REVIEW ARTICLE

Human lung organoids as a model for respiratory virus replication and countermeasure performance in human hosts

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Human respiratory viruses induce a wide breadth of disease phenotypes and outcomes of varying severity. Innovative models that recapitulate the human respiratory tract are needed to study such viruses, understand the virus-host interactions underlying replication and pathogenesis, and to develop effective countermeasures for prevention and treatment. Human organoid models provide a platform to study virus-host interactions in the proximal to distal lung in the absence of a human in vivo model. These cultures fill the niche of a suitable ex vivo model that represents the in vivo lung environment and encapsulates the structure and function of the native human lung. (Translational Research 2022; 250:36–45)

Abbreviations: ARDS = Acute respiratory distress syndrome; AT1 = Alveolar epithelial type 1 cells; AT2 = Alveolar epithelial type 2 cells; ALI = Air-liquid interface; COPD = Chronic obstructive pulmonary disease; HPIV = Parainfluenza; hCoV = Human coronavirus; HA = Hemagglutinin; hPSC = Human pluripotent stem cells; hDLO = Human distal lung organoids; NA = Neuraminidase; RSV = Respiratory Syncytial Virus

HUMAN RESPIRATORY VIRUSES

Human respiratory virus infections cause a wide spectrum of asymptomatic to severe disease phenotypes each year, resulting in high morbidity and mortality outcomes globally. Common respiratory viruses, such as human influenza, respiratory syncytial virus (RSV), parainfluenza (HPIV), rhinoviruses and common cold coronaviruses (CoV), represent diverse families of viruses characterized by different genome organizations, unique transmission patterns and pathogenic outcomes in the upper and lower respiratory tract of human populations. Contemporary human respiratory viruses oftentimes cause seasonal epidemics, such as influenza, RSV, and HPIV, or have widespread endemic transmission across the calendar year, such as the common cold CoV and rhinovirus strains. While infections usually cause mild upper respiratory tract disease, patients may progress to more serious lower respiratory tract infections like bronchiolitis and croup, or to life threatening pneumonias that can progress to acute lung injury and/or organizing chronic pneumonias with fibrosis. Moreover, due to waning immune responses in upper respiratory mucosal compartments, coupled with antigenic changes in response to host immune memory responses, many contemporary respiratory viruses have the potential to cause dramatic spikes in disease prevalence, hospitalization, and

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mortality, associated with epidemic or pandemic spread.[1,2] Alarmingly, extensive zoonotic virus reservoirs exist for many of these virus families, providing unparalleled opportunities for the sudden emergence and spread of "new" strains which have not circulated previously in human populations. Typified by avian influenza viruses like H5N1 and H7N9 and the emerging bat coronaviruses from the Sarbecovirus (Severe Acute Respiratory Coronavirus: SARS-CoV, SARS-CoV-2) and Merbecovirus (Middle East Respiratory Coronavirus: MERS-CoV) lineages, these pathogens have caused repeat outbreaks of disease some of which have progressed to large epidemics or global pandemics in the 21st century.

Seasonal influenza epidemics are caused by two main antigenic types, designated A and B strains, and strain variation is further driven by progressive evolution in the hemagglutinin (HA) gene in the dominant strain of influenza A each year. Influenza virus evolves by yearly antigenic variation (mutation) and periodic antigenic shift (RNA reassortment), where human strains encoding the HA and neuraminidase (NA) can reassort with highly heterologous strains circulating in zoonotic reservoirs and introduce avian or other mammalian HA or NA genes into the human population. Influenza A strains encoding HA1, HA2 and HA3 have predominated over the past 75 years, supported by antigenic drift caused by adaptative mutations in influenza A HA that arise in response to and that ultimately dimmish human herd immunity.[3] Evolution in the neuraminidase (NA) glycoproteins of influenza A also support the emergence and propagation of new viral variants that serve as the basis of both annual seasonal epidemics and the emergence of pandemic strains, such as those in 1918, 1956, 1968 and 2009[3]. Prior to the COVID-19 pandemic, and despite effective public health measures and vaccine campaigns that have substantially curbed the frequency of severe influenza, 35 million flu related illnesses, 380K hospitalizations, and 20K flu related deaths were reported in 2019.[4] While an influenza vaccine exists, the vaccine must be adjusted each year to include the predicted annual strain, sometimes resulting in a mismatch and poor protection against the true circulating strain.[5] In the study of a universal influenza vaccine, models to study vaccine candidates in *ex vivo* human respiratory tissues, such as human lung organoids, are critical and have revealed novel differences in the tropism and pathogenesis of contemporary and avian influenza strain infections.[6]

Paramyxovirus infections, typified by RSV, metapneumoviruses and HPIV, cause seasonal outbreaks but are most commonly diagnosed in infants, with nearly all children affected by the time they reach age 2.[7-9] Seniors are also vulnerable to severe RSV infection.[10] While influenza is typically associated with fever, cough, sore throat, a runny or stuffy nose, as well as frequent lower respiratory tract infections, RSV and HPIV typically cause upper or lower respiratory infection that more frequently progress into bronchiolitis or pneumonia.[7,8] Each year, RSV alone causes 2.1 million outpatient visits and 58K hospitalizations in children <5 years of age.[7] Currently, no vaccine exists for either RSV or HPIV, although critical new developments in RSV vaccine design and the development of therapeutic antibodies offer hope.[11,12]

Endemic human coronaviruses (HCoV) make up a family of "common cold" viruses with similar symptoms, resulting in, on average, 1 billion annual infections in the US alone.[13] While rhinoviruses also cause common cold symptoms, endemic coronaviruses are often associated with more a more severe symptomatic response, especially in young and aged populations. Four endemic coronaviruses are commonly associated with upper respiratory tract infections, including HCoV 229E, HCoV OC43, HCoV NL63, and HCoV HKU1. All contemporary human coronaviruses have origins in wildlife, including bats, cattle, and mice over the past few hundred years.[14,15] The more historic of these viruses, 229E and OC43, were identified in the late 1960s and are highly prevalent in children.[13] HCoV infection typically elicits the common cold, but the very young and old or persons with co-morbidities can develop more severe conditions such as pneumonia and bronchiolitis. Additional studies are needed to determine if some HCoV strains cause more severe disease than others as lethal outbreaks of HCoV OC43 have been reported in retirement communities and other HCoV infections have been associated with life threatening infections in infants and the elderly.[16] Importantly, NL63 and HKU1 were first identified in the early 2000s and are associated with widespread, mild, self-limiting upper respiratory infections.[13] However, HKU1 has also been known to cause a high incidence of febrile seizures in children in addition to cold-like symptoms and was initially isolated from a life-threatening infection in a patient during the SARS-CoV epidemic. As seen with RSV and HPIV, all four endemic coronaviruses lack an effective vaccine or treatment, dictating a need for adequate models and investment to efficiently develop countermeasures using human lung tissue models.

