Supplementary information:

Pharmacokinetics of remdesivir in a COVID-19 patient with end-stage renal disease on intermittent hemodialysis

Supplementary table 1

General patient characteristics and medical history

Age	Mid seventies
Gender	Male
Weight	53.2 kg
Glomerular Filtration Rate	0 mL/min (no residual renal function)
Relevant medical history	End-stage renal disease due to IgA-nephropathy Intermittent hemodialysis for > 20 years Paroxysmal atrial fibrillation Type 2 diabetes mellitus Coronary artery disease

Supplementary table 2

Concomitant medication during remdesivir treatment

Medication	Dose	Indication
Piperacillin/tazobactam	2 x 4.5 g*	Suspected bacterial superinfection
Metoclopramide	3 x 10 mg	Nausea
Dexamethasone	1 x 6 mg	Severe COVID-19
Famotidine	1 x 20 mg	Stress ulcer prophylaxis
Acetylsalicyclic acid	1 x 100 mg	Secondary prevention after NSTEMI
Levothyroxine	1 x 75 µg	Hypothyroidism
Tilidin/naloxone	2 x 100/8 mg	Chronic Pain Syndrome
Calcium acetate	3 x 950 mg	Chronic kidney disease (phosphate binder)
Enoxaparin	1 x 20 mg*	Thromboembolism prophylaxis
Heparine (during hemodialysis)	varying	Hemodialysis

*Dose adjusted to GFR; NSTEMI, non-ST-elevation myocardial infarction

Supplementary table 3

Parameters of remdesivir (200 mg single dose) and its metabolites

	Parameter	Index patient (no renal function)	Humeniuk et al. 2020[a] (healthy volunteers)
Remdesivir (GS-5734)	$AUC_{0-\infty}$*	13.0 µg/mL*h	3.3 µg/mL*h
	C_{max}	19.8 µg/mL	7.8 µg/mL
	Clearance	257 mL/min	1000 mL/min
	Volume of distribution (V_z)*	24.5 L	87 L
	Apparent elimination half-life*	1.10 h	1 h
GS-441524	$AUC_{0-20.1\ h}$	18.4 µg/mL*h	n.a.
	C_{max}	1.15 µg/mL*h	0.2 ng/mL
	Apparent elimination half-life	not estimable (∞)	27 h
GS-704277	Apparent elimination half-life*	2.98 h	1.3 h

[a] values reported for the 30 min infusion of 75 mg extrapolated to a 200 mg dose assuming dose linearity[1]; clearance was calculated from reported parameters as dose/AUC and volume of distribution as half-life * clearance /ln(2); * estimated using the last 3 time points with quantifiable remdesivir concentrations; $AUC_{0-\infty}$, area under the concentration vs. time profile extrapolated to infinity; C_{max} , maximal observed plasma concentration; V_z, volume of distribution at pseudo-equilibrium

Supplementary table 4

Quantification of systemic remdesivir prodrug (GS-5734) and GS-441524 metabolite, relative quantification of remdesivir intermediate metabolite (GS-704277)

Sample ID	Sampling time	Time after start infusion (h)	Remdesivir (ng/mL)	GS-441524 (ng/mL)	Ratio GS-704277/IS
DH-1	24.09.2020 - 13:02	0	BQL	BQL	-
DH-2	24.09.2020 - 13:22	0.25	19845.3	46.208	0.955
DH-3	24.09.2020 - 13:36	0.48	15513.7	145.65	3.028
DH-4	24.09.2020 - 13:50	0.72	8525.0	264.93	4.392
DH-5	24.09.2020 - 14:05	0.97	2918.6	375.16	4.234
DH-6	24.09.2020 - 14:35	1.47	1484.9	460.43	3.227
DH-7	24.09.2020 - 15:32	2.42	439.80	883.10	1.989
DH-8	24.09.2020 - 17:32	4.42	127.69	958.27	1.613
DH-9	24.09.2020 - 19:43	6.60	31.729	859.15	0.756
DH-1	25.09.2020 - 07:06	17.98	BQL	1152.7	0.229
DH-11	25.09.2020 - 09:14	20.12	BQL	876.44	0.148
DH-12	25.09.2020 - 10:59	21.87	BQL	856.22	-
DH-15	25.09.2020 - 13:55	24.80	BQL	589.44	-
DH-18	25.09.2020 - 16:45	27.63	BQL	442.62	-
DH-19	25.09.2020 - 17:03	27.93	8327.6	430.08	0.358
DH-20	25.09.2020 - 17:15	28.13	6548.0	404.21	2.272
DH-21	25.09.2020 - 17:29	28.37	3225.5	611.62	2.953
DH-22	26.09.2020 - 16:00	50.88	BQL	1659.5	-
DH-24	27.09.2020 - 16:30	75.38	BQL	1613.5	-
DH-26	28.09.2020 - 09:55	91.80	BQL	1787.0	-
DH-27	28.09.2020 - 11:34	93.45	BQL	1118.1	-
DH-30	28.09.2020 - 13:40	95.55	BQL	835.26	-
DH-33	28.09.2020 - 14:45	96.63	BQL	810.75	-
DH-36	28.09.2020 - 15:05	96.97	BQL	549.99	-
Dialysate	25.09.2020 - 17:25	-	BQL	197..93	-

