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LIST OF ABBREVIATIONS, ACRONYMS AND DEFINITION OF TERMS

Abbreviation/Acronym	Definition
AUC	Area Under the Concentration-Time Curve
$AUC_{0\text{-last}}$	Area Under the Concentration-Time Curve from Time Zero to the Last Quantifiable Concentration
$AUC_{0\text{-inf}}$	Area Under the Concentration-Time Curve Extrapolated to Infinity
BCRP	Breast Cancer Resistance Protein Efflux Transporter
BDC	Bile Duct-Cannulated
BID	Twice a Day
BQL	Below the Quantifiable Limit
Caco-2	Colorectal Adenocarcinoma Cells
CES	Carboxylesterase
CL_{int}	Intrinsic Clearance
CL_p	Plasma Clearance
C_{max}	Maximum Concentration
CNT	Concentrative Nucleoside Transporter
CTP	Cytidine Triphosphate
CYP	Cytochrome P450
DDI	Drug-Drug Interaction
EIDD	Emory Institute for Drug Development
EMA	European Medicines Agency
ENT	Equilibrative Nucleoside Transporter
FDA	Food and Drug Administration
GLP	Good Laboratory Practice
hBTEC	Human Bronchial Tracheal Epithelial Cells
HIM	Human Intestinal Microsomes
HIS9	Human Intestinal S9
HLM	Human Liver Microsomes
HLS9	Human Liver S9
HPLC	High-Performance Liquid Chromatography
hr	Hour(s)
HRMS	High-Resolution Mass Spectrometry
IC_{50}	Half-Maximal Inhibitory Concentration
IP	Intraperitoneal
IV	Intravenous
K_2EDTA	Dipotassium Ethylenediaminetetraacetic Acid
K_{obs}	Observed Rate Constant
K_m	Michaelis Constant
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LLC-PK1	Lilly Laboratories Cell Porcine Kidney
LLOQ	Lower Limit of Quantitation
LSC	Liquid Scintillation Counting
mARC	Mitochondrial Amidoxime Reducing Component
MATE	Multidrug and Toxin Extrusion Protein
MDCKII	Madin-Darby Canine Kidney II Cells
MDR1	Multidrug Resistance Protein 1
min	Minute(s)
MHLW	Ministry of Healthy and Labour Welfare

MOV	Molnupiravir (MK-4482, EIDD-2801)
mRNA	Messenger Ribonucleic Acid
MRP2	Multidrug Resistance-Associated Protein-2
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NHC	N-Hydroxycytidine
NHC-TP	N-Hydroxycytidine-5′-Triphosphate
OAT	Organic Anion Transporter
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter
P_{app}	Apparent Permeability Coefficient
P-gp	P-glycoprotein
PMSF	Phenylmethylsulfonyl Fluoride
P.O.	Per Oral
QC	Quality Control
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SD	Sprague Dawley
Sf9	Spodoptera Frugiperda Cells
$t_{½}$	Terminal Half-Life
T_{max}	Time of Maximum Concentration
TP	Triphosphate
ULOQ	Upper Limit of Quantitation
UTP	Uridine Triphosphate
Vd_{ss}	Distribution Volume at Steady State
V_{max}	Maximal Rate

1 BRIEF SUMMARY

Molnupiravir (MOV, known as MK-4482 and EIDD-2801) is a 5'-isobutyrate ester prodrug designed to be rapidly metabolized by serum/cellular esterases to deliver the nucleoside metabolite NHC (formerly EIDD-1931) into systemic circulation following oral administration (only very low levels of MOV detected in plasma). Following uptake into cells, NHC is phosphorylated by host kinases to the pharmacologically active triphosphate NHC-TP (formerly EIDD-2601). NHC-TP acts as a competitive alternative substrate for the viral RNA polymerase and after incorporation into nascent viral RNA can pair with either guanosine or adenosine, and consequently substitute for either CTP or UTP, respectively. This mis-matched pairing results in an accumulation of mutations in the viral genome causing viral error catastrophe and inhibition of replication.

An extensive preclinical evaluation of the pharmacokinetics and metabolism of MOV and the nucleoside NHC, as well as the distribution and exposure of NHC-TP in tissues was completed to support administration of MOV in clinical studies.

When NHC itself was orally administered to cynomolgus macaques, the systemic plasma exposure to NHC was low. The prodrug MOV was designed to increase the systemic bioavailability of NHC, and in subsequent studies it delivered an approximate 6-fold improvement in NHC plasma exposure (AUC) in cynomolgus macaques. In rats and dogs, the bioavailability of NHC after orally administered MOV was high (NHC bioavailability of 52% and ≥77%, respectively). Additionally, the low recovery of radioactivity in feces after oral dosing [^{14}C]MOV to rats and dogs suggests >90% absorption of [^{14}C]MOV-related radioactivity in both of these species. NHC and NHC-TP were observed in all tissues analyzed from each species tested. MOV is hydrolyzed in plasma, especially in rodents, and MOV exposure was generally <1% of NHC after oral dosing in all species tested except dogs. While MOV was hydrolyzed the slowest in dog plasma ($t_{½}$ >3 hr measured *in vitro*), the circulating MOV plasma levels in dogs were still <15% of NHC levels, indicating most of the prodrug was hydrolyzed during absorption/first pass. *In vitro*, MOV is hydrolyzed to NHC in both intestinal and liver microsomes, and intracellularly after uptake by cells from all tissues tested. The widely distributed high-capacity carboxylesterases CES1 and CES2 were both capable of converting MOV to NHC *in vitro*, and likely play a significant role in converting MOV to NHC during absorption/first pass.

While NHC is relatively stable in plasma and liver/intestinal microsomes, intracellularly it is anabolized (phosphorylated) to NHC-TP. Based on *in vitro* metabolism data, NHC and/or its metabolites can also be metabolized in cells to endogenous pyrimidine nucleosides (uridine, cytidine, and their respective phosphorylated metabolites). *In vivo* these mix with the endogenous nucleosides, explaining [^{14}C]MOV-associated radioactivity being retained in the body in animal excretion studies (low recovery of [^{14}C] in excreta was observed). In addition, following oral dosing of [^{14}C]MOV in rats and dogs, small amounts of [^{14}C]-labeled cytidine, uridine, or metabolites thereof were observed in plasma and/or urine, demonstrating conversion to endogenous pyrimidine nucleosides is a route of NHC elimination *in vivo*. Similarly, in both human plasma and urine from subjects dosed with MOV (unlabeled), NHC, and the nucleosides cytidine and/or uridine increased dose dependently, indicating MOV was also converted to these endogenous pyrimidine nucleosides in humans. Both

cytidine deaminase and the mitochondrial amidoxime reducing components mARC1 and mARC2 have demonstrated the capability to convert NHC to endogenous pyrimidines *in vitro*. Currently, based on both *in vitro* and *in vivo* preclinical data, metabolism to endogenous pyrimidine anabolites is most likely the dominant route of elimination of MOV/NHC-related drug material in animals and humans, and renal excretion of MOV or NHC is relatively minor.

Based on the totality of preclinical data, the disposition of MOV in preclinical species following oral administration can be characterized as rapid hydrolysis of the prodrug during absorption/hepatic first pass resulting in near complete drug absorption and high NHC bioavailability. Circulating NHC is then rapidly taken up from systemic circulation into cells potentially via nucleoside transporters, where it is anabolized by host kinases to the pharmacologically active tri-phosphate, NHC-TP. The predominant elimination pathway of NHC appears to be conversion to either uridine or cytidine which mix with the endogenous nucleoside pool. Given the hydrolysis of MOV to NHC is likely by high capacity esterases (CES1 and CES2), and the uptake of NHC and formation of NHC-TP are by host transporters and kinases important in the regulation of pyrimidine nucleosides, it is unlikely that other drugs will impact the exposure of NHC-TP in tissues. While the similarity of NHC to cytidine (one hydroxyl different) allows recognition by the nucleoside transporters and kinases which confer its intracellular uptake and activity, it is also consistent with the observed lack of either inhibitory or inductive activity of MOV/NHC towards any of the xenobiotic metabolic enzymes and transporters tested *in vitro*, suggesting that the potential for DDI's between MOV/NHC and co-medications is low.

2 METHODS OF ANALYSIS

2.1 Quantification of MOV and NHC in Biological Matrices

For toxicokinetic studies in mouse, rat, rabbit, dog, and monkey, the MOV and NHC concentrations in plasma and tissue samples were determined using protein precipitation followed by LC-MS/MS methods. These bioanalytical methods are summarized in [Sec. 2.6.5: 2].

For non-GLP pharmacokinetic and toxicokinetic studies, plasma samples were not treated to stabilize MOV during sample collection, unless where specifically indicated. MOV, NHC, or NHC-TP concentrations were determined in various animal matrices by protein precipitation followed by LC-MS/MS methods qualified according to laboratory procedures for non-GLP study support. Tissue samples were collected by snap frozen in liquid nitrogen to stabilize NHC-TP (and presumably MOV) and stored at -80°C until analysis.

For GLP toxicology studies, the bioanalytical methods were validated in accordance with FDA Guidance for Industry, Bioanalytical Method Validation 2018 [Ref. 4.3: 05GX6H] for the quantitation of MOV and NHC in plasma from mice, rats, rabbits, and dogs. Plasma samples were treated with potassium oxalate/sodium fluoride acetonitrile during sample collection to stabilize MOV. The LLOQ of MOV GLP plasma assays ranged from 1.0 to 10 ng/mL and the ULOQ ranged from 100 to 10,000 ng/mL. The LLOQ of NHC GLP plasma assays ranged from 5.0 to 20 ng/mL and the ULOQ ranged from 5000 to

20,000 ng/mL. Inter-run accuracy of QC samples ranged from -6.4% to 9.0%, and precision ranged from 1.7% to 13.7%. The analytical method validation reports are summarized in [Sec. 2.6.5: 3].

2.2 Determination of Total Radioactivity and Metabolite Profiling

The total radioactivity in plasma, urine, bile, and feces samples was determined by LSC. Metabolites in liver microsomes, hepatocytes, plasma, urine, and feces were separated using a reversed-phase HPLC column, and MOV and its metabolites were detected by HRMS and on-line radiometric detection. The proposed metabolite structures were based on the exact mass and MS/MS fragmentation patterns or compared with synthetic standards.

3 ABSORPTION

MOV is a 5′-isobutyrate ester prodrug cleaved by esterases (including both CES1 and CES2) present in the intestine and liver during absorption/hepatic first pass, delivering the nucleoside metabolite NHC into systemic circulation following oral administration of MOV. Near complete absorption was observed in mass balance studies where [^{14}C]MOV-related radioactivity appears to be >90% absorbed in rats and dogs. The bioavailability of NHC after an oral dose of MOV in rats and dogs was 52% and ≥77%, respectively, at oral doses ranging from 30 to 300 mg/kg.

Facilitated by nucleoside transporters (CNT's and ENT's), NHC rapidly distributes into cells and is phosphorylated by host kinases to the active triphosphate NHC-TP. NHC plasma exposure is dependent on the extent of absorption of MOV and conversion to NHC, as well as the rate of NHC uptake into tissues and extent of NHC-TP formation (an elimination mechanism of plasma NHC). Oral dosing studies with MOV have been completed in mouse, rat, dog, ferret, and monkey.

3.1 Permeability of MOV and NHC *In Vitro*

The permeability of MOV and NHC was evaluated in Caco-2 cell monolayers and both MOV and NHC exhibited low P_{app} (0.29×10^{-6} cm/s and 1.1×10^{-6} cm/s, respectively) [Sec. 2.6.5: 30.1]. Their permeability was also evaluated in LLC-PK1 cells, where the P_{app} of MOV and NHC was 2.7×10^{-6} cm/s and 2.9×10^{-6} cm/s, respectively [Sec. 2.6.5: 30.2]. Due to the potential for intracellular metabolism of both MOV and NHC during these assays, the *in vitro* assessment of permeability may be an underestimation.

3.2 Pharmacokinetics in Mice

The plasma concentrations of MOV and NHC were determined after single IP (32 mg/kg) or P.O. (64, 190, and 635 mg/kg) administration of MOV to female CD-1 mice [Table 2.6.4: 1]. Negligible levels of MOV were observed in the plasma at all doses. Oral absorption was rapid with the T_{max} of NHC occurring at 0.5 hr post-dose at all three dose levels. The plasma AUC of NHC following oral administration was approximately dose-proportional and close to half that observed following IP administration (dose normalized). The oral $t_{½}$ values were

≤4.3 hr at doses up to 190 mg/kg indicating accumulation after multiple-dose is not expected [Sec. 2.6.5: 4].

Table 2.6.4: 1 Pharmacokinetics of NHC in Mice After IP and Oral Administration of MOV

Route	IP	P.O.	P.O.	P.O.
Dose (mg/kg)	32	64	190	635
Vehicle/Formulation	Sterile saline	10% PEG 400, 2.5% Cremophor RH 40 in Water (v/v/v)		
PK parameters				
CL_p (L/hr/kg)	2.8	NA	NA	NA
$t_{½}$ (hr)	3.0	1.5	4.3	8.2
$AUC_{0\text{-}inf}$ (µM*hr)	35.1	31.3	77.8	277.5
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	0.36	0.16	0.13	0.14
C_{max}(µM)	46.6	30.8	52.0	187.4
C_{max} /Dose (kg/L)	0.48	0.16	0.09	0.10
T_{max} (hr)	0.25	0.5	0.5	0.5

Abbreviations: IP = intraperitoneal; P.O. = per oral; hr = hour; MOV = molnupiravir; NHC = N-hydroxycytidine; PEG 400 = polyethylene glycol 400; PK = pharmacokinetics; CL_p = plasma clearance; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$ /Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; T_{max} = time to maximum concentration; NA = not applicable.
[Sec. 2.6.5: 4].

In a separate study where MOV was orally administered to CD-1 mice at 130 mg/kg, NHC again had a T_{max} of 0.5 hr, a terminal oral $t_{½}$ of 1.9 hr, and a similar C_{max} of 22.6 µM and an $AUC_{0\text{-}inf}$ of 48.1 µM*hr as the previous study (when dose normalized) [Sec. 2.6.5: 4].

3.3 Pharmacokinetics in Rats

The plasma concentrations of MOV and NHC were determined after administration of MOV IV at 32 mg/kg and P.O. at 32, 95, and 320 mg/kg to male SD rats [Table 2.6.4: 2]. MOV exposure in plasma was negligible at all doses. Oral absorption was rapid with T_{max} of NHC occurring between 1-2 hr post-dose. The AUC and C_{max} of NHC in plasma after oral dosing were approximately 2-fold greater than dose-proportional at the highest dose, and the oral $t_{½}$ of NHC was 2.8-4.0 hr, indicating accumulation after multiple-doses is not expected [Sec. 2.6.5: 5].

Table 2.6.4: 2 Pharmacokinetic Parameters of NHC in Rats Following IV or Oral Administration of MOV

Route	IV[a]	P.O.	P.O.	P.O.
Dose (mg/kg)	32	32	95	320
CL_p (L/hr/kg)	2.6 ± 0.2	NA	NA	NA
$t_{½}$ (h)	1.1 ± 0.1	2.8 ± 1.0	4.0 ± 0.8	3.1 ± 0.2
$AUC_{0\text{-}inf}$ (µM*hr)	37.0 ± 3.0	12.8 ± 1.8	64.0 ± 5.4	280.0 ± 40.5
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	0.38 ± 0.03	0.13 ± 0.02	0.22 ± 0.02	0.29 ± 0.04
C_{max}(µM)	72.6 ± 3.2	4.4 ± 1.1	16.4 ± 2.2	98.5 ± 28.5
C_{max} /Dose (kg/L)	0.75 ± 0.03	0.04 ± 0.01	0.06 ± 0.01	0.10 ± 0.03
T_{max} (hr)	0.1 ± 0.0	1.8 ± 0.5	1.5 ± 0.6	1.0 ± 0.0

Abbreviations: IV = intravenous; P.O. = per oral; hr = hour; MOV = molnupiravir; NHC = N-hydroxycytidine; CL_p = plasma clearance; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$/Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; T_{max} = time to maximum concentration; NA = not applicable.
Values represent mean ± standard deviation (n=4).
[a] IV bolus.
[Sec. 2.6.5: 5]

The pharmacokinetics of MOV and NHC were also studied in male SD rats following IV administration of NHC (10 mg/kg) and oral administration of MOV (30 mg/kg) [Table 2.6.4:3]. While the plasma samples were stabilized to prevent hydrolysis of MOV, the concentrations of MOV in plasma were below the limit of concentration at all timepoints collected. Following IV administration of NHC to rats, CL_p was 2.8 L/hr/kg, the volume of distribution was 2.8 L/kg. An effective $t_{½}$ of 3.5 hr indicated accumulation after multiple-doses is not anticipated. The mean oral bioavailability of NHC following oral administration of MOV was estimated at 52% [Sec. 2.6.5: 5].