While endemic coronaviruses make up a large proportion of human respiratory infections each year, numerous highly pathogenic epidemic coronaviruses have emerged in the last two decades, exacerbating the need for human respiratory models to study prophylactic and therapeutic treatments, such as vaccines and antivirals. In 2002, the emergence of SARS-CoV caused over 8K cases, 800 deaths, and approximately 11% mortality in the Asian-Pacific region and Canada.[17,18] In 2012, MERS-CoV was discovered in

Saudi Arabia and quickly spread to multiple countries in the Middle East outside the Arabian Peninsula, resulting in over 2,500 cases, 800 deaths, and ~35% mortality from 27 countries.[17] Finally, in 2019, the emergence of SARS-CoV-2, the virus responsible for COVID-19, rapidly evolved into an on-going global pandemic that has caused over 500 million cases and over 6 million deaths thus far.[19] Symptomatically, these three emerging epidemic coronaviruses share common clinical features such as sore throat, cough, fever, headaches, myalgia, and possible progression to acute respiratory distress syndrome (ARDS) in severe cases.[17,20] However, these viruses vary in other prominent symptoms, such as malaise and loss of taste and smell following SARS-CoV-2 infection, a large proportion of patients experiencing diarrhea following SARS-CoV infection, and possible renal injury and failure following MERS-CoV and SARS-CoV-2 infection.[20,21] Given the highly plastic nature of coronavirus genomes, it is not surprising that these viruses have evolved and emerged well adapted to human hosts. However, the recent SARS-CoV-2 virus has continued to adapt throughout the pandemic, resulting in a number of recent variants, including 9 variants of interest and 5 variants of concern.[22,23] The variability of this virus presents a challenge to developing universal vaccines and therapeutics that remain effective against current and future variants or viruses.[24]

In the human lung, SARS-CoV and SARS-CoV-2 primarily target airway epithelial cells reflecting high levels of hACE2 receptors and TMPRSS2 protease entry components.[25-27] In addition, low levels of virus replication are oftentimes seen in secretory goblet cells and club cells lining the smaller airways. In the gas exchange region of the lung, these Sarbecoviruses primarily target alveolar epithelial type 2 (AT2) cells, and to a lesser extent alveolar epithelial type 1 (AT1) cells.[28,29] In contrast, MERS-CoV targets non-ciliated airway epithelial cells in the conducting airways and AT2 and AT1 cells in the alveoli. MERS-CoV also replicates efficiently in lung fibroblasts and lung endothelial cells, reflecting the wider distribution of the dipeptidyl peptidase receptor and TMPRSS2 protease distributions in the human lung.[30,31] Organoid models that allow for emerging coronavirus replication in the presence of multiple tissues and inflammatory cells of the human lung have the potential to offer exciting new insights into virus-host interactions and pathogenesis.

TARGETING OF LUNG CELLS BY RESPIRATORY VIRUSES

Recent advances in technologies, like single-cell RNA sequencing, GeoMx, and CODEX Multiplexed Tissue Staining and Image Acquisition and related technologies allow for detailed characterization of human host innate and acquired immunologic responses at single cell resolution. Although biopsies and bronchial alveolar lavage fluids provide for infrequent but targeted longitudinal sampling opportunities, however, most of these genomic analyses focus on samples derived from end stage lung samples associated with lethal outcomes, thereby missing many of the critical time-ordered events associated with infection, clearance, disease progression and/or repair of the lung. Consequently, the virologic, host and immunologic factors that contribute to altered disease outcomes in humans remains a critical question in viral pathogenesis, the development of treatments and vaccines. Moreover, direct acting antivirals have limited opportunities for reversing disease progression in human patients unless given early in infection. Especially evident after influenza, RSV, and emerging coronavirus infections, disease severity is associated with complex immune pathologic and host response patterns, which can progress to end stage lung diseases. Therefore, later stage pathologic features require treatment modalities that focus on mitigating disease enhancing host response networks and/or immunopathologic signatures, dictating the need for host and immune based interventions that do not directly target live virus replication. Variation in disease severity can be attributed to microvariation in virus strain and sequence variants, changes in virus antigenicity, cell tropism, mutations that enhance virus replication/gene expression and the presence of viral genes that antagonize host innate immune responses. Moreover, natural genetic variation in outbred human populations, which present either as strong monogenic or polygenic traits, can regulate virus disease severity across individuals and promote pathogenic or protective immune/host responses that promote lethal disease.[32] While many immune responses occur within the airway epithelium, multiple subtypes of lymphoid cells and myeloid cells play critical roles in lung development, homeostasis, immunity and disease, including acute lung injury, lung fibrosis, and chronic obstructive pulmonary disease (COPD).[33] As human studies provide discovery and correlative insights into the virologic, immunologic and host responses that associate with disease severity, more definitive model platforms are needed to validate host and immunologic response patterns associated with severe in vivo disease. Recent developments in long term expansion of primary human cells and complex organoid models derived from the proximal to distal lung provide a suitable strategy to study cell and tissue specific host response patterns after infection, the role of host genetics on virus replication efficiency and a platform to perturbate key host responses predicted to regulate disease severity. This review focuses on new discoveries in lung organoid biology and

biochemistry related to viral pathogenesis associated with contemporary and newly emerging viral diseases of humans.

HUMAN PRIMARY CELL AND ORGANOID MODEL SYSTEMS OF INFECTION

Human organoid models provide a reliable platform to study virus-host interactions in the proximal to distal lung in the absence of a human in vivo model. Alternatively, these cultures provide an ex vivo model that presents the in vivo environment and recapitulates the structure and function of the native human lung. While commonly used 2D human airway epithelial cultures provide a resource for understanding viral fitness and potential efficacy of clinical interventions, they fail to represent the 3D lung structure and interactions between cells that is conferred by organoid models. Additionally, such *ex vivo* models can be adapted to represent differing compartments of the respiratory tract, providing novel opportunities to study virus tropism, virus tissue specific host response patterns, lung function, disease pathology, and provide a suitable platform to target therapeutics of various viruses by focusing on distinct cell types or areas of the lung.

