

**DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
CLINICAL VIROLOGY REVIEW**

EUA-000046

DATE REVIEW COMPLETED: 05/04/2020

Reviewers: Eric Donaldson, Ph.D.

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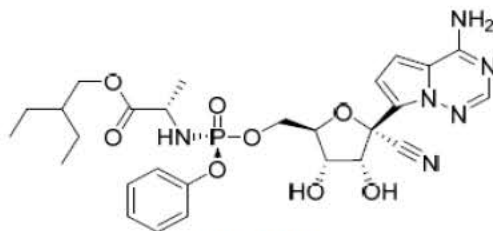
Regulatory History (Virology-Related Submissions Reviewed):

Submission	Received	Assigned	Content
Pre-EUA-000046 SDN 002	04/08/2020	04/08/2020	Pre EUA submission
Pre-EUA-000046 SDN 003	04/10/2020	04/13/2020	Response to Clinical comments communicated to the sponsor on 4/7/2020.
Pre-EUA-000046 SDN 007	4/24/2020	4/27/2020	Response to Clinical comments communicated to the sponsor on 4/7/2020; revised manuscript describing severe Chinese randomized controlled trial.
Pre-EUA-000046 SDN 008	4/29/2020	4/29/2020	Response to comments on HCPFS communicated to the sponsor on 4/28/2020; topline summary of GS-US-540-5773, "A Multicenter, Adaptive, Randomized Blinded Controlled Trial of the Safety and Efficacy of Investigational Therapeutics for the Treatment of COVID-19
Pre-EUA-000046 SDN 012	4/30/2020	5/1/2020	Response to comments on HCPFS communicated to the sponsor on 4/30/2020.
Pre-EUA-000046 SDN 013	4/30/2020	5/1/2020	Response to comments on HCPFS communicated to the sponsor on 4/30/2020 (second set).
EUA-000046 SDN 000	4/17/2020	4/17/2020	Request for Emergency Use Authorization.
EUA-000046 SDN 006	4/22/2020	4/23/2020	Response to comments on the HCPFS communicated to the sponsor on 4/17/20; Interim 1 of Summary Report for IN-US-540-5755 (Compassionate Use).
EUA-000046 SDN 007	4/24/2020	4/23/2020	Revised manuscript: "A Phase 3 Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy and Safety of Remdesivir in Hospitalized Adult Patients with Severe 2019-nCoV Respiratory Disease".

Product Names: remdesivir (GS-5734) and other investigational agents

Chemical Names: 2-Ethylbutyl (2S)-2-[[[(S)-{[(2R,3S,4R,5R)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methoxy}(phenoxy)phosphoryl]amino}propanoate

Structure:



GS-5734

Molecular Formula: C₂₇H₃₅N₆O₈P

Molecular Weight: 602.6

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Drug Category: antiviral

Indication: Treatment of SARS-CoV-2 (aka 2019 nCoV) infection

Dosage Form/Route of administration: liquid/intravenous

Supporting documents: IND1 (b) (4) IND 1 (b) (4) Pre-IND 1 (b) (4) submissions

Abbreviations: CoV, coronavirus; COVID-19, coronavirus infectious disease originating in 2019; CU, compassionate use; ECMO, extracorporeal membrane oxygenation; EUA, emergency use authorization; ExoN, exoribonuclease; MERS, Middle East respiratory syndrome; OP, oropharyngeal; RCT, randomized controlled clinical trial; RDV, remdesivir; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2;

EAU SUBMISSION SUMMARY

This review documents the Emergency Use Authorization (EUA) request for the use of remdesivir (RDV) for the treatment of coronavirus infectious disease originating in 2019 (COVID-19), which is caused by the severe acute respiratory syndrome (SARS) coronavirus (CoV) type 2 (SARS-CoV-2). The sponsor, Gilead Sciences, noted that this request is in accordance with the January 2017 Guidance for Industry, “*Emergency Use Authorization of Medical Products and Related Authorities*,” for RDV for the treatment of COVID-19.

This EUA request for remdesivir was submitted on the basis that the sponsor believes the below statutory criteria for an EUA in Section 564(c) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) are met:

- First, COVID-19 is a serious or life-threatening disease caused by SARS-CoV-2 infection
- Second, based on the totality of the scientific evidence available at this time, it is reasonable to believe that remdesivir may be effective for the treatment of COVID-19 and that the known and potential benefits outweigh the known and potential risks
- Third, there are no adequate, approved, and available alternatives to treat COVID-19. An authorization for the emergency use of remdesivir will meet an urgent and unmet need during this unprecedented public health emergency.

The sponsor submitted a Pre-EUA request [EUA-000046](#) on April 8, 2020. On April 17, 2020 the sponsor submitted a formal EUA request under [EUA-000046](#). From the initial pre-EUA request through the approval of the EUA on May 1, 2020, there were several rounds of revisions to the EUA template, the Health Care Professionals Fact Sheet, and the Patient/Caregiver Fact Sheet. This review provides the issues and sections edited for each of these documents from a Clinical Virology perspective. The submissions that were assigned and reviewed by Clinical Virology are presented in the table above under [Regulatory History \(Virology-Related Submissions Reviewed\)](#).

BACKGROUND

Remdesivir is a nucleotide prodrug that is intracellularly metabolized into its active form GS-441524, which is an analog of adenosine triphosphate that inhibits viral RNA synthesis. The GS-441524 adenosine nucleotide analog is incorporated into the nascent RNA chain by the coronavirus viral RNA polymerase (nsp12) and evades proofreading by the coronavirus exoribonuclease, resulting in a decrease in coronavirus RNA production. It is currently unknown whether it terminates RNA chains or causes mutations in them ([Agostini et al., 2018](#)). For an overview of the Clinical Virology data submitted for remdesivir, please see [Appendix 1](#).

PRE-EUA AND EUA REVIEW

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The sections of each document that were edited by Clinical Virology are copied below. Of note, there were no Clinical Virology comments or edits suggested for the Fact Sheet for Patients/Caregivers.

Fact Sheet for HCP

Clinical Virology comments made to the original document:

(b)(5)/internal comments on pre-EUA Fact Sheet from April 2020; (b)(4)/pre-EUA Fact Sheet from April 2020

EDITED SECTIONS OF THE FACT SHEET FOR HEALTH CARE PROFESSIONALS

This section shows all of the changes that were made and accepted from the original document (see [Appendix 2](#)) to the final approved Fact Sheet (see [Appendix 3](#)).

14.1 Mechanism of Action

Remdesivir ~~broadly and rapidly~~ is an adenosine nucleotide prodrug that distributes into cells where it is ~~efficiently~~ metabolized to form the pharmacologically active nucleoside triphosphate metabolite. ~~Efficient metabolism~~ Metabolism of remdesivir to remdesivir triphosphate has been demonstrated in multiple cell types. Remdesivir triphosphate acts as an analog of adenosine triphosphate (ATP) and competes with the natural ATP substrate for incorporation into nascent RNA chains by the SARS-CoV-2 RNA-dependent RNA polymerase, which results in delayed chain termination during replication of the viral RNA. Remdesivir triphosphate is a weak inhibitor of mammalian DNA and RNA polymerases with low potential for mitochondrial toxicity.

15. MICROBIOLOGY/RESISTANCE INFORMATION

Antiviral Activity

Remdesivir ~~shows potent in vitro activity against 571 the human pathogenic CoVs MERS-CoV and SARS-CoV in multiple relevant human~~ exhibited cell types. Initial in vitro testing showed that ~~remdesivir has potent~~ culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary human airway epithelial (HAE) cells with a 50% effective concentration (EC₅₀) of 9.9 nM after 48 hours of treatment. The EC₅₀ values of remdesivir against SARS-CoV-2 in Vero cells (EC₅₀ = 0.137 μM after 24 hours and 0.750 μM after 48 hours of post-treatment).

