Tamoxifen protects breast cancer patients from COVID-19: first evidence from real world data

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Research Article

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Abstract

Background COVID-19 severity is uneven between genders. We hypothesized a role of hormonal therapies in the severity of COVID-19 in breast cancer (BC) patients via the modulation of SARS-CoV-2 susceptibility genes.

Patients and Methods We mined the Emilia Romagna region (Italy) registries to compare the rates of hospitalization and mortality for COVID-19 in 2020 amongst 24628 BC patients. Next, we analyzed the modulation of ACE2, TMPRSS2 and NRP1 gene expression and the susceptibility to SARS-CoV-2 infection by tamoxifen, fulvestrant and 17 β -estradiol on human ER+ MCF-7 cells in vitro.

Results The hospitalization rate observed for 4784 tamoxifen treated BC patients was the lowest (OR, 0.41; 95% CI, 0.18-0.94; p=0.04) among hormonal therapies and no fatalities occurred. A standard mortality rate reduction has been observed also for patients treated with aromatase inhibitors (SMR: 0.73; 95% CI, 0.45-0.90). In vitro experiments showed that fulvestrant, but not tamoxifen, increases ACE2, TMPRSS2 and NRP1 gene expression and susceptibility to SARS-CoV-2 infection and that 17 β -estradiol reduces significantly TMPRSS2 and NRP1 expression.

Conclusions Tamoxifen treated BC patients showed a reduced rate of hospitalization and strikingly no fatalities for COVID-19. In vitro experiments confirmed a protective role of tamoxifen while an increased susceptibility to SARS-CoV-2 infection of ER+ cells treated with fulvestrant was observed.

Introduction

SARS-CoV-2 entry into target cells is mediated by the transmembrane protease serine 2 (TMPRSS2) that primes the fusogenic SARS-CoV-2 spike protein following its interaction with ACE2 receptor and viral internalization [1]. TMPRSS2 expression level critically determines SARS-CoV-2 infectivity and production in cells [2] and through pH-dependent endocytosis allows the fusion of the viral envelope with the cell membrane [1]. Endolysosome de-acidification can restrict replication of SARS-CoV-2 as acidic conditions are necessary for SARS-CoV-2 to enter into and be released from host cells. Besides ACE2 and TMPRSS2, neuropilin-1 (NRP1) has been recently characterized as a third SARS-CoV-2 susceptibility factor and co-receptor influencing viral tropism and infectivity [3,4]. Androgens enhance transcriptionally the expression of TMPRSS2 and ACE2 [5,6]. This implies that patients undergoing anti-androgen receptor (AR) treatment may be less susceptible to viral entry due to a systemic downregulation of TMPRSS2 expression as recent data on prostate cancer suggest [7]. The severity of COVID-19 symptoms and mortality is uneven among genders with females being less affected [8,9]. However, the molecular basis of this gender bias is unknown. Interestingly, an epidemiological study on SARS-CoV-1, that similarly to SARS-CoV-2 enters cells via ACE2, showed that mortality rates were lowest in young women compared to men and that the protective effect mediated by gender declines progressively with age suggesting that hormones like estrogens could be involved [10]. Accordingly, ovariectomized female mice developed a more severe form of infection compared to controls and to animals treated with the anti-estrogen receptor (ER) fulvestrant

[11]. Hormone receptor (HR) positive breast cancer (BC) patients represent 70-80% of the whole BC population and are principally treated with hormonal therapies including tamoxifen, fulvestrant and aromatase inhibitors (AIs) such as anastrozole, letrozole and exemestane to interfere with estrogen dependent cues according to age and disease stage. Tamoxifen is a selective estrogen receptor (ER) modulator (SERM) acting as an antagonist or agonist depending on the specific tissue context with several pleiotropic activities [12]. Fulvestrant is a strict selective ER degrading (SERD) antagonist, whereas AIs induce estrogen deprivation in [postmenopausal](#) BC women by inhibiting the key aromatization step in the synthesis of [estrogens](#) from androgens thereby acting upstream of ER signaling. Given the distinct mechanisms of action of current hormonal therapies, we hypothesized that they may differently affect COVID-19 severity and mortality in BC patients. Hence, we mined real-world data from Emilia Romagna region (Italy) throughout 2020 to evaluate whether hospitalization and mortality for COVID-19 rates differed according to therapy in BC patients. Furthermore, we performed confirmatory in vitro experiments to investigate the molecular mechanisms involved in the therapy-related susceptibility alteration to SARS-CoV-2 infection at the cell level.

Patients And Methods

Study population

The cohort of the study included all BC patients resident in Emilia Romagna region and treated in 2020 with one of the following drugs alone (monotherapy): tamoxifen, fulvestrant, AIs (anastrozole, letrozole and exemestane) and anti-HER-2 (trastuzumab or pertuzumab). We retrieved information on whether and when patients were hospitalized for COVID-19 during the same period. Patients treated after the date of hospitalization have been excluded. The study was done using record-linkage processes between multiple databases (FED and AFT - direct and territorial drugs distribution, SDO Hospital discharge cards and REM Mortality detection) of the Emilia Romagna region. The following ATC codes were used to mine the databases for drug delivery to patients: AIs (L02BG03 - ANASTROZOLO, L02BG04 - LETROZOLO, L02BG06 - EXEMESTANE); fulvestrant (L02BA03 - FULVESTRANT); tamoxifen (L02BA01 - TAMOXIFENE) and anti-HER2 (L01XC03 TRASTUZUMAB, L01XC13 PERTUZUMAB) and to retrieve the date of treatments or drug administration. Admission for COVID-19 data were retrieved from hospital discharge cards contained in the regional hospital database (SDO) filtering for the following COVID-19 diagnosis codes: 079.82 SARS-CORONAVIRUS ASSOCIATO, 480.3 POLMONITE DA SARS-CORONAVIRUS ASSOCIATO, V01.82 ESPOSIZIONE A SARS-CORONAVIRUS ASSOCIATO. Deaths from COVID-19 were identified within the REM through the search for the following pathologies identified as the cause of death: U071, U072 (COVID-19, identified and not identified), J841 and J849 (Other interstitial lung diseases).

