Estrogen and Androgen Receptor Inhibitors: Unexpected Allies in the Fight Against COVID-19

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Translational Relevance
No prophylactic treatments for COVID-19 have been clearly proven and found. In this pandemic context, cancer patients constitute a particularly fragile population that would benefit the best from such treatments, a present unmet need. TMPRSS2 is essential for COVID-19 replication cycle and it is under androgen control. Estrogen and androgen receptor dependent cues converge on TMPRSS2 regulation through different mechanisms of action that can be blocked by the use of hormonal therapies. We believe that there is enough body of evidence to foresee a prophylactic use of hormonal therapies against COVID-19 and this hypothesis can be easily tested on cohorts of breast and prostate cancer patients who follow those regimens. In case of pandemic, if the protective effect of hormonal therapies will be proven on cancer patients, the use of specific hormonal therapies could be extended to other oncological groups and to healthy individuals to decrease the overall risk of infection by SARS-CoV-2.

Abstract
Given the COVID-19 coronavirus emergency, a special focus is needed on the impact of this rapidly spreading viral infection on cancer patients. Androgen receptor (AR) signaling in the transmembrane protease serine 2 (TMPRSS2) regulation is emerging as an important determinant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) susceptibility. In our study, we analyzed AR and TMPRSS2 expression in 17,352 normal and 9,556 cancer tissues from public repositories and stratified data according to sex and age. The emerging picture is that some patient groups may be particularly susceptible to SARS-CoV-2 infection and may benefit from antiandrogen- or tamoxifen-based therapies. These findings are relevant to choose proper treatments in order to protect cancer patients from concomitant SARS-CoV-2 contagion and related symptoms and put forward the idea that hormonal therapies could be used as prophylactic agents against COVID-19.

Keywords
SARS-CoV-2, breast and prostate cancer patients, TMPRSS2, hormonal therapy, tamoxifen

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Introduction

In the context of COVID-19 emergency, a special focus is needed on the impact of this rapidly spreading viral infection on cancer patients. Patients with cancer are more vulnerable to infections than other individuals because malignancy and anticancer therapies result in an immunosuppressive status, with a higher likelihood to manifest severe adverse events and to die of COVID-19.

Recently, Wambier et al. postulated that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is androgen driven1 since the expression of the transmembrane protease serine 2 (TMPRSS2), required to prime SARS-CoV-2 spike protein for entry into target cells, is controlled by androgen receptor (AR) signaling2. The authors affirmed that androgen signaling could explain the observed disparities in individual susceptibility (very low in children), viral transmission, and in mortality among genders. Future studies are required to validate this theory and to evaluate the therapeutic and prophylactic potential of medications targeting androgen activity. AR inhibitors like nonsteroidal antiandrogen compounds (enzalutamide, bicalutamide, apalutamide, and darolutamide), steriodogenesis inhibitors, 5-alpha reductase inhibitors, and chemical castration with gonadotropin-releasing hormone analogs could be valid options in this fight3. AR inhibitors are already used for the treatment of all prostate cancers that in the initial phase of the disease are sensitive to androgen hormone therapy. Therefore, the use of these compounds should lead to a downregulation of TMPRSS2 gene expression in normal and cancer tissues and to a reduced susceptibility to COVID-19 infection, adverse events, and mortality. Emerging clinical data seem to corroborate this hypothesis4. Intriguingly, the downregulation of TMPRSS2 and ACE2 expression by enzalutamide in prostate and lung cells and the consequent reduction of SARS-CoV-2 infectivity have been recently reported at the AACR VIRTUAL MEETING: COVID-19 AND CANCER by Prof. Irfan A. Asangani from UPenn.

