Effect of antiandrogens on the tropism of seasonal human coronaviruses.

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ABSTRACT

Human coronaviruses (HCoVs) are positive-sense, single-stranded RNA viruses. The HCoVs: OC43 and 229E, HKU1 and NL63 cause 10-30% of common colds with mild respiratory symptoms and more severe disease in immunocompromised patients or the elderly. The emergence and severity of COVID-19 has heightened the need to study the seasonal HCoVs (sHCoVs) to comprehend the pathogenicity mechanisms of coronaviruses. Cellular entry of SARS-CoV-2 is largely dependent on the viral spike (S) protein which binds to the cell surface receptor Angiotensin-Converting Enzyme 2 (ACE2) and is proteolytically cleaved by Transmembrane Serine Protease 2 (TMPRSS2) into two subunits. Studies have shown that TMPRSS2 can also facilitate entry of the HCoV-229E and -NL63. Hence, TMPRSS2 is an attractive therapeutic target as knockout of this protein causes no overly detrimental phenotype unlike ACE2 which is critical to regulating processes such as blood pressure . The sequence alignment analysis shows that the TMPRSS2 cleavage site is well conserved across all sHCoVs therefore TMPRSS2 can potentially cleave the S protein of the sHCoVs. This work further demonstrates that TMPRSS2 at least partially facilitates cell entry of HCoV-229E and NL63. TMPRSS2 targets the androgen receptor and drugs antiandrogens, e.g. enzalutamide that target this transcription factor have been have been found to reduce TMPRSS2 expression and prevent viral entry. Hence, it is hypothesised that similar to SARS-CoV-2, antiandrogens can be used against sHCoVs. Importantly enzalutamide successfully reduced entry of HCoV: 229E and NL63 pseudotype virus, which are enveloped viral particles comprised of a viral capsid surrounded by a cell derived membrane bearing the desired viral envelope protein. Therefore highlighting TMPRSS2 as a potential therapeutic target and antiandrogens as a potential therapy for sHCoVs. In addition, using an androgen response element driven reporter, HCoV-229E infection was found to induce androgen receptor activity and differential expression of androgen regulated genes, indicating a crosstalk between infection and AR signalling

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ABBREVIETIONS .

ACE2	Angiotensin converting enzyme-2.
AR	Androgen.
CoVs	Coronaviruses.
GTEx	Genotype Tissue Expression.
HAE	Human airway epithelial.
HCoVs	Human corona viruses.
HIV-1	Human immunodeficiency virus 1.
HRSV	Human respiratory syncytial virus .
MOI	Multiciplity of infection.
PV	Pseudoviruses.
S	Spike protein.
SHCoVs	Seasonal human corona viruses.
TMPRSS2	Transmembrane serine protease 2.
TMPRSS4	Transmembrane serine protease 4.
9-O-Ac-Sia	9-O-acetylated-sialic acid.

CHAPTER 1: INTRODUCTION

Coronaviruses (CoVs) named in reference to their distinct crownlike morphology when viewed under an electron microscope, are a group of enveloped single stranded RNA viruses that infect various animal species as well as humans (Andersen *et al.*, 2020). The Coronaviridae family is divided into two subfamilies, the CoVs and the Toroviruses. The CoVs are further divided into four genera: Alpha, Beta, Gamma and Delta coronaviruses. The Alpha and Beta subfamily consist of viruses specific to humans: human coronaviruses (HCoV)-229E and HCoV-NL63, Betacoronavirus includes HCoV-OC43, Severe Acute Respiratory Syndrome human coronavirus SARS-CoV, HCoV-HKU1, Middle Eastern respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2; while the Gamma and Delta groups includes viruses of whales, birds and pigs and birds respectively (**Figure 1)** (Masters, 2006; Shi, 2021). The initial reports of a CoV infection in animals go back to the 1920's when an acute respiratory infection was observed in domesticated chickens in North America (Abubakar *et al.*, 2016). The CoVs specifically possess a positive sense genomes and have been known to be consequential pathogens, seasonally endemic in many regions causing the common cold and epidemics as we have seen over the last couple of years (Dimmock, 2003; Fabricant, 1998).

1.1 Human Coronaviruses (HCoVs)

Over time a total of seven HCoVs have been identified HCoV-229E, OC43, HKU1, NL63, SARS-CoV, MERS- CoV and SARS-CoV2. HCoV; 229E, OC43, HKU1 and NL63 all cause common cold and are referred to as the seasonal HCoV (sHCoVs) (Pene *et al.*, 2003). HCoV-229E and OC43 were the first set of HCoVs to be identified in 1966 and 1967 respectively (Hamre and Procknow, 1966; McIntosh *et al.*, 1967). Bradburne *et al.*, (1967) and Callow *et al.*, (1990) later identified them as the causative agents of the common cold in humans after inoculating healthy adult volunteers. Years later in November 2002, the first known case of SARS-CoV occurred in Foshan, China causing subsequent outbreaks of SARS in Hong Kong, Vietnam, Canada and Singapore (Booth *et al.*, 2003; Hsu *et al.*, 2003; To *et al.*, 2021). Reported SARS-CoV cases totalled about 8000 with ~800 deaths worldwide and a ~10% overall mortality rate prior to successful containment of the epidemic. Mortality rates following SARS-CoV infection approached 50% and patients aged over 65 developed lung and cardiac complications (Han *et al.*, 2003).

The emergence and severity of SARS-CoV alerted the scientific community about the possible pandemic potential of coronaviruses, hence efforts to identify and characterize new HCoVs increased. This led to the discovery of HCoV-NL63 and HCoV-HKU1 in 2004 and 2005 respectively (Vabret *et al.*, 2006; van der Hoek *et al.*, 2004; Woo *et al.*, 2005) (**Figure 1)**. First reported case of HCoV-NL63 was first isolated in 2004 from a seven-month-old child; the patient presented with symptoms suggesting respiratory tract infection while chest X-ray showed typical features of bronchiolitis. Even though HCoV-NL63 was first described in 2004, it soon became clear that the virus was not novel in humans, as literature from the mid-1960s and 1980s described a HCoV believed to be HCoV-NL63. Subsequent investigations showed a 98.8% nucleotide similarity with the previously described HCoV-NL63 driving the conclusion that the two virus isolates represent the same species other than HCoV-229E and HCoV-OC43 in circulation (van der Hoek *et al.*, 2006; van der Hoek *et al.*, 2004).

In 2012, The first reported case of MERS-CoV was isolated from the sputum of a male patient who developed severe pneumonia and acute renal failure in a hospital in Jeddah, Saudi Arabia after infection. (de Groot *et al.*, 2013; Zaki *et al.*, 2012). Since 2012 until 31 October 2019, the total number of laboratory-confirmed MERS-CoV infection cases reported globally to WHO was 2484, with 857 associated deaths (WHO, 2019). SARS-CoV-2 is the virus that causes the respiratory illness responsible for the COVID-19 pandemic (Gorbalenya *et al.*, 2020). The virus was first identified in the city of Wuhan, China, due to the rapid prevalence and spread the WHO initially declared the outbreak a Public Health Emergency of International Concern on 30 January 2020, and a pandemic on 11 March 2020 (WHO, 2020; Gorbalenya *et al.*, 2020).

A study by Billah *et al.*, 2020 estimate that each infectious case causes an average of 2 to 3 new cases in communities with little or no preventive measures or no immunity. The original source of coronavirus transmission to humans remains unclear, various studies points to zoonotic origin to be bats due to the extensive similarities to bat coronaviruses (Andersen *et al.*, 2020). Epidemiological data from a COVID-2019 patient early in the outbreak revealed that SARS-CoV-2 is similar to, but distinct from SARS-CoV. The genome of SARSCoV-2 bears 82% resemblance to that of human SARS-CoV and has 89% nucleotide similarity with bat SARS-like-CoV2 strain RaTG13 (Chan *et al.*, 2020). In March 2021 WHO report stated that the spill over via an intermediate animal host was the most likely explanation, with direct spill over from bats next most likely. Introduction through the food supply chain and the Huanan

Seafood Market was considered another possible, but less likely, explanation (WHO 2020; Andersen *et al.*, 2020; Zhou *et al.*, 2020a). Scientists have released studies that speculate about how the COVID-19 pandemic started. Two of the reports trace the outbreak back to a massive market that sold live animals, among other goods, in Wuhan, China and a third suggests that the coronavirus SARS-CoV-2 spilled over from animals — possibly those sold at the market — to humans at least twice in November or December 2019 (Maxmen, 2022)

HCoVs are known for their ability to cause a range of respiratory diseases with varying severity, including common cold, pneumonia and bronchiolitis HCoVs infection can cause mild, chronic ailments in adults and children, with a possibility of death in the elderly or immunocompromised individuals (Pene *et al.*, 2003; Walsh *et al.*, 2013). Besides respiratory illnesses, they may also cause enteric and neurological diseases (Elmashala *et al.*, 2020). HCoVs are frequently co-detected with other respiratory viruses, particularly with human respiratory syncytial virus (HRSV) in a study by Gaunt *et al.*, 2010 a total of 11 to 41% of HCoVs were detected in positive samples for other respiratory viruses, although clinical presentations of coronavirus mono-infections were comparable to those of viruses which have an established role in respiratory disease, such as respiratory syncytial virus, influenza virus, and parainfluenza viruses. Whether severe disease outcomes are related to such co infections is still unclear (Kuypers *et al.*, 2007; Resta *et al.*, 1985b).

Over time HCoVs have been hypothesised to be associated with multiple sclerosis, hepatitis, or enteric disease in infants, but none of this have been proven (Burks *et al.*, 1980; Resta *et al.*, 1985a; Zuckerman *et al.*, 1970). HCoV-229E, NL63, HKU1 are found endemic in human populations as they are in circulation during the winter months in temperate climates causing 10 to 30% of seasonal cold infections in humans while the other three (MERS, SARS CoV 1 and 2) are more pathogenic (Fouchier *et al.*, 2004; Shang *et al.*, 2021; Walsh *et al.*, 2013). The epidemiology and clinical characteristics of HCoV-229E, -HKU1, -NL63, and -OC43 are not clearly described and often rely on case reports. To know more about the HCoVs a large-scale comprehensive screening for the four coronaviruses was conducted by Gaunt *et al.*, (2010), analysing 11,661 diagnostic respiratory samples collected in Edinburgh, UK between 2006 and 2009 using a novel four-way multiplex real-time reverse transcription-PCR (RT-PCR) assay. Generally, all HCoVs detected except HCoV-229E showed their usual patterns of winter seasonality particularly between December and April and were not detected in summer

months while HCoV-229E was detected in the winter of 2008 but was sporadic in the following year. Detection frequencies and dominance between seasons also varied with HCoV-OC43 dominant in the first and third seasons while HCoV-HKU1 dominated the second (Forni *et al.*, 2021). HCoV-OC43 was the highest detected coronavirus, followed by HCoV-NL63 and HCoV-HKU1, which showed moderate detection frequencies, and HCoV-229E, with a comparatively low detection frequency in line with another report by Lau *et al.*, 2006. As RNA viruses HCoVs are referred to as evolving viruses as they are more prone to developing errors or mutations most of which do not lead to anything and are classed as evolutionary dead ends while occasionally some of these genetic errors cause changes advantageous to the viruses herby creating variants of concern (V'Kovski *et al.*, 2021). Their genomic evolution has been thought to be expedited by factors such as rapid urbanisation and extensive bird farming, which led to the increased mixing of species and facilitated spill over to humans and species (V'Kovski *et al.*, 2021). For example viruses related to HCoV-229E and HCoV-NL63 detected in Africa and South-East Asia in hipposiderid bats and rhinonycterid bats share up to 91% and 78% sequence identity at the genome level with HCoV-229E and HCoV-NL63 respectively (Ruiz-Aravena, 2021).

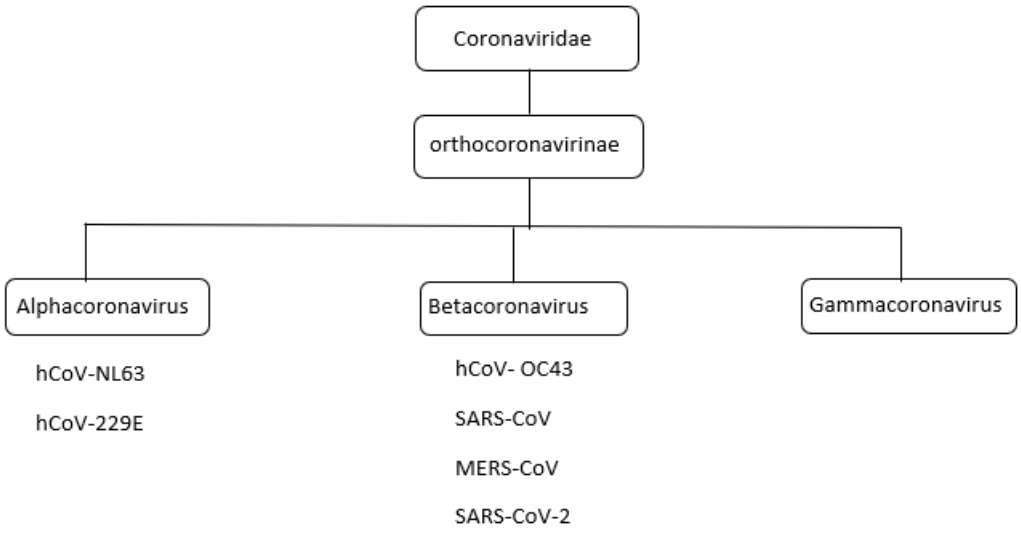


Figure 1: Taxonomy of HCoVs by the International Committee on Taxonomy of Viruses. All seven HCoVs belong to either the alphacoronavirus or betacoronavirus Genus. HCoV-NL63

and 229E belong to the alphacoronavirus genuses while HCoV-HKU1,OC43,MERS-CoV, SARS-CoV and SARS-CoV- 2 belong to the betacoronavirus genus.

1.1.1 HCoV Genome and Virion structure

General genome organization and life cycle of all HCoVs are similar but they differ in their cell receptors, internalization mechanism and the number and types of accessory genes. CoV viral particles appear to be amorphous, with distinct club-shaped peplomers (glycoprotein spike) formed by the spike (S) protein pointing outwards from the viral envelope. The virion possess a helically symmetrical nucleocapsid, which hosts a single-stranded and positive sense RNA viral genome of about 26 to 32 kilobases. The coronavirus genome is the largest known among RNA viruses (Marsolais *et al.*, 1971; Masters, 2006; Stadler *et al.*, 2003).

The positive sense viral genomic RNA acts as a messenger RNA (mRNA), comprising a 5' terminal cap structure and a 3' poly-A tail. The mRNA serves as the initial RNA of the infectious cycle, template for replication and transcription and as a substrate for packaging into the progeny virus (Hyodo and Okuno, 2014). The first two thirds of the viral RNA encode the proteins, which are translated into two large polyproteins, pp1a and pp1ab, that are processed into 15 or 16 non-structural proteins (nsp). This make up the viral replicase–transcriptase complex via proteolytic cleavage by cleaved by two proteases. Papain like protease (PLpro; corresponding to nsp3) and a main protease, 3C-like protease (3CLpro; corresponding to nsp5) (Perlman and Netland, 2009).The remaining one third of the genome contains ORFs (Open reading frame) for the structural proteins in the order 5'-spike (S)→envelope (E)→membrane (M)→nucleocapsid (N)-3', the N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope and are considered as major drug/vaccine targets **(Figure2)** (Liu *et al.*, 2014; Redondo *et al.*, 2021).

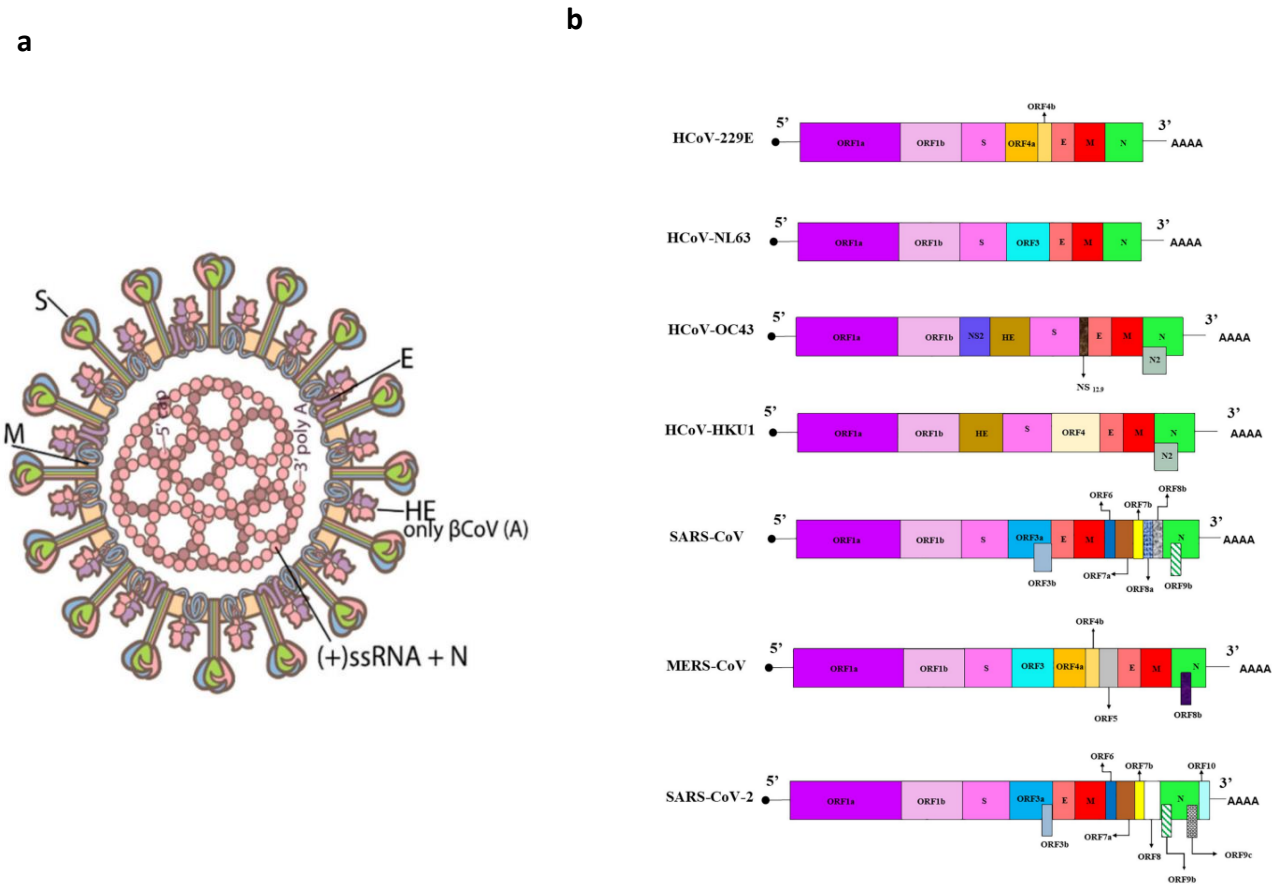


Figure 2: A schematic representation of coronavirus virion structure: Virions contain a linear positive-sense, single-stranded RNA (+ssRNA) with a 5' cap and a 3' poly(A) tail enclosed in the nucleocapsid (N) proteins. The lipid envelope houses Membrane (M), Spike (S) (16-21nm) proteins. The βCoV lineage possess additional short haemagglutinin esterase (HE) spikes (5-8nm in length), which are homodimeric type I transmembrane glycoproteins forming spikelike projections. B) Genomic organisation of HCoVs. The genome is arranged in the order of 5' -ORF1a-ORF1b-S-E-M-N-3. The overlapping open reading frames encode all the components necessary for viral RNA synthesis. The remaining one-third of the genome at the 3' end encodes structural and non-structural proteins.

1.1.2 HCoV membrane, nucleocapsid and envelope proteins

The M protein is a type III membrane protein and the most abundant structural protein in the virion which defines the shape of the viral envelope. It has a small N-terminal glycosylated

ectodomain, a triple-spanning transmembrane domain and a much larger C-terminal endodomain that extends 6–8 nm into the viral particle. The C-terminal domain forms a matrix-like lattice that adds to the extra-thickness of the envelope (Armstrong *et al.*, 1984; Nal *et al.*, 2005; Neuman *et al.*, 2011). The E protein is the smallest (~8–12 kDa) of structural proteins and have been found to be highly variable across HCoVs. Each coronavirus particle possesses an average of 20 copies of the E protein molecule individually made up of 76 to 109 amino acids (Neuman *et al.*, 2011). The E protein facilitates assembly and release of the virus and budding, but also has other functions. For instance, the ion channel activity in SARS-CoV E protein is required for viral replication and pathogenesis. CoVs lacking E have exhibit significantly reduced viral titres, stunted viral maturation and yield incompetent progeny, highlighting the importance of E protein in virion assembly (DeDiego *et al.*, 2007; Nieto-Torres *et al.*, 2011). Unlike other structural proteins, recombinant viruses missing the E protein becomes non-viable depending on virus type for example the deletion of SAR-CoV E protein attenuates the virus (Godet *et al.*, 1992; Nieto-Torres *et al.*, 2014). The N protein is the only protein present in the nucleocapsid, N protein interactions likely help bind the viral genome to the replicase–transcriptase complex (RTC), and subsequently package the encapsulated genome into viral particles (Kuo and Masters, 2013). In addition to its role as structural protein, N protein plays a role in transcription and also in pathogenesis. Expression of N protein is necessary for efficient recovery of virus from infectious SARS-CoV cDNA clones (Yount *et al.*, 2003; Yount *et al.*, 2002; Hurst *et al.*, 2009; Schelle *et al.*, 2005)

1.1.3 HCoV spike protein function and structure.

Infection of target cells by HCoVs is driven by the S-protein, which mediates attachment to the target cell receptor and viral and host cell membrane fusion. This thereby aids the entry, promotes cell-to-cell spread, and determines tissue tropism. They are also the most variable components in CoVs and are responsible for host specificity The S protein is a very large, N-exo, C-endo transmembrane protein that folds into trimers held by thermostable disulfide bonds to form the distinctive surface spikes of CoVs. S protein is inserted into the endoplasmic reticulum (ER) via a cleaved, amino-terminal signal peptide. The ectodomain makes up most of the molecule, with only a small carboxy-terminal segment constituting the transmembrane domain and endodomain (Song *et al.*, 2004).

The HCoV S-proteins are organized into an S1 subunit (outer subunit), which houses the receptor binding domain (RBD), and a transmembrane subunit (S2), which contains all elements necessary for membrane fusion (Hofmann and Poehlmann, 2004). The S1 subunit forms the head of the spike and has signal peptide, followed by an N -terminal domain (NTD) and the receptor-binding domain (RBD). The S2 subunit forms the stem which anchors the spike in the viral envelope and on protease activation enables fusion it contains conserved fusion peptide (FP), heptad repeat (HR) 1 and 2, transmembrane domain (TM), and cytoplasmic domain (CP).

Activation of HCoV S is an intricate process that requires proteolytic cleavage of S at two site, S1/S2 and S2. Cleavage at the SARS- CoV S2' site, upstream of FP, has been proposed by Madu *et al*., 2009 to trigger the membrane fusion activity of S which is believed to occur sequentially, with cleavage at the S1/S2 site. Cleavage at the S1/S2 site begins the conformational changes necessary for receptor binding and/or subsequent exposure of the S2' site to host proteases at the stage of virus entry (Bestle *et al.*, 2020)**.** The two subunits remain noncovalently linked as they are exposed on the viral surface until they attach to the host cell membrane (Chan *et al.*, 2020; Zheng *et al.*, 2006).

1.2 HCoV receptor and protease usage.

Viral entry revolves around an interplay between the virion and the host cell. Host receptors widely determine pathogenicity, tissue tropism and host range of the virus. HCoV begins by the attachment of the S protein to specific host cellular receptors, the ability of HCoVs to replicate in a particular cell type depends solely on the ability to interact with its receptors (Brian and Baric, 2005). Irrespective of the high conservation between amino acid sequence in the S1 subunit of the HCoV 229E and NL63 S protein both HCoVs utilise different cell receptors; Human Aminopeptidase N (CD13) and angiotensin-converting enzyme 2 (ACE2) respectively (Hofmann *et al.*, 2005; Yeager *et al.*, 1992) (**Table 1**). Similar to NL63, SARS -CoV1 and SARS-CoV 2 also utilise ACE2 for cell entry (Li et al. 2003; Wang et al. 2004). MERS-CoV uses human dipeptidyl peptidase 4 (DPP4) (Dveksler et al. 1991; Williams et al. 1991) while HCoV-OC43 and HKU1 both utilize 9-O-acetylated sialic acid (Gomez *et al.*, 2020; Hofmann *et al.*, 2005; Wu *et al.*, 2009; Yeager *et al.*, 1992; Zhou *et al.*, 2020b) (**Table 1**). In addition to cellular receptors endosomal cell entry is facilitated by host proteases such as

transmembrane protease serine 2 (TMPRSS2) and airway trypsin-like protease TMPRSS11D, cathepsin L, furin and trypsin (Murgolo *et al.*, 2021).

The TMPRSS2 gene encodes a predicted protein of 492 amino acids anchored to the plasma membrane (Wettstein *et al.*, 2022). It achieves its form through autocatalytic cleavage between Arg255 and Ile256 (Wettstein *et al.*, 2022). After cleavage, most of the mature TMPRSS2 remain membrane-bound, while the un-bound are liberated outside the cell (Gomez *et al.*, 2020; Lin *et al.*, 1999). TMPRSS2 is predominantly expressed in the prostate, with other organs such as lungs, colon, liver, kidneys and pancreas showing lower levels making other organs potentially susceptible to infection by viruses that utilise this protease (Song *et al.*, 2020). For example the SARS-CoV-2 virus is also known to infect kidney and liver cells (Gavriatopoulou, 2020). TMPRSS2 activates protease activated receptor 2 (PAR-2), a G-protein coupled receptor, and the activation causes the upregulation of matrix metalloproteinase-2 (MMP-2) and MMP-9, both of which are key proteases in the metastasis of tumour cells (Lucas *et al.*, 2014). Furthermore, TMPRSS2-activated hepatocyte growth factor (HGF) promotes c-Met receptor tyrosine kinase signaling and induces a pro-invasive epithelial-mesenchymal transition phenotype in prostate cancer cells(Lucas *et al.*, 2014; Lucas *et al.*, 2008). In addition to its role in cancer and SARS-CoV 2 cell entry, TMPRSS2 is known to enable cell entry of SARS-CoV (Tarnow *et al.*, 2014), MERS CoV (Shirato *et al.*, 2013) **Table 1**, Asian H7N9 influenza virus and several H1N1 subtype Influenza A viruses infections (Limburg *et al.*, 2019). Hence TMPRSS2 is a viable target as an antiviral strategy to treat CoVs and low pathogenic influenza virus infection (Cheng *et al.*, 2015; Matsuyama *et al.*, 2010; Sakai *et al.*, 2014; Tarnow *et al.*, 2014)

Viral attachment begins with a modification in the S protein that triggers the fusion of viral and cellular membranes. In a study conducted by Bestle *et al.*, 2020 it was demonstrated that the SARS-CoV-2 S protein is cleaved by Furin at the S1/S2 and TMPRSS2 S2' site. Cleavage by both proteases have been identified to be crucial, occurring sequentially first at the S1/S2 and then S2'. Cleavage at the S1/S2 site is believed to be instrumental in activating conformational changes required for receptor binding and/or subsequent exposure of the S2' site to host proteases for virus entry (Bestle *et al.*, 2020). After fusion, the viral RNA is released into the cell and takes over the cell machinery to replicate more virions which infect more cells. Virus-

infected cells rearrange their endo membranes to establish viral factories, where the viral genome is replicated and transcribed, infection leads to the formation of double-membrane vesicles (DMVs) that are continuous with ER-derived convoluted membranes. These membrane covered vesicles protect the viral RNA from recognition by host cell innate immunity mechanisms, thus providing safe havens for viral RNA replication. At the point of assembly after replication and transcription of viral RNAs, the genomic and sub-genomic mRNAs exit the DMVs. In the cytoplasm they are translated in ER-associated ribosomes to create the viral envelope proteins E, M, and S, which contain N-terminal signal sequences for ER translocation while the nucleocapsid protein N is synthesized using free ribosomes (V'kovski *et al.,* 2020). Translated structural proteins then translocate into endoplasmic reticulum (ER) membranes moving through the ER-to-Golgi intermediate compartment (ERGIC), where interaction with the N-encapsidated newly produced genomic RNA activates budding into the lumen of secretory vesicular compartments. Virions are secreted from the infected cell by exocytosis. In addition to the endosomal route of entry, some CoVs may also utilise the non-endosomal pathway, or a combination of both **Figure 3** (Burkard *et al.*, 2014).