Historically, human primary cells have been used to reveal mechanisms essential to virus-host interactions and as a platform to evaluate drug performance in key cell types within the human respiratory tract that are targeted by virulent viruses.[34-36] However, these models fail to fully replicate the native environment of the human lung, including complex cellular interactions between virus and host epithelium and the host immune response. These models do, however, provide a strong foundation for understanding viral pathogenesis and potential targets for clinical interventions in vitro. These cells can be harvested and cultured to represent the nasal cavity, the large airway consisting of the bronchi, and the small airway consisting of the bronchioles and alveoli (Fig 1).[37] Human airway cultures are ground on porous membranes at an air-liquid interface (ALI) that mimics that air-liquid barrier of the epithelium lining to respiratory tract.[38,39] Once differentiated and matured, these cells as a whole can provide clues about viral pathogenesis and the innate immune response from the proximal to distal airway. Additional human primary cell types that do not require an air-liquid interface, such as fibroblasts and microvascular endothelial cells, can be cultured in order to further tease apart cellular tropism and phenotype following viral infection.

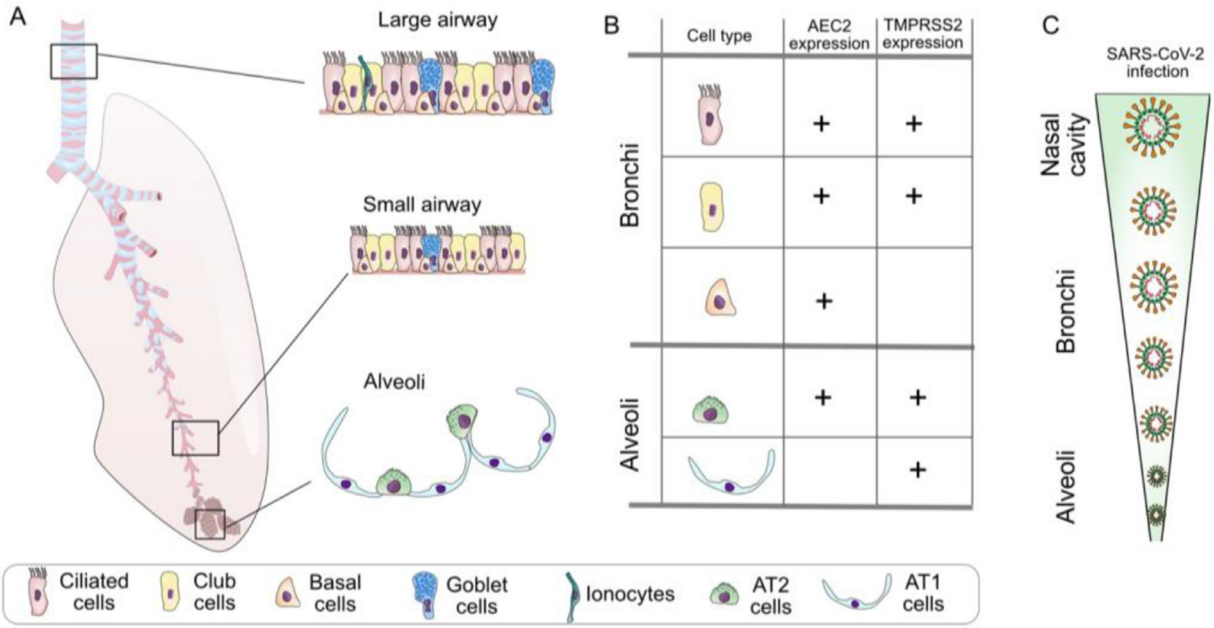

Fig 1. **A**, Human primary lung cells can be harvested from the proximal to distal lung to represent various lung compartments including large airway, small airway, and alveoli. Harvested primary cells represent a heterogeneous population of key lung cells important in virus-host interactions, as well as natural human lung function **B**, Each of these cell types express either ACE2, TMPRSS2, or both, indicating a strong model for studying human epidemic coronaviruses such as SARS-CoV and SARS-CoV-2. **C**, SARS-CoV-2 has been studied in these lung models and depicts a gradients of replication efficiency from the proximal to distal lung.

3D models, such as human organoids, involve arranged complex structures of multiple cells that can self-organize to replicate in vivo tissue structure and morphology while mimicking cellular interactions and the dynamic regulation of signaling pathways in an ex vivo culture. Organoids derived from human pluripotent stem cells (hPSC) are the most common and well-known type of human organoids that can be derived from peripheral blood and differentiated towards airway and alveolar epithelial cells.[40,41] hPSC derived organoids have a broad use as they can be induced to differentiate into specific cell lineages and form tissue-specific organoids using biochemical signaling and cellular markers that orchestrate the process during embryonic development in which a zygote matures into a self-organized unit of cells as an organ.[42,43] These cells are then co-cultured with mesenchymal and endothelial cells to stimulate the generation and maturation of a 3D organoid structure that represents a section of the human lung. Once matured, hPSC derived organoids can possess both upper and lower respiratory-like epithelium containing basal cells, ciliated cells or alveolar-like structures, respectively. However, hPSC-derived organoids may not completely recapitulate the functionality and gene expression profiles compare to mature cells of adult human lungs. Despite this limitation, hPSC remain a reliable tool for studying both viral-host interactions, pathogenesis, and clinical interventions as they better replicate the human airway than previously developed models of the human respiratory tract.[44,45]

While hPSC derived lung organoids can be broadly differentiated to represent both the proximal and distal airway and alveolar regions, harvesting and propagation of cells from the distal airway specifically have gained traction in recent years for the ability to explore terminal airway that had been previously difficult to target for testing clinical interventions, such as prophylactic and therapeutic antivirals. Human distal lung organoids (hDLOs) are derived specifically from the distal lung parenchyma and demonstrate complex bronchial structures with mature cilia that drive mucus movement and clearing of airway debris.[46] Major cell markers present in the distal lung are also present in hLDOs, including ciliated cells, goblet cells, basal cells, AT2 and AT1 cells, allowing these organoids to efficiently mimic infection processes in multiple cell types across the distal lung. Recently, much effort has been made to develop an alveolar organoid model from primary human AT2 cells[47,48]. Previously, AT2 organoids required co-culturing with fibroblasts from alveolar stem cell niche or endothelial cells from fetal tissues, in medium containing FBS and other unknown co-factors.[49,50] Due to these undefined conditions, it was difficult to control growth rate as well as AT2 cell fate decisions. However, in a novel feeder free, chemically defined modular culture system, AT2 cells can be propagated to generate alveolospheres and adequately differentiate further into AT1 cells.[47] This advancement allows for a dynamic environment that more closely recapitulates the alveolar space of the human lung and fills a critical niche for understanding viral pathogenesis and host interaction.

