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Review

RdRp inhibitors and COVID-19: Is molnupiravir a good option?

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ABSTRACT

Rapid changes in the viral genome allow viruses to evade threats posed by the host immune response or antiviral drugs, and can lead to viral persistence in the host cells. RNA-dependent RNA polymerase (RdRp) is an essential enzyme in RNA viruses, which is involved in RNA synthesis through the formation of phosphodiester bonds. Therefore, in RNA viral infections such as SARS-CoV-2, RdRp could be a crucial therapeutic target. The present review discusses the promising application of RdRp inhibitors, previously approved or currently being tested in human clinical trials, in the treatment of RNA virus infections. Nucleoside inhibitors (NIs) bind to the active site of RdRp, while nonnucleoside inhibitors (NNIs) bind to allosteric sites. Given the absence of highly effective drugs for the treatment of COVID-19, the discovery of an efficient treatment for this pandemic is an urgent concern for researchers around the world. We review the evidence for molnupiravir (MK-4482, EIDD-2801), an antiviral drug originally designed for Alphavirus infections, as a potential preventive and therapeutic agent for the management of COVID-19. At the beginning of this pandemic, molnupiravir was in preclinical development for seasonal influenza. When COVID-19 spread dramatically, the timeline for development was accelerated to focus on the treatment of this pandemic. Real time consultation with regulators took place to expedite this program. We summarize the therapeutic potential of RdRp inhibitors, and highlight molnupiravir as a new small molecule drug for COVID-19 treatment.

1. Introduction

Three novel coronaviruses have emerged as human infectious pathogens during the last twenty years or so [1,2]. As evidenced by the emergence of SARS-CoV-2 and SARS-CoV, group 2b coronaviruses will continue to pose a health-threat in the future. The SARS-like CoV family from bats are zoonotic viruses able to grow in human airway cells, by recognizing angiotensin-converting enzyme 2 (ACE2) receptors, and show variations in vaccine gene sequence as well as therapeutic targets [3–7]. Therefore, to tackle the present COVID-19 global health emergency and to enhance preparedness for future pandemics, the discovery of drugs and vaccines that are more effective against high-risk RNA viruses, is required.

Small-molecule antiviral drugs can work via several pathways, such as interfering with a replication-related host factor, inhibiting the production of new viral particles, suppressing a virally encoded enzyme, or blocking viral entry into host cells [8]. Currently, many antiviral drugs are being evaluated in human clinical trials for the management of COVID-19, including ritonavir/lopinavir, remdesivir, and hydroxychloroquine [9–11].

When SARS-CoV-2 emerged as a global pandemic, molnupiravir was being investigated for seasonal influenza treatment under an IND application. Because of the evidence of activity in ferret influenza models [12–14], and favorable ADMET (absorption, distribution,
metabolism, excretion, and toxicity) results in lung tissue in several animal models, the path to drug development of molnupiravir was facilitated. Consultations with funding organizations, including NIAID (National Institute for Allergy and Infectious Disease) and DTRA (Defense Threat Reduction Agency) supported the development of molnupiravir (EIDD-2801). Development for the treatment and prophylaxis of aerosolized Venezuelan equine encephalitis virus (VEEV) was investigated in animal models. Existing in vivo and in vitro evidence suggested the effectiveness of EIDD-2801 (and its active metabolite EIDD-1931) against SARS, MERS and other human coronaviruses [15]. Therefore it was hypothesized that EIDD-2801 would also be useful for prophylaxis and treatment of infections caused by SARS-CoV-2. Herein, we summarize the therapeutic potential of RdRp inhibitors and highlight molnupiravir as a new small molecule drug for COVID-19 therapy.

2. Pathogenesis of COVID-19

To understand the pathogenic mechanisms and treatment targets of SARS-CoV-2, we need to review some aspects, such as the viral replication cycle, structure, and genome. The structure of a positive-stranded RNA CoV virion consists of an envelope and a nucleocapsid [16]. A SARS-CoV-2 virion possesses a 29.9-kb +ssRNA genome with a diameter of 50–200 nm [17]. It is the largest recognized RNA virus with a 3′-polynucleotide and 5′-cap structure, having fourteen open reading frames (ORFs) encoding 27 separate proteins [18,19]. The structural proteins of the virion, include spike (S), envelope (E), nucleocapsid (N) and membrane (M). The RNA genome and the N protein are surrounded by the M, S and E proteins [20]. The virus-ACE2 receptor binding is facilitated by the S protein, which allows the virus to fuse with the host cell membrane [21]. SARS-CoV-2 utilizes the host transmembrane serine protease 2 (TMPSRSS2) to modify the S protein in order to target the cells after binding to ACE2 [21] (Fig. 1). To carry out the virion-receptor binding leading to membrane fusion, the S protein of SARS-CoV-2 has two subunits; receptor-binding S1 and fusion protein S2. Cellular proteases cleave the spike protein at the cleavage site of S1-S2 to allow the attachment of the virus to the receptor and fusion with the cell membrane (Fig. 1). Analysis of the SARS-CoV-2 S protein showed a S1/S2 site insertion, which is absent in other strains of SARS-CoV [22]. It seems that, for effective spreading throughout the human host, as well as simple cell infection, this specific integration provides a gain-of-function alteration.

