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Pfizergate 2.0 – Active actions against competitive anti-COVID drugs? The case of the anti-androgens.

Flavio A. CAdegiani, MD, MSc, PhD[1*]

[1]Corpometria Institute, Brasilia, Brazil

*Corresponding author:
Flávio A. Cadegiani, MD, MSc, PhD
Corpometria Institute; SGAS 915 Centro Clínico Advance, Rooms 260/262/264, Brasília, DF, Brazil, f.cadegiani@gmail.com, flavio.cadegiani@unifesp.br, flavio@flccc.net
+55 61 996506111

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NO ABSTRACT AVAILABLE.

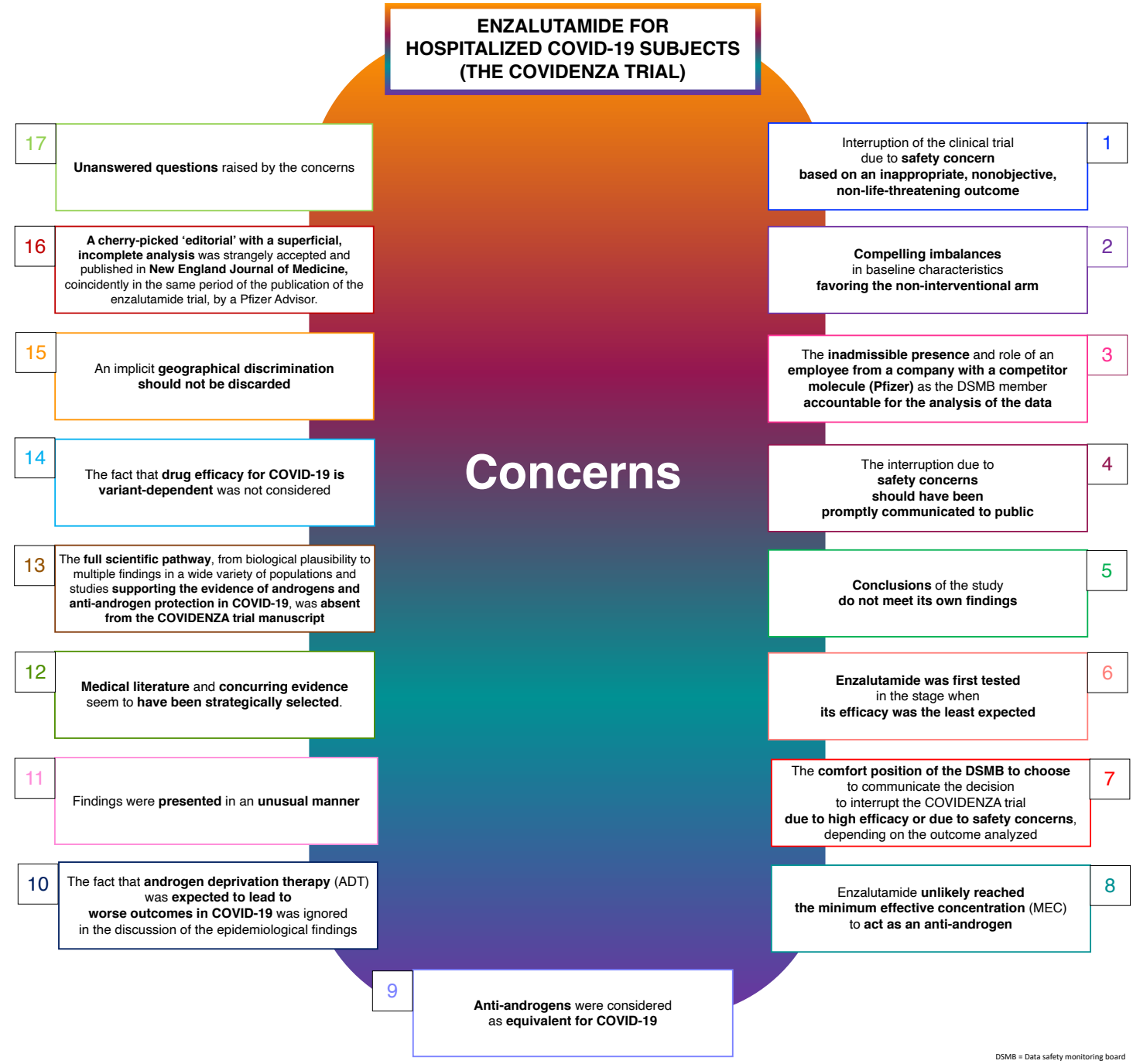
Dear all,

I read with interest an article of a pilot open label trial randomized clinical trial (RCT) that tested enzalutamide, an anti-androgen, in hospitalized COVID-19 patients (The 'COVIDENZA' trial [1]. I also read with interest the two letters to the editor [2,3] regarding this article and their respective responses from the authors of the study [4,5].

These readings, in combination with additional factors that will be listed and described below, generated an intriguing question regarding the underlying impartiality and symmetry required for RCTs in the case of COVIDENZA.