Resistance

No clinical data are available on the development of SARS-CoV-2 resistance to remdesivir. The ~~in vitro~~ cell culture development of SARS-CoV-2 resistance to remdesivir has not been assessed to date.

~~In vitro~~ Cell culture resistance profiling of remdesivir using the rodent CoV murine hepatitis virus identified 2 ~~mutations~~ substitutions (F476L and V553L) in the viral RNA-dependent RNA polymerase

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at residues conserved across CoVs that conferred moderate 5.6 fold reduced susceptibility to remdesivir. The mutant viruses showed reduced viral fitness in vitro cell culture and introduction of the analogous mutations corresponding substitutions (F480L and V557L) into SARS-CoV resulted in 6-fold reduced susceptibility to remdesivir in cell culture and attenuated SARS-CoV pathogenesis in a mouse model.

17. ANIMAL PHARMACOLOGIC AND EFFICACY DATA

It is unknown, at present, how the observed efficacy antiviral activity of remdesivir in animal models of coronavirus SARS-CoV-2 infection will translate into clinical efficacy in patients with symptomatic disease. Key attributes of the remdesivir nonclinical profile supporting its development for the treatment of COVID-19 are provided below:

- Remdesivir inhibited the in vitro replication showed cell culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary HAE cells (EC₅₀ value= 9.9 nM). The EC₅₀ values of remdesivir against SARS-CoV-2 in Vero cells with an EC₅₀ value of 0 has been reported to be 137 μM nM at 24 hours and 0.750 μM after 24 and nM at 48 hours of post-treatment, respectively {Study PC-540-2001}.
- Remdesivir demonstrated prophylactic and therapeutic efficacy showed antiviral activity in a mouse model of SARS-CoV pathogenesis. Administration of 25 mg/kg remdesivir subcutaneously twice daily beginning 1 day before or 1 day after SARS-CoV inoculation resulted in significantly reduced lung viral load and improved clinical signs of disease and lung function {Sheahan 2017}.
- In a mouse model of MERS-CoV pathogenesis, both prophylactic and therapeutic administration of 25 mg/kg remdesivir subcutaneously twice daily improved pulmonary function and reduced lung viral loads and severe lung pathology. In contrast, prophylactic lopinavir/ritonavir and interferon beta (LPV/RTV-IFNβ) slightly reduced viral loads without impacting other disease parameters. Therapeutic LPV/RTV-IFNβ improved pulmonary function but did not reduce virus replication or severe lung pathology {Sheahan 2020}.
- Remdesivir also showed prophylactic and therapeutic efficacy in MERS647 CoV-2-infected rhesus monkeys. Administration of remdesivir at 10/5 mg/kg or (10 mg/kg first dose, followed by 5 mg/kg once daily for 7 days thereafter) using IV bolus injection beginning 1 day prior to MERS-CoV initiated 12 hours post-inoculation with SARS-CoV-2 resulted in a significant reduction of clinical scores, in clinical signs of respiratory disease, lung pathology and gross lung lesions, and lung viral RNA levels compared to with vehicle-treated animals. Therapeutic remdesivir treatment of 5 mg/kg once daily using IV bolus injection initiated 12 hours post inoculation also resulted in reduced clinical signs, reduced virus replication in the lungs, and decreased presence and severity of lung lesions {De Wit 2020}.

EDITED SECTIONS OF THE PRE-EUA TEMPLATE

This section shows all of the changes that were made and accepted from the original document (see [Appendix 4](#)) to the final approved EUA template (see [Appendix 5](#)). The template instructions for these sections is provided in [Appendix 6](#).

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Identifying Information

Therapeutic Class	Coronavirus nucleoside analog RNA polymerase inhibitor
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XIII. Nonclinical Data to Support Efficacy

- Remdesivir is a nucleotide prodrug that is intracellularly metabolized into its active form GS-441524, which is an analog of adenosine triphosphate that inhibits viral RNA synthesis.
- The GS-441524 adenosine nucleotide analog is incorporated into the nascent RNA chain by the coronavirus viral RNA polymerase ([nsp12](#)) and evades proofreading by the coronavirus exoribonuclease, resulting in a decrease in coronavirus RNA production. It is currently unknown whether it terminates RNA chains or causes mutations in them ([Agostini et al., 2018](#)).
- Remdesivir ~~has broad~~[exhibited](#) antiviral activity against ~~multiple, unrelated~~[several](#) human RNA viruses including, [SARS-CoV and](#) Middle East Respiratory Syndrome (MERS) ~~coronavirus and SARS-CoV~~, Ebola virus, Marburg virus, Junin virus, and Lassa fever virus.
- The EC₅₀ values of remdesivir against the SARS-CoV-2 grown in Vero cells has been reported to be ~~0.13714~~ [0.75](#) μM at 24 hours and 0.75 μM at 48 hours post-treatment ([Chinese CDCPC-540-2001](#)).
- An EC₅₀ value of 0.77 μM in Vero cells has been reported for RDV against SARS-CoV-2 by the Wuhan Institute of Virology ([Wang et al., 2020](#)).
- Remdesivir inhibited a recombinant chimeric virus expressing the RdRp gene (nsp12) of SARS-CoV-2 in a backbone of SARS-CoV with a ~~luciferase~~[fluorescent](#) reporter ~~protein~~ in Huh7 cells with an EC₅₀ value = 0.003 μM. ([PC-540-2002](#)).
- [Cell culture antiviral assessments against other human and animal coronaviruses are summarized below:](#)
 - Remdesivir and GS-466547 (an opposite diastereomer) were tested in cell culture antiviral activity assessments ~~against human pathogenic coronaviruses, specifically MERS-CoV and SARS-CoV, and MERS -CoV.~~ Remdesivir and GS-466547 inhibited replication of MERS-CoV in Vero E6 cells, with mean EC₅₀ values of 0.52 and 0.42 μM, respectively. No cytotoxicity was observed at 10 μM, the highest concentration tested, indicating selective inhibition of virus replication with selectivity indices of >19 and >24, respectively.
 - ~~In addition,~~ GS-466547 inhibited ~~both SARS-CoV and~~ MERS-CoV ~~and SARS-CoV~~ replication in human airway epithelial (HAE) cultures as ~~observed~~[measured](#) by the reduction in the expression of ~~the RFP or GFP~~[a fluorescent](#) reporter ~~genes~~[protein](#) at compound concentrations ranging from 0.1 to 1.1 μM.
 - The activity of remdesivir against ~~SARS-CoV and~~ MERS-CoV ~~and SARS-CoV~~ was ~~also~~ assessed using recombinant viruses expressing a fluorescent reporter protein in a continuous human lung epithelial cell line, 2B4 (Calu-3; MERS-CoV only) and primary ~~human airway epithelial~~[HAE](#) cells (~~SARS-CoV and MERS-CoV and SARS-CoV~~). Remdesivir inhibited MERS-CoV replication in Calu-3 cells, with a mean EC₅₀ value of 0.025 μM ([Sheahan et al., 2017](#)). In HAE cells, remdesivir inhibited both ~~SARS-CoV and~~ MERS-CoV ~~and SARS-CoV~~ replication with EC₅₀ values of

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0.074069 and 0.069074 μM , respectively. In both HAE and Calu-3 cells, no cytotoxicity was observed at 10 μM of remdesivir, the highest concentration tested, indicating that remdesivir has selectivity indices >100 in these cell culture systems ([Sheahan et al., 2017](#)).