The viral RNA employs the host cell machinery to begin the synthesis of polypeptides and the replication of the viral genome, as well as creating the transcription-replication complex required to produce sub-genomic RNAs and structural proteins (nucleocapsid, envelope) (Fig. 1). The envelope plays an essential role in the release, assembly and pathogenesis of the virus [23]. The precise roles of several viral peptidases have not yet been identified. Further studies are required to determine how the structural characteristics of SARS-CoV-2 affect the different pathogenic pathways. The symptoms of SARS-CoV-2 to some degree resemble those of the common cold, such as shortness of breath, coughing, and fever [24]; however, the infection may also result in SARS, multi-organ failure, and pneumonia, culminating in death in patients with co-existing conditions or advanced age (Fig. 2) [25]. In comparison with children and younger adults (6%), people aged over 60 years and those suffering from underlying conditions are more susceptible to the severe stage of COVID-19 (18.5%) [26]. The clinical information on deceased patients showed that the most frequent comorbidities of COVID-19 were diabetes mellitus (16.2–35%) and hypertension (24–75%) [24,27]. Of note, in COVID-19 patients treated with ACE inhibitors, more common comorbidities were reported [27,28]. The host cell-SARS-CoV-2 binding occurs via ACE2 receptor expressed on epithelial cells in the lungs, brain, kidney, intestines, and blood vessel endothelial cells [29]. ACE2 expression is significantly higher in hypertensive and diabetic patients treated with ACE inhibitors or angiotensin receptor blockers (ARBs) [29], thus increasing the overall severity of SARS-CoV-2.

The first pathological report on the severe form of COVID-19 showed that diffuse alveolar damage on both sides of the lungs led to the development of cellular fibromyxoid exudates [30]. The right lung showed hyaline membrane formation and lung cell shedding, suggesting ARDS (acute respiratory distress syndrome). The left lung revealed the formation of hyaline membrane as well as pulmonary edema, which implied early ARDS. In both lungs, interstitial mononuclear inflammatory infiltrates were also detected. Another investigation demonstrated that, during the progression of COVID-19, proteinuria and acute kidney injury could also occur. It was observed that ACE2 was higher in patients with COVID-19, and positive SARS-CoV nucleoprotein immunostaining was found in kidney tubules using a specific antibody [31]. In addition, limited interstitial mononuclear inflammatory infiltrates in the heart tissue were detected, indicating that cardiac impairment is not directly induced by this virus [30]. Aside from ARDS, during the infection process, inflammatory reactions were also triggered, leading to unrestrained inflammation in the lung. Because of cell damage and rapid viral replication, along with the virus-mediated downregulation of ACE2 and antibody-SARS-CoV-2 binding, these processes might result in excessive inflammatory responses [32]. The initial stages of rapid viral replication can cause endothelial and epithelial cell death, leading to the secretion of proinflammatory cytokines and chemokines (Fig. 3) [33]. Recently, an investigation evaluated the transcriptional responses caused by SARS-CoV-2 compared to other viral respiratory infections, to identify the transcriptional features responsible for the COVID-19 pathogenic effects [34]. They showed that SARS-CoV-2 affected the transcription of many genes. Despite viral replication, strong responses of IFN-1 and IFN-III were not triggered by SARS-CoV-2, however the increased level of chemokines needed for recruitment of effector cells was detected. This inflammatory response was characterized by low levels of IFN-1 and IFN-III in addition to increased chemokines and high levels of IL-6. Lowered innate antiviral defense plus increased inflammatory cytokine secretion is probably the driving force behind COVID-19 progression [34]. Furthermore, because the weakened immune response contributes to more replication of the virus, this can explain why the more severe forms of COVID-19 are commonly found in individuals suffering from comorbidities [35].

3. RdRp structure and function, and approved anti-RdRp drugs

A complex mechanism governs the replication of the viral genome involving multiple host and viral factors. When the virus has been introduced into the host cell, after uncoating, the virus begins to replicate, and subsequently to translate the viral proteins. Completion of these processes occurs in the cytoplasm for the majority of viruses via an RNA-dependent RNA polymerase (RdRp). There are some exceptions, one of the most important of which is the influenza virus. The RdRp is encoded as a multivalent enzyme by all members of the RNA virus family (except family Retroviridae), and acts as a nucleotidyl transferase (EC 2.7.7.48) [36]. RdRp produces nascent RNA strands by appending ribonucleotides to the 3′-hydroxyl end, so it synthesizes RNA molecules in a 5′-3′ direction. Factors required for RdRp activity are one RNA template, two magnesium ions at the active site required for phosphodiester bond formation, ribonucleoside 5′-triphosphates (ATP, CTP, UTP and GTP) that are the constituents of nascent RNA. The RdRp structure contains two channels that connect with each other at the active site, including the primary channel to position the RNA template, and the secondary channel to position the input nucleoside triphosphate (NTP) [37–39].

