scientific reports

Check for updates

OPEN

First real-world evidence from the Emilia-Romagna region suggests tamoxifen protects breast cancer patients from COVID-19

Sara Bravaccini[2,10], Fabio Nicolini[3], William Balzi[4], Irene Azzali[5], Luigi Pasini[3], Lucia Mazzotti[3], Anna Gaimari[3], Anna De Lucia[3], Matteo Zurlo[3], Roberta Maltoni[4], Francesca Pirini[1], Michela Cortesi[1], Michele Zanoni[1], Maria Maddalena Tumedei[3], Oriana Nanni[5], Claudio Cerchione[6], Vittorio Sambri[7,8], Patricia Borges de Souza[3], Giovanni Martinelli[9] & Massimiliano Mazza[3,10]✉

COVID-19 severity is uneven between genders. A role for sex hormones is possible and has been postulated. Considering this idea, we hypothesized hormonal therapies might influence the severity of COVID-19 in breast cancer (BC) patients. We mined the Emilia-Romagna region (Italy) registries to compare the rates of hospitalization and mortality by COVID-19 in 2020 amongst 22,987 BC patients treated with tamoxifen, aromatase inhibitors (AIs), fulvestrant, and anti-HER2 therapies (trastuzumab and pertuzumab) in 2020, the latter used as reference group. The hospitalization rate observed in 4719 tamoxifen-treated BC patients was the lowest (0.61%, OR, 0.38; 90% CI, 0.20–0.76; $p = 0.03$) among hormonal therapies. Importantly, only one COVID-19 fatality was observed in tamoxifen-treated BC patients, who showed a striking 0.02% COVID-19 death frequency as compared to 0.24% observed for the whole BC patients population. In addition, tamoxifen emerged in the sex and age adjusted analysis as the only agent significantly decreasing the standard mortality rate (SMR) by COVID-19 as compared to the regional population consisting of 2,296,559 female residents from the Emilia Romagna region in 2020. Our results show that BC patients are at increased risk of COVID-19 hospitalization and that COVID-19 mortality is hindered by SERM-based therapies like tamoxifen. These findings are relevant to choose adequate treatments in order to protect cancer patients from concomitant SARS-CoV-2 contagion and related symptoms and contribute to the idea that SERMs could be used as prophylactic agents and therapeutics to prevent the progression of severity of COVID-19.

Keywords COVID-19 severity, Breast cancer, Tamoxifen, Fulvestrant, Aromatase inhibitors, COVID-19 protection

Abbreviations

Ais	Aromatase Inhibitors
BC	Breast Cancer
CRS	Cytokine Release Syndrome
DHT	Dihydrotestosterone
HER2	Human Epidermal Growth Factor Receptor 2

[1]Biosciences Laboratory, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy. [2]Department of Medicine and Surgery, University of Enna "Kore", 94100 Enna, Italy. [3]Advanced Cellular Therapies and Rare Tumors Unit, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy. [4]Healthcare Administration, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy. [5]Unit of Biostatistics and Clinical Trials, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy. [6]Hematology Unit, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy. [7]Unit of Microbiology, The Great Romagna Area Hub Laboratory, Cesena, Italy. [8]Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy. [9]Scientific Directorate, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy. [10]Sara Bravaccini and Massimiliano Mazza contributed equally to this work. ✉email: massimiliano.mazza@irst.emr.it

ER	Estrogen Receptor
HL	Half Life
OR	Odds Ratios
SERD	Estrogen Receptor Degrader
SERM	Selective Estrogen Receptor Modulator
SHBG	Sex hormone-binding globulin
T1D	Type 1 Diabetes
TMPRSS2	Transmembrane Protease Serine 2

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[3], neuropilin-1 (NRP1) has been recently characterized as a third SARS-CoV-2 susceptibility factor and co-receptor influencing viral tropism and infectivity[4,5]. Importantly, SARS-COV-2 has been reported to induce DNA damage in infected cells through a coordinated action of several viral proteins[6], while the spike was found to interact with human estrogen receptor (ER) and to alter estrogen-dependent signaling by maintaining ERa in the cytoplasm in alveolar macrophages assessed in SARS-CoV-2 infected human and murine specimens[7]. The impact of SARS-CoV-2 as a mutational agent during COVID-19 pandemic is not clear yet. However, it is conceivable that it will impact negatively on tumor incidence worldwide in the future. Therefore, it is of utmost importance to provide the population with measures that prevent cell infection and consequently restrain the potential for mutational impact caused by the infection. Besides a direct effect on the genomic mutation rate in infected cells, health systems are witnessing a significant increase in the diagnosis of tumors at more advanced stages related to the delay in tumor diagnosis during the pandemics[8–11], although there are notable exceptions as reported by Mangone and colleagues that for example did not observe any change in breast cancer diagnosis after a 3-month cessation of mammographic screening[12]. Therefore, COVID-19 and tumor biology are intertwined at multiple levels. In particular, sex hormones have been shown to influence the expression of COVID-19 susceptibility genes such as TMPRSS2[13–15] and ACE2[16], although to a different extent and way according to the tissue examined. Hence, hormone concentration can influence the tropism of SARS-CoV-2 in vivo towards different tissues and organs, thereby modifying gender-related COVID-19 symptomatology and mutational impact.

