MDL-001: An Oral, Safe, and Well-Tolerated Broad-Spectrum Inhibitor of Viral Polymerases

Virgil Woods*[1], Tyler Umansky*[1], Sean M. Russell[1], Briana L. McGovern[2,3], Romel Rosales[2,3], M. Luis Rodriguez[2,3], Harm van Bakel[2,4,5,6], Emilia Mia Sordillo[7], Viviana Simon[2,3,7,8,9], Adolfo García-Sastre[2,3,7,9,10], Kris M. White[2,3], Davey Smith[11], and Daniel Haders[1]**

[1]Model Medicines, La Jolla, CA, USA

[2]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[3]The Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[4]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[5]Icahn Institute for Data Science and Genomic Technology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[6]Department of Artificial Intelligence and Human Health, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[7]Department of Pathology, Molecular and Cell based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[8]Center for Vaccine Research and Pandemic Preparedness, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[9]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[10]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[11]School of Medicine, University of California, San Diego, La Jolla, CA, USA

*These authors contributed equally to this work.

**Corresponding author (Tools@modelmedicines.com)

ABSTRACT

The death toll and financial stress posed by the recent COVID-19 pandemic have highlighted the pressing need to develop safe and effective, broad-spectrum inhibitors to treat viral infections. To accelerate the antiviral drug discovery process, we developed GALILEO™, a computational platform that interfaces with a customizable bioinformatics pipeline with a geometric deep learning algorithm we named ChemPrint™ for in silico drug screening. Combining these algorithms with a large chemical repositioning library, we discovered MDL-001, which interacts with the Thumb pocket 1 subdomain of multiple single-stranded RNA viruses. For MDL-001, we demonstrate potent in vitro activity against a broad spectrum of pathogenic viruses, and we demonstrate potent in vivo efficacy in a mouse model of SARS-CoV-2 infection. In clinical trials, orally administered MDL-001 has been shown to be safe and well tolerated. These data underline both the effectiveness of the GALILEO™ platform for drug discovery and the promise of MDL-001 as a novel broad-spectrum antiviral clinical candidate.

Introduction

Pandemic and epidemic viral outbreaks have occurred throughout documented history, but never as frequently as in the past 25 years[1], including the recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[2]. The COVID-19 pandemic caused by SARS-CoV-2 is widely regarded as the worst viral outbreak since the Great Flu in 1918[1] resulting in millions of deaths worldwide and unspeakable financial losses.

As a result of the COVID-19 pandemic, high-stakes viral surveillance and drug development projects have been established to prevent another devastating pandemic[4]. Special attention was placed on viruses that have caused recent epidemics[4,5], including Ebola virus (EBOV)[6], Influenza virus types A and B (IAV and IBV, respectively)[7], Norovirus (NoV)[8], SARS-CoV-1/2[9,10], and Zika virus (ZIKV)[11].

Unfortunately, oral, safe, broad-spectrum antivirals are not available should a new outbreak of these or new viruses occur[12]. This lack of antiviral availability is likely because the paradigm for treating viral diseases is the “one bug, one drug” paradigm, which necessitates diagnosing the type of viral infection before treatment can begin[13]. This is problematic even when there is not a pandemic, as in regular clinical practice pathogen identification often takes time and can be marred by false positive or negative identifications[13]. Analogous to broad-spectrum antibiotics used after clinical diagnosis, broad-spectrum antivirals could obviate the need for testing before starting treatment. A broad-spectrum antiviral, especially one that can be administered orally, would allow for earlier and wider spread treatment, and such a drug would greatly augment our preparedness for future viral pandemics[14].

To overcome these and other therapeutic roadblocks, the National Institute of Allergy and Infectious Diseases (NIAID) has developed a target product profile (TPP) to guide the development of future antivirals (**Table 1**), emphasizing the need for broad-spectrum activity against emergent viruses with pandemic potential and the importance of drug leads with novel

inhibitory mechanisms[15]. Leading experts have also described the ideal antiviral Target Product Profile (TPP), advocating for an orally available medication that can be administered as a single daily dose for acute infections, supporting rapid and straightforward administration in urgent or initial outbreak scenarios[12]. Accordingly, the Center for Disease Control (CDC) emphasizes the need for new oral antivirals with no monitoring requirements[16].

Due to its essential role in genome replication, the catalytic pocket in viral polymerases has long been an antiviral drug target[17,18]. Because the catalytic pocket of viral polymerases is highly conserved across many pathogenic viruses, molecules developed to target this site are expected to have broad-spectrum activity. However, recent epidemic and pandemic outbreaks have unveiled the limitations of this approach[19].

Motivated by the need to develop new viral polymerase inhibitors, we recently developed a geometric deep learning algorithm (ChemPrint™)[20], as part of a suite of bioinformatics tools that interface with a computational platform, we named GALILEO™ [21]. Using GALILEO™, we identified, from a large chemical repositioning library, the small molecule we named MDL-001 that interacts *in silico* with Thumb pocket 1, an allosteric subdomain in viral polymerases[21]. This prior study also revealed that Thumb pocket 1 is structurally conserved across many positive-sense single-stranded RNA viruses, suggesting that inhibitors of this domain may have broad-spectrum potential[21]. Small molecule inhibitors targeting the HCV Thumb pocket 1 have been discovered over the past 10 years, with several having entered clinical trials[22-25]. Unfortunately, these small molecule inhibitors were only developed for HCV[22-25], and not for other similar viruses, which share a conserved Thumb pocket 1 structure.

In this study, we assessed the *in vitro* antiviral potency of MDL-001 in a diverse panel of pathogenic viruses, presenting strong evidence that this compound is a broad-spectrum antiviral drug lead. In mice, we found that MDL-001 kills a mouse-adapted SARS-CoV-2 variant and that it accumulates in the target tissue (lung) at concentrations far higher than its *in vitro* EC_{50}.