- Remdesivir ~~also~~ showed cell culture antiviral activity against human betacoronavirus OC43 and alphacoronavirus 229E as well as the animal betacoronavirus murine hepatitis virus and the genetically divergent porcine deltacoronavirus with submicromolar EC_{50} values ranging from 0.02 to 0.15 μM in various cell types ([Agostini et al., 2018](#); [Brown et al., 2019](#); [Sheahan et al., 2017](#)).
- Remdesivir inhibited the replication of SARS-CoV-2 and SARS-CoV with EC_{50} values of 0.0099 μM and 0.0066 μM , respectively, in HAE cells after 48 hours of treatment. No cytotoxicity has been observed for remdesivir in HAE cells at concentrations up to 10 μM (CC_{50} value ~~$>10 \mu\text{M}$~~ $10 \mu\text{M}$) ([PC-540-2002](#)). The dose response curve of RDV against SARS-CoV-2 exhibited a shallow dose-dependent increase in inhibition compared to the response against SARS-CoV, indicating that RDV may be more active against SARS-CoV. Alternatively, there could be a lag in formation of the active diphosphate partially overcome by higher concentrations due to slow uptake by the cells, slow metabolism of the prodrug to the monophosphate, or a slow phosphorylation step.
- The development of resistance to RDV in coronaviruses has been assessed by cell culture passaging of murine hepatitis virus (MHV), a coronavirus, in the presence of the remdesivir parent nucleoside, GS-441524. After 23 passages, two substitutions were selected in the nsp12 polymerase at residues conserved across coronaviruses: F476L and V553L.
- Compared to wild-type virus, recombinant MHV containing the F476L substitution showed 2.4-fold reduced susceptibility to RDV, and MHV containing the V553L substitution demonstrated 5-fold reduced susceptibility, while the double mutant conferred 5.6-fold reduced susceptibility to RDV in cell culture. ~~The potential relevance of this finding to SARS-CoV-2 is unknown.~~
- ~~Remdesivir demonstrated antiviral activity in a nonlethal mouse model of SARS-CoV pathogenesis. Administration of 25 mg/kg RDV subcutaneously twice daily beginning 1 day before or 1 day after SARS-CoV inoculation resulted in significantly reduced lung viral load and improved clinical signs of disease as well as lung function ([Sheahan et al., 2017](#)).~~
- ~~In a lethal mouse model of MERS-CoV pathogenesis, both prophylactic and therapeutic administration of 25 mg/kg RDV subcutaneously twice daily improved pulmonary function and reduced lung viral loads and severe lung pathology ([Sheahan et al., 2020](#)). Treatment with RDV did not improve survival.~~
- ~~A 7-day treatment regimen of 10 mg/kg GS-5734 administered once daily by IV bolus injection beginning 1 day prior to virus exposure significantly reduced viral lung burden and clinical disease signs in the nonlethal MERS-CoV infected rhesus macaque model. In several of the MERS-CoV infected animals, the treatment with GS-5734 adversely affected renal function.~~
- There are no directly relevant animal studies showing that remdesivir inhibits SARS-CoV-2 or improves outcomes in an animal model to date using a treatment paradigm.

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- In a nonlethal non-human primate model of SARS-CoV-2 pathogenesis under a post-exposure prophylaxis paradigm that initiated treatment 12 hours after challenge with SARS-CoV-2, administration of a loading dose of 10 mg/kg remdesivir, followed by a 5 mg/kg dose 12 hours after the loading dose, and then a daily maintenance dose of 5 mg/kg for 5 additional days delivered as a slow intravenous bolus injection resulted in a marginal clinical benefit during SARS-CoV-2 infection in rhesus macaques. There were a number of limitations to this study, including the lack of an adequately characterized and validated model, use of a non-lethal model that cannot be used to assess mortality or severe respiratory disease, and lack of clarity related to the optimal route and dose of the viral challenge. Additionally, there were differences in RDV prodrug and metabolite exposures between infected NHPs, healthy NHPs, and healthy humans that further impede extrapolation of these results to humans with COVID-19.
- In a non-lethal mouse model of SARS-CoV pathogenesis, administration of 25 mg/kg remdesivir subcutaneously twice daily beginning 1 day before or 1 day after SARS-CoV inoculation resulted in reduced lung viral load and improved clinical signs of disease and lung function (Sheahan et al., 2017).
- In a mouse model of MERS-CoV pathogenesis, administration of 25 mg/kg remdesivir subcutaneously twice daily beginning 1 day before or 1 day after MERS-CoV inoculation improved pulmonary function and reduced lung viral loads and lung pathology (Sheahan et al., 2020). Of note, this mouse model was not uniformly lethal at the challenge doses used in these studies, which resulted in 50% mortality by Day 6. Treatment with RDV did not improve survival.
- In MERS-CoV-infected rhesus monkeys, administration of remdesivir at 10 mg/kg or 5 mg/kg once daily for 7 days using IV bolus injection beginning 1 day prior to MERS-CoV inoculation resulted in a reduction of clinical scores, clinical signs of respiratory disease, and viral RNA levels compared to vehicle-treated animals (De Witt et al., 2020).

Comment: None of the animal model data rise to the level of direct relevance to support antiviral activity of remdesivir against SARS-CoV-2. Therefore, the following sections are not relevant to the EUA.

CLINICAL DATA TO SUPPORT EUA ACTIONS

The pre-EUA

(b)5/internal comments on pre-EUA from April 2020; (b)(4)/pre-EUA from April 2020

A draft memo stating DAV's position on the EUA was drafted by the CDTL and can be found in [Appendix 7](#).

On April 29, 2020, NIAID, part of the National Institutes of Health, provided topline data from a Phase 3 adaptive, randomized, double-blind, placebo-controlled trial for RDV as a potential treatment for hospitalized adult patients diagnosed with COVID-19 (Gilead study number CO-US-540-5776 [[NCT 04280705](#)]). This clinical trial evaluated remdesivir 200 mg once daily for 1 day followed by remdesivir 100 mg once daily for 9 days (for a total of up to 10 days of intravenously administered therapy) in hospitalized adult patients with COVID-19. The trial enrolled 1063 hospitalized patients in a 1:1 manner to receive remdesivir or placebo. The primary clinical endpoint was time to recovery within 28 days after randomization. In a preliminary analysis of the primary endpoint performed after 606 recoveries were attained, the median time to recovery was 11 days in the remdesivir group compared to 15 days in the placebo group (hazard ratio 1.31; 95% CI 1.12 to 1.54, $p < 0.001$). Mortality was 8.0% for the remdesivir group versus 11.6% for the placebo group ($p = 0.059$).

Top line results were also available from a Gilead-sponsored trial, GS-US-540-5773 ([NCT04292899](#)), entitled "*A Phase 3 Randomized Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734TM) in Participants with Severe COVID-19*" that compared 5-day and 10-day remdesivir durations in patients with severe COVID-19. Remdesivir was administered intravenously at a dose of 200 mg on Day 1 followed by 100 mg on subsequent days. There was no placebo or standard of care group. A total of 401 patients were randomized in a 1:1 ratio to the 5-day and 10-day remdesivir groups, and the primary analysis set included 200 patients in the 5-day group and 197 patients in the 10-day group. Overall, results in this trial were suggestive of similar treatment effects with 5-day and 10-day regimens in this patient population, with appropriate caveats related to the open-label design.

The data from clinical trial CO-US-540-5776 ([NCT 04280705](#)) was considered to be sufficient to officially grant an EUA for remdesivir, with support from clinical trial GS-US-540-5773 ([NCT04292899](#)), and the EUA was officially approved on May 1, 2020.

In general, Clinical Virology is supportive of the EUA approval based on the topline results from CO-US-540-5776 (NCT 04280705) which showed that the median time to recovery was 11 days in the remdesivir group compared to 15 days in the placebo group (hazard ratio 1.31; 95% CI 1.12 to 1.54, $p < 0.001$). Mortality was 8.0% for the remdesivir group versus 11.6% for the placebo group ($p = 0.059$). Of note, no clinical virology data have been submitted from the NIAID clinical trial at this time.

REPLY TO CLINICAL VIROLOGY COMMENT

In EUA-000046 SDN 003, the sponsor responded to a Clinical Virology comment that was communicated to the sponsor during discussion of the compassionate use data that the sponsor presented and planned to use as a basis for the EUA.