However, the role of hormonal therapies is controversial. For example, some authors reported tamoxifen and clomiphene as inhibiting the infectivity of the virus in vitro and in preclinical models[17]. Accordingly, breast cancer patients treated with anti-estrogenic hormone therapy have been reported to be protected from infection[18]. In addition, raloxifene, an analogue molecule of tamoxifen, showed protective effects against COVID-19 in clinical trials[19,20]. On the contrary, other studies reported that estrogens exert a protective role in terms of COVID-19 severity. Accordingly, Wang et al.. reported clinical observations where high expression of estrogen appears to protect patients from SARS-CoV-2 infection and related mortality[21]. These results suggest estrogens play a protective role in the pathophysiology of COVID-19. Similarly, estrogen-based hormone replacement therapy seems to protect against serious adverse events of COVID-19[22,23].

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[24]. Fulvestrant is a strict selective ER Degrader (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 the Emilia-Romagna region (Italy) in 2020 in order to have homogeneous and consistent information from the beginning of the pandemic ruling out the confounding factor of COVID-19 vaccinations to evaluate whether the rates of hospitalization and of mortality for COVID-19 differed according to therapy in BC patients.

Results

In order to investigate if hormonal therapies influence COVID-19 susceptibility and the severity of symptoms, we mined real-world clinical data from the BC population of the Emilia-Romagna region assessing the frequency of hospitalization and death for COVID-19 in patients treated with tamoxifen, AIs, fulvestrant (+/- CDK4/6 inhibitors) and anti-HER2 therapies (trastuzumab and pertuzumab) in 2020, the latter used as non-hormonal therapy treated BC patients as control group (Supplementary Table S1). Logistic models were applied to compare the odds of 1-year COVID-19 hospitalization and mortality between treatment groups, adjusting for age, sex, Charlson Comorbidity Index (CCI), metastasis, and previous treatments. Out of a total of 22,987 BC patients (22662 females, 325 males), 254 (249 females, 5 males) were hospitalized for COVID-19 (1.10%) while the overall rate of hospitalization for COVID-19 observed for residents in Emilia-Romagna during 2020 was 0.57% (25448/4474292 residents). As expected, age was a significant determinant of hospitalization (OR, 1.05; 90% CI, 1.04 to 1.07; $p < 0.001$) and death (OR, 1.13; 90% CI, 1.10 to 1.16; $p < 0.001$) for COVID-19 in the BC patient cohort. However, the most relevant factor associated with COVID-19 mortality was the presence of metastatic disease (OR, 2.75; 90% CI, 1.64–4.50; $p = 0.002$; Table 1). Concerning therapy groups, the model revealed a significant decrease in the hospitalization rate for tamoxifen-treated patients (0.61%; OR, 0.38; 90%

	Therapies	OR	90% CI	Count	COVID-19 hospitalization/death (%)	*p*-value
COVID-19 hospitalization	Anti-HER2	reference		409	10 (2.44)	
	Tamoxifen	**0.38**	**0.20–0.76**	**4719**	**29 (0.61)**	**0.03**
	Aromatase inhibitors	**0.35**	**0.20–0.68**	**17,365**	**206 (1.19)**	**0.01**
	Fulvestrant (± CDK4/6 (inhibitors)	0.47	0.21–1.07	494	9 (1.82)	0.13
	Age	**1.05**	**1.04–1.07**			**< 0.001**
	Males	1.17	0.51–2.30			0.73
	High CCI	1.35	1.00–1.82.00.82			0.10
	Metastasis	1.10	0.81–1.48			0.60
	Previous treatment	0.78	0.62–1.00.62.00			0.11
COVID-19 mortality	Anti-HER2	reference		409	2 (0.49)	
	Tamoxifen	**0.07**	**0.01–0.37**	**4719**	**1 (0.02)**	**0.01**
	Aromatase inhibitors	*0.22*	*0.08–0.84*	*17,365*	*50 (0.29)*	*0.07*
	Fulvestrant (± CDK4/6 inhibitors)	0.35	0.08–1.60	494	3 (0.61)	0.24
	Age	**1.13**	**1.10–1.16**			**< 0.001**
	Males	1.92	0.32–6.50			0.49
	High CCI	1.31	0.59–3.24			1.00
	Metastasis	**2.75**	**1.64–4.50**			**0.002**
	Previous treatment	0.82	0.50–1.38			0.52

Table 1. **Clinical determinants associated with COVID–19 mortality.** COVID-19 hospitalization and mortality rates in patients under monotherapy in Emilia-Romagna region from January 1 st to December 31 st, 2020. Anti-HER2 therapy is designed as the standard of reference to compare the effect of the other therapies. Statistically significant values are indicated in bold. Association of the aromatase inhibitor therapy with COVID-19 mortality is indicated as close to significance (italic). The logistic model applied is described in the Methods. OR, odds ratio; 90% CI, 90% confidence interval; CDK, cyclin-dependent kinase.

