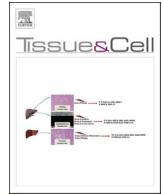




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## Clinically relevant cell culture models and their significance in isolation, pathogenesis, vaccine development, repurposing and screening of new drugs for SARS-CoV-2: a systematic review

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### ABSTRACT

**Background:** *In-Vitro*/Cellular evidence is the backbone and vital proof of concept during the development of novel therapeutics as well as drugs repurposing against COVID-19. Choosing an ideal *in-vitro* model is vital as the virus entry is through ACE2, CD147, and TMPRSS2 dependant and very specific. In this regard, this is the first systematic review addressing the importance of specific cell lines used as potential *in-vitro* models in the isolation, pathogenesis, and therapeutics for SARS–COV-2.

**Methods:** We searched 17 literature databases with appropriate keywords, and identified 1173 non-duplicate studies. In the present study, 71 articles are included after a careful, thorough screening of the titles and their abstracts for possible inclusion using predefined inclusion/exclusion criteria (PRISMA Guidelines).

**Results:** In the current study, we compiled cell culture-based studies for SARS-CoV-2 and found the best compatible *In-Vitro* models for SARS-CoV-2 (Vero, VeroE6, HEK293 as well as its variants, Huh-7, Calu-3 2B4, and Caco2). Among other essential cell lines used include LLC-MK2, MDCKII, BHK-21, HepG2, A549, T cell leukemia (MT-2), stems cells based cell line DYRO100for differentiation assays, and embryo-specific NIH3T3 cell line for vaccine production.

**Conclusion:** The Present study provides a detailed summary of all the drugs/compounds screened for drug repurposing and discovery purpose using the *in-vitro* models for SARS-CoV-2 along with isolation, pathogenesis and vaccine production. This study also suggests that after careful evaluation of all the cell line based studies, Kidney cells (VeroE6, HEK293 along with their clones), liver Huh-7cells, respiratory Calu-3 cells, and intestinal Caco-2 are the most widely used *in-vitro* models for SARS-CoV-2.

**Abbreviations:** SARS-CoV-2, Severe Acute Respiratory Syndrome-Coronavirus-2; 2019-nCoV, 2019-novel Corona virus; ACE-2, Angiotensin-Converting Enzyme-2; TMPRSS2, Transmembrane Protease Serine 2.

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## 1. Introduction

### 1.1. Overview of Corona viruses and the current outbreak of COVID-19

Corona viruses (CoVs) have a long history and are the part of family Coronaviridae (subfamily Coronaviridae). They spread infection especially through the respiratory tract's involvement (both lower and upper respiratory tract) with the symptoms of common cold, pneumonia, bronchiolitis, rhinitis, pharyngitis, sinusitis, diarrhea (Chang et al., 2016; Paules et al., 2020). Till date, a total of seven human Corona virus strains have been identified, [229E, NL63, OC43, HKU1, severe acute respiratory syndrome- Corona virus (SARS-CoV), middle east respiratory syndrome-corona virus (MERS-CoV), and severe acute respiratory syndrome - corona virus 2 (SARS-CoV-2) or 2019-novel Corona virus (2019-nCoV) (Paules et al., 2020). Though CoVs were known to cause milder symptoms, the outbreak of these three strains were deadly with high mortality and shown very adaptive potential as per environmental conditions and classified as "emerging viruses." COVID-19 was initially named "2019-nCoV." but later on, it was changed to "SARS-CoV-2" due to high similarity with severe acute respiratory syndrome corona virus (SARS-CoV) as per the recommendation of the Corona virus Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) (Coronaviridae Study Group of the International Committee on Taxonomy of V, 2020). Corona viruses (CoVs) are single-stranded positively sense RNA viruses of the family Coronaviridae (subfamily Coronavirinae). Their genome is the largest among all RNA viruses, with a size range between 26.2 and 31.7 kb. They are either pleomorphic or spherical with a diameter of 80–120 nm and distinguished by bears club-shaped projections of glycoproteins on their surface. CoVs genome consists of six to ten open reading frames (ORFs) (Belouzard et al., 2012), and their genetic material is highly susceptible to frequent recombination process, resulting in new strains with alteration in their virulence (Hilgenfeld, 2014). The most important structural proteins of SARS-COV-2 are spike (S) protein, which is a trimeric, membrane (M) protein, envelop (E) protein, and the nucleocapsid (N) protein, and all of them are potential drug development targets. Some viruses, such as beta-COVs, also have hemagglutinin esterase (HE) glycoprotein (Belouzard et al., 2012). The RNA genome of CoV has seven conserved genes (ORF1a, ORF1b, S, ORF3, E, M, and N) arranged in 5' to 3': ORF1a/b alone covers two-third part of its RNA genome. It is responsible for the production of two viral replicase polyproteins which are PP1a and PP1ab. Further processing gives rise to sixteen mature nonstructural proteins (NSPs) and play a crucial role in various viral functions, including the formation of the replicase transcriptase complex (McBride et al., 2014). The rest of the virus's genomic part encodes the mRNA, which produces its other essential structural proteins, including spike, envelope, membrane, and nucleocapsid (McBride et al., 2014). HE is another essential envelop-associated protein that is expressed by specific CoV strains (Structure, 2016). The entire RNA genome of corona virus is packed with nucleocapsid protein under covered with envelope (Guo et al., 2008).

