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Current Opinion in Virology

Developing a direct acting, orally available antiviral agent in a pandemic: the evolution of molnupiravir as a potential treatment for COVID-19

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Despite the availability of vaccines, there remains an urgent need for antiviral drugs with potent activity against SARS-CoV-2, the cause of COVID-19. Millions of people are immune-suppressed and may not be able to mount a fully protective immune response after vaccination. There is also an increasingly critical need for a drug to cover emerging SARS-CoV-2 variants, against which existing vaccines may be less effective. Here, we describe the evolution of molnupiravir (EIDD-2801, MK-4482), a broad-spectrum antiviral agent originally designed for the treatment of Alphavirus infections, into a potential drug for the prevention and treatment of COVID-19. When the pandemic began, molnupiravir was in pre-clinical development for the treatment of seasonal influenza. As COVID-19 spread, the timeline for the development program was moved forward significantly, and focus shifted to treatment of coronavirus infections. Real time consultation with regulatory authorities aided in making the acceleration of the program possible.

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Introduction

In January of 2020 the WHO declared the outbreak of coronavirus disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) a Public Health Emergency of International Concern, and by March it was declared a pandemic [1–5]. In January of 2020, we at the Emory Institute of Drug Development (EIDD) and Drug Innovation Ventures at Emory (DRIVE), a wholly owned subsidiary of Emory University, were planning for a June submission of an Investigational New Drug application (IND) on a ribonucleoside analog, designated internally as EIDD-2801 (subsequently given the generic name molnupiravir), for the treatment of influenza. Because of the emerging pandemic, our near-term timelines were moved ahead by 3 months and the focus shifted to treatment of highly pathogenic coronaviruses. Both the coronavirus and influenza indications were supported by extensive work done in cell culture models of infection and in animal models of influenza, SARS and MERS and ultimately SARS-CoV-2 [6,7•,8,9••,10••,11,12]. But the discovery and early development of molnupiravir actually began in 2013 with a focus on finding an orally available, direct acting antiviral agent for the treatment of infection by the encephalitic New World alphavirus VEEV (Venezuelan equine encephalitis virus).

The search for a specific countermeasure against VEEV arose in response to reports that VEEV was weaponized for delivery as an aerosol during the cold war [13,14]. However, VEEV is only one of three viruses in the genus Alphavirus (family *Togaviridae*) endemic to the Americas that under natural conditions are transmitted by the bite of an infected mosquito and can cause outbreaks of severe encephalitic disease in humans [15]. The other two are Eastern and Western equine encephalitis virus, EEEV and WEEV respectively. Although most infections in adult humans are asymptomatic or produce a mild illness characterized by fever, chills, headache, nausea, vomiting and myalgia, neuroinvasive disease can occur. EEEV infection, which is occurring with increasing frequency in the United States, is the most virulent of the New World alphaviruses with a mortality rate ranging between 50 and 75% in people developing neurologic disease [16]. Moreover, vector expansion (*Culex* spp. of mosquitoes) that is possibly the result of climate change has been documented and underscores that the threat of EEEV may continue to expand [17,18]. Clearly the availability of a direct acting antiviral agent for treating alphavirus infections is becoming of increasing importance in the public health sector.

Identification of a broadly active antiviral agent for the treatment of RNA viral infections

The pathophysiology of encephalitic alphavirus disease and the circumstances around the potential use of an antiviral agent in either a combat or a public health setting dictated the desired product profile. First and foremost, the drug candidate needed to show a high level of activity in cell culture models of infection and in animal models of alphavirus disease. It was also desirable that, at a minimum, the drug candidate be active against all three encephalitic alphaviruses. However, a broader spectrum of activity to address multiple RNA virus threats would be highly desirable. To treat VEEV, the drug candidate would have to cross the blood–brain barrier and achieve adequate concentrations to suppress viral replication in the brain and arrest progression to encephalitis. The onset of antiviral activity would need to be rapid since animal studies have shown that VEEV invades the CNS as early as 18 hours post aerosol exposure [19,20]. Additionally, under the adverse conditions that might be encountered by a soldier exposed to aerosolized VEEV, or in a widespread zoonotic outbreak of VEEV, it is preferable that the drug be orally available and suitable for self-administration. Development of resistance to the drug should be difficult so that viral breakthrough and loss of activity does not occur quickly. From a counterterrorism perspective, it is desirable that genomic changes purposefully introduced or developed by passaging studies to confer resistance to the antiviral agent should be difficult to generate. Finally, the risk/benefit profile of the drug needs to be acceptable given the severity of the morbidity and mortality associated with the infection.

