**ABSTRACT**
The COVID-19 pandemic, caused by the SARS-CoV-2 coronavirus, attacks various organs but most destructively the lung. It has been shown that SARS-CoV-2 entry into lung cells requires two host cell surface proteins: ACE2 and TMPRSS2. Downregulation of one or both of these is thus a potential therapeutic approach for COVID-19. TMPRSS2 is a known target of the androgen receptor, a ligand-activated transcription factor; activation of the androgen receptor increases TMPRSS2 levels in various tissues, most notably the prostate. We show here that treatment with the antiandrogen enzalutamide—a well-tolerated drug widely used in advanced prostate cancer—reduces TMPRSS2 levels in human lung cells. Further, enzalutamide treatment of mice dramatically decreased Tmprss2 levels in the lung. In support of this new experimental data, analysis of existing datasets shows striking co-expression of AR and TMPRSS2, including in specific lung cell types that are targeted by SARS-CoV-2. Together, the data presented provides strong evidence to support clinical trials to assess the efficacy of antiandrogens as a treatment option for COVID-19.

**KEYWORDS**
COVID-19, SARS-CoV-2, androgen receptor, antiandrogens, lung, TMPRSS2, ACE2

**INTRODUCTION**
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the COVID-19 pandemic, is a positive-sense single-stranded RNA coronavirus highly related to SARS-CoV, which caused the 2002 SARS pandemic [1, 2]. Like SARS, COVID-19 primarily affects the respiratory system (although other organs can also be affected): symptoms are mild in some, but in others the infection can result in pneumonia, Acute Respiratory Distress Syndrome (ARDS) and death [3]. Risk factors associated with poor prognosis include age, diabetes and cardiovascular disease [4]. It has also been shown that gender is a prognostic factor, with approximately 60-70% of deaths being in men [5, 6], suggesting that steroid hormones may be a contributing factor to the severity of the disease. In further support of this, recent studies have shown that men with male pattern hair loss (caused by elevated androgen signalling [7]), are at higher risk of suffering more severe COVID-19 symptoms [8, 9].

Coronaviruses have structural (Spike, Nucleocapsid, Matrix and Envelope) and non-structural (e.g. the proteases nsp3 and nsp5) proteins [10]. Viral entry is reliant on two host proteins present on the surface of epithelial cells in the target organ: Transmembrane Serine Protease 2 (TMPRSS2) and Angiotensin-Converting Enzyme 2 (ACE2) [11]. TMPRSS2 primes the viral Spike (or S) protein by cleaving it at two sites; this facilitates fusion of the viral and host membranes [12-14]. Cellular entry is subsequently facilitated by ACE2, a terminal carboxypeptidase and type I transmembrane glycoprotein [15]. Thus, viral entry may be prevented or slowed by inhibition of ACE2 and/or TMPRSS2. TMPRSS2 is an attractive target for therapeutic intervention.
as knockout of this protein causes no overt detrimental phenotype [16], whereas ACE2 downregulation is associated with increased severity of SARS-induced lung injury [17]. Further, TMPRSS2 expression levels have been shown to be associated with disease severity in mouse models of coronavirus infection [18], and its inhibition was recently shown to inhibit SARS-2-S-driven entry in lung cells [11].

Multiple studies have shown that TMPRSS2 is an androgen receptor (AR) target gene in prostate cancer cells (e.g. [19-21]). The AR is a nuclear receptor and member of the steroid receptor family. It is a transcription factor activated by ligand binding, upon which it translocates from the cytoplasm to the nucleus where it binds to regulatory regions of target genes as a homodimer. Following the recruitment of accessory proteins and the basal transcriptional machinery, the active receptor promotes gene transcription [22]. In the case of prostate cancer, active AR promotes tumour growth and so treatment options for prostate cancer often target this signalling axis, through the use of androgen deprivation and hormonal therapies such as antiandrogens [23, 24]. Antiandrogens (e.g. Bicalutamide and Enzalutamide) interact with the AR in the ligand binding pocket and hold the receptor in an inactive conformation, unable to form an active transcriptional complex, and thus inhibit its activity.