### 1.2. Mechanism of SARS-CoV-2 entry into target cells and the importance of cell culture models

When SARS-COV-2 enters into the body, the primary target cells are the enterocytes and pneumocytes (Guo et al., 2008; Gu and Korteweg, 2007; Hoffmann et al., 2020; Chan et al., 2020a; Corman et al., 2019; Iwata-Yoshikawa et al., 2019; Kleine-Weber et al., 2019). In contrast, other target cells include kidney tubular epithelial cells, cerebral neuronal cells, and immune cells (Guo et al., 2008; Gu and Korteweg, 2007). SARS-CoV-2 entry and infection into the host cells take place through host cell factors, angiotensin-converting enzyme-2 (ACE2), and subsequent proteolytic cleavage on the Spike protein by transmembrane protease serine 2 (TMPRSS2), which is responsible for membrane fusion (Hoffmann et al., 2020). In the case of ACE-2, after recognizing the

receptor, the virus genome and its nucleocapsid are released in the cytoplasm of the target cells. Two viral polyproteins (pp1a and pp1b) encoded by ORF1a and ORF1b genes are further processed by proteases into 16 NSPs and play a crucial role in the formation of the replication transcription complex (teVelthuis et al., 2012). This takes command over host translational machinery for the production of their own proteins (Stobart et al., 2013). All NSPs have their specific functions required starting from their entry into the target cell to suppression of target gene expression to replicate and translate their genome and necessary proteins and specific functions of NSPs and their cellular production (teVelthuis et al., 2012; Stobart et al., 2013; Wang et al., 2016; Egloff et al., 2004; Hu et al., 2017; Bouvet et al., 2014; Narayanan et al., 2000; de Wit et al., 2016; Nieto-Torres et al., 2011; Prajapat et al., 2020). Similarly, a recent study suggested that entry and viral spread among other organs such as lungs of the infected host also depend on serine protease TMPRSS-2 activity in S protein priming of SARS-CoV-2 (Hoffmann et al., 2020). It is suggested that SARSCoV infection further leads to a surge of pro-inflammatory cytokines and chemokines, and causes severe pulmonary tissue damage (Ding et al., 2004), deteriorating lung function and cause lung failure (Du et al., 2009). Both host-directed therapies and virus-directed therapies are under investigation with variable success. The most important among the virus directed therapies, are Lopinavir/Ritonavir combination, hydroxy-chloroquine, chloroquine, remdesivir as we discussed in our previous studies (Prajapat et al., 2020; Sarma et al., 2020a, b; Sarma et al., 2021). Owing to the ease of the development process, repurposed drugs are taking the lead role in the fight against the covid with preliminary evidence generated in *in-vitro*, leading to further preclinical and clinical trials. *In-vitro* experimental model systems are required to confirm the safety, efficacy, standardization and validation of new potential drug in combinatorial doses of existing anti-viral drugs for COVID-19. Being the cornerstone of the preliminary evidences generation/preliminary proof of concept studies/repurposing of existing drugs/evaluation of new chemical entities, *in-vitro* studies are taking a lead role in the evidence generation process against SARS-CoV-2. The importance of detailed knowledge of the *in-vitro* systems would help the best system for optimal drug evaluation. Towards this effort, it is the first systematic review addressing the utility of different cell culture systems as potential *in-vitro* models for repurposing and drugs development, vaccine development, isolation and pathogenesis of SARS-COV-2.

## 2. Methods

### 2.1. Objective

Elucidation of potential *In-Vitro* models to combat and understand the pathogenesis of SARS-COV-2

### 2.2. Criteria for including studies in this review

In the systematic review, we included *in-vitro* studies (both primary culture and cell line experiments), which involved viral culture of SARS-CoV-2 for any purpose. Only original studies providing details of the culture process were included. On the other hand, review articles and other study designs were excluded.

### 2.3. Search strategy

A total of 17 literature databases (PubMed, Embase, Wiley Online Library, OVID, SCOPUS, Google Scholar, Epistemonikos, CINAHL, Web of Science, TRIP, Cochrane CENTRAL, Science Direct, Virtual Health Library, CNKI and journals including Nature, Mediterranean-infection. com/pre-prints-ihuan and SSRN preprints) were searched using the keywords COVID-19, 2019-nCoV, novel corona virus, SARS-CoV-2, *in-vitro*, cell lines, cell culture and culture techniques. We included all search studies 19th April 2020, without any language restriction. We also

searched references of all the included articles for identification of articles with possible inclusion.

#### 2.4. Selection of studies

After searching databases and removing duplicates, two authors (HK and SK) independently screened the titles/abstracts using predefined inclusion/exclusion criteria. For relevant articles, full-texts were obtained for further evaluation. In case of any discrepancy, BM was consulted, and the issue was resolved.

#### 2.5. Data extraction

Data extraction was done separately by two authors (HK and SK). In articles published in a language other than english, google translate was used to identify relevant data.

### 3. Results and discussion

#### 3.1. Details of included studies

After searching a total of 17 literature databases, we identified 1173 relevant articles, which were screened for title and abstract. Following which 272 articles were selected for full-text screening and finally a total of 71 articles were selected for the systematic review. For the review process, various cell lines have been used for SARS-CoV-2 isolation, pathogenesis and therapeutic purpose. The details of the selection process and PRISMA chart are included (Fig. 1). For studying the pathogenesis and evaluation of vaccines and therapeutics against SARS-CoV-2, suitable, rapid, and safe experimental models are a current necessity

that could combat the present clinical disease (Gretebeck and Subbarao, 2015; Yong et al., 2019). Various animal models are utilized for SARS-CoV studies, such as golden Syrian hamsters, rabbits, guinea pigs, mouse, ferrets, and non-human primates like rhesus macaques, marmosets, and cats (Lu et al., 2013; Martina et al., 2003; Lamirande et al., 2008; Roberts et al., 2008; Falzarano et al., 2014; Du et al., 2016; Enjuanes et al., 2016). The virus specificity to the ACE-2 receptor and TMPRSS-2 (both SARS-CoV-2 and SARS-CoV) was found to be a significant hindrance in developing the animal models for SARS-CoV-2. In this regard, based on the literature available regarding SARS-CoV-2 cellular binding and viral spread in the infected host, we tried to compile the available compatible *in-vitro* experimental model systems. Although there are some studies where the viral load of SARS-CoV was either not detected or identified very low, all cells are not susceptible to these viruses (Chan et al., 2013). In the present study, we compiled various human and other primates based cell lines used since the outbreak of SARS-CoVs and the compiled list of these cell lines is given in Table 1.