Results

In vitro antiviral potency of MDL-001 against a diverse panel of pathogenic viruses

The *in vitro* validation of drug candidates identified in computational approaches is a hallmark in the drug development pipeline. Based on our previously reported *in silico* studies[21], MDL-001 was hypothesized to be a broad-spectrum antiviral small molecule inhibitor because the Thumb pocket 1 region in viral RNA-dependent RNA polymerase (RdRp) is well-conserved across many pathogenic viruses[26,27].

To test this hypothesis, we assessed the *in vitro* potency of MDL-001 in a panel of 13 cell line-based pseudovirus expression assays. These included 10 +ssRNA viruses (six SARS-CoV-2 variants, two coronavirus types, plus HCV and NoV), and 2 -ssRNA viruses (IAV-H1N1 and IAB) (**Figure 1** and **Supplementary Table 1**). MDL-001 had submicromolar potency against all six SARS-CoV-2 variants (Wuhan isolate WA1, Alpha, Beta, Delta, and Omicron variants, as well as a mouse-adapted variant SARS-CoV-2 MA), and the two coronaviruses tested (Alphacoronavirus and Betacoronavirus). Our results also showed that MDL-001 had lesser but still significant potency (EC_{50} = 3-8 μM) against the remaining +ssRNA viruses (HCV, and NoV), and the -ssRNA viruses (IAV and IAB) (**Figure 1** and **Supplementary Table 1**).

***In vivo* antiviral efficacy of MDL-001 in mice infected with MA-SARS-CoV-2**

We next investigated the *in vivo* antiviral efficacy of MDL-001 in mice infected with a mouse-adapted (MA) viral variant, named MA-SARS-CoV-2. We found that MDL-001 ameliorated body weight loss and viral accumulation in the lung in a dose-dependent manner (**Figure 2**). The reduction in body weight loss by MDL-001 compared to vehicle became statistically significant on days 2 and 3. Furthermore, the two highest MDL-001 doses (250 and 375 mg/kg BID) resulted in a body weight loss reduction similar in magnitude to our findings for

remdesivir (**Figure 2A**). Noticeably, the decrease in viral lung titers upon treatment with MDL-001 mirrored the dose-dependent pattern observed for weight loss, supporting the notion that a reduction in weight loss provoked by viral infection was correlated to virus clearance (**Figure 2B**).

Direct-acting small molecules are expected to exert a dose-dependent response effect, upon interaction with their receptor. The dose-dependent reduction in body weight loss and the decrease in viral levels in the lungs, upon MDL-001 treatment, were in accordance with our prediction of a specific MDL-001/Thumb pocket 1 interaction[21]. Significantly, MDL-001 prevention of body weight loss mirrored those observed for remdesivir, even though drugs administered subcutaneously (remdesivir) undergo a faster biodistribution than following oral administration (MDL-001)[28].

In vivo pharmacokinetics (PK)

To determine the pharmacokinetics (PK) of MDL-001 in rats, we first assessed MDL-001 biodistribution in plasma and lung following single oral doses. The maximum plasma concentration (C_{max}) and bioavailability (AUC_{last}) upon administration of the highest dose (1000 mg/kg) of MDL-001 increased by approximately 1.5-fold and 2-fold, respectively, compared to the lowest treatment (250 mg/kg) (**Figure 3**). The mean absorption/clearance (T_{max}) and half-life ($t_{1/2}$) in the lowest and highest concentrations had a time bracket of 5–6 h (**Supplementary Table S3**). In rat lungs, MDL-001 reached remarkably high levels, compared to those in plasma, with C_{24h} lung/plasma ratios between 97–122 across the dose range (**Supplementary Table S4**).

To determine the pharmacokinetics (PK) of MDL-001 in mice, drug concentrations in plasma and lung were measured following administration of two dose concentrations, each in two different drug dosage regimens. One regimen entailed a single dose of each concentration. The other regimen comprised a daily administration of each dose concentration over the course

of five days (**Supplementary Table S5**). In all cases, the plasma C_{max} increased in a dose-dependent manner. Overall, we found that MDL-001 absorption and clearance were faster in mice, compared to rats. MDL-001 plasma levels were quantifiable at the first time point (0.5 h); and the $t_{1/2}$ was 2-4 h, approximately 2-fold faster than the one in rats ($t_{1/2}$ in rats 5–6 h). (**Figure 4** and **Supplementary Table S5**).

Like in mouse plasma, MDL-001 accumulation in mouse lungs was remarkably high and very similar in the two drug administration regimens. The lung/plasma AUC_{last} ratios showed a similar pattern in the two dose regimens tested. In the low-dose experiments, the lung/plasma AUC_{last} ratio was 65 in the single-dose experiment and 56 in the 5-day regimen. In the high-dose experiments, the lung/plasma AUC_{last} ratio was 36 in the single-dose experiment and 44 in the 5-day regimen. (**Supplementary Table S6**). Compared to its value in plasma (T_{max} = 2-6 h), the T_{max} in the lung after the last dose on day 5 was between 4-8 h. This suggested a slower biodistribution to the lung than we observed in rats.

In vivo safety and tolerability

MDL-001 is an investigational new drug (IND), which had previously undergone preclinical and clinical evaluation in rodents and humans, respectively[29,30]. In the present study, we further investigated the safety and tolerability of MDL-001 in rodents to assess potential adverse effects when used at doses as high as 1000 mg/kg (**Supplementary Table S2**). Rats received single oral doses of MDL-001 at four concentrations: 250 mg/kg, 500 mg/kg, 750 mg/kg, or 1000 mg/kg and after drug administration, clinical signs of toxicity, body weight changes, and clinical chemistry parameters were recorded. No significant toxicity was observed in these assays and all animals behaved normally during the experiment, even when rats were treated with 1000 mg/kg, corroborating the tolerability of MDL-001 (**Supplementary Table S2**).

We also collected safety and tolerability data in mice. In these experiments, mice were treated with a range of MDL-001 dose concentrations, repeated once or twice daily, as indicated

in **Supplementary Table S2**. In all cases, mice were monitored for adverse effects, and detailed observations were recorded. Overall, no adverse reactions were observed in these studies and all animals behaved normally. **Table 2** summarizes the drug concentrations, method of administration, and duration over which animals were observed for the tolerability studies of MDL-001 in mice and rats performed by us and published studies.