Clinical Virology comment: We note that the median duration of symptoms before initiation of RDV was 13 days (IQR 9, 15) in CU patients described in the draft manuscript entitled, *Compassionate Use of Remdesivir for Patients with Severe Covid-19*. Early data from published reports (Zou et al., 2020; Xu et al., 2020; To et al., 2020; Yu and Li, 2020; Lescure et al., 2020) indicate that 13 days after onset of

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symptoms may be very late in the CoV infection cycle, and potentially beyond the therapeutic window for RDV. Moreover, viral RNA detected by real time RT-PCR assays at that time may represent remnants of viral RNA genomes and not infection-competent viral particles (Wolfel et al., 2020). Viral loads are likely declining at this time due to the activation of the adaptive immune response. Please comment on how the mechanism of action of RDV is contributing to a clinical benefit at this late stage of the infection cycle.

Gilead's Response: Details on the literature review, including the subset of patients used for the comparisons provided on 04 April 2020 (IND 1 (b) (4) /SN 0033), are included in Attachment 3. The corresponding literature sources with yellow highlights to indicate the subset of patient used are provided in Module 5.4. Gilead acknowledges the Agency's comment that a proportion of CU patients were treated in the 2nd week of infection. The body of data on the infection cycle of SARS-CoV-2 and utility of monitoring viral RNA in the upper respiratory tract in this disease is growing. It is important to note that the literature on viral RNA or active viral replication in the lower respiratory tract, the site of disease in these patients with pneumonia, is limited at this time.

Clinical Virology response: The sponsor's reply did not address the original comment, but they noted that data on the viral RNA or active viral replication in the lower respiratory tract are lacking. It is clear that prolonged replication occurs in some subjects for several weeks; however, it is unclear if all subjects with severe disease are experiencing prolonged viral replication in the lower respiratory tract. No additional regulatory information is required at this time.

Clinical Virology Outstanding Issues

Remdesivir is an analog of adenosine triphosphate that inhibits viral RNA synthesis, and as such, the drug would most likely work early in the infection cycle when SARS-CoV-2 replication is occurring at a high level. Most patients who are hospitalized with COVID-19 are entering the hospital during the second week of infection when viral loads are in decline and the underlying disease is associated with severe lung pathology driven by a hyperactive immune response and cytokine release syndrome. It is not clear that remdesivir will have much of an impact on viral replication this late into the infection cycle.

CONCLUSIONS

- This review documents the EUA request for the use of remdesivir for the treatment of COVID-19, which caused by SARS-CoV-2 infection. The EUA request was for remdesivir for treatment of severe COVID-19 in hospitalized patients.
- The sponsor noted that this request is in accordance with the January 2017 Guidance for Industry, "Emergency Use Authorization of Medical Products and Related Authorities," for RDV for the treatment of COVID-19.
- This EUA request for remdesivir was submitted on the basis that the sponsor believes the below statutory criteria for an EUA in Section 564(c) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) are met:
 - First, COVID-19 is a serious or life-threatening disease caused by SARS-CoV-2 infection
 - Second, based on the totality of the scientific evidence available at this time, it is reasonable to believe that remdesivir may be effective for the treatment of COVID-19 and that the known and potential benefits outweigh the known and potential risks
 - Third, there are no adequate, approved, and available alternatives to treat COVID-19. An authorization for the emergency use of remdesivir will meet an urgent and unmet need during

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this unprecedented public health emergency.

- The sponsor submitted a Pre-EUA request [EUA-000046](#) on April 8, 2020. On April 17, 2020 the sponsor submitted a formal EUA request under [EUA-000046](#). From the initial pre-EUA request through the approval of the EUA on May 1, 2020, there were several rounds of revisions to the EUA template, the Health Care Professionals Fact Sheet, and the Patient/Caregiver Fact Sheet.
- This review covered the issues and sections edited for each of these documents from a Clinical Virology perspective.
- In general, Clinical Virology is supportive of the EUA approval based on the topline results from CO-US-540-5776 ([NCT 04280705](#)) which showed that the median time to recovery was 11 days in the remdesivir group compared to 15 days in the placebo group (hazard ratio 1.31; 95% CI 1.12 to 1.54, $p < 0.001$). Mortality was 8.0% for the remdesivir group versus 11.6% for the placebo group ($p = 0.059$).
- Clinical Virology remains concerned about the disconnect regarding this drugs mechanism of action and the timing of treatment administration. Remdesivir is an analog of adenosine triphosphate that inhibits viral RNA synthesis, and as such, the drug would most likely work early in the infection cycle when SARS-CoV-2 replication is occurring at a high level. Most patients who are hospitalized with COVID-19 are entering the hospital during the second week of infection when viral loads are in decline and the underlying disease is associated with severe lung pathology driven by a hyperactive immune response and cytokine release syndrome. It is not clear that remdesivir will have much of an impact on viral replication this late into the infection cycle.
- No additional regulatory action is required for this submission.

SIGNATURES

Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer

CONCURRENCES

_____ **Date:** _____
HFD-530/Clin Micro TL/J O'Rear

cc:
HFD-530/preIND
HFD-530/Division File
HFD-530/RPM/Kim

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Appendix 1

Overview of Remdesivir

- Remdesivir is a nucleotide prodrug that is intracellularly metabolized into its active form GS-441524, which is an analog of adenosine triphosphate that inhibits viral RNA synthesis.
- The GS-441524 adenosine nucleotide analog is incorporated into the nascent RNA chain by the coronavirus viral RNA polymerase (nsp12) and evades proofreading by the coronavirus exoribonuclease, resulting in a decrease in coronavirus RNA production. It is currently unknown whether it terminates RNA chains or causes mutations in them ([Agostini et al., 2018](#)).
- Remdesivir exhibited antiviral activity against several human RNA viruses including, SARS-CoV and Middle East Respiratory Syndrome (MERS) CoV, Ebola virus, Marburg virus, Junin virus, and Lassa fever virus.
- Remdesivir exhibited cell culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary human airway epithelial (HAE) cells with a 50% effective concentration (EC₅₀) of 9.9 nM after 48 hours of treatment (PC-540-2001). The EC₅₀ values of remdesivir against SARS-CoV-2 in Vero cells was 137 nM at 24 hours and 750 nM at 48 hours post-treatment (PC-540-2001).
- An EC₅₀ value of 0.77 µM in Vero cells has been reported for RDV against SARS-CoV-2 by the Wuhan Institute of Virology ([Wang et al., 2020](#)).
- Remdesivir inhibited a recombinant chimeric virus expressing the RdRp gene (nsp12) of SARS-CoV-2 in a backbone of SARS-CoV with a fluorescent reporter protein in Huh7 cells with an EC₅₀ value = 0.003 µM (PC-540-2002).
- Cell culture antiviral assessments against other human and animal coronaviruses are summarized below:
 - Remdesivir and GS-466547 (an opposite diastereomer) were tested in cell culture antiviral activity assessments against SARS-CoV and MERS -CoV. Remdesivir and GS-466547 inhibited replication of MERS-CoV in Vero E6 cells with mean EC₅₀ values of 0.52 and 0.42 µM, respectively. No cytotoxicity was observed at 10 µM, the highest concentration tested, indicating selective inhibition of virus replication with selectivity indices of >19 and >24, respectively.
 - GS-466547 inhibited SARS-CoV and MERS-CoV replication in human airway epithelial (HAE) cultures as measured by the reduction in the expression of a fluorescent reporter protein at compound concentrations ranging from 0.1 to 1.1 µM.
 - The activity of remdesivir against SARS-CoV and MERS-CoV was assessed using recombinant viruses expressing a fluorescent reporter protein in a continuous human lung epithelial cell line, 2B4 (Calu-3; MERS-CoV only) and primary HAE cells (SARS-CoV and MERS-CoV). Remdesivir inhibited MERS-CoV replication in Calu-3 cells, with a mean EC₅₀ value of 0.025 µM ([Sheahan et al., 2017](#)). In HAE cells, remdesivir inhibited both SARS-CoV and MERS-CoV replication with EC₅₀ values of 0.069 and 0.074 µM, respectively. In both HAE and Calu-3 cells, no cytotoxicity was observed at 10 µM of remdesivir, the highest concentration tested, indicating that remdesivir has selectivity indices >100 in these cell culture systems ([Sheahan et al., 2017](#)).
 - Remdesivir showed cell culture antiviral activity against human betacoronavirus OC43 and alphacoronavirus 229E as well as the animal betacoronavirus murine hepatitis virus

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and the genetically divergent porcine deltacoronavirus with submicromolar EC₅₀ values ranging from 0.02 to 0.15 µM in various cell types ([Agostini et al., 2018](#); [Brown et al., 2019](#); [Sheahan et al., 2017](#)).