	COVID-19 hospitalization				COVID-19 mortality			
Therapies	SHR	90% CI	Exp.	COVID-19 hospitalized (%)	SMR	90% CI	Exp.	COVID-19 deaths (%)
Tamoxifen	1.26	0.88–1.76	21	26 (0.58)	**0.21**	**0.01–0.98**	**5**	**1 (0.02)**
Aromatase inhibitors	**1.36**	**1.21–1.52**	**151**	**205 (1.19)**	0.96	0.75–1.23	52	50 (0.29)
Fulvestrant (± CDK4/6 inhibitors)	1.74	0.91–2.31	5	9 (1.84)	1.52	0.41–3.92	2	3 (0.61)
Breast cancer cohort	**1.36**	**1.22–1.51**	**176**	**240 (1.08)**	0.92	0.72–1.15	59	54 (0.24)

Table 2. **COVID-19 hospitalization and mortality rates in female patients treated with a single hormone therapy in Emilia-Romagna region.** Data were collected from January 1 st to December 31 st, 2020 and compared to the regional risks. Statistically significant values are indicated in bold. The logistic model applied is described in the Methods. SHR, standard hospitalization ratio; SMR, standard mortality ratio; 90% CI, 90% confidence interval; Exp., expected number of cases; CDK, cyclin-dependent kinase.

CI, 0.20–0.76; $p = 0.03$). A similar result was observed also for AIs treated patients (1.19%, OR, 0.35; 90% CI, 0.20–0.68; $p = 0.01$) while a 2.44% frequency for COVID-19 hospitalization was observed in the anti-HER-2 therapy reference group. The analysis of mortality for COVID-19 confirmed a significant protective role for tamoxifen (0.02%; OR, 0.07; 90% CI, 0.01–0.37; $p = 0.01$) and for AIs (0.29%; OR, 0.22; 90% CI, 0.08–0.84; $p = 0.07$), as compared to the anti-HER2 therapy reference group. Strikingly, only one COVID-19 fatality out of 4719 tamoxifen-treated patients occurred. To get more insight into COVID-19 hospitalization and mortality rates for BC patients treated with hormone therapies as compared to the whole female population resident in the same region (reference group), we compared 22,275 female BC patients with 2,296,559 female residents from the Emilia-Romagna region in 2020 (Supplementary Table S2). Table 2 indicates a significant increase in the Standard Hospitalization Ratio (SHR, 1.36; 90%-CI, 1.22–1.51) while a reduced but not significant Standard Mortality Ratio (SMR, 0.92; 90%-CI, 0.72–1.15) in the comparison matched by age for the whole breast cancer patient cohort as compared to the regional population. The deconvolution of the rates according to the different types of treatment highlights striking differences in the mortality by COVID-19. In particular, only 1 fatality was observed in the tamoxifen arm out of the 5 expected, considering the regional rates and the age of the patients during the same period. Tamoxifen-treated patients showed a striking 0.02% COVID-19 death frequency, as compared to 0.24% observed for the whole BC patients population. In addition, tamoxifen was the only agent

decreasing the risk of mortality compared to that of the control group (SMR: 0.21, 90%-CI, 0.01–0.98, Table 2) although this data will need to be confirmed in a larger cohort of patients. Our results support a model where estrogen signaling is important in protecting BC patients from COVID-19 severity and that tamoxifen can exert its protective role by different pleiotropic mechanisms of action, either related to ER signaling or not (Fig. 1).

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[25]. 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[26]. In addition, a recent revision of the studies examining the risk factors associated with disease severity showed that preexisting conditions like chronic obstructive pulmonary disease (COPD), cardiovascular disease, diabetes mellitus, hypertension, chronic kidney failure, cancer and a history of smoking are tightly and positively associated with a more severe course of COVID-19[27].