Recently researchers have turned to studying RdRps as a promising therapeutic target against viral infections for multiple reasons: (1) the critical function of this enzyme in replication of the majority of RNA viruses; (2) the enzyme has been conserved during the evolution of such viruses [40]; (3) lack of any homologous counterpart of this enzyme in mammalian cells; (4) availability of comprehensive information on the
Fig. 1. Schematic representation of the structure and genome of coronavirus (A) and the replication cycle of SARS-CoV-2 (B). The structural proteins of the virus include proteins S, E, M, and N. Coronaviruses are approximately 100–160 nm in diameter, and enveloped with a lipid bilayer. This virus family has a large genome (positive-sense single-stranded RNA about 30 Kb length) that is bound to the nucleocapsid. The S proteins consist of subunits S1 and S2 and also TMPRSS2 and furin-related cleavage locations. S proteins are responsible for host cell-virus attachment following TMPRSS2-mediated activation. The S protein structure in the pre-fusion conformation, and genome plus C-terminal domain crystal structure of S protein combined with human ACE2 are shown in (A). The stages of coronavirus life cycle include (B) attachment and penetration (1), uncoating (2), gene expression (3 and 5–6) and replication (4), assembly (7–8), and release (9).
structure and function of this enzyme; (5) easy development and subsequent access to rapid biochemical screening using large libraries of compounds. On the other hand, there may be limitations to the use of RdRp inhibitors due to the emergence of drug-resistant variants of this enzyme, which highlights the potential need for other drugs to be used in combination approaches. Some successes in the field of RdRp inhibitors include, sofosbuvir, and remdesivir. Clinically approved sofosbuvir is prescribed to treat hepatitis C virus (HCV) infection. Remdesivir was first developed to treat Ebola virus infection, and was recently introduced as a treatment for hospitalized patients with severe COVID-19 [41]. Table 1 summarizes the investigational drugs in clinical trials, and antiviral drug approved by the US Food and Drug Administration (FDA), which target viral RdRP.
was orally administrated (for one week, beginning one hour after the
study, favipiravir was used as a post-exposure prophylactic agent, and
reportedly decreased the rate of virus positivity in the brain. In addition, in
terms of post-exposure prophylactic efficacy, the agent showed similar
efficacy to equine rabies virus immunoglobulin. It was concluded that, as
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4. Antiviral drugs for COVID-19 treatment
4.1. RdRp inhibitors
RNA-dependent RNA polymerase (RdRp) is a conserved enzymes in RNA viruses, with a crucial function in RNA virus replication [48–51].
Because of the conserved protein structure of RdRp, its inhibitors all
show various degrees of antiviral activity [52–55]. When compared to
coronaviruses, the RdRp protein of SARS-CoV-2 reveals an identical
binding site with the same amino acid residues [39]. For instance, the
structural similarity of RdRp in SARS-CoV-2 and SARS-CoV is 96%.
Therefore, available potent compounds that inhibit RdRp have been
extensively studied for the treatment of SARS-CoV-2.

4.2. Favipiravir
Favipiravir undergoes phosphoribosylation of the molecule within
the tissue to produce the active form of the drug, favipiravir-RTP. The
antiviral activity of favipiravir occurs by the following mechanisms.
Firstly, favipiravir can serve as a RdRp substrate, because the enzyme
recognizes it as a purine [56], therefore resulting in termination of
protein synthesis in the virus (Figure 4). Secondly, favipiravir can be
integrated into the viral RNA sequence thus preventing further repli-
cation [57]. These mechanisms of action, along with the conservation of
the RdRp enzyme catalytic domain across different RNA viruses, ex-
plains the broad activity of this drug. Recently, favipiravir has been
reported to trigger deadly mutagenesis during influenza virus replica-
tion in vitro, making it a virucidal agent [58]. Whether similar activity
occurs against SARS-CoV-2 or not is unclear. Yamada and colleagues investigated the ability of favipiravir to
prevent SS-RNA virus replication under in vivo conditions [59]. In their
study, favipiravir was used as a post-exposure prophylactic agent, and
was orally administrated (for one week, beginning one hour after the
inoculation of virus) in rabies virus-infected mice. Favipiravir consid-
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VHFVs: Viral hemorrhagic fevers, RSV: Respiratory syncytial virus, HCV: hepatitis C virus,

| Anti-RdRp drug | Approval Date or clinical status | Approved clinical used | Mechanism | Ref |
|---------------|---------------------------------|------------------------|-----------|-----|
| Ribavirin     | Approved in 1985                | RSV, VHF, HCV          | Inhibits viral RNA polymerase and mRNA capping | [42] |
| Favipiravir   | Approved in 2014 and randomized trial for SARS-CoV-2 | Influenza A, influenza B and influenza C | Binds to catalytic site of viral RdRp and prevents incorporation of Nts for viral genome replication | [43, 44] |
| Baloxavir     | Approved in 2018                | Influenza A and influenza B | Binds to cap-dependent endonuclease in the PA subunit of the viral RdRp | [45] |
| Sofosbuvir    | Approved in 2013                | Genotype-2 or – 3 HCV  | Inhibits HCV replication by binding to Mg2+ ions of viral RdRp | [46] |
| Remdesivir    | Emergency use Authorization for SARS-CoV-2 in 2020 | SARS-CoV-2 | Competes with ATP and Nts for incorporation by viral RdRp | [47] |
| Dasabuvir     | Approved in 2014 for use in combination with ritonavir/ombitasvir/paritaprevir | Genotype-1 of HCV | Non-nucleoside RdRp inhibitor which binds to the palm domain of HCV RdRp and induces a conformational change | [48] |

4.3. Remdesivir
Remdesivir is a RdRp inhibitor that has been tested against corona-
viruses, which was initially introduced for the treatment of Ebola virus.
According to one experimental study, early administration of remdesivir
considerably decreased pulmonary damage and the viral load in SARS-
CoV-2-infected monkeys [67–69]. Remdesivir interacts with the RdRp enzyme of the virus to induce delayed chain termination. It was shown to be active against several coronaviruses in vitro (e.g. MERS, SARS, bat-CoVs, contemporary human CoVs) [68]. Remdesivir is active against model β-coronavirus SARS, MERS, and murine hepatitis virus (MHV), suggesting a pan-CoV RdRp activity, even in the presence of proofreading exonuclease activity [70]. The delayed chain-termination is the main mechanism of ac-
tion, based on biochemical findings using recombinant RdRp in respiratory syncytial virus [71–73]. Remdesivir suppresses viral repli-
cation (RSV and Ebola) in cellular experiments with an IC50 of 100 nM, while RNA polymerase II (Pol II) and mitochondrial RNAP in humans
were not inhibited in the laboratory [71], showing about 500-fold

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Therefore, available potent compounds that inhibit RdRp have been
extensively studied for the treatment of SARS-CoV-2.
selectivity. The fact that nucleoside analogues are weak substrates for human polymerases, partly explains this selectivity [58]. In comparison with natural nucleotide pools, the triphosphate nucleotide analog, remdesivir was incorporated at high levels [74], probably explaining the potent antiviral performance by termination of premature RNA production.