Statistical analysis

Age was summarized as medians and interquartile ranges, while COVID-19 hospitalizations and deaths were summarized by counts and percentages. Logistic regression models were fitted on the entire cohort and on both male and female sub-cohorts with COVID-19 hospitalization or COVID-19 death as

outcomes. The models were stratified according to the therapy received in 2020 and age adjusted. Age was considered a potential confounder, since elderly patients are at increased risk for COVID-19 hospitalization and death [13], and were not evenly distributed in different therapy groups. Furthermore, Firth (1993) profile penalized log likelihood approach was adopted to obtain robust parameter estimates in the presence of rare events such as the present dataset. We obtained odds ratios (OR) and 95% confidence intervals (95% CI) from these models. We used a Wald test to assess the null hypothesis of no difference between the rates of COVID-19 hospitalization/death among the different groups. Additional descriptive analyses were conducted in the COVID-19 hospitalized/died sub-cohorts to explore the time to hospitalization/death from last treatment administration normalized to the drug's half-life (HL). Age standardized hospitalization and mortality ratio (SHR and SMR respectively) were calculated to compare the risk of hospitalization/death for COVID-19 in the cohorts against the general REM population. 95% CI of SHR and SMR were calculated assuming a Poisson distribution of the observed numbers. All statistical analyses were performed using R version 4.0.0 (R Foundation for Statistical Computing) or GraphPad Prism; package logistf version 1.23 was used to compute the estimates of the odds ratios. Anti-HER-2-treated patients were used as a reference group since their age distribution was not skewed and they were not treated with hormonal therapy. T-test was used to analyze the significance of gene expression data. Kruskal-Wallis test was used to compare age distribution across therapies.

Expression analysis for *ACE2*, *TMPRSS2* and *NRP1* in human cells

MCF7 cells (HTB-22, ATCC) were cultured in RPMI-1640 or high glucose DMEM and MDA-MB-231 (HTB-26, ATCC) were cultured in L-15 medium in standard conditions with or without 10nM 17 β -estradiol (E2) as indicated. Cells were seeded into six well plates at a density of 1 $\times$ 10⁵ cells/well for 24 hours in duplicate. Monolayers were treated for 48 hours with 10nM or 1uM fulvestrant, 4-hydroxytamoxifen (4-OHT) or vehicle with or without 1nM E2. 10nM and 1uM were chosen since are the steady-state plasmatic concentrations of fulvestrant and tamoxifen in patients, respectively [14,15], RNA was extracted using miRNeasy (Qiagen) and retro transcribed using iScript cDNA synthesis kit (BioRad). Real-time PCR was performed using SYBR Selected Master mix and 7500 Real-Time PCR System (Thermo Scientific) following manufacturer's instructions. Primers used in RT-PCR are listed in the following table:

Target gene	Primer	Sequence (DNA)
ACE2	ACE2 forward	TCCATTGGTCTTCTGTCAACCCG
	ACE2 reverse	AGACCATCCACCTCCACTTCTC
NRP1	NRP1 forward	AACAACGGCTCGGACTGGAAGA
	NRP1 reverse	GGTAGATCCTGATGAATCGCGTG
TMPRSS2	TMPRSS2 forward	CCTCTAACTGGTGTGATGGCGT
	TMPRSS2 reverse	TGCCAGGACTTCCTCTGAGATG
HPRT	HPRT forward	CATTATGCTGAGGATTTGGAAAGG
	HPRT reverse	CTTGAGCACACAGAGGGCTACA

Experiments with SARS-CoV-2 virus

MCF-7 cells were maintained for 72 hours in 10% FBS DMEM either with vehicle or supplemented with 1 μ M 4-OHT or 1 μ M fulvestrant, detached and counted. All steps were performed maintaining 1 μ M 4-OHT, 1 μ M fulvestrant or vehicle respectively. Pretreated MCF-7 cells were seeded in 24 well plates (250,000 cells/well) and infected with SARS-CoV-2 (2019-nCoV/Italy-INMI1) (MOI = 1 PFU/well). Cells were subsequently incubated in medium supplemented with 2% FBS and the drugs. Forty-eight hours post-infection MCF-7 cell supernatants were harvested, viral RNA was extracted (QIAamp Viral RNA mini kit, QIAGEN) and subjected to reverse transcription and amplification by Real-Time PCR, as previously described [16]. Specifically, the N gene of SARS-CoV-2 was adopted to quantify the amount of virus. In parallel, the extraction and amplification steps were controlled by amplification of a spike in control for the albumin gene cloned harbored in a plasmid and added to the samples as 1.1pg DNA [17]. Furthermore, total RNA was extracted from MCF-7 cell pellets (PureLink™ RNA Mini Kit - Thermo Fisher Scientific) and normalized amounts of total RNA were subjected to reverse transcription and Real-Time PCR assay as described above. The endogenous albumin transcript was adopted as extraction and normalization control. Finally, MCF-7 supernatants were also evaluated for the presence of infectious viral particles by plaque assays on Vero E6 cells (ATCC® CRL-1586), as previously described [18]. N gene expression for 4-OHT, fulvestrant and vehicle-treated cells was normalized to albumin gene expression. The reported fold changes were calculated between mean values.