Abbreviations: BQL, Below Quantification Limit (remdesivir: 3.60 ng/mL, GS-441524: 10.8 ng/mL); IS, internal standard

Supplementaty table 5

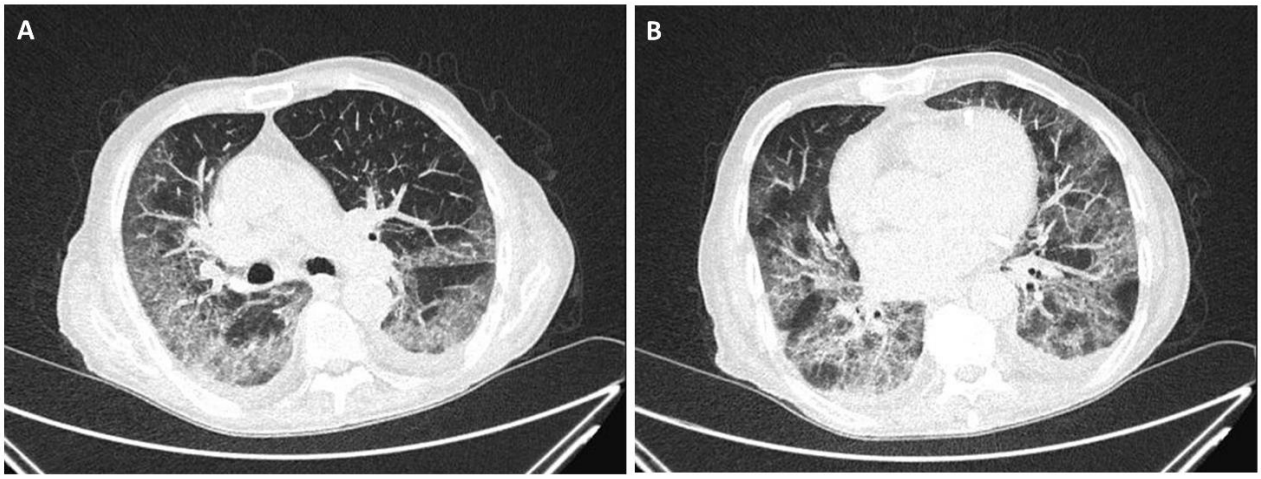
Quantification of remdesivir prodrug (GS-5734) and GS-441524 metabolite in arterial (inlet) and venous (outlet) lines of the hemodialysis system

Sample ID	Sampling time	Time after start infusion (h)	Remdesivir (ng/mL)	GS-441524 (ng/mL)
DH-13 HDA	25.09.2020 - 11:05	21.97	BQL	792.30
DH-14 HDV	25.09.2020 - 11:05	21.97	BQL	334.94
DH-16 HDA	25.09.2020 - 13:55	24.80	BQL	540.66
DH-17 HDV	25.09.2020 - 13:55	24.80	BQL	229.09
DH-28 HDA	28.09.2020 - 11:34	93.45	BQL	1217.3
DH-29 HDV	28.09.2020 - 11:34	93.45	BQL	395.95
DH-31 HDA	28.09.2020 - 13:40	95.55	BQL	862.97
DH-32 HDV	28.09.2020 - 13:40	95.55	BQL	459.22
DH-34 HDA	28.09.2020 - 14:45	96.63	BQL	920.17
DH-35 HDV	28.09.2020 - 14:45	96.63	BQL	549.99

