 (50%) having 4 lines or more. Most patients (70%) had also undergone chemotherapies, with 20 (50%) receiving one line and 8 (20%) receiving 2 or more lines of chemotherapies.
Disposition: The distribution of 40 patients by dose cohort was as follows: 3 at the 50 mg, 6 at the 100 mg, 6 at the 200 mg, 7 at the 300 mg, 7 at the 400 mg, 6 at the 500 mg and 5 at the 600 mg per day dose levels. Of the 40 patients, 1 (2.5%) did not complete 1 cycle, 14 (35%) completed 1 cycle, 10 (25%) completed 2 cycles, 3 (7.5%) completed 3 cycles, 3 (7.5%) completed 4 cycles, 5 (12.5%) completed 5 cycles, and 4 (10%) completed at least 6 cycles of treatment. The primary reason for discontinuation was progressive disease (28/40 patients, 70%). Other discontinuation reasons included the

following: unacceptable toxicity or AE (6/40, 15%), withdrawal of consent (4/40, 10%) and patient lost to follow-up (2/40, 5%).

*Efficacy***:** Of the 40 patients, no PSA response with more than 50% reduction from baseline was observed. CR or PR are not required as per phase 1 study data analyses. Treating physicians will do imaging scan to see if patients have with SD allowing for continuing the treatment. The mean number of dosing duration was 12.5 weeks (87.6 days) across all dose cohorts from 50 mg/day to 600 mg/day, and the mean dosing duration for individual dose cohorts ranged from 8.3 weeks (57.7 days) to 15.6 weeks (109.4 days). Patients with treatment duration longer than 22.9 weeks (160 days) were from the following dose cohorts: 1 in the 50 mg/day, 1 in the 100 mg/day, and 2 in the 400 mg/day cohorts. A total of 9 patients had completed between 4 and 6 cycles (28 days per cycle) of treatment, belonging to these dose cohorts: 200 mg/day (1/6), 400 mg/day (4/7), 500 mg/day (2/6), and 600 mg/day (2/5).

*Safety***:** The results from this study showed that the safety profile of GT0918 was generally favorable in patients with mCRPC whose disease progressed after multi-lines of therapies. The mean number of dosing duration was 87.6 days across all dose cohorts from 50 mg/day to 600 mg/day, and the mean dosing duration for individual dose cohorts ranged from 57.7 to 109.4 days. Of the 40 patients, 39 (98%) experienced at least 1 TEAE during the study, with the most frequent AEs being fatigue, nausea, decreased appetite, anemia, weight decrease, diarrhea, constipation, back pain and dizziness. Most of patients reported TEAEs that were considered related to the study drug, with the most common drug-related AEs being fatigue (42.5%), decreased appetite (20%), nausea (15%), dizziness (12.5%), constipation (12.5%), anemia (10%), weight decrease (10%), dysgeusia (10%), and diarrhea (7.5%). Most TEAEs were Grade 1 or 2. Twenty patients across all dose cohorts reported TEAEs of Grade 3 or higher. Each individual TEAE of Grade 3 or higher occurred sporadically in 1 or 2 patients, except for the following: anemia (7/40), fatigue (5/40) and sepsis (3/40). The majority of Grade 3 or higher TEAEs were considered not related to the study drug. Overall, nine patients (9/40, 22.5%) reported at least one SAE. The nine patients were distributed in nearly all dose cohorts except the 100 mg/day cohort. The majority of SAEs were Grade 3 or 4, and two Grade 5 deaths were reported. Both deaths were due to disease progression and they were not related to the study drug. Most SAEs were not drug-related, except for one event of Grade 4 increased creatine phosphokinase (CK). Five patients with SAEs, including sepsis, worsening dehydration, pneumonia and increased CK, were permanently discontinued from the study. Other drug-related AEs that led to study discontinuation included: fatigue (Grade 3 and Grade 2), anemia (Grade 3) and decreased white blood cell (Grade 3). No DLT was reported in any of the dose cohort. Therefore, MTD was not established in this study. Overall, GT0918 was generally well-tolerated in mCRPC patients.

A summary of AEs are given in the following tables:

Table 12.3 Overview of Treatment-Emergent Adverse Events, Safety Analysis Set

	Escalation Cohort							
	50 mg/day (N=3) n (%)	100 mg/day (N=6) n (%)	200 mg/day (N=6) n (%)	300 mg/day (N=7) n (%)	400 mg/day (N=7) n (%)	500 mg/day (N=6) n (%)	600 mg/day (N=5) n (%)	Total (N=40) n (%)
Adverse Events	3 (100.0%)	5 (83.3%)	6 (100.0%)	7 (100.0%)	7 (100.0%)	6 (100.0%)	5 (100.0%)	39 (97.5%)
Drug-Related Adverse Events	0	3 (50.0%)	4 (66.7%)	6 (85.7%)	6 (85.7%)	6 (100.0%)	5 (100.0%)	30 (75.0%)
Deaths	0	0	0	1 (14.3%)	1 (14.3%)	0	0	2 (5.0%)
Serious Adverse Events	1 (33.3%)	0	1 (16.7%)	2 (28.6%)	3 (42.9%)	1 (16.7%)	1 (20.0%)	9 (22.5%)
Drug-Related Serious Adverse Events	0	0	0	0	0	1 (16.7%)	0	1 (2.5%)
Adverse Events Leading to Permanent Discontinuation of Study Drug	0	0	0	3 (42.9%)	2 (28.6%)	2 (33.3%)	2 (40.0%)	9 (22.5%)
Drug-Related Adverse Events Leading to Permanent Discontinuation of Study Drug	0	0	0	2 (28.6%)	0	2 (33.3%)	2 (40.0%)	6 (15.0%)
Grade 3 or Higher Treatment-Emergent Adverse Events	2 (66.7%)	1 (16.7%)	3 (50.0%)	3 (42.9%)	7 (100.0%)	2 (33.3%)	2 (40.0%)	20 (50.0%)

Table 12.4 Treatment-Emergent Adverse Events by System Organ Class >20%, Safety Analysis Set