Considering the differential COVID-19 responses across genders for all these symptoms, our study aimed to verify through real-world clinical and epidemiological evidence whether hormonal treatments impact the severity of the illness in female BC patients. Hence, we assessed the rate of hospitalization and mortality for COVID-19 in BC patients, providing evidence that tamoxifen can actually protect from COVID-19 mortality. Tamoxifen is the first-line adjuvant endocrine therapy for premenopausal women with early-stage, hormone receptor–positive breast cancer, who present with a more favourable performance status and fewer comorbidities. On the opposite, patients treated with AIs - agents aimed to block peripheral estrogen synthesis - are typically post-menopausal and may have a higher baseline risk for severe COVID-19 due to age and associated comorbidities. Patients treated with fulvestrantare almost exclusively metastatic, which may contribute to increased susceptibility to the infection. It is clear, however, that different treatments influence patients' outcomes differently[28]. Although the risk of COVID-19 mortality increases with age in both sexes, there is a known disparity between males and females. For these reasons, and to minimize the confounding effect of age, age-adjusted analyses were conducted both for the comparisons within the BC population and between BC patients and the overall regional female population. Although the different endocrine therapies used in clinical practice may potentially introduce differences in baseline risk for COVID-19 complications, our results did not seem to be significantly affected by associated confounding factors. Fulvestrant and AIs that interfere with estrogen signaling albeit with different mechanisms did show an increased risk of hospitalization as compared to the regional female population, reinforcing the idea that estrogen signaling is important to restrain COVID-19 severity. In particular, fulvestrant leads to ER degradation in cells, thereby preventing both estrogen-dependent and independent ER signaling while AIs hinder estrogen production from androgens by inhibiting CYP19 aromatase enzymatic activity. ER signaling outcome is influenced by the expression of co-regulators (co-factors and co-repressors) at the single cell level and by the activation signals and post-translational modifications ER perceives, either via estrogens or estrogen-independent cues[29–31]. It has been shown that growth factor receptor signaling such as epidermal growth factor 1, insulin-like growth factor receptors[32] and human epidermal growth factor receptor 2 (HER2)[33] can lead to: ER phosphorylation through the action of downstream kinases[34], estrogen insensitivity and to hormonal therapy resistance in breast cancer[35]. 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. 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[36]. It is conceivable that those patients with low IGF1 plasmatic levels may be at increased risk for COVID-19 complications and mortality due to altered estrogen receptor signaling. In addition, the absence or the interference with ER phosphorylation could take place also in other pathological or pharmacological settings. For example, we observed that anti-HER2 treated BC patients, who are impaired in HER2 signaling at the systemic level, and not only in breast cancer tissue, showed an increased risk of COVID-19 hospitalization and mortality. It is then evident that the type of treatment impacts COVID-19 protection differently, probably due to additional pleiotropic effects. Importantly, tamoxifen, toremifene and bazedoxifene[37–39] reduce SARS-CoV-2 infectivity in monkey Vero E6 cells while the related SERM raloxifene, was demonstrated to benefit mild COVID-19 patients[20] as recently reported by Allegretti and colleagues[19,40]. Two studies 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[41]. 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[41]. Drug repurposing screens to select agents with anti-SARS-CoV-2 activity identified many sigma receptors (Sig-Rs) ligands[42]. Tamoxifen is a Sig-R ligand[42–44] 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[45–48]. In addition, tamoxifen alters endosomal trafficking and increases the pH of endolysosomes[49], 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[50]. SHBG is produced by the liver and binds to testosterone, dihydrotestosterone (DHT) and 17-β-estradiol (E2) in the blood, regulating sex hormones availability to cells. Therefore, tamoxifen may exert its anti-viral effect in vivo also by altering sex hormones bioavailability. Those results suggest that tamoxifen tackles SARS-CoV-2 infection in multiple ways at once and potentially at different stages of its life cycle, explaining its superiority in COVID-19