Cathepsins are non-specific proteases which exhibit endopeptidase and exopeptidase activities and are activated in protein degradation in the late endosomes and lysosomes. They are mainly classified by their structure, catalytic mechanism, and types of proteins they cleave (Turk *et al.*, 2012). They are generally distributed into three classes based on their catalytic activity: aspartic (D and E), serine (G) and cysteine (B, C, K, L, S and V) proteases. Of these, cysteine proteases (cathepsins B, L and S) contribute the most to viral entry (Jackson *et al.*, 2022). Cathepsin-L cleaves the SARS-CoV-1 S protein and proteolytically activates membrane fusion while unlike the SARS-CoV-1 S protein, the SARS-CoV-2 S protein is pre-cleaved by furin at the S1/S2 cleavage site making the effects of cathepsin L in SARS-CoV-2 relatively replaced by those of furin (Simmons *et al.*, 2005). However, Zhao *et al.*, 2021 directly detected the cleavage of purified SARS-CoV-2 S protein by cathepsin L where addition of Cathepsin L resulted in cleavage of purified SARS-CoV-2 S protein, likewise cathepsin L also efficiently cleaved purified SARS-CoV-2 S protein in a dose-dependent manner suggesting the involvement of cathepsin L in tropism of SARS-CoV-2. This is further confirmed by Bayati *et al.*, 2021 who explained that if the target cells express insufficient TMPRSS2 or if a virus–ACE2 complex does not encounter TMPRSS2, ACE2-bound virus is internalized via clathrin-

mediated endocytosis into the late endolysosome, where the S2' site is cleaved by cathepsin L further diversifying cellular infection route. The ability of cathepsin L in processing the S2' site is supported by partial inhibition of PV entry by cathepsin L inhibitors in cells overexpressing TMPRSS2 further affirming a non-endosomal entry of SARS-CoV-2 (Hoffmann *et al.*, 2020a; Ou *et al.*, 2021). The low pH in the cellular environment and cathepsins may help to facilitate membrane fusion and endosomal CoV cell entry **Figure 3** (Zumla *et al.*, 2016).

Targeting host proteases as treatment for CoV infection appears to have fewer side effects than targeting cellular receptors as the latter play significant roles in regulating various metabolic activities (Kaur *et al.,* 2021). For example ACE2 the receptor for HCoV- NL63, SARS-CoV and SARS-CoV-2 regulates several vital parameters, such as blood pressure while proteases have fewer manageable side effects. (Turner *et al.*, 2004). Therefore, drugs like camostat mesylate, nafamostat mesylate which blocks TMPRSS2 activity and E-64d a cathepsin L inhibitor have been put forward as COVID-19 antiviral candidates (Hoffmann *et al.*, 2020a; Hoffmann *et al.*, 2020b).

Hydroxychloroquine has been reported to prevent SARS-CoV-2 cell entry by blocking the endocytic pathway where it suppresses the clathrin-mediated viral entry route and also prevents endosomal acidification, the latter being a necessary condition for cathepsin L activity (Hu *et al.*, 2020; Ou *et al.*, 2021; Sanders *et al.*, 2020). While studies suggest that hydroxychloroquine therapy on its own may be inadequate, its efficacy in combination with camostat mesylate is under clinical evaluation. Similarly inhibition of cell entry of SARS-CoV and SARS-CoV-2 when cells are treated with a combination of camostat mesylate and E-64d suggest the effectiveness of their use in combination therapy rather than as monotherapy (Hoffmann *et al.*, 2021).

Table 1. Human coronaviruses receptors and proteases: The Alpha-CoVs include 229E and NL63, whilst the Beta-CoVs include OC43, HKU1, and SARS-CoV-2. HCoV-229E utilise cellular

receptor CD13. NL63, SARS-CoV 1 and SARS-CoV 2 use receptor ACE2, while OC43 and HKU1 use receptor 9-O-Acetylated sialic acid and MERS uses cellular receptor DPP4. Cellular receptors include TMPRSS2, Cathepsin L, Furin Clathrin and HAT.

Genera	**Strains**	**Cellular receptor**	**Protease**	**Host**	**References**
Alpha-CoV	HCoV-229E	Human Aminopeptidase N (CD13)	TMPRSS2, CathepsinL, Trypsin	Bats	Bertram *et al*.,2013; Kawase *et al*.,2009
Alpha-CoV	HCoV-NL63	ACE2	TMPRSS2, Clathrin	Palm civets, Bats	Milewska *et al*.,2018
Beta-CoV	HCoV-OC43	9-O-Acetylated sialic acid	?	cattle	Tang *et al*., 2020
Beta-CoV	HCoV-HKU1	9-O-Acetylated sialic acid	?	Mice	Gierer et al., 2013; Qian *et al*., 2013
Beta-CoV	SARS-CoV 1	ACE 2	TMPRSS2, Furin, Cathepsin L	Palm Civets, Bats	Simons *et al*., 2005; Bosh *et al*., 2008
Beta-CoV	MERS-CoV	DPP4	TMPRSS4, Furin, TMPRSS2, Cathepsin L, HAT	Camels, Bats	Gierer *et al*., 2013; Qian *et al*., 2013
Beta-CoV	SARS-CoV 2	ACE2	TMPRSS2, Furin, Cathepsin L	Bats	Bestle *et al*., 2020; Shnag *et al*., 2020

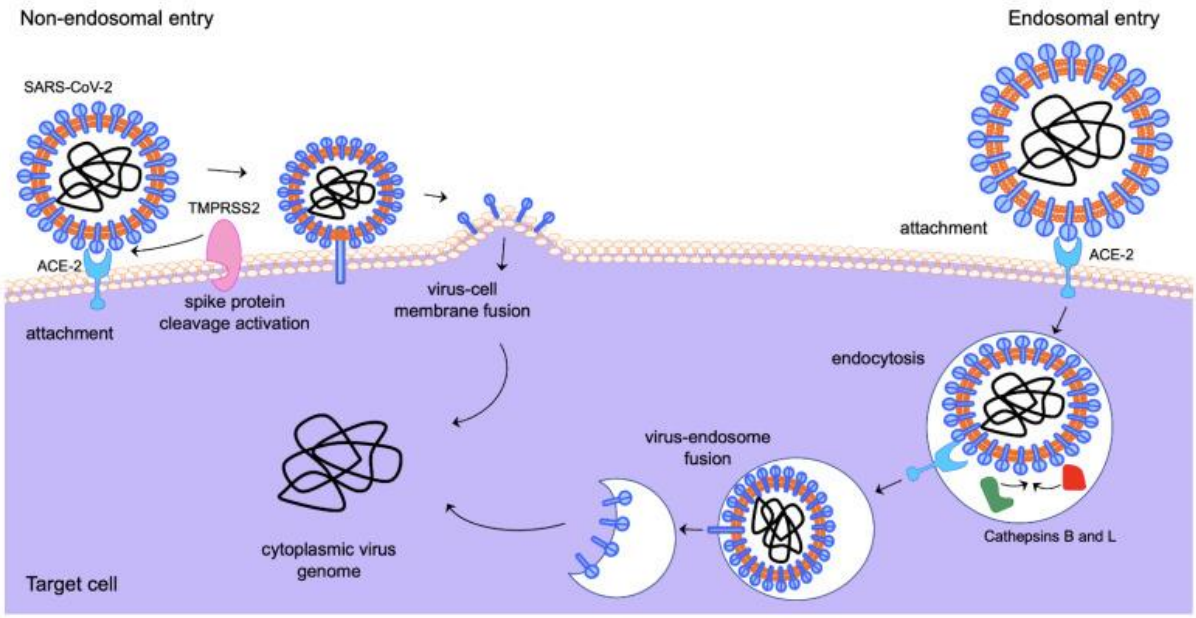


Figure 3: Schematic representation showing endosomal and non-endosomal entry routes of SARS-CoV-2 into target cells. SAR -CoV-2- Spike protein binds to cellular receptor ACE2 and is cleaved by protease TMPRSS2. In target cell with low TMPRSS2 expression the virus and ACE2complex is internalized via clathrin mediated endocytosis then Spike protein is cleaved by cathepsins B and L. Upon successful entry via both pathways both viral genome is released into cell cytoplasm for new viron replication and assembly schematic representation. Figure extracted from Giotis *et al.,* 2022.

1.3 Steroid receptors

Steroids are complex four-ringed organic molecules derived from cholesterol through a specific enzymatic pathway in endocrine organs, they are involved in a wide range of roles and functions in multicellular organisms (Miller and Auchus, 2011). Steroids become activated by binding to specific steroid receptors. These receptors are part of the nuclear receptor family and function as transcription factors. There are six known steroid receptors: oestrogen receptor-α (NR3A1, ESRα), oestrogen receptor-β (NR3A1, ESR), glucocorticoid receptor (GR, NR3C1), mineralocorticoid receptor (MR; NR3C2), progesterone receptor (PR; NR3C3/PGR) and androgen receptor (AR; NR3C4) (Evans, 1988; Mangelsdorf *et al.*, 1995; Novac and Heinzel, 2004; Schwabe *et al.*, 1993). The receptors are usually located in the nucleus where they are activated or in the cytoplasm where they move into the nucleus following activation

by the steroid hormones (Beato and Klug, 2000). For example, oestrogen enters the cytoplasm where it interacts with intracellular ERα and Erβ. Upon activation the receptors bind to specific DNA sequences, termed oestrogen response elements (Wierman, 2007). The ligands for the MR and GR are usually produced in the adrenal cortex while ER, AR and PG are circulated by the gonads (Wierman, 2007). The steroid hormones control a wide range of physiological and cellular processes such as sexual differentiation, growth, and reproduction to immunity, brain function, and behaviour. In light of the current COVID-19 pandemic it has been observed that the severity of cases is unequal in different patients, with worse outcomes reported in men (Onder *et al.*, 2020; Shim *et al.*, 2020). In addition to various biological differences in the immune system and behavioural factors, sex chromosomes and hormones have been identified to explain sex differences in COVID-19 morbidity and mortality (Acheampong *et al.*, 2020). For example the presence of an additional X chromosomes in females is said to benefit females by causing a sex specific phenotype which increases with age which may offer protection against deleterious mutations (Schurz, 2019). Furthermore studies show that steroid hormones control the differentiation of B and T lymphocytes and innate immune activity (Gilliver, 2010), generally from cumulative research androgens are considered as predominantly immunosuppressive while oestrogens are classed as immunoprotective (Taneja, 2016; Khan and Ansar,2015; Bouman *et al.,* 2005).

1.3.1 Oestrogen structure and function.

The biological effects of oestrogen were initially believed to be mediated singularly by ERα until the cloning of ERβ. Even though similar, both receptors are products of different genes differentially expressed in different tissues and both play distinctive roles in oestrogen signaling (Kuiper *et al.*, 1996). The varying susceptibility to CoVs in women when compared to men has been linked to the effect of oestrogen upon the immune activity of B and T-helper cells (Taneja, 2018). ERα directly affects Th1, Th2, Th17, and T regulatory cells, as well as follicular helper T (TFH) cells. A study by Rehman *et al.*, 2021 identified that NP-conjugated ovalbumin immunisation produces specific antibodies that are elevated in CD4-ERα knock-out mice, in the presence of oestrogen. Hence, oestrogen is a key factor that enhances women's immune system against viruses (Goggins, 2004). Studies conducted on COVID-19 patients, have found that 51% of patients died due to cardiac injury (Guo *et al.*, 2020). This could be due to the regulation of ACE2 in the heart by 17β-estradiol. This occurs by increasing

the locally existing ACE2 effect on the cardiac tissue and suppressing the renin-angiotensin system RAS through catalytic cleavage of a particular residue of angiotensin II. To increase the release of cardioprotective angiotensin and enhance anti-oxidative and anti-inflammatory effects. Oestrogen level is also related to the regulation of cardiac troponin secreted during ischemic or anoxic conditions, leading to detrimental injury in cardiac cells (Joyner *et al.*, 2007; Mingels and Kimenai, 2018). In non-lung cells, such as prostate, breast and kidney, oestrogen has been demonstrated to inhibit TMPRSS2 expression, Lemes *et al.*, 2021 demonstrated that previous and 24-hours post-infection, cells treated with 17β-estradiol revealed a reduction in the viral load.

1.3.2 Progesterone structure and function.

Progesterone is an endogenous steroid involved in the menstrual cycle, pregnancy, brain function and embryogenesis of humans and other species (Baulieu and Schumacher, 2000). It is also an importsant metabolic intermediate in the production of other steroids, such as the sex hormones and the corticosteroids. The role of progesterone in HCoV infection remains to be explored. A research study in female mice observed that administration of progesterone to female mice provides defense against lethal and sublethal influenza A virus infection (IAV) by inducing production of the epidermal growth factor and amphiregulin (Hall *et al.*, 2016). Specifically, PR reduces pulmonary inflammation, enhances lung function, repairs damages to lung epithelium, and promotes faster recovery after IAV infection. Another study by Shilpa and Roby, 2019 also recorded the anti-inflammatory and analgesic effects of PR. In the case of SARS CoV 2 the direct impact of PR is difficult to quantify as PR have no obvious direct effect on infection however a number of indirect effects have been explained. For example PR inhibits androgens blocking the upregulation of TMPRSS2 by androgens (De Leo *et al.*, 2016) .

Sigma receptors found in locations such as the lung, liver and intestines have been highlighted to play a role in the infectivity of SARS-CoV-2 (Gordon *et al.*, 2020; Hayashi and Su, 2005).When an agonist binds to the sigma receptor, it modulates a voltage-gated ion channel that accelerates ER stress (Miguel Vela, 2020). Replication of CoVs occurs in membranous compartments which creates stress in the hosts and activates pathways that adapt the host cell machinery to viral needs. Progesterone blocks sigma receptors and the receptor-

mediated modulation of the voltage-gated ion channel (Johannessen *et al.*, 2013). Hence creating a disadvantage for the CoVs by controlling ER stress.

1.3.3 Androgen structure and function

Androgens are steroid hormones that regulate the development and maintenance of male features in vertebrates by binding to AR receptors (Chen *et al.*, 2019). Previous studies show that AR in male mice reduce the severity of IAV infection by promoting the resolution of pulmonary inflammation, similarly data acquired by vom Steeg *et al.*, 2020 also suggest that AR receptor signaling downregulates inflammatory immune response and confers protection against IAV (vom Steeg *et al.*, 2020a; vom Steeg *et al.*, 2016). Multiple androgen pathways in COVID-19 infection have been identified, one of them is that of ACE2 expressed in different tissues such as the lungs, liver, kidneys, and prostate (Xu *et al.*, 2020) has been shown to have reduced expression in the presence of low levels of androgen hormones (Dalpiaz *et al.*, 2015). The TMPRSS2 gene is expressed principally in the adult prostate,and localised and metastatic prostate cancers. This gene is also expressed in other tissues such as the colon, small intestine, pancreas, kidney, lung, and liver (Lucas *et al.*, 2014; Lucas *et al.*, 2008; Wambier *et al.*, 2020). Androgen activity is believed to be soely responsible for the transcription of TMPRSS2 gene and no other TMPRSS2 regulating pathway has been described in humans to date (Wambier *et al.*, 2020). Androgen treatment has been shown to increase TMPRSS2 expression and zymogen activation in cell culture and in a mouse xenograft model, suggesting AR regulates TMPRSS2 at the transcription and post translation in an intrinsically dependent manner (Afar *et al.*, 2001).

Androgen sensitivity may be a relevant contributor to COVID -19 disease severity, which explains the notably severe outcomes in female patients who present with low metabolic syndrome, or are using birth control methods containing progestogen hormones that bind to AR receptor (Wambier *et al.*, 2020). Several studies have demonstrated that androgen activity is affected by the length of the CAG repeat in the first exon of the androgen gene. Shorter CAG repeat length pre-dispose men to develop androgenetic alopecia, acne and oily skin. Therefore, it is belived that the CAG repeat in the AR gene affecting the expression of essential cofactors neccesary for ARE (Androgen response element) may be associated with increased COVID-19 disease severity and mortality (Wambier *et al.*, 2020; Heemers and

Tindal 2007; McCoy *et al.,* 2021). Similarly short CAG repeat have been associated with severe postrate cancer prognosis (Tirabassi, 2015).

1.4 Pseudotyped viruses and their uses.

Due to the complexities of assessing and having appropriate laboratory facilities in studying highly infectious viruses alternatives to authentic viruses called pseudotyped viruses (PVs) are widely used tools (Bentley *et al*., 2015). Discovered when co-infection of Rous sarcoma virus and a helper avian leukosis virus; Rous associated virus (RAV) produced and RSV particle enclosed within an RAV outer membrane. (Rous, 1911; Rubin, 1965; Hanafusa *et al.,* 1963). A PV is a recombinant enveloped virus particle comprising a virus core surrounded by a cell-derived membrane bearing envelope proteins derived from a different virus (Clapham, 1992; Sanders, 2002; King *et al* .,2015). Since the genes inside a PV are typically changed, they do not encode viral envelope proteins, limiting their ability to do no more than just transduce a target cell, initiating one round of nucleic acid replication without producing infectious particles (Zhang *et al.,* 2017; King *et al.,* 2015). When compared with authentic viruses PVs can be safely used in BSL2 laboratories making them a widely distributed system used for studying viral tropism, receptor recognition, virus inhibition and vaccine development (Bartosch *et al., 2003*; Radoshitzky *et al .,* 2007). PVs have been used extensively to study the neutralization of highly infectious viruses such as Ebola, Marburg, Lassa Fever, Rabies and the recent culprit of the Covid-19 pandemic SARS-CoV2 (Bentley *et al.,* 2015; Steffen and Simmons, 2016) The Human immunodeficiency virus (HIV) Vesicular stomatitis virus (VSV) and Murine Leukaemia virus (MLV) are the most used backbones for viral production over the years, the back bones have undergone series of alterations including deletion of promoter sequences and accessory proteins (Suzuki and Suzuki, 20011; Sandrin and Cosset, 2006). The preferred production system choice For PV backbone when pseudotyping a new virus is usually decided by comparison of successful systems published for related viruses. For example, the VSV system was used to study the Ebola virus while the HIV-1 system is mostly used for the HCoVs (Takada *et al.,* 1997; Sampson *et al.,* 2021). To create HCoV PVs, producer cells are co-transfected with plasmids encoding a CoV spike protein, a lentiviral Gag-pol construct and the pCSFLW firefly luciferase reporter (King *et al.,* 2015). Efficacy of PVs are quantified using luciferase assays where the pCSFLW reporter is integrated and expressed

after spike protein mediated entry. Luminescence is then measured from transduced cells by a luminometer (Sampson *et al.,* 2021).

1.5 Antiandrogens, TMPRSS2 and SARS-CoV-2

Antiandrogens belong to a class of drugs that prevent androgens from mediating their natural effects in the body. They act by binding to and inhibiting androgens (Mowszowicz, 1989). Antiandrogens are used to treat an assortment of androgen-dependent conditions (Student *et al.*, 2020). In men, antiandrogens are widely used in the treatment of enlarged prostate, hair loss, early puberty, high sex drive, problematic sexual urges, and prostate cancer. In women, antiandrogens are employed in the treatment of acne, seborrhea, excessive hair growth, scalp hair loss (Student *et al.*, 2020; Wirth *et al.*, 2007). They are also administerd during feminising hormone therapy for transgender women and as puberty blockers in transgender girls (Student *et al.*, 2020).

As discussed earlier the regulation of TMPRSS2 by AR offers a probable path to the development of therapies for SARS CoV-2 as knockout of TMPRSS2 in mice shows no detrimental phenotype, whereas downregulation of ACE2 is associated with increased severity of SARS-induced lung injury (Kim *et al.*, 2006). Furthermore, TMPRSS2 expression levels have been shown to be associated with disease severity in mouse models of coronavirus infection, and its inhibition was shown to inhibit SARS-2-S-driven entry in lung cells (Iwata-Yoshikawa *et al.*, 2019). In a recent study by Leach *et al.*, (2021) antiandrogens were shown to reduce TMPRSS2 levels in human lung cells and mouse lung therfore reducing SARS-CoV-2 entry in lung cells (**Figure 3)**. Other alternative mechanisms of antiandrogens have also been described for example, Qiao *et al.*, 2021 observed that antiandrogens can also downregulate expression of ACE2. This suggested that there might be more that one pathway used by antiandrogens to interfere with the lifecycle of CoVs. Therefore antiandrogens have been proposed as a treatment option for COVID-19.

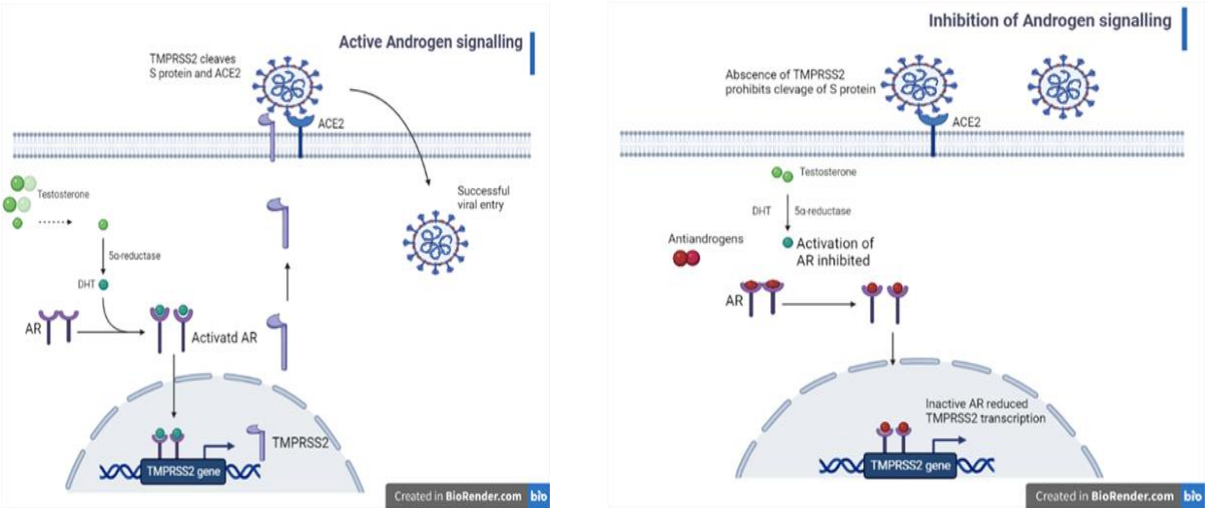


Figure 4: Schematic representation of how AR is activated inducing TMPRSS2 expression in cytoplasm (**a**). While schematic illustration of how inactivation of AR by antiandrogens, inhibiting TMPRSS2 transcription and reducing SARS-CoV-2 entry shown in **b.** The pressence of theraputic antiandrogens effectively inactivates AR therefore reducing TMPRSS2 transcription and ultimately reducing host cell susceptibility to SARS-CoV-2. Schematic inspired by (Leach *et al.*, 2021) and created using https://biorender.com/.

1.6 Hypothesis

In addition to TMPRSS2 mediated cell entry of SARS CoV2 studies by Bertram *et al.*, 2013; Shirato *et al.*, 2018 indicate that TMPRSS2 can activate the spike protein of the sHCoVs for entry into cells that are naturally susceptible to infection. Similarly TMPRSS2 has been identified as an androgen regulated gene in the lung cells and antiandrogens downregulate TMPRRS2 reducing cell entry of SARS-CoV-2 in lung cells (Leach *et al.,* 2021). Hence it is hypothesised that similar to SARS-CoV-2, antiandrogens can be used to prevent and or reduce viral entry of other sHCoVs.

1.7 Objectives

The objectives of the study are:

- To establish and confirm whether TMPRSS2 is essential for cell entry of HCoVs.
- To assess if the antiandrogen enzalutamide can block cell entry of HCoVs by using pseudotyped and further confirmed by natural viruses.

- To investigate the effect of HCoVs on androgen receptor signaling, using publicly available and in-house transcriptome data.

CHAPTER TWO: MATERIALS AND METHODS.

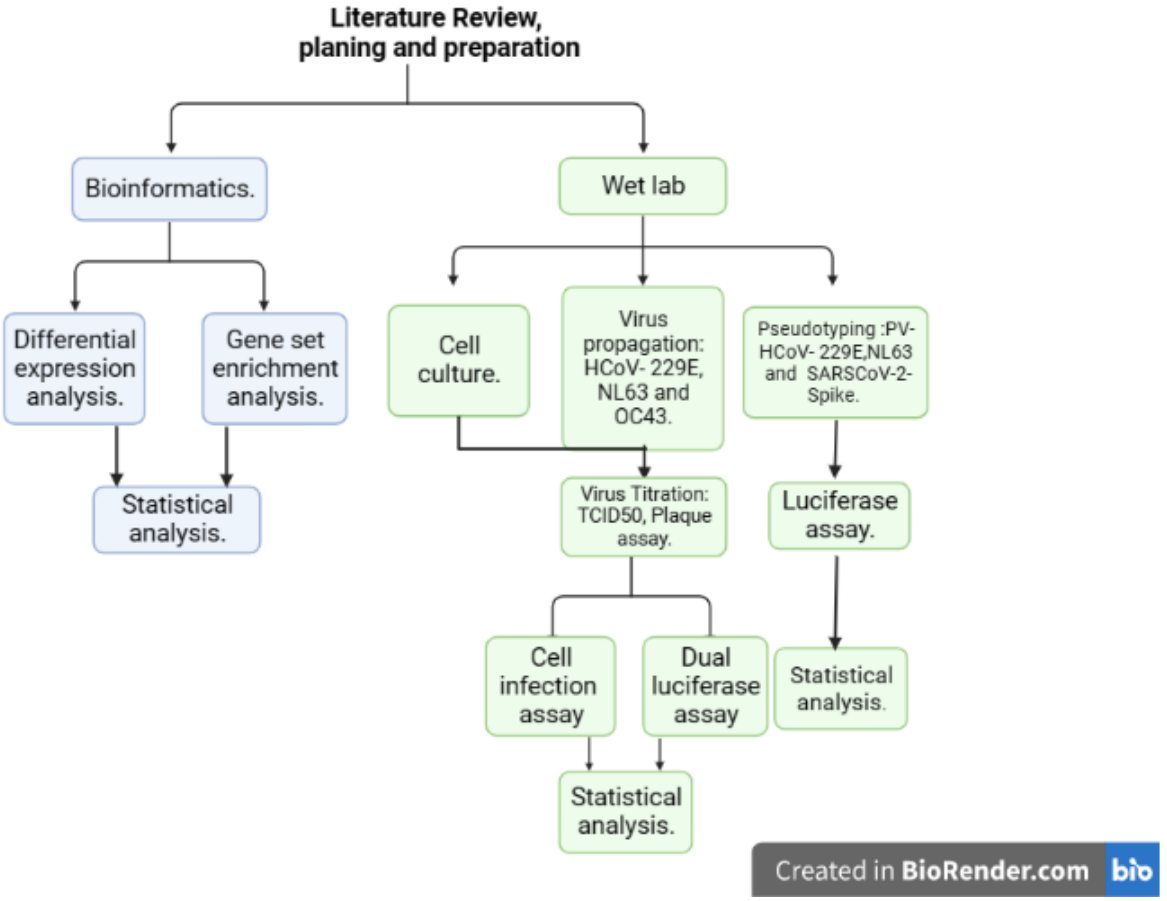


Figure 5: Schematic representation of method flow, highlighting the step by step process of methods used during this study. Diagram created using BioRender.com

2.1 Cell lines.

All cell subculture was performed within the Biosafety Level 2 (BSL-2) laboratory under University of Essex health and safety guidelines. Human embryonic kidney cell line 293 expressing the SV40 T-antigen (HEK293T/27), HEK239T cells stably expressing human ACE2 (HEK293T+ACE2), Chinese hamster ovary cells (CHO), CHO cells stably expressing ACE2 and TMPRSS2. Adenocarcinomic human alveolar basal epithelial cells (A549), A549 stably expressing ACE2 /+TMPRSS, LNCAP, VERO E6+ACE2+TMPRSS2 (VAT), MRC-5, HRT-18G and A549+ACE2 and A459+ACE2+TMPRSS2 were stored in liquid nitrogen and thawed in 37°C

water bath before use. All cell lines were grown in 37°C and in a humidified atmosphere of 5% CO_2 using various medium as stated in **Table 2**.