	Escalation Cohort							Total
System Organ Class *Preferred Term*	GT0918 50 mg/day (N=3) n (%)	GT0918 100 mg/day (N=6) n (%)	GT0918 200 mg/day (N=6) n (%)	GT0918 300 mg/day (N=7) n (%)	GT0918 400 mg/day (N=7) n (%)	GT0918 500 mg/day (N=6) n (%)	GT0918 600 mg/day (N=5) n (%)	Total (N=40) n (%)
Overall	3 (100.0%)	5 (83.3%)	6 (100.0%)	7 (100.0%)	7 (100.0%)	6 (100.0%)	5 (100.0%)	39 (97.5%)
General Disorders And Administration Site Conditions	2 (66.7%)	3 (50.0%)	4 (66.7%)	6 (85.7%)	3 (42.9%)	6 (100.0%)	4 (80.0%)	28 (70.0%)
Fatigue	2 (66.7%)	1 (16.7%)	2 (33.3%)	4 (57.1%)	3 (42.9%)	6 (100.0%)	3 (60.0%)	21 (52.5%)
Musculoskeletal And Connective Tissue Disorders	2 (66.7%)	3 (50.0%)	4 (66.7%)	5 (71.4%)	7 (100.0%)	4 (66.7%)	1 (20.0%)	26 (65.0%)
Arthralgia	1 (33.3%)	0	3 (50.0%)	1 (14.3%)	2 (28.6%)	0	0	7 (17.5%)
Back Pain	0	2 (33.3%)	0	4 (57.1%)	2 (28.6%)	2 (33.3%)	0	10 (25.0%)
Gastrointestinal Disorders	1 (33.3%)	3 (50.0%)	4 (66.7%)	3 (42.9%)	5 (71.4%)	5 (83.3%)	4 (80.0%)	25 (62.5%)
Constipation	0	2 (33.3%)	2 (33.3%)	2 (28.6%)	2 (28.6%)	2 (33.3%)	0	10 (25.0%)
Diarrhoea	0	0	1 (16.7%)	1 (14.3%)	5 (71.4%)	3 (50.0%)	0	10 (25.0%)
Nausea	1 (33.3%)	1 (16.7%)	2 (33.3%)	1 (14.3%)	4 (57.1%)	2 (33.3%)	2 (40.0%)	13 (32.5%)
Nervous System Disorders	2 (66.7%)	4 (66.7%)	4 (66.7%)	4 (57.1%)	3 (42.9%)	2 (33.3%)	3 (60.0%)	22 (55.0%)
Dizziness	0	1 (16.7%)	0	2 (28.6%)	3 (42.9%)	2 (33.3%)	1 (20.0%)	9 (22.5%)

Table 12.4 Treatment-emergent Adverse Events by System Organ Class >20% page 2 of 2

System Organ Class *Preferred Term*	Escalation Cohort							Total
	GT0918 50 mg/day (N=3) n (%)	GT0918 100 mg/day (N=6) n (%)	GT0918 200 mg/day (N=6) n (%)	GT0918 300 mg/day (N=7) n (%)	GT0918 400 mg/day (N=7) n (%)	GT0918 500 mg/day (N=6) n (%)	GT0918 600 mg/day (N=5) n (%)	Total (N=40) n (%)
Metabolism And Nutrition Disorders	1 (33.3%)	2 (33.3%)	2 (33.3%)	4 (57.1%)	5 (71.4%)	4 (66.7%)	3 (60.0%)	21 (52.5%)
Decreased Appetite	0	1 (16.7%)	1 (16.7%)	2 (28.6%)	3 (42.9%)	3 (50.0%)	2 (40.0%)	12 (30.0%)
Investigations	1 (33.3%)	1 (16.7%)	1 (16.7%)	4 (57.1%)	3 (42.9%)	4 (66.7%)	1 (20.0%)	15 (37.5%)
Weight Decreased	1 (33.3%)	0	1 (16.7%)	1 (14.3%)	3 (42.9%)	3 (50.0%)	1 (20.0%)	10 (25.0%)
Respiratory, Thoracic And Mediastinal Disorders	2 (66.7%)	1 (16.7%)	2 (33.3%)	3 (42.9%)	4 (57.1%)	1 (16.7%)	2 (40.0%)	15 (37.5%)
Blood And Lymphatic System Disorders	1 (33.3%)	1 (16.7%)	2 (33.3%)	3 (42.9%)	4 (57.1%)	1 (16.7%)	1 (20.0%)	13 (32.5%)
Anaemia	1 (33.3%)	1 (16.7%)	2 (33.3%)	3 (42.9%)	3 (42.9%)	1 (16.7%)	0	11 (27.5%)
Infections And Infestations	2 (66.7%)	0	0	2 (28.6%)	4 (57.1%)	0	3 (60.0%)	11 (27.5%)
Vascular Disorders	0	3 (50.0%)	1 (16.7%)	2 (28.6%)	4 (57.1%)	0	1 (20.0%)	11 (27.5%)
Renal And Urinary Disorders	1 (33.3%)	0	2 (33.3%)	3 (42.9%)	1 (14.3%)	1 (16.7%)	0	8 (20.0%)
Cardiac Disorders	1 (33.3%)	0	0	2 (28.6%)	3 (42.9%)	1 (16.7%)	1 (20.0%)	8 (20.0%)

Study 4: Phase II dose escalating study of Proxalutamide (100-300 mg) in 108 patients with prostate cancer.

A table of adverse events observed in the trial are described below.