Results

Analysis of hospitalization and death for COVID-19 in breast cancer patients

In order to test the hypothesis that hormonal therapies may influence COVID-19 susceptibility and severity of symptoms, we mined real-world clinical data from the BC population of Emilia Romagna region assessing the frequency of hospitalization and death for COVID-19 in patients treated with tamoxifen, AIs, fulvestrant and anti-HER2 therapies (trastuzumab and pertuzumab) the latter used as a control group

as non-hormonal therapy treated BC patients. Out of a total of 24628 BC patients (24252 females, 376 males), 195 (187 females, 8 males) were hospitalized for COVID-19 in 2020. The rate of hospitalization for patients was higher than the overall rate of hospitalization for COVID-19 observed for residents in Emilia Romagna (0.79% vs 0.57%; 25448/4474292 residents) during 2020. As expected, age was an important determinant of hospitalization (OR, 1.06; 95% CI, 1.04 to 1.07; $p<0.001$) and death (OR, 1.12; 95% CI, 1.08 to 1.15; $p<0.001$) for COVID-19 in the BC patients cohort and its distribution differed among therapy groups ($p<0.001$). Statistical analysis applying an age-adjusted logistic model to the cohort stratified by therapy (single agent) revealed significant decreases in the hospitalization rate in tamoxifen (0.38%; OR, 0.26; 95% CI, 0.13-0.56; $p=0.001$; Table 1) and Als treated patients (0.84%, OR, 0.31; 95% CI, 0.18- 0.61; $p=0.001$; Table 1) as compared to 1.56% observed in the anti-HER-2 therapy reference group. In contrast, fulvestrant-treated BC patients showed no significant difference as compared to the reference group (1.65%; OR, 0.55; 95% CI, 0.23-1.31; $p=0.18$). The analysis of mortality for COVID-19 confirmed a significant protective role for tamoxifen (0%; OR, 0.03; 95% CI, 0-0.34; $p=0.005$; Table 1), as compared to anti-HER2 therapies. Strikingly, no COVID-19 fatalities on 4784 tamoxifen-treated patients occurred. The mortality by COVID-19 in Als and fulvestrant-treated patients was not significantly different ($p>0.05$) as compared to anti-HER2 treated patients although Als p -value was very close to significance (0.21%; OR, 0.23; 95% CI, 0.07-1.14; $p=0.07$; Table 1). Following analyses focused on the comparison of COVID-19 hospitalization and mortality among female patients treated with hormonal therapies. Tamoxifen treated female patients showed a significantly lower rate of hospitalization for COVID-19 (0.31%; OR, 0.41; 95% CI, 0.18-0.94; $p=0.04$; Table 2) compared to fulvestrant treated female patients used as reference group while Als did not (0.84%; OR, 0.56; 95% CI, 0.31-1.12; $p=0.10$; Table 2). Interestingly, we observed a similar picture for COVID-19 mortality in tamoxifen (0%; OR, 0.09; 95% CI, 0-1.08; $p=0.06$; Table 2) and Als treated female patients (0.21%; OR, 0.65; 95% CI, 0.22-3.19; $p=0.54$; Table 2).

Then we compared the COVID-19 hospitalization and mortality rates for female patients treated with the different hormone therapies with those expected from the regional rates. In tamoxifen female treated patients we observed only 14 hospital admissions for COVID-19 out of the 21 expected according to the regional risk matched by age suggesting that the risk of hospitalization in tamoxifen female treated patients was lower (SHR: 0.67; 95% CI, 0.61-1.32; Table 3). Conversely, none of the 4580 tamoxifen treated female BC patients died for COVID-19 while 5 were expected according to the regional mortality risk. Importantly, Als did not show a decreased hospitalization risk (SHR: 0.98; 95% CI, 0.81-1.12; Table 3) but a significantly lower mortality risk (SMR: 0.73; 95% CI, 0.45-0.90; Table 3) compared to the region. Fulvestrant-treated female BC patients showed a higher hospitalization rate for COVID-19 as compared to the region with 10 hospital admissions on 6 expected according to the regional risk adjusted by age (SHR: 1.67; 95% CI, 0.00-2.00; Table 3). Mortality by COVID-19 in the fulvestrant treated female cohort instead was as expected, with 2 fatalities observed based on the regional risk (SMR: 0.89; 95% CI, 0.00-1.92; Table 3).

Interestingly, also anti-HER2 female treated patients showed an increased risk of hospitalization (SHR: 2.50; 95% CI, 0.00-2.72) for COVID-19 with 8 hospital admissions on 3 expected according to the regional risk adjusted by age.

Table 1. COVID-19 hospitalization and mortality rates in patients under monotherapy in Emilia Romagna region from January 1st to December 31st, 2020. OR=odds ratio, 95% CI= 95% confidence interval. Statistically significant values are indicated in bold. The logistic model applied is described in the Methods.

COVID-19	Therapy	OR	95% CI	Count	COVID-19 hospitalization / death (%)	<i>p-value</i>
hospitalization	Anti-HER2	reference		705	11 (1.56)	
	Tamoxifen	0.26	0.13-0.56	4784	18 (0.38)	0.001
	Aromatase inhibitors	0.31	0.18-0.61	18533	156 (0.84)	0.001
	Fulvestrant	0.55	0.23-1.31	606	10 (1.65)	0.18
	Age	1.06	1.04-1.07			< 0.001
COVID-19 mortality	Anti-HER2	reference		705	2 (0.28)	
	Tamoxifen	0.03	0.00-0.34	4784	0 (0)	0.005
	Aromatase inhibitors	0.23	0.07-1.14	18533	39 (0.21)	0.07
	Fulvestrant	0.35	0.05-2.32	606	2 (0.33)	0.26
	Age	1.12	1.08-1.15			< 0.001

Table 2. COVID-19 hospitalization and mortality rates in female patients treated with a single hormone therapy in Emilia Romagna region from January 1st to December 31st, 2020. OR=odds ratio, 95% CI= 95% confidence interval. Statistically significant values are indicated in bold. The logistic model applied is described in the Methods.