Table 2

Laboratory	Days experiment	Concentrations	Administration route	Specimen number	Study type
Model Medicines	3	67 mg/kg QD	Oral gavage	6 mice	Pharmacology mice
Model Medicines	6-8	66.67 or 250 mg/kg QD	Oral gavage	22 mice	Pharmacology mice
Model Medicines	3	125, 250 or 375 mg/kg QD	Oral gavage	27 mice	Pharmacology mice
Model Medicines	1 or 5	50 mg/kg or 250 mg/kg QD	Oral gavage Single dose or repeated daily	96 mice	Pharmacokinetics mice
Model Medicines	1	250, 500, 750, or 1000 mg/kg QD	Oral gavage	12 rats	Pharmacokinetics rats
Patent: US 20020045638A1	35	20 mg/kg QD alone or combined temsirolimus (CCI-779) 5 mg/kg	Oral gavage	10 mice	Pharmacology mice
Patent: US 20020028805A1	3	0.2-20 mg/kg QD	Subcutaneous injection	6 rats	Pharmacology rat
Patent: US 2003OO65008A1	9	0.05-1.5 mg/kg QD	Oral gavage	5 mice	Pharmacology mice

Discussion

In the present study, we show that MDL-001 has broad-spectrum antiviral activity against four RNA viral families, all of which have pandemic potential (**Figure 1**). Our results revealed that MDL-001 has submicromolar potency against the eight +ssRNA Coronaviruses tested (six SARS-CoV-2 variants, plus Alphacoronavirus, and Betacoronavirus), with sub-10uM potency against HCV, NoV (+ssRNA viruses), and two Influenza types, H1N1 and B (-ssRNA viruses) (**Figure 1**). The potency in all cases is orders of magnitude lower than the C_{max} obtained in lung (**Figures 3-5**).

In the past, successful antiviral drug development programs have targeted primarily two types of viral proteins, proteases (e.g., nirmatrelvir in Paxlovid™) or the the catalytic pocket in viral polymerases (remdesivir in Veklury™). These inhibitors target a single or a limited number of virus families and have been plagued by toxicities. Our data demonstrate that MDL-001, which targets Thumb pocket 1, a novel allosteric subdomain in viral polymerases[21], is a

direct-acting, truly broad-spectrum antiviral. With this evidence we have provided validation of the GALILEO™ platform[24] for fast and effective drug discovery.

In a preclinical setting our results indicate that MDL-001 also has *in vivo* antiviral efficacy comparable to that of remdesivir (Veklury) in a mouse model of SARS-CoV-2 infection (**Figure 2**). Moreover, the magnitude with which MDL-001 reduced viral accumulation in the lung (2.9 Log_{10}) was significantly higher than the one recently reported for nirmatrelvir (1.4 Log_{10} with 300 mg/kg and 1.9 log_{10} with 1000 mg/kg), which is the active ingredient in Paxlovid [31]. Thus, MDL-001 has preclinical antiviral efficacy attributes similar if not greater than two FDA-approved drugs to treat COVID-19, Veklury [32] and Paxlovid [33].

In pharmacokinetic studies using mice and rats, we show that MDL-001 is tolerable at concentrations as high as 1000 mg/kg, and that it accumulates in lung (the site of infection) at levels 10-40 times higher than the submicromolar EC_{50} values obtained in *in vitro* assays (**Figures 3-5**). The rapid and sustained accumulation of MDL-001 in the lung, provides for exceptional EC_{50}/C_{max} ratios and minimizes viral infection.

MDL-001 was initially developed in another indication and was previously investigated in Phase I human clinical trials[29,30]. Its chemical name is pipendoxifene[34] and was labeled ERA-293 in clinical trials[29,30]. In both human studies, with dosing up to 200 mg/day for 28 days, MDL-001 was well tolerated with only mild transient, adverse events observed at all doses (**Table 3**). In these studies MDL-001 was tested in nearly 100 human volunteers with no Grade 3 or 4 adverse events, supporting its safety. These studies concluded that MDL-001 is orally available, has once-a-day dosing potential, and did not show any significant safety risks, such as genotoxicity or liver/hepatic toxicity.

Table 3

Trial	Subjects	Dosing/Days	Safety and Tolerability
Safety and pharmacokinetic evaluation of ERA-923 in healthy postmenopausal women in two double masked phase I trials[29].	46 Healthy postmenopausal women	Single test article dose or placebo followed by a single higher dose after a 20-day washout period (1/5, 5/25, 25/50, 50/75, 75/100, 100/150, 150/200 and 200 mg p.o.	MDL-001 was well tolerated with only mild transient adverse events observed at all doses. No clinically significant changes in laboratory values or laboratory toxicities at any MDL-001 dose compared to placebo.
Multiple-Dose, Safety, Pharmacokinetics, and Pharmacodynamics of a New Selective Estrogen Receptor Modulator, ERA-923[30].	50 Healthy postmenopausal women	Dose concentrations: 10, 50, 100, 150 or 200 mg once daily for 28 days	MDL-001 was well tolerated, Adverse events were mild, reversible and unrelated to dose. The most common adverse events associated with the agent included headache (28% vs. 30% in placebo), pain (23% vs. 10% in placebo) and hot flashes (20% vs. 20% in placebo). No clinically relevant changes in laboratory parameters.

In conclusion, MDL-001 is a promising broad-spectrum antiviral drug with the characteristics of an "ideal target product profile (TPP)", as laid out by the NIAID[15]. (**Table 1**). The clinical antiviral efficacy of MDL-001 should be pursued to provide additional therapeutic options for the next viral pandemic and future preclinical studies should focus on assessing a larger collection of RNA viruses, to determine the breadth of MDL-001's antiviral efficacy. Last, the results presented here help consolidate GALILEO™ and ChemPrint™, as useful AI tools, for the discovery of new chemistry and biology, as a preamble to the development of much-needed drugs against intractable diseases.