Table 2: List of all cell lines used and their respective growth medium. All cells were subculture bi-weekly and grown at 37°C and in a humidified atmosphere of 5% CO_2. All cell culture media used were gotten from Gibco Thermo Fisher Scientific.

Cell Lines	**Growth media**
A549 (Hypotriploid alveolar basal epithelial cells), 293T (Human embryonic kidney cells), HRT-18G (human rectal cancer cell line)	Dulbecco's Modified Eagle's medium (DMEM), 10% fetal bovine serum, 1% penicillin+ streptomycin and 1%Penstrep
293T+ACE2 (Human embryonic kidney cells stably expressing ACE2)	DMEM , 10% FBS, 1%Penstrep and 1µg/ml puromycin
MRC-5 (Human Fetal Lung Fibroblast Cells)	MEM 10% FBS, 1% P/S.
Vero+ACE2+TMPRSS2 (African green monkey cells stably expressing ACE2 and TMPRSS2)	DMEM 500 mL, FBS 10%, Hygr B 2mL , Geneticin 10 mL, Non-essential Amino Acid 5 mL
CHO+TMPRSS2 +ACE2 (chines hamster ovary cells stably expressing ACE2 and TMPRSS2)	Ham's F12 media 10%, 1% P/S, 2 ug/mL Puromycin, 100 ug/mL Hygromycin.
A549+ACE2+TMPRSS2 (Hypotriploid alveolar basal epithelial cells stably expressing ACE2 and TMPRSS2)	F-12K Nutrient mixture Heat inactivated Foetal bovine serum, 10% 2mM Glutamine 2mg/ml Geneticin (G418) 200 µg/ml Hygromycin B 100 Units Penicillin and 100ug Streptomycin/ml
A549+ACE2 (Hypotriploid alveolar basal epithelial cells stably expressing ACE2 and TMPRSS2)	F-12K Nut Mix Heat inactivated Foetal bovine serum, 10% 2mM Glutamine 200 µg/ml Hygromycin B 100 Units Penicillin and 100ug Streptomycin/ml

2.2 Plasmids

Plasmids used include pCAGGs-gag/pol packaging construct derived from lentiviral vector of human immunodeficiency virus-1 (HIV-1) (cat.#: VC101743) (ORIGENE) (Giotis *et al.,*2019), and pCSFLW firefly luciferase expression vector (cat.#: TR30004) (ORIGENE) (Giotis *et al.,*2019). Plasmids of pcDNA3.1 envelope proteins include VSV-G (Vesicular stomatitis virus envelope G pantropic envelope protein) (cat.#: V79020) (Addgene), SARS-CoV-2 S (cat.#: VC102557) (Amsbio) (Leach *et al.,* 2021), HCoV-229E and NL63. Plasmids were transformed into *Escherichia coli* competent cells (Kind donations from Lynwen James University of Essex) by heat shock 45°C for 1 minute, followed by addition of standard microbial growth medium (2XYT) and incubation at 37°C with constant shaking (250rpm) for 30min. Then, bacterial cells were cultured in 2XYT agar plates supplemented with ampicillin overnight at 37°C. Freshly transformed bacterial cell colonies were inoculated into 100ml 2XYTbroth supplemented with ampicillin then incubated at 37°C with constant shaking (250rpm) for 16 hrs. Harvesting of the bacterial cell cultures was performed by centrifugation at 3000 x g for 10 minutes to remove supernatant. Total DNA plasmid was extracted with Jena Bioscience, Germany Midi and Mini -Prep kit. Lentiviral Packaging constructs were kind donations from Professor Nigel Temperton (Viral Pseudotype Unit, Kent).

2.3 Virus stocks production.

All work involving the use of HCoV- NL63,229E and OC43 was performed within BSL-2 laboratory under Health and Safety guidelines. While work involving authentic SARS-CoV-2 was performed by Dr Stathis Giotis at Imperial college London. The HCoV-NL63 reference strain (isolate Amsterdam 1 [Ams-001]) was obtained from Dr. Lia van der Hoek and the HCoV-OC43 and 229E strain was obtained from ATCC (VR-1558™, VR-740™) and propagated in MRC5 cells. MRC-5 cells were infected with a multiplicity of infection (MOI 1) and supplemented with MEM and 2% heat-inactivated FBS, 1% penicillin and 1% streptomycin. The HCoV-229E and HCoV-OC43 cultures were incubated at 5% CO_2 and 35℃, respectively for optimal virus production. The cytopathic effect (CPE) of infected cultures was daily monitored by optical microscopy. The HCoV-229E and OC43 containing supernatant was harvested at 7 days post-infection. Supernatants were centrifuged for 5 min at 1500rpm to allow any cell debris settle and then filtered using a 45um filter. Aliquots stored at −80°C (Lei *et al.*, 2021).

2.4 Virus titration.

MRC5 and HRT-18G cells were seeded in 6-well plates at 1 x10^6 to achieve full plate confluency the day after. Sub-confluent monolayer was washed using PBS and cells infected with 100ul of virus solution (HCoV 229E and OC43) diluted in 1/10, 1/100, 1/1000, 1/10000, 1/100000, 1/10000000 folds using suitable media containing 2%FBS. The plates were incubated for 2 h at 35°C, 5% CO_2 with plates being gently rocked every 30 minutes. The inoculum was aspirated, and cells were maintained in 3ml of 0.8% methylcellulose semi solid overlay in equal amount of suitable media. Cells were incubated at 35°C for 7days. To visualise the plaques, the overlay was removed, and cells were fixed with 2% crystal violet in 10% formalin in PBS for 2hrs. Plates were washed gently under tap water. For $TCID_{50,}$ confluent 96 well plates were doubly diluted with virus in row 1-8 across 4 columns. Virus was left on the cells and incubated at 35°C, 5% $CO_2$for 7days after which plates where visualised with crystal violet. The final viral titre was reported by calculation of a 50% tissue culture infective dose per ml ($TCID_{50}$/ml) according to the Reed–Muench formula (Reed & Muench, 1938).

2.5 Production of Lentiviral Pseudotypes (PV) for HCoV-229E, NL63 and SARS-CoV-2

PV's were produced in HEK293T/17 cells. HEK293T/17 producer cells were sub-cultured in T-25 flasks (Thermo Fisher Scientific, Waltham, MA, USA) and co-transfected at 80% confluence with 700 ng of the p8.91 lentiviral packaging plasmid, 1100 ng of the pCSFLW firefly luciferase reporter plasmid and 700ng of HCoV-229E, NL63 and SARS-CoV-2 S-protein expression plasmid per individual flask. The transfection complex was prepared in 150 µL Opti-MEM™ (Thermo Fisher Scientific, Loughborough, UK) using 7.5 µL FuGENE®-HD (Promega, Madison, WI, USA) transfection reagent in ratio 1:3 (DNA: Fugene). The cell supernatant was collected after 72 h using sterile 2.5 mL syringes and passed through a 0.45 µm cellulose acetate (Millipore Sigma, Burlington, MA, USA) prior to storage in microcentrifuge tubes at −80 °C (Di Genova, 2021).

2.6 PV titration and luciferase assay

100µl of the pseudotyped virus supernatant stock was pipetted into row A of Nunc™ MicroWell™ 96-Well, Nunclon Delta-Treated, Flat-Bottom Microplate (Cat # 136101) and 50µl was taken to perform doubling dilutions through to row D containing 50µl complete media. Dilutions were properly mixed by pipetting up and down for a minimum of 7 times with tips

replaced after mixing. Subsequently, 4×10^4 target cells was added to each dilution, seeded in a total volume of 50µl per well. Following a 48-hrs incubation at 37°C with 5% CO_2, the supernatants were carefully aspirated. Each well was lysed with 50µl Bright-Glo™ (luciferase substrate) and PBS mix prepared at a ratio 1:1. All PVs encode the luciferase reporter gene, which is expressed only upon successful entry into host cells. Pseudotyped virus particles which lacked viral envelope glycoproteins, served as a negative control (Δ ENV) in order to identify the baselines of background signals in target cells PVs transfected with VSV-G surface glycoprotein served as positive control. For experiments involving protease inhibitors E-64d; 10, 25, 50µM Camostat mesylate; 50, 100, 200 µM, cells were treated 24 hrs before transduction.

2.7 Luciferase and Renilla reporter assay.

MRC-5 cells were seeded in 96well plates at $1x10^4$ cells/well in hormone depleted DMEM media (hormone depleted media used to study the actual effect of the viruses without misleading effects of hormones endogenous to the growth medium) for 24hrs prior to transfection. Each well was transfected with 0.3ul TAT-GRE-EIB-LUC (500ng/ul), 0.1ng pSV-AR (100ng/ul) and 0.1ul of renilla (100ng/ul) using FuGENE HD transfection reagent. 24hr post infection, cells were treated with 1nM Mibolerone at 48hrs and infected with authentic virus at an MOI of 1 for 2hrs at 72hrs. At 98hrs cells were lysed using 25ul Dual-Glo® Luciferase Assay System (Promega) and incubated for 15minutes with gentle shaking. Substrate was the transferred into 96-well microplates and luminescence was measured, next 20ul of Dual-Glo Stop and Glo reagent (Promega) was added and incubated as before. Renilla luminescence was measured for normalisation using FLUOstar® Omega plate reader BMG Labtech, Germany.

2.8 Cell infection and stimulation

MRC-5 cells were seeded in a 12- well plate and incubated at 37°C with 5% CO_2, until they reached 70% confluency. Uninfected (mock) sample were included in comparison to HCoV-229E and OC43. The cell media were initially replaced with fresh culture media and cells were pre-treated with antiandrogen Enzalutamide (1ug/ml) 48hrs prior to infection. Then, cell media were removed and wells were washed once with PBS and then treated with virus inoculum at an MOI of 1. Cells were incubated at 35°C with 5% CO_2 for 4hrs, and virus

inoculum was removed. All wells were washed with PBS and replenished with 2ml cell culture media. The plates were incubated at 35°C and 5% CO_2 for 48 hrs. At the selected time point supernatant containing cell free progeny virus was harvested, cells were washed once with PBS, spun at 3000 x g for 5 minutes, and then stored in 350µl RNA later in the -80 freezer.

2.9 Extraction of RNA

Total cellular RNA was extracted from infected cells using RNeasy mini kit (QIAGEN, Germany) following the manufacturer's provided instructions. Viral RNA from the cell supernatant was extracted with TRIzol™ Reagent-Thermo Fisher Scientific as per the manufacturer instructions. The concentration of extracted RNA was quantified by using a NanoDrop ND-1000 Spectrophotometer ThermoScientific.

2.30 Reverse transcription

RNA samples extracted from cells were thawed on ice and for reverse transcription to produce complementary viral DNA. The QuantiTect Reverse Transcription Kit (QIAGEN) was used. For every 100ng RNA : 2µl genomic DNA wipeout buffer and RNase-free water to top up for a total of 14µl genomic elimination reaction, which was incubated at 42°C for 2 minutes and placed immediately on ice. Then, an RT master mix was prepared using 1µl Quantiscript Reverse Transcriptase, 1µl RT primer mix, and 4µl Quantiscript RT buffer. Following, 6µl of the RT master mix was added to each sample for a 20µl total volume and incubated at 42°C for 30 minutes and 95°C for 3 minutes. The aliquots of generated cDNA samples were used directly for Real-time PCR (RT-PCR) or stored at -20°C

2.31 qPCR

The cDNA aliquots and primers were thawed in ice and reactions were set in duplicate on a 96-well plate. Each 2µl cDNA was mixed with 5µl PowerUp SYBR Green master mix (Applied Biosystems, US), 0.3µl forward primer (10µM), 0.3µl reverse primer (10µM) and 2µl ddH2O. A PCR plastic film was applied to cover the plate which was spun once after adding the primer mix and once before adding the cover. PCR cycle temperatures in **Table 3.**

Table 3: Thermal cycling conditions for PowerUp SYBR Green master Mix.

Step	Temperature	Time	Cycles
UDG activation	50˚C	2min	Hold
Polymerase activation	95˚C	2min	Hold
Denaturation	95˚C	15sec	40
Annealing	60˚C	1min	

2.32 Quantification of virus replication by qRT-PCR

Reverse transcription of RNA samples from virus in harvested supernatant was performed using SuperScript III platinum one step qRT-PCR kit (Thermo Fisher Scientific) to collect viral RNA. The mRNA was isolated using TRIzol™ Reagent (Thermo Fisher Scientific). Tenfold serial dilutions ranging from 10^5 to 10^0 copies for HCoV OC43 and 10^4 to 10^0 copies of HCoV-229E were tested in duplicate to create a standard curve. Forward and reverse primers and a Taqman probe targeting a conserved region of the HCoV-OC43 and 229E N-gene sequence were used to amplify the template. The template, primers and probe were added to a platinum Taq Mix as stated by the manufacturer. Cycling conditions include: cDNA synthesis and pre denaturation : 55˚ C for 20 min, 94˚C for 2minute, denature anneal and Extend 40 cycles of 94˚C for 15 s; 65˚C for 30s and 68˚C for 1 min; 95 C for 15s with a final extension cycle of 68˚C for 5minutes. All target gene expression levels were normalized to the housekeeping gene and compared to the standard controls using the slope and intercept of curve to calculate viral copies. Primers of androgen regulated genes used for validation include: FKBP5 (forward: ATTATCCGGAGAACCAAACG, reverse: CAAACATCCTTCCACCACAG), NKX3-1 (forward- GTACCTGTCGGCCCCTGAACG, reverse: GCT- GTTA TACACGGAGACCAGG), HCoV-229E (forward: 5'-CAGTCAAATGGGCTGATGCA, reverse: 5'-AAAGGGCTATAAAGAGAATAAGGTATTCT, Probe: 5'-FAM-CCCTGACGACCACGTTGTGGTTCA-TAMRA) HCoV-OC43 (forward:5'CGATGAGGCTATTCCGACTAGGTTAMRA reverse: 3' CCTTCCTGAGCCTTCAATATAGTAACC, probe: FAM-TCCGCCTGGCACGGTACTCCCT-TAMRA).

2.33 Differential expression analysis and Protein sequence alignment

In this study, in-house data of human primary airway epithelial cells and immortalised MRC-5 cells infected with SARS-CoV-2 variants (B.1.1.7 (United Kingdom), B.1.351 (South Africa), B.1.258 (IC19)) Infection, RNA isolation and RNA sequencing was done by other members of the Giotis Lab and HCoV-229E infected MRC-5 cells (GEO repository accession number GSE155986) were used. Cells were infected with 0.01 multiplicity of infection (MOI) HCoV-229E after a 24-hrs culture of MRC-5 cells at 80–90% confluency, grown in a humidified incubator with 5% CO2, at 35°C. Total RNA was extracted at 0, 6, 24, 48, 72 and 96 hrs post-HCoV-229E infection, using NucliSENS® easyMag® bioMerieux. The resulting RNA-Seq data was analysed using the *Partek® Flow®* software, v10. Reads were aligned using STAR aligner and differential expression derived using DESqe2 algorithm with FDR and fold change set at ≤ 0.05 and ≥ ±2 respectively. Gene ontology of most significant differentially regulated transcripts were processed with ShinyGO, DAVID while GSEA software was used to enrich significant genes against known AR and ES signalling genes. (http://bioinformatics.sdstate.edu/go/, https://david.ncifcrf.gov/tools.jsp, https://www.gsea-msigdb.org/gsea/msigdb/genesets.jsp). Resulting data was then further grouped and visualised using Venny venn diagram tool Venny 2.1.0 (csic.es). Amino acid sequences for S-protein of the HCoVs were retrieved from the NCBI Gene bank (https://www.ncbi.nlm.nih.gov/gene) and aligned using EMBL-EBI Clustal Omega tool (Clustal Omega < Multiple Sequence Alignment < EMBL-EBI).

2.34 Statistical analysis

All analyses and graphs were performed using GraphPad Prism software version 9.2.0 (GraphPad, US). The cycle threshold (Ct) values collected from RT-PCR were normalized according to the mean Ct values of Actin for every sample. Fold changes were calculated with the $2^{(-\Delta\Delta Ct)}$ method (Pfaffl, 2001), relative to the mean of mock cells. All data are presented as mean with standard deviation (SD). Statistical analysis was carried out where possible to determine whether data was significant. Shapiro-Wilk normality tests were performed and based on the results were performed one/two-way ANOVA tests with Tukey's multiple

comparison tests, Kruskal–Wallis tests with Dunn's multiple comparison tests, and unpaired t-tests. p-values were set at $p \leq 0.05$ (*), <0.01 (**), and <0.001 (***).

CHAPTER THREE: RESULTS

3.1 TMPRSS2 S2' cleavage site is well conserved across all seven HCoV.

Amino acid sequences were retrieved from the NCBI Gene bank (https://www.ncbi.nlm.nih.gov/gene) and aligned using EMBL-EBI Clustal Omega tool (Clustal Omega < Multiple Sequence Alignment < EMBL-EBI). Results show that the S2' cleavage site of TMPRSS2 at single arginine or lysine residues (R/K*) in the S protein is well conserved by all HCoV (**Figure 6**) suggesting that the different HCoV spike protein can be cleaved by TMPRSS2**.**

VIRUS	S1/S2 site	S2' site	GenBank ID
HCoV-229E	SIIAVQP**R** NVS	GS**R**VAG**R** SAIEDI	APT69890.1
HCoV-NL63	SLIPVRP**R** NSS	SSRIAG**R** SALEDL	AFV53148.1
HCoV-OC43	SKT**RR**SR**R** AIT	CSKASS**R** SAIEDL	AMK59677.1
HCoV-HKU1	SSS**RR**KRR SIS	CGS-SS**R** SFFEDL	AAT98580.1
MERS-CoV	TLTP**R**SVR SVP	TGSRSA**R** SAIEDL	QFQ59587.1
SARS-CoV-2	TNSP**RR**A**R** SVA	PSKPSK**R** SFIEDL	YP_009724390
SARS-CoV	S----LL**R** STS	PLKPTK**R** SFIEDL	AAP13441.1

Figure 6: Alignment of the amino acid sequences at the S1/S2' cleavage site of the S protein of the seven pathogenic HCoVs. Alignments were done using Clusta omega with default parameters.

3.2 Cell lines show varying susceptibility to PV HCoV- NL63, 229E and SARS-CoV-2

To investigate cell tropism, a range of cell lines stated in **Table 2** were transduced with PVs bearing the spike protein of HCoV-NL63, 229E and SARS-CoV2 while PV that lacked viral envelope glycoproteins (Δ-env), served as a negative control to identify the baselines of background luminescence signals in target cells. As expected, all the cell lines tested were highly susceptible to entry driven by the positive control (VSV-G) with luciferase signals ranging from 10^6 to 10^7 RLU/ml and negative control which signals ranging from 10^3 to 10^4 RLU/ml. HEK293T/27 cells (**Figure 7a)** were susceptible to pseudotyped virus bearing HCoV-NL63 S (10^4 RLU/ml), whereas PV bearing SARS-CoV-2 S protein did not show entry above background level ($1x10^3$ RLU/ml). Initial attempts failed to show virus entry into 293T+ ACE2

cells (293T cells stably overexpressing ACE2) where only PV HCoV NL63 showed infectivity values above the negative control at an average of 10^4 RLU/ml. On subsequent attempt PVs optimised by making new plasmids using midi prep instead of mini-prep kits changing plasmid concentration levels for transfection and transfecting for a total of 48hrs instead of 72hrs yielded better PVs as evidenced by luciferase signal. HCoV-NL63-S PV and SARS- CoV-2-S PV average transduction levels increased to 10^6 RLU/ml **Figure 5b**. MRC5 showed susceptibility to HCoV-229E, NL63 and SARS-CoV-2 S PV at an average of 10^3 RLU/ml all above Δ-env with HCoV-229E S PV being the highest **Figure 7c**. VAT cells did not permit entry as all PVs showed transduction levels within background levels $10^3/10^4$ RLU/ml, this is expected as VAT cells are African Monkey cells stably overexpressing ACE2 and TMPRSS2 that are not susceptible to lentiviruses. **Figure 7d.** Similarly CHO cells were not susceptible to any of the PVs as all PVs were within the negative control range **Figure 7e.**

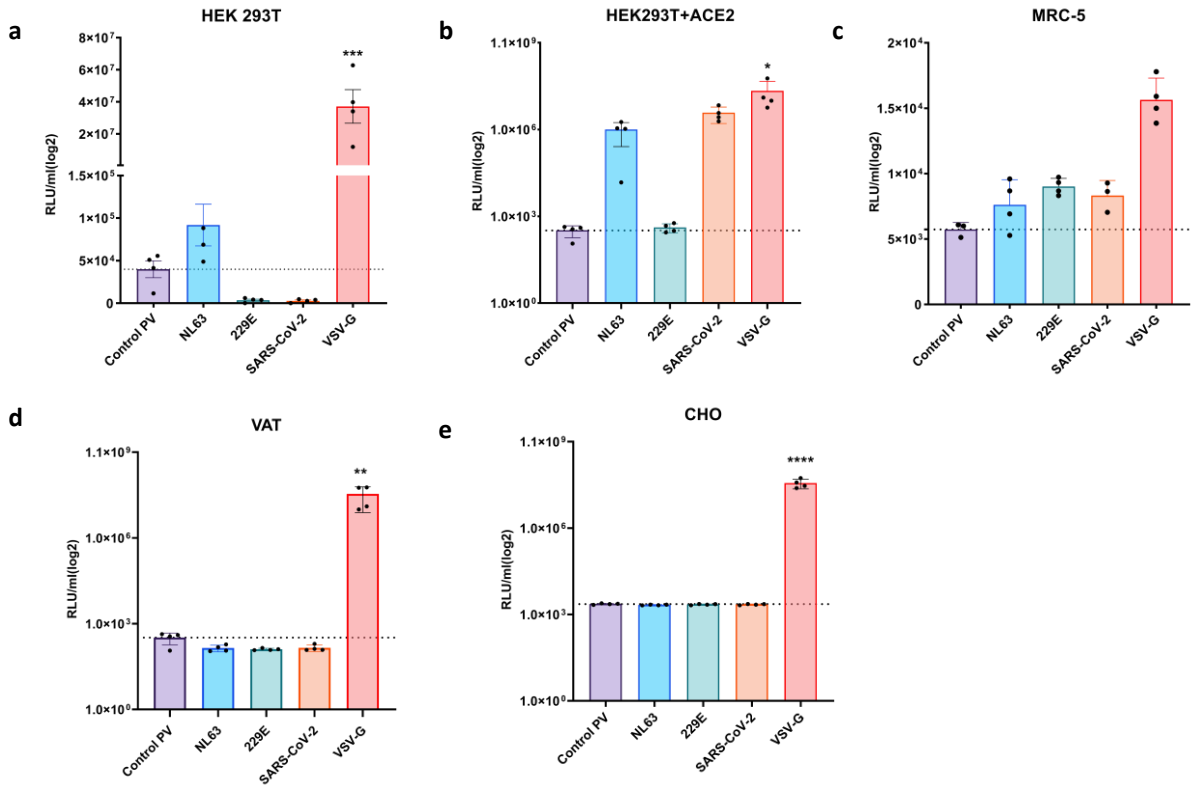


Figure 7: Tropism of HCoV-NL63,229E, and SARS-CoV-2 S-bearing lentiviral PV visualised by transduction efficiencies. In **a** HEK293T/17 shows susceptibility to PV HCoV-NL63 while the other PV are bellow control (Δ-env). HEK 293T+ACE2 cells **(b)** were susceptible to PV HCoV-NL63 and SARS-CoV-2. MRC-5 cells were susceptible to all PV **(c)** while VAT and CHO cells were susceptible to only PV VSVG as all other values remained within background range **(d)**.

The luciferase-expression indicated entry is shown as Relative Luminescence Unit per ml (RLU/ml). Significance determined using one-way ANOVA with Dunnet's multiple comparison test when compared against control: Δ-env, ** p<0.0021, *** p<0.0001. Comparisons not shown are not significant (ns). Control PV (Δ-env) contained vehicle control.

3.3 Protease inhibitors Camostat and E64-d reduce cell entry of HCoVs.

To investigate the role of TMPRSS2 in cell entry of the HCoVs A549+ACE2 cells were treated with Camostat and E64-d which are drugs known to block TMPRSS2 and Cathepsin-L respectively prior to transduction Camostat reduced HCoV- NL63, 229E and SARS-CoV-2 PV by 74% (P<0.0001) ,61%(P<0.0001) and 72% (P<0.0001) respectively. E64-d reduced HCoV-NL63 PV entry by 21% (P0.3067) 229E by 55% (P<0.0001) and SARS-CoV-2 by 29%(0.0026). Treatment combining both E64-d and Camostat further reduced HCoV-NL63, 229E and SARS-CoV-2 entry by 87% (P<0.0001), 68% (P<0.0001), and 82%(P<0.0001) respectively. Positive control VSVG showed no significant reduction (**Figure 8)**.