COVID-19	Therapy	OR	95% CI	Count	COVID-19 hospitalization / death (%)	<i>p-value</i>
hospitalization	Fulvestrant	reference		601	10 (1.66)	
	Tamoxifen	0.41	0.18-0.94	4580	14 (0.31)	0.04
	Aromatase inhibitors	0.56	0.31-1.12	18425	155 (0.84)	0.10
	Age	1.06	1.05-1.07			< 0.001
COVID-19 mortality	Fulvestrant	reference		601	2 (0.33)	
	Tamoxifen	0.09	0.00-1.08	4580	0 (0)	0.06
	Aromatase inhibitors	0.65	0.22-3.19	18425	39 (0.21)	0.54
	Age	1.12	1.08-1.16			< 0.001

Table 3. COVID-19 hospitalization and mortality rates in female patients treated with a single hormone therapy in Emilia Romagna region from January 1st to December 31st, 2020 and compared to the regional risks. SHR=standard hospitalization ratio SMR=standard mortality ratio, 95% CI= 95% confidence interval, Exp.= expected number of cases. Statistically significant values are indicated in bold. The logistic model applied is described in the Methods.

Therapy	COVID-19 hospitalization				COVID-19 mortality			
	SHR	95% CI	Exp.	COVID-19 hospitalized (%)	SMR	95% CI	Exp.	COVID-19 deaths (%)
Tamoxifen	0.67	0.61-1.32	21	14 (0.31)	-	-	5	0 (0)
Aromatase inhibitors	0.98	0.81-1.12	158	155 (0.84)	0.73	0.45-0.90	54	39 (0.21)
Fulvestrant	1.67	0.00-2.00	6	10 (1.66)	0.89	0.00-1.92	2	2 (0.33)
Breast cancer patients cohort	0.97	0.83-1.11	-	179 (0.76%)	0.67	0.47-0.88	-	41 (0.17)

Next, we analyzed the distribution of hospitalization occurrence for COVID-19 normalized to drug half-life (HL), calculating the number of HL equivalents between the date of last treatment or drug administration and the hospitalization, to estimate the duration of protection or of susceptibility for each drug. Strikingly, 80% of hospitalizations were observed within the first HL since the last administration of fulvestrant (Figure 1) suggesting that a correlation between the plasmatic concentration of fulvestrant in BC patients and an increased hospitalization risk may exist. Conversely, the COVID-19 hospitalization rates increased over time after treatment discontinuation with tamoxifen and AIs showing respectively 50% and 85% of admissions occurring after the equivalent of 4 HLs (<6.25% putative drug blood concentration) (Figure 1) suggesting a fade of a protective action as drug concentration lowers.

Tamoxifen treatment protects human cells while fulvestrant makes them more vulnerable to SARS-CoV-2 infection in vitro

In order to gain insights into the mechanism of protection from SARS-CoV-2 infection mediated by tamoxifen and of increased susceptibility by fulvestrant, we performed in vitro experiments on human cells. Specifically, breast cancer cell lines expressing or not ER, MCF7 and MDA-MB-231 cells respectively, were selected as an *in vitro* model. First, cell susceptibility to SARS-CoV-2 was tested. Once assessed low levels of viral replication in both cell lines, we determined basal levels of *ACE2*, *TMPRSS2* and *NRP1* expression and whether 4-OH-tamoxifen (4-OHT), an active metabolite of tamoxifen, and fulvestrant affect the expression of those genes. In MCF7 cells, treatment with fulvestrant at 10nM and 1uM concentration significantly increased the expression of *ACE2* (fold change>1.8; p=0.025), *NRP1* (fold

change>4.7; p=0.015) and *TMPRSS2* (fold change>9.0; p=0.007) at 48 hours post treatment while 4-OH-T did not (Figure 2a). This effect correlates with ER expression since fulvestrant cannot modulate the expression of those genes in triple negative MDA-MB-231 cells (data not shown). Importantly, we tested whether 17 β -estradiol (E2) can modulate *ACE2*, *NRP1* and *TMPRSS2* expression in MCF7 cells at physiological concentrations corresponding to pre and postmenopausal ranges (30-400 pg/ml = 0.11-1.47nM; <15 pg/ml = <0.055nM), respectively, and found both *NRP1* and *TMPRSS2* genes repressed at premenopausal concentrations while not significantly affected below 0.05nM E2 (Figure 2b). This suggests that the decline in E2 concentration during aging may promote their expression in tissues. Importantly, E2 treatment does not alter the expression of *ACE2* while fulvestrant treatment does, suggesting that fulvestrant may exert its action also through estrogen-independent ER mechanisms. Then, we tested whether 4-OHT and fulvestrant affect cell susceptibility to SARS-CoV-2 infection. MCF7 cells, that in our conditions are largely refractory to infection, were pretreated for 72hr with 4-OHT, fulvestrant or vehicle, re-plated at the same concentration, infected with SARS-CoV-2 and cultured in the presence of the drugs. At 48hr from infection, we measured viral RNA amount inside cells (Figure 2c) and in the culture supernatant (Figure 2d) by Q-PCR and found that upon fulvestrant treatment, the quantity of viral RNA was increased about 24 times inside cells and about 4 times in the supernatant consistent with an altered susceptibility to SARS-CoV-2 infection as expected by *ACE2*, *TMPRSS2* and *NRP1* overexpression. Conversely, 4-OHT pretreatment decreased the amount of viral RNA of about 2 fold inside cells and 4.2 fold in the supernatant (p=0.002) suggesting that tamoxifen interferes with SARS-CoV-2 replication. Coherently, infectious particles could be retrieved only from the supernatant of fulvestrant-treated MCF7 cells and not from the 4-OHT- or vehicle-treated cells (Figure 2e).