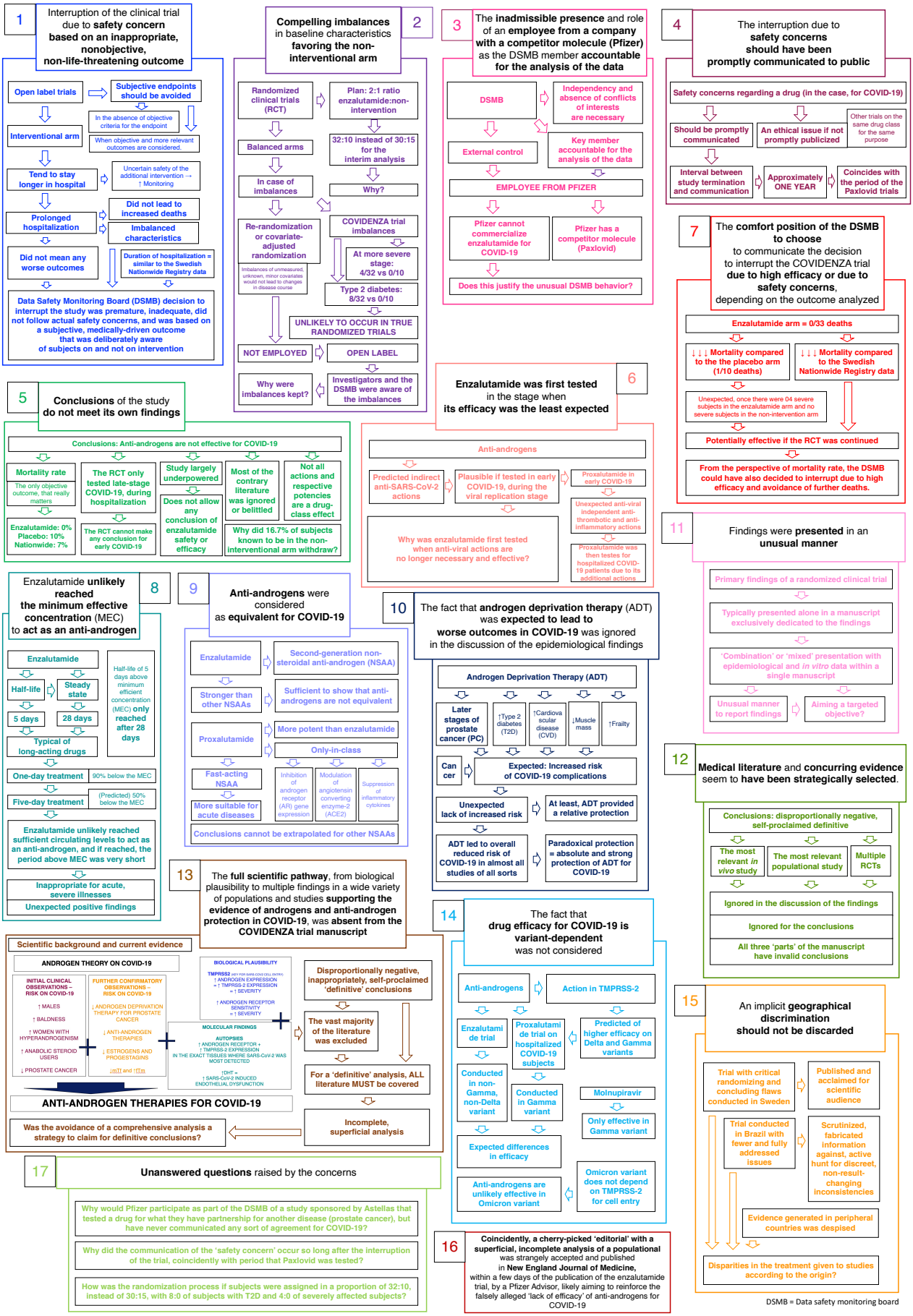
Critical concerns were raised from the thread of coincidental detections from the trial, and require further clarification, rectifications, and other actions. There extensive number of concerns can be better understood if they are ordered in a logical, intuitive manner, that must be depicted individually. Concerns and their portrayal are described below. **Figure 1** summarizes the concerns regarding the RCT discussed in this trial and **Figure 2** summarizes the supporting data for each of the concerns.

Figure 1. Summary of the concerns and questions raised from the COVIDENZA trial.



A cascade of self-feeding system to undermine a certain therapeutical class for COVID-19

Figure 2. Summary of the supporting data for each concern.



1. Interruption of the clinical trial based on an artificial safety concern.

The COVIDENZA trial is an open label study where investigators are inherently influenced by the intervention, since they are aware of subjects that were allocated to the intervention and to the non-intervention arm. Said that, outcomes that depend on subjective evaluation should not be accepted as a determinant outcome, expect for indicating a certain effect that can only be measured objectively to be used as an outcome to compare arms. The outcome of duration of hospitalization is one of the subjective outcomes and is an inappropriate efficacy or safety outcome for decision-making in open label studies when no objective criteria is established for hospital discharge [6]. On the contrary, subjects that are known to be under an experimental additional intervention tend to stay longer in hospital due to uncertain adverse effects and unfamiliarity of the assisting healthcare providers and investigators.

The decision to interrupt the trial based on increased hospitalization stay, determined by the Data Safety Monitoring Board (DSMB), can be considered as being misleading, based on the arguments above. However, additional elements reinforce the atypical behavior from the DSMB.

First, prolonged hospitalization was definitely non-life-threatening outcome. Indeed, in-hospital mortality rate was the lowest in enzalutamide arm, as described later in this article. Hence, no actual safety concerns were present.

Second, it was assumed that baseline characteristics were balanced when the DSMB concluded that the intervention was the likely cause of the increased hospitalization stay. However, not only subjects in the enzalutamide arm were at higher risk and presented higher severity state upon randomization, which could potentially fully justify the differences in the inappropriately measured outcome chosen by the DSMB to interrupt the study, but also both investigators and DSMB were aware of the differences. Why differences in baseline characteristics and actually relevant, objective and essential outcomes were not considered has not been addressed at any point between the manuscript and responses to letters.

Considering that for open label studies non-life-threatening, non-objective outcomes are not encompassed in the measures of safety profile, it would be expected that prolonged hospitalization stay would be described in the original safety monitoring plan for the trial, which was not the case.

Interruptions due to safety concerns should not only be based on statistical significance through calculations of p-values. The question that must be addressed by a DSMB is whether the observed differences will impact in risk of deaths for the intervention or non-intervention arms, in case of harm of high efficacy, respectively. If achieving a significant p-value was sufficient, very few trials would be sufficiently powered for allowing conclusions regarding efficacy and safety.

It is noteworthy that while DSMB planned meetings on a weekly basis, a minimum of 45 subjects was required for the first interim analysis. Any interruption below this number should have been based on undisputable and truly impacts in the chances of been discharged alive. Not only an insufficient number of subjects were recruited, but there is no doubt that absolutely any criteria for earlier interruption was achieved.

In trials where interruption occurred prematurely due to safety concerns with fewer than 50 to 100 subjects included, authors should depict the full process since enrollment and randomization until outcomes and withdraws and provide reports on an unidentified and individual level, so the process that justified the interruption and the subsequent conclusions can be fully transparent, since the decision was atypical.

While the number of subjects that complied with the study in the non-intervention arm was seemingly unreasonably small, authors could rely on the comparisons with the Swedish nationwide COVID-19 hospitalization outcomes to determine the differences compared with the subjects in the enzalutamide arm. However, not only hospitalization stay was not longer than the average duration in the nationwide registry, but also mortality rate was overwhelmingly lower in enzalutamide arm compared to the nationwide in-hospital mortality rate during the period of the trial [7]. Hence, there is no substantiation to justify an open label trial that provided strong conclusions against the intervention based on 10 subjects. This has not been successfully justified in any of the documents published to date.