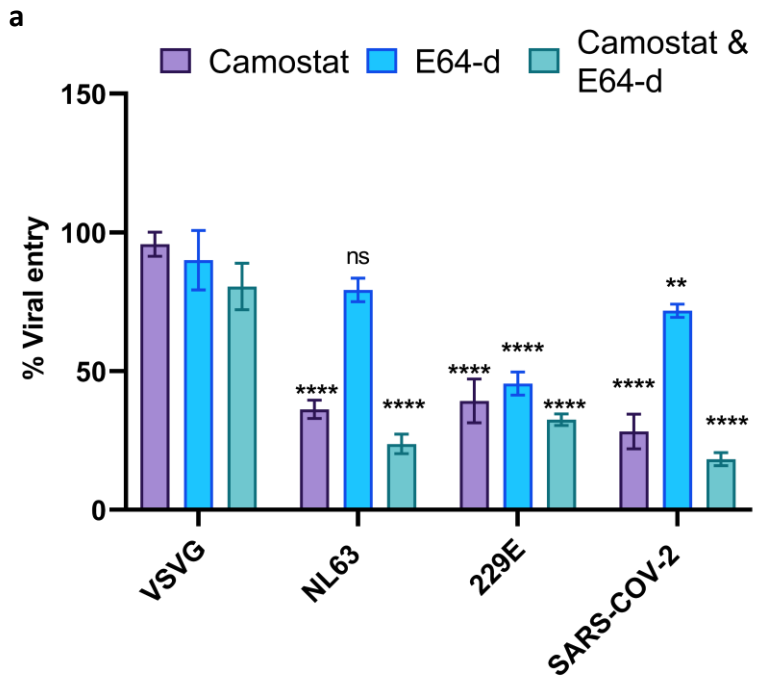


Figure 8: Importance of TMPRSS2 for host cell entry of HCoVs was evaluated by adding inhibitors Camostat (200µM) and E64-d (25µM) to target cells prior to transduction. Entry into cells not treated with inhibitor was set as 100%. Significance determined using two-way

ANOVA with Tukey's multiple comparison test when compared against mock: ns p = 0.3067 ** p = 0.0026, **** p = <0.0001. Mock indicates cells with no treatment.

3.4 TMPRSS2 promotes cell entry in HCoV-229E/NL63 and SARS-CoV-2.

To further examine the effect of TMPRSS2 on cell entry, CHO cells stably overexpressing ACE2 and TMPRSS2 (CHO+ACE2+TMPRSS2) was used, to observe if the expression of TMPRSS2 will boost entry when compared to the parental CHO cells. CHO+ACE2+TMPRSS2 were susceptible to HCoV-NL63 and SARS-CoV-2 PV with average transduction measured around 10^6 and 10^5 RLU/ml unlike CHO cells which showed no susceptibility to the PVs as all values were less than control PV (**Figure 9a)**. Similarly in A549 + ACE 2 susceptibility to HCoV- NL63, 229E and SARS-CoV2 PV were observed at 10^4 10^5 , 10^6 RLU/ml respectively but in comparison to A549+ACE2 +TMPRSS2, transduction efficiencies were significantly higher at 10^6 $10^7$and 10^8 in HCoV-NL63, -229E and SARS-CoV2 respectively indicating the relevance of TMPRSS2 in cell entry. (**Figure 9b**)

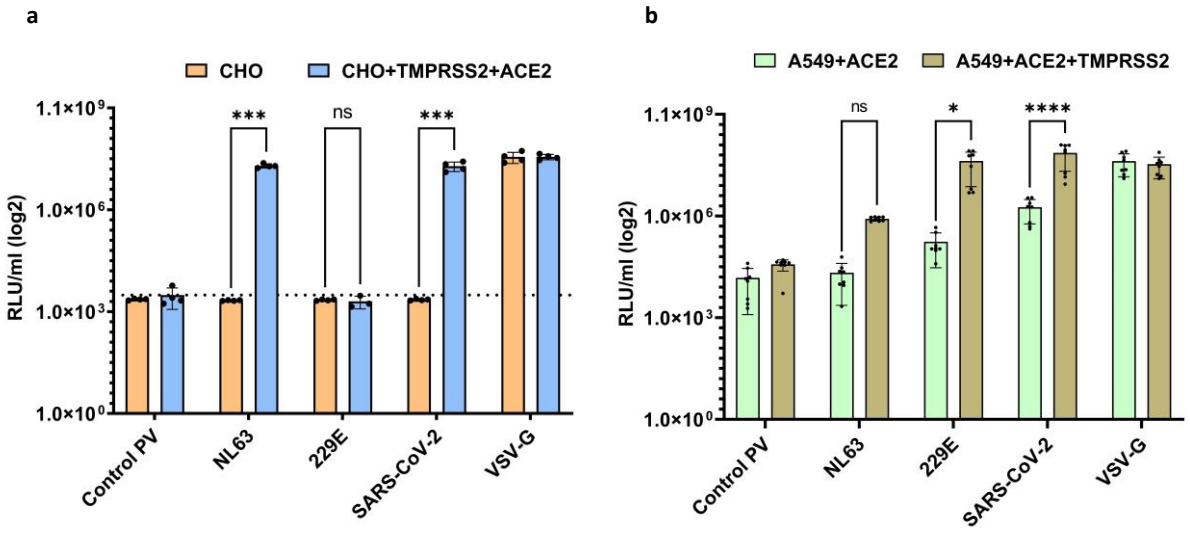


Figure 9a-b. Transduction result of CHO and CHO+ACE2+TMPRSS2 in **a** shows an increase in transduction efficiencies observed in HCoV-NL63 and SARS-CoV-2 from bellow negative control levels of 10^3 to 10^7 and 10^7 RLU/ml respectively **(a).** Similarly in A549+ ACE2 HCoV-NL63, 229E and SARS-CoV2 transduction efficiencies increased from 10^4 10^5 and 10^6 RLU/ml to 10^6 10^8 , 10^8 RLU/ML in A549+ACE2 +TMPRSS2 **(b).** Significance determined using two-way ANOVA with Tukey's multiple comparison test between cell lines: ns (>0.9999) * (0.0131) ***(p <0.0005) **** (p <0.0001).

3.5 Enzalutamide reduces gene expression levels of TMPRSS2 and ACE2 in A549+ACE2 and A549+ACE2+ TMPRSS2 cells.

Next, we wanted to test whether antiandrogen ENZA affects mRNA levels of Cathepsin L TMPRSS2 and ACE2, A549+ACE2 and A549+ACE2+ TMPRSS2 cells were treated with ENZA for 72hrs after which the cells were collected, and qPCR performed to quantify alterations in gene expression. In A549+ACE2, TMPRSS2and cathepsin L where not significantly expressed unlike ACE2. ACE2 expression showed significantly decrease after ENZA treatment (p=0.0027) **(Figure 10a)**. For A549+ACE2+TMPRSS2 cathepsin L was similarly not significantly expressed while ACE2 and TMPRSS2 was significantly decreased by ENZA (p = 0.0040, 0.0003 respectively) (**Figure 8b).**

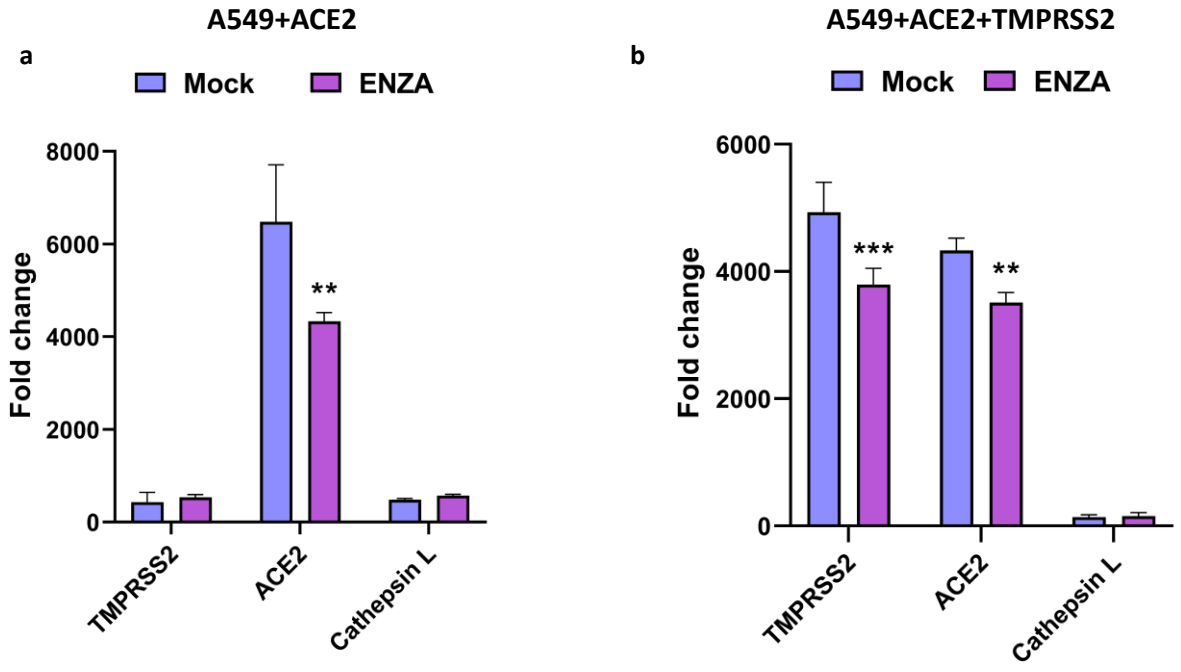


Figure 10:a,b. Following treatment with ENZA A549+ACE2 and A549+ACE2+TMPRSS2 cells quantified for ACE2,TMPRSS2 and Cathepsin L expression using qPCR. ENZA significantly reduced TMPRSS2 and ACE2 levels while changes in Cathepsin L was not significant. Significance determined using two-way ANOVA with Sidak's multiple comparison test. ** p = 0.0040 *** p = 0.0003.

3.6 ENZA reduces cell entry in HCoV- NL63,229E and SARS-CoV-2

Having found that the cell entry of HCoV- NL63,229E and SARS-CoV-2 is at least partially TMPRSS2 mediated, we then wanted to investigate the effect of ENZA on cell entry using PVs. A549+ACE2 and A549+ ACE2+ TMPRSS2 cells treated with ENZA for 72 h prior to transduction for 48 h with the PVs. In A549+ACE2 cells ENZA significantly reduced viral entry of PV HCoV-NL63,229E and SARS-CoV-2 by approximately 74, 85 and 50% respectively. This was also repeated in cells stably expressing TMPRSS2. By using the stably transfected cells, the androgen regulation of TMPRSS2 was partly inhibited i.e the endogenous TMPRSS2 remained androgen receptor (AR) regulated, but the expression of the exogenous TMPRSS2 was independent of AR signalling, further proving the role of TMPRSS2 for the entry of these viruses in target cells. Cell entry in A549+ACE2+TMPRSS2 by HCoV -NL63, -229E and SARS-CoV-2 was reduced by approximately 60, 55 and 31% respectively, showing that expression of TMPRSS2 by a non-AR-regulated promoter reduced the effect of ENZA. In contrast, cellular entry driven by VSV-G PV was unaffected by ENZA, demonstrating that the effects of these drugs are specific for the SARS-CoV-2 non-endosomal mechanism of cell entry (**Figure 11a,b)**. Furthermore viral copy quantification using supernatant of MRC-5 cells infected with native HCoV-229E and treated with ENZA in comparison with mock shows a reduction in viral genomic copies from 3.64×10^7 to 9.29×10^6 further confirming the effect of ENZA on viral entry.

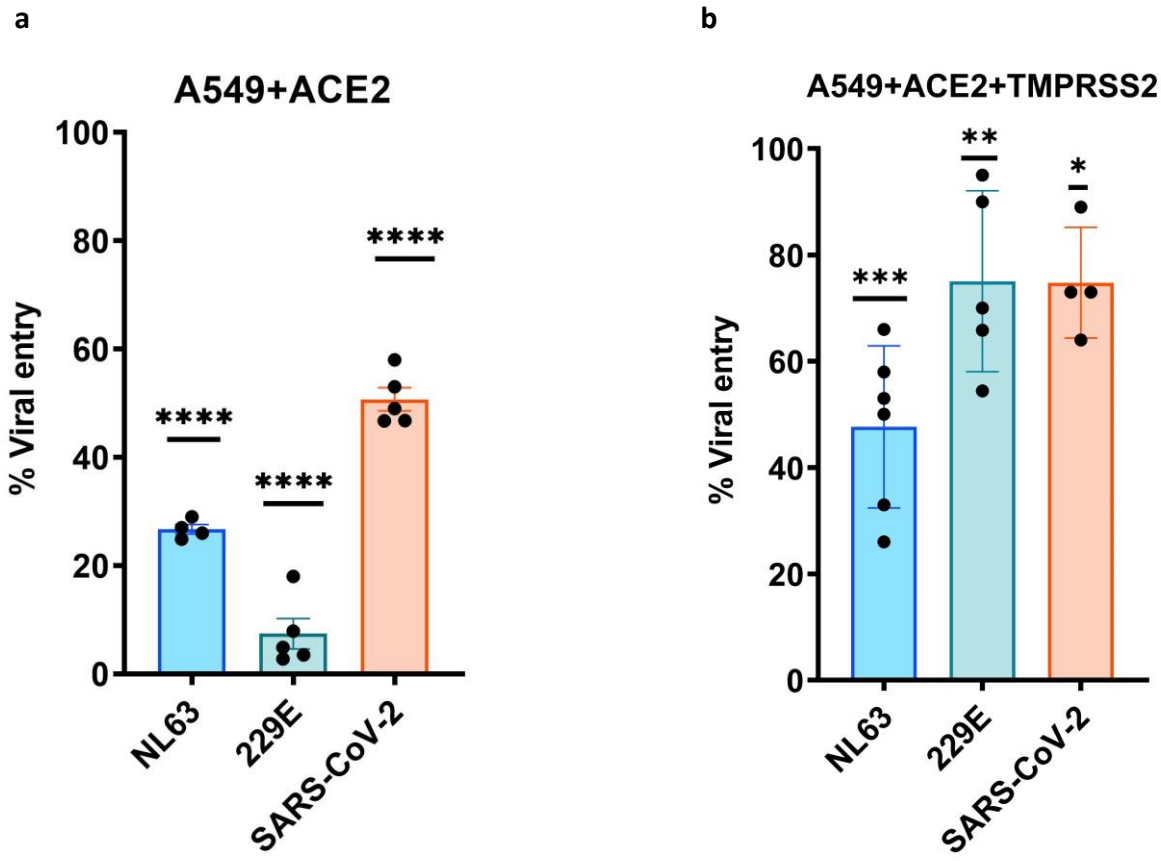


Figure 11 a,b. Following treatment with enzalutamide for 72hrs PVs where transduced into A549+ACE2 and A549+ACE2+TMPRSS2. In A549+ACE2 ENZA significantly reduced viral entry of HCoV-NL63,-229E and SARS-CoV-2 by approximately 74, 85 and 50% respectively **(a).** While in A549+ACE2+TMPRSS2 HCoV-NL63, -229E and SARS-CoV-2 PV transduction levels was reduced by approximately 60, 55 and 31% respectively **(b)**. Significance determined using one-way ANOVA with Tukey's multiple comparison test between ENZA treated and mock. * $p < 0.0119$ ** $p < 0.0074$ **** $p < 0.0001$.

3.7 Enzalutamide does not reduce cell entry of HCoV-OC43.

Viral copy quantification using supernatant of MRC-5 cells infected with native HCoV-OC43 ± ENZA treatment were calculated to be 5.36x 10^6 and 6.37 x 10^6 respectively showing a small but significant decrease in viral copies after ENZA treatment. q-PCR results using RNA extracted from cells treated with ENZA and infected with authentic HCoV-OC43 virus show a significant increase in HCoV-OC43 levels when compared with the infected + ENZA. Furthermore, similarly 10-fold serial dilution $TCID_{50}$ assay using virus containing supernatant ± ENZA treatment show no difference in viral titre levels when compared **(Figure 12)**.

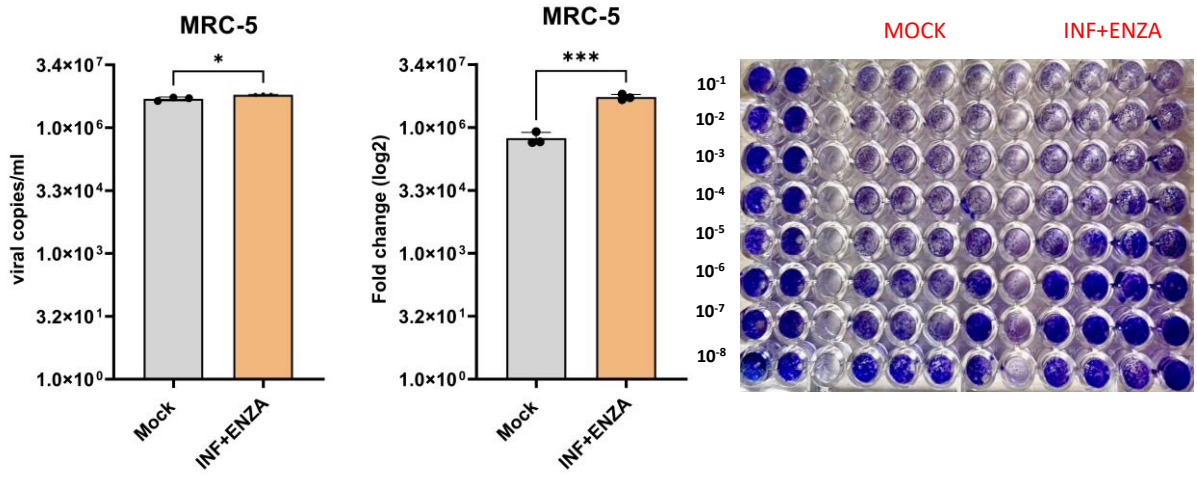


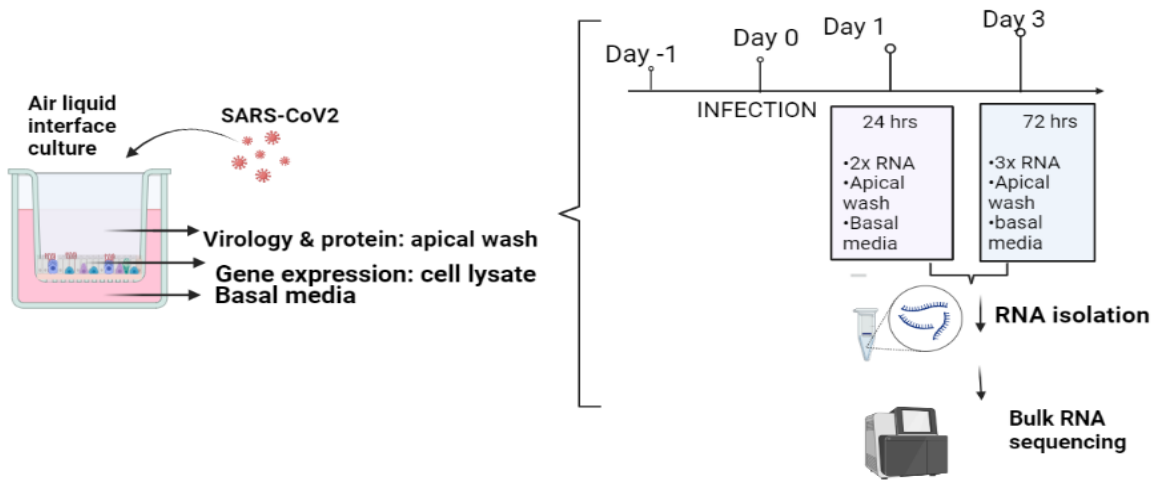
Figure 12: MRC-5 cells were infected with authentic HCoV-OC43 virus viral quantification using infected cells and supernatant show that ENZA does not reduce cell entry for HCoV-OC43 (**a,b**). Supernatant from cells infected and treated with ± ENZA no difference in viral titre when used for TCID 50 assay (**b**) Significance determined using the unpaired T-test * *p*= 0.0129, *** *p*= 0.0006. Mock indicates cells without ENZA treatment.

3.8 SARS-CoV-2 and HCoV-229E infection induce a subset of Androgen receptor signalling genes.

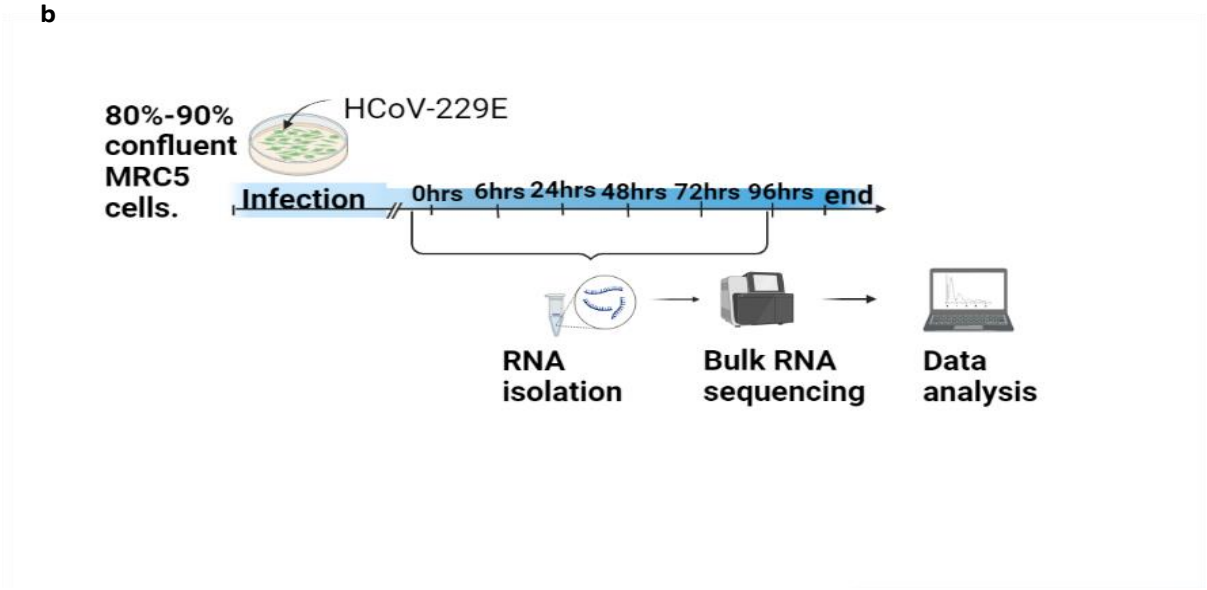
To better understand the relationship between HCoV infection and AR signalling at the cellular level, in-house and publicly available RNA-Seq data of human primary airway epithelial cells and immortalised MRC-5 cells infected with SARS-CoV-2 native variants (B.1.1.7 (UK), B.1.351 (SA), B.1.258 (IC19)) and HCoV-229E (Freidman *et al.,* 2021) (ascension number: GSE155986) (**Figures 13a, b**) was analysed respectively using the Partek flow software. All reads from the MRC-5 infection had length of 50.96bp, average quality of 35.50 and GC content of 50%. Total amount of cellular genes differentially expressed varied at different time point: 2391,4019, 9387 and 8844 at 6,24, 48 and 72hrs respectively (**Figure 13e**). With false discovery rate (FDR) and fold change set at ≤ 0.5 and ≥ ±2 respectively, 224 genes were similarly upregulated at all time point while 489 genes were jointly downregulated at all time points (**Figure: 13c,d**). Gene enrichment analysis of all the differentially expressed genes against known androgen signalling genes (GO:0030521) (Nelson *et al.,*2002), revealed that genes involved in AR signalling genes were significantly represented in our comparisons which suggests a crosstalk between CoV infection and AR signalling. At 6 and 24hrs AR signalling genes were found to be

downregulated in the data set (**Figure 14a)** while AR signalling genes are upregulated at 48 and 72hrs (**Figure 14b**). Unlike HCoV-229E infection which caused downregulation at 6 and 24hrs then followed by upregulation of a subset of AR genes at 48hrs and 72hrs. SARS-CoV2 infection shows both downregulation and upregulation of genes subsets in different time points and variant. These data sets were also enriched for oestrogen signalling genes. Significant oestrogen regulated genes were found to be upregulated at 6hrs (**Figure 14c**) and down regulated at 48 and 72hrs during HCoV- 229E infection. Oestrogen genes were not enriched at 24hrs. While on the other hand ES genes expressed during SARS-CoV2 infection were predominantly up regulated (**Figure 14d**). In order to validate the induction of AR-subsets MRC-5 cells were infected with 229E and chose two AR regulated genes which RNA-seq showed high induction. qPCR results from MRC-5 cells infected with HCoV-229E show that Homeobox protein Nkx-3.1 (NKX3.1) expression was induced during HCoV-229E infection when compared to mock. FK506 binding protein 5 (FKBP5) showed a slight increase in expression levels during ENZA treatment. During HCoV-OC43 infection FKBP5 an NX3-1 expression were upregulated during infection and then reduced by ENZA treatment (**Figure 14)**.

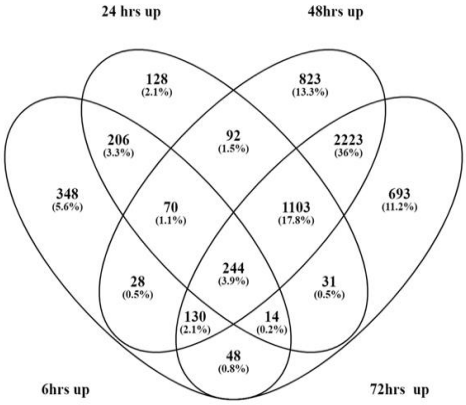
a



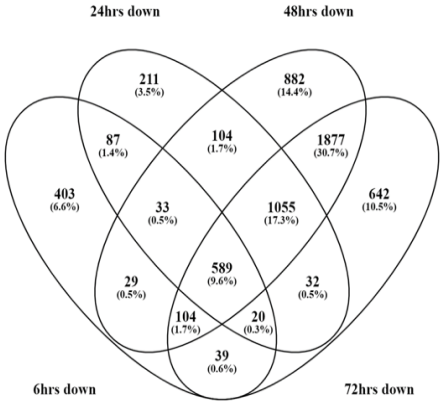
b



c



d



e

	Up regulated	Down regulated	Total
6hrs	1088	1303	391
24hrs	1888	2131	4019
48hrs	4713	4674	9387
72hrs	4486	4358	8844

Figures 13 a,b, Schematic diagram of experiment carried out by Dr Stathis Giotis showing how primary airway cells and MRC-5 cells infected with SARS-CoV-2 and 229E respectively**.** **Figure c:** shows the total number of genes differentially regulated (up and down) during

HCoV-229E infection at 6,24,48 and 72hrs, RNA-Seq data was analysed using Partek flow software with FDR and fold change set at ≤ 0.5 and ≥ ±2 respectively. **d,e**: Comparison of differential expressed genes at different time points Venn diagram shows that 17.8% of all upregulated genes during HCoV-229E infection are expressed at all time points and 17.3% of downregulated genes are expressed at all timepoint.