After the findings of the NIAID and SIMPLE investigations, on May 1, 2020, the FDA approved remdesivir for use in hospitalized patients with severe COVID-19 under an Emergency Use Authorization (EUA). On August 28, 2020, the license was re-issued allowing the use of this drug in patients with non-severe COVID-19. On October 22, 2020, remdesivir became the first FDA-approved drug for management of the COVID-19 pandemic [75,76]. Findings from the Spinner et al. trial, SIMPLE, and NIAID all supported the final approval [77–79].

Shortly after the Chinese reports, the first COVID-19 patients appeared in the USA. One patient reported to urgent care in Snohomish County, Washington (January 20, 2020) with a 4-day history of cough and subjective fever, later to be established as the first positive COVID-19 patient in the USA [80]. On the seventh day of hospitalization and due to the worsening clinical condition, IV remdesivir was administered to the patient and no adverse effects were seen after infusion [80]. The next day, the clinical status improved, but any direct attribution to remdesivir was confounded by concurrent therapy, with supplemental oxygen, cefepime, vancomycin, guaifenesin, ibuprofen, and acetaminophen.

Of twelve cases diagnosed with definite SARS-CoV-2 infection, seven were hospitalized, and after worsening in COVID-19 severity, three were administered remdesivir. The duration of therapy was 4–10 days, the initial dose was 200 mg IV and the patients were given 100 mg by injection on each following day. All cases experienced gastrointestinal symptoms, including rectal bleeding, gastraprosis, vomiting, or nausea; however, treatment was maintained until respiratory symptoms improved, with all adverse effects showing resolution of signs [81]. Analysis of the safety or clinical efficacy of the treatment was precluded due to the absence of controlled randomized and the tiny sample size.

NIAID initiated a placebo-controlled, double-blind randomized phase III trial to assess the efficacy and safety of remdesivir compared to a placebo (NCT04280705) [82]. This investigation was developed on the basis of available Chinese trial evidence plus consultation with the World Health Organization (WHO) [83]. This study is still enrolling participants, assessing a primary outcome of disease severity on an eight-point ordinal scale, plus several secondary outcomes. Seventy-five clinical locations are predicted to take part in the trial, mainly in the US. Gilead Sciences conducted two comparative clinical trials to evaluate remdesivir versus standard care in patients with moderate or severe COVID-19 NCT04292899 [83]. The primary clinical outcomes including oxygen saturation and fever, as well as safety and efficiency of remdesivir (over five or ten days) compared to standard treatments, alone or combined, in the trial NCT04292730 [84].

After the FDA approval of remdesivir in the management of COVID-19, the WHO Solidarity trial detected no significant effect of remdesivir on death rates in patients with COVID-19 [85]. Furthermore, the study performed by Wang and colleagues showing no benefit after administration of remdesivir to patients with COVID-19 was not included during the process of remdesivir approval by FDA. Moreover, the occurrence of remdesivir-based adverse events has been reported in numerous hospitalized COVID-19 patients, thereby raising questions about the efficacy and safety of remdesivir in the management of COVID-19 [86].

4.4. Nitazoxanide

Nitazoxanide contains both a salicylamide and nitrothiazole moiety [87,88]. The properties of nitazoxanide could be explained by these moieties. For example, acetylbenzamide or salicylamide can function as an anti-infective against viruses, parasites, bacteria and fungi. It can also act as antipolymerase in hepatitis virus, and inhibit penetration of influenza virus, and the fusion with host cell membrane. It can act as an antioxidant, analgesic, affect interleukin generation, and modulate immune response [89]. The nitrothiazole moiety also shows anti-inflammatory, anti-proliferative and anti-protozoal activity [90]. Nitrothiazole can inhibit oxidative stress [91] and impair redox reactions mediated by the pyruvate/ferredoxin oxidoreductase necessary for survival and energy metabolism in bacteria and anaerobic protozoa [87,88,92]. Moreover, nitazoxanide can potentiate host innate immunity and also inhibit different stages of the viral multiplication cycle [93,94]. In addition, nitazoxanide could boost the antiviral defense of host, with a low risk of antiviral resistance compared to drugs solely acting on viral proteins [89,95]. Several trials have tested nitazoxanide alone or combined with other antiviral drugs with promising results [94,96]. Nineteen COVID-19 clinical trials (ten trials using nitazoxanide alone and nine trials in combination with other antiviral drugs) have recently been registered in Brazil, Argentina, Mexico, Egypt, and US between April and August 2020 [clinicaltrials.gov] (Nitazoxanide clinical trials, 2020). The dose of nitazoxanide is 600 mg two times per day, or even higher in most of the trials. Furthermore, nitazoxanide has been tested in combination with other potent drugs including ribavirin, ivermectin, and hydroxychloroquine. A recently performed in silico modelling study arrived at 73 combinations resulting from 32 potent drugs against SARS-CoV-2 and verified them in vitro investigations. The findings showed synergy between nitazoxanide combined with umifenovir, amodiaquine, or remdesivir, and strong antagonism in the combination of hydroxychloroquine and remdesivir [97]. Moreover, a combination therapy of nitazoxanide plus azithromycin [98] or hydroxychloroquine has been suggested [93].