PT name	100 mg (N=37)%	200 mg (N=35)%	300 mg (N=36)%	Total (N=108)%	P
Asthenia/ fatigue	2 (5.4)	7（20.0）	14（38.9	23（21.3）	<0.05
Aspartate aminotransferase	5 (13.5)	5 (14.3)	6 (16.7)	16 (14.8)	>0.05
Anaemia	3 (8.1)	7 (20.0)	6 (16.7)	16 (14.8)	>0.05
Alanine aminotransferase increased	6 (16.2)	4 (11.4)	4 (11.1)	14 (13.0)	>0.05
Decreased appetite	3 (8.1)	3 (8.6)	8 (22.2)	14 (13.0)	>0.05
White blood cell count decreased	2 (5.4)	5 (14.3)	6 (16.7)	13 (12.0)	>0.05
Proteinuria	4 (10.8)	4 (11.4)	5 (13.9)	13 (12.0)	>0.05
Constipation	1 (2.7)	3 (8.6)	6 (16.7)	10 (9.3)	>0.05
Oedema peripheral	1 (2.7)	4 (11.4)	3 (8.3)	8 (7.4)	>0.05
Blood fibrinogen increased	2 (5.4)	2 (5.7)	3 (8.3)	7 (6.5)	>0.05
Blood triglycerides increased	5 (13.5)	2 (5.7)	0	7 (6.5)	>0.05
Neutrophil count decreased	0	4 (11.4)	3 (8.3)	7 (6.5)	>0.05
Weight decreased	1 (2.7)	2 (5.7)	4 (11.1)	7 (6.5)	>0.05
Hypertriglyceridaemia	1 (2.7)	3 (8.6)	3 (8.3)	7 (6.5)	>0.05
Nausea	1 (2.7)	0	6 (16.7)	7 (6.5)	<0.05
Supraventricular extrasystoles	1 (2.7)	3 (8.6)	2 (5.6)	6 (5.6)	>0.05
Haematuria	2 (5.4)	3 (8.6)	1 (2.8)	6 (5.6)	>0.05
Platelet count decreased	2 (5.4)	1 (2.9)	2 (5.6)	5 (4.6)	>0.05

Drug Interactions

Inhibition Potential Assessment of Proxalutamide on Cytochrome P450 Enzymes in Pooled Human Liver Microsomes

The inhibition of human liver CYPs: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5, was assessed with Proxalutamide at concentrations of 0.2, 0.8, 2, 10, 50, 100 and 200 μM. The metabolite formation from CYP catalyzed probe substrate metabolism was analyzed with LC-MS/MS. The results showed that Proxalutamide showed no significant inhibition on CYP1A2 and CYP2E1, weak inhibition on CYP2D6, CYP2C9, CYP2C19 and CYP3A (midazolam), but marked inhibition on CYP3A4 (testosterone).

The results indicate that there is an inhibitory effect of Proxalutamide on CYP3A4 in vitro and the corresponding in vivo drug interaction potential needs to be further investigated.

*No interaction has been reported between Proxalutamide, nitazoxanide, and azithromycin.

Table 1.2.3: Inhibitory Effect of Proxalutamide on CYP450 Enzymes

CYP450s	Probe substrate	Characterized Metabolic Pathway	Characterized Metabolite	IC_{50} (μM)
CYP1A2	Phenacetin	Deethylation	Acetaminophen	>200
CYP2C9	Tolbutamide	Hydroxylation	4-Hydroxy-Tolbutamide	8
CYP2C19	Omeprazole	Hydroxylation	5-Hydroxy- Omeprazole	8
CYP2D6	Dextromethorphan	Demethylation	Dextrorphan	5
CYP3A4	Midazolam	Hydroxylation	1-Hydroxy-Midazolam	56
CYP3A4	Testosterone	Hydroxylation	6β-Testosterone	1
CYP2E1	Chlorzoxazone	Hydroxylation	6-Hydroxy-Chlorzoxazone	>200

Evaluation of Cytochrome P450 Induction Potential of Proxalutamide in Human Hepatocytes

Proxalutamide was evaluated for induction of drug metabolizing enzymes in primary human hepatocytes. No inductive effects on CYP1A2 and CYP3A4 were observed in the level of enzymatic activity.

Table 10: Effect of Proxalutamide on the Enzyme Activity of CYP1A2

	Activity of CYP1A2 (%)			Mean	SD	Activity compared to positive control (%)
Negative control	109	82.4	108	100	15.2	NA
Positive control	1174	1246	1207	1209	36.3	100
Proxalutamide 1 μM	98.9	98.2	95.6	97.6	1.74	<40
Proxalutamide 10 μM	92.3	98.9	99.6	96.9	4.01	<40

Table 11: Effect of PROXALUTAMIDE on the Enzyme Activity of CYP3A4

Control	Activity of CYP3A4 (%)			Mean	SD	Activity compared to positive control (%)
Negative control	97.3	103	99.6	100	2.97	NA
Positive control	805	976	825	869	93.4	100
Proxalutamide 1 μM	24.2	26.0	27.7	26.0	1.73	<40
Proxalutamide 10μM	19.7	21.3	19.4	20.2	1.05	<40

Specific Populations

Pediatric

Proxalutamide pharmacokinetics have not been investigated in subjects younger than 18 years.

Geriatric

No dose adjustment is necessary in the elderly. The pharmacokinetics and pharmacodynamics of Proxalutamide were evaluated in 40 patients with an average age of 70.1 (Study 2, Section 1.1.2.4.).

Gender

Proxalutamide is not indicated for use in women. The palnned interventional study will be conducted in men only.

Race

The effect of race on Proxalutamide pharmacokinetics has not been studied.

Renal Impairment

The effect of renal impairment on Proxalutamide pharmacokinetics has not been studied.

Hepatic Impairment

The effect of hepatic impairment on Proxalutamide pharmacokinetics has not been studied.

1.1.3 Prior Pre-clinical Efficacy Data

No prior pre-clinical data exists as to the use of Proxalutamide as a treatment for COVID-19.; however, two studies highlight the possible benefit of the dual anti-androgen activity of Proxalutamide .