Importantly, previous studies have demonstrated that the AR is expressed in the lung [25] and studies using mice have confirmed that AR is functional in this organ [26, 27]. In corroboration, in vitro studies have shown that multiple lung lines express functional AR [26, 28-30]. It is therefore possible that inhibition of androgen signalling, in response to antiandrogens, will reduce TMPRSS2 expression in the lung and reduce viral entry. For this reason, antiandrogens have been proposed as a treatment option for COVID-19 [31-33]. Here we review and reanalyse available data to investigate AR and TMPRSS2 in the lung, and provide additional pre-clinical data to support the use of antiandrogens for the treatment of COVID-19.

METHODS

Ligands
Mibolerone was purchased from Perkin Elmer (MA, USA) and dissolved in ethanol. Enzalutamide was from Sigma Aldrich (MO, USA) and was resuspended in DMSO.

Cell Culture
A549 and LNCaP were purchased from the ATCC (VA, USA) and were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) and Roswell Park Memorial Institute (RPMI) medium 1640 (Invitrogen, Strathclyde, UK) respectively, as previously described [34].

Immunoblotting
Cells were lysed in RIPA buffer (150 mM NaCl, 1% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 25 mM Tris pH 7.4) containing freshly added protease inhibitors (Halt Protease Cocktail, ThermoFisher, MA, USA). Samples were incubated on ice for 10 min, sonicated 4 cycles of 30 sec on/off (Biorupter, Diagenode, NJ, USA) and centrifuged (13,000 rpm, 10 min, 4 °C). The DC protein assay (BioRad) was used to quantify protein concentrations. 30 µg of protein was separated using SDS-PAGE and immunoblotting performed, as previously described [35]. Rabbit anti-AR (ab74272, Abcam, Cambridge, UK) and mouse α-tubulin (B-5-1-2, Sigma Aldrich) primary antibodies were used at a concentration of 1:1000 and 1:10,000 respectively. Secondary HRP-conjugated antibodies (Sigma Aldrich) were used at 1:2000.
Proteins were visualised using Immobilon Forte HRP substrate (Merck Millipore, MA, USA) and a Fusion FX Chemi Imager (Vilber Lourmat, Collégien, France).

Quantitative PCR analysis of gene expression in cell lines
Cells were seeded in 12 well plates and incubated + 10 nM mibolerone ± 10 µM enzalutamide for 48 or 72 hrs. RNA was extracted using Trizol (Thermo Fisher), following the manufacturer’s instructions. 250 ng of RNA was reverse transcribed using a LunaScript RT SuperMix Kit (NEB, MA, USA). Alterations in gene expression were quantified using the Luna Universal qPCR Master Mix (NEB) and a Roche 96 qPCR machine (Basel, Switzerland). TMPRSS2 data were normalised to L19 data and the 2^(-ΔΔCT) method was used to calculate gene expression changes. Human qPCR primers: (5’-3’) TMPRSS2 forward – CTGCTGGATTTCCGGTG; TMPRSS2 reverse – TTCTGAGGTCCTCCCTTCT; L19 forward – GCGGAAGGGTACAGCCAAT, L19 reverse GCAGCCGGCGCAAA.