To date, there is no other known decision based on safety concerns from DMSB in other trials based on so few subjects, fewer than the planned for the first interim analysis, with debatable differences in a non-objective, non-life-threatening and non-risky outcome.

Correspondingly, the decision to interrupt the trial based on hospital length of stay in an open label trial with insufficient number of subjects and unavoidable differences in baseline characteristics between arms is unusual and lacks support. The substantiation for

the first concern is sufficient to indicate that the COVIDENZA trial was bounded to a non-fully impartial analysis.

2. Compelling imbalances in baseline characteristics favoring the placebo arm.

In a rigorous randomization process, differences between baseline characteristics are unlikely to occur, although slight imbalances may occur in small trials. Whenever imbalances by chance occur, there must be inherent mechanisms of detection for rebalancing the arms, through either re-randomization or covariate-adjusted randomization processes [8]. Despite the potential for altering the unmeasured covariates through co-adaptive randomization, which may also occur in re-randomization, this would affect the results for imbalanced unmeasured covariates, since the covariates measured by the COVIDENZA encompass almost all the relevant factors that predict COVID-19 outcomes [except for the presence of androgenetic alopecia (AGA) in males and polycystic ovary syndrome (PCOS) in females]. Certainly, if groups were balanced for all the characteristics encompassed by the trial, the remaining potential characteristics would play a marginal role, unable to change the disease course. Any of these processes or any other solution do not need to be in the original research plan. Instead, it should have been considered as inherent as a correcting mechanism in any randomization process, and would mitigate or reduce the differences that matter.

Mechanisms of detection of imbalances between arms are particularly important in double-blind RCTs, when neither direct investigators nor subjects know in which arm each enrolled subject is. In open label RCTs, such as the COVIDENZA, differences in imbalances are naturally promptly corrected in the upcoming allocations or through re-allocations, since differences are noticeable at all levels of the investigation. From this perspective, the hypothesis of occurrence of imbalances would be inconsiderable.

However, not only there were differences, but these were overwhelming and favoring the placebo arm. Since COVIDENZA was transformed into a pilot study, with very few subjects in each arm, the allocation of each subject is of great relevance. All the four subjects that were at the highest COVID-19 severity accepted for the trial were randomized to the enzalutamide arm, and eight subjects with type 2 diabetes (T2D) were

allocated to the enzalutamide arm, while none was allocated to the placebo arm. In the case of T2D, statistics calculations show that the chances of a distribution of 8:0 to occur in a 2:1 randomized distribution by chance is extremely low, and indicates, not only correcting mechanisms would ignored, but the randomization process *per se* may not have been appropriately performed, and should be further investigated.

The baseline COVID-19 clinical severity is amongst the most determinant factors that influence outcomes, since not only more severe states are inherently harder to overcome in any disease, but also more severe states in COVID-19 tend to reflect a progression of the dysfunctions and the occurrence of new complications that may occur during the COVID-19 disease course. For these reasons, larger RCTs of drugs for COVID-19 provide further COVID-19 severity-stratified results, in particular when conducted in hospitalized COVID-19 patients.

While males, in particular males with AGA, females with PCOS, hypertension and obesity are known risk factors for progression to severe COVID-19 and related complications, after aging, T2D is the likely the second strongest predictor of poorer COVID-19 prognosis [9].

Not only each of the two highly imbalanced characteristics are questionable, but the concurrence of both favoring placebo arm is of major concern.

Critical imbalances are particularly problematic once they were known by direct and indirect investigators, as well as by the DSMB, and failed to be corrected at any point of the trial.

In case of the unusual occurrence of imbalanced RCTs, propensity score matching (PSM) could be employed as a tool to alleviate differences and provide more befitting findings [10]. However, in the present case, when sample size is small, PSM may mislead results, instead of balancing.

Differences may have resulted from imbalances in baseline characteristics, and, if neutralized, there could be no longer substantiation to interrupt the study. However, in this peculiar case, due to the subjectivity and open label characteristic of the study, it would be expected that, even after removal of subjects in more severe COVID-19 states, duration of hospitalization stay would remain significantly higher among subjects in enzalutamide arm.

Despite the additional barriers other than the imbalances in baseline characteristics for non-directed results, when more severe subjects, only present in the enzalutamide arm,

are removed for re-analysis, differences in duration hospitalization stay, which was key for the study interruption, loses its statistical significance.

Finally, the approved project for the COVIDENZA trial previewed a 2:1 enzalutamide:placebo ratio. However, instead of 30 subjects in the enzalutamide arm and 15 subjects in the non-interventional arm (28 and 14, in case of 42 subjects), 30 and 12 subjects were included, respectively. This is not justified by sex as a covariate to determine randomization, since there were nine females in the enzalutamide arm and two females in the placebo arm. The discrepancy was not detailed and justified in a CONSORT flowchart, which is atypical, since RCTs tend to present a CONSORT flowchart to detail the randomization and enrollment process.

The randomization process deserves further elucidation. The balance is unusual and are unlikely to occur in a rigorous randomization process, demonstrating an apparent failure of the process.

The combination of forced conclusion of safety concerns and unfavorable, seemingly deliberate imbalances requests further clarifications due to the gravity of the issue.

Importantly, in case of allegations that differences between arms by could by chance because the sample size of the trial was too small, the same allegation must be automatically extended for the evaluation of endpoints, which invalidates the COVIDENZA's own conclusions.

3. *The inadmissible presence and role of an employee from a company with a competitor molecule (Pfizer) as the DSMB member accountable for the analysis of the data.*

In RCTs, DSMBs are a key external control of the study, and should not only be completely independent from the investigators, but must not have any direct or indirect, present or past conflicts of interest of any sort [11,12]. It may be acceptable that investigators and DSMBs have or had in the past scientific contacts or collaboration, in particular in smaller countries, in case they no longer present direct relationships or any other sort of influences that could mislead the analysis from the DSMB.

However, in the COVIDENZA trial, the key member of the DSMB responsible for the analysis of the data, Mr. Martin Eklund, *i.e*., the person who ultimately presented the data to support the decision of interruption, is an employee from Pfizer, Inc. [13].

Pfizer has an interest against the use of enzalutamide for COVID-19, since they can only commercialize this molecule for indications related to prostate cancer only, as per the currently disclosed documents of partnership between Pfizer and the owner of the molecule, showing that Pfizer is still not licensed to commercialize enzalutamide for COVID-19, in case of efficacy (Astellas Pharma, Inc., Japan) [14,15]. Whether these companies planned for the expansion of the partnership for other diseases is unknown and undisclosed.