Discussion

Hospitalization for COVID-19 is motivated by breathing difficulties, extreme weakness, loss of appetite, diarrhea, dizziness, confusion or a sudden change in mental state especially in older adults. The worst cases manifest with serious respiratory, cardiovascular insufficiency necessitating intensive care or other life threatening complications that lead to death or a severely compromised health status especially in elderly patients. Recent studies have shown that severe COVID-19 symptomatology is related to an overwhelming production of cytokines in a process called cytokine release syndrome (CRS) where, especially IL-6, plays a central role [20]. Our study, based on real world clinical and epidemiological data, assessed the rate of hospitalization and mortality for COVID-19 in BC patients and provides evidence that tamoxifen and, to some extent Als, can protect from COVID-19 mortality. Interestingly, fulvestrant and Als did not provide the same protection despite they both interfere with estrogen signaling albeit with different mechanisms. In particular, fulvestrant leads to ER degradation in cells thereby preventing both estrogen dependent and independent ER signaling while Als hinder estrogen production from androgens by inhibiting CYP19 aromatase enzymatic activity. This difference is mirrored also at the molecular level where fulvestrant treatment in ER expressing MCF7 cells is able to significantly increase the expression of 3 genes involved in SARS-CoV-2 susceptibility (*ACE2*, *NRP1*, *TMPRSS2*) while E2 treatment can only repress *NRP1* and *TMPRSS2* but not *ACE2*, the first driver of SARS-CoV-2 infectivity. Als efficiently

decrease E2 below 5pM concentration in 85% of postmenopausal patients [21]. However, at this very low E2 concentration as well as at postmenopausal levels (<50pM) neither *NRP1* nor *TMPRSS2* genes are significantly repressed in MCF7 cells suggesting that, if the same holds true in patients, Als treatment should not increase further the expression of those genes in post menopausal patients where Als are principally used. On the other hand such a low estrogen concentration in women post menopause and treated with Als could divert farther the equilibrium from estrogen dependent to independent ER signaling resulting in *ACE2* repression. Conversely, typical premenopausal E2 concentrations (>100pM) significantly downregulated *NRP1* and *TMPRSS2* in MCF7 cells suggesting that women in their fertile age may express lower levels of both genes in tissues expressing ERs and possibly being less susceptible as compared to women at the postmenopausal stage. Fulvestrant and Als discrepancies suggest that the former may increase the susceptibility to SARS-CoV-2 infection by removing also an estrogen-independent repression mediated by ER. In addition, fulvestrant treatment exerts a stronger inductive effect as compared to the absence of E2 in MCF7 cells in culture. Interestingly, ER signaling outcome is influenced by the expression of co-regulators (cofactors and corepressors) at the single cell level and by the activation signals it perceives either via estrogens or estrogen-independent cues [22,23]. It has been shown that growth factor receptor signaling such as epidermal growth factor 1, insulin-like growth factor receptors [24] and epidermal growth factor receptor 2 (HER2) [25] can lead to: ER phosphorylation through the action of downstream kinases [26], estrogen insensitivity and to hormonal therapy resistance in breast cancer [27]. ERs are in equilibrium between estrogen responsive and non-responsive forms in tissues and specific external cues and conditions such as the presence of estrogens and growth factors, drugs interfering with their signaling and specific pathological conditions (i.e. diabetes, obesity) can alter this equilibrium. Interestingly, the COVID-19 mortality risk is especially high in patients with type 1 diabetes (T1D) that are characterized by extremely low physiological concentrations of insulin and reduced IGF-1 [28]. It is conceivable that those patients with low IGF1 plasmatic levels may be at increased risk for COVID-19 complications and mortality due to a reduced repression of COVID-19 susceptibility genes by poor estrogen-independent ER signaling. Coherently with a view where receptor tyrosine kinases (RTKs) and ER crosstalk to prevent *ACE2*, *NRP1* and *TMPRSS2* expression, we observed that anti-HER2 treated BC patients, who are likely impaired in HER2 signaling at systemic level, and not only in breast cancer tissue, showed an increased risk of COVID-19 hospitalization and mortality. In this subset of BC patients the activity of anti-HER2 therapies may similarly prevent ER phosphorylation, activation and consequently induce SARS-CoV-2 susceptibility genes. Fulvestrant leads to ER degradation uncovering the net effect of estrogen dependent and independent signaling pathways in the repression of SARS-CoV-2 susceptibility genes. This action cannot be recapitulated by Als or by tamoxifen alone since they can affect only the estrogen dependent pathway (Figure 3). Moreover, the observation that 80% of COVID-19 hospitalizations occurred within the duration of one drug HL equivalent after last treatment in fulvestrant-treated patients further suggests that high fulvestrant plasmatic concentration is linked to higher risk of hospitalization for COVID-19. Epidemiological data are supported by *in vitro* experiments showing that 4-OHT decreased SARS-CoV-2 infectivity in MCF7 cells, while fulvestrant increased the susceptibility to SARS-CoV-2 infection and, consequently, the production of viral particles. Those results deserve special attention from a clinical standpoint because fulvestrant may expose already fragile