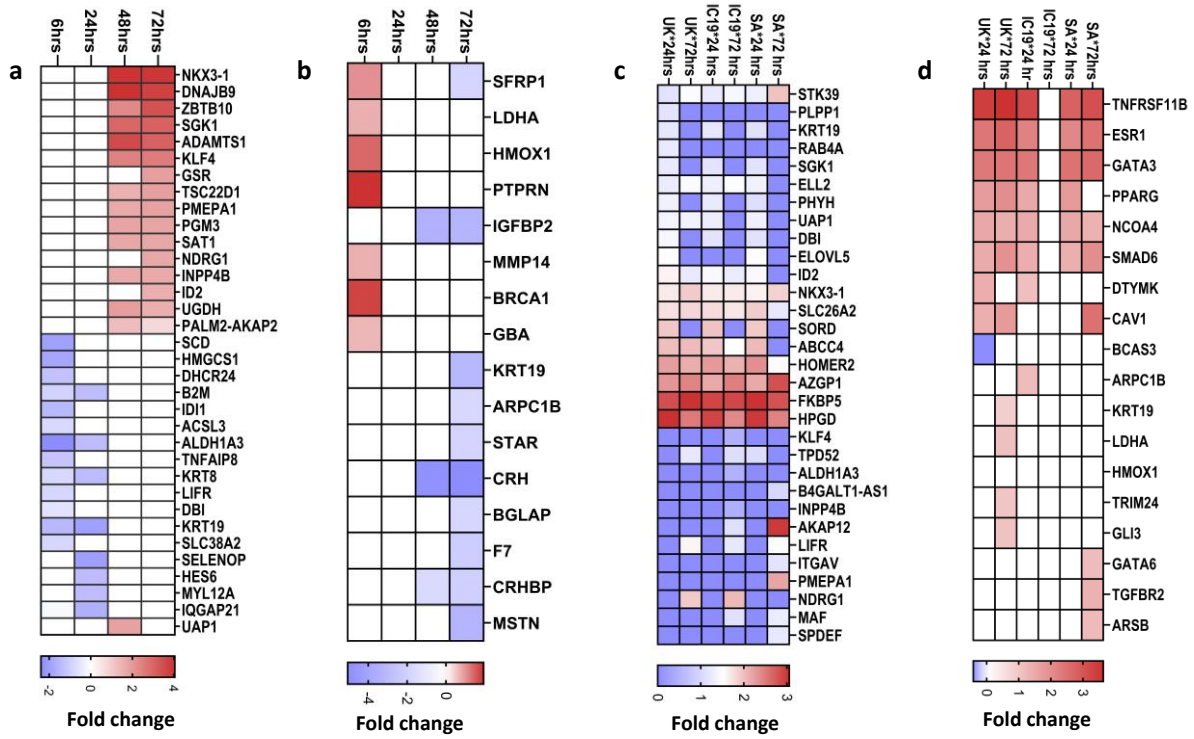


Figure 14a-d: Heat map visualization of the expression pattern of AR and ES during infection of MRC-5 cells by HCoV-229E. During HCoV- 229E infection all AR signalling genes were downregulated at 6-24hrs while other AR significant genes not expressed at 6 and 24hrs become upregulated (**Figure 10a**). In **Figure 10c**, SARS-CoV- infection induces a subset of AR signalling genes similar in expression across the variants. Enrichment against ES signalling genes shows a fewer number of genes with no ES genes at 24hrs (**Figure 10b**). Infection by SARS-CoV-2 variants show similarly upregulated genes at all time points (**Figure 10d**). red columns denote upregulation, while blue indicates downregulation. Small bars bellow the heat map denoted fold changes

3.9 Gene ontology results

Enriched AR signalling genes were analysed using the Shinny GO software and FDR cut off set to 0.05. AR genes upregulated in MRC5 / HCoV-229E infection at 48 and 72hrs was most enriched in nucleotide sugar biosynthetic process and nucleotide-sugar metabolic process, while the down regulated genes were mostly involved in Isoprenoid biosynthetic process, cholesterol biosynthetic process, sterol biosynthetic process and secondary alcohol biosynthetic process. The upregulated significant AR genes were mostly involved in negative regulation of lipoprotein metabolic process, androgen receptor signalling pathway, Positive regulation of smooth muscle cell proliferation and muscle cell proliferation, lipid metabolic process and regulation of apoptotic process**.** NKX3-1 is an androgen-regulated, prostate-specific homeobox gene expressed in the prostate epithelium (Griffin *et al.*, 2022). NKX3-1 expression acts as a transcription factor found important in prostate development and tumour suppression. The absence of NKX3-1 expression is usually observed in prostate tumorigenesis and has been seen to be a result of allelic loss, methylation, and post transcriptional silencing (Griffin *et al.*, 2022). The protein encoded by FKBP5 is a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking (Ising *et al.*, 2019). Zinc-alpha-2-glycoprotein (AZGP1) gene expresses a soluble protein that stimulates lipolysis, induces a reduction in body fat in mice, is associated with the cachexia related to cancer, and is known to be expressed in secretory cells of lung epithelium (Lv *et al.*, 2017). Serum and glucocorticoid-regulated kinase 1 (SGK1) expression is characterised by oxidative stress and an increase in glucocorticoids, which are common components of the neurodegenerative process (Ghani, 2022). SGK1 is an important player in cell death processes underlying neurogenerative diseases (Ghani, 2022). Downregulated AR gene SCD encodes enzyme Stearoyl-CoA desaturase which acts as an important metabolic control component. Inhibition of its expression may enhance the treatment of a host of metabolic diseases elevated expression levels of SCD is found to be correlated with obesity (Zhou *et al.*, 2020c).

qPCR results from MRC-5 cells infected with HCoV-229E show that NX3-1 expression was induced during HCoV-229E infection when compared to mock while FKBP5 showed a slight increase in expression levels during ENZA treatment. While during HCoV-OC43 infection

FKBP5 an NKX3-1 expression were upregulated during infection and then reduced by ENZA treatment (**Figure 15)**.

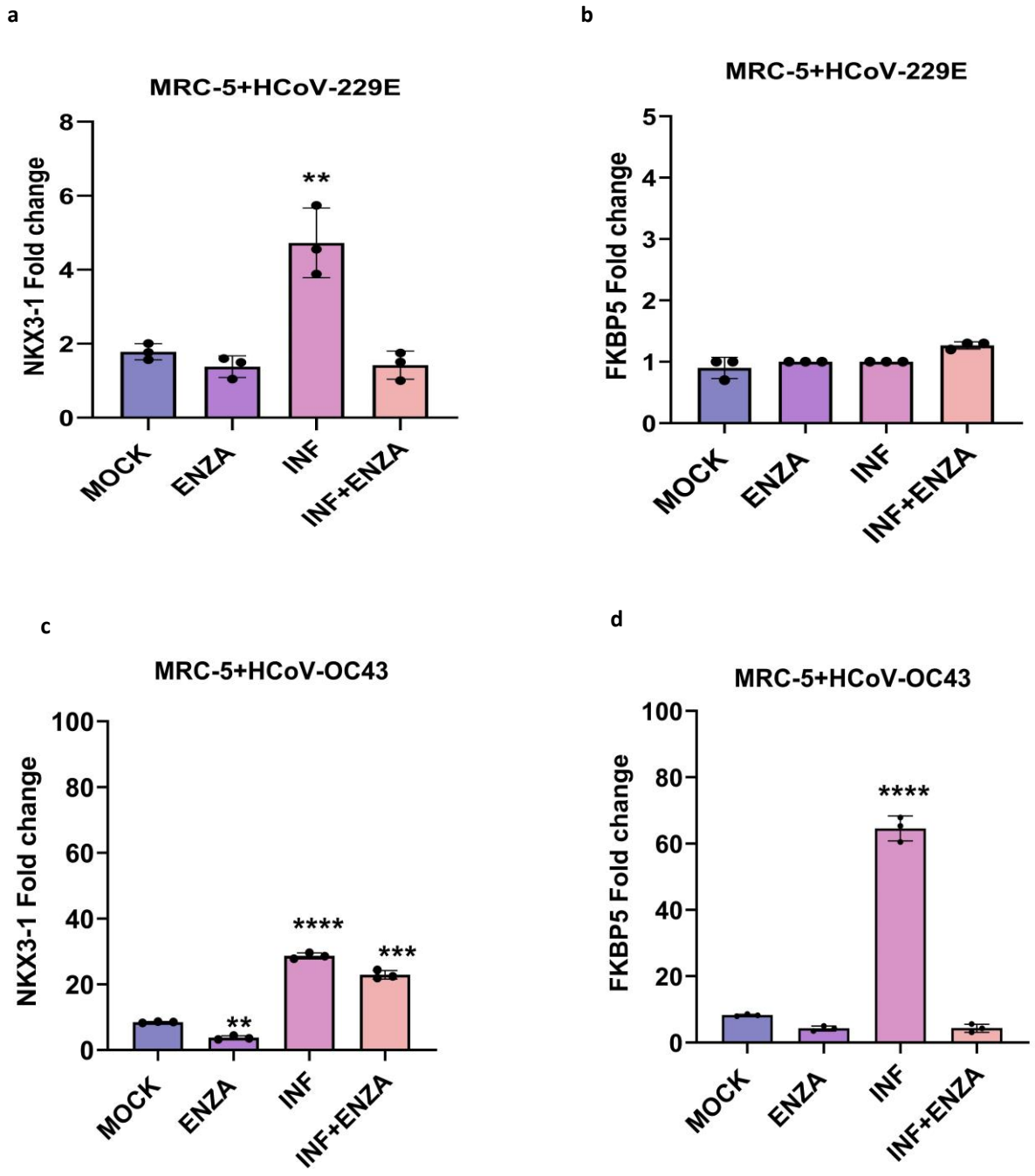


Figure 13: MRC-5 cells were treated with 10 µM ENZA +/-(with and without) then infected at an MOI of 1 with HCoV-OC43 and 229E. RNA was harvested, reverse transcribed and qPCR performed to quantify AR regulated genes NKX3-1 and FKBP5 expression. Means from at least

three independent experiment are represented. Significance determined using two-way ANOVA with a Tukey's multiple comparison test ** $p < 0.0034$, *** $p < 0.0007$ **** $p < 0.0001$.

3.10 HCoV-229E induces androgen signalling.

To assess the effect of HCoVs on AR receptor signalling the dual luciferase assay system with the luciferase gene under the promotional control of androgen response element (ARE) was used. Mibolerone (MIB) a synthetic androgen was used as a stimulant for ARE (Positive control). The uninfected stimulated cells which serves as control show a significant induction of ARE compared to unstimulated cells (**Figure 16**). During HCoV-229E infection ARE was induced in the unstimulated cells and its stimulation was enhanced in the MIB stimulated cells. While HCoV-OC43 infection blocks the MIB-stimulated induction of ARE. These results show that HCoV infection have different effects on AR signalling further indicating a correlation between AR signalling and HCoV 229E infection at the cellular level. Experiments were done in both full media and androgen stripped media and both experiments showed similar results.

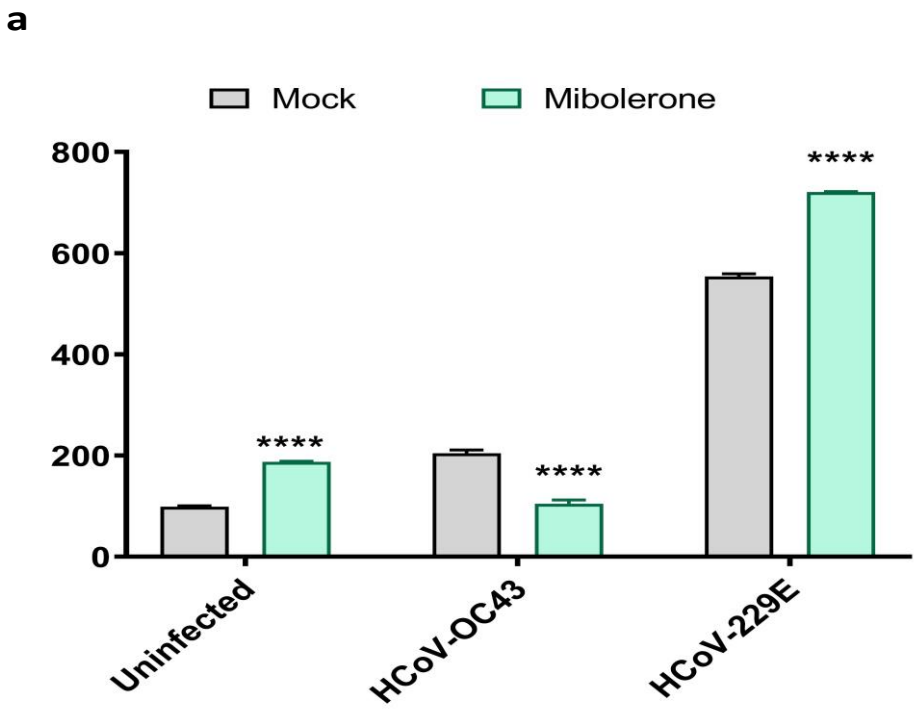


Figure 16: MRC-5 cells transiently expressing luciferase reporter renilla were induced with synthetic androgen Mibolerone +/- during HCoV infection. Luciferase activity was measured

using Dual-Glo assay system. Significance determined using two-way ANOVA with Tukeys's multiple comparison test **** p <0.0001.

CHAPTER FOUR: DISCUSSION

4.1 Technical approaches.

PV production is a widely utilised alternative to pathogenic viruses and is a versatile tool for viral studies. This approach to studying viruses is particularly employed because it allows study of cell entry of the viruses without the limitations of active infection. The produced PVs are usually replication-defective, therefore they can be used in a BSL-2 laboratory which most universities have, in addition the use of PVs are not capital or labour intensive (Bentley et al., 2015; Carnell et al., 2015). PVs for this study were produced by transfecting packaging cells with the surface viral proteins of interest , reporter and packaging system (HIV-1) (Di Genova *et al.*, 2021). The titre of PVs in target cells were then quantified by measuring the reporter gene expression using a luciferase assay. Results were further confirmed by using natural virus as PVs may not always reflect the native state and tropism of the natural virus as they do not contain the full viral genome (Chen & Zhang, 2021). Initially the first challenge that arose was from low transduction titer values of HCoV PV created. To increase the lentiviral PV titer values, the transfection conditions had to be optimised to increase yield. Next, titration of natural HCoVs was performed using $TCID_{50}$ assay to quantify virus titres. Furthermore, protease inhibitors Camostat and E64-d as well as lung cells stably overexpressing ACE2 and TMPRSS2 were utilized to explore the role of TMPRSS2 in the in viral entry of the HCoVs. The effect of Enzalutamide was then evaluated using both PV and WT virus while the relationship between AR signaling and HCoV infection was assessed using publicly available and in house transcriptome data. Results were then confirmed in-vitro using a dual luciferase assay.

4.2 TMPRSS2 is at least partially responsible for cell entry during HCoV-229E, NL63 and SARS-CoV-2 infection.

One of the goals in this study was to ascertain the role of TMPRSS2 in viral entry of the seasonal HCoVs. For this, the TMPRSS2 cleavage site of the S proteins was explore by aligning the respective amino acid sequences of the cleavage site. The results showed that the TMPRSS2 cleavage site at the S2' contains single arginine or lysine residues (R/K↓) necessary for activating viral fusion proteins at the monobasic cleavage sites (Bestle *et al.*, 2020). Several cell lines such as A549, MRC-5, Vero, HEK 293T types have been shown to allow HCoV productive replication (Djomkam *et al.*, 2020; Hoffmann *et al.*, 2020a; Schirtzinger *et al.*,

2022). To further study the role of TMPRSS2 and cathepsin L, HIV-1 based PVs bearing HCoV - NL63, 229E and SARS-CoV-2 spike protein were employed to evaluate the role of the cell surface TMPRSS2 and cathepsins in the cell entry mechanisms of HCoVs (HCoV – HKU1 and -OC43 PV were unavailable due to technical problems). Using the known TMPRSS2 inhibitor camostat and cathepsin L inhibitor E64-d to selectively inhibit TMPRSS2 and cathepsin-L respectfully as they both can facilitate cell entry for some HCoVs (Bertram *et al.,* 2013). MRC-5, a cell line known to be susceptible to HCoV-NL63 and 229E (Friedman *et al.*, 2021; Hofmann *et al.*, 2005) was used to allow a substantive parallel comparison between the HCoVs. The results show that treatment with camostat, reduces HCoV-NL63,229E and SARS-CoV-2 - S PV entry by 74, 61 and 72% and further by 87, 68 and 62% when cells were treated with both camostat & E64-d. These results suggest that TMPRSS2 and cathepsin L are essential for full cell entry of these viruses although the TMPRSS2 route is more preferable. These results are in line with previous reports from Djomkam *et al.*, 2020 and Hoffmann *et al.*, 2021 where camostat and E64-d significantly reduced the entry of SARS-CoV-2 in Calu-3, Caco-2 and 293T +ACE2 cells. Similarly in a study by Yamaya *et al.*, 2020 using human nasal epithelial (HNE) cells treated with camostat and infected with HCoV-229E, showed a reduction in viral titres when compared to infected. This is also the same for HCoV-NL63, as studies show that camostat treatment of airway tissues infected with HCoV-NL63 reduces viral progeny number at both the 48 and 96 hrs time point (Gard *et al.*, 2021).

Cells overexpressing TMPRSS2 were used to further address how the presence or absence of TMPRSS2 influences HCoVs infection, by comparing cell lines expressing, overexpressing and not expressing this protease. Compared to CHO cells, the CHO cells stably overexpressing ACE2 and TMPRSS2 demonstrated increased cell uptake of PV. Similarly, the presence of overexpressed TMPRSS2 in A549 cells also increased cell entry levels of HCoV-NL63,229E and SARS-CoV-2 S PV. Together these results demonstrate the ability of TMPRSS2 to facilitate cell entry in HCoVs. This is in line with other published reports for example, Bestle *et al.*, 2020 demonstrated the importance of TMPRSS2 in activation of SARS-CoV-2 S in Calu-3 human airway epithelial cells through antisense-mediated knockdown of TMPRSS2 expression. For HCoV-229E our results suggest that the virus actively uses both the cell-surface pathway using TMPRSS2 and the endosomal pathway using cathepsin L to activate the spike protein, this is corroborated by Bertram *et al.*, 2013 and Shirato *et al.*, 2017. However a study by Shirato *et*

al., 2018 reported that clinical isolates of HCoV-229E preferred the TMPRSS2 pathway to the cathepsin pathway for cell entry. When compared with HCoVs: 229E, OC43 and HKU1 , HCoV-NL63 has been described as a better surrogate for studying SARS-CoV-2 due to its similarities in receptor and protease usage (Chakraborty, 2020). Studies by Milewska *et al.*, 2018 further observed that although TMPRSS2 is important for HCoV- NL63 tropism, inhibition of TMPRSS2 hampers virus infection in human airway epithelial (HAE) cultures. Its function does not enable fusion on the cell surface, and the acidification of the microenvironment is necessary for its usage. Although further confirmatory experiments are needed to fully understand the role of TMPRSS2 in cellular entry of the HCoVs the results herein provide a substantial base for the continuation of this study.

4.3 Enzalutamide reduces cell entry for HCoV- NL63, 229E and SARS-CoV-2 but not in HCoV-OC43.

The AR via androgens increase TMPRSS2 expression in the prostate, and non-prostatic tissues, such as kidney, colon, small intestine and most notably the lung (Horl and Heidland, 1984). Antiandrogens work by inhibiting the androgen receptor holding it in an inactive conformation inhibiting its activity, thus preventing the effects of hormones like testosterone and dihydrotestosterone in the prostate gland and elsewhere in the body (Leach *et al.,* 2021, Gibbons *et al*., 2015). TMPRSS2 has been previously reported to be expressed and downregulated by ENZA in the A549 alveolar lung cell line (Leach *et al.*, 2021). Results from this study also shows that ENZA similarly reduces ACE2 levels significantly as presented in the results section. An important thing to note about these cells (A549+ACE2, A549+ACE2+TMPRSS2) is that they have been transduced to constitutively express ACE2 and TMPRSS2 under the selection of antibiotics and because of this, the overexpressed genes are not under the promotional control of the AR response element, therefore the effects of ENZA observed in the results is independent of the activity of the overexpressed genes. Therefore, in addition to ENZA reducing the cell entry in the HCoVs, our results also further establish the role of TMPRSS2 in cell entry. From this study ENZA was shown to reduce cell entry of HCoV-229E, NL63 and SARS-CoV-2 S PV in alveolar lung cells either overexpressing ACE2 or both TMPRSS2 and ACE2. These results are similar to that observed in studies by Leach *et al.,* (2021) and Deng *et al.,* (2021) in which antiandrogens have been shown to inhibit viral entry and infection. This is at least in part, caused by the downregulation of TMPRSS2. On the other

hand another study using human lung organoids expressing AR and TMPRSS2 recorded no effect of enzalutamide on TMPRSS2 expression or on infection by SARS-CoV-2 (Li *et al.*, 2021).

Unlike SARS-CoV-2, in-depth research looking into the tropism or therapies of the seasonal HCoVs is lacking broadly. This is because they mostly cause mild disease and challenges with isolation and poor propagation in conventional cell lines (Liu et al., 2021). Collectively, the data presented here demonstrates that the antiandrogen ENZA inhibits viral entry and infection of the HCoVs, a result at least partially connected with the downregulation of TMPRSS2 and by extension ACE2. Studies by Li *et al.*, 2021 and Qiao *et al.*, 2021 identified co-expression of ACE2, TMPRSS2, and AR in human alveolar and bronchial epithelial cells, suggesting that AR not only modulates TMPRSS2 expression in the lung but that of ACE2 expression as well. The incomplete block of viral entry could be a result of viral entry via alternate routes, proteases which are not AR target genes or dose effect. For example, HCoV-NL63 and SARS-CoV-2 are also known to use the non-endosomal cathepsin L route and TMPRSS4 respectively (Zang *et al.*, 2020; Zhao *et al.*, 2021). Using WT virus, the Effect of ENZA was further investigated on HCoV-OC43 tropism using qPCR and $TCID_{50}$ as described in the materials and methods section. The results show that ENZA does not reduce cell entry. Studies highlighting the entry mechanisms of HCoV-OC43 is limited. Owczarek *et al.*, 2018 showed that in addition to other potential entry routes HCoV OC43 cell entry uses the caveolin-1 dependent endocytosis for the viral entry from the cell surface. This route is dynamin-dependent with the vesicle internalization process requiring actin cytoskeleton rearrangements. Hence the potential usage of TMPRSS2 for cell entry of HCoV - OC43 requires further investigation.

4.4 SARS-CoV-2 and HCoV-229E infection induce a subset of AR signalling genes.

The main function of the Androgen receptor (AR) is to regulate gene expression of testosterone and this occurs via AR nuclear translocation, response element binding and recruitment of, or crosstalk with, transcription factors (Eder *et al.*, 2001). The relationship between SARS-CoV-2, TMPRSS2, ACE2 and AR have been widely investigated during the COVID-19 pandemic (Baratchian *et al.,* 2021). Exploration of Genotype Tissue Expression (GTEx) dataset show that AR and TMPRSS2 are not only co-expressed in a number of tissues but TMPRSS2 is only expressed in tissues that also show detectable levels of the AR (Leach *et al.*, 2021). From the result presented in the current study RNA-Seq analysis show that HCoV

229E and SARS-CoV-2 infection induce the differential expression of genes associated with AR signalling, inferring that a kind of yet to be described crosstalk is going on at the cellular level. This was further confirmed by quantifying expression of some known AR genes such as NKX3-1 a known prostate cancer biomarker (Gurel *et al.*, 2010) and FKBP5, an Hsp90-associated co-chaperone that regulates the responsiveness of steroid hormone receptors (O'Leary *et al.*, 2013).

The results show that HCoV-229E and OC43 infection induce an upregulation in gene expression of NKX3-1 while FKBP5 expression was increased after HCoV-OC43 infection and then reduced by ENZA treatment. The reduction of FKBP5 expression by ENZA was similarly reported by(Leach *et al.*, 2021). Ultimately the dual luciferase assay showed that HCoV-229E infection significantly induces androgen signalling unlike HCoV-OC43. Although research exploring AR and HCoV infection is limited, the role of AR signalling in relation to other viruses has been explored. For example a study by vom Steeg *et al.*, 2020b reported that the AR signaling in the lungs mitigates inflammation and improves the outcome of influenza in mice while SARS-Cov-2 infection is known to cause lung inflammation (li *et al.,* 2020). The AR has also been reported to Promote Hepatitis B induced hepatocarcinogenesis by modulating Hepatitis B transcription (Wu *et al.*, 2010). Nevertheless, since a crosstalk between the HCoVs and AR signalling has been identified, future work should aim to clarify whether they contribute to the cell tropism of HCoVs.

4.5 Conclusion and future perspectives.

HCoVs have been known to exist over the years. However, it was the Covid-19 pandemic caused by SARS-CoV-2 that fully thrust them into the spotlight of the research field making most of the HCoV research today predominantly towards SARS-CoV-2. Although the seasonal HCoVs cause mild respiratory disease they still cause more severe outcomes in the immuno-compromised or new populations (Forni *et al.*, 2021) . This study was designed to gain more insights into the tropism of HCoVs following the success of ENZA in reducing SARS-CoV-2 entry in lung cells (Leach *et al.*, 2021). Some limitations of this study include the lack of western blotting to confirm reduced protein levels from ENZA and camostat, inability to test other antiandrogens and as well as the unavailability of HCoV- OC43 and -HKU1 PV due to technical constraints and time. In summary, from this work we have confirmed that TMPRSS2 plays a role in cell entry of HCoV-229E and NL63. These results are in line with those of

Bertram *et al.*, 2013 in human airway epithelium cultures. Further studies involving HCoV optimised cell lines should be used to fully ascertain this. Furthermore, in addition to SARS-CoV-2 the antiandrogen enzalutamide was also demonstrated to reduce cell entry of HCoV-229E and NL63 in lung cell lines and these needs to be further investigated in human airway epithelium cells and mouse models in other to obtain more robust information. Contrary to the results from this study the Li *et al.*, 2021 group recorded the inability of ENZA to reduce SARS-CoV-2 infectivity in human lung organoid models. Similarly clinical trial (COVIDENZA) using ENZA as a treatment showed no significant therapeutic value for COVID-19 implying that it is ineffective for treatment of infected individuals (Welen *et al.*, 2021). However the use of Proxalutamide another experimental antiandrogen, significantly accelerated viral clearance on Day 7 in mild to moderate COVID-19 therefore reducing hospitalisation rates for outpatients (Cadegiani *et al.*, 2021b; Frontiers Editiorial, 2022).

Although the successful use of anti-androgens as a monotherapy for COVID-19 has been limited in clinical trials its use in combination therapies for pre- or post-exposure prophylaxis has shown some prospects and should be further explored. For example clinical trial results by Cadegiani *et al.*, (2021a) suggest that that the inclusion of an early antiandrogen therapy with Dutasteride to Nitazoxanide and Azithromycin for COVID-19 effectively reduces SARS-CoV-2 viral shedding, improve clinical symptoms, and prevent inflammatory responses. Interestingly in addition to the above results we also discovered that HCoV- 229E and SARS-CoV-2 infection causes differential expression of AR signalling genes, hinting at a complex crosstalk at the cellular level between the two events. Although the relationship is still unclear and worth investigating, subsequent research using molecular and cell biology tools such as western blotting and next generation sequencing are invaluable for the detailed characterisation of all HCoV tropism, most importantly it will also allow for a robust testing of ENZA and other antiandrogens as therapeutic options in relevant cell models.

REFERENCES

Acheampong, D. O., Barffour, I. K., Boye, A., Aninagyei, E., Ocansey, S. and Morna, M. T. (2020) Male predisposition to severe COVID-19: Review of evidence and potential therapeutic prospects. *Biomedicine & Pharmacotherapy,* **131**, 110748-Article No.: 110748.

Afar, D. E. H., Vivanco, I., Hubert, R. S., Kuo, J., Chen, E., Saffran, D. C., Raitano, A. B. and Jakobovits, A. (2001) Catalytic cleavage of the androgen-regulated TMPRSS2 protease results in its secretion by prostate and prostate cancer epithelia. *Cancer Research,* **61**, 1686-1692.

