Supplementary Material for

Why Remdesivir Failed: Preclinical Assumptions Overestimate the Clinical Efficacy of Remdesivir for COVID-19 and Ebola

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GEO accession identifier for Vero E6 RNA-seq data in Figure 2c:

GSM4658803, GSM4658804, GSM4658805.

Gene IDs for prodrug bioactivating enzymes:

CES1 (ENSCSAG00000023840)

CTSA (ENSCSAG00000014583)

HINT1 (ENSCSAG00000014000)

ADK (ENSCSAG00000009045)

AK2 (ENSCSAG00000001068)

ABCB1 (ENSCSAG00000012391)

ABCC1 (ENSCSAG00000008018)

SLC29A3 (ENSCSAG00000008424)

A

		RDV 10 mg/kg IV		GS-441524 20 mg/kg IV
		AGM	Cyno	Cyno
All metabolites (nmol/g)	Liver	17.3	18.4	2.44
	Kidney	39.8	65.7	3.44
	Ratio (kidney : liver)	2.3	3.6	1.4

B

Cell type	CC_{50} (μM)	
	RDV	GS-441524
PHH	2.5 ± 0.6	>100
RPTEC	12.9 ± 6.2	>100

Supplementary Figure S1. Captisol® formulation results in significant distribution of RDV metabolites to the kidneys. (A) NHP (African green monkeys, cynomolgus macaques) were intravenously administered a single dose of either RDV (10 mg/kg) or its parent nucleoside, GS-441524 (20 mg/kg) using two different formulations as indicated. RDV was formulated as administered in the clinic, in 12% sulfobutylether-ß-cyclodextrin in water (pH 3.5; Captisol®) and administered at a constant rate over 30 minutes at 2 mL/kg. GS-441524 was formulated in 5% EtOH, 3% propylene glycol, 45% PEG 400, and 20% water with 1 equiv. HCl and administered at a constant rate of 0.5 mg/mL. Metabolites quantified in liver and kidney represent the sum of GS-441524, mono-, di-, and triphosphates from either RDV or GS-441524. While both RDV and GS-441524 generate the same metabolic intermediates, the ratio of metabolites (kidney : liver) is higher for RDV than it is for GS-441524. Data are adapted from Mackman and Cihlar et al. *J. Med. Chem.* (2021) (1). **(B)** Considering the CC_{50} for RDV is much lower than that of GS-441524 in primary human hepatocytes (PHH) and renal proximal tubule epithelial cells (RPTECs), Captisol®-mediated distribution of RDV could explain concerns of RDV's nephrotoxicity. Data are adapted from Xu et al. *Antimicrob. Agents Chemother.* (2020) (2).

References

1. Mackman RL, Hui HC, Perron M, Murakami E, Palmiotti C, Lee G, Stray K, Zhang L, Goyal B, Chun K, Byun D, Siegel D, Simonovich S, Du Pont V, Pitts J, Babusis D, Vijjapurapu A, Lu X, Kim C, Zhao X, Chan J, Ma B, Lye D, Vandersteen A, Wortman S, Barrett KT, Toteva M, Jordan R, Subramanian R, Bilello JP, Cihlar T. 2021. Prodrugs of a 1′-CN-4-Aza-7,9-dideazaadenosine C -Nucleoside Leading to the Discovery of Remdesivir (GS-5734) as a Potent Inhibitor of Respiratory Syncytial Virus with Efficacy in the African Green Monkey Model of RSV . J Med Chem https://doi.org/10.1021/acs.jmedchem.1c00071.
2. Xu Y, Barauskas O, Kim C, Babusis D, Murakami E, Kornyeyev D, Lee G, Stepan G, Perron M, Bannister R, Schultz BE, Sakowicz R, Porter D, Cihlar T, Feng JY. 2020. Off-target In Vitro Profiling Demonstrates that Remdesivir Is a Highly Selective Antiviral Agent. Antimicrob Agents Chemother https://doi.org/10.1128/aac.02237-20.