Mouse studies
Pten<sup>loxp/loxp</sup>;Pb-Cre4 mice (The Jackson Laboratory), which have prostate-specific PTEN deletion [36], were treated with 50 mg/kg Enzalutamide (in 5% DMSO +1% CMC +0.1% P80 oral gavage) or vehicle control every day for three days. All work was carried out in accordance with the provisions of the Animals (Scientific Procedures) Act 1986 of the United Kingdom and under an appropriate Home Office license. Organs were snap frozen until RNA was extracted using a Monarch RNA extraction kit (NEB) following disruption of frozen tissue utilizing mechanical disruption and enzymatic digestion with Proteinase K. 2 µg of RNA was reversed transcribed using Precision Nanoscript2 Rt Kit (Primer Design, Southampton, UK). Changes in gene expression were measured using SYBR Green Fast Master Mix (Life Technologies, CA, USA) and QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, CA, USA). Gene expression data were normalised to L19, Gapdh, and Actb data and the 2^(-ΔΔCT) method used to calculate changes in expression. Mouse qPCR primers (5’-3’): Tmprss2 forward – GAGAACCGTTGTGTTGC; Tmprss2 reverse – GCCTGCGCTGGATTC; Ace2 forward – TGGATATAGCGGCTGGATG; Ace reverse – ATTCTGAGACCTCCGTGCC; Ar forward – TGGACCTGGAGGACA; Ar reverse – CTCCGATGTACGACCGAGA; Gapdh forward – GCAAGGTGAGATGGCTG; Gapdh reverse – CCTGACTGTGCCGTG; L19 forward – GAAATCGCCAATGC; L19 reverse – TCTAGACTCGCAGCTCA; β-actin forward – CCTCTATGCCAAC; β-actin reverse – CCTGCTTGCTGATCCCA.

Analysis of TMPRSS2 expression in tissue and cell line datasets
RNA-sequencing dataset v8 was downloaded from the Genotype-Tissue Expression (GTEx) Project Online Portal [37]. Gene expression was normalized (inverse normal transformation) across samples, and medians for AR and TMPRSS2 expression across each tissue was calculated. Data from RNA sequencing of isolated single nuclei, performed on surgical specimens of healthy, non-affected lung tissue from twelve lung adenocarcinoma (LADC) patients, was analysed for AR, TMPRSS2, and ACE2 expression using Eils Lab UCSC Cell browser (https://eils-lung.cells.ucsc.edu) [38]. Sequencing data from MCF7 cell lines treated with 10 nM DHT or equivalent control (GSE99626), T47D cells treated with 10 nM DHT (GSE62243) [39], and data from lungs of castrated or intact mice (GSE31341) [40] were log2 transformed. Significance was determined by ANOVA.
Identification of potential AREs

The AR binding motif, MA0007.2, was obtained from the JASPAR online curated motif database [41]. Segments of DNA around the TMPRSS2 gene were scanned for potential matches for presence of the MA0007.2 AR motif using FIMO [42]. Of the 40 possible MA0007.2 matches in regulatory region 1 (P<0.0001) and 34 possible matches in regulatory region 2 (P<0.0001), only response elements that correlated with binding peaks were selected.

Analysis of ChIP-sequencing data

ChIP sequencing data (receptors in the presence of their cognate ligands) were analysed using Cistrome Analysis Pipeline and visualised with WashU Epigenome Browser (v51.0.5). Datasets used: LNCaP - AR (GSE94682) [43], H3K27ac (GSE73783) [44], FOXA1 (GSE94682) [43] and NR3C1/GR (GSE39880) [45]; A549 - H3K27ac (GSE29611) [46], FOXA1 (GSE32465) [47], JUN (GSE92812) [46] and NR3C1/GR (GSE91313) [46]; MC7 - AR (GSE104399) [48], H3K27ac (GSE94804) [49], FOXA1 (GSE112969) [50], JUN (GSE102410) [51] and NR3C1/GR (GSE39879) [45].

RESULTS AND DISCUSSION

TMPRSS2 is an androgen and antiandrogen-regulated gene

ACE2 and TMPRSS2 are crucial for SARS-CoV-2 entry into cells [11], and hence these receptors represent potential therapeutic targets for COVID-19. TMPRSS2 has been shown to be an AR target gene in prostate cancer (e.g. [19-21]) and we therefore hypothesised that the expression of the gene could be down-regulated in response to antiandrogens. To confirm this, the AR-positive prostate cancer cell line LNCaP was seeded in hormone-depleted media for 72 hrs and treated with the synthetic androgen mibolerone (MIB, 1 nM) and/or the antiandrogen enzalutamide (ENZA, 10 µM) for 24 hrs. Alterations in gene expression were quantified using qPCR. As expected, the addition of androgen significantly increased TMPRSS2 expression (approximately 35-fold, Figure 1A). Importantly, enzalutamide successfully blocked this androgen-induced up-regulation, resulting in an almost complete inhibition of TMPRSS2 expression. To determine whether AR regulation of TMPRSS2 also occurs in other cell types, gene expression was investigated in two breast cancer cell lines, MCF-7 (GSE99626) and T47D (GSE62243) [39]. In agreement with the LNCaP results, TMPRSS2 was also found to be upregulated in response to androgen in the breast lines, albeit weakly (Figure 1B and C).