Proxalutamide inhibition of androgen binding to AR and AR protein expression

A study by Zhou et al[19] reported that: "GT0918 inhibited the binding of androgen to AR in a dose-dependent manner, and the Ki value of GT0918 (1.4 x 10^{-8} M) in binding to AR was 3.4-fold lower than that of MDV3100 (4.8 x 10^{-8} M) (Fig. 1A). It indicated that GT0918 was more potent than MDV3100 in inhibiting the binding of androgen to AR." Additionally, in cultures of C4-2B cells, "the protein expression of AR was significantly reduced by GT0918". Data depicting the inhibition androgen binding to AR and the reduced AR protein expression in C4-2B cells is shown below:

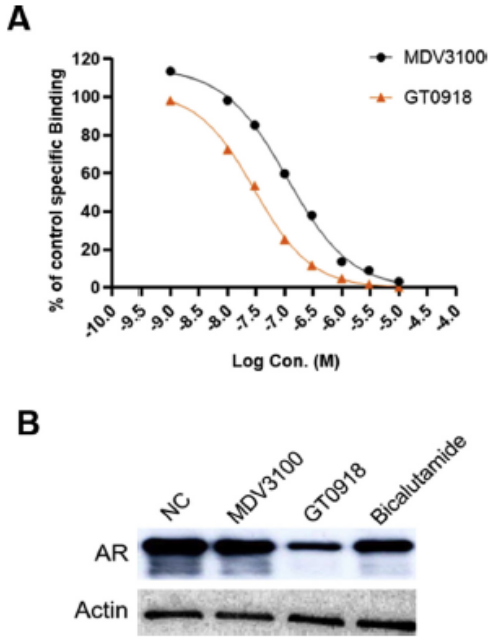
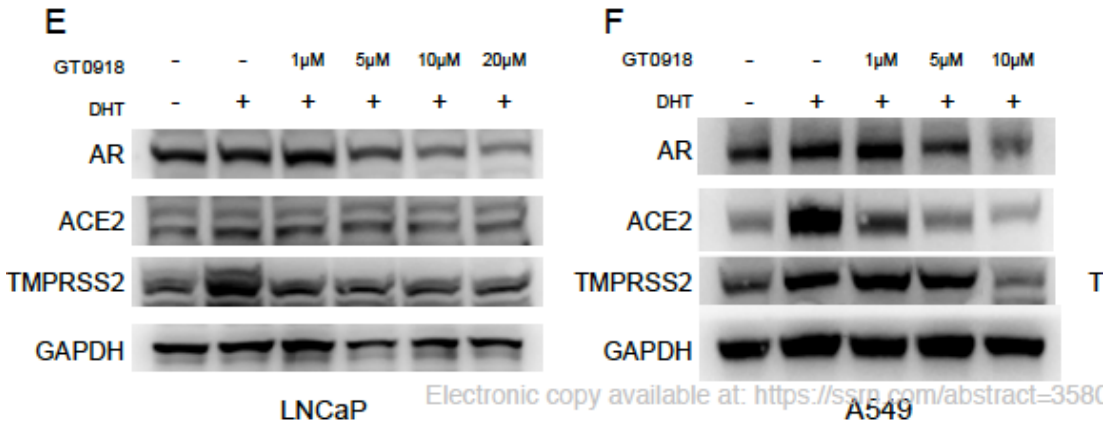


Fig. 1. GT0918 effectively decreases androgen binding to AR. (A) IC_{50} values for GT0918 and MDV3100 in inhibiting androgen from binding to AR. (B) GT0918 reduces the protein expression of AR in C4-2B cells.

Proxalutamide suppression ACE2 and TMPRSS2 in A549 lung cells

A study by Wu et al [20] reported that "In LNCaP and A549 cells, we showed that androgen induced the ACE2 and TMPRSS2 expression, and GT0918 could suppress the ACE2 and TMPRSS2 expression". The data from the study is depicted in the figure below.



1.1.4 Justification for Dosage

According to a phase I study of Proxalutamide conducted by Zhou et al[19] pK parameters showed that drug exposure increased with dose proportionally from 50 to 300 mg and a saturation was observed between 300mg and 400 mg. In addition, according to Zhou et al: "Of note, patients treated with a 400-mg dose in the present study showed greater PSA response (100%) than those treated with a 300-mg dose (50%) or a 200-mg dose (67%). Although several previous studies demonstrated that a 30% PSA decline at 3 months was associated with a decreased risk of death from PCa"

Assuming the PSA reduction is linearly correlated with the dosage of Proxalutamide; to achieve a clinically meaningful PSA reduction, a dosage of 200mg should suffice for the treatment of PCa. As we have no prior data on dosing of Proxalutamide for the treatment of COVID-19, we chose the same dosage used for the treatment of PCa.

1.1.5 Other Data

The PI has not identified any additional data related to the safety or efficacy of this study.

1.2. Risks/Benefits

This study is designed as a prospective, interventional, placebo controlled, double-blinded, randomized parallel assignment study to assess the efficacy of Proxalutamide as a treatment for COVID-19; therefore, we assess below the risks/benefits for the proposed study.

Benefit(s) of the Proposed Clinical Study

The study is intended to explore the theory that COVID-19 infection and disease severity is driven by androgens. As such, anti-androgen therapy is hypothesized to provide protection against COVID-19 disease progression. Provided anti-androgen therapy is effective, subjects enrolled in this study will possibly experience mild COVID-19 related symptoms.

Risk(s) of the Proposed Clinical Study

Treatment with any drug carries risk. Treatment with Proxalutamide caries the risk of the adverse events reported in Phase I clinical trials with Proxalutamide; however, due to the short treatment duration of 15 days, we believe the risk of serious adverse events is lower than described in Phase I studies.

1.3. Trial Conduct

This study will be conducted in compliance with the protocol approved by the Institutional Review Board (Ethics Committee), and according to Good Clinical Practice standards. No deviation from the protocol will be implemented without the prior review and approval of the IRB (EC) except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the IRB (EC) as soon as possible.

1.4. Population

This is a multi-center study to be conducted at outpatient clinics. This exact protocol will be followed at each site. There will be one PI. The study will be approved by the national Ethics Committee.

The population for this study will be non-hospitalized SARS-CoV-2 confirmed subjects previously presented to an outpatient hospital and/or clinic participating in the local health care electronic record exchange (SUS). Approximately, 260 male subjects who meet all the eligibility criteria will be enrolled. The estimated time from screening to end of the study for each subject is approximately 30 days.

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2. Trial Objectives

The primary purpose of this study is to evaluate the efficacy of Proxalutamide as a treatment for COVID-19.