- Remdesivir inhibited the replication of SARS-CoV-2 and SARS-CoV with EC₅₀ values of 0.0099 µM and 0.0066 µM, respectively, in HAE cells after 48 hours of treatment. No cytotoxicity has been observed for remdesivir in HAE cells at concentrations up to 10 µM (CC₅₀ value >10µM) (PC-540-2002). The dose response curve of RDV against SARS-CoV-2 exhibited a shallow dose-dependent increase in inhibition compared to the response against SARS-CoV, indicating that RDV may be more active against SARS-CoV. Alternatively, there could be a lag in formation of the active diphosphate partially overcome by higher concentrations due to slow uptake by the cells, slow metabolism of the prodrug to the monophosphate, or a slow phosphorylation step.
- The development of resistance to RDV in coronaviruses has been assessed by cell culture passaging of murine hepatitis virus (MHV), a coronavirus, in the presence of the remdesivir parent nucleoside, GS-441524. After 23 passages, two substitutions were selected in the nsp12 polymerase at residues conserved across coronaviruses: F476L and V553L.
- Compared to wild-type virus, recombinant MHV containing the F476L substitution showed 2.4-fold reduced susceptibility to RDV, and MHV containing the V553L substitution demonstrated 5-fold reduced susceptibility, while the double mutant conferred 5.6-fold reduced susceptibility to RDV in cell culture. The potential relevance of this finding to SARS-CoV-2 is unknown.
- There are no directly relevant animal studies showing that remdesivir inhibits SARS-CoV-2 or improves outcomes in an animal model of SARS-CoV-2 to date.
- In a non-lethal mouse model of SARS-CoV pathogenesis, administration of 25 mg/kg remdesivir subcutaneously twice daily beginning 1 day before or 1 day after SARS-CoV inoculation resulted in reduced lung viral load and improved clinical signs of disease and lung function ([Sheahan et al., 2017](#)).
- In a mouse model of MERS-CoV pathogenesis, administration of 25 mg/kg remdesivir subcutaneously twice daily beginning 1 day before or 1 day after MERS-CoV inoculation improved pulmonary function and reduced lung viral loads and lung pathology ([Sheahan et al., 2020](#)). Of note, this mouse model was not uniformly lethal at the challenge doses used in these studies, which resulted in ~50% mortality by Day 6. Treatment with RDV did not improve survival.
- Remdesivir showed antiviral activity in SARS-CoV-2-infected rhesus monkeys. Administration of remdesivir at 10/5 mg/kg (10 mg/kg first dose, followed by 5 mg/kg once daily thereafter) using IV bolus injection initiated 12 hours post-inoculation with SARS-CoV-2 resulted in a reduction in clinical signs of respiratory disease, lung pathology and gross lung lesions, and lung viral RNA levels compared with vehicle-treated animals (De Witt et al., 2020).

Note: None of the animal model studies assessed RDV in a robust treatment paradigm where treatment was initiated 3 or 4 (or more) days after CoV infection.

- The development of resistance to RDV in coronaviruses has been assessed by cell culture passaging of murine hepatitis virus (MHV) in the presence of the RDV parent nucleoside, GS-441524. After 23

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passages, two substitutions were selected in the nsp12 polymerase at residues conserved across coronaviruses: F476L and V553L.

- Compared to wild-type virus, recombinant MHV containing the F476L substitution showed 2.4-fold reduced susceptibility to RDV, and MHV containing the V553L substitution demonstrated 5-fold reduced susceptibility, while the double mutant conferred 5.6-fold reduced susceptibility to RDV in cell culture.
- RDV has been studied in Phase 1 healthy volunteer studies and has been evaluated in patients with EBOV infection (including in the PALM RCT).
- Two Gilead-sponsored Phase 3 clinical studies, GS-US-540-5773 ([NCT 04292899](#)) and GS-US-540-5774 ([NCT 04292730](#)) are ongoing to evaluate the safety and efficacy of RDV in adults and adolescents diagnosed with COVID-19.
- Study GS-US-540-5773 Part A will evaluate the safety and efficacy of both a 5-day and a 10-day dosing regimen of RDV, in addition to standard of care, for patients with severe COVID-19.
- Study GS-US-540-5774 Part A will evaluate the safety and efficacy of the same dosing regimens of RDV, in addition to standard of care, for patients with moderate manifestations of COVID-19, compared with standard of care alone. Part A of these studies taken together are expected to enroll approximately 1,000 patients, globally. Both studies have a Part B, which will be extension treatment groups.
- Health authorities in China initiated 2 clinical trials in COVID-19 patients. These trials are coordinated by the China-Japan Friendship Hospital and are being conducted at multiple sites in the Hubei province.
 - GS-US-540-5774 evaluated RDV in patients with confirmed disease with more severe clinical manifestations such as a requirement for supplemental oxygen (Gilead study number CO-US-540-5758 [[NCT 04257656](#)]). Topline results from this study indicate that RDV did not have a treatment effect beyond standard of care.
 - The other study is evaluating RDV in patients with confirmed COVID-19 infection who have been hospitalized but are not displaying significant clinical manifestations of disease such as an oxygen requirement (Gilead study number CO-US-540-5764 [[NCT 04252664](#)]). The current status of these trials is unknown due to reported enrollment issues in China.
- NIAID, part of the National Institutes of Health, conducted a Phase 3 adaptive, randomized, double-blind, placebo-controlled trial for RDV as a potential treatment for hospitalized adult patients diagnosed with COVID-19 (Gilead study number CO-US-540-5776 [[NCT 04280705](#)]). This clinical trial evaluated remdesivir 200 mg once daily for 1 day followed by remdesivir 100 mg once daily for 9 days (for a total of up to 10 days of intravenously administered therapy) in hospitalized adult patients with COVID-19. The trial enrolled 1063 hospitalized patients in a 1:1 manner to receive remdesivir or placebo. The primary clinical endpoint was time to recovery within 28 days after randomization. In a preliminary analysis of the primary endpoint performed after 606 recoveries were attained, the median time to recovery was 11 days in the remdesivir group compared to 15 days in the placebo group (hazard ratio 1.31; 95% CI 1.12 to 1.54, $p < 0.001$). Mortality was 8.0% for the remdesivir group versus 11.6% for the placebo group ($p = 0.059$).
- Gilead has provided RDV on a compassionate use basis to more than 1,000 patients with COVID-19.
 - The sponsor has published their interpretation of patients hospitalized for severe Covid-19 who were treated with compassionate-use remdesivir, where they suggest that clinical improvement was observed in 36 of 53 patients (68%) ([Grein et al., 2020](#)).

APPENDIX 2

Original Fact Sheet for Health Care Workers submitted with the pre-EUA on April 8, 2020

14.1 Mechanism of Action

Remdesivir broadly and rapidly distributes into cells where it is efficiently metabolized to form the pharmacologically active nucleoside triphosphate metabolite. Efficient metabolism of remdesivir to remdesivir triphosphate has been demonstrated in multiple cell types. Remdesivir triphosphate acts as an analog of adenosine triphosphate (ATP) and competes with the natural ATP substrate for incorporation into nascent RNA chains by the SARS-CoV-2 RNA dependent RNA polymerase, which results in delayed chain termination during replication of the viral RNA. Remdesivir triphosphate is a weak inhibitor of mammalian DNA and RNA polymerases with low potential for mitochondrial toxicity.