Methods

In vitro potency

We assessed the *in vitro* antiviral potency of MDL-001 with a panel of 14 viral variants from eight pathogenic viruses across four ssRNA viral families (Figure 1). Coronaviridae (+ssRNA): SARS-CoV-2 variants, including Alpha, Beta, Delta, Omicron, the initial Wuhan isolate (WA1), a mouse-adapted (MA) variant, HCV and NoV. Flaviviridae Orthomyxoviridae (-ssRNA): Influenza A H1N1/WSN/33 and Influenza B Yamagata/Brisbane and Victoria/Florida variants.

We determined antiviral potency based on half maximal effective concentration (EC_{50}) and 50% cytotoxicity concentration (CC_{50}), which in turn allowed us to determine the half maximal selective index (SI_{50}), after dividing CC_{50} by EC_{50}. The antiviral assays were cell line based and consisted in transfecting a pseudovirus version of each of the viruses tested, in a compatible cell line. Upon infection and after drug treatment, viral survival and cell toxicity were reported from the readout of three different assays, cytopathic effect (CPE)[35], viral yield Reduction (VYR)[36], or immunofluorescence (IF)[37]. A breakdown of the results obtained can be found in **Supplementary Table S1.**

In vivo efficacy

The *in vivo* efficacy of MDL-001 was evaluated using a mouse-adapted (MA) SARS-CoV variant to simulate human disease in mice. The goal was to assess its therapeutic potential in a preclinical setting. We quantitated the readout values for two features: reduction in body weight loss, as a surrogate of symptomatic relief, and viral load. Data was analyzed by two-way ANOVA (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

The symptomatic relief method tracks changes in body weight as an indicator of disease progression and treatment efficacy, as described elsewhere[38]. The dose range tested included 125 mg/kg BID, 250 mg/kg QD, 250 mg/kg BID, and 375 mg/kg BID. The viral yield reduction

assays determine viral titers were quantified to measure the reduction in viral load following treatment with MDL-001, as described elsewhere[39]. The dose range tested included 125 mg/kg BID, 250 mg/kg QD, 250 mg/kg BID, and 375 mg/kg BID.

In vivo pharmacokinetics

To determine the pharmacokinetics (PK) of MDL-001 in mice and rats, we assessed its accumulation in plasma and lung along a longitudinal time course as described below. In SD rats we first assessed MDL-001 biodistribution in plasma and lung following single oral doses at four concentrations: 250 mg/kg, 500 mg/kg, 750 mg/kg, and 1000 mg/kg. In 129S mice, plasma and lung MDL-001 concentrations were measured following administration of two dose concentrations: 50 mg/kg and 250 mg/kg each in two different drug dosage regimens. One regimen entailed a single dose of each concentration. The other regimen comprised a daily administration of each dose concentration over the course of five days.

In vivo safety and tolerability

Safety and tolerability of MDL-001 were assessed in rat and mouse model experiments to determine any potential adverse effects upon drug treatment. In rat studies, 12 male Sprague Dawley (SD) specimens were used. We collected safety and tolerability data from male Sprague Dawley (SD) rats. Briefly, rats received single oral doses at four concentrations: 250 mg/kg, 500 mg/kg, 750 mg/kg, or 1000 mg/kg. Upon infection, clinical signs of toxicity, body weight changes, and clinical chemistry parameters were recorded.

In mouse studies, the *in vivo* safety assays upon MDL-001 treatment were assessed in 151 mice. We also collected safety and tolerability data in female 129S mice. In these experiments, mice were treated with a range of MDL-001 dose concentrations (67, 125, 250, 375, 500, 750, or 1000 mg/kg), repeated once or twice daily. In all cases, mice were monitored for adverse effects, and detailed observations were recorded.

Acknowledgments

We would like to extend our sincere gratitude to Dr. Brett Hurst from Utah State University for his invaluable insights and contributions to this work. His expertise has been instrumental in advancing this research.

Competing Interests

The authors affiliated with Model Medicines declare the existence of a financial competing interest. Davey Smith reports the following competing interests: Consulting fees from Bayer, Lucira, Pharma Holdings, Evidera, Vx Biosciences, Gilead, and Red Queen Biosciences. Stock options from Fluxergy, Linear Therapies, and Model Medicines. Payments to his institution from the NIH. These entities have provided financial compensation, stock options, or institutional support within the past 36 months, potentially influencing, or that could give the appearance of potentially influencing the submitted work.

Figures and Table 1

Table 1. Comparison of MDL-001 to the NIAID Target Product Profile for SARS-COV-2. Minimal desired attributes are shown in blue, while optimal attributes are in red. The properties of MDL-001 have been colored to convey whether they more closely reflect the minimal attributes (blue), the optimal attributes (red), somewhere between minimal and optimal (purple), or excede even the optimal attributes (green).

Categories	Minimal Attributes	Optimal Attributes	MDL-001
Clinical Outcomes/Efficacy	• Decrease in progression to severe disease/hospitalization OR decrease in duration of symptoms, similar to current COVID-19 SOCs	• Decrease in long-term symptoms and/or complications • Decrease in risk of ongoing transmission demonstrated by reduction in viral titers to undetectable levels	• Remains to be investigated
Target Population	• U.S. adults with diagnosis of SARS-CoV-2 infection with particular focus on those > 60 years of age and/or at high risk for complications	• Pediatric patients • Pregnant women • Global population	• U.S. adults with diagnosis of SARS-CoV-2 infection with particular focus on those > 60 years of age and/or at high risk for complications • Safety in children or pregnant women remains to be investigated
Treatment Regimen, Duration, Dosage, and Treatment Window	• Up to two times per day • Administered within mild to moderate phase of symptomatic presentation • Maximum 10 days treatment course	• Single dose or once per day • Maximum 5 days treatment course	• Up to two times per day • Administered within mild to moderate phase of symptomatic presentation • Maximum 10 days treatment course
Route of Administration	• Oral or inhaled/intranasal formulation, ability to self-administer with minimal skill level	• Pediatric formulation • No adjustment for renal/hepatic impairment	• Oral formulation, ability to self-administer with minimal skill level
Safety and Tolerability	• Broadly acceptable risk/benefit profile in target population	• Well-established safety profile with AEs that do not require monitoring and do not compromise patient compliance • Acceptable safety profile for use in pediatrics and pregnant women	• Broadly acceptable risk • Well-established safety profile with AEs that do not require monitoring and do not compromise patient compliance
Drug Interactions/DDI	• No significant DDI with products licensed for COVID-19 and/or common anti-inflammatory, analgesic, and antipyretic agents • Dose adjustment permitted with concomitant medications	• No dose adjustment needed with concomitant medications	• No known published DDI