3. Trial Design

3.1 Primary Study Endpoints/Secondary Endpoints

Primary Outcome Measures:

1. COVID-19 hospitalization [Time Frame: 30 days]
   Percentage of subjects hospitalized due to COVID-19

Secondary Outcome Measures:

1. COVID-19 Ordinal Outcomes Scale on Day 15, 30 [Time Frame: assessed on study day 15, 30]

We will determine the COVID Ordinal Scale for all patients on study day 15, 30

COVID Ordinal Scale defined as:

8. Death;
7. Hospitalized, on invasive mechanical ventilation or ECMO;
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;
1. Not hospitalized, no limitations on activities

3.2 Study Design/Type

This study is designed as a prospective, interventional, placebo controlled, double-blinded, randomized parallel assignment study. The study will have 2 arms:

For the first 15 days:

Arm 1: Subjects administered Proxalutamide 200mg q.d for 15 days (or until remission) + standard of care as determined by the PI at the site

Arm 2: Subjects administered Proxalutamide placebo q.d for 15 days (or until remission) + standard of care as determined by the PI at the site

Starting at day 15 +/- 3 (if patients have not experienced full remission)

Arm 1: Subjects administered Proxalutamide placebo 200mg q.d for 15 days (or until remission) + standard of care as determined by the PI at the site

Arm 2: Subjects administered Proxalutamide placebo 200mg q.d for 15 days (or until remission) + standard of care as determined by the PI at the site

Study Environment:

This is a multi-center study to be conducted at multiple outpatient clinics (the sites). This exact protocol will be followed during the study. There will be one or more PIs. The study will be approved by the appropriate Ethics Committees (IRBs). Data collection will be performed at each site by study personnel under the supervision of the PI.

Study Design:

Phase I: Enrollment (first site visit or consultation by phone):

1. Each subject will be evaluated for the inclusion and exclusion criteria

2. Each subject will undergo a physical examination

3. Each subject (or legally authorized representative) will complete and sign the Informed Consent Form

4. Each subject will be assigned a subject study number

5. Each subject will be randomly assigned to an Arm (Section 3.3)

6. Based on the Arm assignment, each subject will be given a 15 days supply of the intervention

7. All information will be recorded in the appropriate CRFs

Phase II: Treatment Administration at Home (day 0-15)

1. Each subject will self-administer the assigned treatment orally once per day

Phase III: Outcome Assessment at Site (day 15+/-3):

1. Each subject will be evaluated by the PI for the primary and secondary outcomes

2. The PI will assess each subject for any treatment related adverse events

3. All information will be recorded in the appropriate CRFs

Phase IV: Treatment Administration at Home (day 16-30)

1. Each subject will self-administer the assigned treatment orally once per day

Phase V: Outcome Assessment at Site (day 30+/-3):

1. Each subject will be evaluated by the PI for the primary and secondary outcomes

2. The PI will assess each subject for any treatment related adverse events

3. All information will be recorded in the appropriate CRFs

3.3 Randomization

Subjects will be randomized into 1 of 2 arms each will receive an interventional treatment.

For the first 15 days:

Arm 1: Subjects administered Proxalutamide 200mg q.d for 15 days (or until remission) + standard of care as determined by the PI at the site

Arm 2: Subjects administered Proxalutamide placebo q.d for 15 days (or until remission) + standard of care as determined by the PI at the site

Starting at day 15 +/- 3 (if patients have not experienced full remission)

Arm 1: Subjects administered Proxalutamide placebo 200mg q.d for 15 days (or until remission) + standard of care as determined by the PI at the site

Arm 2: Subjects administered Proxalutamide placebo 200mg q.d for 15 days (or until remission) + standard of care as determined by the PI at the site

During the enrollment phase (admission to hospital), each subject will be assigned a subject study number. The first subject will be assigned the number 001 and each subject thereafter will be assigned a consecutive number i.e., 002, 003, etc. The randomization plan is based on a 1:1 ratio for each arm. Since the study is double-blinded, the following randomization schedule will be used but the identification of the Arm assignment will be known only to the sponsor:

Subjects 001-020 will be assigned to Arm 1
Subjects 021-040 will be assigned to Arm 2
Subjects 041-060 will be assigned to Arm 1
Subjects 061-080 will be assigned to Arm 2
Subjects 081-100 will be assigned to Arm 1
Subjects 101-120 will be assigned to Arm 2
etc……..

3.4 Records

Each subject will be assigned a number. The numbers will be consecutive starting at 001.

A record will be created for each subject. Each record will contain a medical history, and the subject's efficacy parameters copied from the subject's charts and documented in the appropriate CRF.

The subjects' records will be stored and handled in the same manner as the PI's other clinical study patients' records are stored i.e., in a locked research storage area.

No other data will be recorded. No personal information will be kept outside the study site.

Data compiled at the end of the study will not include any information that can be used to identify individuals.

3.5 Duration

The duration of the study is 30 days. There will be no study related follow-up treatment.

3.6 Discontinuation

In the event that a subject experiences a SAE or an AE grade 3 or 4 (defined in Section 6) we will discontinue the study for that particular subject.

In the event a subject in any arm experiences a SAE defined as death not due to respiratory failure (presents with clear lung CT and no ARDS symptoms) the study will be discontinued. The reminder of the study shall continue.

3.7 Product Accountability

All interventional treatments for this study will be stored and monitored according to each hospital standard protocol.

3.8 Data Identification

The subject records kept by the PI will be stored and handled in the same manner as the PI's other clinical research subject records. Only authorized personnel named in this study, or medical professional retained by the PI in case of an adverse event, will have access to the subject records.

No other data will be recorded. No personal information will be kept outside the study site.

Data compiled at the end of the study will not include any information that can be used to identify individuals.