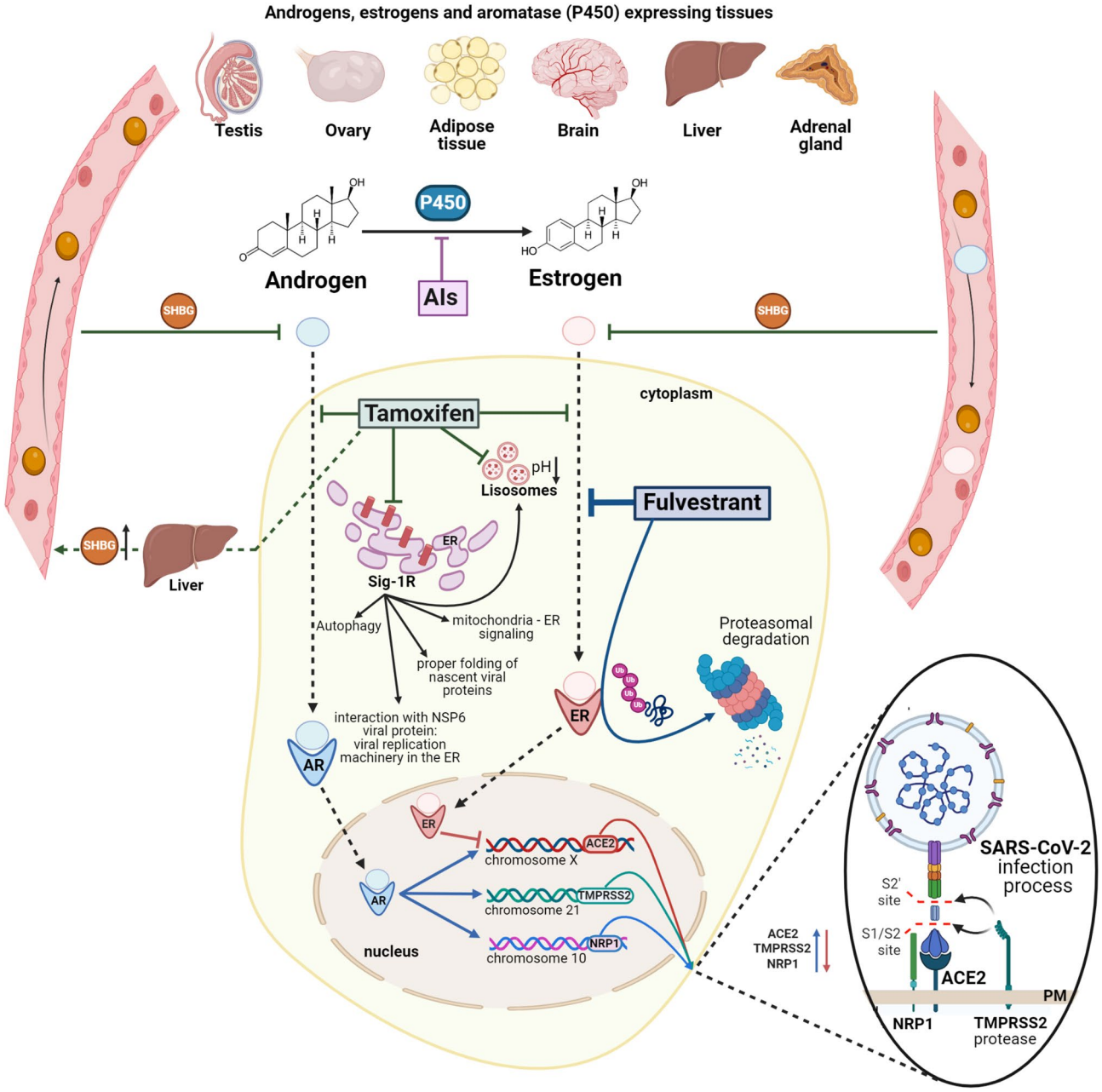

Fig. 1. **Schematic representation of hormonal therapy actions at cellular and systemic level**. Androgen and estrogen signaling can target multiple organs to regulate the expression of key coronavirus disease (COVID-19)-related genes, like the angiotensin-converting enzyme 2 (ACE2), the transmembrane protease, serine 2 (TMPRSS2), and the neuropilin-1 (NRP1) gene. While activation of the androgen receptor (AR) promotes the expression of these genes, facilitating the infection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), estrogen receptor (ER) can inhibit ACE2 expression, lowering the chances of the virus accessing target cells and possibly restraining COVID-19 severity. Breast cancer patients are mostly treated with hormonal therapies that include tamoxifen, fulvestrant and aromatase inhibitors (AIs). AIs block estrogens conversion from androgens by inhibiting the catalytic activity of aromatase, an enzyme from the cytochrome P450 group. Fulvestrant leads to ER degradation through the proteasome. Patients treated with AIs and fulvestrant, may exhibit an increased risk of hospitalization by COVID-19 because estrogens lowering and the impossibility of acting through the ER respectively hinder protection. On the opposite, tamoxifen can exert a protective role through various mechanisms, including agonism on ER signaling, impairment of lysosomal acidification, alteration of free sex hormones availability via increased production of the sex hormone binding globulin (SHBG) which would alter the expression of genes associated with COVID-19 susceptibility, binding and modulation of Sigma-1 receptor (Sig-1R) involved in several intracellular processes.

protection as compared to other treatments in the setting of hormone-positive BC patients. As mentioned above, we can envision several mechanisms of action for tamoxifen like: ER agonism[51], antagonism against spike by binding to ER[7,52], direct binding to the spike protein as shown for raloxifene[40], effects on receptor expression[53], potential effects on lysosome acidification[49] and on the bioavailability of sex hormones at the systemic level[50]. Patients receiving AIs exhibit greater COVID-19 hospitalization risk, consistent with estrogens' protective function, as various studies indicate[22,54]. Fulvestrant appears to be a factor promoting severity by COVID-19, although the results are not statistically significant due to the limited cohort size and the rarity of the events. The increase in COVID-19 severity could be due to fulvestrant mechanism of action, which leads to the degradation of the ER and therefore to the inability of endogenous estrogens to exert a protective role in BC treated patients[55]. We emphasise that the comparison of the age and sex-adjusted COVID-19 mortality risk between the regional female population and the tamoxifen-treated BC patients shows a lower risk for the latter, although BC patients are more fragile and susceptible to COVID-19 hospitalization (1.10% vs. 0.57%). This observation should prompt a discussion about the potential use of tamoxifen for a limited period in otherwise healthy individuals. However, we recognise several limitations in our study. First, the retrospective nature of the analysis introduced potential biases for patient selection and unmeasured confounding factors that may be associated with specific patient subgroups and, consequently, with the therapeutic decision. Second, we could not take into account disease stage and menopausal status for all the almost 23,000 BC patients in the database, due to data entry limitations Another shortcoming of the study is that we cannot determine whether a reduced rate of hospitalization and mortality for COVID-19 in tamoxifen-treated patients is whether due to a decreased rate of infection, or a mitigation of symptoms, or both. However, Montopoli and colleagues reported the analysis of a cohort of 926 BC patients for 2 months, indicating a decreased prevalence of SARS-CoV-2 infection in SERM-treated patients[18]. Data that can support the hypothesis that the decreased hospitalization and mortality observed in tamoxifen-treated patients is actually related to the infection rate. Still, we wish to highlight the strengths of our study, which examines the largest cohort of BC patients (N = 22,987) evaluated to date for the impact of COVID-19 severity, as measured by hospitalization and mortality rates in the Italian population across the 2020. To note, our cohort comprises hormone receptor-positive (luminal) BC patients, representing 70 to 80% of the whole BC population. Considering the incidence of this tumour subtype, our conclusions may apply to most BC patients. In addition, by comparing the SMR of BC patients treated with different drugs and that are characterised by an increased risk of COVID-19 severity, including the whole regional female population of about 2.3 million citizens, we showed that COVID-19 mortality in tamoxifen-treated BC patients is even lower than in the general population matched by age. It is vital to clarify the molecular regulation mechanisms of anti-hormonal therapies in disease models, identifying which SARS-CoV-2 replication stages are impeded and how cells are protected from infection. The emerging picture is that some patient groups may be particularly susceptible to SARS-CoV-2 infection and may benefit from SERM-based therapies, including tamoxifen. These findings may aid in selecting treatments to safeguard cancer patients from SARS-CoV-2 infection and its symptoms, suggesting hormonal therapies as potential COVID-19 prophylactics[56]. Furthermore, prophylactic and temporary use of tamoxifen in healthy people, especially in those high-risk groups for COVID-19 infection, can reduce the spread of the disease and the worsening of symptoms, in low-income countries, for example, where vaccine availability may be limited. In summary, our study provides for the first time real-world evidence that tamoxifen protects BC patients from COVID-19 mortality, suggesting that tamoxifen could be repurposed for COVID-19 patients at risk of developing severe symptoms similar to what reported for raloxifene. This protection from disease severity seems somewhat to parallel the observations from Montopoli et al[18]. where the prevalence of COVID-19 in SERM-treated BC patients is reduced, suggesting that tamoxifen may also interfere with SARS-CoV-2 infection susceptibility. To note, it is known that tamoxifen can lead to an increased risk of venous thromboembolism (VTE) 2.8% vs. 0.8% in premenopausal BC patients compared to the general population[57]. However, this is observed in patients treated with tamoxifen for years in combination with chemotherapy, and it is conceivable to assume that this risk becomes irrelevant in healthy individuals considering the short period of exposure necessary to protect from COVID-19 mortality. However, additional clinical studies should be performed to assess the risk-benefit profile of tamoxifen and other SERMs in the context of COVID-19 pandemic and the short and long-term implications also on healthy individuals that have not been investigated to date.