PK/PD	• C_{min} > EC_{50} (protein-binding adjusted) for the entire dosing interval OR rationale for alternate PK/PD profile • Evidence of appropriate distribution and exposure at primary and secondary sites of infection (upper respiratory tract, lungs)	• C_{min} > EC_{90} • Rapid attainment (< 24 h) of efficacious drug levels	• C_{min} > EC_{50} (protein-binding adjusted) for the entire dosing interval • Evidence of appropriate distribution and exposure at primary and secondary sites of infection (upper respiratory tract, lungs) • Rapid attainment (< 24 h) of efficacious drug levels
Logistical Supportability and Manufacturing	• Readily available manufacturing and distribution network, appropriate to phase of project	• Capability to rapidly scale-up production at cost/dose level that allows broad use with minimal manufacturing lag time • Proof of commercial viability (low cost of goods, easily reproducible chemical formulation)	• Originally manufactured by Wyeth/Pfizer • Capability to rapidly scale-up production at cost/dose level that allows broad use with minimal manufacturing lag time • Proof of commercial viability (low cost of goods, easily reproducible chemical formulation)
Product Stability and Storage	• Stability ≥ 2 years • Short term cold chain (at 4°C) storage with stability • Long term storage at -20°C with stability	• Stability ≥ 7 years • Ambient storage with stability	• In lyophilized form, the chemical is stable for 36 months. • In solution, store at -20ºC and use within 3 months to prevent loss of potency
Spectrum of Activity	• Demonstrated activity against SARS-CoV-2 • No evidence of emergence of resistance in clinical trials	• Broad activity against betacoronaviruses • Acceptable level of resistance development with understanding of potential cross-resistance	• Activity against SARS-CoV-2 • Activity against alpha and betacoronaviruses • Activity against four RNA virus families
Nonclinical Evidence of Antiviral Activity	• Demonstrated activity against clinical variants at concentrations achievable in vivo • Acceptable selectivity of antiviral activity with CC_{50}/EC_{50} (SI) > 10 • Evidence of viral load reduction in appropriate animal model] with treatment dosing modality	• Acceptable selectivity of antiviral activity with CC_{50}/EC_{50} (SI) > 100 • Resolution of clinical endpoints in appropriate animal model with treatment dosing modality	• Demonstrated activity against clinical variants at concentrations achievable in vivo • Acceptable selectivity of antiviral activity with CC_{50}/EC_{50} (SI) > 10 • Evidence of viral load reduction in appropriate animal model with treatment dosing modality

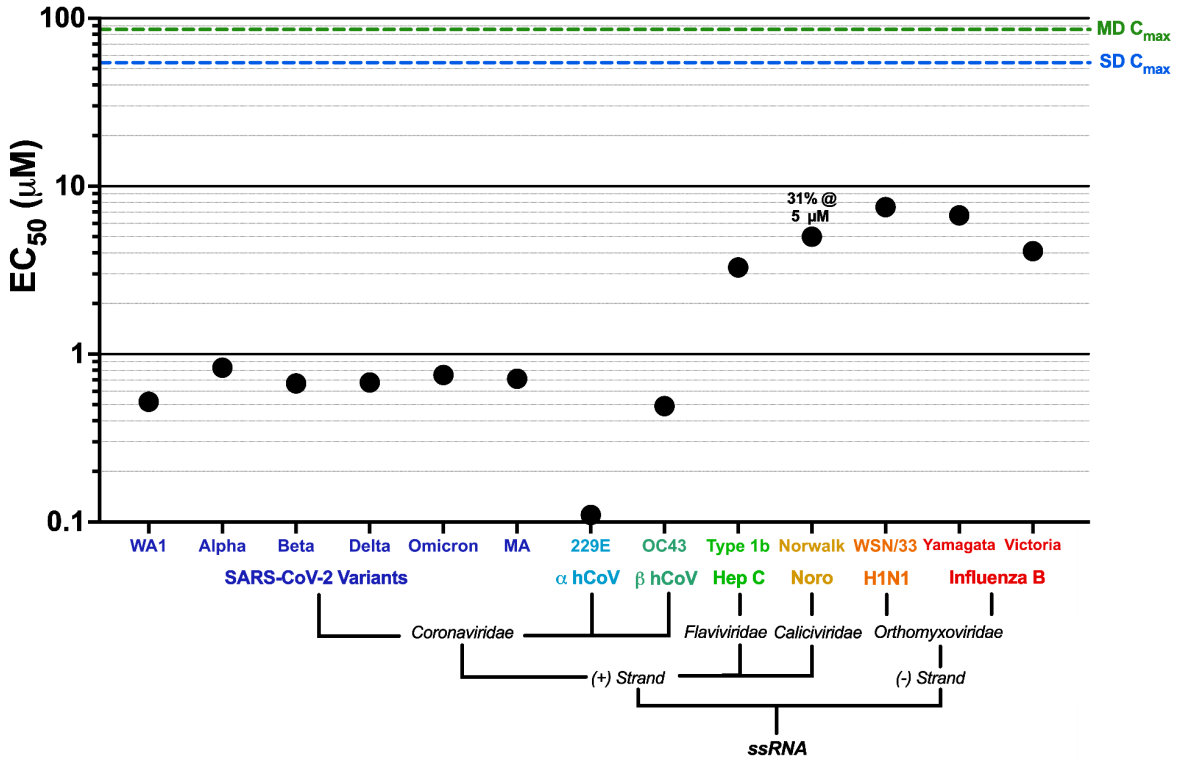

Figure 1. Family tree diagram of 4 viral families, 13 viruses and variants for which MDL-001 exhibited sub 10 µM antiviral activity. The best EC_{50} values obtained for each virus/strain/variant obtained across 29 assays are plotted. Complete results including SI_{50} calculations can be found in **Supplementary table S1**. MDL-001 Lung, C_{max} concentrations in mice after Multiple Dose (MD) and Single Dose (SD) of 250 mg/kg QD dosing as measured in PK studies reported below are shown as green and blue dashed lines, respectively.