Table 2.6.4: 3 Pharmacokinetic Parameters of NHC in Rats Following IV Administration of NHC or Oral Administration of MOV

Compound Dosed	Route	Dose (mg/kg)	$AUC_{(0\text{-}inf)}$ (µM*hr)	CL_p (L/hr/kg)	T_{max} (hr)	C_{max} (µM)	Vd_{ss} (L/kg)	$t_{½}$ (hr)	F[a] (%)
NHC	IV	10	14.0 ± 2.2	2.8 ± 0.5	NA	NA	2.8 ± 0.3	3.5 ± 0.2	NA
MOV	P.O.[b]	30	22.0 ± 15.5	NA	0.7 ± 0.3	16.7 ± 10.6	NA	NA	52.3 ± 36.6

Abbreviations: IV = intravenous; P.O. = per oral; hr = hour; MOV = molnupiravir; NHC = N-hydroxycytidine; CL_p = plasma clearance; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; C_{max} = maximum concentration; F = oral bioavailability; T_{max} = time to maximum concentration; Vd_{ss} = volume of distribution at steady state; NA = not applicable.
[a] Bioavailability (F) was derived from dose-normalized plasma exposure of NHC after oral dosing of MOV at 30 mg/kg relative to dosed IV with NHC at 10 mg/kg.
[b] Plasma samples were stabilized with potassium oxalate/sodium fluoride to prevent esterase activity.
Values shown are means ± standard deviation (n=3)
[Sec. 2.6.5:5]

3.4 Pharmacokinetics in Dogs

The plasma concentrations of MOV and NHC were determined after oral administration of a single dose of MOV at 30, 100, and 300 mg/kg to male dogs [Table 2.6.4: 4]. While MOV was detected in dog plasma during the first 8-12 hr, the AUC was low, corresponding to only 1.8-7.0% that of NHC. Although the plasma samples were not stabilized to prevent esterase activity during collection, the half-life of MOV in dog plasma measured *in vitro* is >3hr

suggesting the low levels measured in plasma were not due to *ex-vivo* instability post sample collection. The T_{max} of NHC in plasma was between 1-2 hr at all dose levels, and overall, NHC $AUC_{0\text{-}inf}$ and C_{max} values were approximately dose-proportional. NHC was also administered to dogs intravenously (8 mg/kg) to establish the bioavailability of NHC when administering MOV orally. The estimated bioavailability at 30, 100, and 300 mg/kg was 77%, 128%, and 89%, respectively, and NHC plasma clearance was 0.9 L/hr/kg [Sec. 2.6.5: 6].

Table 2.6.4: 4 Pharmacokinetic Parameters of NHC and MOV in Dogs Following IV Administration of NHC or Oral Administration of MOV

Route of administration	**IV[a]**	**P.O.**					
Dose (mg/kg)	8	30		100		300	
Analyte	NHC	NHC	MOV	NHC	MOV	NHC	MOV
CL_p (L/hr/kg)	0.9 ± 0.1	NA	NA	NA	NA	NA	NA
$t_{½}$ (hr)	27.0 ± 7.0	9.2 ± 2.8	0.7 ± 0.2	6.1 ± 0.5	0.7 ± 0.8	5.6 ± 1.0	1.1 ± 0.7
$AUC_{0\text{-}inf}$ (µM*hr)	34.6 ± 4.0	78.5 ± 9.5	4.3 ± 0.7	475.0 ± 39.3	8.4 ± 9.7	905.4 ± 86.4	63.1 ± 8.1
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	1.1 ± 0.1	0.9 ± 0.1	0.05 ± 0.01	1.6 ± 0.1	0.03 ± 0.03	1.0 ± 0.1	0.07 ± 0.01
C_{max} (µM)	50.7 ± 2.0	22.7 ± 5.1	3.0 ± 0.5	109.4 ± 10.2	9.5 ± 2.9	166.3 ± 22.2	42.0 ± 15.2
C_{max} /Dose (kg/L)	1.6 ± 0.1	0.2 ± 0.1	0.03 ± 0.01	0.4 ± 0.03	0.03 ± 0.01	0.2 ± 0.02	0.05 ± 0.02
T_{max} (hr)	0.083	1.0 ± 0.0	0.6 ± 0.3	1.8 ± 0.5	0.6 ± 0.3	2.0 ± 0.0	0.7 ± 0.4
F (%)[b]	NA	77.0 ± 0.5	NA	128.0 ± 1.0	NA	89.0 ± 3.0	NA

Abbreviations: IV = intravenous; P.O. = per oral; hr = hour; CL_p = plasma clearance; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$/Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; F = oral bioavailability; T_{max} = time to maximum concentration; NA = not applicable; NHC = N-hydroxycytidine; MOV = molnupiravir.
Values represent mean ± standard deviation (n=4).
[a] IV bolus.
[b] Bioavailability was derived from dose-normalized plasma exposure to NHC after oral dosing with MOV at different dose levels relative to dosed IV with NHC at 8 mg/kg.
[Sec. 2.6.5: 6]

Male and female dogs were also dosed with 80 mg/kg NHC and 100 mg/kg MOV (approximately equivalent molar doses of NHC) by oral gavage to compare resulting plasma exposure to NHC. Dosing MOV yielded C_{max} and AUC values for NHC in plasma of 152 µM and 548 µM*hr, which were similar to dosing NHC which resulted in a C_{max} and AUC values of 128 µM and 518 µM*hr in plasma. The terminal oral half-life values of NHC after dosing either NHC or MOV were also similar; 7.0 and 6.6 hr, respectively [Sec. 2.6.5: 6].

3.5 Pharmacokinetics in Monkeys

3.5.1 Single Dose

The plasma concentrations of MOV and NHC were determined after single doses of MOV were administered P.O. at 300 and 1000 mg/kg and IV at 100 mg/kg to 4 male and 4 female cynomolgus macaques [Table 2.6.4: 5]. Overall, exposures in males and females were similar. After oral dosing, the C_{max} and $AUC_{0\text{-}inf}$ of MOV in plasma were low, <1% compared to NHC. Oral dosing resulted in approximately dose-proportional increases in NHC $AUC_{0\text{-}inf}$ and C_{max} in plasma. After an IV dose with MOV at 100 mg/kg, T_{max} for NHC

was 5 min post-dose and the terminal half-life of MOV in plasma was only 0.1 hr, while the terminal $t_{½}$ of NHC was 11.1 hr [Sec. 2.6.5: 7].

Table 2.6.4: 5 Pharmacokinetic Parameters of MOV and NHC in Monkeys Following IV or Oral Administration of MOV

Route of administration	IV[a]		P.O.			
Dose (mg/kg)	100		300		1000	
Analyte	NHC	MOV	NHC	MOV	NHC	MOV
$t_{½}$ (hr)	11.1 ± 10.1	0.1 ± 0.1	4.4 ± 2.2	0.2 ± 0.3	3.2 ± 1	1.0 ± 0.5
$AUC_{0\text{-inf}}$ (µM*hr)	246.4 ± 32.6	3.7 ± 1.5	277.6 ± 125.4	0.02 ± 0.03	887.2 ± 365.3	0.41 ± 0.11
$AUC_{0\text{-inf}}$ /Dose (kg*hr/L)	0.81 ± 0.11	0.01 ± 0.00	0.30 ± 0.14	0.00 ± 0.00	0.29 ± 0.12	0.00 ± 0.00
C_{max} (µM)	405.0 ± 50.1	26.3 ± 10.2	91.5 ± 63.6	0.02 ± 0.03	270.1 ± 193.6	0.2 ± 0.16
C_{max} /Dose (kg/L)	1.33 ± 0.16	0.09 ± 0.03	0.10 ± 0.07	0.00 ± 0.00	0.09 ± 0.06	0.00 ± 0.00
T_{max} (hr)	0.083	0.083	1.4 ± 0.7	0.22 ± 0.36	2.5 ± 2.3	0.88 ± 0.73

Abbreviations: IV = intravenous; P.O. = per oral; hr = hour; $t_{½}$ = terminal half-life; $AUC_{0\text{-inf}}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-inf}}$ /Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; T_{max} = time to maximum concentration; MOV = molnupiravir; NHC = N-hydroxycytidine.
Values represent mean ± standard deviation of combined male and female monkeys (n=8).
[a] IV bolus.
[Sec. 2.6.5: 7].

3.5.2 MOV Prodrug Assessment

NHC was orally administered at 100 mg/kg to cynomolgus macaques in the fed or fasted state and no significant difference in plasma NHC exposure was observed. Four male and 4 female animals were then dosed by oral gavage with 100 mg/kg NHC and 130 mg/kg of MOV which result in approximately equimolar doses of NHC. The NHC plasma $AUC_{0\text{-inf}}$ was approximately 6-fold higher when dosing MOV compared to NHC, suggesting an approximately 6-fold improvement in bioavailable NHC was achieved when administering NHC as the prodrug MOV [Sec. 2.6.5: 7].

3.6 Pharmacokinetics in Ferrets

3.6.1 Single Dose

The plasma exposure of NHC were determined after single P.O. doses of MOV were administered to ferrets at 4, 20, 128, and 512 mg/kg [Sec. 2.6.5: 8] [Table 2.6.4: 6]. NHC reached maximal levels within 1.8 hr post-dose for the 4, 20, and 128 mg/kg dose levels. NHC exhibited dose-proportional increases in C_{max} and $AUC_{0\text{-inf}}$. While the C_{max} and $AUC_{0\text{-inf}}$ at the 512 mg/kg dose increased dose-dependently, it did so less than dose-proportionally.

Table 2.6.4: 6 Pharmacokinetic Parameters of NHC in Ferrets Following Oral Administration of MOV

Dose (mg/kg)	4	20	128	512
$t_{½}$ (hr)	8.2 ± 1.7	4.7 ± 1.3	5.1 ± 0.8	4.2 ± 0.6
$AUC_{0\text{-}inf}$ (µM*hr)	13.2 ± 4.8	72.5 ± 32.1	322.4 ± 42.6	791.4 ± 390.7
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	1.1 ± 0.4	1.2 ± 0.5	0.8 ± 0.1	0.5 ± 0.3
C_{max} (µM)	3.5 ± 1.5	15.4 ± 1.9	100.1 ± 22.3	209.2 ± 106.0
C_{max} /Dose (kg/L)	0.3 ± 0.1	0.3 ± 0.03	0.3 ± 0.1	0.1 ± 0.1
T_{max} (hr)	1.7 ± 0.6	1.8 ± 1.9	1.7 ± 0.0	2.5 ± 1.0

Abbreviations: hr = hour; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$ /Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; T_{max} = time to maximum concentration; MOV = molnupiravir; NHC = N-hydroxycytidine.
Values represent mean ± standard deviation (n=3).
[Sec. 2.6.5: 8].

The concentrations of MOV and NHC in plasma were also determined 4hr after a single P.O. dose of MOV at 20 mg/kg. MOV was not detected in plasma, and the levels of NHC were 9.1 µM [Sec. 2.6.5: 8].

3.6.2 Repeat Dose

The levels of MOV and NHC in plasma were determined in influenza A/California/07/2009 infected ferrets that were administered a total of 6 oral doses of MOV every 12 hr (BID) at dose levels of 0.8, 2.3, 7, and 21 mg/kg per dose. Plasma was collected 8 hr after the sixth dose at each dose level. Plasma levels of MOV were negligible at any dose. The levels of NHC in plasma increased dose-dependently and were 0.015, 0.17, 0.28, and 1.0 µM, for the 0.8, 2.3, 7, or 21 mg/kg dose levels, respectively [Sec. 2.6.5: 9].

4 DISTRIBUTION

4.1 Tissue Distribution in Mice

Single doses of 64, 190, and 635 mg/kg MOV were orally administered by gavage to CD-1 mice and the concentrations of NHC and NHC-TP in lung, spleen, kidney, liver, heart, and brain tissue were determined. NHC and NHC-TP AUC in all tissues increased in an approximately dose-proportional manner. NHC-TP was observed in all tissue types sampled, with the highest exposure (AUC) in spleen followed by lung, and the lowest was in liver. Pharmacokinetic parameters for NHC and NHC-TP in tissues are summarized in [Sec. 2.6.5: 10] [Table 2.6.4: 7].

Table 2.6.4: 7 Tissue Distribution of NHC and NHC-TP in Mice

Tissue	Dose mg/kg	Analyte	T_{max} (hr)	C_{max}[a] (µg/g)	$AUC_{0\text{-}last}$[a] (µg*hr/g)	$AUC_{0\text{-}inf}$[a] (µg*hr/g)	$t_{½}$ (hr)
Lung	64	NHC	0.5	9.6	14.2	14.2	1.2
Lung	64	NHC-TP	2	0.9	6.9	7.3	5.5
Lung	190	NHC	0.5	16.2	31.9	32.0	3.1
Lung	190	NHC-TP	2	2.1	20.6	22.8	7.0
Lung	635	NHC	0.5	54.8	105.0	105.2	1.9
Lung	635	NHC-TP	3	6.9	63.8	67.8	5.6
Brain	64	NHC	0.5	1.3	4.1	4.2	4.4
Brain	64	NHC-TP	4	0.8	7.3	7.6	5.1
Brain	190	NHC	0.5	2.6	10.6	11.0	5.5
Brain	190	NHC-TP	2	1.5	15.5	16.7	6.1
Brain	635	NHC	0.5	8.2	31.2	32.3	5.5
Brain	635	NHC-TP	4	4.0	43.5	49.5	7.4
Liver	64	NHC	0.5	3.3	3.5	ND	ND
Liver	64	NHC-TP	1	1.2	4.1	5.9	16.6
Liver	190	NHC	0.5	0.6	1.2	ND	ND
Liver	190	NHC-TP	2	0.4	6.0	11.0	20.7
Liver	635	NHC	0.5	8.7	9.3	9.3	1.1
Liver	635	NHC-TP	0.5	7.1	20.2	28.1	16.6
Spleen	64	NHC	0.5	35.2	47.3	47.4	1.4
Spleen	64	NHC-TP	2	5.7	49.2	54.6	7.1
Spleen	190	NHC	0.5	77.9	126.4	126.7	3.2
Spleen	190	NHC-TP	2	9.1	94.3	97.3	4.8
Spleen	635	NHC	0.5	165.9	320.4	322.1	3.7
Spleen	635	NHC-TP	2	24.1	297.3	310.4	5.4
Kidney	64	NHC	0.5	9.5	10.7	10.7	1.0
Kidney	64	NHC-TP	1	1.0	4.2	4.8	8.5
Kidney	190	NHC	0.5	25.6	32.0	32.0	3.7
Kidney	190	NHC-TP	1	1.9	8.1	9.6	9.9
Kidney	635	NHC	0.5	92.1	126.0	126.3	3.4
Kidney	635	NHC-TP	1	4.4	26.6	28.9	6.7
Heart	64	NHC	0.5	8.6	11.4	11.4	1.8
Heart	64	NHC-TP	1	1.1	6.5	6.6	4.3
Heart	190	NHC	0.5	15.4	28.5	28.5	2.0
Heart	190	NHC-TP	2.0	1.3	12.7	13.6	5.8
Heart	635	NHC	0.5	58.1	91.2	91.8	3.9
Heart	635	NHC-TP	3	4.0	40.0	44.5	7.0

Abbreviations: $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}last}$ = area under the concentration-time curve from time zero to the last quantifiable concentration; C_{max} = maximum concentration; hr = hour; T_{max} = time of maximum concentration; $t_{½}$ = terminal half-life; ND = not determined; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Values represent mean (n=3).
[a] Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
[Sec. 2.6.5: 10].

4.2 Tissue Distribution in Rats

4.2.1 Single Dose

A single dose of 320 mg/kg MOV was orally administered by gavage to SD rats and the concentrations of MOV, NHC, and NHC-TP in lung, spleen, kidney, liver, heart, and brain tissue were determined. MOV was not observed in kidney or brain and was only observed at

low levels near the limit of detection in other tissues. NHC-TP was observed in all tissue types sampled, with the highest exposure (AUC) in lung and spleen, and the lowest was in liver. Pharmacokinetic parameters for NHC and NHC-TP in tissues are summarized in [Sec. 2.6.5: 11.1] [Table 2.6.4: 8].