Importantly, the use of high-dose estrogens in prostate cancer patients is beneficial in restraining AR signaling and tumor proliferation highlighting the importance of estrogen receptor (ER) and AR interplay5. The clinical trial NCT04359329 is based on this rationale. It seeks to assess if estrogen given as a patch placed on the skin of COVID-19 positive or presumptive positive patients for 7 days can reduce the severity of COVID-19 symptoms compared to regular care. In principle, these preventive and therapeutic approaches in a COVID-19 pandemic context could be applied and extended to healthy individuals at increased risk of infection, such as militaries and healthcare professionals and to cancer patients harboring AR expressing tumors such as lung, bladder, kidney, breast, and liver cancer whether beneficial or nondetrimental effects will be demonstrated. Side effects of antiandrogens depend on the specific antiandrogen in question. However, common side effects of antiandrogens include breast enlargement, feminization, hot flashes, sexual dysfunction, infertility, and osteoporosis. Those side effects depend on the duration of treatment and are completely reversible therefore their use seems justified.

Breast Cancer and Hormonal Therapies

Malignant breast tumors often express AR (up to 80%) with 84% to 95% in luminal, 50% to 63% in HER2 amplified and 10% to 53% in triple negative breast cancer (TNBC)6.

Ongoing clinical trials are already testing anti-AR compounds (Darolutamide) efficacy in TNBC patients expressing AR (i.e., NCT0338367). A positive outcome of the study would suggest a role for AR inhibitors in this subset of patients that would likely benefit from both the antitumor and the anti-COVID-19 prophylactic/protective effects.

Moreover, the crosstalk between ARs and ERs in breast cancer (BC) is well known7. The balance between the activity of these two hormone receptors (HRs) and their interplay in different clinical settings is influenced by many coregulators. This comprises a dynamic regulatory network enhancing or limiting the activity of AR-directed treatments in breast and prostate tumorigenesis and sensitivity to anti-AR drugs7. There are different potential mechanisms by which ERb isoforms can modulate AR activity during carcinogenesis and this may affect treatment efficacy in prostate and BCs. Among them, the most important is the competition for DNA binding that may alter the recruitment of transcription coregulators, depending on the cancer stage7. The most used therapies in hormone-positive patients with advanced or metastatic BC are tamoxifen, fulvestrant, and aromatase inhibitors. The choice of the specific treatment depends upon several factors, for example, the disease setting (adjuvant vs. metastatic), age, disease-free interval, disease progression, etc. Among its functions, tamoxifen can bind directly to AR8, thereby inhibiting its activity9. These observations suggest that also patients with ER/PGR positive BC would potentially benefit from tamoxifen treatment in relation to infection and severity of symptoms by COVID-19. Interestingly, the clinical trial NCT04353180 is testing additional functions of tamoxifen in COVID-19 patients in combination with isoretinoin and trimethoprime following a different rationale. In fact, tamoxifen was shown to increase endolysosomal pH and alter endosomal dynamics similarly or even better compared to the antimarial chloroquine10, thereby potentially interfering with SARS-CoV-2 entry. Fulvestrant is used to treat HR-positive metastatic BC postmenopausal women with disease progression as well as HR-positive, HER2-negative advanced BC in combination with palbociclib in women with disease progression after endocrine therapy. Fulvestrant binds specifically to ER and leads to its degradation acting as selective estrogen receptor downregulator, thereby preventing estrogen signaling without affecting the binding of androgens to AR, as tamoxifen does. The benefit of fulvestrant treatment on BC patients in the context of COVID-19 pandemic should be investigated with caution since, on the one hand, in vitro treatment of MCF7 BC cells significantly upregulates TMPRSS2, while
coherently estrogen and anti-AR treatments lead to TMPRSS2 downregulation in MCF7 as well as in A549 lung adenocarcinoma cells\textsuperscript{11}.

The different mechanisms of action between tamoxifen and fulvestrant therefore could affect COVID-19 susceptibility and severity of effects in BC patients. Tamoxifen can act against SARS-CoV-2 by restraining AR signaling directly and by promoting endolysosomal alkalinization and endosomal dynamics. If our hypothesis holds true, tamoxifen-treated BC patients should be protected, less susceptible and will manifest less severe COVID-19 symptoms than fulvestrant-treated individuals.