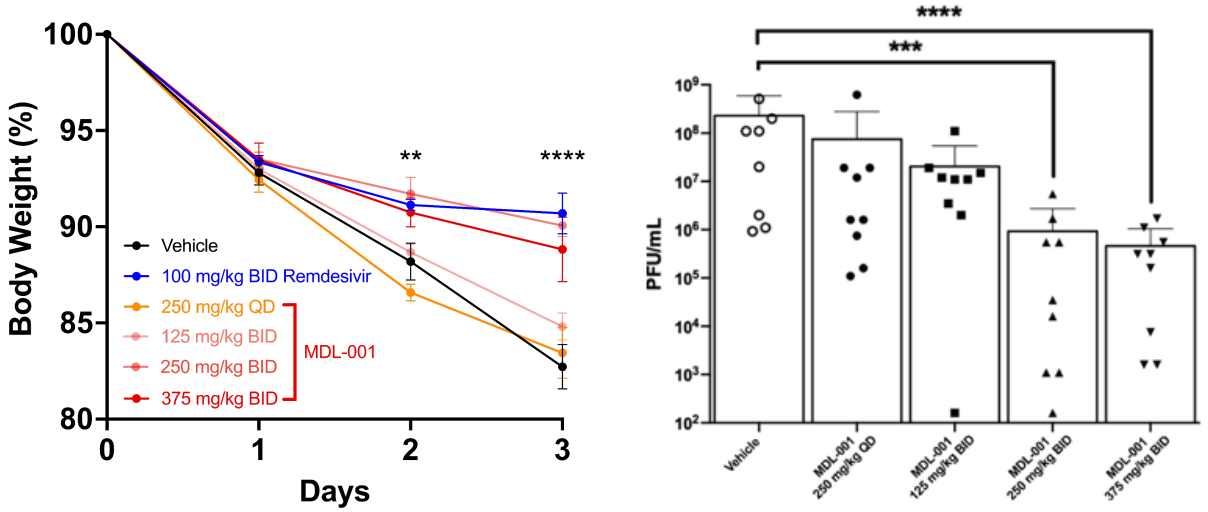

Figure 2. *In vivo* antiviral efficacy of MDL-001 in mice infected with MA-SARS-CoV-2 (A) 129/S mice were intranasally infected with 2.5×10^4 PFU of MA-SARS-CoV-2 and treated orally with MDL-001 or subcutaneously with 100 mg/kg remdesivir twice daily for 3 days. Animal weights were monitored daily. N = 9 per group. **(B)** SARS-CoV-2 titers in the lungs were determined on day 3 post-infection. N = 9. (A-B) Data was analyzed by two-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001)

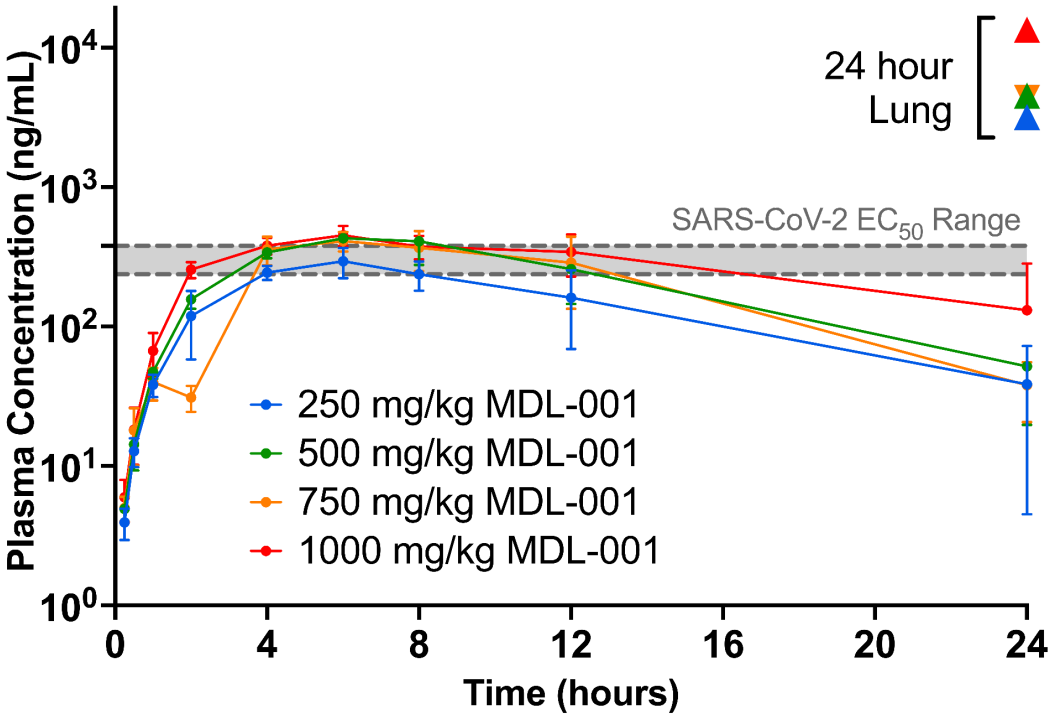

Figure 3. Plasma and Lung concentrations of MDL-001 in male SD rats. Rats were given a single administration of 250 mg/kg (Group 1), 500 mg/kg (Group 2), 750 mg/kg (Group 3), 1000 mg/kg in male SD rats (Group 4). MDL-001's EC_{50} range (0.52 μM - 0.83 μM; 237 ng/mL - 379 ng/mL, gray) against SARS-CoV-2 variants is labeled.