Conclusions

Our study features the largest cohort of Italian BC patients analyzed for the impact of COVID-19 hospitalization and mortality in Italy in 2020, leveraging on data from the Emilia-Romagna region, and supporting the notion that tamoxifen protects treated patients from the most severe COVID-19 symptoms. Importantly, our cohorts comprise hormone receptor-positive (luminal) BC patients, thereby our conclusions apply to the most considerable fraction of BC patients. Tamoxifen offers significant protection against COVID-19 mortality, likely due to its combined pleiotropic effects, and this evidence further supports the idea that tamoxifen could be beneficial not only for BC patients but also for healthy individuals during a COVID-19 pandemic resurgence.

Materials 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: tamoxifen, fulvestrant (+/- CDK4/6 inhibitors), AIs (anastrozole, letrozole and exemestane) and anti-HER-2 (trastuzumab or pertuzumab). We retrieved information on whether and when patients were hospitalized and/or died for COVID-19 within 1 year from the start of the treatment. 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). The study was approved by the local ethical committee (CEROM, protocol number IRST 100.51 ACT4COVID; informed consents were previously collected from all participants and/or their legal guardians, and all data completely anonymized). The authors confirm that the methods were performed in accordance with the relevant guidelines and regulations, and the research was performed in accordance with the Declaration of Helsinki.

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 female under hormone therapy sub-cohort,21 with COVID-19 hospitalization or COVID-19 death as outcomes. The models were stratified according to the therapy received in 2020 and adjusted for age, sex, Charlson Comorbidity Index (CCI), presence of metastasis and previous BC treatment as potential confounders. 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 90% confidence intervals (90% 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. 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. 90% CI of SHR and SMR were calculated assuming a Poisson distribution of the observed numbers. All statistical analyses were performed using R version 4.0.4 (R Foundation for Statistical Computing) or GraphPad Prism; package logistf version 1.23 was used to compute the estimates of the odd ratios. T-test was used to analyze the significance of gene expression data.

Data availability

All data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Correspondence to: massimiliano.mazza@irst.emr.it (Tel.: +39 0543739934).

Received: 21 January 2025; Accepted: 29 September 2025
Published online: 05 November 2025