#### 3.2. In-vitro model platforms for SARS-CoV-2

Cell culture models can replicate the different properties and functions of the various organs specific cells in *in-vitro* conditions and are the key to successfully translating research findings into real-world medical applications. Based on the receptor-specific cellular infection of SARS-CoV-2, the selection of a suitable experimental model becomes essential for further studies. SARS-CoV-2 and SARS-CoV represent highly similar genetic makeup with a similarity of approximately 70%. Both SARS-CoV and SARS-CoV-2 share a similar entrance system in the target cells as all the target cells with ACE-2, CD147 and TMPRSS-2, are susceptible to SARS-CoV-2 infection. In a recent study, the expression of

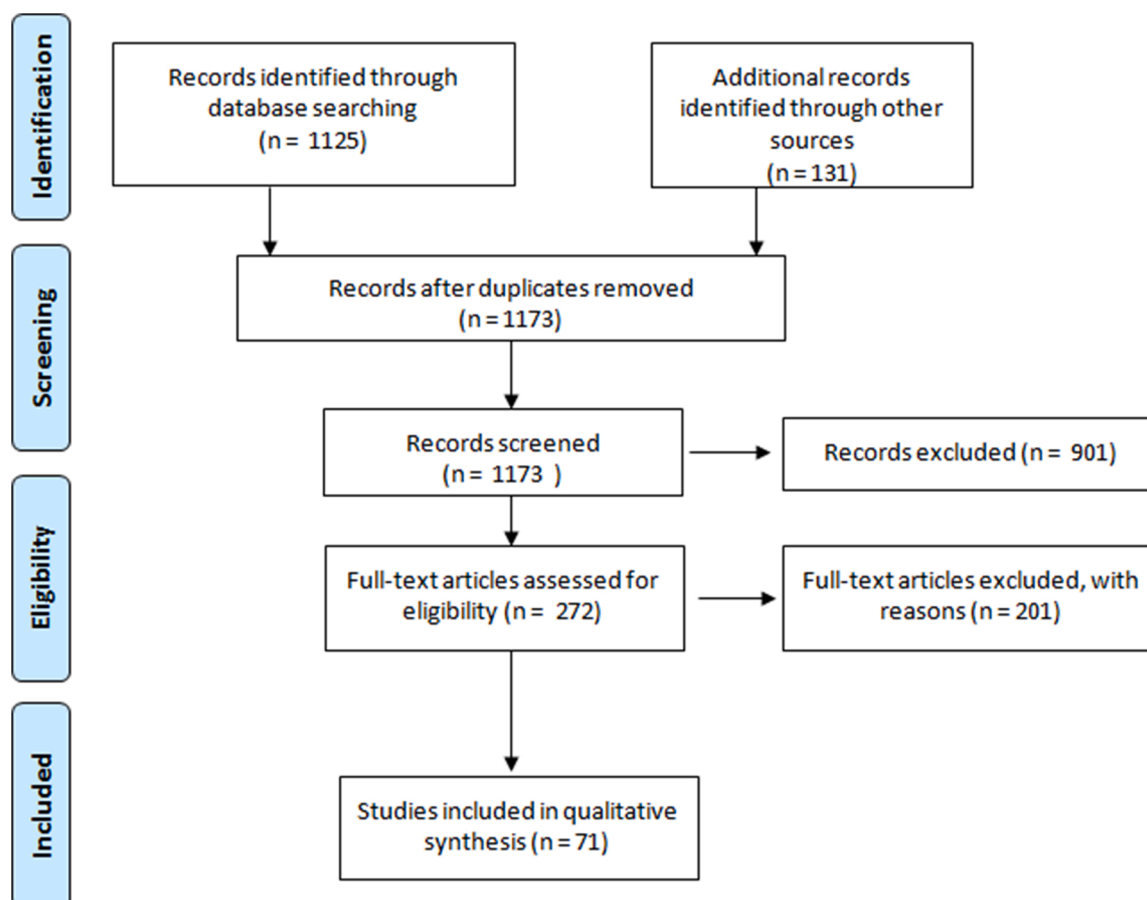


Fig. 1. Prisma flow chart of the included studies.