More recently, distal lung organoids, derived from AT2 cells or basal appear to form intact lumens lined with differentiated ciliated and club cells, providing new insights into complex virus-host interactions with respiratory cell epitheliums.[51] Basal cell organoids are mostly characterized by two major, but distinct subpopulations of cells that express proliferation and cell-cycle related expression patterns or display expression patterns associated with vesicular transport and squamous cell markers, respectively.[51] After differentiation into mature cultures, cultures can be evaluated as either an apical in or apical out polarity ("flipped" organoids), the latter orientation presents critical host receptors, such as ACE2 or DPP4, on exposed organoid surfaces that promote efficient infection by respiratory viruses.[51]

Receptor expression for various respiratory viruses on the surface of human organoids is critical to modeling of viral replication in these cultures and understanding the virus-host interactions. However, the expression of these receptors in the human respiratory tract is cell and organoid dependent. For example, while α2,3 and α2,6 linked sialic acids, the receptors for avian and human influenza viruses, respectively, are abundant on most airway cells, ACE2 and DPP4, the receptors for epidemic human coronaviruses, are more localized.[52-54] α2,3 and α2,6 linked sialic acids are expressed on ciliated and non-ciliated cells, such as goblet cells, as well as abundantly on AT2 cells and cells of the distal trachea.[53,55] The broad expression of this receptors provides ample opportunity to utilize human organoids of multiple origins to study virus-host factors that drive replication and pathogenesis. Alternatively, ACE2, the receptor for SARS-CoV, SARS-CoV-2, and endemic hCoV NL63, is more prominently localized to the apical surface of ciliated cells, as well as with greater expression in the proximal vs distal airway.[52,56-59] Importantly for organoid formation and differentiation, ACE2 is expressed in airway epithelium cultures differentiation from basal stem and progenitor cells, such that receptor expression is prevalent on fully differentiated organoids.[58] However, ACE2 is also expressed in AT2 cells, indicating that AT2 derived organoids serve as a strong ex vivo model to study these viruses and potential

interventions. Lastly, expression of DPP4, the receptor for epidemic hCoV MERS-CoV, follows a more specific pattern and is rarely found on the surface epithelium of the proximal airway with a slight increase in expression in the distal airway.[54] Additionally, DPP4 is predominantly found on AT1 and AT2 cells of the alveoli, as well as macrophages and fibroblasts, indicating a unique role of such primary cells to study MERS-CoV replication, pathogenesis, and repair in human organoids of various derivation.[54,60]

As indicated, systems of human lung organoids provide reproducible models for applications in viral replication, and pathogenesis, including understanding virus-host interactions, exploring viral induced tissue damage and innate immune pathways and subsequent tissue regeneration responses, and the testing of novel clinical interventions such as prophylactic and therapeutic drugs that target viral genes or host pathways essential for efficient virus growth (Fig 2). Human lung organoids that recapitulate the human respiratory environment provide a model to explore the function of lung-related genes and signaling pathways, as well as immune responses following viral infection. With this tool, modeling of lung diseases becomes a multi-factorial process that allows for the further understanding of age-dependent viruses and outcomes, such as the predominance of RSV in infant lungs or age-exacerbated adverse events following coronavirus infection, by modifying the age at which organoids are used following stem cell proliferation. Further, these interactions