In addition, Pfizer also has a competitor molecule (nirmatrelvir-Paxlovid), that, at the time of the interruption of the study, the efficacy of nirmatrelvir for COVID-19 was certain and its respective RCTs were in early stages.

The statistician, the key member upon the decision to interrupt the study, has an acceptable level of conflicts of interest. Due to this issue, investigators and the other DSMB members must have required prompt additional external analysis before the decision for the interruption of the study [11,12], which has not been reported.

Finally, it is astonishing that this indisputably inappropriateness has not been disclosed at any point in the manuscript or in the supplements of the trial, which strengthens the suspicions over the objectives of the COVIDENZA trial.

As shown, there is sufficient questions to hypothesize that the DSMB behavior follows patterns expected for a company (in case, Pfizer) that battles against a molecule. The first three points of concern would be, together, sufficiently satisfactory to characterize the management of the processes of this RCT as highly concerning.

4. ***The interruption due to safety concerns should have been promptly communicated to public.***

When RCTs detect that the molecule (or molecules, or any other intervention) being tested may cause more harm than good for the condition proposed, besides the study interruption, the safety concern should be communicated to public promptly, in particular when the findings are of great medical relevance, which is the present case.

However, the safety concern raised by the DSMB of the COVIDENZA trial has only become public upon the publication of the trial. The interval of more than one year between the study interruption and the communication is unusual. It was notorious that other trials on the same drug class were ongoing, that could require further actions if the communication was performed timely.

In addition, the authors' conclusions, claiming that RCTs on anti-androgens for COVID-19 should no longer continue, is a strong conclusion resulted from the safety concern, and reinforces the previous need for the communication more than one year before its disclosure.

5. Conclusions of the study do not meet its own findings.

COVID-19 is a disease for which drug efficacy is highly dependent on the disease stage. None of the molecules tested for COVID-19 to date showed equal efficacy throughout all the stages of the disease The COVIDENZA trial was conducted in hospitalized subjects, i.e., in subjects at latter stages of COVID-19. However, the conclusions extrapolated and affirmed that enzalutamide was not effective for COVID-19, without specifying the stage, which conveys the message of inefficacy for all stages of COVID-19.

The RCT is largely underpowered and does not allow conclusions regarding efficacy. This should have been addressed in the conclusions. Oppositely, despite the insignificant number of subjects in the placebo arm, conclusions were inadequately strong and premature, without one single grade 5 severe adverse event (SAE) in the enzalutamide arm, despite the higher severity of these subjects compared to the placebo arm, and without any statistical differences in all grades 3 or 4 SAEs, since prolonged hospitalization stay not supported by worsening or lack of recovery of objective parameters in subjects already hospitalized upon randomization is not usually considered a SAE.

A sort of anxiety to find a minimum plausibility to interrupt the COVIDENZA trial with pre-established objectives and conclusions seem to be a hypothesis that must be considered.

Finally, it is unexpected that almost 20% of subjects (2 out of 12 subjects) from the non-intervention arm in an open label study, *i.e.,* subjects were aware they received standard-of-care only, withdraw the RCT. Since the high withdraw rate in a small sample largely influences the results, this should have been highlighted and thoroughly analyzed.

6. *Enzalutamide was first tested in the stage when its efficacy was the least expected.*

The pathophysiology of COVID-19 is complex and sequential, i.e., the mechanisms that cause the SARS-CoV-2-induced pathology change throughout the disease course. To hypothesize that a specific drug or drug class could be repurposed for COVID-19, the expected mechanisms of action that support the hypothesis must meet the mechanisms of the pathophysiology according to the COVID-19 stage. As further explained, anti-androgens are expected to act as indirect anti-viral (anti-SARS-CoV-2) agents. Consequently, the rationale supports anti-androgens for early COVID-19, during the viral replication stage, not during a latter, inflammatory stage, that coincides with the occurrence of hospitalizations. Since it would be less expected that enzalutamide would be effective for hospitalized COVID-19 than early, mild COVID-19 subjects, it is unclear why enzalutamide was first tested when viral replication is no longer a major factor in almost all SARS-CoV-2 variants.

Proxalutamide is another second-generation non-steroidal anti-androgen (NSAA) that has also been tested for COVID-19. As per the rationale, the molecule was first tested during the first seven days of symptoms [16], and only then, once its potential additional anti-inflammatory and anti-thrombotic actions were identified [17], further trials in hospitalized COVID-19 patients were conducted [18].

7. ***The comfort position of the DSMB to choose to communicate the decision to interrupt the COVIDENZA trial due to high efficacy or due to safety concerns, depending on the outcome analyzed.***

While the subjective, investigator-largely influenced outcome of duration of hospitalization stay was alleged to be longer among subjects in the enzalutamide arm, none of the 33 subjects allocated to this arm died. The lack of deaths is far below the expected following the Swedish nationwide data [7].

In result, DSMB could also have optionally ordered the study interruption due to high efficacy based on a reduction in in-hospital mortality, aiming to prevent further deaths. This situates the DSMB in a comfort position to choose the direction of the trial. The approximate one-year interval coincides with the period that the RCTs on Paxlovid for COVID-19 were conducted.

8. *Enzalutamide unlikely reached the minimum effective concentration (MEC) to act as an anti-androgen.*

Authors from the COVIDENZA trial claim that the half-life of enzalutamide is five to six days and therefore a 5-day enzalutamide treatment would lead to an exposure to enzalutamide for 10 to 11 days [4,5]. However, half-life of a drug to maintain efficacy is only calculated when its steady state is reached. In the case of enzalutamide, the steady state is only reached after 28 days of therapy to act as an androgen receptor (AR) blocker [19,20]. One-day therapy only reaches approximately 10% of the estimated Minimum Effective Concentration (eMEC), and five days should not reach 50% to 60% of the eMEC. The pharmacokinetic profile of enzalutamide meets the needs for chronic treatments but is not timely for critically acute illnesses. Drugs with longer half-life tend to take longer to achieve a steady state and an attack-dose could've been given up to 600mg/day in the short term with extensive safety profile, as supported by the literature [21], which was not the case of the present trial.

Hence, effective therapy with enzalutamide is unlikely reached at any point with 160mg 5-day enzalutamide treatment [3], and, in the optimistic scenario of enzalutamide eMEC as being reached, the duration of circulating enzalutamide above the eMEC would be as short as one to two days. Definitely, the proposed treatment to test efficacy of enzalutamide for hospitalized COVID-19 subjects was insufficient to for its expected protective roles.