individuals to unwanted increased health risks at the time of COVID-19 pandemics. Moreover our results are coherent with other studies showing that tamoxifen and toremifene [29-31], and related SERMs like raloxifene and bazedoxifene [30], reduce SARS-CoV-2 infectivity in monkey Vero E6 cells. Interestingly, while increased susceptibility mediated by fulvestrant correlates with the transcriptional upregulation of SARS-CoV-2 susceptibility genes, 4-OHT treatment does not change their expression in MCF7 cells suggesting that tamoxifen protects most likely through the interference with other steps of SARS-CoV-2 life cycle. Recently, two articles have shown that the SIGMA-1 receptor located in the endoplasmic reticulum plays an important role in SARS-CoV-2 replication using a comparative viral-human protein-protein interaction map [29]. Knockout and knockdown of *SIGMAR1* gene caused robust reductions in SARS-CoV-2 production identifying SIGMAR1 as a key therapeutic target for SARS-CoV-2 replication [32]. Drug repurposing screens to select agents with anti-SARS-CoV-2 activity identified many sigma receptors (Sig-Rs) ligands [31]. Tamoxifen is a Sig-R ligand [34,35] and was retrieved together with related molecules (i.e. toremifene) in screens for antiviral compounds against RNA viruses (i.e. HCV, SARS-CoV, MERS-CoV, EBOV) suggesting it may block the early steps of the viral replication cycle [36-39]. In addition, tamoxifen alters endosomal trafficking and increases the pH of endolysosomes [40] thereby hindering protease mediated membrane fusion events. Besides host cell-specific effects, tamoxifen treatment systemically doubles the sex hormone-binding globulin (SHBG) concentration in BC patients [41]. SHBG is produced by the liver and binds to testosterone, dihydrotestosterone (DHT) and E2 in the blood regulating sex hormones availability to cells. Tamoxifen may therefore exert its anti-androgen effect in vivo also by altering sex hormones bioavailability in females. Our results suggest a model where tamoxifen tackles SARS-CoV-2 infection at different stages of its life cycle and in multiple ways at once (Figure 3). Sex hormones balance is known to influence key cell functions. Our experiments indeed highlight the role of E2 at premenopausal concentrations in restraining the expression of *TMPRSS2* and *NRP1* in human cells suggesting that E2 protection from severe COVID-19 in fertile females exists, in part may be due to this regulation and progressively diminishes with menopause onset. In addition, E2 concentration critically affects pro versus anti-inflammatory responses in humans and animals. High E2 concentrations (periovular to pregnancy levels) produce immune suppressive actions while the opposite occur for low E2 concentrations with the production of proinflammatory cytokines (IL-1, IL-6, TNF- α , IL-4, IL-10 and IFN) through NF- κ B regulation [42,43]. Acute respiratory distress syndrome (ARDS), a severe complication in COVID-19, has been investigated in experimental studies and in agreement with our model a protective role of E2 in preventing systemic and pulmonary release of cytokines upon acute lung injury [44], viral infection with H1N1 [45] and SARS-CoV-1 [11] has been documented. Interestingly, estrogens are known repressors of IL-6 and are used to treat osteoporosis in women [46]. Similarly, E2 treatment inhibits IL-6 expression in MCF7 cells and tamoxifen treatment does not interfere with this repression. If the same regulation takes place in patients, the putative protective effect of E2 in restraining IL-6 production from cells should not be hindered by tamoxifen therapy. Therefore, premenopausal BC patients treated with tamoxifen may be characterized by a triple protection mediated by estrogens, by estrogen independent mechanisms (ER phosphorylation and activation) and last by the pleiotropic anti-viral effects of tamoxifen.

The conclusions of our study on the outcome of COVID-19 hospitalization and mortality are supported by observations on a very large sample size of BC patients and are also compared with the whole population of Emilia Romagna region across 2020. In addition those data are coherent with *in vitro* experiments on the expression of SARS-CoV-2 susceptibility genes and virus infectivity. We emphasize that we tested the effects of 4-OHT and fulvestrant at their therapeutic concentrations and demonstrated that they can significantly influence the susceptibility of human ER expressing MCF7 cells to SARS-CoV-2 infection. Our results envision a therapeutic use of tamoxifen in infected people to restrain the severity of the symptoms, COVID-19 mortality and the vicious circle induced by SARS-CoV-2 infection on ACE2 and TMPRSS2 expression [47]. Potentially also a prophylactic use could reduce the risk of infection and spread of the disease. A shortcoming of the study is that we cannot distinguish whether a reduced rate of hospitalization and mortality for COVID-19 in tamoxifen treated patients is due to a decreased rate of infection, whether it is due to the mitigation of symptoms or both. However, in a recently published letter [48], the authors analyzed a small number of BC patients (926) for a short period of time (2 months) and suggested what we have first envisioned [49] on SERMs activity in the prevention of SARS-CoV-2 transmission by the modulation of SARS-CoV-2 susceptibility genes, the latter we have documented here. Our findings may inform future measures and treatment options for cancer patients and warrant an investigation into the prophylactic properties of tamoxifen in healthy individuals.

References

1. Hoffmann M, Kleine-Weber H, Schroeder S et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; 181(2):271–280.e8.
2. Matsuyama S, Nao N, Shirato K et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. *Proc Natl Acad Sci USA* 2020; 117(13):7001.
3. Cantuti-Castelvetri L, Ojha R, Pedro LD et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* 2020; 370(6518):856–860.
4. Daly JL, Simonetti B, Klein K et al. Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science* 2020; 370(6518):861.
5. Clinckemalie L, Spans L, Dubois V et al. Androgen regulation of the TMPRSS2 gene and the effect of a SNP in an androgen response element. *Mol. Endocrinol.* 2013; 27(12):2028–2040.
6. Qiao Y, Wang X-M, Mannan R et al. Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and TMPRSS2. *Proc. Natl. Acad. Sci. U.S.A.* 2021. doi:10.1073/pnas.2021450118.
7. Lazzeri M, Duga S, Azzolini E et al. Impact of anti-androgen therapies on COVID-19 susceptibility: a case-control study in male population from two COVID-19 regional centers of Lombardy (Italy). *medRxiv* 2020:2020.04.20.20068056.
8. Marik PE, DePerrior SE, Ahmad Q, Dodani S. Gender-based disparities in COVID-19 patient outcomes. *J Investig Med* 2021. doi:10.1136/jim-2020-001641.
9. Josa-Laorden C, Crestelo-Vieitez A, García Andreu MDM et al. Gender-Based Differences by Age Range in Patients Hospitalized with COVID-19: A Spanish Observational Cohort Study. *J Clin Med*

2021. doi:10.3390/jcm10050899.