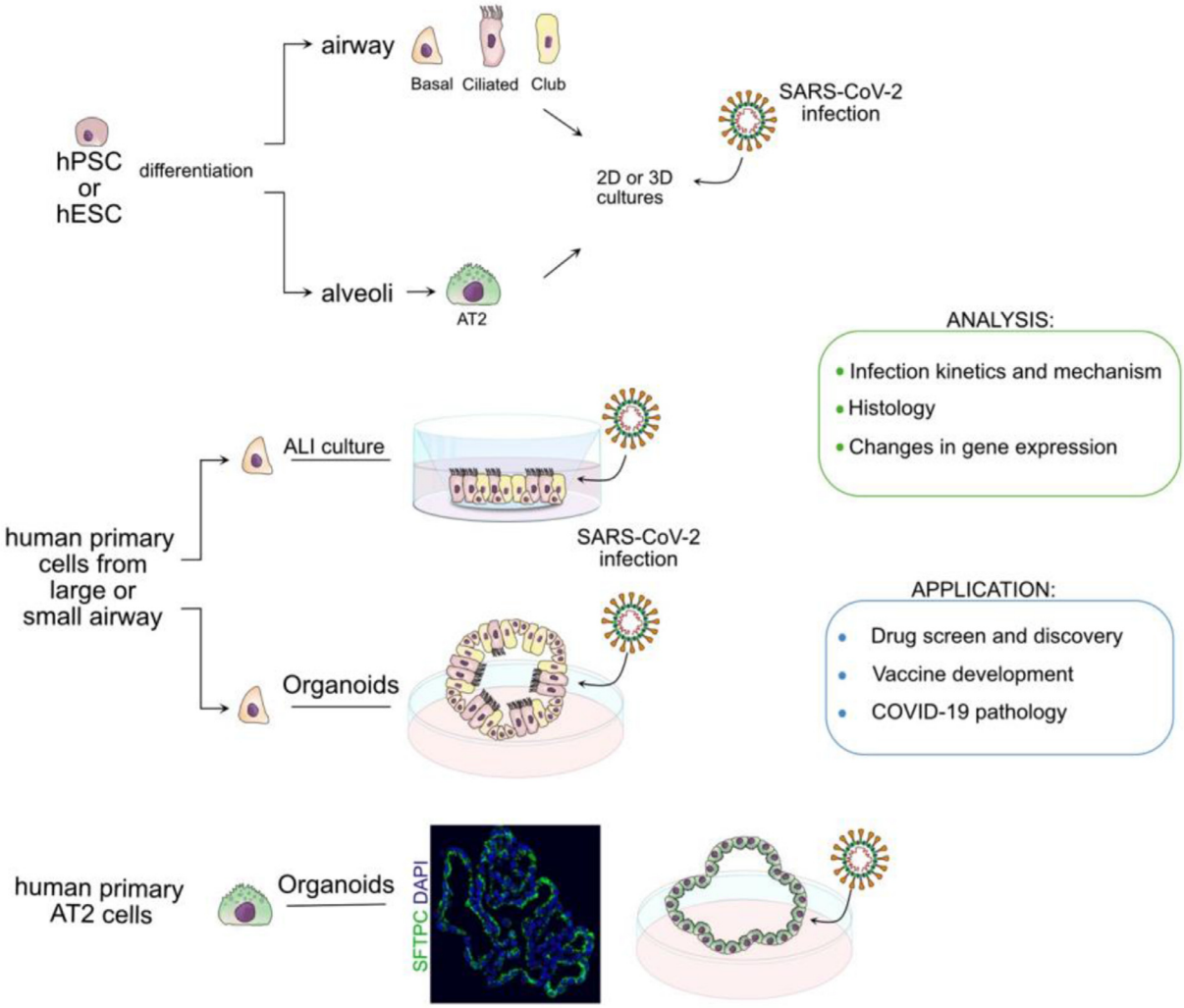

Fig 2. Human pluripotent (hPSC) or embryonic (hESC) stem cells can be cultured and differentiated into primary airway cells that can generate 2D and 3D cultures. Additionally, human primary cells fr om the large airway, small airway, and alveoli can be used to develop models of human lung tissue including ALI models, organoid models, or both. Each of these models has unique analytical and clinical testing applications that fill a critical niche in studying human respiratory viruses.

between virus infection and respiratory epithelial tissue can be teased out to understand the underlying mechanisms that drive viral induced tissue damage. Additionally, as organoids derived from stem cells and progenitor cells retain their programming, by capturing the regenerative process of in vivo lung processes, researchers can study, and mimic processes of lung repair suggested from an in vivo study or autopsy of afflicted human lung tissue. Lastly, the multiple types of human organoid models provide a platform for screening drugs to prevent or alleviate respiratory disease by understanding disease pathology in ex vivo tissues and applying these findings to effective clinical interventions.

DRUG TESTING AND DEVELOPMENT

Prior to clinical trial testing of potential clinical interventions, drug development and testing in vitro must be completed and demonstrate potential efficacy. Historically, pre-clinical testing of these drugs has been completed in 2D human primary cells, such as large bronchial cultures. These cultures have provided a pathway to in vivo small animal models and ultimately human clinical trials of many antivirals, such as those used against the pandemic virus SARS-CoV-2, including remdesivir, molnupiravir, and pegylated IFNλ, which have proven effective in clinical trials.[35,36,61-63] However, in future studies involving the development of antivirals, human organoid systems could provide a more robust environment for pre-clinical testing, which provides detailed insights into drug delivery, especially in the gas exchange region of the lung that is oftentimes associated with life threatening disease.

Remdesivir, an approved nucleoside analog inhibitor effective at blocking SARS-CoV-2 infection in human patients, was first tested in primary lung cell cultures, such as large airway cultures, followed by robust mouse models of human disease.[64] A strength of these early studies were applications to show that broad antiviral activity exists against an array of unique coronaviruses that could replicate efficiently in primary cells, supporting its application as a potentially effective antiviral against a newly emerged coronavirus. In animal models, the drug was effective, although efficacy waned in concert with reducing virus titers later in infection. Similar findings became evident in human trials. Upon successful development and approval of remdesivir to inhibit infection, it became widely used as a control for testing novel prophylactic and therapeutic treatments.[64] Interestingly, this drug was also utilized as confirmation of the development of human tonsillar epithelial organoids as an ex vivo model to study viral infections, such as SARS-CoV-2.[65] It was found that remdesivir could successfully decrease viral RNA in these organoid cultures in a dose-dependent manner, indicating that these novel type organoids could be used as a preclinical and translational research platform for testing of future clinical interventions.

Similarly, molnupiravir is a second anti-viral authorized by the FDA for treatment against SARS-CoV-2 and causes error catastrophe following treatment of virally infected cells.[37] This orally available antiviral effectively inhibited SARS-CoV-2 replication in human airway epithelial cultures, as was seen with remdesivir.[37] The broad-spectrum activity of this ribonucleoside analog successfully inhibited all three emergent epidemic coronaviruses, SARS-CoV, MERS-CoV, and SARS-CoV-2, when administered both prophylactically and therapeutically.[37] Moreover, molnupiravir was also tested in human organoid models, in conjunction with an in vivo mouse model to demonstrate the efficacy in complex human lung tissue, as well as robust SARS-CoV, SARS-CoV-2, and MERS-CoV mouse models of human disease.[66] Later, immunodeficient mice were implanted with human lung tissue in the form of organoids and again infected with all three emergent coronaviruses, as well as two SARS-like bat coronaviruses that have yet to infect human hosts but remain poised for potential zoonosis. These human lung organoids demonstrated robust infection of AT2 cells and ciliated airway cells but effectively blocked or reduced infection when treated prophylactically or therapeutically with molnupiravir, respectively.[66] Molnupiravir is a prime example of the utilization of human respiratory organoids to further demonstrate the efficacy of clinical interventions in human lung tissue, following 2D models, and the ability to use this data to propagate potential interventions into human clinical trials. It has been approved for use in some human patients.

Lastly, pegylated type III interferon, or peg-IFN-λ1, has recently completed final phase III clinical trials for the inhibition of SARS-CoV-2 in human patients.[67] Similarly, it has been suggested that peg-IFN-λ1 can potently block replication in human airway cells, both prophylactically and therapeutically, as also demonstrated in the molnupiravir studies. Studies with peg-IFN-λ1 would largely benefit from testing in human organoid models as the type III interferon receptors are largely found in epithelial cells, including the cells that compromise the lung epithelium prominent in lung organoids models.[62] As seen with the previous pre-clinical and clinical candidates, in vitro testing of peg-IFN-λ1a was confirmed by successful inhibition of infection in vivo in small animal models.[62]

Additionally, peg-IFN-λ1a was demonstrated to block SARS-CoV replication in human primary cells, a finding that could be further studied in human lung organoids and have important potential to successfully block future emergent or zoonotic coronaviruses. Importantly, all of these antiviral drugs work well if delivered early in infection during peak rises in virus replication and spread. After acute virus infection (eg, day 7), direct acting antivirals and therapeutic antibodies provide little benefit as pathogenic host responses and immunopathologic phases of disease do not depend on significant levels of virus replication and spread.