Table 2.6.4: 8 Tissue Distribution of NHC and NHC-TP in Rats

Tissue	Dose mg/kg	Analyte	T_{max} (hr)	C_{max} [a] (µg/g)	$AUC_{0\text{-}last}$ [a] (µg*hr/g)	$AUC_{0\text{-}inf}$ [a] (µg*hr/g)	$t_{½}$ (hr)
Lung	320	NHC	2	43.0	253.4	254.8	3.4
Lung	320	NHC-TP	4	19.5	237.8	243.3	4.3
Brain	320	NHC	2	9.7	96.3	99.8	5.1
Brain	320	NHC-TP	6	4.5	69.3	81.6	7.8
Liver	320	NHC	1	22.9	51.2	51.3	2.8
Liver	320	NHC-TP	1	1.5	20.1	22.4	7.4
Spleen	320	NHC	2	84.1	533.9	535.5	3.0
Spleen	320	NHC-TP	4	18.8	204.2	207.1	3.8
Kidney	320	NHC	2	44.6	218.8	219.0	2.5
Kidney	320	NHC-TP	2	4.5	33.6	34.0	3.9
Heart	320	NHC	1	48.3	228.3	228.7	2.7
Heart	320	NHC-TP	4	4.4	50.4	51.2	4.0

Abbreviations: $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}last}$ = area under the concentration-time curve from time zero to the last quantifiable concentration; C_{max} = maximum concentration; hr = hour; T_{max} = time of maximum concentration; $t_{½}$ = terminal half-life; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Values represent mean (n=3).
[a] Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
[Sec. 2.6.5: 11.1].

Male SD rats received a single oral gavage dose of 50, 150, or 500 mg/kg MOV. Bone marrow tissue was collected at 3 and 24 hr post-dose and tissue levels of MOV, NHC, and NHC-TP were determined by LC-MS/MS [Table 2.6.4: 9]. MOV was below the quantitation limit of detection in all samples. At 3 hr post-dose, the levels of NHC and NHC-TP in bone marrow were approximately dose-proportional. At 24 hr post-dose, the levels of NHC decreased significantly (>100-fold) and were only detectable in the highest dose group. NHC-TP concentrations at 24 hr also decreased 20 to 30-fold compared to the 3-hr levels in all dose groups and remained dose-dependent [Sec. 2.6.5: 11.3].

Table 2.6.4: 9 Rat Bone Marrow Concentrations of MOV, NHC, and NHC-TP After a Single Oral Dose of MOV

Time Post-Dose (hr)	Dose Level (mg/kg)	Concentrations in Bone Marrow (µg/g)		
		MOV	NHC	NHC-TP
3	50	BQL	2.85	1.92
	150	BQL	7.57	4.71
	500	BQL	36.39	15.12
24	50	BQL	BQL	0.09
	150	BQL	BQL	0.15
	500	BQL	0.25	0.50

Abbreviations: BQL = below quantitation limit; hr = hour; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Value represent mean (n=3).
[Sec. 2.6.5: 11.3]

4.2.2 7-Day Repeat Dose

MOV was orally administered by gavage to rats once daily at doses of 200, 700, and 2000 mg/kg/day for seven consecutive days, and the concentrations of MOV, NHC, and NHC-TP in liver, heart, brain, kidney, lung, and spleen tissue at 2, 4, and 24 hr after the 1st and last (7th) dose were determined. MOV was not detectable or was near the limit of quantitation in all tissues with the two lower doses. At 2000 mg/kg/day, MOV was generally ≤1% of the NHC levels present in tissues. NHC-TP exposure was approximately dose-dependent in all organs, with no meaningful accumulation after multiple-dose, except in the liver at 2000 mg/kg/day there was approximately 4-fold accumulation [Sec. 2.6.5: 11.2]. The tissue concentrations of NHC and NHC-TP at the lowest dose of 200 mg/kg are summarized in [Table 2.6.4: 10].

Table 2.6.4: 10 Tissue Distribution of NHC and NHC-TP in Rats After a Single P.O. or 7 QD P.O. Doses of MOV at 200 mg/kg

Tissue	Analyte	Day 1[a]			Day 7[a]		
		2 hr (µg/g)	4 hr (µg/g)	24 hr (µg/g)	2 hr (µg/g)	4 hr (µg/g)	24 hr (µg/g)
Liver	NHC	2.7 ± 2.4	1.1 ± 0.8	< 0.1	2.5 ± 1.5	0.9 ± 0.8	< 0.1
Liver	NHC-TP	2.0 ± 1.6	1.2 ± 0.9	0.4 ± 0.4	1.1 ± 0.2	0.9 ± 0.5	0.2 ± 0.0
Heart	NHC	15.1 ± 5.5	29.2 ± 26.0	0.0 ± 0.0	16.7 ± 8.0	4.6 ± 4.7	0.2 ± 0.1
Heart	NHC-TP	2.2 ± 0.7	10.9 ± 12.7	< 0.1	1.4 ± 0.4	0.7 ± 0.2	0.1 ± 0.0
Brain	NHC	10.7 ± 1.6	6.7 ± 1.9	0.4 ± 0.1	9.0 ± 0.9	4.1 ± 2.0	0.3 ± 0.2
Brain	NHC-TP	1.8 ± 0.1	2.0 ± 1.0	0.4 ± 0.1	1.0 ± 0.4	0.7 ± 0.3	0.8 ± 0.5
Kidney	NHC	21.9 ± 21.1	5.5 ± 4.4	< 0.1	17.7 ± 13.1	5.6 ± 5.7	0.2 ± 0.1
Kidney	NHC-TP	1.9 ± 3.2	0.6 ± 0.5	0.1 ± 0.0	1.1 ± 0.6	0.7 ± 0.5	1.0 ± 0.3
Lung	NHC	18.2 ± 16.0	5.7 ± 3.8	0.1 ± 0.1	13.8 ± 5.3	8.3 ± 5.5	0.1 ± 0.0
Lung	NHC-TP	1.4 ± 0.6	3.4 ± 2.2	0.1 ± 0.1	1.2 ± 0.5	1.0 ± 0.4	0.3 ± 0.2
Spleen	NHC	100.2 ± 64.8	29.6 ± 17.7	0.5 ± 0.5	98.8 ± 12.9	50.0 ± 24.9	1.0 ± 0.5
Spleen	NHC-TP	3.3 ± 1.2	3.8 ± 1.1	0.2 ± 0.2	2.4 ± 0.3	1.6 ± 0.8	0.3 ± 0.1

Abbreviations: hr = hour, MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; P.O. = per oral; QD = once a day.
Values represent mean ± standard deviation (n = 4, two male and two female rats).
MOV was administered once daily by oral gavage to male and female rats for seven days at doses of 200 mg/kg/day. Liver, heart, brain, kidney, lung, and spleen samples were collected from 2 rats/sex at 2, 4, and 24 hr after the 1st and last (7^{th}) dose.
[a] Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
[Sec. 2.6.5: 11.2].

4.3 Tissue Distribution in Dogs

4.3.1 Single Dose

A single dose of 100 mg/kg MOV was orally administered to dogs, and the concentrations of MOV, NHC, and NHC-TP in brain (olfactory bulb), brain (limbic system), spleen, lung, kidney, liver, and heart tissue at 3 hr and 24 hr post-dose were determined. MOV was observed only in kidney at 3 hr post-dose at a relatively low level. NHC-TP was observed in all tissue types sampled, with the highest exposure in spleen and lowest in brain. The concentrations of MOV, NHC, and NHC-TP are summarized in [Sec. 2.6.5: 12.1] [Table 2.6.4: 11].

Table 2.6.4: 11 Tissue Distribution of MOV, NHC, and NHC-TP in Dogs

Tissue	Analyte	3 hr (µg/g)	24 hr (µg/g)
Brain-Olfactory bulb	NHC	3.7 ± 0.1	0.6 ± 0.3
Brain-Olfactory bulb	NHC-TP	0.3 ± 0.3	0.4 ± 0.3
Brain-Olfactory bulb	MOV	BQL	BQL
Brain-Limbic system	NHC	1.1 ± 0.6	0.3 ± 0.2
Brain-Limbic system	NHC-TP	0.1 ± 0.0	0.1 ± 0.1
Brain-Limbic system	MOV	BQL	BQL
Spleen	NHC	12.8 ± 1.4	0.9 ± 0.0
Spleen	NHC-TP	31.1 ± 4.2	18.8 ± 5.3
Spleen	MOV	BQL	BQL
Lung	NHC	19.8 ± 3.8	3.2 ± 1.5
Lung	NHC-TP	2.4 ± 2.8	2.5 ± 1.3
Lung	MOV	BQL	BQL
Kidney	NHC	38.0 ± 8.4	0.5 ± 0.2
Kidney	NHC-TP	2.1 ± 0.2	0.3 ± 0.0
Kidney	MOV	0.3 ± 0.1	BQL
Liver	NHC	15.0 ± 4.9	0.2 ± 0.0
Liver	NHC-TP	4.4 ± 0.8	0.4 ± 0.1
Liver	MOV	BQL	BQL
Heart	NHC	15.0 ± 1.0	0.4 ± 0.1
Heart	NHC-TP	5.9 ± 0.6	1.4 ± 0.3
Heart	MOV	BQL	BQL

Abbreviations: hr = hour; BQL = below the quantifiable limit; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Values represent mean or mean ± standard deviation (n=2).
Data were recalculated by multiplying molar unit concentrations from study report by molecular weight of analyte.
Molecular weight: MOV = 329.31; NHC = 259.22; NHC-TP = 499.157.
[Sec. 2.6.5: 12.1].

4.3.2 7-Day Repeat Dose

MOV was administered orally to male and female dogs once daily up to seven days at doses of 100, 300, and 1000 mg/kg/day and the concentrations of MOV, NHC, and NHC-TP in brain (olfactory bulb), brain (limbic system), spleen, lung, kidney, liver, and heart tissue were determined. Tissue samples were collected 24 hr after the 7th oral dose at 100 mg/kg/day, 27-28 hr after the 6th oral dose at 300 mg/kg/day (unscheduled termination due to toxicity), and 28-30 hr after the 3rd oral dose at 1000 mg/kg/day (unscheduled termination due to toxicity). MOV exposure was negligible in all tissues. NHC-TP was observed in all tissue types sampled, with the highest level in spleen and lung, and lowest in brain. The tissue concentrations of NHC and NHC-TP are summarized in [Sec. 2.6.5: 12.2] [Table 2.6.4: 12].

Table 2.6.4: 12 Tissue Distribution of NHC and NHC-TP in Dogs After Repeated QD P.O. Dose of MOV at 100, 300, and 1000 mg/kg/day

Tissue	Analyte	100 mg/kg/day 24 hr (µg/g)[a]	300 mg/kg/day 27-28 hr (µg/g)[b]	1000 mg/kg/day 28-30 hr (µg/g)[c]
Brain (Limbic system)	NHC	0.8 ± 0.3	1.1 ± 0.3	3.1 ± 0.8
Brain (Limbic system)	NHC-TP	1.0 ± 0.3	2.1 ± 1.4	5.7 ± 2.5
Brain (Olfactory Bulb)	NHC	1.7 ± 1.0	2.0 ± 0.2	5.1 ± 5.1
Brain (Olfactory Bulb)	NHC-TP	4.1 ± 6.0	2.2 ± 1.3	6.6 ± 2.6
Spleen	NHC	2.2 ± 0.4	8.5 ± 6.1	13.9 ± 6.9
Spleen	NHC-TP	101.2 ± 12.6	175.4 ± 61.0	177.5 ± 61.6
Lung	NHC	7.9 ± 6.8	28.7 ± 30.2	25.4 ± 24.1
Lung	NHC-TP	14.4 ± 13.1	108.2 ± 58.2	96.7 ± 46.5
Kidney	NHC	1.0 ± 0.6	2.6 ± 2.1	2.7 ± 0.9
Kidney	NHC-TP	4.9 ± 2.3	34.0 ± 42.5	20.8 ± 18.2
Liver	NHC	1.7 ± 1.3	1.0 ± 0.8	1.9 ± 0.9
Liver	NHC-TP	13.9 ± 7.3	14.4 ± 10.2	35.2 ± 4.3
Heart	NHC	1.9 ± 0.9	2.4 ± 2.4	3.6 ± 2.7
Heart	NHC-TP	8.0 ± 1.5	10.5 ± 7.8	24.1 ± 14.0

Abbreviations: hr = hour, MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; P.O. = per oral; QD = once a day.
Values represent mean ± standard deviation (n = 4, two male and two female dogs).
Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte.
Molecular weight: NHC = 259.22; NHC-TP = 499.15.
[a] MOV was administered once daily by oral gavage to male and female dogs for seven days at doses of 100 mg/kg/day. Brain (olfactory bulb), brain (limbic system), spleen, lung, kidney, liver, and heart tissues were collected 24 hr after the 7th oral dose at 100 mg/kg/day at the end of the study.
[b] Tissues were collected at 27-28 hr after the 6th oral dose at 300 mg/kg/day (unscheduled termination due to toxicity).
[c] Tissues were collected at 28-30 hr after the 3rd oral dose at 1000 mg/kg/day (unscheduled termination due to toxicity).
[Sec. 2.6.5: 12.2].

4.4 Tissue Distribution in Monkeys

An IV dose of 100 mg/kg MOV was administered to cynomolgus macaques (4 males and 4 females) and concentrations of NHC and NHC-TP in brain (olfactory bulb), brain (limbic system), spleen, lung, kidney, liver, and heart tissue were determined. Tissue samples were collected at 2 and 12 hr post-dose from two monkeys per gender per time point. MOV was not detected in all tissues tested. NHC and NHC-TP were present in all tissues and there were no meaningful differences in exposure between male and female. The highest concentrations of NHC-TP were in spleen and then lung at 12 hr post-dose, while brain had the lowest levels. The tissue concentrations of NHC and NHC-TP are summarized in [Sec. 2.6.5: 13] [Table 2.6.4: 13].

Table 2.6.4: 13 Tissue Distribution of NHC and NHC-TP in Monkeys

Tissue	NHC (µg/g)		NHC-TP (µg/g)	
	2hr	12hr	2hr	12hr
Brain (Olfactory Bulb)	2.7 ± 1.5	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
Brain (Limbic System)	2.5 ± 0.8	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Spleen	22.6 ± 5.5	3.7 ± 1.9	7.3 ± 3.7	11.3 ± 14.0
Lung	10.8 ± 5.5	6.6 ± 9.1	0.6 ± 0.1	0.9 ± 0.9
Kidney	1.0 ± 0.4	0.0 ± 0.0	0.5 ± 0.1	0.4 ± 0.3
Liver	2.6 ± 0.9	0.2 ± 0.3	0.6 ± 0.3	0.7 ± 0.6
Heart	1.1 ± 0.4	0.1 ± 0.0	0.5 ± 0.3	0.3 ± 0.1

Abbreviations: hr = hour; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Values represent mean or mean ± standard deviation (n=4).
Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte.
Molecular weight: NHC = 259.22; NHC-TP = 499.15.
[Sec. 2.6.5: 13]

4.5 Tissue Distribution in Ferrets

A single dose of 20 mg/kg MOV was orally administered to ferrets and the concentrations of MOV, NHC, and NHC-TP in brain, spleen, heart, lung, kidney, and liver tissue (4 hr post-dose) were determined. MOV was not detected in any tissue except the kidney where it was low. NHC-TP levels were highest in the spleen followed by lung, and lowest in brain. The tissue concentrations of NHC and NHC-TP are summarized in [Sec. 2.6.5: 14] [Table 2.6.4: 14].

Table 2.6.4: 14 Tissue Distribution of MOV, NHC and NHC-TP in Ferrets

Tissue	NHC (µg/g)	NHC-TP (µg/g)	MOV (µg/g)
Lung	2.6 ± 0.5	7.0 ± 0.7	BQL
Heart	1.5 ± 0.5	1.4 ± 0.2	BQL
Liver	0.5 ± 0.3	3.5 ± 3.2	BQL
Spleen	4.2 ± 1.4	10.8 ± 2.1	BQL
Kidney	1.1 ± 0.9	0.7 ± 0.1	0.01 ± 0.01
Brain	0.5 ± 0.1	0.5 ± 0.1	BQL

Abbreviations: BQL = below the quantifiable limit; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Values represent mean ± standard deviation (n=3).
Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte.
Molecular weight: MOV = 329.31; NHC = 259.22; NHC-TP = 499.15.
[Sec. 2.6.5: 14].