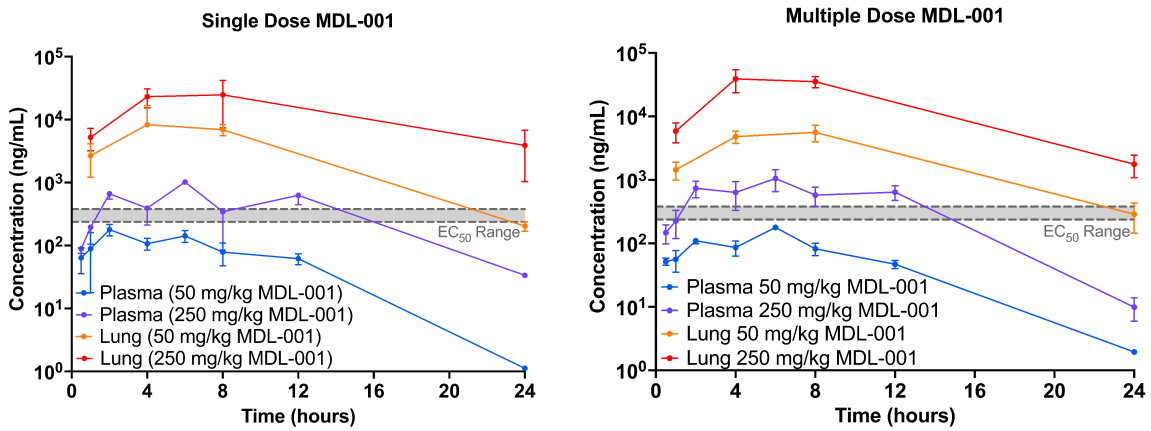

Figure 4. The plasma and lung tissue concentrations and pharmacokinetics of MDL-001 in female 129S1 mice. Plasma and lung concentrations of MDL-001 were evaluated following **(A)** single and **(B)** 5-day repeat oral (PO) administrations at 50 mg/kg and 250 mg/kg. MDL-001's EC_{50} range (0.52 μM - 0.83 μM; 237 ng/mL - 379 ng/mL, gray) against SARS-CoV-2 variants is labeled.

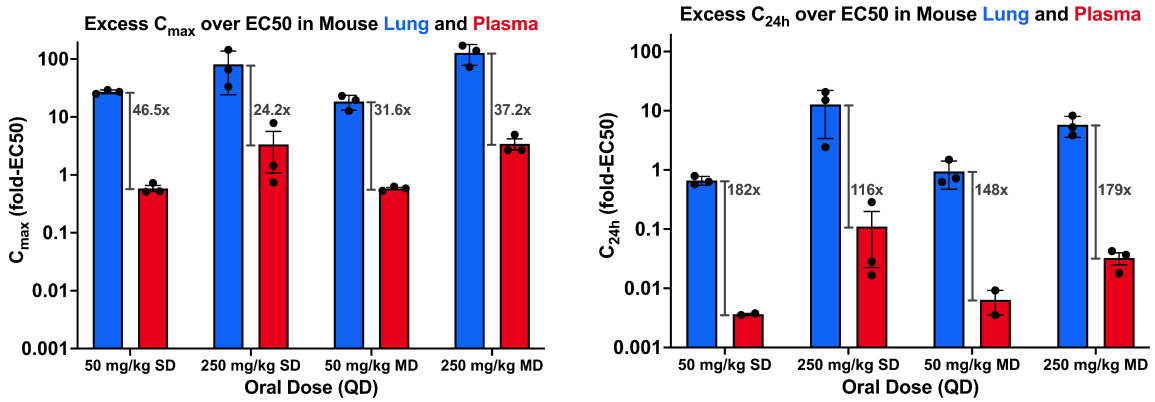

Figure 5. MDL-001 concentration in respiratory tissue is many fold greater than the EC_{50} against ILI-viruses at safe doses. The fold differences are shown in **(A)** mice and **(B)** rats. MDL-001's EC_{50} = 0.668 μM (305 ng/mL) against the SARS-CoV-2 Beta variant was chosen for normalization as it is the lowest EC_{50} that also shows an SI>10.

References

1. Baker, R. E. *et al.* Infectious disease in an era of global change. *Nature reviews. Microbiology* **20**, 193–205 (2022).

2. Piret, J. & Boivin, G. Pandemics Throughout History. *Frontiers in microbiology* **11**, 631736 (2020).

3. Government Accountability Office (GAO). *Infectious Disease Preparedness: Assessing Global Efforts and Opportunities for Improvement* . https://www.gao.gov/products/gao-23-106089 (2023).

4. Bird, B. H. & Mazet, J. A. K. Detection of Emerging Zoonotic Pathogens: An Integrated One Health Approach. *Annual review of animal biosciences* **6**, 121–139 (2018).

5. Grubaugh, N. D. *et al.* Tracking virus outbreaks in the twenty-first century. *Nature microbiology* **4**, 10–19 (2019).

6. Jacob, S. T. *et al.* Ebola virus disease. *Nature reviews. Disease primers* **6**, 13 (2020).

7. Uyeki, T. M., Hui, D. S., Zambon, M., Wentworth, D. E. & Monto, A. S. Influenza. *Lancet (London, England)* **400**, 693–706 (2022).

8. de Graaf, M., van Beek, J. & Koopmans, M. P. G. Human norovirus transmission and evolution in a changing world. *Nature reviews. Microbiology* **14**, 421–33 (2016).