TMPRSS2 and the AR are co-expressed in the lung

Androgen signalling is known to be important in multiple tissues/organs. To better characterise this signalling, we previously created the AR-LUC transgenic mouse in which luciferase expression is under the control of an androgen responsive promoter, allowing for visualisation of both in vivo and ex vivo AR activity. AR signalling was found to be active in a number of tissues/organs, including the prostate, seminal vesicles, uterus and ovaries – and importantly, AR signalling was also found to be active in the lungs of male and female mice, although activity was weaker than in the reproductive organs [27]. Other studies have also demonstrated that AR signalling is active in the lung. For example, Mikonnnen et al. found the AR to be predominantly expressed in type II pneumocytes and the bronchial epithelium and microarray analysis of the murine lung, and demonstrated that genes involved in oxygen
transport (among other pathways) are up-regulated in murine lung in response to androgen [26].

To investigate AR and TMPRSS2 expression in different human tissues, we interrogated the Genotype Tissue Expression (GTEx) dataset [52]. We found that AR and TMPRSS2 are co-expressed in a number of tissues, and generally, TMPRSS2 is only expressed in tissues that also show detectable levels of the AR, with the exception of the pancreas (Figure 2A). Importantly, AR and TMPRSS2 were found to be co-expressed in the lung (highlighted red, also highlighted are prostate, breast (both co-expressing) and pancreas). Analysis of single cell sequencing data from lung tissue [38], demonstrated that the AR is expressed in most cell types, with highest expression in club (bronchiolar exocrine cells), alveolar type 2 (AT2), fibroblast and endothelial cells. TMPRSS2 was found to have a fairly uniform expression across cell types, but was most highly expressed in the AT1 and AT2 luminal cells (Figure 2B,C). Significantly, these are the cell types targeted by SARS-CoV-2 [53]. AT1 and AT2 cells also demonstrated highest ACE2 expression, and measurable AR expression.

**TMPRSS2 expression in the lung is higher in men**

In adults, men have on average 7-8 times higher levels of circulating testosterone compared to women [54]. It was therefore hypothesised that TMPRSS2 expression would be higher in male lungs compared to females. Analysis of the GTEx dataset confirmed this, with TMPRSS2 expression significantly higher in the male lung (Figure 3). Interestingly, there was no significant difference in AR expression levels between men and women, suggesting the higher levels of TMPRSS2 expression are a result of increased AR activity due to circulating androgen levels, rather than higher AR levels. This would support the theory that the observed worse prognosis in men following SARS-CoV-2 infection (60-70% of COVID-19-related deaths are in men [5, 6]) is at least in part due to elevated expression of TMPRSS2 as a consequence of higher levels of androgen. In light of the recent studies linking male pattern hair loss with more severe COVID-19 symptoms [8, 9], it would be of interest to compare TMPRSS2 expression levels in men with and without this androgen-associated form of hair loss.

**TMPRSS2 expression is reduced by enzalutamide in A549 cells**

As discussed above, the AR is expressed in human and murine lung, and has been shown to be active. To investigate AR regulation of TMPRSS2 in the lung, we used the A549 human type II pneumocyte cell line; a cell type targeted by SARS-CoV-2 [53]. Immunoblotting confirmed AR expression: in agreement with the GTEx data, the AR is expressed in the lung cell line and levels are approximately 10-fold lower than in the prostate LNCaP line (Figure 4A).