10. Karlberg J, Chong DSY, Lai WYY. Do men have a higher case fatality rate of severe acute respiratory syndrome than women do? *Am J Epidemiol* 2004; 159(3):229–231.
11. Channappanavar R, Fett C, Mack M et al. Sex-Based Differences in Susceptibility to Severe Acute Respiratory Syndrome Coronavirus Infection. *J. Immunol.* 2017; 198(10):4046.
12. Bogush TA, Polezhaev BB, Mamichev IA et al. Tamoxifen Never Ceases to Amaze: New Findings on Non-Estrogen Receptor Molecular Targets and Mediated Effects. *Cancer Invest.* 2018; 36(4):211–220.
13. Dudley JP, Lee NT. Disparities in Age-specific Morbidity and Mortality From SARS-CoV-2 in China and the Republic of Korea. *Clin Infect Dis* 2020; 71(15):863–865.
14. Robertson JFR, Erikstein B, Osborne KC et al. Pharmacokinetic profile of intramuscular fulvestrant in advanced breast cancer. *Clin Pharmacokinet* 2004; 43(8):529–538.
15. Sanchez-Spitman A, Dezentjé V, Swen J et al. Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. *JCO* 2019; 37(8):636–646.
16. Corman VM, Landt O, Kaiser M et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 2020. doi:10.2807/1560-7917.ES.2020.25.3.2000045.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} Method. *Methods* 2001; 25(4):402–408.
18. van den Worm SHE, Eriksson KK, Zevenhoven JC et al. Reverse genetics of SARS-related coronavirus using vaccinia virus-based recombination. *PLoS ONE* 2012; 7(3):e32857.
19. Lønning P, Pfister C, Martoni A, Zamagni C. Pharmacokinetics of third-generation aromatase inhibitors. *Semin. Oncol.* 2003; 30(4 Suppl 14):23–32.
20. Leisman DE, Ronner L, Pinotti R et al. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. *The Lancet Respiratory* 2020; 8(12):1233–1244.
21. Faltinová M, Vehmanen L, Lyytinen H et al. Monitoring serum estradiol levels in breast cancer patients during extended adjuvant letrozole treatment after five years of tamoxifen: a prospective trial. *Breast Cancer Res Treat* 2021. doi:10.1007/s10549-021-06168-w.
22. Fuentes N, Silveyra P. Estrogen receptor signaling mechanisms. *Adv Protein Chem Struct Biol* 2019; 116:135–170.
23. Bennesch MA, Picard D. Minireview: Tipping the balance: ligand-independent activation of steroid receptors. *Mol. Endocrinol.* 2015; 29(3):349–363.
24. Klotz DM, Hewitt SC, Ciana P et al. Requirement of estrogen receptor-alpha in insulin-like growth factor-1 (IGF-1)-induced uterine responses and in vivo evidence for IGF-1/estrogen receptor cross-talk. *J. Biol. Chem.* 2002; 277(10):8531–8537.
25. Giuliano M, Trivedi MV, Schiff R. Bidirectional Crosstalk between the Estrogen Receptor and Human Epidermal Growth Factor Receptor 2 Signaling Pathways in Breast Cancer: Molecular Basis and

- Clinical Implications. *Breast Care (Basel)* 2013; 8(4):256–262.
26. Nilsson S, Mäkelä S, Treuter E et al. Mechanisms of estrogen action. *Physiol Rev* 2001; 81(4):1535–1565.
 27. Pietras RJ, Arboleda J, Reese DM et al. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. *Oncogene* 1995; 10(12):2435–2446.
 28. Teppala S, Shankar A. Association between serum IGF-1 and diabetes among U.S. adults. *Diabetes Care* 2010; 33(10):2257–2259.
 29. Daniloski Z, Jordan TX, Wessels H-H et al. Identification of Required Host Factors for SARS- CoV-2 Infection in Human Cells. *Cell* 2021; 184(1):92–105.e16.
 30. Jeon S, Ko M, Lee J et al. Identification of Antiviral Drug Candidates against SARS-CoV-2 from FDA-Approved Drugs. *Antimicrob Agents Chemother* 2020; 64(7):e00819–20.
 31. Weston S, Coleman CM, Haupt R et al. Broad Anti-coronavirus Activity of Food and Drug Administration-Approved Drugs against SARS-CoV-2 *In Vitro* and SARS-CoV *In Vivo*. *J. Virol.* 2020; 94(21):e01218–20.
 32. Gordon DE, Jang GM, Bouhaddou M et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* 2020; 583(7816):459–468.
 33. Vela JM. Repurposing Sigma-1 Receptor Ligands for COVID-19 Therapy? *Front Pharmacol* 2020; 11:582310.
 34. Kedjouar B, Daunes S, Vilner BJ et al. Structural similitudes between cytotoxic antiestrogen-binding site (AEBS) ligands and cytotoxic sigma receptor ligands. Evidence for a relationship between cytotoxicity and affinity for AEBS or sigma-2 receptor but not for sigma-1 receptor. *Biochem. Pharmacol.* 1999; 58(12):1927–1939.
 35. Moebius FF, Reiter RJ, Hanner M, Glossmann H. High affinity of sigma 1-binding sites for sterol isomerization inhibitors: evidence for a pharmacological relationship with the yeast sterol C8-C7 isomerase. *Br. J. Pharmacol.* 1997; 121(1):1–6.
 36. Gastaminza P, Whitten-Bauer C, Chisari FV. Unbiased probing of the entire hepatitis C virus life cycle identifies clinical compounds that target multiple aspects of the infection. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107(1):291–296.
 37. Mingorance L, Friesland M, Coto-Llerena M et al. Selective inhibition of hepatitis C virus infection by hydroxyzine and benztropine. *Antimicrob Agents Chemother* 2014; 58(6):3451–3460.
 38. Dyall J, Coleman CM, Hart BJ et al. Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. *Antimicrob Agents Chemother* 2014; 58(8):4885–4893.
 39. Johansen LM, Brannan JM, Delos SE et al. FDA-approved selective estrogen receptor modulators inhibit Ebola virus infection. *Sci Transl Med* 2013; 5(190):190ra79.