Even when assuming that the flaws of the treatment regimen in the COVIDENZA trial were not present and that enzalutamide circulating levels were above its eMEC since the first day of treatment until its half-life, the treatment duration, considering the clearance of enzalutamide, was still below 14 days. It has been noticed that subjects that used proxalutamide for less than 14 days not only failed to present reduction but presented increase in mortality rate when compared to placebo, as a sort of relapse if treatment is interrupted before the 14-day treatment course. The proposal of 14 days of therapy was based on the relapses observed in outpatients. These reports were made publicly available and have been publicized aiming to warn investigators from other trials. Unexpectedly, this warning has not been mentioned in the discussion of the manuscript.

Due to the short undertreatment, positive effects of enzalutamide in the subjects allocated to the interventional arm would be unexpected. However, positive data from enzalutamide has been obtained, depending on the outcome analyzed.

Altogether, enzalutamide undertreatment tested in the population that was expected to be the least benefited from anti-androgen treatments (hospitalized COVID-19 subjects) with unusual DSMB behavior, unsupported decision and forced conclusions strengthens the hypothesis that COVIDENZA trial was a trial designed and conducted with directed, undisclosed objectives.

9. Anti-androgens were considered as equivalent for COVID-19.

Enzalutamide is a strong second-generation NSAA that was developed for males with castration-resistant prostate cancer that did not respond to previous NSAAs [22]. Consequently, this is sufficient to demonstrate that efficacy of NSAAs is not a drug-class effect.

However, in a head-to-head comparison, proxalutamide, another second-generation NSAA, was shown to be more powerful than enzalutamide to inhibit androgen expression, and only proxalutamide was able to inhibit AR expression (24) Indeed, proxalutamide is the only-in-class to demonstrate inhibition of AR expression, modulation of angiotensin converting enzyme-2 (ACE2) and suppression of inflammatory cytokines, in particular the Tumor Necrosis Factor-alpha (TNF-alpha).

Not only proxalutamide was a molecule expected to exert stronger anti-COVID actions than other NSAAs, but proxalutamide reached more than 70% of the steady state in the first 24 hours [25], which is critical for acute maladies when prompt-action is needed.

For an acute and severe situation, which requires the opposite characteristics from its original use for prostate cancer, in addition to the notable differences between enzalutamide and proxalutamide, not only regarding pharmacokinetics, but also stronger and broader actions found with proxalutamide, these differences become highly relevant and may have been determinant for the alleged lack of positive results.

In conclusion, findings with enzalutamide cannot be extrapolated and assumed to be a drug-class level of effect. Any effort in this direction may represent attempts not fully scientifically-driven.

10. The fact that androgen deprivation therapy (ADT) was expected to lead to worse outcomes in COVID-19 was ignored in the discussion of the epidemiological findings.

In a large study that aimed to identify risk and protective factors for COVID-19 hospitalization and death, prostate cancer (PC) was surprisingly identified as one of the strongest predictors of better outcomes for both hospitalization and death [26]. From a rationale perspective, males with PC were expected to be at higher risk for worse COVID-19-related outcomes due to two main reasons: 1. The presence of overall cancer alone is associated with increased COVID-19 incidence, progression and mortality [27], i.e., it seems that PC is an exception among different types of cancer; and 2. Males with PC tend to have higher AR sensitivity, which is, alone, an independent risk factor for COVID-19 [28]. The paradoxical protective role of PC for COVID-19 finds in testosterone suppression the sole plausible explanation, since the majority of subjects with PC undergo chronic and continuous or everlasting modalities to mitigate testosterone production and/or action.

Within males with PC, two subgroups can be compared between them: those under androgen deprivation therapy (ADT) and those not under ADT. Among males with PC, it is expected that subjects under ADT would be at higher COVID-19 risk, since these subjects are typically at later stages of prostate cancer, including castration-resistant prostate cancer (CRPC) and metastatic CRPC (mCRPC), refractory to other therapies, while the stronger androgen blockade that males under ADT experiment leads to increased risk of multiple metabolic abnormalities, including insulin resistance, type 2 diabetes, cardiovascular disease, partially resulted from the androgen-deprivation-induced increase in body fat and decrease in muscle mass [29-33]. In addition, decrease of cognitive function, sexual function and libido, and, finally, increased frailty, are, altogether, independent risk factors for COVID-19 complications.

Since it is expected that males with PC under ADT would perform worse in COVID-19, a lack of increased risk of COVID-19 and COVID-19 complications in this population should be interpreted as a relative protection provided by ADT, while reduced risk of COVID-19 and its progression allows the interpretation that ADT provides a strong protection against COVID-19.

Although the majority of the populational analyses identified ADT as a protective factor [1,35,36], some demonstrated a lack of decreased risk, while also demonstrated a

lack of increased risk [1]. However, the largest analysis that was performed through the whole system of the Veteran Affairs Hospitals across the United States of America (USA), that evaluated approximately 250,000 males, provided a definitive answer showing ADT as being protective for both COVID-19 infection and COVID-19 progression to the need to intensive care unit (ICU), mechanical ventilation, and death [37]. Although published after peer review recently, its preprint has been made available in May 2021 [38].

Finally, whether populational analyses showed decreased risk of COVID-19 and related outcomes in males under ADT or not, none of them identified increased risk in this population. Hence, the conclusion regarding ADT as being protective is unanimous.

The difference is based on the level of protection: ADT protection could be interpreted either as: 1. Relative, when risk of COVID-19 and related complications in males under ADT were similar than those not under ADT, i.e., ADT evened the risk between these two populations, since, as mentioned before, the inherent profile of males with ADT and the metabolic consequences of ADT would, together, lead to increased risk of COVID-19 progression and death; or 1. Absolute and strong, when ADT led to reduced risk of COVID-19 and its complications, paradoxically to the expected, when not considering the rationale for the protection conferred by ADT against SARS-CoV-2.

11. Findings were presented in an unusual manner.

The importance of the presentation of the primary results of a RCT is unquestionable. It provides an answer to an already well-established hypothesis and confirms preliminary evidence from observational, epidemiological and molecular studies that support the hypothesis. It is expected, then, that findings are presented in a manuscript dedicated to the RCT.

However, in the case of the COVIDENZA trial, a combination of the primary results of the trial, epidemiological findings and *in vitro* findings was presented within a single manuscript, which is atypical and rare to occur in the medical literature.