A single dose of 128 mg/kg MOV was orally administered to ferrets, and the concentrations of NHC and NHC-TP in brain, spleen, heart, lung, kidney, and liver tissue at 3 and 12 hr post-dose (one animal collected at 10 hr) were determined. NHC-TP was observed in all tissue-types sampled throughout the study, with the highest exposure in spleen and the lowest in brain. The concentrations of NHC and NHC-TP are summarized in [Table 2.6.4: 15] [Sec. 2.6.5: 14].

Table 2.6.4: 15 Tissue Distribution of NHC and NHC-TP in Ferrets

Collection Time (number of animals)	Analyte	Lung (µg/g)	Heart (µg/g)	Liver (µg/g)	Spleen (µg/g)	Kidney (µg/g)	Brain (µg/g)
3 hr (3)[a]	NHC	23.0 ± 7.1	19.1 ± 9.5	14.9 ± 10.1	16.2 ± 6.9	24.8 ± 13.8	3.9 ± 2.0
10 hr (1)[b]	NHC	6.7	0.4	0.5	4.6	1.6	0.6
12 hr (2)[c]	NHC	2.8 ± 0.2	0.3 ± 0.0	0.3 ± 0.0	1.0 ± 0.1	0.5 ± 0.0	0.2 ± 0.1
3 hr (3)[a]	NHC-TP	4.4 ± 1.1	8.6 ± 2.1	6.4 ± 6.5	39.6 ± 14.8	4.0 ± 2.3	2.1 ± 1.1
10 hr (1)[b]	NHC-TP	2.9	2.7	0.9	16.4	0.6	0.1
12 hr (2)[c]	NHC-TP	1.6 ± 0.4	1.3 ± 0.2	0.4 ± 0.1	12.3 ± 1.1	0.6 ± 0.0	0.1 ± 0.0

Abbreviations: hr = hour; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte.
Molecular weight: NHC = 259.22; NHC-TP = 499.15.
[a] Values represent mean or mean ± standard deviation (n=3).
[b] One animal was sacrificed 10 hr post-dose instead of the intended 12 hr post-dose.
[c] Values represent mean or mean ± standard error (n=2).
[Sec. 2.6.5: 14].

4.6 Plasma Protein Binding

The binding of NHC in CD-1 mouse, SD rat, beagle dog, cynomolgus monkey, and human plasma, and in human alpha1-acid glycoprotein and human serum albumin was measured using rapid equilibrium dialysis for 6 hr at 37°C. Concentrations of 2, 20, and 100 µM were tested in each matrix. The unbound fraction of NHC was approximately 1 in all matrices and at all concentrations tested [Sec. 2.6.5: 15]. The plasma protein binding of MOV was not assessed since it is not stable in plasma.

5 METABOLISM

5.1 *In Vivo* Metabolism

The exposures to the MOV metabolites NHC and NHC-TP in mice, rats, dogs, ferrets, and monkeys have been presented in the Distribution Section [Sec. 2.6.4: 4].

The *in vivo* metabolism of MOV was studied in male BDC Wistar Han rats and male intact beagle dogs following oral administration of [^{14}C]MOV formulated as a solution in 1% methylcellulose at 30 mg/kg. A semi-quantitative analysis was also conducted with pooled urine and pooled plasma samples obtained from healthy subjects (P004 and P012) treated with multiple oral doses of MOV (unlabeled). The proposed structures of the metabolites identified in excreta and plasma from nonclinical species or human are presented in [Figure 2.6.4: 1].

5.1.1 *In Vivo* Metabolism in Rats

Following oral administration of [^{14}C]MOV to BDC rats at 30 mg/kg, the mean total recovery of administered dose was 67% over the period of 72 hr, with 0.2, 5.5, and 6.8% of the dose recovered in bile, urine, and feces, respectively. The majority of the radioactive dose was retained in the body, with 54% recovered from the animal carcasses [Sec. 2.6.5: 19]. The low recovery in feces (6.8%) indicates MOV-related radioactivity was well absorbed in rats,

likely >90%. Given the short $t_{½}$ of NHC in rat plasma when dosing MOV [Table 2.6.4: 2], as well as the low levels of NHC and NHC-TP in tissues observed after 24 hr in a single dose distribution study [Table 2.6.4: 10], the retained radioactivity in the carcass suggests that the majority of the dose was ultimately metabolized to pyrimidine metabolites (uridine, cytidine, etc.) as was observed *in vitro* [Table 2.6.4: 22] which then mix with the endogenous pyrimidine pool. Metabolite profiling analysis of excreta showed that the major radioactive components detected in urine were the metabolites of uridine, uracil, and 3-ureidopropionic acid, which accounted for 1.4 and 1.8% of administered dose, respectively. Relatively small amounts of cytidine, NHC, a glucuronide metabolite of NHC and a methylation metabolite of NHC were also detected in rat urine (each <1% of the dose). Uracil was also the major radioactive component in feces (4.6% of the dose), along with a small amount of 3-ureidopropionic acid (<1% of the dose) and an unknown metabolite (1.4% of the dose). MOV was not detected in any excreta in rats. The major circulating radioactive components in rat plasma were cytidine, uridine and 3-ureidopropionic acid, which accounted for 53, 21, and 14% of the total circulating radioactivity, respectively. Additionally, small amounts of uracil and NHC were also detected in rat plasma (each <10% of the radioactivity) [Sec. 2.6.5: 16] [Table 2.6.4: 16].

The presence of [^{14}C]-labeled uracil and 3-ureidopropionic acid in rats suggests that an unknown amount of the [^{14}C]MOV dose in this study was ultimately released as [^{14}C]-labeled CO_2 as this is a known pathway of endogenous uracil metabolism *in vivo* [Ref. 4.3: 05Q3MZ].

5.1.2 *In Vivo* Metabolism in Dogs

Following oral administration of [^{14}C]MOV to intact dogs at 30 mg/kg, the total recovery of radioactivity in excreta (urine and feces) and cage wash was only 9.9% of the administered dose within 96 hr post-dose, with 9% of the dose recovered in urine, and 0.5% and 0.4% of the dose recovered in feces and cage wash, respectively [Sec. 2.6.5: 19]. The low recovery in feces (0.5%) indicates MOV-related radioactivity was well absorbed in dogs, likely >90%. Given the short $t_{½}$ of NHC in dog plasma when dosing MOV [Table 2.6.4: 4], as well as the low levels of NHC and NHC-TP in tissues observed after 24 hr in a single dose distribution study [Table 2.6.4: 11], the low recovery suggests that the majority of the dose was retained in the body as a result of ultimately being metabolized to pyrimidine metabolites (uridine, cytidine, etc.) as was observed *in vitro* [Table 2.6.4: 22] which then mix with the endogenous pyrimidine pool. Metabolite profiling analysis of dog urine showed that NHC was the major radioactive component in urine (8% of the dose). A relatively small amount of 3-ureidopropionic acid was also detected (<1% of the dose), along with a small amount of an unknown metabolite (<1% of the dose). NHC was also the major circulating radioactive component in dog plasma at 2 hr post-dose (96% of the radioactivity), with a small amount of cytidine and trace level of MOV detected in dog plasma [Sec. 2.6.5: 16] [Table 2.6.4: 16].

The presence of [^{14}C]-labeled 3-ureidopropionic acid in dogs suggests that some unknown amount of the [^{14}C]MOV dose in this study was ultimately released as [^{14}C]-labeled CO_2 since this is a known pathway of endogenous uracil metabolism *in vivo* [Ref. 4.3: 05Q3MZ].

5.1.3 *In Vivo* Metabolism in Humans

A semi-quantitative analysis was conducted with pooled human urine samples (0-12 hr samples, Day 1) from human healthy subjects treated with multiple oral doses of 100 mg BID or 800 mg BID MOV (P004) [Sec. 2.6.5: 16]. NHC, cytidine, uridine, and NHC-glucuronide were all detected in urine from both dose groups with concentrations that increased in an approximate dose-dependent manner. The levels of NHC and NHC-glucuronide in urine increased approximately 18- and 13-fold, respectively, in the 800 mg BID dose group compared to the 100-mg BID dose group. Uridine increased approximately 6-fold in the 800 mg BID dose, and while the fold-increase in cytidine could not be calculated because little to no cytidine was detected at the lower dose, the increase was likely as much or greater than that observed for uridine. The dose-dependent increases of these two natural pyrimidine bases in human urine suggest that some amount was derived from the oral dose of MOV.

A semi-quantitative analysis was also conducted with pooled human plasma samples (0-12 hr samples, both Day 1 and Day 11) from healthy subjects treated with multiple oral doses of 400 mg BID or 800 mg BID MOV for 10.5 days (P012) [Sec. 2.6.5: 16]. NHC and uridine were detected in human plasma from both dose groups, and their exposures increased in an approximately dose-dependent manner. The range of fold changes for uridine from pre-dose, baseline levels to 400 mg was approximately 1.2-1.5 and from 400 mg to 800 mg approximately was 1.6-1.7 on Day 1 and 11. The dose-dependent increases of uridine suggest that some amount was derived from the oral dose of MOV. In addition, trace levels of a glucuronide metabolite of NHC and a glucose conjugate of NHC were detected in both dose groups. Cytidine was not detected in either dose groups. All of the major metabolites detected in human plasma have also been observed previously in either rats and/or dogs.

These data can be compared qualitatively to the metabolites found in [^{14}C]MOV mass balance studies in rats and dogs where the recovery of dose in excreta was low (<13% of dose in 3 or 4 days in rats and dogs, respectively). In these studies, low amounts of either [^{14}C]-labeled cytidine, uridine, uracil, or uracil metabolites were observed in urine and/or in plasma [Table 2.6.4: 16].

Overall, these data are consistent with the expectation that the majority of the MOV-related dose in animals and humans is converted to NHC, NHC-TP, and (or ultimately to) uridine and/or cytidine which then mix with the endogenous nucleoside pool.

Table 2.6.4: 16 Summary of MOV and its Metabolites in Plasma and Excreta from Nonclinical Species and Humans Following Oral administration of [^{14}C]MOV or Unlabeled MOV

Species	Rat[a] 30 mg/kg			Dog[b] 30 mg/kg		Human[c]		Human[d]			
Matrix	Presence of MOV and Metabolites in Plasma and Excreta[e]					Peak area/IS ratio[f]		Peak area/IS ratio[f]			
	Urine	Feces	Plasma	Urine	Plasma	Urine (100 mg BID)	Urine (800 mg BID)	Plasma (400 mg BID)		Plasma (800 mg BID)	
Sample Pooling Interval (hr)	0-48	0-24	0-24	0-24	2	0-12 (Day 1)	0-12 (Day 1)	0-12 (Day 1)	0-12 (Day 11)	0-12 (Day 1)	0-12 (Day 11)
MOV	-	-	-	-	+	-	-	-	-	-	-
NHC	0.4	-	3	8	96	0.034	0.62	0.128	0.152	0.265	0.285
3-ureidopropionic acid	1.8	0.5	14	0.4	-	-	-	-	-	-	-
Uracil	1.4	4.6	6	-	-	-	-	-	-	-	-
Cytidine	0.5	-	53	-	4	Trace	0.042	-	-	-	-
Uridine[g]	-	-	21	-	-	0.0085	0.053	0.189	0.236	0.307	0.398
M1 (NHC-Glu)	0.4	-	-	-	-	0.0049	0.064	Trace	Trace	Trace	Trace
M2 (NHC-methylation)	0.5	-	-	-	-	-	-	-	-	-	-
M3 (NHC-glucose)	-	-	-	-	-	-	-	Trace	Trace	Trace	Trace
Unknown1	-	1.4	-	-	-	-	-	-	-	-	-
Unknown2	-	-	-	0.4	-	-	-	-	-	-	-

Abbreviations: Glu = glucuronide; MOV = molnupiravir; NHC = N-hydroxycytidine; BID = twice daily; BDC = bile duct-cannulated; hr = hour; IS = internal standard; AUC = area under the concentration-time curve; + = detected by mass spectrometry only; - = not detected.

[a] Metabolite profiling was conducted in pooled urine, feces and plasma samples from BDC rats following oral administration of [^{14}C]MOV at 30 mg/kg. Rat bile samples were not analyzed due to low radioactivity recovered in samples.

[b] Metabolite profiling was conducted in pooled urine and plasma samples from intact dogs following oral administration of [^{14}C]MOV at 30 mg/kg. Dog fecal samples were not analyzed due to low radioactivity recovered in samples.

[c] Metabolite profiling was conducted in pooled urine (0-12 hr, Day 1) samples from healthy subjects treated with multiple oral doses at 100 mg BID or 800 mg BID (P004).

[d] Metabolite profiling was conducted in AUC pooled plasma samples (0-12 hr, Day 1 and Day 11) from healthy subjects treated with multiple oral doses at 400 mg BID or 800 mg BID for 10.5 days (P012).

[e] Values represent percentage relative to the total integrated radioactivity in the plasma or percentages relative to the administered dose in urine and feces.

[f] All estimations of metabolites were semi-quantitative and assumed that matrix effect was the same for the metabolites in the samples from the two dose groups in each matrix.

[g] The peak area/IS ratio of uridine in pre-dose human plasma samples from average of two dose groups was 0.158.

[Sec. 2.6.5: 16].

5.2 *In Vitro* Metabolism

5.2.1 Metabolic Stability of MOV and NHC

The stability of MOV (1 µM) was assessed in plasma and liver microsomes from mouse, rat, dog, monkey, and humans. MOV was relatively unstable in mouse, rat, and monkey plasma (all $t_{½}$ ≤0.4 hr), while more stable in human and dog plasma ($t_{½}$ 1.05 and 3.2 hr, respectively). MOV was relatively unstable in mouse, rat, dog, and monkey liver microsomes ($t_{½}$ 0.02 - 0.08 hr) while more stable in human liver microsomes ($t_{½}$ 1.2 hr). These data are summarized below [Sec. 2.6.5: 17.1] [Table 2.6.4: 17]. MOV was stable in simulated gastric and intestinal fluids ($t_{½}$ >24 hr) [Sec. 2.6.5: 17.2]. NHC was stable when incubated with plasma, whole blood, liver microsomes, and liver S9 extracts and intestinal microsomes from mouse, rat, dog, monkey, and human ($t_{½}$ all ≥3 hr) as shown below [Sec. 2.6.5: 17.3] [Table 2.6.4: 18].

Table 2.6.4: 17 Metabolic Stability of MOV in Plasma and Liver Microsomes

Species	Plasma stability	LM stability
	$t_{1/2}$ (hr)	$t_{½}$ (hr)
Mouse	0.017	0.033
Rat	0.033	0.083
Dog	3.2	0.017
Monkey	0.40	0.017
Human	1.05	1.22

Abbreviations: hr = hour; LM = liver microsomes; MOV = molnupiravir; $t_{½}$ = terminal half-life.
[Sec. 2.6.5: 17.1].

Table 2.6.4: 18 Metabolic Stability of NHC in Plasma, Whole Blood, Liver S9, and Liver and Intestinal Microsomes

Medium	Plasma	Whole Blood[a]	Liver Microsomes	Liver S9	Intestinal Microsomes[a]
Species	$t_{½}$ (hr)	$t_{½}$ (hr)	$t_{½}$ (hr)	$t_{½}$ (hr)	$t_{½}$ (hr)
Mouse	-	>24	>24	-	>24
Rat	17	-	>24	>24	-
Dog	>24	-	-	>24	-
Monkey	6.5	3	>24	>24	>24
Human	10	7	>24	20	>24

Abbreviations: hr = hour; NHC = N-hydroxycytidine; - = not available; $t_{½}$ = terminal half-life.
NHC was tested at 4 µM in plasma, liver microsomes and liver S9, 4.24 µM in whole blood, and 3 µM in intestinal microsomes.
[a] Stability was determined using $^{13}C_5$-NHC ($^{13}C_5$-labeled version of NHC).
[Sec. 2.6.5: 17.3].

5.2.2 Uptake and Anabolism of NHC in Tissue Culture and Primary Cells

MOV and/or NHC are taken up by all tissue culture cells tested and converted to the pharmacologically active NHC-TP. Intracellular NHC-TP levels are generally concentration-dependent, with C_{max} levels approximately 160-2600 pmol/10^6 in various cell lines cells (at 10-20 µM dose in culture media). NHC-TP reaches T_{max} between 1 and 24 hr depending on the cell line and concentration tested [Sec. 2.6.5: 17.4] [Table 2.6.4: 19].