TMPRSS2 expression has been previously shown to be androgen-regulated in the A549 cell line [18]. To replicate these findings, we seeded A549 cells in hormone-depleted media (containing serum that has been charcoal-stripped to remove any traces of hormones) for 3 days and treated with the synthetic androgen MIB. However, under these conditions TMPRSS2 was undetectable by qPCR (data not shown). The experiment was therefore repeated for A549, and LNCaP, in media supplemented with 10% full serum, with/without enzalutamide. Fetal calf serum has been shown to contain castrate levels of testosterone, which LNCaP cells metabolise to produce physiologically relevant intracellular levels of dihydrotestosterone, sufficient to promote their growth [55]. Since this might not be the case in A549 cells, a final concentration of 10nM MIB was also added to both cell lines throughout the experiment. In these conditions, TMPRSS2 was expressed at detectable levels and,
importantly, enzalutamide potently down-regulated \(\text{TMPRSS2}\) expression in both LNCaP and A549 after 48 and (more so) 72 hours (Figure 4B). This therefore confirms that antiandrogens could be used to down-regulate \(\text{TMPRSS2}\) expression in lung cells.

**\(\text{TMPRSS2}\) expression is reduced by enzalutamide in mouse lung**

To investigate the effects of enzalutamide, on \(\text{TMPRSS2}\) expression in vivo, mice were treated for three days with enzalutamide or vehicle control. Following sacrifice, lung tissue was collected and qPCR performed to quantify alterations in gene expression. While there was no significant change in \(\text{Ar}\) or \(\text{Ace2}\) expression, \(\text{Tmprss2}\) expression was significantly decreased after enzalutamide treatment \((P<0.05, \text{Figure \textit{5A}})\). To validate these findings, expression data from intact mice and mice that had been castrated (removal of testicular production of androgen) were interrogated \((\text{GSE31341})\) \([40]\). In agreement with our cell line data, castration significantly reduced \(\text{Tmprss2}\) expression in the mouse lung \((\text{Figure \textit{5B}})\). In the same mice, castration was also associated with an increase in \(\text{Ar}\) expression \((P<0.01)\), expected as \(\text{Ar}\) gene transcription is downregulated in response to androgen \([56]\).

**\(\text{TMPRSS2}\) expression in lung is potentially directly regulated by nuclear receptor proteins and coregulators.**

Although ChIP-Seq data for genomic AR binding in lung tissue or cells is not available, we were able to assess the cistrome of FOXA1 and JUN, known pioneer coregulatory factors for the AR \([57]\) and other nuclear receptors. Binding of the glucocorticoid receptor (GR) was also investigated as this can bind to many of the same response elements as the AR \([58]\), also acetylated histone 27 (H3K27ac) as an indicator of active regulatory regions, all in A549 lung cells \((\text{Figure \textit{6A}})\). Binding profiles for prostate \((\text{LNCaP})\) and breast \((\text{MCF-7})\) cell lines were included for comparison. In LNCaP cells the AR binding pattern correlates with previous findings \([20]\), and confirms that AR and GR bind in the same regions, corresponding also to binding of the pioneer factor FOXA1, and these sites largely correlate with the marker of transcriptionally active regions, H3K27ac. Detailed analysis of these potential response elements by the Claessens lab demonstrated that an androgen response element in the enhancer region \((\text{approximately } -13 \text{ kb from the transcription start site})\) is crucial for optimal androgen regulation of \(\text{TMPRSS2}\) in prostate cells \([20]\).

The binding patterns of GR, pioneer factors and H3K27ac in lung cells, however, differ to what is seen in LNCaP cells \((\text{compare regulatory region 1 and 2})\). To assess if androgen response elements are present in regulatory region 2, the AR binding motif \((\text{MA0007.2, Figure \textit{6B}})\) from the JASPAR database, was used to detect AR target sites using methods previously described \([31]\). This analysis identified potential androgen response elements throughout the 5’ region of the \(\text{TMPRSS2}\) gene \((\text{Figure \textit{6A}} \text{and} \textit{6C})\). Importantly, several of the potential androgen response elements were found to correlate with the GR, FOXA1, JUN, and H3K27ac peaks seen in the A549 regulatory region 2. Together, this suggests that AR \((\text{and associated factors})\) may directly regulate \(\text{TMPRSS2}\) via different regulatory regions in lung and prostate.