**Table 1**  
Showing the list of cell lines, their origin, availability and purpose of use for SARS-CoV-2.

Organ/ Tissue	Experimental Models: Cell Lines	Origin/ Details of culture model	Drug/vaccine Evaluated with reference			Availability	Remark
			Drug Screening	Vaccine/Antibodies Production/Immune Response/Genes Expression	Virus Isolation, Expression /Other purpose		
	Vero E6	<i>Cercopithecus aethiops</i> , Kidney	Chloroquine (Capobianchi et al., 2020) Ritonavir (Xiong et al., 2020a) CVL218 (Weston et al., 2020) HTS of various drugs (Choy et al., 2020) Lopinavir (Liu et al., 2020a) Emetine (Liu et al., 2020a) Homoharringtonine (Liu et al., 2020a) Carmofur (Dai et al., 2020) Peptidomimetic Aldehydes (Liu et al., 2020b) Hydroxychloroquine (Zhijian et al., 2020) Nelfinavir (Xiong et al., 2020b) DHODH Inhibitors (Wang et al., 2020a) Remdesivir (Caly et al., 2020b; Weston et al., 2020; Liu et al., 2020a; Andreani et al., 2020) Lianhuaqingwen (Andreani et al., 2020) EIDD-2801 (Yoshikawa et al., 2010) Atazanavir (Ge et al., 2020) Fluphenazine (Yao et al., 2020) Dihydrochloride (Yao et al., 2020) Benztropine Mesylate (Yao et al., 2020) Amodiaquin Hydrochloride (Yao et al., 2020) Chlorpromazine Hydrochloride (Yao et al., 2020) Toremifene Citrate (Yao et al., 2020) Amodiaquin Dihydrochloride Dihydrate (Yao et al., 2020) Thiethylperazine Maleate (Yao et al., 2020) Mefloquine Hydrochloride (Yao et al., 2020) Triparanol (Yao et al., 2020) Terconazole Vetrinal (Yao et al., 2020) Anisomycin (Yao et al., 2020) Gemcitabine Hydrochloride (Yao	Immune Response (Fukushi et al., 2006) Antibodies Production (Pu et al., 2020) Virus induced Genes Expression reversal (Xiong et al., 2020a) MAbs screening (Fintelman-Rodrigues et al., 2020)	Pathogenesis and Transmission (Hoffmann et al., 2020; Chan et al., 2020b; Zhou et al., 2020; Matsuyama et al., 2020) Viral Enhanced Virus Isolation (Matsuyama et al., 2020) Virus Isolation (Chan et al., 2020b; Xiao et al., 2020; Harcourt et al., 2020)	ATCC Cat #CRL-1586	
Kidney	Vero	<i>Cercopithecus Aethiops</i> , Kidney	Toremifene Citrate (Yao et al., 2020) Amodiaquin Dihydrochloride Dihydrate (Yao et al., 2020) Thiethylperazine Maleate (Yao et al., 2020) Mefloquine Hydrochloride (Yao et al., 2020) Triparanol (Yao et al., 2020) Terconazole Vetrinal (Yao et al., 2020) Anisomycin (Yao et al., 2020) Gemcitabine Hydrochloride (Yao	Immune Response (Pan et al., 2020) Vaccine Production (Mantlo et al., 2020) Type I IFNs as target of Antiviral Therapy (Anderson et al., 2020) MTHFD1 as a target of Antiviral Therapy (Xing et al., 2020)	Pathogenesis and Transmission (Matsuyama et al., 2020) Virus Isolation (Caly et al., 2020a; TT-Y et al., 2020) Viral Virus susceptibility (Thevarajan et al., 2020)	ATCC Cat# CRL-1586; RRID: CVCL_0574	Most commonly used cell lines in case of SARS-CoV-2 are Vero, Vero E6 and HEK293 T Characteristic: High expression of ACE2 and TMPRSS2.

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Table 1 (continued)

Organ/ Tissue	Experimental Models: Cell Lines	Origin/ Details of culture model	Drug/vaccine Evaluated with reference			Availability	Remark
			Drug Screening	Vaccine/Antibodies Production/Immune Response/Genes Expression	Virus Isolation, Expression /Other purpose		
			<a href="#">et al., 2020</a> Imatinib Mesylate ( <a href="#">Yao et al., 2020</a> ) Fluspirilene ( <a href="#">Yao et al., 2020</a> ) Clomipramine Hydrochloride ( <a href="#">Yao et al., 2020</a> ) Hydroxychloroquine Sulfate ( <a href="#">Yao et al., 2020</a> ; <a href="#">Touret et al., 2020</a> ) Promethazine Hydrochloride ( <a href="#">Yao et al., 2020</a> ) Emetine Dihydrochloride Hydrate ( <a href="#">Yao et al., 2020</a> ) Chloroquine Phosphate ( <a href="#">Yao et al., 2020</a> ) Tamoxifen Citrate ( <a href="#">Yao et al., 2020</a> ) Baicalein ( <a href="#">Jin et al., 2020</a> )				
	VeroE6/ TMPRSS2	<i>Cercopithecus aethiops</i> , Kidney			Enhanced Virus Isolation ( <a href="#">Matsuyama et al., 2020</a> )	ATCC Cat #CRL-1586	
	Vero/hSLAM HEK 293	<i>Cercopithecus aethiops</i> , Kidney <i>Homo sapiens</i> , Embryonic Kidney	Ivermectin ( <a href="#">Sheahan et al., 2020a</a> )	Vaccine Production ( <a href="#">Mantlo et al., 2020</a> )	Viral Pathogenesis and Transmission ( <a href="#">Hoffmann et al., 2020</a> )	ATCC Cat #CRL-1586 ATCC Cat #CRL-1573	
	293 T	<i>Homo sapiens</i> , Embryonic Kidney	Teicoplanin ( <a href="#">Wu et al., 2020</a> )	Vaccine Production ( <a href="#">Mantlo et al., 2020</a> ) MAbs screening ( <a href="#">Fintelman-Rodriguez et al., 2020</a> ) Immune Response ( <a href="#">Ye et al., 2020</a> ; <a href="#">Ju et al., 2020</a> ) MAb Production ( <a href="#">Li et al., 2020</a> ; <a href="#">Sun et al., 2020</a> ) Vaccine Production and Antibodies Response ( <a href="#">Mosser et al., 2005</a> ) ACE2 Gene Expression ( <a href="#">Zhang et al., 2020a</a> ) MAbs production ( <a href="#">Li et al., 2020</a> )	Virus Isolation ( <a href="#">Xiao et al., 2020</a> ) Virus susceptibility ( <a href="#">Thevarajan et al., 2020</a> ) MTHFD1 as a target of Antiviral Therapy ( <a href="#">Xing et al., 2020</a> ) Antiviral CRISPR ( <a href="#">Wang et al., 2020b</a> ) Viral Pathogenesis and Transmission ( <a href="#">Gao et al., 2020</a> )	ATCC® CRL-11,268	
	293 FT 293 T-ACE2	<i>Homo sapiens</i> , Embryonic Kidney <i>Homo sapiens</i> , Embryonic Kidney	DHODH Inhibitors ( <a href="#">Wang et al., 2020a</a> )	Vaccine Production and Antibodies Response ( <a href="#">Mosser et al., 2005</a> )	Viral Pathogenesis ( <a href="#">Gao et al., 2020</a> )		
	LLC-MK2 MDCKII	<i>Macaca mulatta</i> , Kidney <i>Canine</i> , Kidney	Virus susceptibility ( <a href="#">Chan et al., 2013</a> ) DHODH Inhibitors ( <a href="#">Wang et al., 2020a</a> )			ATCC CCL-7.1 ATCC Cat# CRL-2936; RRID: CVCL_B034	
	BHK-21	<i>Mesocricetus auratus</i> , Kidney fibroblast		MAbs screening ( <a href="#">Fintelman-Rodriguez et al., 2020</a> )		ATCC Cat# CCL-10; RRID: CVCL_1915	