While this product profile is derived from consideration of encephalitic alphavirus disease, it is clearly applicable to any number of additional RNA viruses. RNA viruses cause an enormous global health burden and are a major source of emerging and reemerging infectious disease [21]. Woolhouse and Brierly have reported a catalogue of 214 human-infective RNA viruses [22]. In addition, they suggested that megatrends including climate change, deforestation and urbanization that are already impacting society will result in more RNA viruses being discovered and more RNA viruses with pandemic potential emerging. Consequently, it has become increasingly clear that achieving the desired target product profile outlined here could add a valuable countermeasure not only against current RNA viral threats but also against novel RNA viruses that will emerge from the expanding pool of human viral pathogens.

Based on the desired target product profile and the general experience with approved products for viral infections, the decision was made to target the RNA-directed RNA polymerase (RdRp) encoded by all RNA viruses. The RdRps are key enzymes in the viral replication cycle with the dual roles of transcribing mRNA from genome templates and acting as a replicase to copy genomic RNA. There are no known mammalian equivalents and therefore in principal the RdRp can be targeted with a high degree of selectivity. These enzymes are the most conserved of the RNA virus encoded proteins [23]. Structurally, the RdRps have the canonical 'right hand' configuration observed for all polymerases with three conserved subdomains referred to as fingers, thumb and palm [24,25]. Within the palm subdomain there is a series of conserved primary sequence motifs that are critical to catalysis. Given the overall structural similarity amongst the RdRps and the conservation of primary, secondary and tertiary structural elements in the palm and thumb subdomains where catalytic function is located, there has been speculation that these enzymes have evolved from a common ancestor and may be the best target for achieving broad spectrum activity across a number of RNA families [23].

We chose to target the RdRp utilizing ribonucleoside analogs that selectively act as competitive alternative substrates and upon integration into nascent chain RNA interrupt viral genomic and/or mRNA synthesis. Nucleoside analogs acting through this mechanism are generally regarded as the backbone of modern antiviral therapy with over thirty approved (either alone or in combination) for the prophylaxis and treatment of viral infections [26]. While there have been a number of excellent reviews on the discovery and development of nucleoside analogs as antiviral agents [27] a few key properties that figure heavily into their utility, particularly in the context of emerging and reemerging RNA viral diseases and public health emergencies, are worth mentioning. The nucleoside analogs are generally quite potent and tend to have higher barriers to the development of resistance than do other classes of direct acting antiviral agents. Additionally, nucleoside analogs tend to be orally available or can be modified to be orally bioavailable using prodrug strategies, which allows for self-administration in emergency settings where the ability to treat a large number of people quickly may be crucial. Importantly and in line with the desired target product profile, the ribonucleoside analog RdRp inhibitors tend to have activity across multiple families of RNA viruses (see Ref. [28] for review). Consequently, the development of a single ribonucleoside analog as a therapeutic agent for one emerging or reemerging RNA virus may result in a countermeasure for multiple RNA viruses, which is what we experienced with molnupiravir and what led to its rapid development for the treatment of COVID-19.