15. MICROBIOLOGY/RESISTANCE INFORMATION

Antiviral Activity

Remdesivir shows potent in vitro activity against 571 the human pathogenic CoVs MERS-CoV and SARS-CoV in multiple relevant human cell types. Initial in vitro testing showed that remdesivir has potent antiviral activity against SARS-CoV-2 in Vero cells ($EC_{50} = 0.137 \mu\text{M}$ after 24 hours and $0.750 \mu\text{M}$ after 48 hours of treatment).

Resistance

No clinical data are available on the development of SARS-CoV-2 resistance to remdesivir. The in vitro development of SARS-CoV-2 resistance to remdesivir has not been assessed to date.

In vitro resistance profiling of remdesivir using the rodent CoV murine hepatitis virus identified 2 mutations in the viral polymerase at residues conserved across CoVs that conferred moderate reduced susceptibility to remdesivir. The mutant viruses showed reduced viral fitness in vitro and introduction of the analogous mutations into SARS-CoV resulted in attenuated SARS-CoV pathogenesis in a mouse model.

18. ANIMAL PHARMACOLOGIC AND EFFICACY DATA

It is unknown, at present, how the observed efficacy of remdesivir in animal models of coronavirus infection will translate into clinical efficacy in patients with symptomatic disease. Key attributes of the remdesivir nonclinical profile supporting its development for the treatment of COVID-19 are provided below:

- Remdesivir inhibited the in vitro replication of SARS-CoV-2 in Vero cells with an EC_{50} value of $0.137 \mu\text{M}$ and $0.750 \mu\text{M}$ after 24- and 48-hours of treatment, respectively {Study PC-540-2001}.
- Remdesivir demonstrated prophylactic and therapeutic efficacy in a mouse model of SARS-CoV pathogenesis. Administration of 25 mg/kg remdesivir subcutaneously twice daily beginning 1 day before or 1 day after SARS-CoV inoculation resulted in significantly reduced lung viral load and improved clinical signs of disease and lung function {Sheahan 2017}.
- In a mouse model of MERS-CoV pathogenesis, both prophylactic and therapeutic administration of 25 mg/kg remdesivir subcutaneously twice daily improved pulmonary function and reduced lung viral loads and severe lung pathology. In contrast, prophylactic lopinavir/ritonavir and interferon beta (LPV/RTV-IFN β) slightly reduced viral loads without impacting other disease parameters. Therapeutic LPV/RTV-IFN β improved pulmonary function but did not reduce virus replication or severe lung pathology {Sheahan 2020}.
- Remdesivir also showed prophylactic and therapeutic efficacy in MERS647 CoV-infected rhesus monkeys. Administration of remdesivir at 10 mg/kg or 5 mg/kg once daily for 7 days using IV bolus injection beginning 1 day prior to MERS-CoV inoculation resulted in a significant reduction of clinical

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scores, clinical signs of respiratory disease, and viral RNA levels compared to vehicle-treated animals. Therapeutic remdesivir treatment of 5 mg/kg once daily using IV bolus injection initiated 12 hours post inoculation also resulted in reduced clinical signs, reduced virus replication in the lungs, and decreased presence and severity of lung lesions {De Wit 2020}.

APPENDIX 3

Final Fact Sheet for Health Care Workers that was finalized upon approval of EUA-000046 on May 1, 2020

14.1 Mechanism of Action

Remdesivir is an adenosine nucleotide prodrug that distributes into cells where it is metabolized to form the pharmacologically active nucleoside triphosphate metabolite. Metabolism of remdesivir to remdesivir triphosphate has been demonstrated in multiple cell types. Remdesivir triphosphate acts as an analog of adenosine triphosphate (ATP) and competes with the natural ATP substrate for incorporation into nascent RNA chains by the SARS-CoV-2 RNA-dependent RNA polymerase, which results in delayed chain termination during replication of the viral RNA. Remdesivir triphosphate is a weak inhibitor of mammalian DNA and RNA polymerases with low potential for mitochondrial toxicity.

15. MICROBIOLOGY/RESISTANCE INFORMATION

Antiviral Activity

Remdesivir exhibited cell culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary human airway epithelial (HAE) cells with a 50% effective concentration (EC_{50}) of 9.9 nM after 48 hours of treatment. The EC_{50} values of remdesivir against SARS-CoV-2 in Vero cells was 137 nM at 24 hours and 750 nM at 48 hours post-treatment.

Resistance

No clinical data are available on the development of SARS-CoV-2 resistance to remdesivir. The cell culture development of SARS-CoV-2 resistance to remdesivir has not been assessed to date.

Cell culture resistance profiling of remdesivir using the rodent CoV murine hepatitis virus identified 2 substitutions (F476L and V553L) in the viral RNA-dependent RNA polymerase at residues conserved across CoVs that conferred a 5.6 fold reduced susceptibility to remdesivir. The mutant viruses showed reduced viral fitness in cell culture and introduction of the corresponding substitutions (F480L and V557L) into SARS-CoV resulted in 6-fold reduced susceptibility to remdesivir in cell culture and attenuated SARS-CoV pathogenesis in a mouse model.

17. ANIMAL PHARMACOLOGIC AND EFFICACY DATA

It is unknown, at present, how the observed antiviral activity of remdesivir in animal models of SARS-CoV-2 infection will translate into clinical efficacy in patients with symptomatic disease. Key attributes of the remdesivir nonclinical profile supporting its development for the treatment of COVID-19 are provided below:

- Remdesivir showed cell culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary HAE cells (EC_{50} value= 9.9 nM). The EC_{50} values of remdesivir against SARS-CoV-2 in Vero cells has been reported to be 137 nM at 24 hours and 750 nM at 48 hours post-treatment.
- Remdesivir showed antiviral activity in SARS-CoV-2-infected rhesus monkeys. Administration of remdesivir at 10/5 mg/kg (10 mg/kg first dose, followed by 5 mg/kg once daily thereafter) using IV bolus injection initiated 12 hours post-inoculation with SARS-CoV-2 resulted in a reduction in clinical signs of respiratory disease, lung pathology and gross lung lesions, and lung viral RNA levels compared with vehicle-treated animals.

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APPENDIX 4

Original EUA Template that was filled out in response to the prEUA submitted on April 8, 2020

Identifying Information

Therapeutic Class	Coronavirus nucleoside analog RNA polymerase inhibitor
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XIII. Nonclinical Data to Support Efficacy