FUTURE DIRECTIONS

In recent years, much progress has been made in developing in vitro primary airway and alveolar culture models. Organoids established using epithelial cells isolated from different regions of the respiratory tract provide a valuable platform for unraveling SARS-CoV-2 virus infection, replication kinetics, and tropism. Although several studies demonstrated many applications of human organoids, the current models lack the complexity and do not fully represent the adult human lung. There is an urgent need to develop human-relevant complex organoid models, incorporating cellular components including endothelial, immune, and mesenchymal cells that closely recapitulate the niche of infected epithelial cells found in human COVID-19 lungs. This approach will provide revolutionary new perspectives for better understanding of SARS-CoV-2 viral infection and cellular responses.

Present studies primarily focused on studying viral entry, replication, and host cell responses. More studies are needed to fully understand the molecular mechanisms and critical factors that control highly pathogenic respiratory tract infections, including SARS-CoV-2 infection and the long-term chronic disease outcomes associated with COVID-19 infection in the upper and lower respiratory tract[68]. Future studies will also need to address how various host factors such as age, sex, and genetic variation influences the potential of stem cells in the airway and alveoli following SARS-CoV-2 infection. Organoid models coupled with genetic perturbations to simulate genetic variants identified in GWAS will be valuable in determining genetic factors that modulate infection efficiency, viral propagation, and immune invasion.

Finally, more research is needed to explore further development and validation of effective antiviral therapeutics for COVID-19 treatment. Recent studies in COVID-19 infected patients and organoid models have shown that, following viral infection, alveolar AT2 cells activate aberrant pathways including interferon signaling and undergo apoptosis. Studies have also shown that AT2s acquire a transitional cell state previously implicated in idiopathic pulmonary fibrosis was also observed in lung specimens collected from COVID-19 autopsies.[68-70] Therefore, there is a need to identify novel therapeutic targets that preserve AT2 cells function and enable them to fully restore the alveolar structure without undergoing transitional state. Organoid platforms that phenocopy the chronic disease states often initiated by viral infections offer novel opportunities for new drug discovery and disease control, especially after virus clearance. With the advent of scalable organoid culture models now it is possible to perform high-throughput genetic and pharmacological screens to identify new therapeutics against COVID-19 and restore lung structure and function.

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REFERENCES

1. Bleier BS, Ramanathan M, Lane AP. COVID-19 vaccines may not prevent nasal SARS-CoV-2 infection and asymptomatic transmission. Otolaryngol Head Neck Surg 2021;164:305–7. https://doi.org/10.1177/0194599820982633.
2. Kissling E, Valenciano M, Larrauri A, et al. Low and decreasing vaccine effectiveness against influenza A(H3) in 2011/12 among vaccination target groups in Europe: results from the I-MOVE multicentre case-control study. Euro Surv 2013;18. https://doi.org/10.2807/ese.18.05.20390-en.
3. Background and epidemiology. Available at: https://www.cdc.gov/flu/professionals/acip/background-epidemiology.htm Accessed April 1, 2022.
4. Estimated flu-related illnesses, medical visits, hospitalizations, and deaths in the United States — 2019−2020 Flu Season. CDC. Available at: https://www.cdc.gov/flu/about/burden/2019-2020.html Accessed March 29, 2022.
5. Krammer F, Smith GJD, Fouchier RAM, et al. Influenza. Nat Rev Dis Primers 2018;4:3. https://doi.org/10.1038/s41572-018-0002-y.
6. Martins de Camargo M, Caetano AR. Ferreira de Miranda Santos IK. Evolutionary pressures rendered by animal husbandry practices for avian influenza viruses to adapt to humans. iScience 2022;25:104005. https://doi.org/10.1016/J.ISCI.2022.104005.