The DNA-binding of AR, GR, FOXA1, JUN, and H3K27ac around the \(\text{TMPRSS2}\) gene in breast cancer cells \((\text{MCF-7})\) appears to be less pronounced than in prostate and lung, and the binding pattern has elements of the binding patterns in both prostate and lung cells. Importantly, AR binding in MCF-7 cells correlates with the H3K27ac, FOXA1, JUN and GR peaks located distally in the A549 regulatory region 2. This therefore provides further evidence that this region contains a functional androgen response element(s). Intriguingly, this region also correlates with a peak for oestrogen receptor-\(\alpha\) \((\text{ESR1})\) binding in MCF-7. This supports the
possibility of TMPRSS2 regulation by other members of the nuclear receptor superfamily, and hence further potential for pharmacological manipulation by their ligands – in this case oestrogens/antioestrogens as well as, via the GR, glucocorticoids, DHT/antiandrogens via the AR.

CONCLUSIONS
The data presented here confirm a role for AR in regulation of TMPRSS2 in the lung, which may at least in part explain why men with COVID-19 have a worse prognosis compared to women. Data from prostate and breast tissue also support regulation in other organs, which may also be targeted by SARS-CoV-2. Importantly, our findings support the hypothesis that therapies to target AR signalling could be used to transcriptionally inhibit lung TMPRSS2 expression. Further, potential regulation of TMPRSS2 by other, related receptors (revealed by cistromic analysis) opens up the possibility of additional potential opportunities for pharmacological inhibition of TMPRSS2 expression. Down-regulation of TMPRSS2 will result in attenuated spike protein priming, reducing SARS-CoV-2 interaction with ACE2, blocking viral entry (summarised in Figure 7). Antiandrogens are used routinely in, or have been trialled for, the treatment of multiple diseases, including prostate cancer, breast cancer, polycystic ovarian syndrome and alopecia [59]. They have been shown to be well tolerated in men and women [59-61] and therefore antiandrogens should be considered as a potential therapeutic strategy for COVID-19.

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**FIGURE LEGENDS**

**Figure 1. TMPRSS2 is an androgen regulated gene in LNCaP cells.** A) LNCaP were incubated in hormone-depleted media for 72 hrs and treated ± 1 nM mibolerone (MIB) ± 10 µM enzalutamide (ENZ) for 24 hrs. RNA was harvested, reverse transcribed and qPCR performed to quantify the expression of TMPRSS2. Mean of 3 independent repeats (± 1SEM). ANOVA, * p<0.05 MIB vs VC (vehicle control), #p<0.05 MIB + ENZ. Data from B) MCF-7 cells (GSE99626, N=2) and C) T47D cells (GSE662243, N=3) treated with 10nM DHT were analysed for TMPRSS2 expression. Significance determined using ANOVA, * p<0.05.

**Figure 2. TMPRSS2 and the AR are expressed in the lung.** A) The GTEx dataset [52] was interrogated and median expression of AR was plotted against median TMPRSS2 expression. Data is expressed as each gene normalised across all tissues (z-score). B) Single-cell analysis of lung tissue [38] was interrogated and cells expressing titled gene are marked in black, negative cells are in light blue. C) Breakdown of AR, TMPRSS2, and ACE2 expression levels in specific lung cell types (X-axis); Y Axis denotes gene mRNA CPM.

**Figure 3. TMPRSS2 is expressed at higher levels in the male lung compared to female lung.** The GTEx dataset [52] was interrogated and expression of AR and TMPRSS2 investigated in lung tissue and dichotomized by gender, M = male (N=220), F = female (N=355). Significance tested via Wilcoxin, * P<0.05. Circles denote statistical outliers (2 x inter quartile range).