The indirect messages conveyed by the authors in the manuscript, when 'unifying' the studies, in additional to all the other flaws and cherry-picked data, are three: 1. Investigators were working targeting a specific objective; and 2. Authors aim to force definitive, closing conclusions, which was what happened in their manuscript. Definitive conclusions are scientifically invalid in this case, even if all the data they presented were truly negative, once previous evidence could not be simply discarded, and this sort of statement is absolutely contrary to the scientific principles, and; 3. The group implicitly consider their work superior to others, since they dismiss the majority of the evidence that supports the androgen hypothesis and anti-androgen protection for COVID-19, as detailed later in this article, and consider themselves as the sole valid evidence, when they concluded as the subject was final despite the contrary evidence. Whether this represents regional, geographical, or racial self-perceptions of superiority is unknown, but cannot be excluded as a hypothesis.

12. Medical literature and concurring evidence seem to have been strategically selected.

When findings from any study, including molecular, epidemiological, or clinical trial, contradict previous findings the discussion of the contrary findings must consider the most relevant literature and evidence that counterbalances the results obtained. This is essential for a critical analysis of the findings and balanced conclusions.

In the COVIDENZA trial, the unacceptable conclusions as claiming to be definitive seem to be purposely resulted from a seemingly strategically cherry-picked data. Conclusions were disproportionally negative in relation to the data of the own article, and ignored much of the medical literature, indicating a potential attempt to force an objective. Indeed, multiple molecular, epidemiological, observational, and clinical studies showing benefits of anti-androgens for COVID-19 were overlooked

None of the findings in the manuscript, including the *in vitro*, epidemiological, or clinical trial, are the most relevant for the discussion of anti-androgens and COVID-19. Unreasonably, regarding two of the three 'parts' of the article, the *in vitro* and the epidemiological findings, were not compared to the most relevant article of the respective findings. The largest epidemiological, populational study that showed ADT as being protective against COVID-19, conducted with approximately 250,000 males [37,38], which was already publicly available, was excluded for the analysis and discussion.

The gold-standard molecular study on the effects of enzalutamide in COVID-19, that demonstrated that enzalutamide, and, at a slightly lower potency, bicalutamide, were able to virtually completely block SARS-CoV-2 replication in both human and mouse lung cells [39], published by a research team from the Imperial College London in Nature Communications, was ignored when concluding that enzalutamide does not act against SARS-CoV-2 in *in vitro* analysis.

The citation and discussion of both articles would be mandatory, considering that the conclusions of the authors were based on a mix of clinical trial, molecular and epidemiological findings. The fact that these two major articles were excluded from the discussion as counterpoints to the findings from the COVIDENZA trial and respective in vitro and epidemiological studies represent they pseudo-balance of the manuscript. While early epidemiological findings that were contrary to the findings have been mentioned, in face of the massive amount of literature showing opposite results from the findings from the manuscript, an undisputable imbalance between the literature for and against the

findings from COVIDEZA is identified in the manuscript. Some preliminary data and a few of the contrary results were smartly included, in order to convey a messages of an impartial, balanced analysis, and that the literature that contradicts was fully covered.

In addition, several additional data that strongly support the evidence of the theory on androgens for COVID-19 and anti-androgen protective roles in COVID-19 have been entirely dismissed. Dismissed data included a detailed description of the mechanisms that strongly support the indirect regulation of SARS-CoV-2 entry by androgens, the observational findings in different populations identified by independent groups, and other RCTs are among the trials forgotten to be included.

For definitive conclusions to be made, all medical literature should have been fully covered and thoroughly analyzed.

In conclusion, authors seemed to have strategically selected articles that supported their claims, selectively yet underreporting claims of the opposite. And, in their response letters [4,5], they are smartly expecting the results of another RCT that tested degarelix, which, as per its indirect actions in androgens, are unexpected to be effective for COVID-19, while dismissing the upcoming phase 3 proxalutamide trials, perhaps due to the geographical origin of the respective trials and industry.

The curious exclusion of imperative studies on anti-androgens and COVID-19 adds evidence to the doubts surrounding the undisclosed objectives of the trial and respective epidemiological and *in vitro* findings.

13. The full scientific pathway, from biological plausibility to multiple findings in a wide variety of populations that unanimously supported the evidence of androgens and anti-androgen protection in COVID-19, was absent from the COVIDENZA trial manuscript.

Science has a beautiful methodology, that initiates with early observational findings, from which molecular mechanisms to support the findings are searched, or from novel molecular mechanisms are detected, from which corresponding clinical observations or tests are performed. From mechanistical plausibility, epidemiological or observational findings, molecular actions, or a combination between them, a hypothesis is constructed. Further observational, epidemiological and/or *in vitro* findings are then actively searched in order to confirm or refute the hypothesis proposed. When most of the data found prior and after the hypothesis are concordant between them, further studies are proposed, and, ultimately, RCTs are conducted to confirm the now strongly supported hypothesis.

In the case of the androgen theory on COVID-19, overwhelming evidence is supported by virtually all studies of all sorts. Mechanistic plausibility has been established early in 2,020, when the 80% to 90% of SARS-CoV-2 cell entry was found to depend on prior priming of the virus by a cell-membrane surface protein, the transmembrane protease, serine,2 (TMPRSS-2) [40]. The only known endogenous regulators are androgens, which are hormones with testosterone-like actions in AR [41].

Early in the pandemic, in addition to aging and metabolic diseases, males were promptly identified as being independent risk factors for COVID-19 complications, i.e., after adjustments for age, presence of comorbidities and use of medications [42]. Correspondingly, as a sort of proof-of-concept, transgenders demonstrated a similar pattern: female-to-male (fTm) transgenders, i.e., genetically born as female but currently phenotypically and, most importantly, hormonally presented as males, had an almost 4-times higher risk of COVID-19 than male-to-female (mTf) transgenders, which were persons born as males but currently phenotypically and hormonally presented as females [43].

Among males, those with androgenetic alopecia (AGA), a clinical sign that represents excess of androgen activity, were demonstrated by different, independent groups, to be an independent predictor of poorer prognosis in COVID-19 [44-48]. The understanding of androgen activity encompasses not only testosterone (T) levels, but levels of other androgenic hormones, in particular the dihydrotestosterone (DHT), an

androgen five times more potent than testosterone, the proportions between T, DHT and estradiol (E), the in-tissue, tissue-specific level of conversion of T into DHT, which means that serum DHT levels not always precisely reflects its levels inside cells from different tissues, and the AR sensitivity to these hormones.