Table 2.6.4: 19 MOV and NHC Uptake and Anabolism to NHC-TP by Cells in Tissue Culture

Cell Line	Drug Incubated	Concentration	Analyte	C_{max} (pmol/10^6 cells)	T_{max} (hr)
A549	NHC	1 µM	NHC	67.0 ± 5.5	6
			NHC-TP	186.8 ± 27.6	24
		10 µM	NHC	571.9 ± 354.1	6
			NHC-TP	1,866.5 ± 75.0	24
		100 µM	NHC	13,215.9 ± 4,067.1	6
			NHC-TP	9,766.6 ± 410.0	6
BEAS-2B	NHC	20 µM	NHC	36.6 ± 8.2	16
			NHC-TP	2,062.6 ± 221.8	24
CEM	NHC	1 µM	NHC	BQL	NA
			NHC-TP	18.8 ± 8.2	3
		10 µM	NHC	BQL	NA
			NHC-TP	158.4 ± 14.5	1
		100 µM	NHC	9.5 ± 1.9	24
			NHC-TP	951.6 ±249.2	3
	MOV	20 µM	MOV	5.4 ± 1.2	4
			NHC	0.3 ± 0.05	3
			NHC-TP	171.3 ± 7.0	24
HepG2	NHC	20 µM	NHC	24.1 ± 1.0	6
			NHC-TP	732.7 ± 2.9	6
	MOV	20 µM	MOV	18.9 ± 2.2	3
			NHC	13.4 ± 0.6	16
			NHC-TP	470.3 ± 14.1	24
Huh7	NHC	20 µM	NHC	133.9 ± 115.5	3
			NHC-TP	2,623.5 ± 487.0	6
	MOV	20 µM	MOV	77.5 ± 73.8	2
			NHC	29.0 ± 1.7	24
			NHC-TP	1,113.3 ± 44.1	24
PC-3	NHC	10 µM	NHC	33.3 ± 7.7	6
			NHC-TP	819.5 ± 16.8	6
Vero	NHC	1 µM	NHC	27.1 ± 11.4	1
			NHC-TP	240.2 ± 90.9	24
		10 µM	NHC	248.2 ± 51.6	6
			NHC-TP	1,629.4 ± 221.0	24
		100 µM	NHC	3,774.1 ± 1,312.1	24
			NHC-TP	5,105.3 ± 933.3	6

Abbreviations: A549 = human adenocarcinomic alveolar basal epithelial cell line; BEAS-2B = human bronchial epithelial cell Line; CEM = T lymphoblastoid cell line; HepG2 = human liver cancer cell line; Huh7 = human hepatoma cell line; PC-3 = human prostatic cancer cell line; Vero = monkey kidney epithelial cell line; BQL = below the quantifiable limit; C_{max} = maximum concentration; hr = hour; T_{max} = time of maximum concentration; NA = not applicable; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Values represent mean or mean ± standard deviation (n=3 replicates).
[Sec. 2.6.5: 17.4].

NHC is also taken up and metabolized to NHC-TP by primary cells [Sec. 2.6.5: 17.5] [Table 2.6.4: 20]. Stable labeled $^{13}C_5$-NHC was utilized to quantify the amount of NHC converted to anabolites in the pyrimidine nucleoside phosphorylation pathway. NHC-TP reached high levels within 1-6 hr. Concentrations of NHC-TP in primary lung cells were significantly higher than in primary hepatocytes. The intracellular stability ($t_{½}$) of NHC-TP was 4-5 hr in human astrocytes and hBTEC, and it was significantly less (0.4-1.1 hr) in primary hepatocytes [Sec. 2.6.5: 17.6] [Table 2.6.4: 21].

Table 2.6.4: 20 NHC Uptake and Anabolism to NHC-TP by Primary Cells

Cell Line	Drug Incubated	Conc.	Analyte	C_{max} (pmol/10^6 cells)	T_{max} (hr)
Normal Human Astrocytes	$^{13}C_5$-NHC	20 µM	$^{13}C_5$-NHC	74.4 ± 23.3	6
			$^{13}C_5$-Uridine	BQL	NA
			$^{13}C_5$-Cytidine	BQL	NA
			$^{13}C_5$-UTP	9.4 ± 0.3	6
			$^{13}C_5$-CTP	21.6 ± 1.0	6
			$^{13}C_5$-NHC-TP	1,673.3 ± 63.8	6
Primary hBTEC	NHC	20 µM	NHC	45.3 ± 7.9	4
			NHC-TP	2,072.1 ± 327.4	6
Primary hBTEC	NHC	20 µM	NHC	92.7 ± 15.0	4
			NHC-TP	4,632.9 ± 285.2	4
Primary Human Hepatocytes	NHC	20 µM	NHC	20.4 ± 0.9	24
			NHC-TP	276 ± 72	4
Primary Human Hepatocytes	$^{13}C_5$-NHC	20 µM	$^{13}C_5$-NHC	84.5 ± 35.9	1
			$^{13}C_5$-Uridine	BQL	NA
			$^{13}C_5$-Cytidine	BQL	NA
			$^{13}C_5$ -UTP	106.4 ± 7.1	6
			$^{13}C_5$-CTP	105.6 ± 7.1	6
			$^{13}C_5$-NHC-TP	118.4 ± 4.1	6
Primary Monkey Hepatocytes	NHC	20 µM	NHC	18.9 ± 8.7	2
			NHC-TP	267 ± 36	1
Primary Monkey Hepatocytes	$^{13}C_5$-NHC	20 µM	$^{13}C_5$-NHC	26.9 ± 4.5	1
			$^{13}C_5$-Uridine	BQL	NA
			$^{13}C_5$-Cytidine	BQL	NA
			$^{13}C_5$-UTP	54.9 ± 0.6	6
			$^{13}C_5$-CTP	655.7 ± 22.1	6
			$^{13}C_5$-NHC-TP	578.2 ± 7.5	3
Primary Mouse Hepatocytes	NHC	20 µM	NHC	129 ± 3.0	24
			NHC-TP	33 ± 11.7	2
Primary Mouse Hepatocytes	$^{13}C_5$-NHC	20 µM	$^{13}C_5$-NHC	20.5 ± 4.0	6
			$^{13}C_5$-Uridine	BQL	NA
			$^{13}C_5$-Cytidine	2.7 ± 0.7	6
			$^{13}C_5$-UTP	90.2 ± 13.4	3
			$^{13}C_5$-CTP	706.5 ± 58.7	3
			$^{13}C_5$-NHC-TP	17.5 ± 2.7	6

Abbreviations: BQL = below the quantifiable limit; C_{max} = maximum concentration; hBTEC = Human Bronchial/Tracheal Epithelial Cells; hr = hour; T_{max} = time of maximum concentration; NA = not applicable; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; CTP = cytidine triphosphate; UTP = uridine triphosphate.
Values represent mean or mean ± standard deviation (n=3 replicates).
[Sec. 2.6.5: 17.5].

Table 2.6.4: 21 Metabolism of NHC in Primary Cells After Drug Withdrawal (Washout)

Cell Type	Drug Incubated	Analyte	$t_{½}$ (hr)
Normal Human Astrocytes	$^{13}C_5$-NHC	$^{13}C_5$-NHC	0.2
		$^{13}C_5$-NHC-TP	4.6
Primary hBTEC	NHC	NHC	0.2
		NHC-TP	4.9
Primary Human Hepatocytes	$^{13}C_5$-NHC	$^{13}C_5$-NHC	0.2
		$^{13}C_5$-NHC-TP	1.1
Primary Monkey Hepatocytes	$^{13}C_5$-NHC	$^{13}C_5$-NHC	0.5
		$^{13}C_5$-NHC-TP	0.7
Primary Mouse Hepatocytes	$^{13}C_5$-NHC	$^{13}C_5$-NHC	BQL[a]
		$^{13}C_5$-NHC-TP	0.4

Abbreviations: hBTEC = Human Bronchial/Tracheal Epithelial Cells; hr = hour; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; $t_{½}$ = terminal half-life.
Values represent mean or mean ± standard deviation (n=3 replicates).
[a] Below quantitation level at first timepoint (0.5 hr) after drug withdrawal.
[Sec. 2.6.5: 17.6].

5.2.3 *In Vitro* Metabolism of MOV in Hepatocytes

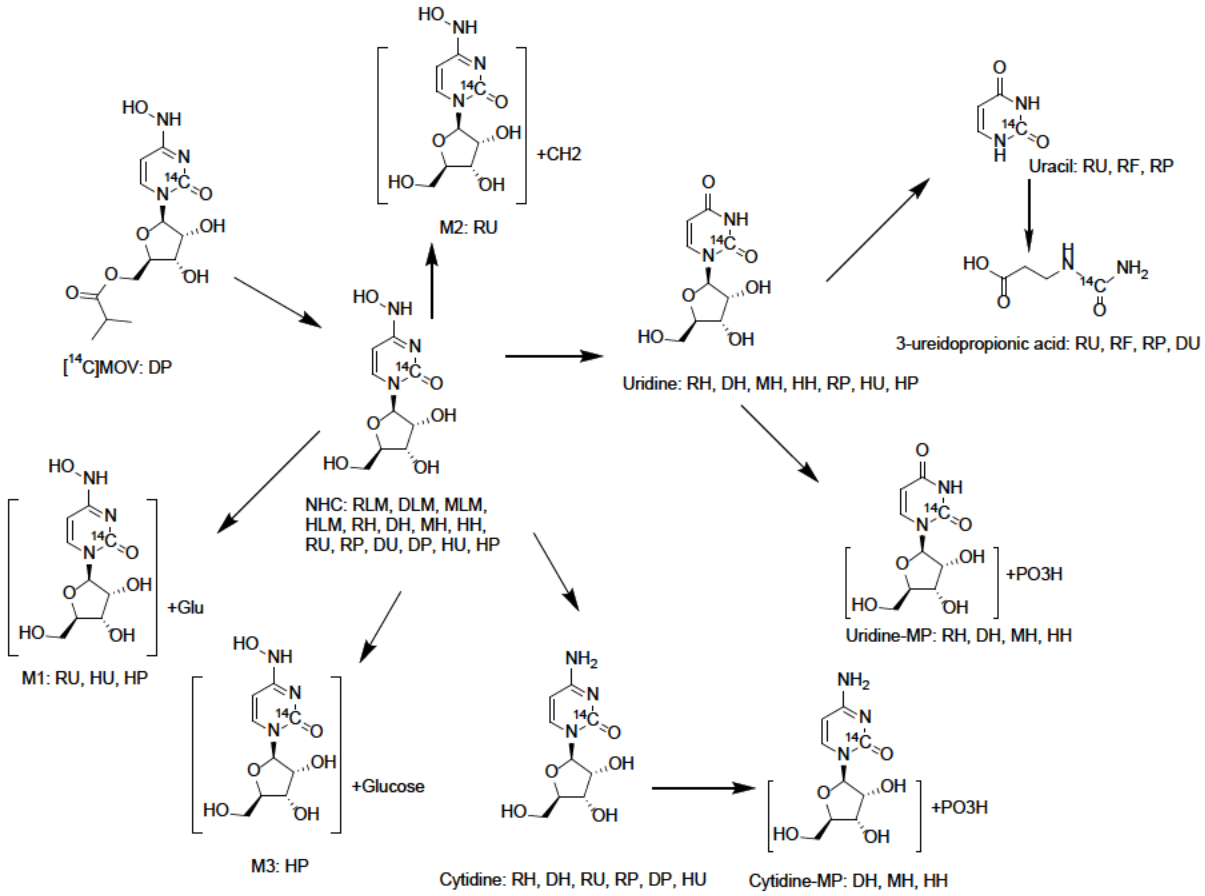
The *in vitro* metabolism of [^{14}C]MOV (10 µM) was studied in pooled cryopreserved hepatocytes from male SD rats, male beagle dogs, male cynomolgus monkey, and humans (mix of genders) [Sec. 2.6.5: 17.7] [Table 2.6.4: 22]. After a 2 hr incubation, [^{14}C]MOV was extensively metabolized in hepatocytes, and its metabolic profiles were qualitatively similar across all species. Hydrolysis to NHC was the major route of metabolism, and NHC accounted for 56, 73, 86, and 71% of the radioactivity in rat, dog, monkey, and human hepatocytes, respectively. Uridine was also a major metabolite detected in human hepatocytes and accounted for 26% of the radioactivity. Cytidine was a major metabolite detected in rat hepatocytes and accounted for 37% of the radioactivity. Minor metabolites (<10% of the radioactivity) detected in incubations from all species included cytidine-monophosphate (except in rat) and uridine-monophosphate. Under the conditions tested and current LC-MS/MS method, NHC-TP was not observed following incubation of MOV in hepatocytes suspensions. All metabolites observed in human hepatocyte incubations were also detected in the nonclinical species [Sec. 2.6.5: 18] [Figure 2.6.4: 1]. The formation of cytidine and uridine are consistent with the *in vitro* metabolism of NHC reported in literature [Ref. 4.3: 05JF07].

Table 2.6.4: 22 Summary of Metabolites of [^{14}C]MOV Detected in Hepatocytes

Percent of Radioactivity of Prominent Metabolites Detected in Hepatocytes				
Metabolite	Rat	Dog	Monkey	Human
MOV	-	-	-	-
NHC	55.8	73.0	85.8	70.5
Cytidine	36.8	4.1	-	-
Uridine	3.1	2.8	6.0	26.1
Cytidine-MP	-	9.7	3.2	2.4
Uridine -MP	2.2	9.1	4.5	1.0
Unknown	-	1.3	-	-

Abbreviations: MOV = molnupiravir; NHC = N-hydroxycytidine; MP = monophosphate; - = Not detected.
Values represent n=1 incubation.
[Sec. 2.6.5: 17.7].

Figure 2.6.4: 1 Proposed Structures of MOV Metabolites *In Vitro* and *In Vivo*



Abbreviations: MOV = molnupiravir; NHC= N-hydroxycytidine; RLM = rat liver microsomes; DLM = dog liver microsomes; MLM = monkey liver microsomes, HLM = human liver microsomes; RH = rat hepatocytes; DH = dog hepatocytes; MH = monkey hepatocytes; HH = human hepatocytes; MP = monophosphate; RU = rat urine; RF = rat feces; RP = rat plasma; DU = dog urine; DP = dog plasma; HU = human urine (unlabeled MOV); HP = human plasma (unlabeled MOV).
[Sec. 2.6.5: 18].

6 EXCRETION

6.1 Rats

The *in vivo* excretion and mass balance of MOV were studied in male BDC Wistar Han rats following oral administration of [^{14}C]MOV formulated as a solution in 1% methylcellulose at 30 mg/kg. The mean total recovery of administered dose was 67% over the period of 72 hr, with 0.2, 5.5, and 6.8% of the dose recovered in bile, urine, and feces, respectively, and 54% of the dose recovered from carcasses, indicating that majority of the dose was retained in the body [Sec. 2.6.5: 19] [Table 2.6.4: 23].

6.2 Dogs

The *in vivo* excretion and mass balance of MOV were studied in male intact beagle dogs following oral administration of [^{14}C]MOV formulated as a solution in 1% methylcellulose at 30 mg/kg. The mean total recovery of administered dose in excreta was only 9.9% over the period of 96 hr, with 9 and 0.5% of the dose recovered in urine and feces, respectively, indicating that the majority of the dose was retained in the body [Sec. 2.6.5: 19] [Table 2.6.4: 23].

The low dose recovery in excreta from rats and dogs was anticipated given a major route of metabolism of [^{14}C]MOV *in vitro* after formation of [^{14}C]NHC was further metabolism to [^{14}C]-labeled uridine and/or cytidine, which then mix with the endogenous host nucleoside pools [Ref. 4.3: 05QZJH]. This is further evidenced by the 54% of dose recovered in the rat carcass. In addition, some release of [^{14}C]-labeled CO_2 is also anticipated to have occurred in these studies as a result of metabolism of [^{14}C]MOV to uracil and/or 3-ureidopropionic acid which are known to be further metabolized to release CO_2 *in vivo* [Ref. 4.3: 05Q3MZ].

Table 2.6.4: 23 Excretion of Radioactivity in BDC Rats and Intact Dogs After an Oral Administration of [^{14}C]MOV

Species	Cannulation status	Percent of Administered Dose					
		Bile	Urine	Feces	Cage Wash	Carcass	Total
Rat[a]	BDC	0.18 ± 0.04	5.48 ± 0.38	6.82 ± 3.59	0.22 ± 0.08	54.26 ± 12.26	66.96 ± 9.66
Dog[b]	Intact	NA	8.92 ± 0.28	0.52 ± 0.21	0.44 ±0.13	NA	9.89 ± 0.48

Abbreviations: BDC = bile duct-canulated; MOV = molnupiravir; NA = not applicable.
[a] Values represent mean ± standard deviation (n = 4).
[b] Values represent mean ± standard deviation (n = 3).
[Sec. 2.6.5: 19].