**Figure 4. AR is expressed in the A549 lung cell line.** A) A549 and LNCaP cells were lysed and proteins separated using SDS-PAGE. Immunoblotting was performed to visualise AR expression levels and α-tubulin was used as a loading control. B) LNCaP and A549 were incubated in full media for 48 hrs or 72 hours + 10 nM mibolerone ± 10 µM enzalutamide. RNA was harvested, reverse transcribed and qPCR performed to quantify the expression of TMPRSS2. Mean of 3 independent repeats (± 1SEM). ANOVA, *** p<0.0005.

**Figure 5. Enzalutamide successfully down-regulates TMPRSS2 in mouse lung.** A) Relative expression of Ar, Tmprss2, and Ace2 mRNA from lung tissue of mice treated with enzalutamide (n=9) or vehicle control (VC, n=8) once daily for 3 days. Expression was normalised to housekeeping genes and made relative to VC. B) Log2 expression of Tmprss2, Ar, and Ace2 in lung tissue from male mice physically castrated (N=3) vs intact male mice (N=3) (GSE31341). Statistical analysis was performed using Student T test, * p< 0.05, ** p<0.01, *** p<0.001.

**Figure 6. Potential regulatory regions in the TMPRSS2 promoter.** ChIP sequencing peaks of AR, FOXA1, GR, and H3K27ac in LNCaP cells, FOXA1, JUN, GR, and H3K27ac in A549 lung cells, AR, H3K27ac, FOXA1, JUN, GR, ESR1 in MCF-7 breast cancer cells. The TMPRSS2 gene is highlighted in the purple shaded box and the potential regulatory region 1 is boxed in yellow, the potential regulatory region 2 is boxed in orange. Potential AREs are marked in blue boxes (MA0007.2) or green (determined by [20]). B) AR motif MA0007.2 from JASPAR curated motif database. C) Position of potential MA0007.2 motifs around the TMPRSS2 gene separated into two regions, the first region (regulatory region 1 covering areas of TSS and promoter/enhancers, the second region (regulatory region 2) covers more distant enhancer regions.

**Figure 7. Schematic representation of how targeting the AR could reduce SARS-CoV-2 entry.**
Leach et al. Figure 1

A) LNCaP

B) MCF-7

C) T47D

Relative TMPRSS2 Exp.

VC  MIB  ENZ  MIB  ENZ

Relative TMPRSS2 Exp.

VC  DHT

Relative TMPRSS2 Exp.

VC  DHT

*  #  *

Bars represent mean ± SEM.
Leach et al. Figure 2

A) 

B) 

C) 

AR in Lung Tissue

TMPRSS2 in Lung Tissue

ACE2 in Lung Tissue

Gene CPM

Gene CPM

Gene CPM

Prostate

Lung

Pancreas

Breast

Other Organs

+ve for expression

-ve for expression
Leach et al. Figure 3

![Box plots comparing TMPRSS2 and AR Z-scores by gender.](image)

- **TMPRSS2 Z-score**
  - Female (F) vs. Male (M) comparison with an asterisk (*) indicating a significant difference.

- **AR Z-score**
  - Female (F) vs. Male (M) comparison.
Leach et al. Figure 4

A) LNCaP A549

AR (Low Exposure)

AR (High Exposure)

Tubulin

B) LNCaP Cells

AR

48 Hours 72 Hours

Relative TMPRSS2 Exp.

0.2 0.4 0.6 0.8 1.0

Full Media

Full Media + Enzalutamide

A549 Cells

48 Hours 72 Hours

Relative TMPRSS2 Exp.

0.2 0.4 0.6 0.8 1.0

***
Nucleus

Primed spike protein interacts with ACE2

Androgen receptor + androgen

Gene transcribed/translated into TMPRSS2

SARS-CoV-2

Cell Membrane

TMPRSS2

Successful viral entry

Nucleus

Androgen receptor + antiandrogen

Spike protein cannot interact with ACE2

SARS-CoV-2

Cell Membrane

ACE2

Inactive androgen receptor cannot promote TMPRSS2 transcription

Leach et al. Figure 7