4. Selection and Withdrawal of Subjects

4.1 Inclusion Criteria

1. Male age ≥18 years old
2. Laboratory confirmed positive SARS-CoV-2 rtPCR test within 7 days prior to randomization
3. Clinical status on the COVID-19 Ordinal Scale (defined in Section 5.1) of 1 or 2
4. Coagulation: INR ≤ 1.5×ULN, and APTT ≤ 1.5×ULN
5. Subject (or legally authorized representative) gives written informed consent prior to any study screening procedures
6. Subject (or legally authorized representative) agree that subject will not participate in another COVID-19 trial while participating in this study

4.2 Exclusion Criteria

1. Subject enrolled in a study to investigate a treatment for COVID-19
2. Subject taking an anti-androgen of any type including: androgen depravation therapy, 5-alpha reductase inhibitors, etc…
3. Patients who are allergic to the investigational product or similar drugs (or any excipients);
4. Subjects who have malignant tumors in the past 5 years, with the exception of completed resected basal cell and squamous cell skin cancer and completely resected carcinoma in situ of any type
5. Subjects with known serious cardiovascular diseases, congenital long QT syndrome, torsade de pointes, myocardial infarction in the past 6 months, or arterial thrombosis, or unstable angina pectoris, or congestive heart failure which is classified as New York Heart Association (NYHA) class 3 or higher, or left ventricular ejection fraction (LVEF) < 50%, QTcF > 450 ms
6. Subjects with uncontrolled medical conditions that could compromise participation in the study(e.g. uncontrolled hypertension, hypothyroidism, diabetes mellitus)
7. Known diagnosis of human immunodeficiency virus(HIV) , hepatitis C, active hepatitis B, treponema pallidum (testing is not mandatory）
8. Alanine Transaminase (ALT) or Aspartate Transaminase (AST) > 5 times the upper limit of normal.
9. Estimated glomerular filtration rate (eGFR) < 30 ml/min

10. Severe kidney disease requiring dialysis
11. Subject unlikely to return for day 15 site visit for reasons other then remission
12. Subject (or legally authorized representative) not willing or unable to provide informed consent

4.3 Subject Withdrawal

Subjects may withdraw at any time for any reason. In the event the principal investigator or the site monitor believes a subject is at risk of injury the subject will be withdrawn from the study and:

(a) If the subject has not completed the study

(b) The subject will be replaced with another subject

(c) The PI will follow-up with the subject every day for 14 days

(d) The information will be reported in the appropriate CRF

4.4 Treatment of Subjects

Once enrolled in the study, each subject will self-administer the assigned treatment at home in the form of a daily oral tablet.

4.5 Medication

Proxalutamide showed no significant inhibition on CYP1A2 and CYP2E1, weak inhibition on CYP2D6, CYP2C9, CYP2C19 and CYP3A (midazolam), but obvious inhibition on CYP3A4 (testosterone). Potential inducer on CYP3A4 at a concentration of 10μM. No inductive effects on CYP1A2 and CYP2B6 were observed in the level of enzymatic activity. Co-administration of strong/moderate CYP3A4 inducer, strong/moderate CYP3A4 inhibitor, sensitive CYP3A4/ CYP2D6 substrates and narrow therapeutic index with Proxalutamide should be used cautiously (See Appendix 1).

Note: No interaction has been reported between Proxalutamide, Nitazoxanide, and azithromycin.

4.6 Monitoring for subject compliance

During each site visit, the PI will ask each subject to confirm if he adhered to the daily administration of the treatment.

5 Assessment of Efficacy

5.1 Efficacy Parameters

The following efficacy parameters will be assessed and recorded in the CRF.

I. COVID-19 Hospitalization:

1. COVID-19 hospitalization [Time Frame: 30 days]

Percentage of subjects hospitalized due to COVID-19

II. COVID-19 Ordinal Outcome Scale:

1. COVID-19 Ordinal Outcomes Scale on Day 15, 30 [Time Frame: assessed on study day 15, 30]
   We will determine the COVID Ordinal Scale for all patients on study day 15, 30

   COVID Ordinal Scale defined as:

   8. Death;
   7. Hospitalized, on invasive mechanical ventilation or ECMO;
   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;
   1. Not hospitalized, no limitations on activities

5.2 Method and Timing

The assessments, as described in section 5.1, will occur as follows:

The PI will assesses each subject and record the efficacy parameters at baseline, days 15 and 30 following enrollment (+/-3 days).

6 Assessment of Safety

6.1 Safety Parameters

Safety parameters will be assessed as follows:

I. Physical Examination:

The PI will conduct a thorough physical examination during screening. The assessment will be recorded in the appropriate CRF.

II. Adverse Events:

Safety will be assessed by summarizing the incidence and type of Adverse Events in a CRF form.

6.2 Method and Timing

The assessments, as described in section 6.1, will occur as follows:

I. Physical Examination

The assessment will occur at screening. The information will be recorded in the appropriate CRF.

II. Adverse Events:

Adverse events will be assessed by the PI at baseline and during the site visits on days 15 and 30 following enrollment (+/-3 days).

The methods employed for completing assessments are described in section 6.1

6.3 Adverse Events

6.3.1 Definition of Adverse Event (AE)

AE means any medical event associated with the use of an intervention, whether or not considered intervention-related. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

Given the nature of severity of the underlying illness, subjects will have many symptoms and abnormalities in vitals and laboratory. All Grade 3 and 4 AEs will be captured as AEs in this trial.

6.3.2 Definition of Serious Adverse Event (SAE)

An SAE is defined as "An AE or suspected adverse reaction is considered serious if, in the view of either the investigator or the sponsor, it results in any of the following outcomes: Death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse." "Life-threatening" refers to an AE that at occurrence represents an immediate risk of death to a subject. An event that may cause death if it occurs in a more severe form is not considered life-threatening. Similarly, a hospital admission for an elective procedure is not considered a SAE. All SAEs, as with any AE, will be assessed for severity and relationship to study intervention. All SAEs will be recorded on the appropriate SAE

CRF. All SAEs will be followed through resolution or stabilization by a licensed study physician (for IND studies, a physician listed on the Form FDA 1572 as the site PI or Sub-Investigator). All SAEs will be reviewed and evaluated by DMID and will be sent to the SMC (for periodic review), and the IRB/IEC.

6.3.3 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A SUSAR is any SAE where a causal relationship with the study product is at least reasonably possible but is not listed in the Investigator Brochure (IB), Package Insert, and/or Summary of Product Characteristics.

6.3.4 Classification of an Adverse Event

The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose AE information, provide a medical evaluation of AEs, and classify AEs based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

6.3.5 Severity of Adverse Events

All AEs and SAEs will be assessed for severity, according to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, version 2.1 (July 2017). For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.

Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

Severe (Grade 4): Events that are potentially life threatening.

AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop Duration of each reported AE will be recorded on the appropriate CRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

6.3.6 Relationship to Study Intervention

For each reported adverse reaction, the PI or designee must assess the relationship of the event to the study product using the following guideline:

Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.

Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

6.3.7 Time Period and Frequency for Event Assessment and Follow-Up

For this study, all Grade 3 and 4 AEs and all SAEs occurring from the time the informed consent is signed through the Day 30 (end of study) visit will be documented, recorded, and reported.

6.3.8 Investigators Reporting of AEs

Information on all AEs should be recorded on the appropriate CRF. All clearly related signs, symptoms, and results of diagnostic procedures performed because of an AE should be grouped together and recorded as a single diagnosis. If the AE is a laboratory abnormality that is part of a clinical condition or syndrome, it should be recorded as the syndrome or diagnosis rather than the individual laboratory abnormality. Each AE will also be described in terms of duration (start and stop date), severity, association with the study product, action(s) taken, and outcome.

6.3.9 Serious Adverse Event Reporting

6.3.9.1 Investigators Reporting of SAEs

Any AE that meets a protocol-defined criterion as a SAE must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US) SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US) SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible. The DMID

Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct. At any time after completion of the study, if the site PI or appropriate sub-investigator becomes aware of an SAE, the site PI or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

6.3.9.2 Regulatory Reporting of SAEs

Following notification from the site PI or appropriate sub-investigator, DMID, as the IND sponsor, will report any SUSAR in an IND safety report to the FDA and will notify all participating site PIs as soon as possible. DMID will report to the FDA any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. If the event is not fatal or life- threatening, the IND safety report will be submitted within 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to the FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request. SAEs that are not SUSARs will be reported to the FDA at least annually in a summary format which includes all SAEs. Sites may have additional local reporting requirements (to the IRB and/or national regulatory authority).

6.3.10 Reporting Events to Subjects

Subjects will be informed of any severe AEs or SAEs that occur as part of their participation in this trial.

6.3.11 Reporting of Pregnancy

Pregnancy is not defined as an AE. However, any pregnancy that occurs during study participation should be reported to the sponsor on the appropriate CRF. Pregnancy should be followed to outcome.

6.4 Unanticipated Problems

6.4.1 Definition of Unanticipated Problems (UP)

An Unanticipated Problem is any event, incident, experience, or outcome that meets the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

6.4.2 Unanticipated Problem Reporting

To satisfy the requirement for prompt reporting, unanticipated problems (UP) will be reported using the following timeline: UPs that are SAEs will be reported to the IRB and to the Statistical and Data Coordinating Center (SDCC)/study sponsor within 24 hours of the investigator becoming aware of the event per the above describe SAE reporting process. Any other UP will be reported to the IRB and to the SDCC/study sponsor within 3 days of the investigator becoming aware of the problem.

6.4.3 Reporting Unanticipated Problems to Subjects

Subjects will be informed of any UPs that occur as part of their participation in this trial.

7 Statistical Plan

7.1 Statistical Methods

I. General Principles:

- Double-blinded, placebo controlled randomized interventional study with a two-sided type I error rate of 0.05.
- Continuous variables will be expressed as median (interquartile range [IQR]) and categorical variables will be expressed as a number
- 95% confidence intervals will be calculated for the primary outcome
- Group comparisons will be analyzed by the Wilcoxon rank sum test or $\chi 2$ test for independent proportions
- The placebo arm will be used as a reference group when calculating treatment effects
- Differences between rates of clinical improvement will be calculated using unadjusted ordinal logistic regression or Cox proportional hazard models
- Statistical analyses will be conducted using XLSTAT version 2020.5.1 (Addinsoft, Inc.)

II. Statistical Hypotheses:

The primary endpoint is the proportion of subjects hospitalized through day 30. The primary outcome will be analyzed using the $\chi 2$ test for independent proportions.

The Null Hypothesis (H_0) is that the proportion of subjects hospitalized due to COVID-19 in Arm 1 (Proxalutamide) is equal to the proportion of subjects hospitalized due to COVID-19 in Arm 2 (standard of care)

The Alternative Hypothesis (H_A) is that the proportion of subjects hospitalized due to COVID-19 in Arm 1 (Proxalutamide) is less than the proportion of subjects hospitalized due to COVID-19 in Arm 2 (standard of care)

Mathematically written as:

H_0: $p_1 - p_2 = 0$
H_A: $p_1 - p_2 < 0$

Where p1 and p2 are the proportion of subjects hospitalized from arm1 and arm2 respectively.

III. Primary Efficacy Analysis:

- The $\chi 2$ test for independent proportions will be used to assess the primary end point.

- P-values <0.05 will be considered significant. All statistical analysis will be based on the intent-to-treat (ITT) population. All missing data will be described.

7.1 Sample Size Estimates

Assumptions:

- Riccardo et al (2020)[1] reported that in Italy the hospitalization rate was as high as 20% among adults above the age of 65 tested positive for SARS-CoV-2. Further, Montopoli et al (2020)[2] reported that males represent 60% of hospitalized patients; therefore, we can estimate that the rate of hospitalization of males over the age of 65% tested positive for SARS-CoV-2 is approximately 30%.
- To estimate the efficacy of Proxalutamide as a treatment for COVID-19, we use the results reported by Montopoli et al (2020)[2]. According to Montopoli et al: "Comparing the total number of SARS-CoV-2 positive cases, patients with prostate cancer receiving ADT had a significantly lower risk of SARS-CoV-2 infections compared to patients who did not receive ADT (OR 4.05; 95% CI 1.55-10.59). Applying the 20% hospitalization rate, we derive a probability of treatment efficacy of 50%

$$p_{treatment} = \frac{OR \times p_{control}}{1 + OR \times p_{control} - p_{control}} :$$

- The study has two arms with randomization at 1:1 ratio
- 80% power to detect the difference in proportions using a two-tailed test with a type I error rate of 5%

- 5% of the subjects will not complete the study.