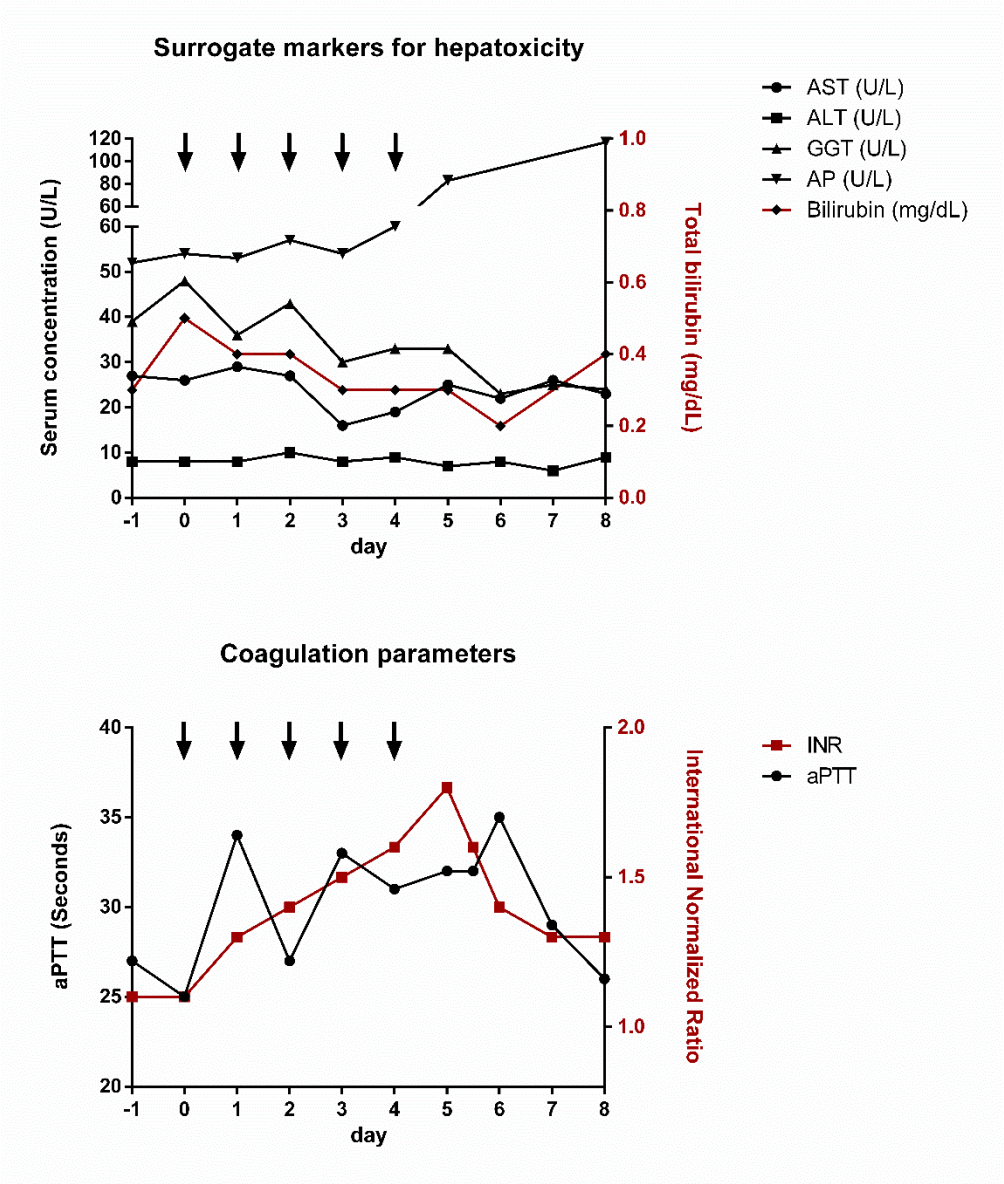
Abbreviations: HDA, hemodialysis arterial line (inlet); HDV, hemodialysis venous line (outlet); BQL, Below Quantification Limit (remdesivir: 3.60 ng/mL, GS-441524: 10.8 ng/mL)

Supplementary figure 1

Chest computed tomography (CT) performed eight days after diagnosis



A (cranial) and B (caudal) pictures from low-dose plain CT scan performed 22.09.2020 showing progressive bipulmonary ground glass opacities and consolidations with a predominantly peripheral distribution pattern. In addition, linear intralobular septal thickening (crazy paving) and small pleural effusions.



Supplementary figure 2. Daily monitoring of toxicity markers during antiviral treatment (day 0-4, ↓) of a patient with end-stage renal disease with the standard regimen of remdesivir (200 mg d0, 100 mg d1-4 IV) and follow up (day 5-8). Hemodialysis was performed on days 1 and 4. Laboratory reference ranges are not shown here but were only exceeded in case of INR which nearly normalized under vitamin K supplementation. Abbreviations: AST, aspartate transaminase; ALT, alanine aminotransaminase; GGT, gamma-glutamyltransferase; AP, alkaline phosphatase; Bilirubin = total bilirubin; INR, International Normalized Ratio, aPTT, activated partial thromboplastin time.