In addition, postmenopausal BC patients are conventionally treated with aromatase inhibitors that inhibit the conversion of androgens to estrogens. In these patients androgen accumulation could potentially increase TMPRSS2 expression in tissues via AR signaling activation. However, estrogens exert complex and pleiotropic functions in human tissues and they have been involved as well on TMPRSS2 transcriptional regulation. In particular, results from Kim et al. suggest that an estrogen-dependent signaling through the ERβ2/Src-IGF-1R/NFkB (p65) axis can upregulate TMPRSS2 expression\textsuperscript{12}. This implies that aromatase inhibitor treatments such as anastrozole, letrozole and exemestane in females, which are exposed to high concentration of estrogens as compared to androgens, may exert a stronger anti-COVID-19 activity by inhibiting estrogen production and the following p65-TMPRSS2 signaling rather than promoting a TMPRSS2 upregulation via marginal androgen/AR signaling. The entity of the two opposite effects in terms of COVID-19 susceptibility, namely, the androgen-driven TMPRSS2 upregulation and antiestrogen-dependent TMPRSS2 downregulation in females, should be investigated. Our hypothesis is that in females, where estrogen signaling is dominant over the androgen one, aromatase inhibitors and tamoxifen should have a strong protective effect. Hence, it would be probably advisable to use tamoxifen or aromatase inhibitors rather than fulvestrant in patients at high risk of infection or already infected by COVID-19.

The majority of the side effects seen with tamoxifen have been mild and do not usually cause BC patients to stop taking the medication. However, approximately 15% of women stop treatment because of side effects. The most common ones are: hot flashes, vaginal discharge or bleeding, menstrual irregularities, cataracts, nausea, and vomiting. Additionally, women may undergo hair loss, skin rashes (itching or peeling skin) or headaches. Rare but serious side effects of tamoxifen are blood clot in the veins, increased stroke, and endometrial cancer rates. Blood clotting can be prevented with heparin or other anticoagulant medications. Since disseminated intravascular coagulation is one of COVID-19 related complications and potentially a life-threatening event, COVID-19 patients are usually treated with anticoagulants. Aromatase inhibitors tend to cause fewer serious side effects than tamoxifen. However aromatase inhibitors can cause heart problems, joint pain, and bone loss when used for a long time. Both treatments are widely used since a long time and the management of the related side effects is common practice. Considering the limited and reversible side effects in BC patients, their manageability from a medical standpoint, and the short period of use as a potentially prophylactic treatment for COVID-19 infection, we believe that the administration of tamoxifen and aromatase inhibitors in healthy women would be acceptable and justified.

Additionally, AR gene polymorphisms can have an impact on AR and TMPRSS2 expression as already shown\textsuperscript{13}. AR polymorphisms in the African population are different from the ones in the Caucasian population implying possible differences in COVID-19 susceptibility. Our previous research experience on African BC patients confirms a high proportion of AR positive TNBC tumors in this ethnic group for which anti-androgen strategies could be important in the concomitant fight to COVID-19\textsuperscript{14}. Treating these patients with anti-AR compounds opens to the possibility to prevent viral infections and probably to reduce the severity of symptoms. Moreover, if our hypothesis is correct, tamoxifen or aromatase inhibitors could be used in those patients regardless of their ER/PGR negativity and in individuals without a diagnosis of cancer as COVID-19 prophylactic regimens.

In order to assess the distribution of AR expression in cancer and normal tissues, we performed a retrospective analysis of available expression data from the The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) portal (dbGaP accession number phs000424.v8.p2 on 04/10/2020). The rationale we followed was to identify patients or groups at high risk of COVID-19 infection due to an increased AR tumor expression, which would likely benefit from anti-AR- and tamoxifen-based treatments.