7 DRUG INTERACTIONS

7.1 Drug Metabolizing Enzymes and/or Transporters Involved in the Elimination of MOV and/or NHC

7.1.1 Drug Metabolizing Enzymes

Following single or multiple-dose administration of MOV in humans, MOV was generally not observed in systemic circulation (P004), indicating complete hydrolysis to NHC likely occurs during absorption/first pass. The high capacity esterases CES1 and CES2 efficiently hydrolyze MOV to NHC *in vitro*, and likely play a role *in vivo* given the abundance of CES2 in intestinal epithelial cells and CES1 in the liver. Any MOV reaching systemic circulation may also be hydrolyzed by either of these esterases given their broad tissue distribution, such that any reduction in first pass hydrolysis likely will not affect the fraction of dose ultimately converted to NHC. Following the uptake of circulating NHC into cells, host kinases and phosphatases involved in the endogenous pyrimidine nucleoside pathways then anabolize/ catabolize NHC to/from NHC-TP. *In vitro* metabolism studies with MOV suggest a major route of elimination of NHC-related material is likely through conversion to endogenous pyrimidine nucleosides (uridine and/or cytidine), and their respective phosphate metabolites. At least three widely distributed cellular enzymes have demonstrated the ability to metabolize NHC to endogenous pyrimidines. The mitochondrial amidoxime reducing components (mARC1 and mARC2) have been reported to convert NHC to cytidine *in vitro* [Ref. 4.3: 06CBPM]. Additionally, cytidine deaminase readily converts NHC to uridine *in vitro*. Studies with CES1 and CES2 and cytidine deaminase are further described in the following paragraphs [Sec. 2.6.5: 20].

The *in vitro* metabolism of [^{14}C]MOV (10 μM) was evaluated using liver microsomes from male SD rats, male beagle dogs, male cynomolgus monkey, and humans (mix of genders). After a 1 hr incubation, [^{14}C]MOV was extensively hydrolyzed to NHC by all species. The hydrolysis was not NADPH dependent and was inhibited by PMSF which is an inhibitor of carboxylesterase activity [Sec. 2.6.5: 20.1] [Table 2.6.4: 24].

Table 2.6.4: 24 Metabolism of [^{14}C]MOV in Liver Microsomes

Percent of Radioactivity of Prominent Metabolites Detected												
Metabolite	**Rat**			**Dog**			**Monkey**			**Human**		
	-NADPH	**+NADPH**	**+PMSF[a]**	**-NADPH**	**+NADPH**	**+PMSF[a]**	**-NADPH**	**+NADPH**	**+PMSF[a]**	**-NADPH**	**+NADPH**	**+PMSF[a]**
MOV	8	-	72	2	-	78	1	3	13	39	51	90
NHC	85	86	21	87	90	16	92	90	80	57	44	6
Unknown	4	4	-	-	-	-	-	-	-	-	-	-

Abbreviation: MOV = molnupiravir; NHC = N-hydroxycytidine; NADPH = reduced nicotinamide adenine dinucleotide phosphate; PMSF = phenylmethylsulfonyl fluoride;
- = not detected by radiometric detection.
[a] [^{14}C]MOV was incubated with liver microsomes containing 0.1 mM PMSF (CES inhibitor) in the presence of NADPH.
[Sec. 2.6.5: 20.1].

The hydrolysis of MOV to NHC was evaluated using microsomes and S9 from human liver and intestine in the presence and absence of the more specific esterase inhibitors arachidonic acid (20 µM, CES1 inhibitor) or loperamide (50 µM, CES2 inhibitor) over a time course of 90 min. MOV (1 µM) was extensively hydrolyzed to NHC in HIM, HIS9, and HLM, and to a lesser extent in HLS9, yielding $CL_{int,unscaled}$ estimations of 34.3, 16.6, 33.3, and 5.7 µL/min/mg, respectively. Either arachidonic acid or loperamide inhibited the hydrolysis in HLM and HLS9 to a similar extent (roughly 50%). In HIM and HIS9, loperamide inhibited hydrolysis almost completely (roughly 90%) and arachidonic acid by approximately 50%. The hydrolysis of MOV (1 µM) to NHC was also evaluated using human recombinant CES1 and CES2, yielding CL_{int} estimations of 1 and 22 µL/min/mg, respectively [Sec. 2.6.5: 20.2] [Table 2.6.4: 25]. The K_m for both CES1 and CES2 was estimated to be >1000 µM, which was the highest concentration tested, and V_{max} was >2000 and >25,000 pmol/min/mg protein, respectively [Sec. 2.6.5: 20.3]. These data are consistent with MOV being a substrate of both CES1 and CES2, where the extent each contribute to the hydrolysis of MOV during first pass is determined by both their enzyme affinities and abundance in either intestine or liver.

Table 2.6.4: 25 Hydrolysis of MOV in HIM, HIS9, HLM, HLS9, and Human Recombinant CES Isoforms

Inhibitor	$CL_{int, unscaled}$ (µL/min/mg)					
	HLM	HLS9	HIM	HIS9	rCES1	rCES2
-	33.3	5.7	34.3	16.6	1.0	21.9
Loperamide (50 µM)	17.6	2.3	3.3	1.2	NA	NA
Arachidonic Acid (20 µM)	17.7	2.2	18.8	7.1	NA	NA

Abbreviations: rCES = recombinant carboxylesterase; CL_{int} = intrinsic clearance; HIM = human intestinal microsomes; HIS9 = human intestinal S9; HLM = human liver microsomes; HLS9 = human liver S9; MOV = molnupiravir; NADPH = reduced nicotinamide adenine dinucleotide phosphate; NA = not applicable; - = no inhibitor.
Additional information: all incubations were conducted in the absence of NADPH.
[Sec. 2.6.5: 20.2].

Conversion of NHC to uridine was studied using [^{14}C]NHC that was generated by incubating [^{14}C]MOV (10 µM) with monkey liver microsomes. In the presence of cytidine deaminase (5 or 50 µg/mL, at 37°C for 20 min), approximately ≥90% of NHC was converted to uridine at either protein concentration, with no other metabolite detected [Sec. 2.6.5: 20.4].

7.1.2 Transporters

MOV and NHC were evaluated as substrates of a series of human nucleoside transporters, BCRP and MDR1 P-gp *in vitro*.

Uptake of MOV and NHC was measured in MDCKII cells and MDCKII cells stably expressing the human concentrative nucleoside transporters CNT1, CNT2, or CNT3, and the human equilibrative nucleoside transporters ENT1, or ENT2 [Sec. 2.6.5: 30]. Under the conditions tested, MOV was a comparatively weak substrate of human CNT1, and was not a substrate of CNT2, CNT3, ENT1, or ENT2. In contrast, NHC was a substrate of CNT1, CNT2, CNT3, and ENT2, and it could not be excluded as a substrate of ENT1. These data are consistent with published data that NHC is a substrate of both ENT1 and ENT2 transporters [Ref. 4.3: 07WZTW]. Based on the high transport capacity and functional redundancy of nucleoside transporters, coupled with the lack of evidence of CNT or ENT

mediated drug-drug interactions reported in the literature, clinically meaningful DDIs mediated by these transporters are not anticipated [Ref. 4.3: 05R0PD] [Ref. 4.3: 05R0PT] [Ref. 4.3: 05R0PK].

Uptake of MOV and NHC was also evaluated in membrane vesicles isolated from baculovirus infected Sf9 cells containing human MDR1 P-gp or human BCRP. Under the conditions tested, MOV and NHC were not substrates of human MDR1 P-gp or BCRP [Sec. 2.6.5: 30].

7.2 Inhibition of Drug Metabolizing Enzymes and Transporters

7.2.1 Inhibition of Drug Metabolizing Enzymes

The potential for MOV or NHC to be reversible inhibitors of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 enzyme activity was evaluated using pooled human liver microsomes. MOV and NHC were tested at concentrations ranging from 0.13 to 1000 µM. At 1000 µM, neither MOV nor NHC inhibited 50% of the marker activity of any CYP tested, therefore their IC_{50} values are all greater than 1000 µM [Sec. 2.6.5: 21].

The potential for MOV or NHC to be time-dependent inhibitors of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 enzyme activity was evaluated using pooled human liver microsomes in a K_{obs} assay. At concentrations of 10 and 50 µM, neither MOV nor NHC demonstrated time-dependent inhibition of any CYP tested [Sec. 2.6.5: 28].

At an 800 mg clinical dose of MOV, the C_{max} of NHC is 10.8 µM, while for MOV the C_{max} in plasma after the final dose on Day 6 was 0.026 µM (P004). Since MOV levels are expected to rapidly decline as a result of hydrolysis to NHC during absorption/first pass, a Gastroplus model was used to estimate maximum portal vein concentrations for MOV and NHC of 1.6 and 24 µM, respectively. The estimated risk assessment parameters for either MOV or NHC to inhibit CYP enzymes were all below the threshold values outlined in the FDA [Sec. 2.6.5: 22] [Sec. 2.6.5: 25], EMA [Sec. 2.6.5: 23] [Sec. 2.6.5: 26], and MHLW [Sec. 2.6.5: 24] [Sec. 2.6.5: 27] DDI guidelines. Therefore, the potential for MOV or NHC to result in either presystemic or systemic CYP inhibition based on FDA, EMA, and MHLW DDI guidelines is low.

7.2.2 Inhibition of Transporters

The potential for MOV or NHC to inhibit the human transporters OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, MATE2K, BCRP, MDR1 P-gp, and MRP2 was studied *in vitro* [Sec. 2.6.5: 31] [Sec. 2.6.5: 35].

In these assays, MOV and NHC did not inhibit OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, MATE2K, and MRP2 indicating IC_{50} values greater than the highest concentration tested of 100 µM. Significantly higher concentrations than 100 µM were not tested in the cellular assays due to the potential for cytotoxicity [Ref. 4.3: 05JF0B]. MOV and NHC also did not inhibit MDR1 P-gp and BCRP when assayed in a membrane vesicle format which afforded testing up to 1000 µM, indicating IC_{50} values greater than the highest concentration tested of 1000 µM.

Using the exposures of MOV and NHC discussed in the section on CYP inhibition above, these transporter IC_{50}'s would be well above 10x the NHC plasma C_{max} (well above 100x for MDR1 P-gp and BCRP) and also well above the maximum portal vein concentrations. Therefore, the potential for NHC to cause either presystemic or systemic transporter inhibition based on FDA, EMA, and MHLW DDI guidelines is low [Sec. 2.6.5: 36] [Sec. 2.6.5: 37] [Sec. 2.6.5: 38]. Given little to no MOV is observed in circulation, only presystemic liabilities were assessed using FDA, EMA, and MHLW DDI guidelines. MOV is not expected to inhibit transporters at exposures associated with the 800 mg clinical dose [Sec. 2.6.5: 32] [Sec. 2.6.5: 33] [Sec. 2.6.5: 34].

7.3 Induction of Cytochrome P450 Enzymes

An *in vitro* study was performed to determine the induction potential of MOV on human CYP isoenzyme (1A2, 2B6, and 3A4) activity using 3 single-donor lots of inducible cryopreserved human hepatocytes. Induction was measured by changes in enzyme activity using probe substrates selective for CYP1A2, CYP2B6, and CYP3A4 and by quantifying changes in CYP1A2, CYP2B6, and CYP3A4 mRNA transcription using RT-PCR. Hepatocytes were incubated with MOV at 2, 10, and 50 µM for 48 hr. Based on *in vitro* metabolism studies in hepatocytes, it is anticipated that the majority of MOV in these incubations was metabolized to NHC during the course of the assay [Table 2.6.4: 22]. MOV and/or its metabolites (mainly NHC) did not produce an induction response in CYP1A2, 2B6, and 3A4 mRNA or enzyme activity across the 3 lots tested.

Additional studies were conducted to evaluate the induction potential of MOV and NHC on human CYP isoenzyme (1A2, 2B6, and 3A4) activity in cultured cryopreserved human hepatocytes from three human donors. At concentrations up to and including the highest concentration tested of 20 µM, neither MOV nor NHC produced an induction response in CYP1A2, 2B6, and 3A4 mRNA or enzyme activity across the 3 lots tested [Sec. 2.6.5: 29].

Significantly higher concentrations were not tested due to the potential for cytotoxicity. These data indicate both MOV and NHC are unlikely to cause DDIs due to the induction of CYPs given concentrations ~2-5x the plasma C_{max} of NHC at 800 mg show no induction response *in vitro*.

8 OTHER PHARMACOKINETIC STUDIES

Currently, no other pharmacokinetics studies with MOV have been conducted.

9 DISCUSSION AND CONCLUSIONS

MOV is an ester prodrug that when orally administered in rats and dogs is well absorbed and resulted in high bioavailability of NHC, and significantly improved the oral exposure to NHC in monkeys (6-fold) when compared to oral administration of NHC itself. MOV generally provided dose-proportional exposures of NHC in all preclinical species after oral dosing. NHC is distributed widely in all animal tissues analyzed where it is converted to the active triphosphate NHC-TP which causes viral error catastrophe and inhibition of replication.

MOV is readily converted to NHC by esterases (including CES1 and CES2) in intestinal and liver microsomes as well as plasma. NHC is more stable than MOV, and in human plasma and liver/intestinal microsomes had a $t_{½}$ >6 and 24 hr, respectively. MOV and NHC can be taken up by a broad array of cell types *in vitro* and converted to the active triphosphate NHC-TP, as well as converted to endogenous uridine, cytidine and their phosphorylated metabolites which appears to be the major route of elimination of MOV related drug material *in vivo*. *In vitro*, MOV and NHC did not inhibit or induce any of the human CYPs or transporters tested. Based on the totality of these data and similarity to cytidine (NHC has one added hydroxyl), MOV and NHC are not likely to be either perpetrators or victims of human CYP or transporter DDIs.

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Sec. 2.6.5.1 Pharmacokinetics: Overview

Type of Study	Test System	Method of Administration	Testing Facility[a]	Study Number
Absorption after a single dose				
Pharmacokinetics	Mouse (CD-1)	IP, P.O.	[redacted]	[Ref. 4.2.2.2: PK001MK4482]
Pharmacokinetics	Mouse (CD-1)	P.O.		[Ref. 4.2.2.2: PK002MK4482]
Pharmacokinetics	Rat (SD)	IV, P.O.		[Ref. 4.2.2.2: PK003MK4482]
Pharmacokinetics	Rat (SD)	IV, P.O.	MRL	[Ref. 4.2.2.2: PK081MK4482]
Pharmacokinetics	Dog (Beagle)	IV, P.O.	[redacted]	[Ref. 4.2.2.2: PK004MK4482]
Pharmacokinetics	Dog (Beagle)	P.O.		[Ref. 4.2.2.2: PK005MK4482]
Pharmacokinetics	Monkey (Cynomolgus)	IV, P.O.		[Ref. 4.2.2.2: PK006MK4482]
Pharmacokinetics	Monkey (Cynomolgus)	P.O.		[Ref. 4.2.2.2: PK007MK4482]
Pharmacokinetics	Ferret	P.O.		[Ref. 4.2.2.2: PK008MK4482]
Pharmacokinetics	Ferret	P.O.		[Ref. 4.3: 05JF0D]
Absorption after a repeated dose				
Pharmacokinetics	Ferret	P.O.	[redacted]	[Ref. 4.2.2.2: PK009MK4482]
Pharmacokinetics	Ferret	P.O.		[Ref. 4.3: 05JF0D]
Distribution				
Single-dose tissue distribution	Mouse (CD-1)	P.O.	[redacted]	[Ref. 4.2.2.2: PK001MK4482]
Single-dose tissue distribution	Rat (SD)	P.O.		[Ref. 4.2.2.3: PK012MK4482]
Single-dose tissue distribution	Rat (SD)	P.O.		[Ref. 4.2.2.3: PK067MK4482]
Single-dose tissue distribution	Rat (SD)	P.O.		[Ref. 4.2.2.3: PK073MK4482]
Single-dose tissue distribution	Dog (Beagle)	P.O.		[Ref. 4.2.2.3: PK068MK4482]
Single-dose tissue distribution	Monkey (Cynomolgus)	IV		[Ref. 4.2.2.3: PK013MK4482]
Single-dose tissue distribution	Ferret	P.O.		[Ref. 4.2.2.2: PK008MK4482]
Single-dose tissue distribution	Ferret	P.O.		[Ref. 4.2.2.3: PK014MK4482]
Repeated-dose tissue distribution	Rat (SD)	P.O.		[Ref. 4.2.2.3: PK067MK4482]
Repeated-dose tissue distribution	Dog (Beagle)	P.O.		[Ref. 4.2.2.3: PK066MK4482]
In vitro reversible binding	Mouse, Rat, Dog, Monkey and Human plasma, Human alpha1-acid glycoprotein and Human serum albumin	*In vitro*		[Ref. 4.2.2.3: PK011MK4482]
Metabolism				
Metabolism *in vivo* (plasma, bile, urine, feces)	Rat (BDC)	P.O.	MRL	[Ref. 4.2.2.5: PK083MK4482]
Metabolism *in vivo* (plasma, urine, feces)	Dog (intact)	P.O.	MRL	[Ref. 4.2.2.5: PK083MK4482]
Metabolism *in vivo* (urine)	Human	P.O.	MRL	[Ref. 4.2.2.4: PK086MK4482]
Metabolism *in vivo* (plasma)	Human	P.O.	MRL	[Ref. 4.2.2.4: PK087MK4482]
Metabolic stability of MOV in plasma	Mouse, Rat, Dog, Monkey, Human	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK031MK4482] [Ref. 4.2.2.4: PK036MK4482]