Hyperandrogenic activity was not only found to be an independent risk for COVID-19 among males, but also in females, which reinforces the hypothesis that androgens play a determinant role for infectivity and pathogenicity levels of SARS-CoV-2. Our preliminary findings of women with hyperandrogenism presenting more severe COVID-19 [49] was further confirmed by a United Kingdom (UK) nationwide database, showing similar findings among females with polycystic ovary syndrome (PCOS) [50]. PCOS is a syndrome present in 15% to 20% of women with a complex pathophysiology, and is the main representative of hyperandrogenic states in females.

The hyperandrogenic state as being a risk factor for COVID-19 complications, independent of the sex, was reinforced by the potential increased risk among anabolic steroid users [51]. The lack of circulating steroids may justify why pre-pubertal children are relatively protected from COVID-19, with milder stays and extremely low mortality rates, whereas babies below one y/o have increased risk of COVID-19, compared to one to 10, pre-pubertal children, since babies below one y/o tend to have circulating steroids, termed as ‘mini-puberty’.

An apparent contradictory observation, that males with lower testosterone levels upon hospital admission had increased risk of disease progression and death [52], led to a misleading understanding that testosterone would be protective for COVID-19. However, these findings allow the opposite conclusion. First, causality was improperly established for this study. Low testosterone upon admission can be considered a mark for poorer prognosis, which meets the understanding of the SARS-CoV-2 pathophysiology. SARS-CoV-2 enters and replicates abundantly in Leydig cells in the testicles [53], which are the cells that produce testosterone in males. In result, sharper decrease in testosterone levels is a precise marker of higher viral infectivity and pathogenicity, which allow the speculation that lower testosterone could predict COVID-19 severity, which corresponds to the findings [52]. In addition, reduction in testosterone level is a universal phenomenon that occurs in severe states of any disease [54], as a potential mechanism of energy-saving by blocking anabolic processes. Consequently, lower testosterone upon hospital admission may indicate current and tendency to progression to more severe states, with inherently poorer prognosis.

This explanation is not only plausible and follows the adaptative physiology of the acute disease and literature, but is reinforced by the fact that, despite higher testosterone levels as being 'predictors' of better outcomes among hospitalized COVID-19 males, increased levels are associated with higher inflammatory markers, at least in females [55].

The supporting molecular findings for the androgen theory on COVID-1 and respective anti-androgen protection against COVID-19 is strong and vast. It has been demonstrated that DHT enhances endothelial dysfunction and stimulates cytokine storm through inflammation in the presence of SARS-CoV-2 [56], which was corroborated by the AR gene determines COVID-19 severity [57]. Definitive findings from autopsies showed strict overlapping locations and concentrations of the virus, TMPRSS-2, androgens and androgen receptors, unlikely to occur by chance [58].

In the case of drugs to be tested for a certain disease, it is noteworthy that RCTs should be designed considering the optimal timing for the treatment and a treatment regimen, dose and duration that can effectively test the proposed hypothesis. In the present case, the optimal timing for COVID-19 treatment using indirect anti-viral agents, which is the proposed actions for anti-androgens, would be in the first days of symptoms, and, in the case of the use of a long-action drug, such as enzalutamide, that inherently takes longer to reach a MEC, higher doses within the safety profile established in the literature should be administered. Not following these two principles may compromise the results of the trial of enzalutamide for COVID-19.

In the case of other trials, in a double-blind, placebo-controlled RCT, dutasteride, a broad potent 5-alpha-reductase, that inhibits the conversion of testosterone into DHT, was able to increase COVID-19 speed recovery, reduce viral load, and prevent inflammatory responses, when compared to placebo [59]. Similarly, proxalutamide accelerates viral clearance and increases recovery speed in COVID-19 outpatients [60], and consequently reduced hospitalization rates by approximately 90% in both males [16] and females [61], analyzed in a sex-stratified manner due to differences in androgen expression and action between sexes. The decision to extend the study on proxalutamide in further trials was based on possible anti-thrombotic and anti-inflammatory roles of proxalutamide, as previously confirmed *in vitro*, that may occur independently of its anti-viral actions [17].

In hospitalized COVID-19 subjects, an important reduction in mortality rates was observed in two entirely independent populations from distinct geographic regions, ethnic

characteristics, and levels of access to healthcare [18]. Recovery speed rates and mortality rates were similar between regions, similar between males and females, and occurred in all levels of COVID-19 severity unless if under mechanical ventilation. Independent radiology experts performed blind, objective analysis of chest computed tomography (CT) from patients upon randomization and approximately five days after, and showed that proxalutamide led to an average of 50% reduction in the percentage of lung parenchyma affected, compared to placebo [62], which substantiates the clinical findings. These findings demonstrate the consistency and reproducibility of the efficacy observed with proxalutamide, as observed between different regions.

An independent RCT on finasteride for hospitalized COVID-19 subjects identified that finasteride statistically significantly improved oxygen parameters and a numerical reduction of 75% in mortality rate [63]. Coincidently, this RCT was not included in the discussion of the results of the COVIDENZA trial.

14. The fact that drug efficacy for COVID-19 is variant-dependent was not considered.

SARS-CoV-2 is a virus that can enter human cells through two different manners: 1. Mediated by ACE2 after priming by TMPRSS-2; and 2. Direct endocytosis entry mediated by cathepsin K. Before the Omicron variant, the prevailing entry was mediated by TMPRSS-2 and ACE2, whereas in Omicron variant endocytosis has become the main mechanism of cell entry [64-67]. Even between other variants, such as Delta and Gamma, differences in pathophysiology were relevant. Consequently, efficacy of drugs against COVID-19 does not only depend on the disease stage, but also change according to the variant, i.e., drug efficacy for COVID-19 is dependent on each variant, and should not be extrapolated for other variants. The example of molnupiravir is representative of this issue: in the RCT [68] that led to molnupiravir's emergency use authorization (EUA) in multiple countries relied in a single country where the Gamma variant was prevailing. If this country is removed from the analysis, its efficacy is no longer present.

The COVIDENZA trial was conducted in 2020, in the first SARS-CoV-2 sub-variants, whereas proxalutamide in hospitalized COVID-19 patients was tested in the Gamma variant, which was confirmed in a subset of patients from the trial [69]. Alone, this is sufficient to justify potential differences between trials, even when conducted with the same molecule.

In the Omicron variant, anti-androgens are unlikely as effective, since their actions are based on the suppression of TMPRSS-2 expression, which is not necessary for the SARS-CoV-2 Omicron variant.

Now that the fact that efficacy of a drug for COVID-19 changes according to the variant is vastly known, discussions regarding this aspects is expected to be present in manuscripts or RCTs of molecules or other modalities such as vaccines for COVID-19.