In particular, we selected PanCancer Atlas studies from TCGA portal that include 35 cancer types. In this dataset, we focused on AR expression data in 9,556 oncologic patients. From the GTEx project we analyzed the expression of AR in 17,382 tissue samples from 948 healthy donors and the related clinical data available. Expression data were analyzed by gender, age (20 to 59, above 60 years old), and tumor versus normal tissue. Aggregated analysis of AR expression in tumors with respect to healthy tissues showed lower expression levels in cancer for both genders (Fig. 1A). Deeper investigation on AR expression according to age and sex subgroups, however, highlighted a more complex picture. Indeed, while both genders in young individuals showed a coherent reduction of AR expression in tumors as compared to healthy tissues, elderly females showed increased AR levels in tumors compared to normal tissues. This difference is gender specific since age-matched elderly males did not show differences in AR expression between tumors and healthy tissues (Fig. 1A). In contrast to elderly females, young females expressed lower levels of AR in their tumors compared to healthy tissues (Fig. 1A). Coherently with AR overexpression, we observed a significant increase in TMPRSS2 gene expression in tumors of elderly females with respect to age-matched normal tissues (Fig. 1B, C). These
findings indicate that cancers of elderly females have typically higher expression of AR and of TMPRSS2 identifying a population of cancer patients that may be particularly susceptible to COVID-19 infection.

Conclusions and Future Perspectives

In conclusion, anti-AR and tamoxifen-based treatments through their downregulation of TMPRSS2 could be used as protective compounds from COVID-19 infection (Fig. 1D). Besides prostate and BC, this concept could be translated also to other AR-expressing tumor types (AR+ TNBC, bladder, kidney, lung, and liver) or to healthy individuals at risk of contracting COVID-19. Despite it has been reported that various tissues express different levels of ACE2 and TMPRSS2\textsuperscript{15}, to the best of our knowledge, it is not clear yet if those features are associated to an increased susceptibility, infectivity, and severity of symptoms in those patients. Moreover, it should be better investigated if a tissue-specific damage in COVID-19 patients correlates with the level of ACE2 and/or TMPRSS2 expression in the same tissue. Due to the pandemic situation, clinicians should pay even more attention to the choice of anti-AR and ER compounds to treat cancer patients given their effects on the regulation of TMPRSS2 expression\textsuperscript{15}, since proper treatments could protect from COVID-19 infection and from the severity of its
symptoms. We want to stress that for BC patients that can be treated with fulvestrant, tamoxifen or aromatase inhibitors, either tamoxifen or aromatase inhibitors rather than fulvestrant (that could result in TMPRSS2 upregulation), should be preferred. This is especially true for postmenopausal elderly female cancer patients that show the highest expression of both AR and TMPRSS2 in tumors. Among those BC patients typically treated with fulvestrant may even worsen their COVID-19 susceptibility and clinical course. In this pandemic context, tamoxifen (with its anti-ER, anti-AR and anti-viral combined functions), aromatase inhibitors and antiandrogen therapies, could be unexpected allies in the fight to COVID-19 for patients with cancer as well as people at increased risk of contagion.

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Authors’ Contributions
SB and MM designed the study. SB, EF, MT, DA, GM, FN, PP, and MM acquired the data. SB and MM analyzed and interpreted the data. SB and MM wrote the manuscript. SB and MM supervised the study. All authors provided final approval to publish and agree to be accountable for all aspects of the manuscript.

Availability of Data and Materials
All the data are available upon request and on public repositories.

Consent for Publication
All authors gave their consent for publication

Ethical Approval
The study has been approved by the local ethical committee (CEROM; protocol number IRST 100.51 ACT4COVID).

Statement of Human and Animal Rights
This article does not contain any studies with human or animal subjects.

Statement of Informed Consent
There are no human subjects in this article and informed consent is not applicable.

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The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: GM has competing interests with Novartis, BMS, Roche, Pfizer, ARIAD, and MSD not related to this manuscript. All the other authors have no conflicts of interest to declare.

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