7. RSV trends and surveillance. CDC 2022:Available at: https://www.cdc.gov/rsv/research/us-surveillance.htmlAccessed March 29, 2022.
8. Clinical overview of human parainfluenza viruses (HPIVs). CDC 2022:Available at: https://www.cdc.gov/parainfluenza/hcp/clinical.htmlAccessed March 29, 2022.
9. Schuster JE, Williams JV. Human metapneumovirus. Microbiol Spectr 2014;2. https://doi.org/10.1128/microbiolspec.AID-0020-2014.
10. Pierangeli A, Scagnolari C, Antonelli G. Respiratory syncytial virus. Minerva Pediatr 2018;70:553–65. https://doi.org/10.23736/S0026-4946.18.05312-4.
11. Simões EAF, Center KJ, Tita ATN, et al. Prefusion F protein-based respiratory syncytial virus immunization in pregnancy. N Engl J Med 2022;386:1615–26. https://doi.org/10.1056/NEJMoa2106062.
12. Hammitt LL, Dagan R, Yuan Y, et al. Nirsevimab for prevention of RSV in healthy late-preterm and term infants. N Engl J Med 2022;386:837–46. https://doi.org/10.1056/NEJMoa2110275.
13. Islam A, Ferdous J, Islam S, et al. Evolutionary dynamics and epidemiology of endemic and emerging coronaviruses in humans, domestic animals, and wildlife. Viruses 2021;13. https://doi.org/10.3390/V13101908.
14. Mulabbi EN, Tweyongyere R, Byarugaba DK. The history of the emergence and transmission of human coronaviruses. Onderstepoort J Vet Res 2021;88:1–8. https://doi.org/10.4102/OJVR.V88I1.1872.
15. Huynh J, Li S, Yount B, et al. Evidence supporting a zoonotic origin of human coronavirus strain NL63. J Virol 2012;86:12816–25. https://doi.org/10.1128/JVI.00906-12.
16. Patrick DM, Petric M, Skowronski DM, et al. An outbreak of human coronavirus OC43 infection and serological cross-reactivity with SARS coronavirus. Canad J Infect Dis Med Microbiol 2006;17:330–6. https://doi.org/10.1155/2006/152612.
17. Abdelrahman Z, Li M, Wang X. Comparative review of SARS-CoV-2, SARS-CoV, MERS-CoV, and influenza A respiratory viruses. Front Immunol 2020;11. https://doi.org/10.3389/fimmu.2020.552909.
18. Kirtipal N, Bharadwaj S, Kang SG. From SARS to SARS-CoV-2, insights on structure, pathogenicity and immunity aspects of pandemic human coronaviruses. Infect Genet Evol 2020;85. https://doi.org/10.1016/J.MEEGID.2020.104502.
19. COVID-19 Map - Johns Hopkins Coronavirus Resource Center. Available at: https://coronavirus.jhu.edu/map.html. Accessed April 19, 2022
20. Pustake M, Tambolkar I, Giri P, Gandhi C. SARS, MERS and CoVID-19: An overview and comparison of clinical, laboratory and radiological features. J Family Med Prim Care 2022;11:10. https://doi.org/10.4103/JFMPC.JFMPC_839_21.
21. Legrand M, Bell S, Forni L, et al. Pathophysiology of COVID-19-associated acute kidney injury. Nat Rev Nephrol 2021 17:11 2021;17:751–64. https://doi.org/10.1038/s41581-021-00452-0.
22. Tracking SARS-CoV-2 variants Available at: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/. Accessed March 31, 2022.
23. Koelle K, Martin MA, Antia R, Lopman B, Dean NE. The changing epidemiology of SARS-CoV-2. Science (1979) 2022;375:1116–21. https://doi.org/10.1126/SCIENCE.ABM4915.
24. DeGrace MM, Ghedin E, Frieman MB, et al. Defining the risk of SARS-CoV-2 variants on immune protection. Nature 2022;31. https://doi.org/10.1038/s41586-022-04690-5 Published online March.
25. Muus C, Luecken MD, Eraslan G, et al. Single-cell meta-analysis of SARS-CoV-2 entry genes across tissues and demographics. Nat Med 2021;27:546–59. https://doi.org/10.1038/s41591-020-01227-z.
26. Deprez M, Zaragosi LE, Truchi M, et al. A single-cell atlas of the human healthy airways. Am J Respir Crit Care Med 2020;202:1636–45. https://doi.org/10.1164/RCCM.201911-2199OC.
27. Sungnak W, Huang N, Bécavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Na Med 2020;26:681–7. https://doi.org/10.1038/s41591-020-0868-6.
28. Hou YJ, Okuda K, Edwards CE, et al. SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. Cell 2020;182:429. https://doi.org/10.1016/J.CELL.2020.05.042.
29. Xu J, Xu X, Jiang L, Dua K, Hansbro PM, Liu G. SARS-CoV-2 induces transcriptional signatures in human lung epithelial cells that promote lung fibrosis. Respir Res 2020;21:1–12. https://doi.org/10.1186/S12931-020-01445-6/FIGURES/7.
30. Scobey T, Yount BL, Sims AC, et al. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. Proc Natl Acad Sci U S A 2013;110:16157–62. https://doi.org/10.1073/PNAS.1311542110/SUPPL_FILE/PNAS.201311542SI.PDF.
31. Sims AC, Mitchell HD, Gralinski LE, et al. Unfolded protein response inhibition reduces middle east respiratory syndrome coronavirus-induced acute lung injury. mBio 2021;12. https://doi.org/10.1128/MBIO.01572-21.
32. Niemi MEK, Karjalainen J, Liao RG, et al. Mapping the human genetic architecture of COVID-19. Nature 2021;600:472–7. https://doi.org/10.1038/s41586-021-03767-x.
33. Ji JJ, Fan J. Discovering myeloid cell heterogeneity in the lung by means of next generation sequencing. Mil Med Res 2019;6:1–10. https://doi.org/10.1186/S40779-019-0222-9/FIGURES/1.
34. Schäfer A, Martinez DR, Won JJ, et al. Therapeutic treatment with an oral prodrug of the remdesivir parental nucleoside is protective against SARS-CoV-2 pathogenesis in mice. Sci Transl Med 2022;22. https://doi.org/10.1126/SCITRANSLMED.ABM3410 Published online March.
35. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun 2020;11. https://doi.org/10.1038/S41467-019-13940-6.
36. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 2017;9. https://doi.org/10.1126/SCITRANSLMED.AAL3653.
37. Sheahan TP, Sims AC, Zhou S, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med 2020;12. https://doi.org/10.1126/SCITRANSLMED.ABB5883.
38. Fulcher ML, Randell SH. Human nasal and tracheo-bronchial respiratory epithelial cell culture. Methods Mol Biol 2013;945:109–21. https://doi.org/10.1007/978-1-62703-125-7_8.
39. Fulcher ML, Gabriel S, Burns KA, Yankaskas JR, Randell SH. Well-differentiated human airway epithelial cell cultures. Methods Mol Med. 2005;107:183–206. https://doi.org/10.1385/1-59259-861-7:183.
40. Konishi S, Gotoh S, Tateishi K, et al. Directed induction of functional multi-ciliated cells in proximal airway epithelial spheroids from human pluripotent stem cells. Stem Cell Reports 2016;6:18–25. https://doi.org/10.1016/J.STEMCR.2015.11.010.
41. Gotoh S, Ito I, Nagasaki T, et al. Generation of alveolar epithelial spheroids via isolated progenitor cells from human