In 2013 we began screening ribonucleoside analogs for activity against alphaviruses and quickly identified N4-hydroxycytidine (identified internally as EIDD-1931) as a lead with activity against the New World encephalitic alphaviruses as well as chikungunya virus. Before this there had been a number of studies demonstrating the

activity and favorable cytotoxicity profile of EIDD-1931 in cell culture models of infection [29–35]. Pharmacokinetic and distribution studies in mice, rats, ferrets and dogs revealed that EIDD-1931 was orally bioavailable, widely distributed to organs including the lungs and appeared to be actively transported into the CNS where it was quickly anabolized to the active 5′triphosphate [7•,8,10••]. Replication of virus in the CNS is important in the pathophysiology of VEEV infection and must be addressed to provide protection from mortality in mouse models of infection [36•]. Broader testing confirmed that EIDD-1931 inhibits replication of multiple RNA viruses, and its antiviral activity was verified in animal models (mice and ferrets) of influenza [8,10••,12], various coronaviruses [9••,11], respiratory syncytial virus (RSV) [12], VEEV) [36•], Chikungunya and Ebola virus infection (unpublished data). However, EIDD-1931 was quickly metabolized in the enterocytes of non-human primates after oral administration. To address this situation a prodrug of EIDD-1931 (designated EIDD-2801) was synthesized that facilitated movement across the gut lining and efficiently delivered EIDD-1931 to the circulating volume of all species tested, including non-human primates [12]. Consequently, EIDD-2801, molnupiravir, became the clinical development candidate.

A shift in clinical development plans in response to a global pandemic

When SARS-CoV-2 emerged as a global health threat in late 2019, an IND for molnupiravir was being prepared for the treatment of seasonal influenza. Based on activity in ferret models of influenza [8,10••,12] and evidence of robust distribution into and anabolism by lung tissue in multiple species, this pathway to developing the drug was agreed upon in consultation with funding agencies that had supported the discovery and development of EIDD-2801, the Defense Threat Reduction Agency (DTRA) and the National Institute for Allergy and Infectious Disease (NIAID). Development for the prophylaxis and treatment of aerosolized VEEV was planned to proceed in parallel under the Animal Efficacy Rule. At that time, there were *in vitro* and *in vivo* data demonstrating that EIDD-1931/2801 was active against SARS, MERS and human coronaviruses [9••], and as a consequence EIDD-2801 was also considered as a potential countermeasure for the prophylaxis and treatment of highly pathogenic coronavirus infections. In January of 2020 as the crisis worsened, BARDA (the Biomedical Advanced Research and Development Authority) opened a portal requesting information on potential countermeasures for COVID-19. In response, DRIVE/EIDD submitted a synopsis and gave a presentation to BARDA in early February. We never received a response from BARDA regarding the presentation or their assessment of molnupiravir as a potential drug. However, we did subsequently receive a notice from the US Food and Drug Administration (FDA) in late February of 2020 requesting all available information on the activity of EIDD-2801 against pathogenic coronaviruses. We made the decision to proceed with filing the IND for influenza as planned in order to facilitate initiating a Phase 1 clinical study to assess the pharmacokinetic, safety and tolerability profile of EIDD-2801. The influenza IND was filed on March 25, 2020 by DRIVE.

As the intensity of the pandemic grew it became apparent to us within DRIVE and the EIDD that additional resource would be needed to accelerate development. To that end a licensing deal was concluded with Ridgeback Therapeutics, a biotechnology company that had recently completed the development of a therapeutic agent for the treatment of Ebola infection in March of 2020. The IND for the treatment of Influenza infection was transferred to Ridgeback Biotherapeutics on April 7, 2020. A second IND for the treatment of pathogenic coronavirus infections was filed by Ridgeback on April 10, 2020 and a safe-to-proceed letter was received from the FDA on April 16, 2020. A project team was immediately formed by Ridgeback Therapeutics that included DRIVE/EIDD as well as representatives of a CRO. The project team developed an expedited Phase 1 single ascending dose (SAD) and food effect (FE) trial design, which included a placeholder for the later addition of multiple ascending dose (MAD) cohorts. This approach greatly facilitated startup of the clinical study.