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C. and Garry, R. F. (2020) The proximal origin of SARS-CoV-2. *Nature medicine,* **26**, 450-452.

Armstrong, J., Niemann, H., Smeekens, S., Rottier, P. and Warren, G. (1984) SEQUENCE AND TOPOLOGY OF A MODEL INTRACELLULAR MEMBRANE-PROTEIN, E1-GLYCOPROTEIN, FROM A CORONAVIRUS. *Nature,* **308**, 751-752.

Baulieu, E. E. and Schumacher, M. (2000) Progesterone as a neuroactive neurosteroid, with special reference to the effect of progesterone on myelination. *Steroids,* **65**, 605-612.

Bayati, A., Kumar, R., Francis, V. and McPherson, P. S. (2021) SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. *Journal of Biological Chemistry,* **296**.

Beato, M. and Klug, J. (2000) Steroid hormone receptors: An update. *Human Reproduction Update,* **6**, 225-236.

Bertram, S., Dijkman, R., Habjan, M., Heurich, A., Gierer, S., Glowacka, I., Welsch, K., Winkler, M., Schneider, H., Hofmann-Winkler, H., Thiel, V. and Poehlmann, S. (2013) TMPRSS2 Activates the Human Coronavirus 229E for Cathepsin-Independent Host Cell Entry and Is Expressed in Viral Target Cells in the Respiratory Epithelium. *Journal of Virology,* **87**, 6150-6160.

Bestle, D., Heindl, M. R., Limburg, H., Van, T. V. L., Pilgram, O., Moulton, H., Stein, D. A., Hardes, K., Eickmann, M., Dolnik, O., Rohde, C., Klenk, H. D., Garten, W., Steinmetzer, T. and Bottcher-Friebertshauser, E. (2020) TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. *Life Science Alliance,* **3**.

Billah, M. A., Miah, M. M. and Khan, M. N. (2020) Reproductive number of coronavirus: A systematic review and meta-analysis based on global level evidence. *PLoS One,* **15**, e0242128-Article No.: e0242128.

Booth, C. M., Matukas, L. M., Tomlinson, G. A., Rachlis, A. R., Rose, D. B., Dwosh, H. A., Walmsley, S. L., Mazzulli, T., Avendano, M., Derkach, P., Ephtimios, I. E., Kitai, I., Mederski, B. D., Shadowitz, S. B., Gold, W. L., Hawryluck, L. A., Rea, E., Chenkin, J. S., Cescon, D. W., Poutanen, S. M. and Detsky, A. S. (2003) Clinical features and short-term outcomes of 144 patients with SARS in

the greater Toronto area. *JAMA (Journal of the American Medical Association),* **289**, 2801-2809.

Bradburne, A. F., Bynoe, M. L. and Tyrrell, D. A. (1967) Effects of a "new" human respiratory virus in volunteers. *British medical journal,* **3**, 767-9.

Brian, D. A. and Baric, R. S. (2005) Coronavirus genome structure and replication. *Current topics in microbiology and immunology,* **287**, 1-30.

Burkard, C., Verheije, M. H., Wicht, O., van Kasteren, S. I., van Kuppeveld, F. J., Haagmans, B. L., Pelkmans, L., Rottier, P. J. M., Bosch, B. J. and de Haan, C. A. M. (2014) Coronavirus Cell Entry Occurs through the Endo-/Lysosomal Pathway in a Proteolysis-Dependent Manner. *Plos Pathogens,* **10**.

Burks, J. S., DeVald, B. L., Jankovsky, L. D. and Gerdes, J. C. (1980) Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. *Science (New York, N.Y.),* **209**, 933-4.

Callow, K. A., Parry, H. F., Sergeant, M. and Tyrrell, D. A. J. (1990) THE TIME COURSE OF THE IMMUNE RESPONSE TO EXPERIMENTAL CORONAVIRUS INFECTION OF MAN. *Epidemiology and Infection,* **105**, 435-446.

Chan, J. F.-W., Kok, K.-H., Zhu, Z., Chu, H., To, K. K.-W., Yuan, S. and Yuen, K.-Y. (2020) Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerging Microbes & Infections,* **9**, 221-236.

Chen, B., Cao, D.-H., Guo, J.-B., Liu, L.-R. and Wei, Q. (2019) Androgen biosynthesis in castration-resistant prostate cancer: Advances in studies. *Zhonghua nan ke xue = National journal of andrology,* **25**, 265-271.

Cheng, Z., Zhou, J., To, K. K.-W., Chu, H., Li, C., Wang, D., Yang, D., Zheng, S., Hao, K., Bosse, Y., Obeidat, M. e., Brandsma, C.-A., Song, Y.-Q., Chen, Y., Zheng, B.-J., Li, L. and Yuen, K.-Y. (2015) Identification of TMPRSS2 as a Susceptibility Gene for Severe 2009 Pandemic A(H1N1) Influenza and A(H7N9) Influenza. *Journal of Infectious Diseases,* **212**, 1214-1221.

Dalpiaz, E. L. M., Lamas, A. Z., Caliman, I. F., Ribeiro, R. F., Jr., Abreu, G. R., Moyses, M. R., Andrade, T. U., Gouvea, S. A., Alves, M. F., Carmona, A. K. and Bissoli, N. S. (2015) Sex Hormones Promote Opposite Effects on ACE and ACE2 Activity, Hypertrophy and Cardiac Contractility in Spontaneously Hypertensive Rats (vol 10, e0127515, 2015). *PLoS One,* **10**, e0133225-Article No.: e0133225.

de Groot, R. J., Baker, S. C., Baric, R. S., Brown, C. S., Drosten, C., Enjuanes, L., Fouchier, R. A. M., Galiano, M., Gorbalenya, A. E., Memish, Z. A., Perlman, S., Poon, L. L. M., Snijder, E. J., Stephens, G. M., Woo, P. C. Y., Zaki, A. M., Zambon, M. and Ziebuhr, J. (2013) Middle East

Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. *Journal of Virology,* **87**, 7790-7792.

De Leo, V., Musacchio, M. C., Cappelli, V., Piomboni, P. and Morgante, G. (2016) Hormonal contraceptives: pharmacology tailored to women's health. *Human Reproduction Update,* **22**, 634-646.

DeDiego, M. L., Alvarez, E., Almazan, F., Rejas, M. T., Lamirande, E., Roberts, A., Shieh, W. J., Zaki, S. R., Subbarao, K. and Enjuanes, L. (2007) A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo. *Journal of Virology,* **81**, 1701-1713.

Dimmock, N. (2003) Cold wars: The fight against the common cold. *Nature,* **422**, 379-380.

Elmashala, A., Chopra, S. and Garg, A. (2020) The Neurologic Manifestations of Coronavirus Disease 2019. *Journal of neurology research,* **10**, 107-112.

Evans, R. M. (1988) The steroid and thyroid hormone receptor superfamily. *Science (New York, N.Y.),* **240**, 889-95.

Fabricant, J. (1998) The early history of infectious bronchitis. *Avian Diseases,* **42**, 648-650.

Fouchier, R. A. M., Hartwig, N. G., Bestebroer, T. M., Niemeyer, B., de Jong, J. C., Simon, J. H. and Osterhaus, A. D. M. E. (2004) A previously undescribed coronavirus associated with respiratory disease in humans. *Proceedings of the National Academy of Sciences of the United States of America,* **101**, 6212-6216.

Gaunt, E. R., Hardie, A., Claas, E. C. J., Simmonds, P. and Templeton, K. E. (2010) Epidemiology and Clinical Presentations of the Four Human Coronaviruses 229E, HKU1, NL63, and OC43 Detected over 3 Years Using a Novel Multiplex Real-Time PCR Method. *Journal of Clinical Microbiology,* **48**, 2940-2947.

Giotis ES, Cil E, Brooke GN. Use of Antiandrogens as Therapeutic Agents in COVID-19 Patients. Viruses. 2022 Dec 7;14(12):2728. doi: 10.3390/v14122728. PMID: 36560732; PMCID: PMC9788624.

Godet, M., Lharidon, R., Vautherot, J. F. and Laude, H. (1992) TGEV CORONA VIRUS ORF4 ENCODES A MEMBRANE-PROTEIN THAT IS INCORPORATED INTO VIRIONS. *Virology,* **188**, 666-675.

Goggins, W. (2004) Re: "Do men have a higher case fatality rate of severe acute respiratory syndrome than women do?". *American journal of epidemiology,* **160**, 925-6.

Gomez, J., Albaiceta, G. M., Garcia-Clemente, M., Lopez-Larrea, C., Amado-Rodriguez, L., Lopez-Alonso, I., Hermida, T., Enriquez, A. I., Herrero, P., Melon, S., Alvarez-Argueelles, M. E., Boga, J. A., Rojo-Alba, S., Cuesta-Llavona, E., Alvarez, V., Lorca, R. and Coto, E. (2020) Angiotensin-converting enzymes (ACE, ACE2) gene variants and COVID-19 outcome. *Gene (Amsterdam),* **762**, 145102-Article No.: 145102.

Gorbalenya, A. E., Baker, S. C., Baric, R. S., de Groot, R. J., Drosten, C., Gulyaeva, A. A., Haagmans, B. L., Lauber, C., Leontovich, A. M., Neuman, B. W., Penzar, D., Perlman, S., Poon, L. L. M., Samborskiy, D. V., Sidorov, I. A., Sola, I., Ziebuhr, J. and Coronaviridae Study, G. (2020) The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology,* **5**, 536-544.

Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., O'Meara, M. J., Guo, J. Z., Swaney, D. L., Tummino, T. A., Huttenhain, R., Kaake, R. M., Richards, A. L., Tutuncuoglu, B., Foussard, H., Batra, J., Haas, K., Modak, M., Kim, M., Haas, P., Polacco, B. J., Braberg, H., Fabius, J. M., Eckhardt, M., Soucheray, M., Bennett, M. J., Cakir, M., McGregor, M. J., Li, Q., Naing, Z. Z. C., Zhou, Y., Peng, S., Kirby, I. T., Melnyk, J. E., Chorba, J. S., Lou, K., Dai, S. A., Shen, W., Shi, Y., Zhang, Z., Barrio-Hernandez, I., Memon, D., Hernandez-Armenta, C., Mathy, C. J. P., Perica, T., Pilla, K. B., Ganesan, S. J., Saltzberg, D. J., Ramachandran, R., Liu, X., Rosenthal, S. B., Calviello, L., Venkataramanan, S., Lin, Y., Wankowicz, S. A., Bohn, M., Trenker, R., Young, J. M., Cavero, D., Hiatt, J., Roth, T., Rathore, U., Subramanian, A., Noack, J., Hubert, M., Roesch, F., Vallet, T., Meyer, B., White, K. M., Miorin, L., Agard, D., Emerman, M., Ruggero, D., Garcia-Sastre, A., Jura, N., von Zastrow, M., Taunton, J., Schwartz, O., Vignuzzi, M., d'Enfert, C., Mukherjee, S., Jacobson, M., Malik, H. S., Fujimori, D. G., Ideker, T., Craik, C. S., Floor, S., Fraser, J. S., Gross, J., Sali, A., Kortemme, T., Beltrao, P., Shokat, K., Shoichet, B. K. and Krogan, N. J. (2020) A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing. *bioRxiv : the preprint server for biology*.

Guo, T., Fan, Y. Z., Chen, M., Wu, X. Y., Zhang, L., He, T., Wang, H. R., Wan, J., Wang, X. H. and Lu, Z. B. (2020) Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). *Jama Cardiology,* **5**, 811-818.

Hall, O. J., Limjunyawong, N., Vermillion, M. S., Robinson, D. P., Wohlgemuth, N., Pekosz, A., Mitzner, W. and Klein, S. L. (2016) Progesterone-Based Therapy Protects Against Influenza by Promoting Lung Repair and Recovery in Females. *Plos Pathogens,* **12**.

Hamre, D. and Procknow, J. J. (1966) A new virus isolated from the human respiratory tract. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.),* **121**, 190-3.

Han, Y., Geng, H., Feng, W., Tang, X., Ou, A., Lao, Y., Xu, Y., Lin, H., Liu, H. and Li, Y. (2003) A follow-up study of 69 discharged SARS patients. *Journal of traditional Chinese medicine = Chung i tsa chih ying wen pan,* **23**, 214-7.

Hayashi, T. and Su, T. (2005) The sigma receptor: evolution of the concept in neuropsychopharmacology. *Current neuropharmacology,* **3**, 267-80.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Muller, M. A., Drosten, C. and Pohlmann, S. (2020a) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell,* **181**, 271-+.

Hoffmann, M., Schroeder, S., Kleine-Weber, H., Muller, M. A., Drosten, C. and Pohlmann, S. (2020b) Nafamostat Mesylate Blocks Activation of SARS-CoV-2: New Treatment Option for COVID-19. *Antimicrobial Agents and Chemotherapy,* **64**.

Hofmann, H. and Poehlmann, S. (2004) Cellular entry of the SARS coronavirus. *Trends in Microbiology,* **12**, 466-472.

Hofmann, H., Pyrc, K., van der Hoek, L., Geier, M., Berkhout, B. and Pohlmann, S. (2005) Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, 7988-7993.

Hsu, L.-Y., Lee, C.-C., Green, J. A., Ang, B., Paton, N. I., Lee, L., Villacian, J. S., Lim, P.-L., Earnest, A. and Leo, Y.-S. (2003) Severe acute respiratory syndrome (SARS) in Singapore: Clinical features of index patient and initial contacts. *Emerging Infectious Diseases,* **9**, 713-717.

Hu, T. Y., Frieman, M. and Wolfram, J. (2020) Insights from nanomedicine into chloroquine efficacy against COVID-19. *Nature Nanotechnology,* **15**, 247-249.

Hurst, K. R., Koetzner, C. A. and Masters, P. S. (2009) Identification of In Vivo-Interacting Domains of the Murine Coronavirus Nucleocapsid Protein. *Journal of Virology,* **83**, 7221-7234.

Hyodo, K. and Okuno, T. (2014) Host factors used by positive-strand RNA plant viruses for genome replication. *Journal of General Plant Pathology,* **80**, 123-135.

Iwata-Yoshikawa, N., Okamura, T., Shimizu, Y., Hasegawa, H., Takeda, M. and Nagata, N. (2019) TMPRSS2 Contributes to Virus Spread and Immunopathology in the Airways of Murine Models after Coronavirus Infection. *Journal of virology,* **93**.

Jackson, C. B., Farzan, M., Chen, B. and Choe, H. (2022) Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology,* **23**, 3-20.

Johannessen, M., Fontanilla, D., Mavlyutov, T., Ruoho, A. E. and Jackson, M. B. (2013) Antagonist action of progesterone at sigma-receptors in the modulation of voltage-gated sodium channels (vol 300, pg C328, 2011). *American Journal of Physiology-Cell Physiology,* **305**, C997-C997.

Joyner, J., Neves, L. A. A., Granger, J. P., Alexander, B. T., Merrill, D. C., Chappell, M. C., Ferrario, C. M., Davis, W. P. and Brosnihan, K. B. (2007) Temporal-spatial expression of ANG-(1-7) and angiotensin-converting enzyme 2 in the kidney of normal and hypertensive pregnant rats.

American Journal of Physiology-Regulatory Integrative and Comparative Physiology, **293**, R169-R177.

Kim, T. S., Heinlein, C., Hackman, R. C. and Nelson, P. S. (2006) Phenotypic analysis of mice lacking the Tmprss2-encoded protease. *Molecular and Cellular Biology,* **26**, 965-975.

Kuiper, G. G. J. M., Enmark, E., Pelto-Huikko, M., Nilsson, S. and Gustafsson, J.-A. (1996) Cloning of a novel oestrogen receptor expressed in rat prostate and ovary. *Proceedings of the National Academy of Sciences of the United States of America,* **93**, 5925-5930.

Kuo, L. L. and Masters, P. S. (2013) Functional Analysis of the Murine Coronavirus Genomic RNA Packaging Signal. *Journal of Virology,* **87**, 5182-5192.

Kuypers, J., Martin, E. T., Heugel, J., Wright, N., Morrow, R. and Englund, J. A. (2007) Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics,* **119**, E70-E76.

Lau, S. K. P., Woo, P. C. Y., Yip, C. C. Y., Tse, H., Tsoi, H. W., Cheng, V. C. C., Lee, P., Tang, B. S. F., Cheung, C. H. Y., Lee, R. A., So, L. Y., Lau, Y. L., Chan, K. H. and Yuen, K. Y. (2006) Coronavirus HKU1 and other coronavirus infections in Hong Kong. *Journal of Clinical Microbiology,* **44**, 2063-2071.

Leach, D. A., Mohr, A., Giotis, E. S., Cil, E., Isac, A. M., Yates, L. L., Barclay, W. S., Zwacka, R. M., Bevan, C. L. and Brooke, G. N. (2021) The antiandrogen enzalutamide downregulates TMPRSS2 and reduces cellular entry of SARS-CoV-2 in human lung cells. *Nature communications,* **12**, 4068-4068.

Lemes, R. M. R., Costa, A. J., Bartolomeo, C. S., Bassani, T. B., Nishino, M. S., Pereira, G. J. D., Smaili, S. S., Maciel, R. M. D., Braconi, C. T., da Cruz, E. F., Ramirez, A. L., Maricatto, J. T., Janini, L. M. R., Prado, C. M., Stilhano, R. S. and Ureshino, R. P. (2021) 17 beta-estradiol reduces SARS-CoV-2 infection in vitro. *Physiological Reports,* **9**.

Limburg, H., Harbig, A., Bestle, D., Stein, D. A., Moulton, H. M., Jaeger, J., Janga, H., Hardes, K., Koepke, J., Schulte, L., Koczulla, A. R., Schmeck, B., Klenk, H. D. and Bottcher-Friebertshauser, E. (2019) TMPRSS2 Is the Major Activating Protease of Influenza A Virus in Primary Human Airway Cells and Influenza B Virus in Human Type II Pneumocytes. *Journal of Virology,* **93**.

Lin, B., Ferguson, C., White, J. T., Wang, S., Vessella, R., True, L. D., Hood, L. and Nelson, P. S. (1999) Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. *Cancer Research,* **59**, 4180-4184.

Liu, D. X., Fung, T. S., Chong, K. K.-L., Shukla, A. and Hilgenfeld, R. (2014) Accessory proteins of SARS-CoV and other coronaviruses. *Antiviral Research,* **109**, 97-109.

Lucas, J. M., Heinlein, C., Kim, T., Hernandez, S. A., Malik, M. S., True, L. D., Morrissey, C., Corey, E., Montgomery, B., Mostaghel, E., Clegg, N., Coleman, I., Brown, C. M., Schneider, E. L., Craik, C., Simon, J. A., Bedalov, A. and Nelson, P. S. (2014) The Androgen-Regulated Protease TMPRSS2

Activates a Proteolytic Cascade Involving Components of the Tumor Microenvironment and Promotes Prostate Cancer Metastasis. *Cancer Discovery,* **4**, 1310-1325.

Lucas, J. M., True, L., Hawley, S., Matsumura, M., Morrissey, C., Vessella, R. and Nelson, P. S. (2008) The androgen-regulated type II serine protease TMPRSS2 is differentially expressed and mislocalized in prostate adenocarcinoma. *Journal of Pathology,* **215**, 118-125.

Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schuetz, G., Umesono, K., Blumnberg, B., Kastner, P., Mark, M., Chambon, P. and Evans, R. M. (1995) The nuclear receptor superfamily: The second decade. *Cell,* **83**, 835-839.

Marsolais, G., Berthiaume, L., DiFranco, E. and Marois, P. (1971) Rapid diagnosis by electron microscopy of avian coronavirus infection. *Canadian journal of comparative medicine : Revue canadienne de medecine comparee,* **35**, 285-8.

Masters, P. S. (2006) The molecular biology of coronaviruses. *Advances in virus research,* **66**, 193-292.

Matsuyama, S., Nagata, N., Shirato, K., Kawase, M., Takeda, M. and Taguchi, F. (2010) Efficient Activation of the Severe Acute Respiratory Syndrome Coronavirus Spike Protein by the Transmembrane Protease TMPRSS2. *Journal of Virology,* **84**, 12658-12664.

McIntosh, K., Dees, J. H., Becker, W. B., Kapikian, A. Z. and Chanock, R. M. (1967) Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proceedings of the National Academy of Sciences of the United States of America,* **57**, 933-40.

Miguel Vela, J. (2020) Repurposing Sigma-1 Receptor Ligands for COVID-19 Therapy? *Frontiers in Pharmacology,* **11**.

Miller, W. L. and Auchus, R. J. (2011) The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine reviews,* **32**, 81-151.

Mingels, A. M. A. and Kimenai, D. M. (2018) Sex-Related Aspects of Biomarkers in Cardiac Disease. *Sex-Specific Analysis of Cardiovascular Function,* **1065**, 545-564.

Mikkonen, L., Pihlajamaa, P., Sahu, B., Zhang, F. P. and Janne, O. A. (2010) Androgen receptor and androgen-dependent gene expression in lung. *Molecular and Cellular Endocrinology,* **317**, 14-24

Mowszowicz, I. (1989) Antiandrogens. Mechanisms and paradoxical effects. *Annales d'endocrinologie,* **50**, 189-99.

Murgolo, N., Therien, A. G., Howell, B., Klein, D., Koeplinger, K., Lieberman, L. A., Adam, G. C., Flynn, J., McKenna, P., Swaminathan, G., Hazuda, D. J. and Olsen, D. B. (2021) SARS-CoV-2 tropism, entry, replication, and propagation: Considerations for drug discovery and development. *PLoS Pathogens,* **17**, e1009225-Article No.: e1009225.

Nal, B., Chan, C. M., Kien, F., Siu, L., Tse, J., Chu, K., Kam, J., Staropoli, I., Crescenzo-Chaigne, B., Escriou, N., van der Werf, S., Yuen, K. Y. and Altmeyer, R. (2005) Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. *Journal of General Virology,* **86**, 1423-1434.

Neuman, B. W., Kiss, G., Kunding, A. H., Bhella, D., Baksh, M. F., Connelly, S., Droese, B., Klaus, J. P., Makino, S., Sawicki, S. G., Siddell, S. G., Stamou, D. G., Wilson, I. A., Kuhn, P. and Buchmeier, M. J. (2011) A structural analysis of M protein in coronavirus assembly and morphology. *Journal of Structural Biology,* **174**, 11-22.

Nieto-Torres, J. L., DeDiego, M. L., Alvarez, E., Jimenez-Guardeno, J. M., Regla-Nava, J. A., Llorente, M., Kremer, L., Shuo, S. and Enjuanes, L. (2011) Subcellular location and topology of severe acute respiratory syndrome coronavirus envelope protein. *Virology,* **415**, 69-82.

Nieto-Torres, J. L., DeDiego, M. L., Verdia-Baguena, C., Jimenez-Guardeno, J. M., Regla-Nava, J. A., Fernandez-Delgado, R., Castano-Rodriguez, C., Alcaraz, A., Torres, J., Aguilella, V. M. and Enjuanes, L. (2014) Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Ion Channel Activity Promotes Virus Fitness and Pathogenesis. *Plos Pathogens,* **10**.

Novac, N. and Heinzel, T. (2004) Nuclear receptors: overview and classification. *Current drug targets. Inflammation and allergy,* **3**, 335-46.

Onder, G., Rezza, G. and Brusaferro, S. (2020) Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *Jama,* **323**, 1775-1776.

Ou, T. L., Mou, H. H., Zhang, L. Z., Ojha, A., Choe, H. and Farzan, M. (2021) Hydroxychloroquine-mediated inhibition of SARS-CoV-2 entry is attenuated by TMPRSS2. *Plos Pathogens,* **17**.

Pene, F., Merlat, A., Vabret, A., Rozenberg, F., Buzyn, A., Dreyfus, F., Cariou, A., Freymuth, F. and Lebon, P. (2003) Coronavirus 229E-related pneumonia in immunocompromised patients. *Clinical Infectious Diseases,* **37**, 929-932.

Perlman, S. and Netland, J. (2009) Coronaviruses post-SARS: update on replication and pathogenesis. *Nature reviews. Microbiology,* **7**, 439-50.

Qiao, Y., Wang, X.-M., Mannan, R., Pitchiaya, S., Zhang, Y., Wotring, J. W., Xiao, L., Robinson, D. R., Wu, Y.-M., Tien, J. C.-Y., Cao, X., Simko, S. A., Apel, I. J., Bawa, P., Kregel, S., Narayanan, S. P., Raskind, G., Ellison, S. J., Parolia, A., Zelenka-Wang, S., McMurry, L., Su, F., Wang, R., Cheng, Y., Delekta, A. D., Mei, Z., Pretto, C. D., Wang, S., Mehra, R., Sexton, J. Z. and Chinnaiyan, A. M. (2021) Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and TMPRSS2. *Proceedings of the National Academy of Sciences of the United States of America,* **118**, e2021450118-Article No.: e2021450118.

Redondo, N., Zaldivar-Lopez, S., Garrido, J. J. and Montoya, M. (2021) SARS-CoV-2 Accessory Proteins in Viral Pathogenesis: Knowns and Unknowns. *Frontiers in immunology,* **12**, 708264-708264.

Rehman, S., Ravinayagam, V., Nahvi, I., Aldossary, H., Al-Shammari, M., Amiri, M. S. A., Kishore, U. and Al-Suhaimi, E. A. (2021) Immunity, Sex Hormones, and Environmental Factors as Determinants of COVID-19 Disparity in Women. *Frontiers in immunology,* **12**, 680845-680845.

Resta, S., Luby, J. P., Rosenfeld, C. R. and Siegel, J. D. (1985a) ISOLATION AND PROPAGATION OF A HUMAN ENTERIC CORONAVIRUS. *Science,* **229**, 978-981.

Resta, S., Luby, J. P., Rosenfeld, C. R. and Siegel, J. D. (1985b) Isolation and propagation of a human enteric coronavirus. *Science (New York, N.Y.),* **229**, 978-81.

Sakai, K., Ami, Y., Tahara, M., Kubota, T., Anraku, M., Abe, M., Nakajima, N., Sekizuka, T., Shirato, K., Suzaki, Y., Ainai, A., Nakatsu, Y., Kanou, K., Nakamura, K., Suzuki, T., Komase, K., Nobusawa, E., Maenaka, K., Kuroda, M., Hasegawa, H., Kawaoka, Y., Tashiro, M. and Takeda, M. (2014) The Host Protease TMPRSS2 Plays a Major Role in In Vivo Replication of Emerging H7N9 and Seasonal Influenza Viruses. *Journal of Virology,* **88**, 5608-5616.

Sanders, J. M., Monogue, M. L., Jodlowski, T. Z. and Cutrell, J. B. (2020) Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19) A Review. *Jama-Journal of the American Medical Association,* **323**, 1824-1836.

Schelle, B., Karl, N., Ludewig, B., Siddell, S. G. and Thiel, V. (2005) Selective replication of coronavirus genomes that express nucleocapsid protein. *Journal of Virology,* **79**, 6620-6630.

Schwabe, J. W. R., Chapman, L., Finch, J. T. and Rhodes, D. (1993) The crystal structure of the oestrogen receptor DNA-binding domain bound to DNA: How receptors discriminate between their response elements. *Cell,* **75**, 567-578.

Shang, Z., Chan, S. Y., Liu, W. J., Li, P. and Huang, W. (2021) Recent Insights into Emerging Coronavirus: SARS-CoV-2. *ACS Infectious Diseases,* **7**, 1369-1388.

Shi, Z. (2021) From SARS, MERS to COVID-19: A journey to understand bat coronaviruses. *Bulletin De L Academie Nationale De Medecine,* **205**, 732-736.