Sample Size Estimate:

Based on the assumptions above, we calculated[3] that at a minimum we would need to recruit 254 subjects i.e., 127 subjects in each arm

References:

1. https://doi.org/10.1101/2020.04.08.20056861
2. Montopoli M, Zumerle S, Vettor R, Rugge M, Zorzi M, Catapano CV, Carbone GM, Cavalli A, Pagano F, Ragazzi E, Prayer-Galetti T, Alimonti A, Androgen-deprivation therapies for prostate cancer and risk of infection by SARS-CoV-2: a population-based study (n=4532), Annals of Oncology (2020), doi: https://doi.org/10.1016/j.annonc.2020.04.479.
3. https://jamanetwork.com/journals/jama/fullarticle/2765184
4. Machin D, Campbell MJ, Tan SB, Tan SH (2009) Sample size tables for clinical studies. 3rd ed. Chichester: Wiley-Blackwell.

7.2 Subject Population(s) for Analysis

- The primary analysis will be based on the intent-to-treat (ITT) population. The data from all subjects enrolled in the study will be analyzed.
- The safety analysis will be based on a modified intent-to-treat (MITT) population i.e., subjects who received at least one dosage of the interventional treatment.
- Subgroup analysis based on: 1) age stratification; and 2) androgen status as defined by the "Gabrin sign" will be conducted for the primary and secondary outcomes.

7.4 Interim Analysis

Interim efficacy and safety data will be made upon recruitment of 50% of the subjects.

7.5 Termination Criteria

Upon occurrence of any one of the events listed below, the study will terminate or be modified accordingly:

- Completion of the study by a sufficient number of subjects (254 subjects) to reach our confidence level

- Termination of Arm 1: Serious adverse event due to treatment with Proxalutamide

- Substantial evidence of treatment difference between the arms. The study design will be modified to an open-label study.

7.6 Accountability Procedure

The data will be analyzed by a bio-statistics expert. The data will also be independently verified by an outside expert consultant.

7.7 Deviation Reporting

No deviation from the plan will be implemented without the prior review and approval of the EC/IRB.

8 Direct Access to Source Data/Documentation

The PI and hospital will permit trial-related monitoring, audits, IRB/IEC(s) review and regulatory inspection(s) by providing direct access to source data/documentation.

9 Quality Control and Quality Assurance

Every effort will be made to keep staff assignments consistent throughout the entire study. The PI who assesses the subject at baseline should follow the subject throughout the completion of the study. This will ensure that this study is conducted – and that data is generated, documented (recorded), and reported - in compliance with this protocol, with GCP, and any other applicable regulatory requirements. The study monitor will audit the study procedures and CRFs throughout the study.

10 Ethical Considerations

This study will be conducted according to US and international standards of Good Clinical Practice (FDA regulations 21 CFR 312 for IND studies and FDA guidance E6) for all studies. Applicable government regulations and hospital research policies and procedures will also be followed.

This protocol and any amendments will be submitted to the Institutional Review Board (IRB) for formal approval to conduct the study. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

11 Data Handling and Record Keeping

During enrollment, each subject will be assigned a number. The numbers will be consecutive starting at 001.

During enrollment a record will be created for each subject. Each record will contain the subject's demographics, subject's efficacy parameters and any adverse events or study related information.

The subjects' records will be stored and handled in the same manner as the PI's patients' records.

The study monitor will keep a separate record at the monitor's office of each subject's identification number, the treatment administered to the subject (arm assignment), outcomes and any laboratory results.

No other data will be recorded. No personal information will be kept outside the study site.

Data compiled at the end of the study will not include any information that can be used to identify individuals.

The site will keep all subject records for a minimum of 3 years after the completion of the study.

12 Finance and Insurance

The Principal Investigator will be responsible for the cost of study. The site carries insurance for accidental injury. There is no other insurance.

APPENDIX 1

Drugs that should be used cautiously with Proxalutamide

Strong CYP3A4 inducer
Avamibe, Carbamazepine, Phenytoin, Rifampicin, Mitotan, Nevirapine, Phenobarbital, Rifabutin, Rifapentine, St. John's wort, Alfentanil, Ryclosporin, Dihydroergotamine / Ergotamine, Fentanyl, Irinotecan, Pimozit, Quinidine, Sirolimus, Tacrolimus
Moderate CYP3A4 inducer
Smacet, Tavern, Bosentan, Efaviron, Etruverin, Lopinavir, Modafinil, Nafcillin, Thalidazine, Tiranavir, Ritonavir
Strong CYP3A4 inhibitor
Posidovir, Clarithromycin, Conivatan, Indinavir, Itraconazole, Ketoconazole, lopinavir / Ritonavir, Mibedil, Nefazodone, Nefenavir, Posaconazole, Ritonavir, Saquinavir, Trapivir, Taliomycin, Voriconazole, Etigavir / Ritonavir, Fluconazole, Tiranavir / Ritonavir, Acetamycin
Moderate CYP3A4 inhibitors
Apronavir, Aripitan, Azanavir, Casopitam, Cimetidine, Ciprofloxacin, Clazotinib, Cyclosporin, Dalunavir, Diltiazem, Dronedarone, Erythromycin, Imatinib, Tofesoyang, Verapamil
Sensitive CYP3A4 substrates
Remifentanil, Aripiptam, Budesonide, Buspirone, Conivatan, Daphnesin, Darunavir, Dasatinib, Dronedarone, Eletroptan, Eplerenone, Everolimus, Felodipine, Indinavir, Fluticasone, Lopinavir, Lovastatin, Lulasidone, Maravelo, Midazolam, Nisoldipine, Quetiapine, Saquinavir, Sildenafil, Simvastatin, Sirolimus, Tolvaptan, Tiranavir, Triazolam, Vardenafil
Narrow therapeutic index CYP3A4 Substrate
Astemizole, Cisapride, Cyclosporine, Dihydroergotamine, Fentanyl, Pethidine, Quinidine, Tacrolimus, Terfenadine
Sensitive CYP2D6 substrates
Tomoxetine, Decipamine, Dextromethorphan, Metoprolol, Nebeprolol, Perphenazine,

Tolterodine, Venlafaxine, Avamibe, Carbamazepine,	Phenytoin sodium, Rifampicin
Narrow therapeutic index CYP2D6 Substrate	
Thioridazine	