40. Altan N, Chen Y, Schindler M, Simon SM. Tamoxifen inhibits acidification in cells independent of the estrogen receptor. *Proc. Natl. Acad. Sci. U.S.A.* 1999; 96(8):4432–4437.
41. Jordan VC. Targeted Hormone Therapy for Breast Cancer. *Hospital Practice* 1993; 28(3):55–62.
42. Galien R, Garcia T. Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF-kappaB site. *Nucleic Acids Res.* 1997; 25(12):2424–2429.
43. Kurebayashi S, Miyashita Y, Hirose T et al. Characterization of mechanisms of interleukin-6 gene repression by estrogen receptor. *J Steroid Biochem Mol Biol* 1997; 60(1-2):11–17.
44. Fantozzi ET, Breithaupt-Faloppa AC, Ricardo-da-Silva FY et al. Estradiol mediates the long-lasting lung inflammation induced by intestinal ischemia and reperfusion. *Journal of Surgical Research* 2018; 221:1–7.
45. Vermillion MS, Ursin RL, Attreed SE, Klein SL. Estriol Reduces Pulmonary Immune Cell Recruitment and Inflammation to Protect Female Mice From Severe Influenza. *Endocrinology* 2018; 159(9):3306–3320.
46. Ray P, Ghosh SK, Zhang DH, Ray A. Repression of interleukin-6 gene expression by 17 beta-estradiol: inhibition of the DNA-binding activity of the transcription factors NF-IL6 and NF-kappa B by the estrogen receptor. *FEBS Lett.* 1997; 409(1):79–85.
47. Lemes RMR, Costa AJ, Bartolomeo CS et al. 17 β -estradiol reduces SARS-CoV-2 infection in vitro. *Physiological Reports* 2021; 9(2):e14707.
48. Montopoli M, Zorzi M, Cocetta V et al. Clinical outcome of SARS-CoV-2 infection in breast and ovarian cancer patients who underwent antiestrogenic therapy. *annonc* 2021. doi:10.1016/j.annonc.2021.01.069.
49. Bravaccini S, Fonzi E, Tebaldi M et al. Estrogen and Androgen Receptor Inhibitors: Unexpected Allies in the Fight Against COVID-19. *Cell Transplant* 2021; 30:0963689721991477.

Declarations

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Authors' Contribution

Conceptualization: SB, MM, GM

Methodology: SB, FN, WB, IA, PP, MA, FF, MTM, ON, SR, FP, MMT, MC, TI, MM

Investigation: FN, CP, AC, AV, MAB

Visualization: FN, LM, AG, IA, SB, MM

Funding acquisition: CP

Project administration: MM, SB, MMT, GM

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Writing – original draft: MM, SB, IA, WB, FN

Writing – review & editing: CP, AC, GM, SB, RM, MZ, CC, VS, MM

Competing interests

GM has competing interests with Novartis, BMS, Roche, Pfizer, ARIAD, and MSD that are not related to the present study. All the other authors have no conflicts of interest to declare.

Ethical Approval

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

Data and materials availability

All data and materials used in the analysis are available upon request. Materials transfer agreements (MTAs) will be issued if required.

Figures

Figure 1

Hospitalization rates expressed as percentage of all COVID-19 hospitalizations occurred since the last drug administration. In order to compare different treatments, the time from the last drug administration is expressed as drug half live equivalents. In particular, 50 days HL for fulvestrant¹⁴, 7 days HL for tamoxifen¹⁵, 2 days HL for Als¹⁹ were considered.

Figure 2

Gene expression and SARS-CoV-2 infection analyses on MCF7 cells treated with 4-OHT, fulvestrant and 17 β -estradiol (E2). (a) Expression analysis for ACE2, NRP1, TMPRSS2 in human MCF7 cells treated with 4-OHT and fulvestrant at 10nM and 1uM concentration and (b) with increasing concentrations of E2 as indicated. Concentration of E2 ≤ 0.05 nM is characteristic of post menopausal women. mRNA expression analysis for target genes was performed by Q-PCR, normalized on HPRT reference expression and represented as fold change compared to expression in vehicle treated cells. SARS-CoV-2 N gene expression inside cells (c) and in the supernatant (d) of SARS-CoV-2 infected MCF7 cells. (e) SARS-CoV-2 titer from the supernatant of MCF7 cells treated with vehicle, 4-OHT and fulvestrant measured on Vero E6 cells and expressed as plaque forming units (PFUs)/ml. N.D. = not detectable. Statistical significance was assessed by t-test (* = p-value<0.05; ** = p-value<0.01)

Figure 3

Schematic representation of hormonal therapies actions at the cell and systemic level