Methods

Administration of remdesivir

Remdesivir was administered as controlled intravenous infusion (500 mL/h) over 30 minutes via femoral vein catheter without any parallel infusions (for doses and timing see figure 1). Markers for hepatotoxicity were monitored at least daily during treatment with remdesivir and for subsequent days (supplementary figure 2).

Pharmacokinetic sampling

Blood samples were drawn from the arterial line after discarding dead space volume. In addition, several blood samples were taken from the inlet and the outlet of the dialysis device to analyze drug elimination during hemodialysis. Polypropylene tubes containing 1.2 mg/mL ethylenediaminetetraacetic acid (EDTA) to prevent clotting and 1 mg/mL of sodium fluoride (Sarstedt, Nümbrecht, Germany) were used and directly placed on ice to prevent *in vitro* hydrolysis of remdesivir [2] and its metabolites before further processing within a maximum of one hour. Plasma was separated by centrifugation at 4°C and subsequently stored at -80°C prior to pharmacokinetic analyses.

Hemodialysis

Hemodialysis was performed using the Fresenius GENIUS® device, a single pass close tank hemodialysis system, containing 90 liters of dialysis fluid. The system was equipped with an FX600 CorDiax® hemodialysis filter (Fresenius) and standard blood lines and tubing. The extracorporeal circuit was established via central venous catheter. Blood flow ranged between 150 and 280 mL/min. According to technical design, dialysate flow equaled blood flow. Ultrafiltration settings ranged between 100 and 200 ml/h. Hemodialysis was performed prior to administration of the daily remdesivir dose on days 2 and 5 of treatment. In the two dialysis sessions a blood volume of 88 and 90 liters was processed. Duration of treatment was 425 minutes and 315 minutes. Ultrafiltration volume was 990 ml and 1037 ml, respectively. For anticoagulation unfractionated heparin was administered as intravenous bolus of 2000 IE at the beginning of hemodialysis and a rate of 1000 IE/h during treatment.

Determination of remdesivir and metabolites

Measurements of drug concentrations were performed at the Institute for Biomedical and Pharmaceutical Research, Nürnberg-Heroldsberg, Germany. Remdesivir and its metabolite GS-441524 were purchased from BIOSYNTH® Carbosynth (Berkshire, UK). Calibration standards and spiked quality control samples were prepared in drug-free human EDTA plasma. Liquid chromatography/mass spectrometry to detect the compounds used transitions REM 603.3→402.0 and 292.2→163.1 (GS-441524) on a SCIEX API 6500TM triple quadrupole mass spectrometer equipped with turbo ion spray interface (SCIEX, Concord, Ontario, Canada). Calibration for both compounds was performed by weighted ($1/concentration^2$) linear regression. Linearity for remdesivir and GS-441524 in human plasma could be demonstrated over a calibration range from 3.600 to 787.4 ng/mL for remdesivir and from 10.84 to 474.5 ng/mL for GS-441524. Quantification of remdesivir and GS-441524 was performed by peak area ratio of analyte to internal standard (remdesivir: piperacillin-d5, GS-441524 amoxicillin-d4). When samples were outside that range a dilution by drug free human plasma was used. No interferences were observed for remdesivir and GS-441524 and the internal standards in human plasma. Since there was no reference standard available for the intermediate metabolite GS-704277, only the time course of plasma concentrations could be described which were obtained from peak heights relative to an internal standard (transition 443.1→202.1).

Pharmacokinetic assessment

Pharmacokinetic parameters following the first dose for remdesivir and its metabolites were calculated by standard non-compartmental methods. Extraction of GS-441524 by dialysis was calculated as (1-outlet concentration [corrected for volume loss] / inlet concentration). The observed decrease of GS-441524 extraction during dialysis was described by linear regression with cumulative blood flow.

References

1. Humeniuk R, Mathias A, Cao H et al. Safety, Tolerability, and Pharmacokinetics of Remdesivir, An Antiviral for Treatment of COVID-19, in Healthy Subjects. *Clin Transl Sci* 2020; **13**: 896-906.

2. Scherf-Clavel O, Kaczmarek E, Kinzig M et al. Tissue level profile of SARS-CoV-2 antivirals in mice to predict their effects in COVID-19 multiorgan failure. *bioRxiv* 2020: 2020.09.16.299537.