pluripotent stem cells. Stem Cell Reports 2014;3:394–403. https://doi.org/10.1016/J.STEMCR.2014.07.005.
42. Tian L, Gao J, Garcia IM, Chen HJ, Castaldi A, Chen Y. Human pluripotent stem cell-derived lung organoids: potential applications in development and disease modeling. WIREs Devel Biol 2021;10. https://doi.org/10.1002/wdev.399.
43. Porotto M, Ferren M, Chen YW, et al. Authentic modeling of human respiratory virus infection in human pluripotent stem cell-derived lung organoids. mBio 2019;10. https://doi.org/10.1128/mBio.00723-19.
44. Hsia GSP, Esposito J, da Rocha LA, Ramos SLG, Okamoto OK. Clinical application of human induced pluripotent stem cell-derived organoids as an alternative to organ transplantation. Stem Cells Int 2021:2021. https://doi.org/10.1155/2021/6632160.
45. Li Y, Wu Q, Sun X, Shen J, Chen H. Organoids as a powerful model for respiratory diseases. Stem Cells Int 2020;2020:1–8. https://doi.org/10.1155/2020/5847876.
46. Wang T, Zhang N, Fan S, et al. Establishment of human distal lung organoids for SARS-CoV-2 infection. Cell Discovery 2021 7:1 2021;7:1–4. https://doi.org/10.1038/s41421-021-00346-2.
47. Katsura H, Sontake V, Tata A, et al. Human lung stem cell-based alveolospheres provide insights into SARS-CoV-2-mediated interferon responses and pneumocyte dysfunction. Cell Stem Cell 2020;27:890–904. https://doi.org/10.1016/J.STEM.2020.10.005 e8.
48. Lamers MM, Vaart J, Knoops K, et al. An organoid-derived bronchioalveolar model for SARS-CoV-2 infection of human alveolar type II-like cells. EMBO J 2021;40. https://doi.org/10.15252/embj.2020105912.
49. McQualter JL, Yuen K, Williams B, Bertoncello I. Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. Proc Natl Acad Sci U S A 2010;107:1414–9. https://doi.org/10.1073/PNAS.0909207107.
50. Barkauskas CE, Chung MI, Fioret B, Gao X, Katsura H, Hogan BLM. Lung organoids: current uses and future promise. Development 2017;144:986–97. https://doi.org/10.1242/DEV.140103.
51. Salahudeen AA, Choi SS, Rustagi A, et al. Progenitor identification and SARS-CoV-2 infection in human distal lung organoids. Nature 2020;588:670–5. https://doi.org/10.1038/S41586-020-3014-1.
52. Zhang H, Rostami MR, Leopold PL, et al. Expression of the SARS-CoV-2 ACE2 receptor in the human airway epithelium. Am J Respir Crit Care Med 2020;202:219–29. https://doi.org/10.1164/RCCM.202003-0541OC/SUPPL_FILE/DISCLOSURES.PDF.
53. Ibricevic A, Pekosz A, Walter MJ, et al. Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. J Virol 2006;80:7469. https://doi.org/10.1128/JVI.02677-05.
54. Meyerholz DK, Lambertz AM, McCray PB. Dipeptidyl peptidase 4 distribution in the human respiratory tract: implications for the middle east respiratory syndrome. Am J Pathol 2016;186:78. https://doi.org/10.1016/J.AJPATH.2015.09.014.
55. Thompson AJ, Paulson JC. Adaptation of influenza viruses to human airway receptors. J Biol Chem 2021;296:100017. https://doi.org/10.1074/JBC.REV120.013309.
56. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020;12:1–5. https://doi.org/10.1038/s41368-020-0074-x.
57. Ekanger CT, Zhou F, Bohan D, et al. Human organotypic airway and lung organoid cells of bronchiolar and alveolar differentiation are permissive to infection by influenza and SARS-COV-2 respiratory virus. Front Cell Infect Microbiol 2022;12:208. https://doi.org/10.3389/FCIMB.2022.841447/BIBTEX.
58. Chen Q, Langenbach S, Li M, et al. ACE2 expression in organotypic human airway epithelial cultures and airway biopsies. Front Pharmacol 2022;13:440. https://doi.org/10.3389/FPHAR.2022.813087/BIBTEX.
59. Jia HP, Look DC, Shi L, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. J Virol 2005;79:14614. https://doi.org/10.1128/JVI.79.23.14614-14621.2005.
60. Zhang T, Tong X, Zhang S, et al. The roles of dipeptidyl peptidase 4 (DPP4) and DPP4 inhibitors in different lung diseases: new evidence. Front Pharmacol 2021;12:3657. https://doi.org/10.3389/FPHAR.2021.731453/BIBTEX.
61. Fischer WA, Eron JJ, Holman W, et al. A phase 2a clinical trial of molnupiravir in patients with COVID-19 shows accelerated SARS-CoV-2 RNA clearance and elimination of infectious virus. Sci Transl Med 2022;14. https://doi.org/10.1126/SCITRANSLMED.ABL7430.
62. Dinnon KH, Leist SR, Schäfer A, et al. A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. Nature 2020;586:560–6. https://doi.org/10.1038/S41586-020-2708-8.
63. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of covid-19 — final report. N Engl J Med 2020;383:1813–26. https://doi.org/10.1056/NEJMOA2007764/SUPPL_FILE/NEJMOA2007764_DATA-SHARING.PDF.
64. Pruijssers AJ, George AS, Schäfer A, et al. Remdesivir inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice. Cell Rep 2020;32. https://doi.org/10.1016/J.CELREP.2020.107940.
65. Kim HK, Kim H, Lee MK, et al. Generation of human tonsil epithelial organoids as an ex vivo model for SARS-CoV-2 infection. Biomaterials 2022;283:121460. https://doi.org/10.1016/J.BIOMATERIALS.2022.121460.
66. Wahl A, Gralinski LE, Johnson CE, et al. SARS-CoV-2 infection is effectively treated and prevented by EIDD-2801. Nature 2021;591:451–7. https://doi.org/10.1038/S41586-021-03312-W.
67. Eiger's single-dose peginterferon lambda for COVID-19 reduced risk of hospitalization or ER visits by 50% in a predominantly vaccinated population in phase 3 TOGETHER study. Eiger BioPharmaceuticals 2022:Available at: https://ir.eigerbio.com/news-releases/news-release-details/eigers-single-dose-peginterferon-lambda-covid-19-reduced-riskAccessed April 28, 2022.
68. Dinnon KH, Leist SR, Okuda K, et al. A model of persistent post SARS-CoV-2 induced lung disease for target identification and testing of therapeutic strategies. bioRxiv 2022;15. https://doi.org/10.1101/2022.02.15.480515 Published online February.
69. Lechowicz K, Drożdżal S, Machaj F, et al. COVID-19: the potential treatment of pulmonary fibrosis associated with SARS-CoV-2 infection. J Clin Med 2020;9:1–20. https://doi.org/10.3390/JCM9061917.
70. Ravaglia C, Doglioni C, Chilosi M, et al. Clinical, radiological, and pathological findings in patients with persistent lung disease following SARS-CoV-2 infection. Eur Respir J 2022;17:2102411. https://doi.org/10.1183/13993003.02411-2021 Published online March.