- Remdesivir is a nucleotide prodrug that is intracellularly metabolized into its active form GS-441524, which is an analog of adenosine triphosphate that inhibits viral RNA synthesis.
- The GS-441524 adenosine nucleotide analog is incorporated into the nascent RNA chain by the coronavirus viral RNA polymerase and evades proofreading by the coronavirus exoribonuclease, resulting in a decrease in coronavirus RNA production. It is currently unknown whether it terminates RNA chains or causes mutations in them ([Agostini et al., 2018](#)).
- Remdesivir has broad antiviral activity against multiple, unrelated human RNA viruses including, Middle East Respiratory Syndrome (MERS) coronavirus and SARS-CoV, Ebola virus, Marburg virus, Junin virus, and Lassa fever virus.
- The EC₅₀ values of remdesivir against the SARS-CoV-2 grown in Vero cells has been reported to be 0.137 µM at 24 hours and 0.75 µM at 48 hours post-treatment (Chinese CDC).
- An EC₅₀ value of 0.77 µM in Vero cells has been reported for RDV against SARS-CoV-2 by the Wuhan Institute of Virology ([Wang et al., 2020](#)).
- Remdesivir inhibited a recombinant chimeric virus expressing the RdRp gene (nsp12) of SARS-CoV-2 in a backbone of SARS-CoV with a luciferase reporter in Huh7 cells with an EC₅₀ value = 0.003 µM.
- Remdesivir and GS-466547 (an opposite diastereomer) were tested in cell culture antiviral activity assessments against human pathogenic coronaviruses, specifically MERS-CoV and SARS-CoV. Remdesivir and GS-466547 inhibited replication of MERS-CoV in Vero E6 cells, with mean EC₅₀ values of 0.52 and 0.42 µM, respectively. No cytotoxicity was observed at 10 µM, the highest concentration tested, indicating selective inhibition of virus replication with selectivity indices of >19 and >24, respectively.
- In addition, GS-466547 inhibited both MERS-CoV and SARS-CoV replication in human airway epithelial (HAE) cultures as observed by the reduction in the expression of the RFP or GFP reporter genes at compound concentrations ranging from 0.1 to 1.1 µM.
- The activity of remdesivir against MERS-CoV and SARS-CoV was also assessed using recombinant viruses expressing a fluorescent reporter protein in a continuous human lung epithelial cell line, 2B4 (Calu-3; MERS-CoV only) and primary human airway epithelial cells (MERS-CoV and SARS-CoV). Remdesivir inhibited MERS-CoV replication in Calu-3 cells, with a mean EC₅₀ value of 0.025 µM ([Sheahan et al., 2017](#)). In HAE cells, remdesivir inhibited both MERS-CoV and SARS-CoV replication with EC₅₀ values of 0.074 and 0.069 µM, respectively. In both HAE and Calu-3 cells, no cytotoxicity was observed at 10 µM of remdesivir, the highest concentration tested, indicating that remdesivir has selectivity indices >100 in these cell culture systems ([Sheahan et al., 2017](#)).
- Remdesivir also showed cell culture antiviral activity against human betacoronavirus OC43 and alphacoronavirus 229E as well as the animal betacoronavirus murine hepatitis virus and the genetically divergent porcine deltacoronavirus with submicromolar EC₅₀ values ranging from 0.02 to 0.15 µM in various cell types ([Agostini et al., 2018](#); [Brown et al., 2019](#); [Sheahan et al., 2017](#)).

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- Remdesivir inhibited the replication of SARS-CoV-2 and SARS-CoV with EC₅₀ values of 0.0099 μM and 0.0066 μM, respectively, in HAE cells after 48 hours of treatment. No cytotoxicity has been observed for remdesivir in HAE cells at concentrations up to 10 μM (CC₅₀ value >10 μM). The dose response curve of RDV against SARS-CoV-2 exhibited a shallow dose-dependent increase in inhibition compared to the response against SARS-CoV, indicating that RDV may be more active against SARS-CoV. Alternatively, there could be a lag in formation of the active diphosphate partially overcome by higher concentrations due to slow uptake by the cells, slow metabolism of the prodrug to the monophosphate, or a slow phosphorylation step.
- The development of resistance to RDV in coronaviruses has been assessed by cell culture passaging of murine hepatitis virus (MHV), a coronavirus, in the presence of the remdesivir parent nucleoside, GS-441524. After 23 passages, two substitutions were selected in the nsp12 polymerase at residues conserved across coronaviruses: F476L and V553L.
- Compared to wild-type virus, recombinant MHV containing the F476L substitution showed 2.4-fold reduced susceptibility to RDV, and MHV containing the V553L substitution demonstrated 5-fold reduced susceptibility, while the double mutant conferred 5.6-fold reduced susceptibility to RDV in cell culture.
- Remdesivir demonstrated antiviral activity in a nonlethal mouse model of SARS-CoV pathogenesis. Administration of 25 mg/kg RDV subcutaneously twice daily beginning 1 day before or 1 day after SARS-CoV inoculation resulted in significantly reduced lung viral load and improved clinical signs of disease as well as lung function ([Sheahan et al., 2017](#)).
- In a lethal mouse model of MERS-CoV pathogenesis, both prophylactic and therapeutic administration of 25 mg/kg RDV subcutaneously twice daily improved pulmonary function and reduced lung viral loads and severe lung pathology ([Sheahan et al., 2020](#)). Treatment with RDV did not improve survival.
- A 7-day treatment regimen of 10 mg/kg GS-5734 administered once daily by IV bolus injection beginning 1 day prior to virus exposure significantly reduced viral lung burden and clinical disease signs in the nonlethal MERS-CoV-infected rhesus macaque model. In several of the MERS-CoV infected animals, the treatment with GS-5734 adversely affected renal function.
- There are no directly relevant animal studies showing that remdesivir inhibits SARS-CoV-2 or improves outcomes in an animal model to date.

Comment: None of the animal model data rise to the level of direct relevance to support antiviral activity of remdesivir against SARS-CoV-2. Therefore, the following sections are not relevant to the EUA.

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APPENDIX 5

Final EUA Template that was finalized upon approval of EUA-000046 on May 1, 2020

Identifying Information

Therapeutic Class	Coronavirus nucleoside analog RNA polymerase inhibitor
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XIII. Nonclinical Data to Support Efficacy

- Remdesivir is a nucleotide prodrug that is intracellularly metabolized into its active form GS-441524, which is an analog of adenosine triphosphate that inhibits viral RNA synthesis.
- The GS-441524 adenosine nucleotide analog is incorporated into the nascent RNA chain by the coronavirus viral RNA polymerase (nsp12) and evades proofreading by the coronavirus exoribonuclease, resulting in a decrease in coronavirus RNA production. It is currently unknown whether it terminates RNA chains or causes mutations in them ([Agostini et al., 2018](#)).
- Remdesivir exhibited antiviral activity against several human RNA viruses including, SARS-CoV and Middle East Respiratory Syndrome (MERS) CoV, Ebola virus, Marburg virus, Junin virus, and Lassa fever virus.
- The EC₅₀ values of remdesivir against the SARS-CoV-2 grown in Vero cells has been reported to be 0.14 µM at 24 hours and 0.75 µM at 48 hours post-treatment (PC-540-2001).
- An EC₅₀ value of 0.77 µM in Vero cells has been reported for RDV against SARS-CoV-2 by the Wuhan Institute of Virology ([Wang et al., 2020](#)).
- Remdesivir inhibited a recombinant chimeric virus expressing the RdRp gene (nsp12) of SARS-CoV-2 in a backbone of SARS-CoV with a fluorescent reporter protein in Huh7 cells with an EC₅₀ value = 0.003 µM (PC-540-2002).
- Cell culture antiviral assessments against other human and animal coronaviruses are summarized below:
 - Remdesivir and GS-466547 (an opposite diastereomer) were tested in cell culture antiviral activity assessments against SARS-CoV and MERS -CoV. Remdesivir and GS-466547 inhibited replication of MERS-CoV in Vero E6 cells with mean EC₅₀ values of 0.52 and 0.42 µM, respectively. No cytotoxicity was observed at 10 µM, the highest concentration tested, indicating selective inhibition of virus replication with selectivity indices of >19 and >24, respectively.
 - GS-466547 inhibited SARS-CoV and MERS-CoV replication in human airway epithelial (HAE) cultures as measured by the reduction in the expression of a fluorescent reporter protein at compound concentrations ranging from 0.1 to 1.1 µM.
 - The activity of remdesivir against SARS-CoV and MERS-CoV was assessed using recombinant viruses expressing a fluorescent reporter protein in a continuous human lung epithelial cell line, 2B4 (Calu-3; MERS-CoV only) and primary HAE cells (SARS-CoV and MERS-CoV). Remdesivir inhibited MERS-CoV replication in Calu-3 cells, with a mean EC₅₀ value of 0.025 µM ([Sheahan et al., 2017](#)). In HAE cells, remdesivir inhibited both SARS-CoV and MERS-CoV replication with EC₅₀ values of 0.069 and 0.074 µM, respectively. In both HAE and Calu-3 cells, no cytotoxicity was observed at 10 µM of remdesivir, the highest concentration tested, indicating that remdesivir has selectivity indices >100 in these cell culture systems ([Sheahan et al., 2017](#)).
 - Remdesivir showed cell culture antiviral activity against human betacoronavirus OC43 and alphacoronavirus 229E as well as the animal betacoronavirus murine hepatitis virus and the

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genetically divergent porcine deltacoronavirus with submicromolar EC₅₀ values ranging from 0.02 to 0.15 μM in various cell types ([Agostini et al., 2018](#); [Brown et al., 2019](#); [Sheahan et al., 2017](#)).