9. Stadler, K. *et al.* SARS--beginning to understand a new virus. *Nature reviews. Microbiology* **1**, 209–18 (2003).

10. Koelle, K., Martin, M. A., Antia, R., Lopman, B. & Dean, N. E. The changing epidemiology of SARS-CoV-2. *Science (New York, N.Y.)* **375**, 1116–1121 (2022).

11. Pierson, T. C. & Diamond, M. S. The emergence of Zika virus and its new clinical syndromes. *Nature* **560**, 573–581 (2018).

12. von Delft, A. *et al.* Accelerating antiviral drug discovery: lessons from COVID-19. *Nature reviews. Drug discovery* **22**, 585–603 (2023).

13. Bekerman, E. & Einav, S. Infectious disease. Combating emerging viral threats. *Science (New York, N.Y.)* **348**, 282–3 (2015).

14. Karim, M., Lo, C.-W. & Einav, S. Preparing for the next viral threat with broad-spectrum antivirals. *The Journal of clinical investigation* **133**, (2023).

15. NIAID. *Target Product Profile (TPP) for APP Antiviral Therapeutics SARS-CoV-2*. https://www.niaid.nih.gov/research/target-product-profiles-antivirals (2023).

16. Spengler, J. R. *et al.* Meeting report: 35th International Conference on Antiviral Research in Seattle, Washington, USA - March 21-25, 2022. *Antiviral research* **211**, 105521 (2023).

17. Venkataraman, S., Prasad, B. V. L. S. & Selvarajan, R. RNA Dependent RNA Polymerases: Insights from Structure, Function and Evolution. *Viruses* **10**, (2018).

18. Palazzotti, D. *et al.* Small Molecule Drugs Targeting Viral Polymerases. *Pharmaceuticals (Basel, Switzerland)* **17**, (2024).

19. Martinez, M. A. Efficacy of repurposed antiviral drugs: Lessons from COVID-19. *Drug discovery today* **27**, 1954–1960 (2022).

20. Umansky, T. *et al.* ChemPrint: An AI-Driven Framework for Enhanced Drug Discovery. *bioRxiv* 2024.03.22.586314 (2024) doi:10.1101/2024.03.22.586314.

21. Woods, V. *et al.* Discovery of RdRp Thumb-1 as a novel broad-spectrum antiviral family of targets and MDL-001 as a potent broad-spectrum inhibitor thereof - Part I: A Bioinformatics and Deep Learning Approach. *bioRxiv* 2024.03.29.587401 (2024) doi:10.1101/2024.03.29.587401.

22. Lemm, J. A. *et al.* Preclinical characterization of BMS-791325, an allosteric inhibitor of hepatitis C Virus NS5B polymerase. *Antimicrobial agents and chemotherapy* **58**, 3485–95 (2014).

23. Zhao, F., Liu, N., Zhan, P., Jiang, X. & Liu, X. Discovery of HCV NS5B thumb site I inhibitors: core-refining from benzimidazole to indole scaffold. *European journal of medicinal chemistry* **94**, 218–28 (2015).

24. Gentile, I., Zappulo, E., Buonomo, A. R., Maraolo, A. E. & Borgia, G. Beclabuvir for the treatment of hepatitis C. *Expert opinion on investigational drugs* **24**, 1111–21 (2015).

25. Beaulieu, P. L. *et al.* Discovery of the first thumb pocket 1 NS5B polymerase inhibitor (BILB 1941) with demonstrated antiviral activity in patients chronically infected with genotype 1 hepatitis C virus (HCV). *Journal of medicinal chemistry* **55**, 7650–66 (2012).

26. Gao, Y. *et al.* Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science (New York, N.Y.)* **368**, 779–782 (2020).

27. Hillen, H. S. *et al.* Structure of replicating SARS-CoV-2 polymerase. *Nature* **584**, 154–156 (2020).

28. Stielow, M. *et al.* The Bioavailability of Drugs-The Current State of Knowledge. *Molecules (Basel, Switzerland)* **28**, (2023).

29. Gandhi, T. *et al.* Safety and pharmacokinetic evaluation of ERA-923 in healthy postmenopausal women in two double masked phase I trials. *Annals of Oncology* **11**, 41 (2000).

30. Cotreau, M. M. *et al.* Multiple‐Dose, Safety, Pharmacokinetics, and Pharmacodynamics of a New Selective Estrogen Receptor Modulator, ERA‐923, in Healthy Postmenopausal Women. *The Journal of Clinical Pharmacology* **42**, 157–165 (2002).

31. Owen, D. R. *et al.* An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19. *Science (New York, N.Y.)* **374**, 1586–1593 (2021).

32. Rubin, D., Chan-Tack, K., Farley, J. & Sherwat, A. FDA Approval of Remdesivir - A Step in the Right Direction. *The New England journal of medicine* **383**, 2598–2600 (2020).

33. Harris, E. FDA Grants Full Approval to Paxlovid, COVID-19 Antiviral Treatment. *JAMA* **329**, 2118 (2023).

34. Sorbera, L. A., Castaner, J. & Silvestre, J. S. Pipendoxifene. *Drugs of the Future* **27**, 0942 (2002).

35. Zaliani, A. *et al.* Cytopathic SARS-CoV-2 screening on VERO-E6 cells in a large-scale repurposing effort. *Scientific Data* **9**, 405 (2022).

36. Balchak, S. K., Hedge, J. M., Murr, A. S., Mole, M. L. & Goldman, J. M. Influence of the drinking water disinfection by-product dibromoacetic acid on rat estrous cyclicity and ovarian follicular steroid release *in vitro*. *Reproductive toxicology (Elmsford, N.Y.)* **14**, 533–9 (2000).

37. Riva, L. *et al.* Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. *Nature* **586**, 113–119 (2020).

38. Boras, B. *et al.* Preclinical characterization of an intravenous coronavirus 3CL protease inhibitor for the potential treatment of COVID19. *Nature Communications* **12**, 6055 (2021).

39. Lohmann, V. *et al.* Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science (New York, N.Y.)* **285**, 110–3 (1999).