Because of a positive Ames test, the potential for genotoxicity has been thoroughly evaluated for molnupiravir both *in vitro* and *in vivo*. Mutagenicity assays required by the US FDA to initiate clinical studies (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s2r1-genotoxicity-testing-and-data-interpretation-pharmaceuticals-intended-human-use) (developed in collaboration with the Expert Working Group (safety) of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals) and run under validated protocols provides strong evidence of lack of relevance of the Ames test for molnupiravir. It is well-recognized that *in vivo* studies are needed to establish the biological significance and potential clinical risk suggested by the results of *in vitro* assays, and that no single *in vitro* test is adequate to asses genotoxicity potential [37]. Consequently, two distinct *in vivo* rodent mutagenicity assays that are recognized as robust tools for evaluating mutagenicity, and for assessing human risk for mutagenicity, the Pig-a mutagenicity assay and the Big Blue® (cII Locus) transgenic rodent assay [38], were conducted. In both assays, which were run at doses and durations significantly greater than those being used in the clinic, the impact of molnupiravir treatment on mutation rates was not differentiable from mutation rates observed in untreated historical control animals. Additionally, molnupiravir was negative for induction of chromosomal damage in *in vitro*

micronucleus (with and without metabolic activation) and *in vivo* rat micronucleus assays. Thus, based on the totality of genotoxicity data molnupiravir is not considered to pose an increased risk of genotoxicity in clinical use.

Rapid implementation and conduct of a Phase 1 study

There were immediate challenges in conducting the Phase 1 study during the pandemic. At the time the study was initiated there were multiple clinical site closures in the United States due to the spread of COVID-19 and uncertainty about the infectivity status of potential normal healthy volunteers. In order to maximize the likelihood of completing the Phase 1 study without interruption due to possible COVID-19-related closure of investigative sites, regulatory submissions were also made in the U.K. where at the time the impact of COVID-19 was not as pronounced. In the U.S. and in the U.K., the influenza IND provided the venue for submission of preliminary data on the activity of molnupiravir/EIDD-2801 against highly pathogenic coronaviruses. The FDA expedited the conduct of a Pre-IND Meeting and review of the coronavirus IND (cross-referencing the influenza IND). In the U.K., the influenza IND plus coronavirus activity data were the basis for submission packages in the U.K. An Expert Working Group for COVID-19 was established by the U.K. Commission on Human Medicines, and the MHRA (Medicines and Healthcare products Regulatory Agency) published guidance on Clinical Trial Applications (CTAs) for COVID-19 products, which specified the procedures to supply rapid scientific advice, review and approval for potential COVID-19 treatments (Guidance: Clinical trials applications for Coronavirus (COVID-19), https://www.gov.uk/guidance/clinical-trials-applications-for-coronavirus-covid-19). MHRA confirmed that the CTA should include all the usual components (i.e. Investigator's Brochure, Study Protocol and Investigational Medicinal Product Dossier), but that review of final draft documents would occur on a rolling basis. Review comments were provided by the MHRA in real-time, permitting the project team to make requested changes before formal CTA submission. The MHRA further advised that Research Ethics Committee (REC) submission should not proceed in the usual fashion, via the Combined Ways of Working pathway (which allows consecutive regulatory and ethics review from a single application), but rather a request for expedited review should be made through the Health Research Authority (HRA) Director of Approvals Service. With regard to the protocol, the MHRA advised that the single ascending dose (SAD) design should be fully defined in the initial submission, but that subsequent components (e.g. multiple ascending dose (MAD) cohorts) could be referenced as placeholders for tailoring at a later date. Protocol amendments received similar expedited review by both the MHRA and the REC.

Single and multiple doses of molnupiravir were evaluated in a first-in-human, Phase 1, randomized, double-blind, placebo-controlled study in healthy volunteers, which included evaluation of the effect of food on pharmacokinetics [39•]. After administration of molnupiravir, EIDD-1931 appeared rapidly in plasma, with a median time of maximum observed concentration of 1.00–1.75 hours, and declined with a geometric half-life of approximately 1 hour, with an apparent slower elimination phase following multiple doses or higher single doses (7.1 hours at the highest dose tested). Mean maximum observed concentration and area under the concentration versus time curve increased in a dose-proportional manner, and there was no accumulation following multiple doses. When administered in a fed state, there was a decrease in the rate of absorption, but no decrease in overall exposure. Molnupiravir was well tolerated. Fewer than half of subjects reported an adverse event, the incidence of adverse events was higher following administration of placebo, and 93.3% of adverse events were mild. One discontinued early due to rash. There were no serious adverse events and there were no clinically significant findings in clinical laboratory, vital signs, or electrocardiography. Plasma exposures exceeded expected efficacious doses based on scaling from animal models; therefore, dose escalations were discontinued before a maximum tolerated dose was reached. Subsequently, Phase 2 and then Phase 2/3 studies were initiated as the accelerated development of molnupiravir continues. Information regarding these studies can be found on CT.gov.