- Remdesivir inhibited the replication of SARS-CoV-2 and SARS-CoV with EC₅₀ values of 0.0099 μM and 0.0066 μM, respectively, in HAE cells after 48 hours of treatment. No cytotoxicity has been observed for remdesivir in HAE cells at concentrations up to 10 μM (CC₅₀ value >10μM) (PC-540-2002). The dose response curve of RDV against SARS-CoV-2 exhibited a shallow dose-dependent increase in inhibition compared to the response against SARS-CoV, indicating that RDV may be more active against SARS-CoV. Alternatively, there could be a lag in formation of the active diphosphate partially overcome by higher concentrations due to slow uptake by the cells, slow metabolism of the prodrug to the monophosphate, or a slow phosphorylation step.
- The development of resistance to RDV in coronaviruses has been assessed by cell culture passaging of murine hepatitis virus (MHV), a coronavirus, in the presence of the remdesivir parent nucleoside, GS-441524. After 23 passages, two substitutions were selected in the nsp12 polymerase at residues conserved across coronaviruses: F476L and V553L.
- Compared to wild-type virus, recombinant MHV containing the F476L substitution showed 2.4-fold reduced susceptibility to RDV, and MHV containing the V553L substitution demonstrated 5-fold reduced susceptibility, while the double mutant conferred 5.6-fold reduced susceptibility to RDV in cell culture. The potential relevance of this finding to SARS-CoV-2 is unknown.
- There are no directly relevant animal studies showing that remdesivir inhibits SARS-CoV-2 or improves outcomes in an animal model to date using a treatment paradigm.
- In a nonlethal non-human primate model of SARS-CoV-2 pathogenesis under a post-exposure prophylaxis paradigm that initiated treatment 12 hours after challenge with SARS-CoV-2, administration of a loading dose of 10 mg/kg remdesivir, followed by a 5 mg/kg dose 12 hours after the loading dose, and then a daily maintenance dose of 5 mg/kg for 5 additional days delivered as a slow intravenous bolus injection resulted in a marginal clinical benefit during SARS-CoV-2 infection in rhesus macaques. There were a number of limitations to this study, including the lack of an adequately characterized and validated model, use of a non-lethal model that cannot be used to assess mortality or severe respiratory disease, and lack of clarity related to the optimal route and dose of the viral challenge. Additionally, there were differences in RDV prodrug and metabolite exposures between infected NHPs, healthy NHPs, and healthy humans that further impede extrapolation of these results to humans with COVID-19.
- In a non-lethal mouse model of SARS-CoV pathogenesis, administration of 25 mg/kg remdesivir subcutaneously twice daily beginning 1 day before or 1 day after SARS-CoV inoculation resulted in reduced lung viral load and improved clinical signs of disease and lung function ([Sheahan et al., 2017](#)).
- In a mouse model of MERS-CoV pathogenesis, administration of 25 mg/kg remdesivir subcutaneously twice daily beginning 1 day before or 1 day after MERS-CoV inoculation improved pulmonary function and reduced lung viral loads and lung pathology ([Sheahan et al., 2020](#)). Of note, this mouse model was not uniformly lethal at the challenge doses used in these studies, which resulted in 50% mortality by Day 6. Treatment with RDV did not improve survival.
- In MERS-CoV-infected rhesus monkeys, administration of remdesivir at 10 mg/kg or 5 mg/kg once daily for 7 days using IV bolus injection beginning 1 day prior to MERS-CoV inoculation resulted in a reduction of clinical scores, clinical signs of respiratory disease, and viral RNA levels compared to vehicle-treated animals (De Witt et al., 2020).

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Comment: None of the animal model data rise to the level of direct relevance to support antiviral activity of remdesivir against SARS-CoV-2. Therefore, the following sections are not relevant to the EUA.

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APPENDIX 6

EUA Template Instructions

Identifying Information

Therapeutic Class	
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XIII. Nonclinical Data to Support Efficacy

- Describe the results of available in vitro data for the product for the disease/condition of interest.
 - Briefly describe what is known about the product’s mechanism of action in support of the proposed clinical indication. Include relevant information, e.g., in vitro data evaluating activity against the target agent, enzyme inhibition, receptor binding, etc.
- Summarize the results of in vitro studies evaluating the activity of the compound (e.g., in vitro metabolic profile, antimicrobial susceptibility results, etc.).
- List relevant in vivo studies of the product in animal models of disease in the following table (may include exploratory studies, dose-response studies, and adequate and well-controlled animal efficacy studies).

Table 3: Relevant Studies of the Product in Animal Models of Disease

Study Number	IND, NDA, or Literature Reference	Type of Study ¹	Species/ Number of Animals Per Group	Study Design and Type of Control	Test Product(s); Dosing Regimens; Dosage Forms; Route(s) of Administration; Duration	Study Status ²

- For the most relevant studies in animal models of disease, describe the relationship of each animal model to the human disease/condition based upon the pathophysiology of the disease or condition in humans as compared to the animal model (e.g., host response, route of exposure to the threat agent, disease manifestations, tissue sites involved, etc.).
- Describe the endpoints evaluated in the animal model studies and their relationship to the desired benefit in humans.
- Discuss the relationship between the dose used in PK/ADME and PD or other relevant animal studies and the proposed human dose. Include information on active metabolites.
- Assessment of the available animal data relevant to activity or efficacy for the treatment/prophylaxis of the disease/condition (include statistical analyses from adequate and well-controlled animal efficacy studies, if available).


¹ May include pharmacokinetic, exposure-response, treatment, post-exposure prophylaxis, pre-exposure prophylaxis, etc.; discuss further as appropriate based on bullet points below. Specify whether GLP.

² Specify whether the study is complete or ongoing.

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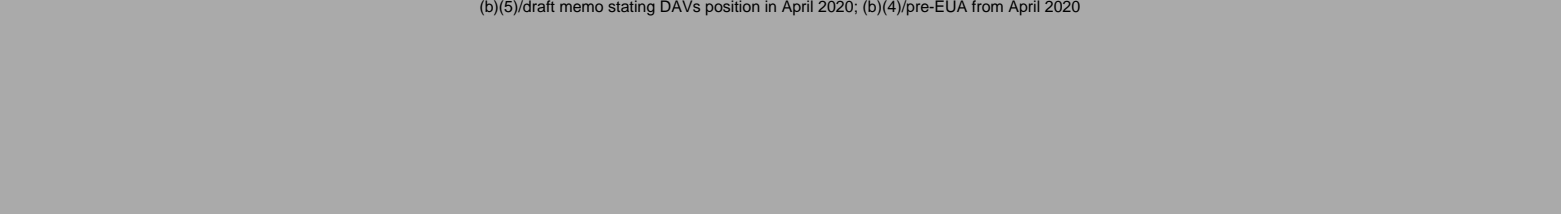
APPENDIX 7

(b)(5)/draft memo stating DAVs position in April 2020; (b)(4)/pre-EUA from April 2020



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EUA-000046 DATE REVIEW COMPLETED: 05/04/2020

(b)(5)/draft memo stating DAVs position in April 2020; (b)(4)/pre-EUA from April 2020



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/s/

ERIC F DONALDSON
05/06/2020 08:21:40 AM

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05/06/2020 08:40:20 AM