4.5. Ribavirin

Ribavirin is a synthetic nucleoside guanosine analog, approved for the management of infections caused by RNA viruses, such as hantavirus, Lassa virus, hepatitis C virus (HCV), hepatitis E virus (HEV), and RSV. The combination of ribavirin and favipiravir has anti-coronavirus activity. The exonuclease enzyme is expressed by coronaviruses as a nonstructural protein 14 (nsp14-ExoN). The nsp14-ExoN has been shown to have RNA proofreading functions [99]. Thus, coronaviruses are speculated to be resistant to nucleoside analogues. Experimental studies have revealed that ribavirin alone has poor anti-coronavirus activity, suggesting it must be combined with NIs against RNA viruses [100]. Synergistic effects of ribavirin combined with IFNα, or MERS-CoV was seen in rhesus macaques, as well as in vitro, suggesting that IFN increases ribavirin activity at lower doses [101,102]. However, a combination of IFNα and ribavirin had no significant therapeutic benefit on clinical symptoms in five MERS-CoV-positive patients [103]. This combination therapy did increase the survival rate in twenty MERS patients on day 14 but not on day 28 [104]. In another study, the combination therapy of MERS patients with IFNα1a or IFNα2a plus ribavirin provided no survival benefit [105]. Therefore, ribavirin does not provide clinical benefits for MERS-CoV or SARS-CoV patients. Although ribavirin therapy has been examined for the treatment of RSV and HCV in MERS and SARS patients, its side effects including anemia may be severe at high concentrations [106]. Based on the findings of a retrospective multicenter cohort investigation, there was no association between RBV/IFN-α treatment and the progression of non-severe COVID-19 to severe disease, or a reduction in the 30-day mortality rate [107]. It should be noted that the duration of hospitalization before discharge in COVID-19 patients might be extended following RBV/IFN-α treatment. Preclinical trials have confirmed the beneficial effects of RBV/IFN-α therapy for SARS and MERS [105,109], whereas this work did not observe any clinical benefits of RBV/IFN-α treatment (RBV and IFN-α alone or in combination) for COVID-19 patients [107]. Moreover, potential complications have been reported after ribavirin [110], which underscores the need for careful clinical investigation of the drug.
4.6. EIDD-1931

EIDD-1931 is a ribonucleoside analog which causes mutations in the RNA virus, and has recently been suggested to be effective for the management of COVID-19 [111,112]. EIDD-2801 is an isopropyl ester prodrug for EIDD-1931, which inhibits coronavirus replication in human and mouse airway epithelial cells [111,112]. Moreover, EIDD-1931 has been found to be useful for COVID-19 treatment [112]. It was also found to suppress remdesivir-resistant coronaviruses [112]. EIDD-1931 exists in two tautomeric forms [112]. One tautomer (EIDD-1931-t1) can mimic cytidine due to a single bonded N-OH group to carbon, whereas the other tautomer (EIDD-1931-t2) can mimic uridine because of an oxime group with a double bonded N-OH group to cytidine and thus places adenosine (instead of guanosine) in front of this base [111,112]. Based on a non-radioactive primer extension assay [102], EIDD-1931-triphosphate was incorporated as a U-analog at position +4, G-analog at position +3, and C-analogue at position +2 in the absence of any competing UTP or GTP [112]. According to recent evidence, the most stable base-pairing of EIDD-1931 occurs with G, and isoenergetic base pairs are formed between EIDD-2801 and G, U and A [113].

5. Molnupiravir structure and biological activity

Molnupiravir (EIDD-2801/MK-4482) is a prodrug for the ribonucleoside analogue β-d-N4-hydroxycytidine (NHC; EIDD-1931), which has been shown to be useful in the treatment of RNA virus infections, such as influenza and pathogenic coronaviruses, as well as encephalitis alphaviruses (such as Eastern, Western and Venezuelan equine encephalitis viruses) [114-116]. In plasma, molnupiravir is rapidly cleaved to EIDD-1931, and after it distributes into different tissues, it is transformed into the 5′-triphosphate (Fig. 5) [114]. EIDD-1931 5′-triphosphate is a substrate for virally encoded RdRp and causes a catastrophic error in replication after integration into the emerging RNA chain [114,117]. When the rate of mutations in the virus goes beyond a tolerable threshold, the virus is extinguished.

Molnupiravir is well absorbed, and the plasma concentration of EIDD-1931 shows dose-dependent, linear pharmacokinetics after administration of 50–1600-mg doses [118]. The absorption rate is slower when taken with food, but the absorption extent (as evaluated by AUCinf) is similar for both fasted and fed states [118]. Thus, concurrent use of molnupiravir with food does not provide synergistic therapeutic effects. The capacity-restricted uptake seen in pharmacokinetic investigations performed in animals, has not been seen in humans [114]. A multiple-ascending-dose study, with administration doses of 50–800 mg (BID), could be predicted from the exposure in a single-ascending-dose investigation [118]. EIDD-1931 has a half-life of 0.907–7.08 h, and is notably dose dependent. However, the use of BID dosage in the multiple-ascending-dose investigation was based on the half-life of the active antiviral compound, EIDD-1931 5′-triphosphate, measured both in vitro and in vivo [114,119]. In these studies, the intracelullar EIDD-1931 5′-triphosphate half life ranged from 3 h in Huh-7 cells to 6.6 h in murine lung tissue. Molnupiravir was tolerated at a single dose up to 1600 mg, and 50–800 mg BID for 5.5 days [118]. In the single-ascending-dose study, headache was the most common adverse effect, with diarrhea in the multiple-ascending-dose study. In the single-ascending-dose study, numerous placebo-treated patients reported headaches (18.8% placebo, 12.5% molnupiravir), and in the multiple-ascending-dose investigation (7.1%), the same number of placebo-treated patients reported diarrhea, as did molnupiravir-administered patients. In this study one patient discontinued treatment due to a rash; however, no other patients reported significant adverse effects [118]. The absence of any dose-dependent changes in clinical laboratory findings, electrocardiography, vital signs, along with the tolerability data, showed the safety of molnupiravir at the duration and dose assessed in the investigation. Recruitment limitations (e.g. variation in body mass index) and possible metabolic differences in patients, such as gastrointestinal disease or diabetes may have led to variability in pharmacokinetic parameters. Although most patients were Caucasian and male due to the inclusion criteria as well as the geographic constraints of the study location, there were no identified race- or sex-based metabolic concerns. These findings supported the advancement of molnupiravir into phase II clinical trial in patients with high-risk RNA-viral infections, such as COVID-19 [118].