References

1. Hoffmann, M. et al. SARS-CoV-2 *Cell* entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* **181**, 271–280e8 (2020).
2. Matsuyama, S. et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. *Proc. Natl. Acad. Sci.* **117**, 7001–7003 (2020).
3. Ravaioli, S. et al. ACE2 and TMPRSS2 potential involvement in genetic susceptibility to SARS-COV-2 in cancer patients. *Cell. Transpl.* **29**, 0963689720968749 (2020).
4. Cantuti-Castelvetri, L. et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* **370**, 856–860 (2020).
5. Daly, J. L. et al. Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science* **370**, 861–865 (2020).
6. Gioia, U. et al. SARS-CoV-2 infection induces DNA damage, through CHK1 degradation and impaired 53BP1 recruitment, and cellular senescence. *Nat. Cell. Biol.* **25**, 550–564 (2023).
7. Solis, O. et al. The SARS-CoV-2 Spike protein binds and modulates Estrogen receptors. *Sci. Adv.* **8**, eadd4150 (2022).
8. Toss, A. et al. Two-month stop in mammographic screening significantly impacts on breast cancer stage at diagnosis and upfront treatment in the COVID era. *ESMO Open*. **6**, 100055 (2021).
9. Yarikkaya, E. et al. Impact of the COVID-19 pandemic on the diagnosis, tumor characteristics, and survival outcomes of colorectal cancer: a retrospective cohort study. *Ann. Saudi Med.* **45**, 169–176 (2025).
10. Marty, S. et al. Impact of the Sars-Cov-2 outbreak on the initial clinical presentation of new solid cancer diagnoses: a systematic review and meta-analysis. *BMC Cancer*. **24**, 143 (2024).
11. Wang, Y. et al. The impact of the COVID-19 pandemic on the diagnosis, treatment, and prognosis of ovarian cancer in the united states: a retrospective cohort study based on the SEER database. *Discov Oncol.* **16**, 545 (2025).
12. Mangone, L. et al. Prompt resumption of screening programme reduced the impact of COVID-19 on new breast cancer diagnoses in Northern Italy. *Cancers* **14**, 3029 (2022).
13. Clinckemalie, L. et al. Androgen regulation of the TMPRSS2 gene and the effect of a SNP in an androgen response element. *Mol. Endocrinol.* **27**, 2028–2040 (2013).
14. Wambier, C. G. & Goren, A. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is likely to be androgen mediated. *J. Am. Acad. Dermatol.* **83**, 308–309 (2020).
15. Lucas, J. M. et al. The Androgen-Regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. *Cancer Discov*. **4**, 1310–1325 (2014).
16. Baratchian, M. et al. Androgen regulation of pulmonary AR, TMPRSS2 and ACE2 with implications for sex-discordant COVID-19 outcomes. *Sci. Rep.* **11**, 11130 (2021).
17. Zu, S. et al. Tamoxifen and clomiphene inhibit SARS-CoV-2 infection by suppressing viral entry. *Signal. Transduct. Target. Ther.* **6**, 435 (2021).
18. Montopoli, M. et al. Clinical outcome of SARS-CoV-2 infection in breast and ovarian cancer patients who underwent antiestrogenic therapy. *Ann. Oncol.* **32**, 676–677 (2021).
19. Allegretti, M. et al. Repurposing the Estrogen receptor modulator raloxifene to treat SARS-CoV-2 infection. *Cell. Death Differ.* **29**, 156–166 (2022).