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Table 1 (continued)

Organ/ Tissue	Experimental Models: Cell Lines	Origin/ Details of culture model	Drug/vaccine Evaluated with reference			Availability	Remark
			Drug Screening	Vaccine/Antibodies Production/Immune Response/Genes Expression	Virus Isolation, Expression /Other purpose		
	COS-7 cells	<i>Cercopithecus aethiops</i> , Kidney Fibroblast, SV0 Transformed		Antibodies Production (Pu et al., 2020)		ATCC CRL-1651	
	PaKi	<i>Chiroptera</i> , Kidney, Primary Pteropus alecto kidney cells			MTHFD1 as a target of Antiviral Therapy (Xing et al., 2020)	NA	
	293 F	Derivative of HEK 293 Cells		MAB Production (Li et al., 2020; Sun et al., 2020)		ThermoFisher, Cat #R79007	
	HK2	<i>Homo sapiens</i> , Human papillomavirus 16 (HPV-16) transformed	Remdesivir (Hamming et al., 2004)			ATCC® CRL-2190	
	NRK-49F	<i>Rattus norvegicus</i> , Kidney Fibroblast Normal	Remdesivir (Hamming et al., 2004)			ATCC® CRL-1570	
	Calu-3	<i>Homo sapiens</i> , Lung Adenocarcinoma Epithelial Type II Cells			Viral Pathogenesis and Transmission (Hoffmann et al., 2020; Matsuyama et al., 2020)	ATCC Cat# HTB-55; RRID: CVCL_0609	
	Calu-3 2B4	<i>Homo sapiens</i> , Lung Adenocarcinoma Epithelial Cells	EIDD-2801 (Yoshikawa et al., 2010)			ATCC Cat# HTB-55; RRID: CVCL_0609	
	A549	<i>Homo sapiens</i> , Lung Adenocarcinoma Epithelial Type II Cells	ATAZANAVIR (Ge et al., 2020) DHODH Inhibitors (Wang et al., 2020a) Teicoplanin (Wu et al., 2020)	ACE2 Gene Expression (Zhao et al., 2020b)		ATCC Cat# CRM-CCL-185; RRID: CVCL_0023	
Respiratory	NCI-H1299	<i>Homo sapiens</i> , Lung, carcinoma Epithelial Cells			Antiviral CRISPR (Wang et al., 2020b) Viral Pathogenesis and Transmission (Hoffmann et al., 2020)	ATCC Cat# CRL-5803; RRID: CVCL_0060	Most commonly used cell lines in case of SARS-CoV-2 are Calu-3 and Calu-3 2B4 Characteristic: High expression of ACE2 and TMPRSS2.
	BEAS-2B	<i>Homo sapiens</i> , Lung, Epithelial virus transformed			Viral Pathogenesis and Transmission (Hoffmann et al., 2020)	ATCC Cat# CRL-9609; RRID: CVCL_0168	
	Hep2	<i>Homo sapiens</i> HeLa contaminant, Carcinoma		Vaccine Production (Mantlo et al., 2020)		ATCC CCL-23	
	Primary culture of type II alveolar cells (AT2)	<i>Homo sapiens</i> , Lung Alveolar Tissue			ACE2 Gene Expression (Poschet et al., 2020)	N/A	
	IB3-1 and its derivative S9	<i>Homo sapiens</i> , Immortalized Bronchial Epithelial Cells	Azithromycin and Ciprofloxacin (Wang et al., 2020c)			ATCC CRL-2777	
	KMB17	<i>Homo sapiens</i> , Lung, human embryonic lung fibroblast-like cells		Vaccine Production (Mantlo et al., 2020)		<a href="http://www.biofeng.com/xibao/xibaozh/ku/KMB-17.html">http://www.biofeng.com/xibao/xibaozh/ku/KMB-17.html</a>	
Liver	Huh-7	<i>Homo sapiens</i> , Lung, human embryonic lung fibroblast-like cells	Teicoplanin (Wu et al., 2020) DHODH Inhibitors (Wang et al., 2020a) Remdesivir (Wu et al., 2020) Chloroquine (Wu et al., 2020) Lianhuaqingwen (Andreani et al., 2020)	Vaccine Production (Mantlo et al., 2020)	Virus Isolation (Xiao et al., 2020) Virus susceptibility (Thevarajan et al., 2020; Fang et al., 2020) Viral Pathogenesis and Transmission (Hoffmann et al., 2020; Gao et al.,	JCRB Cat# JCRB0403; RRID: CVCL_0336	Most commonly used cell lines in case of SARS-CoV-2 is Huh-7 cells Characteristic: High expression of ACE2 and TMPRSS2.