Molnupiravir is now being assessed in a phase III clinical trial for the treatment of COVID-19 [118]. The drug is bioavailable in oral form and could be administered to outpatients with early stages of COVID-19 to decrease the risk of hospitalization. In vitro findings showed that transition mutations of G-to-A and C-to-U were dose-dependently increased.
along with the antiviral activity against coronaviruses [116,117]. Thus, molnupiravir can be categorized as a mutagenic nucleotide analog. Fig. 6 shows the mechanism of the complex between molnupiravir and RdRp in SARS-CoV-2, highlighting the effect on RNA synthesis fidelity and efficiency.

6. Molnupiravir and COVID-19

NHC can suppress SARS-CoV-2, MERS-CoV and SARS-CoV with submicromolar EC$_{50}$, depending upon the cell line [116]. Also, molnupiravir could inhibit SARS-CoV-2 replication in humanized mice [116, 120]. Sheahan and colleagues have reported that the NHC possesses a broad range of antiviral activity against SARS-CoV, SARS-CoV-2, MERS-CoV, and related zoonotic 2b/2c Bat-CoVs. It has good activity against a coronavirus with a resistance mutation to the nucleoside analog inhibitor remdesivir [116]. Both therapeutic and prophylactic use of EIDD-2801 prevented body weight loss and decreased viral load, and improved pulmonary performance in mice with SARS-CoV or MERS-CoV infections. The reduced replication of MERS-CoV under in vivo and in vitro conditions was related to an increased frequency of transition mutation in the viral cell RNA, but not in the host cell RNA, supporting a lethal mutagenesis activity in CoVs. The oral bioavailability and potency of NHC/EIDD-2801 against several coronaviruses suggest its beneficial use as an efficient drug against SARS-CoV-2 and other zoonotic coronaviruses [116].

Fig. 6. Molnupiravir is transformed to EIDD-1931 (NHC) rapidly in plasma, and then to active antiviral agent of EIDD-1931 5'-triphosphate following dispersion in different tissues through host kinases.

Fig. 6. NHC model of mutagenesis versus SARS-CoV-2; (A) Schematic incorporation of nucleotide into RNA primer (gray circles) or template (white circles) mediated by RNA-dependent RNA polymerase (oval) of SARS-CoV-2; minus and plus marks indicate RNA sense; U, G, C and A letters are natural nucleotide bases and M letter is molnupiravir; NTP triphosphate moiety is three small circles; (B) Tautomerization accounts for alternative base-pairing of NHC base moiety. N-hydroxylamine is predominant tautomer in the presence of NHC-TP substrate, but oxime and N-hydroxylamine forms are both found in the presence of template-embedded NHC-MP.; (C) Mechanism of template-embedded NHC-MP action for viral mutagenesis and inhibition. Incorporation of NTP into template NHC-MP is shown as blue circles.; (D) Mutagenic and inhibitory effects of NHC on viral replication. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)
A recent study utilized a platform based on ‘human lung-only’ (LoM) mice to allow effective replication of human coronaviruses, such as MERS-CoV, SARS-CoV, SARS-CoV-2, and two pre-pandemic SARS-like bat coronaviruses in vivo [120]. The replication occurred in the bony human lung tissue within the mice, and needed no modification of host or virus. It was shown that bats harbor endogenous coronaviruses able to be directly transmitted to humans. Further evaluation of SARS-CoV-2 infection in human lung tissue revealed predominant infection of epithelial cells, such as ciliated airway cells and type II pneumocytes. Acute infection was cytopathic and mediated robust type I interferon and inflammatory responses. Moreover, both prophylactic and therapeutic administration of EIDD-2801 inhibited SARS-CoV-2 replication, and was promising for COVID-19 prevention and treatment [120].

Molnupiravir/EIDD-2801 received an IND for influenza in the U.K. and the U.S. as a basis for preliminary data on activity versus strongly pathogenic coronaviruses. Pre-IND sessions and coronavirus IND revisions (cross-referral to influenza IND) were accelerated by the FDA. The data on coronavirus activity combined with the influenza IND were the basis for package submission in the U.K. The U.K. set up an Expert Working Group regarding COVID-19 studies. A guidance was published by the Medicines and Healthcare Products Regulatory Agency (MHRA) and also the Commission on Human Medicines (CHM) on the Clinical Trial Applications (CTAs) for COVID-19 studies (https://www.gov.uk/guidance/clinical-trials-applications-for-coronavirus-covid-19) to identify routes to provide prompt scientific advice, review, and possible approval for COVID-19 treatments. According to the MHRA, all routine elements such as Investigational Medicinal Product Dossier (IMPD), Study Protocol and Investigator’s Brochure should be included in the CTA, and the final draft documents should undergo rolling review. The MHRA provided real-time review comments and authorized the research team to make the requested alterations before formally submitting the CTA. According to the MHRA recommendation, the Research Ethics Committee (REC) should not be convened in a routine way that involves the sequential review of rules and ethics in a single application, but rather Director of the Approvals Service for the Health Research Authority (HRA) should make a request for expedited review. According to the MHRA proposal, the single ascending dose (SAD) should be used entirely for the initial submission, and later elements such as cohorts of multiple ascending doses (MAD) can be added as placeholders to be modified on the next date. The REC and the MHRA both agreed an identical expedited review for the protocol amendment.