Conclusions

The availability of molnupiravir to quickly enter testing as a potential therapeutic agent for the prophylaxis and treatment of COVID-19 is the direct result of the long-standing focus of DRIVE/EIDD on emerging/reemerging infectious diseases and government funding programs to identify and develop countermeasures for biodefense and emerging/reemerging infectious disease that have been ongoing for over 20 years. It is possible to receive funding through various federal agencies in the United States including DTRA, the NIAID and BARDA, to carry medical countermeasures for category A, B and C pathogens through clinical evaluation and FDA approval. Given this level of support, the lack of early focus on the part of government planning groups on facilitating the development of direct-acting antiviral agents for use in blunting the COVID-19 pandemic is puzzling. The target product profile for molnupiravir, for example, is ideal for use in long term care facilities where patients owing to age and/or health status may not be able to mount an effective immune response after vaccination, and in public health circumstances where the logistics and timing of vaccination present critical challenges (such as the current circumstances in India). It also should be apparent to planners that there is a significant portion of the global

population that is resistive to vaccination and consequently represents a sustained pool of virus. Antiviral drugs could be used in this population to help keep viral burden down in order to minimize transmission and to help suppress the development of more virulent strains.

The speed with which the clinical program for molnupiravir was implemented was due a number of factors: 1) the favorable characteristics of the molecule to address public health needs, 2) the thorough nonclinical program that included extensive testing in models of a number of viral disease, and 3) the collaboration between the sponsor, a multinational CRO and regulatory agencies in the United States and the United Kingdom. The efficient study conduct was made possible by the work of many individuals and by coordinated collaboration between stakeholders. Within nine weeks of Phase 1 protocol finalization, molnupiravir was ready for Phase 2 testing in outpatients with COVID-19. Had this study been conducted to standard industry timelines, it would have taken approximately nine months to generate the data necessary to enable a Phase 2 study. Indeed, Phase 2 studies have now been completed (clinicaltrials.gov identifier NCT04405570 and NCT04405739) and Phase 3 studies are starting. This case study demonstrates that urgent, coordinated efforts to support expedited study start-up and execution, including collaboration between sponsor, CRO and regulatory authorities, can greatly accelerate early clinical development of promising drug therapies under extraordinary circumstances, such as the SARS-CoV-2 pandemic.

Conflict of interest statement

Ridgeback Biotherapeutics LP licensed Molnupiravir from Emory University, funded the Phase 1 and 2 clinical studies and has subsequently entered into a collaboration with Merck to jointly develop molnupiravir.

W.P.P. is an employee of Ridgeback Biotherapeutics LP and previously was a consultant to Emory Institute of Drug Development. She has no financial interest in Molnumiravir.

W.H. is a cofounder, owner, and advisor to Ridgeback Biotherapeutics. She has a financial interest in Molnupiravir.

O.C. is an employee of Covance Clinical Research Unit Limited (the drug development division of Laboratory Corporation of America Holdings), which was responsible for the clinical conduct of this study. He has no financial interest in Molnupiravir.

G.R.P. is the director of the Emory Institute of Drug Development, is the chief executive officer of Drug Innovation Ventures at Emory. He is an inventor of record on the patent for molnupiravir and has a financial interest in molnupiravir.

M.G.N. is the director of operations at the Emory Institute of Drug Development. He is an inventor of record on the patent for molnupiravir and has a financial interest in molnupiravir.

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