20. Nicastri, E. et al. A phase 2 randomized, double-blinded, placebo-controlled, multicenter trial evaluating the efficacy and safety of raloxifene for patients with mild to moderate COVID-19. *eClinicalMedicine* **48**, 101450 (2022).
21. Wang, Z. P., Hua, M., Jiu, T., Ge, R. L. & Bai, Z. Biofunctional roles of Estrogen in coronavirus disease 2019: beyond a steroid hormone. *Front. Pharmacol.* **13**, 1003469 (2022).
22. Sund, M., Fonseca-Rodríguez, O., Josefsson, A., Welen, K. & Connolly, A. M. F. Association between pharmaceutical modulation of oestrogen in postmenopausal women in Sweden and death due to COVID-19: a cohort study. *BMJ Open.* **12**, e053032 (2022).
23. Seeland, U. et al. Evidence for treatment with estradiol for women with SARS-CoV-2 infection. *BMC Med.* **18**, 369 (2020).
24. Bogush, T. A. et al. Tamoxifen never ceases to amaze: new findings on Non-Estrogen receptor molecular targets and mediated effects. *Cancer Investig.* **36**, 211–220 (2018).
25. Bravaccini, S. et al. Why the complications of COVID-19 patients differ in elderly and young cancer patients. *Transl Oncol.* **26**, 101541 (2022).
26. Zhang, C., Wu, Z., Li, J. W., Zhao, H. & Wang, G. Q. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist Tocilizumab May be the key to reduce mortality. *Int. J. Antimicrob. Agents.* **55**, 105954 (2020).
27. Indonesia, D., Martono, N., Fatmawati, M. & Mulyanti, S. of, F. Risk Factors Associated with the Severity of COVID-19. *Malays. J. Méd. Sci.: MJMS* 30, 84–92 (2023).
28. Ngcobo, N.N. Influence of Ageing on the Pharmacodynamics and Pharmacokinetics of Chronically Administered Medicines in Geriatric Patients: A Review. *Clin. Pharmacokinet.* **64**, 335–367. https://doi.org/10.1007/s40262-024-01466-0 (2025).
29. Faltinová, M. 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.* **187**, 769–775 (2021).
30. Fuentes, N. & Silveyra, P. Chapter three Estrogen receptor signaling mechanisms. *Adv. Protein Chem. Struct. Biol.* **116**, 135–170 (2019).
31. Bennesch, M. A., Picard, D. & Minireview Tipping the balance: Ligand-Independent activation of steroid receptors. *Mol. Endocrinol.* **29**, 349–363 (2015).
32. Klotz, D. M. et al. Requirement of Estrogen receptor-α 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.* **277**, 8531–8537 (2002).
33. Giuliano, M., Trivedi, M. V. & 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.* **8**, 256–262 (2013).
34. Nilsson, S. et al. Mechanisms of Estrogen action. *Physiol. Rev.* **81**, 1535–1565 (2001).
35. Pietras, R. J. et al. HER-2 tyrosine kinase pathway targets Estrogen receptor and promotes hormone-independent growth in human breast cancer cells. *Oncogene* **10**, 2435–2446 (1995).
36. Teppala, S. & Shankar, A. Association between serum IGF-1 and diabetes among U.S. Adults. *Diabetes Care.* **33**, 2257–2259 (2010).
37. Daniloski, Z. et al. Identification of required host factors for SARS-CoV-2 infection in human cells. *Cell* **184**, 92–105e16 (2021).
38. Jeon, S. et al. Identification of antiviral drug candidates against SARS-CoV-2 from FDA-Approved drugs. *Antimicrob. Agents Chemother.* **64**, e00819–e00820 (2020).
39. Weston, S. 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.* **94** https://doi.org/10.1128/jvi.01218-20 (2020).
40. Iaconis, D. et al. Characterization of raloxifene as a potential Pharmacological agent against SARS-CoV-2 and its variants. *Cell. Death Dis.* **13**, 498 (2022).
41. Gordon, D. E. et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* **583**, 459–468 (2020).
42. Vela, J. M. Repurposing Sigma-1 receptor ligands for COVID-19 therapy? Front. *Pharmacol* **11**, 582310 (2020).
43. Moebius, F. F., Reiter, R. J., 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.* **121**, 1–6 (1997).
44. Kedjouar, B. 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.* **58**, 1927–1939 (1999).
45. Gastaminza, P., Whitten-Bauer, C. & Chisari, F. V. Unbiased probing of the entire hepatitis C virus life cycle identifies clinical compounds that target multiple aspects of the infection. Proc. Natl. Acad. Sci. 107, 291–296 (2010).
46. Mingorance, L. et al. Selective Inhibition of hepatitis C virus infection by hydroxyzine and benztropine. *Antimicrob. Agents Chemother.* **58**, 3451–3460 (2014).
47. Dyall, J. et al. Repurposing of clinically developed drugs for treatment of middle East respiratory syndrome coronavirus infection. *Antimicrob. Agents Chemother.* **58**, 4885–4893 (2014).
48. Johansen, L. M. et al. FDA-Approved selective Estrogen receptor modulators inhibit Ebola virus infection. *Sci. Transl Med.* **5**, 190ra79 (2013).
49. Altan, N., Chen, Y., Schindler, M. & Simon, S. M. Tamoxifen inhibits acidification in cells independent of the Estrogen receptor. *Proc. Natl. Acad. Sci.* **96**, 4432–4437 (1999).
50. Jordan, V. C. Targeted hormone therapy for breast cancer. *Hosp. Pr.* **28**, 55–62 (1993).
51. Gallo, M. A. & Kaufman, D. Antagonistic and agonistic effects of tamoxifen: significance in human cancer. *Semin Oncol* **24**, S1-71-S1-80 (1997).
52. Barbieri, S. S. et al. Relevance of Spike/Estrogen Receptor-α interaction for endothelial-based coagulopathy induced by SARS-CoV-2. *Signal. Transduct. Target. Ther.* **8**, 203 (2023).
53. Li, W. et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450–454 (2003).
54. Yoshida, Y. et al. Effect of menopausal hormone therapy on COVID-19 severe outcomes in women – A population-based study of the US National COVID cohort collaborative (N3C) data. *Maturitas* **170**, 39–41 (2023).
55. Nathan, M.R., Schmid, P. A Review of Fulvestrant in Breast Cancer. *Oncol Ther.* **5**(1), 17-29. https://doi.org/10.1007/s40487-017-0046-2 (2017).
56. Bravaccini, S. et al. Estrogen and androgen receptor inhibitors: unexpected allies in the fight against COVID-19. *Cell. Transpl.* **30**, 0963689721991477 (2021).
57. Saphner, T., Tormey, D. C. & Gray, R. Venous and arterial thrombosis in patients who received adjuvant therapy for breast cancer. *J. Clin. Oncol.* **9**, 286–294 (1991).

Acknowledgements

We thank Alessandro Vagheggini for his support with the statistical methodological approach, Alicja M. Gruszka for the critical reading of the manuscript and Ilaria Massa for administrative support. We also thank Cellnex for support on our COVID-19 projects through ACT4COVID consortium and the Emilia-Romagna registry. We dedicate this study to the late Scientific Director Prof. Emeritus Dino Amadori in awe of the values he transmitted to us and his belief that we would make the difference.

Author contributions

S.B., M.M.: Conceptualization. S.B., F.N., W.B., I.A., M.A., F.F., M.T.M., O.N., S.R., F.P., M.M.T., M.C., T.I., M.M.:

Methodology. F.N., L.M., A.G., I.A., S.B., M.M., A.D.L.: Visualization. M.M., S.B., S.R., G.M.: Project administration. M.M., S.B.: Supervision of the project. M.M., S.B., I.A., W.B., F.N.: Writing – original draft. G.M., S.B., R.M., S.R., M.Z., C.C., V.S., M.M., L.P., P.B.S.: Writing – review & editing. All authors reviewed the manuscript.

Funding
Internal resources.

Declarations

Conflict of interest
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.

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

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-22463-8.

Correspondence and requests for materials should be addressed to M.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.