Multiple and single doses of molnupiravir were assessed in a phase I clinical trial in healthy subjects, which included examination of food effects on pharmacokinetics [118]. After molnupiravir administration, EIDD-1931 was rapidly detected in plasma, with a median time to the peak of 1.00–1.75 h, and thereafter decreased with a geometric t\(_{1/2}\) of about 1 h, along with a significantly slower removal phase after higher single or multiple doses [121]. The mean maximum detected concentration, as well as area under the time-concentration curve increased in a dose-dependent manner, and no further accumulation was found after multiple doses. When given along with food, a reduction was found in the absorption rate, but not in total exposure. Molnupiravir is generally well tolerated. Less than 50% of participants experienced any complication, and the incidence of adverse events was actually greater in the placebo group. About 93% of reported adverse effects were mild [121]. Although no severe adverse effects or significant changes in electrocardiography, heart rate, or clinical laboratory parameters were detected, one patient did not complete the study because of a rash. According to the results in animal models, plasma concentration exceeded the anticipated effective dose; thus before the maximal tolerated dose was reached, the dose escalation was discontinued [121]. Kho and co-workers examined the safety and optimal dose of molnupiravir in patients with early symptomatic infection [122]. After screening and enrollment of 18 patients, they received oral doses of 300, 600 and 800 mg molnupiravir (BID, for five days) or a placebo. A dose was considered unsafe when the likelihood of 30% or higher dose-limiting toxicity (as primary outcome) compared to controls was 25% or more. Safety, virological response, pharmacokinetics, and clinical progression were the secondary outcomes. Molnupiravir was well tolerated at all three doses without any serious adverse effects. Of note, 4 out of 4, 4 out of 4, and 1 out of 4 of patients receiving 300, 600 or 800 mg of molnupiravir, respectively, as well as 5 out of 6 control subjects, reported at most one complication, and all of them were mild (equal to or lower than grade II). The probability of more than 30% excess toxicity over control subjects at 800 mg was predicted to be 0.9%. Because molnupiravir was safe and well-tolerated; 800 mg BID for five days was proposed for the Phase II trial [122].

In a double-blind and randomized phase III trial (MOVe-OUT), the safety and efficacy of molnupiravir were evaluated in non-hospitalized adult COVID-19 patients aged ≥ 18 years (n = 1850; NCT04575597, MK-4482-002) [123]. According to the interim analysis (n = 775) for this trial, the risk of hospitalization or death was significantly decreased up to 50% (p = 0.0012) on the 29th day. Based on the findings, 7.3% (28 of 385 patients receiving molnupiravir) were either hospitalized or deceased, compared with 14.1% (53 of 377) placebo patients, and there were no deaths in the molnopiravir-treated patients on the 29th day compared to 8 deaths in the placebo group. In addition, the time between the onset of illness, the specific variant of SARS-CoV-2 (delta, gamma, or mu), and the presence of underlying conditions had no influence on the molnupiravir efficacy. Possible adverse events (35% for molnupiravir versus 40% for placebo) and drug-induced adverse events (12% for molnupiravir versus 11% for placebo) were almost the same in both groups. Treatment discontinuation was lower in the molnupiravir group (1.3%) versus the placebo group (3.4%) [124,125]. Another double-blind randomized phase III trial (MOVe-IN, NCT04575584) evaluated the safety and efficacy of molnupiravir in hospitalized adult COVID-19 patients aged ≥ 18 years (n = 304; MK-4482–001), but it was terminated because the data from the interim analysis suggested that the clinical benefits of this drug were unlikely to be significant in hospitalized patients [123,126]. At the present time, a randomized, double-blind, placebo-controlled multicenter phase III trial (NCT04939428) with the large sample size of 1332 patients is underway (MOVe-AHEAD) to evaluate the safety and efficacy of molnupiravir to prevent the incidence of COVID-19 in non-infected adults living with an infected person. The trial hypothesized that molnupiravir would be effective in preventing definitive COVID-19 transmission compared to placebo until the 14th day [127]. Various pharmaceutical companies in India have recently reported limited findings from the interim analysis of phase III trials of molnupiravir among Indian COVID-19 patients aged 18–60 years suffering from mild symptoms (upper respiratory tract signs and/or fever with no hypoxia or dyspnea) or moderate symptoms (pneumonia without severe signs but with hypoxia and/or dyspnea, respiratory rate of ≥ 24 breaths per minute, cough with SpO\(_2\) of ≤ 93% within the range of 90–93% on room air, or fever). In an open-label, multicenter randomized clinical trial (CTRI/2021/05/033739) carried out by Hetero Labs Limited of Hyderabad (India), the interim analysis was published on July 9, 2021 including patients with mild COVID-19 (n = 741). They evaluated the safety and efficacy of 800-mg molnupiravir (4 × 200 mg) twice a day (every 12 h) for 5 days in combination with SOC, versus SOC alone (as control) for five days after the onset of symptoms. Their interim findings were as follows: [1] clinically earlier improvement (2-fold reduction on WHO Clinical Progression Scale) in molnupiravir compared to SOC (2 days; p < 0.0001), molnupiravir (79.0%) compared to SOC (49.5%) at the 10th day (p < 0.0001), and molnupiravir (81.6%) compared to SOC (73.2%) at the 14th day (p = 0.02); [2] short-term improvement (p = 0.0001) in molnupiravir (median time = 8 days) compared to SOC (median time = 12 days); [3] more negative RT-PCR tests (p < 0.0001) in molnupiravir (77.4%) compared to SOC (26.1%) at the 5th day, in molnupiravir (94.0%) compared to SOC (57.2%) at the 10th day and in molnupiravir (97.0%) relative to SOC (85.2%) at 14th day; [4]...
significant decrease in the duration of hospitalization (p = 0.003) in molnupiravir (1.9%) compared to SOC (6.2%) within 14 days. Neither group showed any mortality. The most prevalent complications were mild in severity, diarrhea, headache and nausea. All subjects completed the trial [128]. Overall, the interim findings from the ongoing Indian phase III trial indicated that there was a significant reduction in the duration of hospitalization for mild COVID-19 patients treated with molnupiravir compared to those treated with SOC alone; and there were no distinct drug-mediated adverse events, although the two groups exhibited no difference in mortality rate.