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Table 1 (continued)

Organ/ Tissue	Experimental Models: Cell Lines	Origin/ Details of culture model	Drug/vaccine Evaluated with reference			Availability	Remark
			Drug Screening	Vaccine/Antibodies Production/Immune Response/Genes Expression	Virus Isolation, Expression /Other purpose		
	HepG-2	<i>Homo sapiens</i> , Hepatocellular carcinoma  Hep1–6  ATCC® CRL-1830 HL7702  <a href="https://web.expasy.org/cellosaurus/CVCL_6926">https://web.expasy.org/cellosaurus/CVCL_6926</a> RHT6.0	<i>Mus musculus</i> , Hepatoma Epithelial Cells  <i>Homo sapiens</i> , Liver-Cancer Cell Line  <i>macaque</i> liver cells	Vaccine Production (Mantlo et al., 2020) Genes Expression (Xu et al., 2020b)	2020; Kaye, 2006; Zhang et al., 2020a; Zhao et al., 2020c) Virus susceptibility (Thevarajan et al., 2020) Vaccine Production (Mantlo et al., 2020) Vaccine Production (Mantlo et al., 2020) Vaccine Production (Mantlo et al., 2020)	ATCC HB-8065	
X9.0		NA <i>Scandentia</i> , Immortalized Tree shrew liver cells		Vaccine Production (Mantlo et al., 2020)			
X9.5	<i>Scandentia</i> , Immortalized tree shrew liver cells			Vaccine Production (Mantlo et al., 2020)			
C3A	<i>Homo sapiens</i> , Derivative of Hep-G2			Genes Expression (Xu et al., 2020b)		ATCC® CRL-10,741	
Intestinal	Caco-2	<i>Homo sapiens</i> , Colorectal adenocarcinoma Epithelial Cells	Darunavir (Zebin et al., 2020)	Vaccine Production (Mantlo et al., 2020)	Viral Pathogenesis and Transmission (Hoffmann et al., 2020)	ATCC Cat# HTB-37; RRID: CVCL_0025	High ACE2 expression
Immune Cells	MT-2 cell	<i>Homo sapiens</i> , T cell leukemia cells			Viral Pathogenesis (Gao et al., 2020)	Sigma-Aldrich Cat #08,081,401	
Stems Cells	DYR0100	<i>Homo sapiens</i> , Human Induced Pluripotent Stem [IPS] Cells			Differentiation assay (Cong et al., 2020)	ATCC ACS-1011	
Embryo	NIH3T3	<i>Mus musculus</i> , Embryo Fibroblast		Vaccine Production (Mantlo et al., 2020)		ATCC Cat# CRL-1658; RRID: CVCL_0594	
Ovary	CHO-K1	<i>Cricetulus griseus</i> [Hamster] Ovary epithelial-like		Vaccine Production (Mantlo et al., 2020)	Virus susceptibility (Thevarajan et al., 2020)	ATCC CCL-61	
skin	A357	<i>Homo sapiens</i> , malignant melanoma		Vaccine Production (Mantlo et al., 2020)		ATCC CRL-1619	
	HACAT	<i>Homo sapiens</i> , Keratinocyte		Vaccine Production (Mantlo et al., 2020)		<a href="https://clsgmbh.de/pdf/hacat.pdf">https://clsgmbh.de/pdf/hacat.pdf</a>	
Leukemia	LR7	<i>Rattus norvegicus</i> , Murine LR7 cells			Viral Life Cycle (Snijder et al., 2020a,b)	e-WRT-7-LR (RRID: CVCL_J367)	
	HeLa-CEACAM1a cells	<i>Homo sapiens</i> , Variant of HeLa Cells			Viral Life Cycle (Snijder et al., 2020a,b)		
Cervix	HeLa-hACE2	<i>Homo sapiens</i> , Variant of HeLa Cells			Viral Pathogenesis and Transmission (Zhou et al., 2020)		HeLa-hACE2: High ACE2 expression
BALB/c immortalized cell line	17C11	<i>Mus musculus</i> , Breed/subspecies: BALB/c			Viral Replication and Pathogenesis (	RRID:CVCL_VT75	

(continued on next page)



Table 1 (continued)

Organ/ Tissue	Experimental Models: Cell Lines	Origin/ Details of culture model	Drug/vaccine Evaluated with reference			Availability	Remark
			Drug Screening	Vaccine/Antibodies Production/Immune Response/Genes Expression	Virus Isolation, Expression /Other purpose		
		Spontaneously immortalized cell line					Snijder et al., 2020a,b)

ACE-2 has been quantified organ wise explaining these organ/tissue-specific cells as potential *in-vitro* models for drug screening against COVID-19 as its entry into the target cells occur through S-protein-ACE receptor binding (Xu et al., 2020a). ACE-2 is a type I transmembrane metalloproteinase enzyme that plays a key role in the renin-angiotensin system (RAS), and the majority of it expresses in the renal tubular epithelium of the kidney, lungs, gastrointestinal tract, vascular endothelium, and Leydig cells of testes (Kuba et al., 2013; Jiang et al., 2014; Ksiazek et al., 2003; Harmer et al., 2002). SARS-CoV-2 and target cells binding occur through spike protein-ACE-2 receptor interaction similar to SARS-CoV (Hoffmann et al., 2020; Li et al., 2003). Most of the used *in-vitro* models rely on the expression of ACE-2 and TMPRSS-2 by the cellular system and isolation, pathogenesis and all the drugs/new chemical entities evaluated upon them (Table 1).