Type of Study	Test System	Method of Administration	Testing Facility[a]	Study Number
Metabolic stability of MOV in liver microsomes	Mouse, Rat, Dog, Monkey, Human	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK032MK4482] [Ref. 4.2.2.4: PK037MK4482]
Metabolic stability of MOV in SGF and SIF	SGF, SIF	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK033MK4482]
Metabolic stability of MOV in PBS	PBS with FBS	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK035MK4482]
Metabolic stability of NHC in plasma	Rat, Dog, Monkey, Human	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK015MK4482] [Ref. 4.2.2.4: PK024MK4482]
Metabolic stability of NHC in blood	Mouse, Monkey, Human	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK028MK4482] [Ref. 4.2.2.4: PK029MK4482] [Ref. 4.2.2.4: PK034MK4482]
Metabolic stability of NHC in liver microsomes	Mouse, Rat, Monkey, Human	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK019MK4482] [Ref. 4.2.2.4: PK020MK4482] [Ref. 4.2.2.4: PK021MK4482] [Ref. 4.2.2.4: PK022MK4482]
Metabolic stability of NHC in liver S9	Rat, Dog, Monkey, Human	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK016MK4482] [Ref. 4.2.2.4: PK017MK4482] [Ref. 4.2.2.4: PK018MK4482] [Ref. 4.2.2.4: PK023MK4482]
Metabolic stability of NHC in intestinal microsomes	Mouse, Monkey, Human	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK030MK4482]
Uptake of NHC in A549 cells	A549 cells	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK056MK4482]
Uptake of NHC in BEAS-2B cells	BEAS-2B cells	*In vitro*		[Ref. 4.2.2.4: PK046MK4482]
Uptake of MOV in CEM cells	CEM cells	*In vitro*		[Ref. 4.2.2.4: PK052MK4482]
Uptake of NHC in CEM cells	CEM cells	*In vitro*		[Ref. 4.2.2.4: PK058MK4482]
Uptake of MOV in HepG2 cells	HepG2 cells	*In vitro*		[Ref. 4.2.2.4: PK050MK4482]
Uptake of NHC in HepG2 cells	HepG2 cells	*In vitro*		[Ref. 4.2.2.4: PK051MK4482]
Uptake of MOV in Huh7 cells	Huh7 cells	*In vitro*		[Ref. 4.2.2.4: PK053MK4482]
Uptake of NHC in Huh7 cells	Huh7 cells	*In vitro*		[Ref. 4.2.2.4: PK054MK4482]
Uptake of NHC in PC-3 cells	PC-3 cells	*In vitro*		[Ref. 4.2.2.4: PK055MK4482]
Uptake of NHC in Vero cells	Vero cells	*In vitro*		[Ref. 4.2.2.4: PK057MK4482]
Uptake of $^{13}C_5$-NHC in normal human astrocytes cells	Human astrocyte cells	*In vitro*		[Ref. 4.2.2.4: PK045MK4482]
Uptake of NHC in hBTEC cells	hBTEC cells	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK047MK4482] [Ref. 4.2.2.4: PK048MK4482]
Uptake of NHC in primary mouse hepatocytes	Mouse hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK026MK4482]
Uptake of $^{13}C_5$-NHC in primary mouse hepatocytes	Mouse hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK039MK4482]
Uptake of NHC in primary monkey hepatocytes	Monkey hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK027MK4482]

Type of Study	Test System	Method of Administration	Testing Facility[a]	Study Number
Uptake of $^{13}C_5$-NHC in primary monkey hepatocytes	Monkey hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK041MK4482]
Uptake of NHC in primary human hepatocytes	Human hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK025MK4482]
Uptake of $^{13}C_5$-NHC in primary human hepatocytes	Human hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK043MK4482]
Metabolism of $^{13}C_5$-NHC in normal human astrocytes	Normal human astrocyte	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK044MK4482]
Metabolism of NHC in hBTEC cells	hBTEC cells	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK049MK4482]
Metabolism of $^{13}C_5$-NHC in primary mous hepatocytes	Mouse hepatocytes	*In vitro*		[Ref. 4.2.2.4: PK038MK4482]
Metabolism of $^{13}C_5$-NHC in primary monkey hepatocytes	Monkey hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK040MK4482]
Metabolism of $^{13}C_5$-NHC in primary huma hepatocytes	Human hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.4: PK042MK4482]
Metabolism in hepatocytes	Rat, Dog, Monkey and Human hepatocytes	*In vitro*	MRL	[Ref. 4.2.2.4: PK082MK4482]
Excretion				
Excretion (bile, urine, feces)	Rat (BDC)	P.O.	MRL	[Ref. 4.2.2.5: PK083MK4482]
Excretion (urine, feces)	Dog (Intact)	P.O.	MRL	[Ref. 4.2.2.5: PK083MK4482]
Pharmacokinetic Drug Interactions				
Enzymes involved in metabolism of MOV or NHC	Rat, Dog, Monkey and Human Liver Microsomes Human liver and intestinal S9 and microsomes Recombinant Human CES1 and CES2	*In vitro*	MRL	[Ref. 4.2.2.4: PK085MK4482]
Reversible inhibition of human CYP isoforms by MOV and NHC	Human liver microsomes	*In vitro*	[redacted]	[Ref. 4.2.2.6: PK080MK4482]
Reversible inhibition of human CYP isoforms by MOV	Human liver microsomes	*In vitro*	[redacted]	[Ref. 4.2.2.6: PK088MK4482]
Reversible inhibition of human CYP isoforms by NHC	Human liver microsomes	*In vitro*	[redacted]	[Ref. 4.2.2.6: PK089MK4482]
Time-dependent inhibition of human CYP isoforms by MOV and NHC	Human liver microsomes	*In vitro*	[redacted]	[Ref. 4.2.2.6: PK079MK4482]
Induction of human CYP isoforms by MOV	Human hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.6: PK065MK4482]
Induction of human CYP isoforms by MOV	Human hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.6: PK071MK4482]
Induction of human CYP isoforms by NHC	Human hepatocytes	*In vitro*	[redacted]	[Ref. 4.2.2.6: PK072MK4482]

Type of Study	Test System	Method of Administration	Testing Facility[a]	Study Number
Permeability of MOV and NHC	Caco-2 cells	*In vitro*	[redacted]	[Ref. 4.2.2.2: PK010MK4482]
Permeability of MOV and NHC	LLC-PK1 cells	*In vitro*	MRL	[Ref. 4.2.2.6: PK084MK4482]
Evaluation of MOV and NHC as a substrate of human P-gp	Sf9-MDR1 vesicles	*In vitro*	MRL	[Ref. 4.2.2.6: PK063MK4482]
Evaluation of MOV and NHC as a substrate of human BCRP	Sf9-BCRP vesicles	*In vitro*	MRL	[Ref. 4.2.2.6: PK063MK4482]
Evaluation of MOV and NHC as a substrate of human CNT1, CNT2 and CNT3	MDCKII-CNT1 cells MDCKII-CNT2 cells MDCKII-CNT3 cells	*In vitro*	MRL	[Ref. 4.2.2.6: PK063MK4482]
Evaluation of MOV and NHC as a substrate of human ENT1 and ENT2	MDCKII-ENT1 cells MDCKII-ENT2 cells	*In vitro*	MRL	[Ref. 4.2.2.6: PK063MK4482]
Inhibition of human OATP1B1- and OATP1B3- mediated transport by MOV and NHC	HEK293-OATP1B1 cells HEK293-OATP1B3 cells	*In vitro*	MRL	[Ref. 4.2.2.6: PK064MK4482]
Inhibition of human OAT1- and OAT3-mediated transport by MOV and NHC	MDCKII-OAT1 cells MDCKII-OAT3 cells	*In vitro*	MRL	[Ref. 4.2.2.6: PK064MK4482]
Inhibition of human OCT1- and OCT2-mediated transport by MOV and NHC	CHO-K1-OCT1 cells HEK293-OCT2 cells	*In vitro*	MRL	[Ref. 4.2.2.6: PK064MK4482]
Inhibition of human MATE1- and MATE2K- mediated transport by MOV and NHC	CHO-K1-MATE1 cells MDCKII-MATE2K cells	*In vitro*	MRL	[Ref. 4.2.2.6: PK064MK4482]
Inhibition of human MRP2- mediated transport by MOV and NHC	Sf9-MRP2 vesicles	*In vitro*	MRL	[Ref. 4.2.2.6: PK064MK4482]
Inhibition of human P-gp- mediated transport by MOV and NHC	Sf9-MDR1 vesicles	*In vitro*	MRL	[Ref. 4.2.2.6: PK064MK4482]
Inhibition of human BCRP- mediated transport by MOV and NHC	Sf9-BCRP vesicles	*In vitro*	MRL	[Ref. 4.2.2.6: PK064MK4482]

Abbreviations: P.O. = per oral; IV = intravenous; IP = Intraperitoneal; BDC = bile duct-cannulated; CES = carboxylesterase; FBS = fetal bovine serum; SGF = simulated gastric fluid; SIF = simulated intestinal fluid; A549 = human adenocarcinomic alveolar basal epithelial cell line; BEAS-2B = human bronchial epithelial cell line; Caco-2 = colorectal adenocarcinoma cells; CEM = T lymphoblastoid cell line; Huh7 = human hepatoma cell line; hBTEC = human bronchial tracheal epithelial cells, PC-3 = human prostatic cancer cell line; Vero = monkey kidney epithelial cell line; CHO-K1 = Chinese hamster ovary-K1 cell line; HepG2 = human liver cancer cell line; PBS = phosphate buffered saline; CYP = cytochrome P450; BCRP = breast cancer resistance protein; CNT1 = Concentrative Nucleotide Transporter 1; CNT2 = Concentrative Nucleotide Transporter 2; CNT3 = Concentrative Nucleotide Transporter 3; ENT1 = Equilibrative Nucleoside Transporter 1; ENT2 = Equilibrative Nucleoside Transporter 2; LLC-PK1 = Lilly Laboratories Cell Porcine Kidney; MDCKII = Madin-Darby canine kidney; OATP1B1 = organic anion transporting polypeptide 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; HEK293 = human embryonic kidney 293; OAT1 = organic anion transporter 1; OAT3 = organic anion transporter 3; OCT1 = organic cation transporter 1; OCT2 = organic cation transporter 2; MATE1 = multidrug and toxin extrusion protein 1; MATE2K = multidrug and toxin extrusion protein 2K; P-gp = P-glycoprotein; MDR1 = multidrug resistance protein 1; MRP2 = Multidrug resistance-associated protein 2; Sf9 = Spodoptera frugiperda; SD = Sprague Dawley; MOV = molnupiravir; NHC = N-hydroxycytidine.
[a] [redacted] USA; [redacted] USA; [redacted] USA; MRL = Merck Research Laboratory, NJ, USA; [redacted] USA.
Additional information: molnupiravir (MOV) is known as MK-4482 and EIDD-2801; NHC is known as EIDD-1931.

Sec. 2.6.5.2 Overall Summary of Non-Clinical Pharmacokinetic and Toxicokinetic Studies and Associated Analytical Methods

Test Articles: MOV, NHC

Species/Strain	Study Number	Analyte(s)	Type of Analytical Method	Matrix	Bioanalytical Method Reference	Regulatory Study Status
Mouse/CD-1	TT#[redacted]-7850 [Ref. 4.2.3.2: TT[redacted]7850FIN]	NHC	LC-MS/MS	Plasma	Exploratory Assay	Non GLP
Mouse/CD-1	TT#[redacted]-7850 [Ref. 4.2.3.2: TT[redacted]7850FIN]	NHC,NHC-TP	LC-MS/MS	Tissue: heart, lung, spleen, kidney, liver, brain	Exploratory Assay	Non GLP
Mouse/CByB6F1 wild type	TT#[redacted]-9037 [Ref. 4.2.3.2: TT[redacted]9037FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2224	GLP (MOV) Non GLP (NHC)
Mouse/CByB6F1 wild type	TT#[redacted]-9011 [Ref. 4.2.3.7.7: TT[redacted]9011FIN]	NHC	LC-MS/MS	Plasma	SAP.2259	GLP
Rat/SD	TT#[redacted]-7825 [Ref. 4.2.3.2: TT[redacted]7825FIN]	MOV, NHC	LC-MS/MS	Plasma	Exploratory Assay BAM-106 v2 10-10,000 ng/mL	Non GLP
Rat/SD	TT#[redacted]-7814 [Ref. 4.2.3.2: TT[redacted]7814FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2111	GLP
Rat/Fisher 344	TT#[redacted]-9027 [Ref. 4.2.3.7.7: TT[redacted]9027FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2229	Non GLP
Rat /SD	TT#[redacted]-1014 [Ref. 4.2.3.2: TT[redacted]1014FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2229	GLP
Rat /SD	TT#[redacted]-7054 [Ref. 4.2.3.5.2: TT[redacted]7054FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2229	GLP
Rat/ SD, Fisher 344	TT#[redacted]-1015 [Ref. 4.2.3.7.7: TT[redacted]1015FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2229	Non GLP
Rat/SD	TT#[redacted]-7000 [Ref. 4.2.3.5.2: TT[redacted]7000FIN]	NHC	LC-MS/MS	Plasma	SAP.2229	GLP
Rat/SD	TT#[redacted]-9000 [Ref. 4.2.3.5.1: TT[redacted]9000FIN]	NHC	LC-MS/MS	Plasma	SAP.2229	GLP
Rat/SD	TT#[redacted]-9010 [Ref. 4.2.3.5.3: TT[redacted]9010FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2229	GLP
Rat/SD	TT#[redacted]-9012 [Ref. 4.2.3.5.1: TT[redacted]9012FIN]	NHC	LC-MS/MS	Plasma	SAP.2229	GLP
Rabbit/Dutch Belted	TT#[redacted]-7043 [Ref. 4.2.3.7.7: TT[redacted]7043FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2225	Non GLP
Rabbit/Dutch Belted	TT#[redacted]-7064 [Ref. 4.2.3.5.2: TT[redacted]7064FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2225	GLP (MOV) Non GLP (NHC)
Rabbit/Dutch Belted	TT#[redacted]-7010 [Ref. 4.2.3.5.2: TT[redacted]7010FIN]	NHC	LC-MS/MS	Plasma	SAP.2225	GLP
Dog/Beagle	TT#[redacted]-7826 [Ref. 4.2.3.2: TT[redacted]7826FIN]	MOV, NHC	LC-MS/MS	Plasma	Exploratory Assay BAM-106 v1 10-10,000 ng/mL	Non GLP
Dog/Beagle	TT#[redacted]-7815 [Ref. 4.2.3.2: TT[redacted]7815FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2112	GLP
Dog/Beagle	TT#[redacted]-1005 [Ref. 4.2.3.7.7: TT[redacted]1005FIN]	MOV, NHC	LC-MS/MS	Plasma	SAP.2249	Non GLP

Dog/Beagle	TT#[redacted]-1005 [Ref. 4.2.3.7.7: TT[redacted]1005FIN]	NHC-TP	LC-MS/MS	PBMC	Exploratory Assay TM.3205 5 - 2,000 ng/mL	Non GLP
Monkey/Rhesus	TT#[redacted]-7817 [Ref. 4.2.3.7.7: TT[redacted]7817FIN]	MOV, NHC	LC-MS/MS	Plasma	Exploratory Assay BAM-106 v2 10-10,000 ng/mL	Non GLP

Abbreviations: BAM = bioanalytical method; GLP = good laboratory practice; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; PBMC = peripheral blood mononuclear cells; SD = Sprague Dawley; NHC-TP = N-hydroxycytidine-5'-triphosphate.