In another phase III trial examining the efficacy of molnupiravir in Indian patients with mild COVID-19 carried out by Optimus pharma (CTRU/2021/06/033992), the interim findings were reported on July 21, 2021. In a first report on 353 patients, the molnupiravir group exhibited no deaths at the 29th day in the molnupiravir group, but there were 8 (48.4%) at the 5th day [129]. According to a personal communication (CTRI/2021/06/033992), the interim findings were reported on July 2021. Indian patients with mild COVID-19 carried out by Optimus pharma exhibited no difference in mortality rate. Molnupiravir compared to those treated with SOC alone, and there were no distinct drug-mediated adverse events, although the two groups exhibited no difference in mortality rate.

The medicine regulatory authority of the UK has recently authorized the administration of the antiviral molnupiravir to manage mild-to-moderate COVID-19 infections in adult patients with at least one risk factor for severe disease. According to a press release by the manufacturer of the drug (MSD), the interim findings from the phase III trial suggested that the administration of molnupiravir could decrease the risk of hospitalization or mortality up to approximately 50% among non-hospitalized adult patients suffering from mild to moderate COVID-19, who were at risk of poor outcomes [132]. Moreover, 28 (7.3%) patients (out of 385 participants) treated with molnupiravir and 53 (14.1%) patients (out of 377 participants) treated with placebo were hospitalized or died up to the 29th day post randomization. There were no deaths at the 29th day in the molnupiravir group, but there were 8 deaths in the placebo group. The independent data monitoring committee stopped the trial recruitment early owing to the positive results [132]. Table 2 lists various studies on molnupiravir in different models, ranging from in vitro to humans.

7. Conclusions

RdRp is an essential viral protein of RNA viruses, and has been found to be an important target for the development of antiviral drugs. Regarding the similarity of important drug-binding sites between SARS-CoV, MERS-CoV and SARS-CoV-2 RdRps, the repurposing of established RdRp inhibitors for SARS-CoV-2 treatment is a promising approach.

| Table 2 | Pre-clinical and clinical studies on Molnupiravir. |
|---------|--------------------------------------------------|
| Model | Number of patients or animals (F/M) | Intervention | Follow up | Phase | Ref |
| In vivo: hamsters | SG hamsters (F) | Molnupiravir | 4 days | – | [133] |
| In vivo: VeroE6 | Remdesivir | Molnupiravir | – | – | [134] |
| In vivo: Huh7 | AT-511 | Molnupiravir | – | – | [135] |
| In vivo: HtAEC | GS-441524 | Molnupiravir | 2 days | – | [120] |
| In vivo: HsAEC | IFN (β1 and λ1) | Molnupiravir | – | – | [133] |
| In vivo: Immune deficient mice implanted with human lung tissue | Molnupiravir | – | – | – | – |
| Humans | 103 | Molnupiravir | 28-30 days | Phase 1 | [122] |
| Humans | 64 (53 M/11 F) | Molnupiravir | 14 days | Phase 1 | [118] |
| Humans | 202 (98 M/104 F) | Molnupiravir | 28 days | Phase 2a | [137] |
| In vivo: Syrian hamsters | Molnupiravir | 4 days | – | – | [138] |
| In vivo: Ferrets | Molnupiravir | 4 & 10 days | – | – | [139] |
| In vivo: Mice | Molnupiravir | 5 days | – | – | [116] |
| In vitro: Human airway epithelial cell | Molnupiravir | – | – | – | – |

Molnupiravir (formerly called EIDD-2801) can act as a RdRp inhibitor and as a prodrug for beta-D-N4-hydroxyxystidine (EIDD-1931), thereby acquiring the ability to control SARS-CoV-2 infections. A ferret model demonstrated the prophylactic ability of this agent to prevent infection. According to two phase I clinical trials (NCT04746183 and NCT04392219), the oral administration of molnupiravir at therapeutic doses was well-tolerated and safe. In two phase II clinical trials (NCT04405570 and NCT04405739), five-days of oral administration of molnupiravir resulted in acceptable efficacy, shown by removal of nasopharyngeal virus in patients suffering from early and mild COVID-19. There are ongoing clinical trials at phases II/III, including NCT04575584 with a sample size of 304 and NCT04575597 with a sample size of 1850. Based on a recent trial (NCT04575597), molnupiravir produced a significant decrease in the risk of hospitalization or mortality among adult patients suffering from mild-to-moderate COVID-19. For the benefit of individual and general health, molnupiravir may be used clinically to rapidly treat COVID-19 patients and to prevent the transmission of SARS-CoV-2. Molnupiravir could effectively decrease the viral load in the nasopharynx, and displayed good tolerability and safety among COVID-19 patients treated with a short-term (five-day) regimen. The medicine regulators of the UK have recently authorized the administration of the antiviral molnupiravir to manage the mild-to-moderate COVID-19 in adult patients with at least one risk factor for severe disease [132]. Molnupiravir should be evaluated for the optimal dosing regimen to ensure safety and effectiveness.
obtained in the post-marketing phase. Molnupiravir is the first oral antiviral drug that is directly effective in attenuating viral RNA and SARS-CoV-2 in the nasopharynx, which is well tolerated and with good safety. Oral antiviral drugs (such as molnupiravir) have a major advantage over injectable drugs (remdesivir) against COVID-19. The promising results of the initial trials to control COVID-19 give us hope for more successful clinical trials for SARS-CoV-2 vaccines: concept, progress, and challenges. Int. Immunopharmacol. 97 (2021), 107641.

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S.M.R. Hazemian et al.

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13

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