### 3.2.1. Kidney cell lines as *in vitro* model system for Covid19

**3.2.1.1. Vero and Vero E6 cell lines.** Vero and Vero E6 cells are one of the most extensively used kidneys based epithelial cell lines for the culture of SARS-CoV-2 due to the presence of high expression of ACE-2 receptors as we have already discussed that SARS-CoV-2 entry in the cells takes place through S-protein-ACE2 receptors binding. The Vero cell line was established by Japanese scientists Yasumura and Kawakita in 1962 from *Chlorocebus* sp. of African green monkeys. Vero E6 or Vero 1008 cell line is the clone of Vero 76 cells and is a better option than Vero cells as it shows some contact inhibition and imitates properties more like primary cells. Immediately after the outbreak of SARS-CoV-2, several scientific groups used Vero and Vero E6 cells for their identification, pathogenesis as well as transmission studies (Hoffmann et al., 2020; Chan et al., 2020b; Zhou et al., 2020; Matsuyama et al., 2020), isolation (Chan et al., 2020b; Xiao et al., 2020; Caly et al., 2020a; TT-Y et al., 2020; Harcourt et al., 2020; Chan et al., 2020c; Capobianchi et al., 2020; Nie et al., 2020), and virus susceptibility (Thevarajan et al., 2020). Vero and Vero E6 cell lines are used for the immune response (Pan et al., 2020; Fukushi et al., 2006; Zheng et al., 2020), antibodies production (Pu et al., 2020), vaccine production (Mantlo et al., 2020), Type I IFNs as a target of antiviral therapy (Anderson et al., 2020), MTHFD1 as a target of Antiviral Therapy (Xing et al., 2020), virus-induced genes expression reversal (Xiong et al., 2020a), and mAbs screening (Fintelman-Rodrigues et al., 2020). Vero cells were found to be the best screening models for new drugs as well as for already available anti-viral drugs for their repositioning as shown in Table 1 (Xiong et al., 2020a; Ge et al., 2020; Weston et al., 2020; Yao et al., 2020; Touret et al., 2020; Choy et al., 2020; Liu et al., 2020a; Jin et al., 2020; Dai et al., 2020; Liu et al., 2020b; Zhijian et al., 2020; Xiong et al., 2020b; Wang et al., 2020a; Caly et al., 2020b; Sheahan et al., 2020a; Runfeng et al., 2020; Andreani et al., 2020; Su et al., 2020; Ohashi et al., 2020; Yamamoto et al., 2004; Abbott et al., 2020). Some of the drugs, such as Hydroxychloroquine, Ivermectin, Nelfinavir, lopinavir, emetine, Remdesivir, and homoharringtonine have shown effectiveness against SARS-CoV-2 in Vero and Vero E6 cells. TMPRSS-2 is another recently discovered mode of SARS-Cov-2 entry into the target cells and further spread to other organs (Hoffmann et al., 2020). In another recent study on Vero and Vero E6 cells, where Vero E6 cells expressing TMPRSS-2, enhanced the isolation

of SARS-CoV-2 (Matsuyama et al., 2020). However, this is a new and unexplored area, and more studies are required in this direction to target the TMPRSS-2 based viral spread and might be a potential therapeutic target for drug development against SARS-Cov-2.

**3.2.1.2. HEK293 and its variant cell lines.** Other important kidney-specific cell lines that have been used for SRS-CoV2 studies include the HEK293 cell line for vaccine production (Mantlo et al., 2020), viral pathogenesis, and transmission (Hoffmann et al., 2020). 293 T cell line is derived from HEK293 cell line. It is very useful for various studies such as antiviral CRISPR (Wang et al., 2020b), virus isolation, (Dai et al., 2020), MTHFD1 as a target of anti-viral therapy (Xing et al., 2020), virus susceptibility (Thevarajan et al., 2020), viral pathogenesis/transmission (Gao et al., 2020), vaccine production (Mantlo et al., 2020), immune response (Ye et al., 2020; Ju et al., 2020), mAb production (Li et al., 2020; Sun et al., 2020), mAbs screening (Fintelman-Rodrigues et al., 2020), vaccine production and antibodies response (Mossel et al., 2005), ACE-2 gene expression in target cells (Zhang et al., 2020a), for drug screening like Teicoplanin (Wu et al., 2020). Similarly, 293 FT cell line is used for screening of DHODH Inhibitors (Wang et al., 2020a), MAbs production (Li et al., 2020), and 293 F used for monoclonal antibodies production (Li et al., 2020; Sun et al., 2020); whereas, 293 T-ACE-2 cell line for vaccine production and antibodies response (Mossel et al., 2005) and viral pathogenesis (Gao et al., 2020).

**3.2.1.3. Other kidney cell lines as *in vitro* model system.** Other kidney-specific cell lines used for SARS-CoV-2 study include LLC-MK2 for virus susceptibility (Chan et al., 2013); MDCKII cell line for the screening of DHODH Inhibitors (Wang et al., 2020a), virus susceptibility (Pan et al., 2020), and viral pathogenesis and transmission (Hoffmann et al., 2020); BHK-21 cell line for MAbs screening (Fintelman-Rodrigues et al., 2020); COS-7 cells for antibodies production (Pu et al., 2020); PaKi cell line for MTHFD1 as a target of Antiviral Therapy (Xing et al., 2020); and HK2 as well as NRK-49 F cell lines for Remdesivir drug screening (Hamming et al., 2004).

### 3.2.2. Respiratory cell lines as *in vitro* model system for Covid19

In the lungs, the majorities of the ACE-2-expressing cells (~83 %) are alveolar type II cells and may serve as *in vitro* Model system for Covid 19 studies (Zhao et al., 2020a; Zhang et al., 2020b). Similarly, oral and nasal epithelial cells also showed higher expression of ACE-2 receptors of SARS-CoV-2 (Sungnak et al., 2020; Sheahan et al., 2020b).

**3.2.2.1. Calu-3 and Calu-3 2B4 cell line.** Calu-3 is a lung adenocarcinoma epithelial cell line used for various SARS-CoV-2 studies (Matsuyama et al., 2020; Yoshikawa et al., 2010). Among Calu-3 and its clonally derived Calu-3 2B4, ACE-2 expression was checked, and comparatively, 2B4 have higher ACE2 expression (Lokugamage et al., 2020), suggesting them a better model system for SARS-CoV2. It has been used to check the viral pathogenesis and transmission (Hoffmann et al., 2020; Matsuyama et al., 2020; Hikmet et al., 2020) and for drug screening of EIDD-2801 (Yoshikawa et al., 2010).