15. An implicit geographical discrimination should not be discarded.

The progression towards the reduction of the classical geographical discrimination in the scientific community, i.e., the fact that the quality, the evidence, and veracity of a study are at least partially presumed on the origin of the study, is unquestionable. However, the scientific prejudice still exists and are not present in a less explicit manner.

The concerns raised in this article, hard not only hard to be entirely satisfactorily justified, but some of them are clear demonstrations of neglection of the scientific production from other scientists, since authors concluded that anti-androgens should no longer be studied for COVID-19, despising all the extensive contrary scientific literature. This contempt would hardly occur if the other RCTs were conducted in a 'central' region (USA, Canada, Western Europe, Japan, Australia). Hence, there is no plausible explanation other than indirect evidence of prejudice and xenophobia.

Disparities are clear, not only among who conducted the trial, but also how the RCT has been received by scientific community. Inconsistencies in the COVIDENZA trial depicted in this article, that seem to be of higher concern than the alleged ones in the proxalutamide trials, would have already been a matter of prompt retraction, rejection, or an expression of concern. The flaws presented in the concerns in this article would never be acceptable if they occurred in the proxalutamide trials, since alleged minor flaws that have not even been demonstrated to exist were sufficient to lead to expressions of concern. Notably, a few 'data scientists' who raised questions in the proxalutamide trials, and that have been fully clarified, have never questioned the more unusual facts described herein, suggesting a one-side only, partial, pre-determined analysis.

Disparities in the treatments given to each RCT are clear, and only regional discrimination can be the underlying motivation – or undisclosed interests for or against the molecule tested.

16. A cherry-picked 'editorial' with a superficial, incomplete analysis was strangely accepted and published in New England Journal of Medicine coincidently in the same period of the publication of the enzalutamide trial, by a Pfizer Advisor.

Conflicts of interest have been a major determinant of the strength of recommendations and directions of the analyses of all sorts of studies, which has been particularly relevant in COVID-19.

Coincidently, within a few days of interval from the publication of the COVIDENZA trial, a superficial, questionable analysis of a populational study on ADT that showed a relative protection (instead of the conclusion of lack of efficacy, as previously detailed in this article), seemingly aiming to 'reinforce' the results of the trial on enzalutamide, has been strangely accepted and published as an 'editorial' ("JWatch") in New England of Medicine (NEJM) [70]. Also coincidently, the author of the editorial is an advisor for both Pfizer and Astellas with special interest for molecules to treat prostate cancer [71]. Noteworthy, it is notorious that if a certain drug in 'downgraded' for shorter periods of treatment or that would require reduction in sales cost, there may be attempts to avoid the extension of its use.

17. Unanswered questions raised by the concerns

In addition to the concerns brought in this article, some questions are naturally raised. Why would Pfizer be present in a trial sponsored by Astellas, since they have never communicated any partnership for researching enzalutamide for COVID-19? Why was the RCT published so long after its termination? Based on chronological coincidences, could Pfzier have waited to evaluate whether enzlutamide's direct competitor, Paxlovid, would be effective, and base the statistical treatment according to whether enzalutamide needed to be used as a back molecule?

This hypothesis becomes minimally plausible if one considers that the DSMB of the COVIDENZA trial could also have interrupted the RCT based on high efficacy, due to the occurrence of one death among 10 hospitalized subjects not on treatment, whereas there were no deaths among the 32 hospitalized subjects using enzalutamide. We then face a study that could have been interpreted in opposite directions, showing enzalutamide to be effective. In result, an article with a completely opposite content could have written with a strong scientific background to supports its efficacy.

Final discussion

The combination of multiple unusual procedures and facts that coincidently disfavored enzalutamide for COVID-19 conveys a strong message of a potential directed objective. Directed analysis was clear, as observed by the asymmetric covering of previous literature, in particular the molecular study performed by The Imperial College London and the populational study conducted in the US Veteran Affairs Hospitals.

In an open label study where the impartiality of its investigations could be questioned, since assistant doctors and investigators knew the characteristics of each arm, and kept the imbalance, without rerandomizing or employing covariate-adapting randomization, the use of duration of hospitalization stay, a highly subjective outcome based on medical judgement, without objective criteria for hospital discharge, has sufficient substantiation to invalidate duration of hospital stay as an outcome to be employed, measured, or used for study early termination. Coincidently, hospital stay duration was the only manner to determine enzalutamide to have negative effects with a small number of subjects.

An inadequate member played a key role in the analysis of the trial, since Pfizer has open interests against a competitor molecule (enzalutamide). Not only enzalutamide, but Pfizer also needed all other anti-androgens to be ineffective to avoid competitors to Paxlovid. The combination of this fact with the facts that the DSMB could also have interrupted the RCT due to difference in efficacy, when compared to both placebo group and national in-hospital mortality rates, and did not promptly communicate the safety concerns, oppositely, there was an interval of more than one year between the study interruption and its communication, bring several additional questions.

Besides the numerous factors described in this letter, the conclusion that studies on anti-androgens should be interrupted solely based on the authors own data was not only immature, but also seemingly arrogant, with a self-determinism of superiority in the demonstration of results. This requires further ethical assessments since it coincides with the differences between the regions where trials were conducted, which would unlikely have happened if the other RCTs on anti-androgens were conducted in ‘central’ regions. The weaknesses and flaws of this trial show, by itself, that the presumption of regional superiority is not valid.

As the main principal investigators of the already published trials on proxalutamide for COVID-19, I have the right to say that unlike what we have been experiencing with our own trials, that have been questioned through anonymous and/or undisclosed interests by persons that are directed by specific targets, I bring myself as an open voice to discuss the results and concerns of the trial on enzalutamide for hospitalized COVID-19 subjects. It is time that transparency, impartiality, and equity overcome the currently prevailing values.

Conclusions

Altogether, it is unavoidable that we consider that the authors of the trial, together with the supposedly independent DSMB, were biased, which raises concerns regarding the conduct of the trial and the publication of the manuscript.

While emerging data reinforces speculations of Pfizer as an active barrier against potently effective drugs, concrete, documented arguments are necessary for these claims. As presented in this article, the case of this RCT, that represents a potential active action against anti-androgens for COVID-19, presents sufficient evidence for further investigation.

Conflicts of Interest

Flavio A. Cadegiani has voluntarily participated in observational studies and clinical trials on anti-androgens for COVID-19, with no grants or any other sort of support received related to these studies, from any manufacturer of any anti-androgen or competitors.

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