Shim, E., Tariq, A., Choi, W., Lee, Y. and Chowell, G. (2020) Transmission potential and severity of COVID-19 in South Korea. *International Journal of Infectious Diseases,* **93**, 339-344.

Shirato, K., Kawase, M. and Matsuyama, S. (2013) Middle East Respiratory Syndrome Coronavirus Infection Mediated by the Transmembrane Serine Protease TMPRSS2. *Journal of Virology,* **87**, 12552-12561.

Shirato, K., Kawase, M. and Matsuyama, S. (2018) Wild-type human coronaviruses prefer cell-surface TMPRSS2 to endosomal cathepsins for cell entry. *Virology,* **517**, 9-15.

Simmons, G., Gosalia, D. N., Rennekamp, A. J., Reeves, J. D., Diamond, S. L. and Bates, P. (2005) Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, 11876-11881.

Song, H. C., Seo, M.-Y., Stadler, K., Yoo, B. J., Choo, Q.-L., Coates, S. R., Uematsu, Y., Harada, T., Greer, C. E., Polo, J. M., Pileri, P., Eickmann, M., Rappuoli, R., Abrignani, S., Houghton, M. and Han, J. H. (2004) Synthesis and characterization of a native, oligomeric form of recombinant severe acute respiratory syndrome coronavirus spike glycoprotein. *Journal of Virology,* **78**, 10328-10335.

Song, I.-S., Jeong, Y. J., Kim, J., Seo, K.-H., Baek, N.-I., Kim, Y., Kim, C.-S. and Jang, S.-W. (2020) Pharmacological inhibition of androgen receptor expression induces cell death in prostate cancer cells. *Cellular and molecular life sciences : CMLS,* **77**, 4663-4673.

Stadler, K., Masignani, V., Eickmann, M., Becker, S., Abrignani, S., Klenk, H.-D. and Rappuoli, R. (2003) SARS--beginning to understand a new virus. *Nature reviews. Microbiology,* **1**, 209-18.

Student, S., Hejmo, T., Poterala-Hejmo, A., Lesniak, A. and Buldak, R. (2020) Anti-androgen hormonal therapy for cancer and other diseases. *European Journal of Pharmacology,* **866**, 172783-Article No.: 172783.

Taneja, V. (2018) Sex Hormones Determine Immune Response. *Frontiers in immunology,* **9**, 1931-1931.

Tarnow, C., Engels, G., Arendt, A., Schwalm, F., Sediri, H., Preuss, A., Nelson, P. S., Garten, W., Klenk, H.-D., Gabriel, G. and Boettcher-Friebertshaeuser, E. (2014) TMPRSS2 Is a Host Factor That Is Essential for Pneumotropism and Pathogenicity of H7N9 Influenza A Virus in Mice. *Journal of Virology,* **88**, 4744-4751.

To, K. K.-W., Chan, W.-M., Ip, J. D., Chu, A. W.-H., Tam, A. R., Liu, R., Wu, A. K.-L., Lung, K.-C., Tsang, O. T.-Y., Lau, D. P.-L., To, W.-K., Kwan, M. Y.-W., Yau, Y.-S., Ng, A. C.-K., Yip, C. C.-Y., Chan, K.-H., Tse, H., Hung, I. F.-N. and Yuen, K.-Y. (2021) Unique Clusters of Severe Acute Respiratory Syndrome Coronavirus 2 Causing a Large Coronavirus Disease 2019 Outbreak in Hong Kong. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America,* **73**, 137-142.

Turk, V., Stoka, V., Vasiljeva, O., Renko, M., Sun, T., Turk, B. and Turk, D. (2012) Cysteine cathepsins: From structure, function and regulation to new frontiers. *Biochimica Et Biophysica Acta-Proteins and Proteomics,* **1824**, 68-88.

Turner, A. J., Hiscox, J. A. and Hooper, N. M. (2004) ACE2: from vasopeptidase to SARS virus receptor. *Trends in Pharmacological Sciences,* **25**, 291-294.

Vabret, A., Dina, J., Gouarin, S., Petitjean, J., Corbet, S. and Freymuth, F. (2006) Detection of the new human coronavirus HKU1: A report of 6 cases. *Clinical Infectious Diseases,* **42**, 634-639.

van der Hoek, L., Pyrc, K. and Berkhout, B. (2006) Human coronavirus NL63, a new respiratory virus. *FEMS microbiology reviews,* **30**, 760-73.

van der Hoek, L., Pyrc, K., Jebbink, M. F., Vermeulen-Oost, W., Berkhout, R. J. M., Wolthers, K. C., Wertheim-van Dillen, P. M. E., Kaandorp, J., Spaargaren, J. and Berkhout, B. (2004) Identification of a new human coronavirus. *Nature Medicine,* **10**, 368-373.

vom Steeg, L. G., Dhakal, S., Woldetsadik, Y. A., Park, H.-S., Mulka, K. R., Reilly, E. C., Topham, D. J. and Klein, S. L. (2020) Androgen receptor signaling in the lungs mitigates inflammation and improves the outcome of influenza in mice. *PLoS Pathogens,* **16**, e1008506-Article No.: e1008506.

vom Steeg, L. G., Vermillion, M. S., Hall, O. J., Alam, O., McFarland, R., Chen, H., Zirkin, B. and Klein, S. L. (2016) Age and testosterone mediate influenza pathogenesis in male mice. *American Journal of Physiology - Lung Cellular and Molecular Physiology,* **311**, L1234-L1244.

Walsh, E. E., Shin, J. H. and Falsey, A. R. (2013) Clinical Impact of Human Coronaviruses 229E and OC43 Infection in Diverse Adult Populations. *Journal of Infectious Diseases,* **208**, 1634-1642.

Wambier, C. G., Goren, A., Vano-Galvan, S., Ramos, P. M., Ossimetha, A., Nau, G., Herrera, S. and McCoy, J. (2020) Androgen sensitivity gateway to COVID-19 disease severity. *Drug Development Research,* **81**.

Wierman, M. E. (2007) Sex steroid effects at target tissues: mechanisms of action. *Advances in physiology education,* **31**, 26-33.

Wirth, M. P., Hakenberg, O. W. and Froehner, M. (2007) Antiandrogens in the treatment of prostate cancer. *European Urology,* **51**, 306-314.

Woo, P. C. Y., Lau, S. K. P., Chu, C.-M., Chan, K.-H., Tsoi, H.-W., Yi, H., Wong, B. H. L., Poon, R. W. S., Cai, J. J., Luk, W.-K., Poon, L. L. M., Wong, S. S. Y., Yi, G., Peiris, J. S. M. and Yuen, K.-Y. (2005) Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *Journal of Virology,* **79**, 884-895.

Wu, K., Li, W., Peng, G. and Li, F. (2009) Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. *Proceedings of the National Academy of Sciences of the United States of America,* **106**, 19970-19974.

Xu, H., Zhong, L., Deng, J., Peng, J., Dan, H., Zeng, X., Li, T. and Chen, Q. (2020) High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *International journal of oral science,* **12**, 8-8.

Yeager, C. L., Ashmun, R. A., Williams, R. K., Cardellichio, C. B., Shapiro, L. H., Look, A. T. and Holmes, K. V. (1992) HUMAN AMINOPEPTIDASE-N IS A RECEPTOR FOR HUMAN CORONAVIRUS-229E. *Nature,* **357**, 420-422.

Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. M. E. and Fouchier, R. A. M. (2012) Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *New England Journal of Medicine,* **367**, 1814-1820.

Zhao, M. M., Yang, W. L., Yang, F. Y., Zhang, L., Huang, W. J., Hou, W., Fan, C. F., Jin, R. H., Feng, Y. M., Wang, Y. C. and Yang, J. K. (2021) Cathepsin L plays a key role in SARS-CoV-2 infection in humans and humanized mice and is a promising target for new drug development. *Signal Transduction and Targeted Therapy,* **6**.

Zheng, Q., Deng, Y., Liu, J., van der Hoek, L., Berkhout, B. and Lu, M. (2006) Core structure of S2 from the human coronavirus NL63 spike Glycoprotein. *Biochemistry,* **45**, 15205-15215.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Deng, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F. and Shi, Z.-L. (2020a) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature (London),* **579**, 270-273.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Deng, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F. and Shi, Z.-L. (2020b) A pneumonia outbreak associated with a new coronavirus of probable bat origin (vol 579, pg 270, 2020). *Nature (London),* **588**.

Zuckerman, A. J., Taylor, P. E. and Almeida, J. D. (1970) Presence of particles other than the Australia-SH antigen in a case of chronic active hepatitis with cirrhosis. *British medical journal,* **1**, 262-4.

Zumla, A., Chan, J. F. W., Azhar, E. I., Hui, D. S. C. and Yuen, K.-Y. (2016) Coronaviruses - drug discovery and therapeutic options. *Nature reviews. Drug discovery,* **15**, 327-47.

Baratchian, M., McManus, J.M., Berk, M.P. *et al.* Androgen regulation of pulmonary AR, TMPRSS2 and ACE2 with implications for sex-discordant COVID-19 outcomes. *Sci Rep* **11**, 11130 (2021). https://doi.org/10.1038/s41598-021-90491-1

Baulieu, E. E. and Schumacher, M. (2000) Progesterone as a neuroactive neurosteroid, with special reference to the effect of progesterone on myelination. *Steroids,* **65**, 605-612.

Bayati, A., Kumar, R., Francis, V. and McPherson, P. S. (2021) SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. *Journal of Biological Chemistry,* **296**.

Beato, M. and Klug, J. (2000) Steroid hormone receptors: An update. *Human Reproduction Update,* **6**, 225-236.

Bertram, S., Dijkman, R., Habjan, M., Heurich, A., Gierer, S., Glowacka, I., Welsch, K., Winkler, M., Schneider, H., Hofmann-Winkler, H., Thiel, V. and Poehlmann, S. (2013) TMPRSS2 Activates the Human Coronavirus 229E for Cathepsin-Independent Host Cell Entry and Is Expressed in Viral Target Cells in the Respiratory Epithelium. *Journal of Virology,* **87**, 6150-6160.

Bestle, D., Heindl, M. R., Limburg, H., Van, T. V. L., Pilgram, O., Moulton, H., Stein, D. A., Hardes, K., Eickmann, M., Dolnik, O., Rohde, C., Klenk, H. D., Garten, W., Steinmetzer, T. and Bottcher-Friebertshauser, E. (2020) TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. *Life Science Alliance,* **3**.

Billah, M. A., Miah, M. M. and Khan, M. N. (2020) Reproductive number of coronavirus: A systematic review and meta-analysis based on global level evidence. *PLoS One,* **15**, e0242128-Article No.: e0242128.

Booth, C. M., Matukas, L. M., Tomlinson, G. A., Rachlis, A. R., Rose, D. B., Dwosh, H. A., Walmsley, S. L., Mazzulli, T., Avendano, M., Derkach, P., Ephtimios, I. E., Kitai, I., Mederski, B. D., Shadowitz, S. B., Gold, W. L., Hawryluck, L. A., Rea, E., Chenkin, J. S., Cescon, D. W., Poutanen, S. M. and Detsky, A. S. (2003) Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA (Journal of the American Medical Association),* **289**, 2801-2809.

Bradburne, A. F., Bynoe, M. L. and Tyrrell, D. A. (1967) Effects of a "new" human respiratory virus in volunteers. *British medical journal,* **3**, 767-9.

Brian, D. A. and Baric, R. S. (2005) Coronavirus genome structure and replication. *Current topics in microbiology and immunology,* **287**, 1-30.

Burkard, C., Verheije, M. H., Wicht, O., van Kasteren, S. I., van Kuppeveld, F. J., Haagmans, B. L., Pelkmans, L., Rottier, P. J. M., Bosch, B. J. and de Haan, C. A. M. (2014) Coronavirus Cell Entry Occurs through the Endo-/Lysosomal Pathway in a Proteolysis-Dependent Manner. *Plos Pathogens,* **10**.

Burks, J. S., DeVald, B. L., Jankovsky, L. D. and Gerdes, J. C. (1980) Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. *Science (New York, N.Y.),* **209**, 933-4.

Cadegiani, F. A., McCoy, J., Wambier, C. G. and Goren, A. (2021a) Early Antiandrogen Therapy With Dutasteride Reduces Viral Shedding, Inflammatory Responses, and Time-to-Remission in Males With COVID-19: A Randomized, Double-Blind, Placebo-Controlled Interventional Trial (EAT-DUTA AndroCoV Trial - Biochemical). *Cureus Journal of Medical Science,* **13**.

Cadegiani, F. A., McCoy, J., Wambier, C. G., Vano-Galvan, S., Shapiro, J., Tosti, A., Zimerman, R. A. and Goren, A. (2021b) Proxalutamide Significantly Accelerates Viral Clearance and Reduces Time to Clinical Remission in Patients with Mild to Moderate COVID-19: Results from a Randomized, Double-Blinded, Placebo-Controlled Trial. *Cureus Journal of Medical Science,* **13**.

Callow, K. A., Parry, H. F., Sergeant, M. and Tyrrell, D. A. J. (1990) THE TIME COURSE OF THE IMMUNE RESPONSE TO EXPERIMENTAL CORONAVIRUS INFECTION OF MAN. *Epidemiology and Infection,* **105**, 435-446.

Chan, J. F.-W., Kok, K.-H., Zhu, Z., Chu, H., To, K. K.-W., Yuan, S. and Yuen, K.-Y. (2020) Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerging Microbes & Infections,* **9**, 221-236.

Chen, B., Cao, D.-H., Guo, J.-B., Liu, L.-R. and Wei, Q. (2019) Androgen biosynthesis in castration-resistant prostate cancer: Advances in studies. *Zhonghua nan ke xue = National journal of andrology,* **25**, 265-271.

Cheng, Z., Zhou, J., To, K. K.-W., Chu, H., Li, C., Wang, D., Yang, D., Zheng, S., Hao, K., Bosse, Y., Obeidat, M. e., Brandsma, C.-A., Song, Y.-Q., Chen, Y., Zheng, B.-J., Li, L. and Yuen, K.-Y. (2015) Identification of TMPRSS2 as a Susceptibility Gene for Severe 2009 Pandemic A(H1N1) Influenza and A(H7N9) Influenza. *Journal of Infectious Diseases,* **212**, 1214-1221.

Dalpiaz, E. L. M., Lamas, A. Z., Caliman, I. F., Ribeiro, R. F., Jr., Abreu, G. R., Moyses, M. R., Andrade, T. U., Gouvea, S. A., Alves, M. F., Carmona, A. K. and Bissoli, N. S. (2015) Sex Hormones Promote Opposite Effects on ACE and ACE2 Activity, Hypertrophy and Cardiac Contractility in Spontaneously Hypertensive Rats (vol 10, e0127515, 2015). *PLoS One,* **10**, e0133225-Article No.: e0133225.

de Groot, R. J., Baker, S. C., Baric, R. S., Brown, C. S., Drosten, C., Enjuanes, L., Fouchier, R. A. M., Galiano, M., Gorbalenya, A. E., Memish, Z. A., Perlman, S., Poon, L. L. M., Snijder, E. J., Stephens, G. M., Woo, P. C. Y., Zaki, A. M., Zambon, M. and Ziebuhr, J. (2013) Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. *Journal of Virology,* **87**, 7790-7792.

De Leo, V., Musacchio, M. C., Cappelli, V., Piomboni, P. and Morgante, G. (2016) Hormonal contraceptives: pharmacology tailored to women's health. *Human Reproduction Update,* **22**, 634-646.

DeDiego, M. L., Alvarez, E., Almazan, F., Rejas, M. T., Lamirande, E., Roberts, A., Shieh, W. J., Zaki, S. R., Subbarao, K. and Enjuanes, L. (2007) A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo. *Journal of Virology,* **81**, 1701-1713.

Deng, Q., Rasool, R. U., Russell, R. M., Natesan, R. and Asangani, I. A. (2021) Targeting androgen regulation of TMPRSS2 and ACE2 as a therapeutic strategy to combat COVID-19. *Iscience,* **24**.

Di Genova, C., Sampson, A., Scott, S., Cantoni, D., Mayora-Neto, M., Bentley, E., Mattiuzzo, G., Wright, E., Derveni, M., Auld, B., Ferrara, B. T., Harrison, D., Said, M., Selim, A., Thompson, E., Thompson, C., Carnell, G. and Temperton, N. (2021) Production, Titration, Neutralisation, Storage and Lyophilisation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Lentiviral Pseudotypes. *Bio-Protocol,* **11**.

Dimmock, N. (2003) Cold wars: The fight against the common cold. *Nature,* **422**, 379-380.

Djomkam, A. L. Z., Olwal, C. O., Sala, T. B. and Paemka, L. (2020) Commentary: SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Frontiers in Oncology,* **10**.

Eder, I. E., Culig, Z., Putz, T., Nessler-Menardi, C., Bartsch, G. and Klocker, H. (2001) Molecular biology of the androgen receptor: From molecular understanding to the clinic. *European Urology,* **40**, 241-251.

Elmashala, A., Chopra, S. and Garg, A. (2020) The Neurologic Manifestations of Coronavirus Disease 2019. *Journal of neurology research,* **10**, 107-112.

Evans, R. M. (1988) The steroid and thyroid hormone receptor superfamily. *Science (New York, N.Y.),* **240**, 889-95.

Fabricant, J. (1998) The early history of infectious bronchitis. *Avian Diseases,* **42**, 648-650.

Forni, D., Cagliani, R., Arrigoni, F., Benvenuti, M., Mozzi, A., Pozzoli, U., Clerici, M., De Gioia, L. and Sironi, M. (2021) Adaptation of the endemic coronaviruses HCoV-OC43 and HCoV-229E to the human host. *Virus Evolution,* **7**.

Fouchier, R. A. M., Hartwig, N. G., Bestebroer, T. M., Niemeyer, B., de Jong, J. C., Simon, J. H. and Osterhaus, A. D. M. E. (2004) A previously undescribed coronavirus associated with respiratory disease in humans. *Proceedings of the National Academy of Sciences of the United States of America,* **101**, 6212-6216.

Friedman, N., Jacob-Hirsch, J., Drori, Y., Eran, E., Kol, N., Nayshool, O., Mendelson, E., Rechavi, G. and Mandelboim, M. (2021) Transcriptomic profiling and genomic mutational analysis of Human coronavirus (HCoV)-229E-infected human cells. *Plos One,* **16**.

Frontiers Editiorial, O. (2022) RETRACTION: Proxalutamide Reduces the Rate of Hospitalization for COVID-19 Male Outpatients: A Randomized Double-Blinded Placebo-Controlled Trial (Retraction of Vol 8, art no 668698, 2021). *Frontiers in Medicine,* **9**.

Gard, A. L., Luu, R. J., Miller, C. R., Maloney, R., Cain, B. P., Marr, E. E., Burns, D. M., Gaibler, R., Mulhern, T. J., Wong, C. A., Alladina, J., Coppeta, J. R., Liu, P., Wang, J. P., Azizgolshani, H., Fezzie, R. F., Balestrini, J. L., Isenberg, B. C., Medoff, B. D., Finberg, R. W. and Borenstein, J. T.

(2021) High-throughput human primary cell-based airway model for evaluating influenza, coronavirus, or other respiratory viruses in vitro. *Scientific Reports,* **11**.

Gaunt, E. R., Hardie, A., Claas, E. C. J., Simmonds, P. and Templeton, K. E. (2010) Epidemiology and Clinical Presentations of the Four Human Coronaviruses 229E, HKU1, NL63, and OC43 Detected over 3 Years Using a Novel Multiplex Real-Time PCR Method. *Journal of Clinical Microbiology,* **48**, 2940-2947.

Ghani, M. J. (2022) SGK1, autophagy and cancer: an overview. *Molecular Biology Reports,* **49**, 675-685.

Godet, M., Lharidon, R., Vautherot, J. F. and Laude, H. (1992) TGEV CORONA VIRUS ORF4 ENCODES A MEMBRANE-PROTEIN THAT IS INCORPORATED INTO VIRIONS. *Virology,* **188**, 666-675.

Goggins, W. (2004) Re: "Do men have a higher case fatality rate of severe acute respiratory syndrome than women do?". *American journal of epidemiology,* **160**, 925-6.

Gomez, J., Albaiceta, G. M., Garcia-Clemente, M., Lopez-Larrea, C., Amado-Rodriguez, L., Lopez-Alonso, I., Hermida, T., Enriquez, A. I., Herrero, P., Melon, S., Alvarez-Argueelles, M. E., Boga, J. A., Rojo-Alba, S., Cuesta-Llavona, E., Alvarez, V., Lorca, R. and Coto, E. (2020) Angiotensin-converting enzymes (ACE, ACE2) gene variants and COVID-19 outcome. *Gene (Amsterdam),* **762**, 145102-Article No.: 145102.

Gorbalenya, A. E., Baker, S. C., Baric, R. S., de Groot, R. J., Drosten, C., Gulyaeva, A. A., Haagmans, B. L., Lauber, C., Leontovich, A. M., Neuman, B. W., Penzar, D., Perlman, S., Poon, L. L. M., Samborskiy, D. V., Sidorov, I. A., Sola, I., Ziebuhr, J. and Coronaviridae Study, G. (2020) The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology,* **5**, 536-544.

Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., O'Meara, M. J., Guo, J. Z., Swaney, D. L., Tummino, T. A., Huttenhain, R., Kaake, R. M., Richards, A. L., Tutuncuoglu, B., Foussard, H., Batra, J., Haas, K., Modak, M., Kim, M., Haas, P., Polacco, B. J., Braberg, H., Fabius, J. M., Eckhardt, M., Soucheray, M., Bennett, M. J., Cakir, M., McGregor, M. J., Li, Q., Naing, Z. Z. C., Zhou, Y., Peng, S., Kirby, I. T., Melnyk, J. E., Chorba, J. S., Lou, K., Dai, S. A., Shen, W., Shi, Y., Zhang, Z., Barrio-Hernandez, I., Memon, D., Hernandez-Armenta, C., Mathy, C. J. P., Perica, T., Pilla, K. B., Ganesan, S. J., Saltzberg, D. J., Ramachandran, R., Liu, X., Rosenthal, S. B., Calviello, L., Venkataramanan, S., Lin, Y., Wankowicz, S. A., Bohn, M., Trenker, R., Young, J. M., Cavero, D., Hiatt, J., Roth, T., Rathore, U., Subramanian, A., Noack, J., Hubert, M., Roesch, F., Vallet, T., Meyer, B., White, K. M., Miorin, L., Agard, D., Emerman, M., Ruggero, D., Garcia-Sastre, A., Jura, N., von Zastrow, M., Taunton, J., Schwartz, O., Vignuzzi, M., d'Enfert, C., Mukherjee, S., Jacobson, M., Malik, H. S., Fujimori, D. G., Ideker, T., Craik, C. S., Floor, S., Fraser, J. S., Gross, J., Sali, A., Kortemme, T., Beltrao, P., Shokat, K., Shoichet, B. K. and Krogan, N. J. (2020) A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing. *bioRxiv : the preprint server for biology*.

Griffin, J., Chen, Y. Q., Catto, J. W. F. and El-Khamisy, S. (2022) Gene of the month: NKX3.1. *Journal of Clinical Pathology,* **75**, 361-364.

Guo, T., Fan, Y. Z., Chen, M., Wu, X. Y., Zhang, L., He, T., Wang, H. R., Wan, J., Wang, X. H. and Lu, Z. B. (2020) Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). *Jama Cardiology,* **5**, 811-818.

Gurel, B., Ali, T. Z., Montgomery, E. A., Begum, S., Hicks, J., Goggins, M., Eberhart, C. G., Clark, D. P., Bieberich, C. J., Epstein, J. I. and De Marzo, A. M. (2010) NKX3.1 as a Marker of Prostatic Origin in Metastatic Tumors. *American Journal of Surgical Pathology,* **34**, 1097-1105.

Hall, O. J., Limjunyawong, N., Vermillion, M. S., Robinson, D. P., Wohlgemuth, N., Pekosz, A., Mitzner, W. and Klein, S. L. (2016) Progesterone-Based Therapy Protects Against Influenza by Promoting Lung Repair and Recovery in Females. *Plos Pathogens,* **12**.

Hamre, D. and Procknow, J. J. (1966) A new virus isolated from the human respiratory tract. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.),* **121**, 190-3.

Han, Y., Geng, H., Feng, W., Tang, X., Ou, A., Lao, Y., Xu, Y., Lin, H., Liu, H. and Li, Y. (2003) A follow-up study of 69 discharged SARS patients. *Journal of traditional Chinese medicine = Chung i tsa chih ying wen pan,* **23**, 214-7.

Hayashi, T. and Su, T. (2005) The sigma receptor: evolution of the concept in neuropsychopharmacology. *Current neuropharmacology,* **3**, 267-80.

Hoffmann, M., Hofmann-Winkler, H., Smith, J. C., Kruger, N., Arora, P., Sorensen, L. K., Sogaard, O. S., Hasselstrom, J. B., Winkler, M., Hempel, T., Raich, L., Olsson, S., Danov, O., Jonigk, D., Yamazoe, T., Yamatsuta, K., Mizuno, H., Ludwig, S., Noe, F., Kjolby, M., Braun, A., Sheltzer, J. M. and Pohlmann, S. (2021) Camostat mesylate inhibits SARS-CoV-2 activation by TMPRSS2-related proteases and its metabolite GBPA exerts antiviral activity. *Ebiomedicine,* **65**.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Muller, M. A., Drosten, C. and Pohlmann, S. (2020a) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell,* **181**, 271-+.

Hoffmann, M., Schroeder, S., Kleine-Weber, H., Muller, M. A., Drosten, C. and Pohlmann, S. (2020b) Nafamostat Mesylate Blocks Activation of SARS-CoV-2: New Treatment Option for COVID-19. *Antimicrobial Agents and Chemotherapy,* **64**.

Hofmann, H. and Poehlmann, S. (2004) Cellular entry of the SARS coronavirus. *Trends in Microbiology,* **12**, 466-472.

Hofmann, H., Pyrc, K., van der Hoek, L., Geier, M., Berkhout, B. and Pohlmann, S. (2005) Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, 7988-7993.

Horl, W. H. and Heidland, A. (1984) PROTEASES - POTENTIAL ROLE IN HEALTH AND DISEASE - PREFACE. *Advances in Experimental Medicine and Biology,* **167**, R7-R8.

Hsu, L.-Y., Lee, C.-C., Green, J. A., Ang, B., Paton, N. I., Lee, L., Villacian, J. S., Lim, P.-L., Earnest, A. and Leo, Y.-S. (2003) Severe acute respiratory syndrome (SARS) in Singapore: Clinical features of index patient and initial contacts. *Emerging Infectious Diseases,* **9**, 713-717.

Hu, T. Y., Frieman, M. and Wolfram, J. (2020) Insights from nanomedicine into chloroquine efficacy against COVID-19. *Nature Nanotechnology,* **15**, 247-249.

Hurst, K. R., Koetzner, C. A. and Masters, P. S. (2009) Identification of In Vivo-Interacting Domains of the Murine Coronavirus Nucleocapsid Protein. *Journal of Virology,* **83**, 7221-7234.

Hyodo, K. and Okuno, T. (2014) Host factors used by positive-strand RNA plant viruses for genome replication. *Journal of General Plant Pathology,* **80**, 123-135.

Ising, M., Maccarrone, G., Bruckl, T., Scheuer, S., Hennings, J., Holsboer, F., Turck, C. W., Uhr, M. and Lucae, S. (2019) FKBP5 Gene Expression Predicts Antidepressant Treatment Outcome in Depression. *International Journal of Molecular Sciences,* **20**.