**3.2.2.2. Other respiratory cell lines.** Other respiratory systems based In

*In vitro* Model systems are A549 cell line for ACE2 gene expression (Zhao et al., 2020b), and for drug screening of ATAZANAVIR (Ge et al., 2020), Teicoplanin (Wu et al., 2020), DHODH inhibitors (Wang et al., 2020a); NCI-H1299 and BEAS-2B cell lines for viral pathogenesis and transmission cell line for vaccine production (Mantlo et al., 2020); Primary culture of type II alveolar (AT2) cells for ACE2 gene expression (Poschet et al., 2020); IB3-1 and its derivative S9 cells for the screening of Azithromycin and Ciprofloxacin (Wang et al., 2020c); and KMB17 cells for vaccine production (Mantlo et al., 2020). Hep-2 cell line shown significant CoVs viral titer and have ACE2 receptor (Hoffmann et al., 2020; Wang et al., 2020a), and susceptible to SARS-CoV2; however, in another study, A549 cells did not show TMPRSS2 expression, required for S protein processing and viral spread (Matsuyama et al., 2020).

### 3.2.3. Hepatic cell line as a model of SARS-CoV-2 culture

**3.2.3.1. Huh7 cells.** Huh7 cell line is derived from liver carcinoma of a 57-year-old Japanese male in 1982 and is a potential *in vitro* model system for drug screening against many viruses. A recent study shows that SARS-CoV-2 efficiently enters into the Huh-7 cells through ACE-2 receptor binding as a potential *in vitro* study model (Kaye, 2006). In a recent study published in Cell Research, Huh-7 cell line is used as *in vitro* drug screening model to check the therapeutic potential of Remdesivir and chloroquine against SARS-CoV-2 (Caly et al., 2020b). In another study, Li Runfeng et al., used Huh-7 cell line to screen the anti-viral and anti-inflammatory activity of Lianhuaqingwen exerts (Andreani et al., 2020). Based on viral load, SARS-CoVs replication in Huh-7 is comparatively higher (Kaye, 2006; Fang et al., 2020), and suggested to be an ideal *in vitro* model system. In other studies, Huh-7 has been used for Virus Isolation (Xiao et al., 2020), virus susceptibility (Thevarajan et al., 2020; Sungnak et al., 2020; Fang et al., 2020), Viral Pathogenesis and Transmission (Hoffmann et al., 2020; Gao et al., 2020; Zhang et al., 2020a; Kaye, 2006; Zhao et al., 2020c), Vaccine Production (Mantlo et al., 2020), gene expression (Chan et al., 2013), and for drugs screening such as Teicoplanin (Wu et al., 2020), and DHODH Inhibitors (Wang et al., 2020a).

**3.2.3.2. Other liver cell lines.** Other liver-based cell lines that have been used for SARS-CoV2 susceptibility are HepG2, Hep1-6, HL7702, RHT6.0, X9.0, X9.5, and C3A. HepG2 is used to check the SARS-CoV2 Virus susceptibility (Thevarajan et al., 2020), infection-induced target cells gene expression (Xu et al., 2020b) while in another study for vaccine production (Mantlo et al., 2020). Similarly, other cell lines, such as Hep1-6, HL7702, RHT6.0, X9.0, X9.5, and C3A are used for vaccine production (Mantlo et al., 2020).

### 3.2.4. Intestinal based in vitro model system for Covid19

Intestinal ACE-2 receptor expression (De Meyer et al., 2020) and are very susceptible to 2019-nCoV infection. Caco-2 cell line has shown very high CoVs viral titer (Zhang et al., 2020a). It has been used for viral pathogenesis and transmission (Hoffmann et al., 2020), vaccine production (Mantlo et al., 2020), and drug repurposing and screening studies of Darunavir (Zebin et al., 2020) against Covid-19 and has proven to be a good *in vitro* model system.

### 3.2.5. Other cells as in vitro model system for Covid19

There are several other cell lines that have been used for SARS-CoV-2 studies such as T cell leukemia (MT-2) cells derived from co-culturing of human cord leukocytes and leukemic T-cells, shown susceptibility to SARS-CoV2 infection (Wang et al., 2020b); Stems Cells based cell line DYR0100 for Differentiation assay (Zebin et al., 2020); Embryo specific NIH3T3 cell line for vaccine production (Pu et al., 2020); Ovary specific CHO-K1 cell line for vaccine production (Pu et al., 2020) and virus susceptibility (Nie et al., 2020); skin based A357 and HACAT cell lines for vaccine production (Pu et al., 2020); leukemic LR7 cell line for Viral

Life Cycle (Cong et al., 2020); cervix cell line HeLa based clones HeLa-CEACAM1 and HeLa-hACE2 cells for Viral Life Cycle (Cong et al., 2020) and for viral pathogenesis and transmission (Zhou et al., 2020); and BALB/c immortalized cell line 17Cl1 for viral replication and pathogenesis (Snijder et al., 2020a,b).

## 4. Conclusion

Together In a nut-shell, cells expressing the ACE2 receptor and TMPRSS-2 could be potential *In-Vitro* models for all types of studies against SARS-Cov-2, including molecular or biochemical studies of a virus, repurposing of drugs and their dose standardization, vaccine production, efficacy, and safety profile of newly identified lead molecules. In this regard, the knowledge of suitable cell lines becomes essential, and Vero E6, Huh-7, 293 T, Calu-3, and Caco-2 cell lines have shown high potential as *in-vitro* models to combat the COVID-19.

## Authorship contributions

Please indicate the specific contributions made by each author (list the authors' initials followed by their surnames, e.g., Y.L. Cheung). The name of each author must appear at least once in each of the three categories below.

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