Iwata-Yoshikawa, N., Okamura, T., Shimizu, Y., Hasegawa, H., Takeda, M. and Nagata, N. (2019) TMPRSS2 Contributes to Virus Spread and Immunopathology in the Airways of Murine Models after Coronavirus Infection. *Journal of virology,* **93**.

Jackson, C. B., Farzan, M., Chen, B. and Choe, H. (2022) Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology,* **23**, 3-20.

Johannessen, M., Fontanilla, D., Mavlyutov, T., Ruoho, A. E. and Jackson, M. B. (2013) Antagonist action of progesterone at sigma-receptors in the modulation of voltage-gated sodium channels (vol 300, pg C328, 2011). *American Journal of Physiology-Cell Physiology,* **305**, C997-C997.

Joyner, J., Neves, L. A. A., Granger, J. P., Alexander, B. T., Merrill, D. C., Chappell, M. C., Ferrario, C. M., Davis, W. P. and Brosnihan, K. B. (2007) Temporal-spatial expression of ANG-(1-7) and angiotensin-converting enzyme 2 in the kidney of normal and hypertensive pregnant rats. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* **293**, R169-R177.

Kim, T. S., Heinlein, C., Hackman, R. C. and Nelson, P. S. (2006) Phenotypic analysis of mice lacking the Tmprss2-encoded protease. *Molecular and Cellular Biology,* **26**, 965-975.

Kuiper, G. G. J. M., Enmark, E., Pelto-Huikko, M., Nilsson, S. and Gustafsson, J.-A. (1996) Cloning of a novel oestrogen receptor expressed in rat prostate and ovary. *Proceedings of the National Academy of Sciences of the United States of America,* **93**, 5925-5930.

Kuo, L. L. and Masters, P. S. (2013) Functional Analysis of the Murine Coronavirus Genomic RNA Packaging Signal. *Journal of Virology,* **87**, 5182-5192.

Kuypers, J., Martin, E. T., Heugel, J., Wright, N., Morrow, R. and Englund, J. A. (2007) Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics,* **119**, E70-E76.

Lau, S. K. P., Woo, P. C. Y., Yip, C. C. Y., Tse, H., Tsoi, H. W., Cheng, V. C. C., Lee, P., Tang, B. S. F., Cheung, C. H. Y., Lee, R. A., So, L. Y., Lau, Y. L., Chan, K. H. and Yuen, K. Y. (2006) Coronavirus HKU1 and other coronavirus infections in Hong Kong. *Journal of Clinical Microbiology,* **44**, 2063-2071.

Leach, D. A., Mohr, A., Giotis, E. S., Cil, E., Isac, A. M., Yates, L. L., Barclay, W. S., Zwacka, R. M., Bevan, C. L. and Brooke, G. N. (2021) The antiandrogen enzalutamide downregulates TMPRSS2 and reduces cellular entry of SARS-CoV-2 in human lung cells. *Nature communications,* **12**, 4068-4068.

Lei, C. F., Yang, J., Hu, J. and Sun, X. L. (2021) On the Calculation of TCID50 for Quantitation of Virus Infectivity. *Virologica Sinica,* **36**, 141-144.

Lemes, R. M. R., Costa, A. J., Bartolomeo, C. S., Bassani, T. B., Nishino, M. S., Pereira, G. J. D., Smaili, S. S., Maciel, R. M. D., Braconi, C. T., da Cruz, E. F., Ramirez, A. L., Maricatto, J. T., Janini, L. M. R., Prado, C. M., Stilhano, R. S. and Ureshino, R. P. (2021) 17 beta-estradiol reduces SARS-CoV-2 infection in vitro. *Physiological Reports,* **9**.

Li, F., Han, M., Dai, P. F., Xu, W., He, J., Tao, X. T., Wu, Y., Tong, X. Y., Xia, X. Y., Guo, W. X., Zhou, Y. J., Li, Y. G., Zhu, Y. Q., Zhang, X. Y., Liu, Z., Aji, R., Cai, X., Li, Y. T., Qu, D., Chen, Y., Jiang, S. B., Wang, Q., Ji, H. B., Xie, Y. H., Sun, Y. H., Lu, L. and Gao, D. (2021) Distinct mechanisms for TMPRSS2 expression explain organ-specific inhibition of SARS-CoV-2 infection by enzalutamide. *Nature Communications,* **12**.

Limburg, H., Harbig, A., Bestle, D., Stein, D. A., Moulton, H. M., Jaeger, J., Janga, H., Hardes, K., Koepke, J., Schulte, L., Koczulla, A. R., Schmeck, B., Klenk, H. D. and Bottcher-Friebertshauser, E. (2019) TMPRSS2 Is the Major Activating Protease of Influenza A Virus in Primary Human Airway Cells and Influenza B Virus in Human Type II Pneumocytes. *Journal of Virology,* **93**.

Lin, B., Ferguson, C., White, J. T., Wang, S., Vessella, R., True, L. D., Hood, L. and Nelson, P. S. (1999) Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. *Cancer Research,* **59**, 4180-4184.

Liu, D. X., Fung, T. S., Chong, K. K.-L., Shukla, A. and Hilgenfeld, R. (2014) Accessory proteins of SARS-CoV and other coronaviruses. *Antiviral Research,* **109**, 97-109.

Lucas, J. M., Heinlein, C., Kim, T., Hernandez, S. A., Malik, M. S., True, L. D., Morrissey, C., Corey, E., Montgomery, B., Mostaghel, E., Clegg, N., Coleman, I., Brown, C. M., Schneider, E. L., Craik, C., Simon, J. A., Bedalov, A. and Nelson, P. S. (2014) The Androgen-Regulated Protease TMPRSS2 Activates a Proteolytic Cascade Involving Components of the Tumor Microenvironment and Promotes Prostate Cancer Metastasis. *Cancer Discovery,* **4**, 1310-1325.

Lucas, J. M., True, L., Hawley, S., Matsumura, M., Morrissey, C., Vessella, R. and Nelson, P. S. (2008) The androgen-regulated type II serine protease TMPRSS2 is differentially expressed and mislocalized in prostate adenocarcinoma. *Journal of Pathology,* **215**, 118-125.

Lv, S. X., Wang, Y. H., Yang, F., Xue, L. J., Dong, S. Y., Zhang, X. H. and Ye, L. C. (2017) Up-regulated AZGP1 promotes proliferation, migration and invasion in colorectal cancer. *International Journal of Clinical and Experimental Pathology,* **10**, 2794-2803.

Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schuetz, G., Umesono, K., Blumnberg, B., Kastner, P., Mark, M., Chambon, P. and Evans, R. M. (1995) The nuclear receptor superfamily: The second decade. *Cell,* **83**, 835-839.

Marsolais, G., Berthiaume, L., DiFranco, E. and Marois, P. (1971) Rapid diagnosis by electron microscopy of avian coronavirus infection. *Canadian journal of comparative medicine : Revue canadienne de medecine comparee,* **35**, 285-8.

Masters, P. S. (2006) The molecular biology of coronaviruses. *Advances in virus research,* **66**, 193-292.

Matsuyama, S., Nagata, N., Shirato, K., Kawase, M., Takeda, M. and Taguchi, F. (2010) Efficient Activation of the Severe Acute Respiratory Syndrome Coronavirus Spike Protein by the Transmembrane Protease TMPRSS2. *Journal of Virology,* **84**, 12658-12664.

McIntosh, K., Dees, J. H., Becker, W. B., Kapikian, A. Z. and Chanock, R. M. (1967) Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proceedings of the National Academy of Sciences of the United States of America,* **57**, 933-40.

Miguel Vela, J. (2020) Repurposing Sigma-1 Receptor Ligands for COVID-19 Therapy? *Frontiers in Pharmacology,* **11**.

Milewska, A., Nowak, P., Owczarek, K., Szczepanski, A., Zarebski, M., Hoang, A., Berniak, K., Wojarski, J., Zeglen, S., Baster, Z., Rajfur, Z. and Pyrc, K. (2018) Entry of Human Coronavirus NL63 into the Cell. *Journal of Virology,* **92**.

Miller, W. L. and Auchus, R. J. (2011) The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine reviews,* **32**, 81-151.

Mingels, A. M. A. and Kimenai, D. M. (2018) Sex-Related Aspects of Biomarkers in Cardiac Disease. *Sex-Specific Analysis of Cardiovascular Function,* **1065**, 545-564.

Mowszowicz, I. (1989) Antiandrogens. Mechanisms and paradoxical effects. *Annales d'endocrinologie,* **50**, 189-99.

Murgolo, N., Therien, A. G., Howell, B., Klein, D., Koeplinger, K., Lieberman, L. A., Adam, G. C., Flynn, J., McKenna, P., Swaminathan, G., Hazuda, D. J. and Olsen, D. B. (2021) SARS-CoV-2 tropism, entry, replication, and propagation: Considerations for drug discovery and development. *PLoS Pathogens,* **17**, e1009225-Article No.: e1009225.

Nal, B., Chan, C. M., Kien, F., Siu, L., Tse, J., Chu, K., Kam, J., Staropoli, I., Crescenzo-Chaigne, B., Escriou, N., van der Werf, S., Yuen, K. Y. and Altmeyer, R. (2005) Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. *Journal of General Virology,* **86**, 1423-1434.

Neuman, B. W., Kiss, G., Kunding, A. H., Bhella, D., Baksh, M. F., Connelly, S., Droese, B., Klaus, J. P., Makino, S., Sawicki, S. G., Siddell, S. G., Stamou, D. G., Wilson, I. A., Kuhn, P. and Buchmeier, M. J. (2011) A structural analysis of M protein in coronavirus assembly and morphology. *Journal of Structural Biology,* **174**, 11-22.

Nieto-Torres, J. L., DeDiego, M. L., Alvarez, E., Jimenez-Guardeno, J. M., Regla-Nava, J. A., Llorente, M., Kremer, L., Shuo, S. and Enjuanes, L. (2011) Subcellular location and topology of severe acute respiratory syndrome coronavirus envelope protein. *Virology,* **415**, 69-82.

Nieto-Torres, J. L., DeDiego, M. L., Verdia-Baguena, C., Jimenez-Guardeno, J. M., Regla-Nava, J. A., Fernandez-Delgado, R., Castano-Rodriguez, C., Alcaraz, A., Torres, J., Aguilella, V. M. and Enjuanes, L. (2014) Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Ion Channel Activity Promotes Virus Fitness and Pathogenesis. *Plos Pathogens,* **10**.

Novac, N. and Heinzel, T. (2004) Nuclear receptors: overview and classification. *Current drug targets. Inflammation and allergy,* **3**, 335-46.

O'Leary, J. C., Zhang, B., Koren, J., Blair, L. and Dickey, C. A. (2013) The Role of FKBP5 in Mood Disorders: Action of FKBP5 on Steroid Hormone Receptors Leads to Questions About its Evolutionary Importance. *Cns & Neurological Disorders-Drug Targets,* **12**, 1157-1162.

Onder, G., Rezza, G. and Brusaferro, S. (2020) Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *Jama,* **323**, 1775-1776.

Ou, T. L., Mou, H. H., Zhang, L. Z., Ojha, A., Choe, H. and Farzan, M. (2021) Hydroxychloroquine-mediated inhibition of SARS-CoV-2 entry is attenuated by TMPRSS2. *Plos Pathogens,* **17**.

Owczarek, K., Szczepanski, A., Milewska, A., Baster, Z., Rajfur, Z., Sarna, M. and Pyrc, K. (2018) Early events during human coronavirus OC43 entry to the cell. *Scientific Reports,* **8**.

Pene, F., Merlat, A., Vabret, A., Rozenberg, F., Buzyn, A., Dreyfus, F., Cariou, A., Freymuth, F. and Lebon, P. (2003) Coronavirus 229E-related pneumonia in immunocompromised patients. *Clinical Infectious Diseases,* **37**, 929-932.

Perlman, S. and Netland, J. (2009) Coronaviruses post-SARS: update on replication and pathogenesis. *Nature reviews. Microbiology,* **7**, 439-50.

Qiao, Y., Wang, X.-M., Mannan, R., Pitchiaya, S., Zhang, Y., Wotring, J. W., Xiao, L., Robinson, D. R., Wu, Y.-M., Tien, J. C.-Y., Cao, X., Simko, S. A., Apel, I. J., Bawa, P., Kregel, S., Narayanan, S. P., Raskind, G., Ellison, S. J., Parolia, A., Zelenka-Wang, S., McMurry, L., Su, F., Wang, R., Cheng, Y., Delekta, A. D., Mei, Z., Pretto, C. D., Wang, S., Mehra, R., Sexton, J. Z. and Chinnaiyan, A. M. (2021) Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and TMPRSS2. *Proceedings of the National Academy of Sciences of the United States of America,* **118**, e2021450118-Article No.: e2021450118.

Redondo, N., Zaldivar-Lopez, S., Garrido, J. J. and Montoya, M. (2021) SARS-CoV-2 Accessory Proteins in Viral Pathogenesis: Knowns and Unknowns. *Frontiers in immunology,* **12**, 708264-708264.

Rehman, S., Ravinayagam, V., Nahvi, I., Aldossary, H., Al-Shammari, M., Amiri, M. S. A., Kishore, U. and Al-Suhaimi, E. A. (2021) Immunity, Sex Hormones, and Environmental Factors as Determinants of COVID-19 Disparity in Women. *Frontiers in immunology,* **12**, 680845-680845.

Resta, S., Luby, J. P., Rosenfeld, C. R. and Siegel, J. D. (1985a) Isolation and propagation of a human enteric coronavirus. *Science (New York, N.Y.),* **229**, 978-81.

Resta, S., Luby, J. P., Rosenfeld, C. R. and Siegel, J. D. (1985b) ISOLATION AND PROPAGATION OF A HUMAN ENTERIC CORONAVIRUS. *Science,* **229**, 978-981.

Sakai, K., Ami, Y., Tahara, M., Kubota, T., Anraku, M., Abe, M., Nakajima, N., Sekizuka, T., Shirato, K., Suzaki, Y., Ainai, A., Nakatsu, Y., Kanou, K., Nakamura, K., Suzuki, T., Komase, K., Nobusawa, E., Maenaka, K., Kuroda, M., Hasegawa, H., Kawaoka, Y., Tashiro, M. and Takeda, M. (2014) The Host Protease TMPRSS2 Plays a Major Role in In Vivo Replication of Emerging H7N9 and Seasonal Influenza Viruses. *Journal of Virology,* **88**, 5608-5616.

Sanders, J. M., Monogue, M. L., Jodlowski, T. Z. and Cutrell, J. B. (2020) Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19) A Review. *Jama-Journal of the American Medical Association,* **323**, 1824-1836.

Schelle, B., Karl, N., Ludewig, B., Siddell, S. G. and Thiel, V. (2005) Selective replication of coronavirus genomes that express nucleocapsid protein. *Journal of Virology,* **79**, 6620-6630.

Schirtzinger, E. E., Kim, Y. and Davis, A. S. (2022) Improving human coronavirus OC43 (HCoV-OC43) research comparability in studies using HCoV-OC43 as a surrogate for SARS-CoV-2. *Journal of Virological Methods,* **299**.

Schwabe, J. W. R., Chapman, L., Finch, J. T. and Rhodes, D. (1993) The crystal structure of the oestrogen receptor DNA-binding domain bound to DNA: How receptors discriminate between their response elements. *Cell,* **75**, 567-578.

Shang, Z., Chan, S. Y., Liu, W. J., Li, P. and Huang, W. (2021) Recent Insights into Emerging Coronavirus: SARS-CoV-2. *ACS Infectious Diseases,* **7**, 1369-1388.

Shi, Z. (2021) From SARS, MERS to COVID-19: A journey to understand bat coronaviruses. *Bulletin De L Academie Nationale De Medecine,* **205**, 732-736.

Shim, E., Tariq, A., Choi, W., Lee, Y. and Chowell, G. (2020) Transmission potential and severity of COVID-19 in South Korea. *International Journal of Infectious Diseases,* **93**, 339-344.

Shirato, K., Kanou, K., Kawase, M. and Matsuyama, S. (2017) Clinical Isolates of Human Coronavirus 229E Bypass the Endosome for Cell Entry. *Journal of Virology,* **91**.

Shirato, K., Kawase, M. and Matsuyama, S. (2013) Middle East Respiratory Syndrome Coronavirus Infection Mediated by the Transmembrane Serine Protease TMPRSS2. *Journal of Virology,* **87**, 12552-12561.

Shirato, K., Kawase, M. and Matsuyama, S. (2018) Wild-type human coronaviruses prefer cell-surface TMPRSS2 to endosomal cathepsins for cell entry. *Virology,* **517**, 9-15.

Simmons, G., Gosalia, D. N., Rennekamp, A. J., Reeves, J. D., Diamond, S. L. and Bates, P. (2005) Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, 11876-11881.

Song, H. C., Seo, M.-Y., Stadler, K., Yoo, B. J., Choo, Q.-L., Coates, S. R., Uematsu, Y., Harada, T., Greer, C. E., Polo, J. M., Pileri, P., Eickmann, M., Rappuoli, R., Abrignani, S., Houghton, M. and Han, J. H. (2004) Synthesis and characterization of a native, oligomeric form of recombinant severe acute respiratory syndrome coronavirus spike glycoprotein. *Journal of Virology,* **78**, 10328-10335.

Song, I.-S., Jeong, Y. J., Kim, J., Seo, K.-H., Baek, N.-I., Kim, Y., Kim, C.-S. and Jang, S.-W. (2020) Pharmacological inhibition of androgen receptor expression induces cell death in prostate cancer cells. *Cellular and molecular life sciences : CMLS,* **77**, 4663-4673.

Stadler, K., Masignani, V., Eickmann, M., Becker, S., Abrignani, S., Klenk, H.-D. and Rappuoli, R. (2003) SARS--beginning to understand a new virus. *Nature reviews. Microbiology,* **1**, 209-18.

Student, S., Hejmo, T., Poterala-Hejmo, A., Lesniak, A. and Buldak, R. (2020) Anti-androgen hormonal therapy for cancer and other diseases. *European Journal of Pharmacology,* **866**, 172783-Article No.: 172783.

Taneja, V. (2018) Sex Hormones Determine Immune Response. *Frontiers in immunology,* **9**, 1931-1931.

Tarnow, C., Engels, G., Arendt, A., Schwalm, F., Sediri, H., Preuss, A., Nelson, P. S., Garten, W., Klenk, H.-D., Gabriel, G. and Boettcher-Friebertshaeuser, E. (2014) TMPRSS2 Is a Host Factor That Is Essential for Pneumotropism and Pathogenicity of H7N9 Influenza A Virus in Mice. *Journal of Virology,* **88**, 4744-4751.

To, K. K.-W., Chan, W.-M., Ip, J. D., Chu, A. W.-H., Tam, A. R., Liu, R., Wu, A. K.-L., Lung, K.-C., Tsang, O. T.-Y., Lau, D. P.-L., To, W.-K., Kwan, M. Y.-W., Yau, Y.-S., Ng, A. C.-K., Yip, C. C.-Y., Chan, K.-H., Tse, H., Hung, I. F.-N. and Yuen, K.-Y. (2021) Unique Clusters of Severe Acute Respiratory Syndrome Coronavirus 2 Causing a Large Coronavirus Disease 2019 Outbreak in Hong Kong. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America,* **73**, 137-142.

Turk, V., Stoka, V., Vasiljeva, O., Renko, M., Sun, T., Turk, B. and Turk, D. (2012) Cysteine cathepsins: From structure, function and regulation to new frontiers. *Biochimica Et Biophysica Acta-Proteins and Proteomics,* **1824**, 68-88.

Turner, A. J., Hiscox, J. A. and Hooper, N. M. (2004) ACE2: from vasopeptidase to SARS virus receptor. *Trends in Pharmacological Sciences,* **25**, 291-294.

V'Kovski, P., Kratzel, A., Steiner, S., Stalder, H. and Thiel, V. (2021) Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews Microbiology,* **19**, 155-170.

Vabret, A., Dina, J., Gouarin, S., Petitjean, J., Corbet, S. and Freymuth, F. (2006) Detection of the new human coronavirus HKU1: A report of 6 cases. *Clinical Infectious Diseases,* **42**, 634-639.

van der Hoek, L., Pyrc, K. and Berkhout, B. (2006) Human coronavirus NL63, a new respiratory virus. *FEMS microbiology reviews,* **30**, 760-73.

van der Hoek, L., Pyrc, K., Jebbink, M. F., Vermeulen-Oost, W., Berkhout, R. J. M., Wolthers, K. C., Wertheim-van Dillen, P. M. E., Kaandorp, J., Spaargaren, J. and Berkhout, B. (2004) Identification of a new human coronavirus. *Nature Medicine,* **10**, 368-373.

vom Steeg, L. G., Dhakal, S., Woldetsadik, Y. A., Park, H.-S., Mulka, K. R., Reilly, E. C., Topham, D. J. and Klein, S. L. (2020a) Androgen receptor signaling in the lungs mitigates inflammation and improves the outcome of influenza in mice. *PLoS Pathogens,* **16**, e1008506-Article No.: e1008506.

vom Steeg, L. G., Dhakal, S., Woldetsadik, Y. A., Park, H. S., Mulka, K. R., Reilly, E. C., Topham, D. J. and Klein, S. L. (2020b) Androgen receptor signaling in the lungs mitigates inflammation and improves the outcome of influenza in mice. *Plos Pathogens,* **16**.

vom Steeg, L. G., Vermillion, M. S., Hall, O. J., Alam, O., McFarland, R., Chen, H., Zirkin, B. and Klein, S. L. (2016) Age and testosterone mediate influenza pathogenesis in male mice. *American Journal of Physiology - Lung Cellular and Molecular Physiology,* **311**, L1234-L1244.

Walsh, E. E., Shin, J. H. and Falsey, A. R. (2013) Clinical Impact of Human Coronaviruses 229E and OC43 Infection in Diverse Adult Populations. *Journal of Infectious Diseases,* **208**, 1634-1642.

Wambier, C. G., Goren, A., Vano-Galvan, S., Ramos, P. M., Ossimetha, A., Nau, G., Herrera, S. and McCoy, J. (2020) Androgen sensitivity gateway to COVID-19 disease severity. *Drug Development Research,* **81**.

Welen, K., Overby, A. K., Ahlm, C., Freyhult, E., Robinsson, D., Henningsson, A. J., Stranne, J., Bremell, D., Angelin, M., Lindquist, E., Buckland, R., Carlsson, C. T., Pauksens, K., Bill-Axelsson, A., Akre, O., Ryden, C., Wagenius, M., Bjartell, A., Nilsson, A. C., Styrke, J., Repo, J., Balkhed, A. O., Niward, K., Gisslen, M. and Josefsson, A. (2021) COVIDENZA - A prospective, multicenter, randomized PHASE II clinical trial of enzalutamide treatment to decrease the morbidity in patients with Corona virus disease 2019 (COVID-19): a structured summary of a study protocol for a randomised controlled trial. *Trials,* **22**.

Wettstein, L., Kirchhoff, F. and Muench, J. (2022) The Transmembrane Protease TMPRSS2 as a Therapeutic Target for COVID-19 Treatment. *International Journal of Molecular Sciences,* **23**.

Wierman, M. E. (2007) Sex steroid effects at target tissues: mechanisms of action. *Advances in physiology education,* **31**, 26-33.

Wirth, M. P., Hakenberg, O. W. and Froehner, M. (2007) Antiandrogens in the treatment of prostate cancer. *European Urology,* **51**, 306-314.

Woo, P. C. Y., Lau, S. K. P., Chu, C.-M., Chan, K.-H., Tsoi, H.-W., Yi, H., Wong, B. H. L., Poon, R. W. S., Cai, J. J., Luk, W.-K., Poon, L. L. M., Wong, S. S. Y., Yi, G., Peiris, J. S. M. and Yuen, K.-Y. (2005) Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *Journal of Virology,* **79**, 884-895.

Wu, K., Li, W., Peng, G. and Li, F. (2009) Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. *Proceedings of the National Academy of Sciences of the United States of America,* **106**, 19970-19974.

Wu, M. H., Ma, W. L., Hsu, C. L., Chen, Y. L., Ou, J. H. J., Ryan, C. K., Hung, Y. C., Yeh, S. and Chang, C. (2010) Androgen Receptor Promotes Hepatitis B Virus-Induced Hepatocarcinogenesis Through Modulation of Hepatitis B Virus RNA Transcription. *Science Translational Medicine,* **2**.

Xu, H., Zhong, L., Deng, J., Peng, J., Dan, H., Zeng, X., Li, T. and Chen, Q. (2020) High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *International journal of oral science,* **12**, 8-8.

Yamaya, M., Nishimura, H., Deng, X., Kikuchi, A. and Nagatomi, R. (2020) Protease Inhibitors: Candidate Drugs to Inhibit Severe Acute Respiratory Syndrome Coronavirus 2 Replication. *Tohoku Journal of Experimental Medicine,* **251**, 27-30.

Yeager, C. L., Ashmun, R. A., Williams, R. K., Cardellichio, C. B., Shapiro, L. H., Look, A. T. and Holmes, K. V. (1992) HUMAN AMINOPEPTIDASE-N IS A RECEPTOR FOR HUMAN CORONAVIRUS-229E. *Nature,* **357**, 420-422.

Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. M. E. and Fouchier, R. A. M. (2012) Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *New England Journal of Medicine,* **367**, 1814-1820.

Zang, R. C., Castro, M. F. G., McCune, B. T., Zeng, Q. R., Rothlauf, P. W., Sonnek, N. M., Liu, Z. M., Brulois, K. F., Wang, X., Greenberg, H. B., Diamond, M. S., Ciorba, M. A., Whelan, S. P. J. and Ding, S. Y. (2020) TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Science Immunology,* **5**.

Zhao, M. M., Yang, W. L., Yang, F. Y., Zhang, L., Huang, W. J., Hou, W., Fan, C. F., Jin, R. H., Feng, Y. M., Wang, Y. C. and Yang, J. K. (2021) Cathepsin L plays a key role in SARS-CoV-2 infection in

humans and humanized mice and is a promising target for new drug development. *Signal Transduction and Targeted Therapy,* **6**.

Zheng, Q., Deng, Y., Liu, J., van der Hoek, L., Berkhout, B. and Lu, M. (2006) Core structure of S2 from the human coronavirus NL63 spike Glycoprotein. *Biochemistry,* **45**, 15205-15215.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Deng, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F. and Shi, Z.-L. (2020a) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature (London),* **579**, 270-273.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Deng, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F. and Shi, Z.-L. (2020b) A pneumonia outbreak associated with a new coronavirus of probable bat origin (vol 579, pg 270, 2020). *Nature (London),* **588**.

Zhou, Y. P., Zhong, L., Yu, S. J., Shen, W., Cai, C. and Yu, H. H. (2020c) Inhibition of stearoyl-coenzyme A desaturase 1 ameliorates hepatic steatosis by inducing AMPK-mediated lipophagy. *Aging-Us,* **12**, 7350-7362.

Zuckerman, A. J., Taylor, P. E. and Almeida, J. D. (1970) Presence of particles other than the Australia-SH antigen in a case of chronic active hepatitis with cirrhosis. *British medical journal,* **1**, 262-4.

Zumla, A., Chan, J. F. W., Azhar, E. I., Hui, D. S. C. and Yuen, K.-Y. (2016) Coronaviruses - drug discovery and therapeutic options. *Nature reviews. Drug discovery,* **15**, 327-47.