Sec. 2.6.5.3 Analytical Methods and Validation Reports

Test Articles: MOV and NHC

Validation Report Number	[Ref. 4.2.2.1: 05K65T]		[Ref. 4.2.2.1: 05K663] [Ref. 4.2.2.1: 07WLX8][a]		[Ref. 4.2.2.1: 05PJ69] [Ref. 4.2.2.1: 07WLY4][a]		[Ref. 4.2.2.1: 05PJ7C] [Ref. 4.2.2.1: 07WLY6][a]	
Bioanalytical Method Number	SAP.2111		SAP.2112		SAP.2229		SAP.2225	
Activity	Validation rat plasma	Validation rat plasma	Validation dog plasma	Validation dog plasma	Validation rat plasma	Validation rat plasma	Validation rabbit plasma	Validation rabbit plasma
Species	Rat	Rat	Dog	Dog	Rat	Rat	Rabbit	Rabbit
Analyte	MOV	NHC	MOV	NHC	MOV	NHC	MOV	NHC
Matrix	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Method	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Regression Model	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$
Range of Quantitation (LLOQ-ULOQ) (ng/mL)	10 - 10,000	10 - 10,000	10 - 10,000	10 - 10,000	1 - 100	5 – 5,000	1 - 100	5 – 5,000
QC Nominal Concentration (ng/mL)	10, 30, 5,000, 7,500	10, 30, 5,000, 7,500	10, 30, 5,000, 7,500	10, 30, 5,000, 7,500	1, 3, 50, 75	5, 15, 2,500, 3,750	1, 3, 50, 75	5, 15, 2,500, 3,750
QC Inter-day Accuracy Range (%Bias)	-6.2 - 5.0	-3.9 - 4.7	-6.4 - 1.3	-3.6 - 1.6	0.5 - 9.0	-2.4 - 3.3	0.0 - 2.3	-5.8 - 0.7
QC Inter-day Precision Range (%CV)	1.9 - 6.4	2.1 - 7.8	3.6 - 10.4	3.3 - 13.7	2.2 - 7.7	2.8 - 9.6	2.3 - 6.9	2.7 - 13.3
Matrix LTS (at -80°C)	15 days	15 days	153 days	153 days	122 days	122 days	125 days	125 days
Dilution Integrity QC (Dilution Factor) (ng/mL)	20,000 (10)	20,000 (10)	20,000 (10)	20,000 (10)	400 (10)	20,000 (10)	400 (10)	20,000 (10)

Abbreviations: CV = coefficient of variation; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; QC = quality control; LTS = long term stability; LLOQ = lower limit of quantitation; ULOQ = upper limit of quantitation.
[a] Reference for LTS extension.

Sec. 2.6.5.3 Analytical Methods and Validation Reports (Cont.)

Test Articles: MOV and NHC

Validation Report Number	[Ref. 4.2.2.1: 05QXHM]		[Ref. 4.2.2.1: 06FDQD]	[Ref. 4.2.2.1: 07WLRL]	
Bioanalytical Method Number	SAP.2224		SAP.2259	SAP.2249	
Activity	Validation mouse plasma	Validation mouse plasma	Validation mouse plasma	Validation dog plasma	Validation dog plasma
Species	Mouse	Mouse	Mouse	Dog	Dog
Analyte	MOV	NHC	NHC	MOV	NHC
Matrix	Plasma	Plasma	Plasma	Plasma	Plasma
Method	LC- MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Regression Model	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$	Quadratic $1/x^2$
Range of Quantitation (LLOQ-ULOQ) (ng/mL)	1 - 100	5 – 5,000	20 -20,000	1 - 100	5 – 5,000
QC Nominal Concentration (ng/mL)	1, 3, 50, 75	5, 15, 2,500, 3,750	20, 60, 10,000, 15,000	1, 3, 50, 75	5, 15, 2,500, 3,750
QC Inter-day Accuracy Range (%Bias)	1.0 - 2.7	-6.0 - -0.8	-0.7 - 1.0	0.4 - 4.0	-3.2 - 1.8
QC Inter-day Precision Range (%CV)	2.4 - 7.0	3.2 - 9.7	2.7 - 4.1	1.7 - 5.9	2.8 - 7.0
Matrix LTS (at -80°C)	94 days	8 days	29 days	40 days[a]	40 days[a]
Dilution Integrity QC (Dilution Factor) (ng/mL)	400 (10)	20,000 (10)	800,000 (100)	400 (10)	20,000 (10)

Abbreviations: CV = coefficient of variation; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; QC = quality control; LTS = long term stability; LLOQ = lower limit of quantitation; ULOQ = Upper limit of quantitation.
[a] Refer to TT#[redacted]-1005 [Ref. 4.2.3.7.7: TT[redacted]1005FIN] for LTS in dog plasma.

Sec. 2.6.5.4 Pharmacokinetics: Pharmacokinetics Following a Single Dose in Mice

Test Article: MOV
Report Number: [Ref. 4.2.2.2: PK001MK4482] [Ref. 4.2.2.2: PK002MK4482]

Report Number	[Ref. 4.2.2.2: PK001MK4482]				[Ref. 4.2.2.2: PK002MK4482]
Species/Strain	Mouse/CD-1				Mouse/CD-1
Number of animals/Gender	3 per time point/F	3 per time point/F	3 per time point/F	3 per time point/F	3 per time point/F
Feeding condition	NS	NS	NS	NS	NS
Vehicle/Formulation	Sterile saline	10% PEG 400, 2.5% Cremophor RH 40 in Water (v/v/v)			45% PEG 400, 5% Tween 80, 15% Solutol HS 15, 5% DMA in water
Route of administration	IP	P.O.	P.O.	P.O.	P.O.
Dose (mg/kg)	32	64	190	635	130
Sample	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	NHC	NHC			NHC
Assay	LC-MS/MS	LC-MS/MS			LC-MS/MS
PK parameters					
CL_p (L/hr/kg)	2.8	NA	NA	NA	8.2
$t_{½}$ (hr)	3.0	1.5	4.3	8.2	1.9
$AUC_{0\text{-}inf}$ (µM*hr)	35.1	31.3	77.8	277.5	48.1
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	0.36	0.16	0.13	0.14	0.12
C_{max}(µM)	46.6	30.8	52.0	187.4	22.6
C_{max} /Dose (kg/L)	0.48	0.16	0.09	0.10	0.06
T_{max} (hr)	0.25	0.5	0.5	0.5	0.5

Abbreviations: F = female; DMA = N, N-dimethylacetamide; PEG 400 = polyethylene glycol 400; IP = intraperitoneal; P.O. = per oral; hr = hour; MOV = molnupiravir; NHC = N-hydroxycytidine; LC-MS/MS = liquid chromatography-tandem mass spectrometry; PK = pharmacokinetics; CL_p = plasma clearance; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$ /Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; T_{max} = time to maximum concentration; NA = not applicable; NS = not specified.
Values represent means (n=3).
Additional Information: Plasma was obtained by terminal bleeding (n=3 per time point). Plasma samples were not stabilized to prevent esterase activity during the sample collection in this study PK parameters were calculated from the average plasma concentration vs time profile. As such, standard deviation could not be calculated. Negligible levels of MOV were observed in plasma at all doses.

Sec. 2.6.5.5 Pharmacokinetics: Pharmacokinetics Following a Single Dose in Rats

Test Article: MOV and NHC
Report Numbers: [Ref. 4.2.2.2: PK003MK4482] [Ref. 4.2.2.2: PK081MK4482]

Report Number	[Ref. 4.2.2.2: PK003MK4482]	[Ref. 4.2.2.2: PK081MK4482]		[Ref. 4.2.2.2: PK003MK4482]		
Species/Strain	Rat/SD	Rat/SD		Rat/SD		
Number of animals/Gender	4/M	3/M	3/M	4/M	4/M	4/M
Feeding condition	Fed	Fasted	Fasted	Fed	Fed	Fed
Vehicle/Formulation	10% PEG 400, 2.5% Cremophor RH 40, 87.5% Saline (v/v/v)	Saline	1% methylcellulose	10% PEG 400, 2.5% Cremophor RH 40, 87.5% Water (v/v/v)		
Route of administration	IV[a]	IV[a]	P.O.	P.O.	P.O.	P.O.
Dose (mg/kg)	32	10	30	32	95	320
Test Article	MOV	NHC	MOV	MOV	MOV	MOV
Sample	Plasma	Plasma	Plasma[b]	Plasma	Plasma	Plasma
Analyte	NHC	NHC	NHC	NHC	NHC	NHC
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters						
CL_p (L/hr/kg)	2.6 ± 0.2	2.8 ± 0.5	NA	NA	NA	NA
Vd_{ss} (L/kg)	NA	2.8 ± 0.3	NA	NA	NA	NA
$t_{½}$ (hr)	1.1 ± 0.1	3.5 ± 0.2	NA	2.8 ± 1.0	4.0 ± 0.8	3.1 ± 0.2
$AUC_{0\text{-}inf}$ (µM*hr)	37.0 ± 3.0	14.0 ± 2.2	22.0 ± 15.5	12.8 ± 1.8	64.0 ± 5.4	280.0 ± 40.5
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	0.38 ± 0.03	0.36 ± 0.06	0.24 ± 0.17	0.13 ± 0.02	0.22 ± 0.02	0.29 ± 0.04
C_{max} (µM)	72.6 ± 3.2	NA	16.7 ± 10.6	4.4 ± 1.1	16.4 ± 2.2	98.5 ± 28.5
C_{max} /Dose (kg/L)	0.75 ± 0.03	NA	0.18 ± 0.12	0.04 ± 0.01	0.06 ± 0.01	0.10 ± 0.03
T_{max} (hr)	0.1 ± 0.0	NA	0.7 ± 0.3	1.8 ± 0.5	1.5 ± 0.6	1.0 ± 0.0
Bioavailability (%)	NA	NA	52.3 ± 36.6[c]	NA	NA	NA

Abbreviations: M = male; PEG 400 = polyethylene glycol 400; IV = intravenous; P.O. = per oral; hr = hour; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; PK = pharmacokinetics; CL_p = plasma clearance; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$ /Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; SD = Sprague Dawley; T_{max} = time to maximum concentration; Vd_{ss} = volume of distribution at steady state; NA = not applicable.
Values represent mean ± standard deviation.
[a] IV bolus.
[b] Plasma samples were stabilized with potassium oxalate/sodium fluoride to prevent esterase activity.
[c] Bioavailability was derived from dose-normalized plasma exposure of NHC after oral dosing of MOV relative to dosed IV with NHC from the same study [Ref. 4.2.2.2: PK081MK4482].
Additional Information: Negligible levels of MOV were observed in plasma at all doses with MOV.

Sec. 2.6.5.6 Pharmacokinetics: Pharmacokinetics Following a Single Dose in Dogs

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.2: PK004MK4482]

Species/Strain	Dog/Beagle	Dog/Beagle		Dog/Beagle		Dog/Beagle	
Number of animals/Gender	4/M	4/M		4/M		4/M	
Feeding condition	Fed	Fed		Fed		Fed	
Vehicle/Formulation	Sterile saline	1% methylcellulose in water					
Route of administration	IV[a]	P.O.					
Dose (mg/kg)	8	30		100		300	
Test Article	NHC	MOV		MOV		MOV	
Sample	Plasma	Plasma		Plasma[b]		Plasma	
Analyte	NHC	NHC	MOV	NHC	MOV	NHC	MOV
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters							
CL_p (L/hr/kg)	0.9 ± 0.1	NA	NA	NA	NA	NA	NA
Vd_{ss} (L/kg)	35.0 ± 7.0	NA	NA	NA	NA	NA	NA
$t_{½}$ (hr)	27.0 ± 7.0	9.2 ± 2.8	0.7 ± 0.2	6.1 ± 0.5	0.7 ± 0.8	5.6 ± 1.0	1.1 ± 0.7
$AUC_{0\text{-}inf}$ (µM*hr)	34.6 ± 4.0	78.5 ± 9.5	4.3 ± 0.7	475.0 ± 39.3	8.4 ± 9.7	905.4 ± 86.4	63.1 ± 8.1
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	1.1 ± 0.1	0.9 ± 0.1	0.05 ± 0.01	1.6 ± 0.1	0.03 ± 0.03	1.0 ± 0.1	0.07 ± 0.01
C_{max} (µM)	50.7 ± 2.0	22.7 ± 5.1	3.0 ± 0.5	109.4 ± 10.2	9.5 ± 2.9	166.3 ± 22.2	42.0 ± 15.2
C_{max} /Dose (kg/L)	1.6 ± 0.1	0.2 ± 0.1	0.03 ± 0.01	0.4 ± 0.03	0.03 ± 0.01	0.2 ± 0.02	0.05 ± 0.02
T_{max} (hr)	0.083	1.0 ± 0.0	0.6 ± 0.3	1.8 ± 0.5	0.6 ± 0.3	2.0 ± 0.0	0.7 ± 0.4
Bioavailability (%)[c]	NA	77.0 ± 0.5	NA	128.0 ± 1.0	NA	89.0 ± 3.0	NA

Abbreviations: M = male; IV = intravenous; P.O. = per oral; hr = hour; LC-MS/MS = liquid chromatography-tandem mass spectrometry;
CL_p = plasma clearance; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$ /Dose = AUC normalized to dose;
C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; T_{max} = time to maximum concentration; Vd_{ss} = volume of distribution at steady state; NA = not applicable;
MOV = molnupiravir; NHC = N-hydroxycytidine; PK = pharmacokinetics.
Values represent mean ± standard deviation (n=4, except as noted).
[a] IV bolus.
[b] Plasma samples from 0-3 hr post-dose were collected from four dogs, remaining samples (4- 24 hr post-dose) were collected from two dogs only. AUC and $t_{½}$ were calculated only from dogs with the full set of samples (n=2).
[c] Bioavailability was derived from dose-normalized plasma exposure to NHC after oral dosing with MOV at different dose levels relative to dosed IV with NHC at 8 mg/kg.
Additional Information: Plasma samples were not stabilized to prevent esterase activity during sample collection in this study.

Sec. 2.6.5.6 Pharmacokinetics: Pharmacokinetics Following a Single Dose in Dogs (Cont.)

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.2: PK005MK4482]

Species/Strain	Dog/Beagle	Dog/Beagle		Dog/Beagle	
Number of animals/Gender	4/M/4/F	4/M/4/F		4/M/4/F	
Feeding condition	Fed	Fed		Fed	
Vehicle/Formulation	240 mM citrate buffer in distilled Water, pH 3	80% PEG 400 and 20% DMA (v/v)		16.7% Ethanol (v/v), 1.67% Cremophor RH 40 (v/v), 0.67% Methylcellulose in Water (w/v)	
Route of administration	P.O.	P.O.		P.O.	
Dose (mg/kg)	80	100		100	
Test Article	NHC	MOV		MOV	
Sample	Plasma	Plasma		Plasma	
Analyte	NHC	NHC	MOV	NHC	MOV
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters					
$t_{½}$ (hr)	7.0 ± 2.0	6.2 ± 1.9	1.1 ± 0.8	6.6 ± 1.3	1.6 ± 1.4
$AUC_{0\text{-}inf}$ (µM*hr)	517.7 ± 44.2	509.4 ± 113	21.6 ± 7.5	548.0 ± 75.2	15.5 ± 8.2
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	1.7 ± 0.1	1.7 ± 0.4	0.07 ± 0.02	1.8 ± 0.2	0.05 ± 0.03
C_{max} (µM)	127.7 ± 22.9	97.0 ± 16.6	7.2 ± 2.4	152.3 ± 33.9	15.5 ± 8.8
C_{max} /Dose (kg/L)	0.4 ± 0.07	0.3 ± 0.1	0.02 ± 0.01	0.5 ± 0.1	0.05 ± 0.03
T_{max} (hr)	1.1 ± 0.4	2.3 ± 0.5	1.8 ± 0.5	1.3 ± 0.5	0.5 ± 0.2

Abbreviations: F = female; M = male; PEG 400 = polyethylene glycol 400; DMA = N, N-dimethylacetamide; P.O. = per oral; hr = hour; LC-MS/MS = liquid chromatography- tandem mass spectrometry; $AUC_{0\text{-}inf}$ = area under the concentration-time curve from 0 to infinity; $AUC_{0\text{-}inf}$ /Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; $t_{½}$ = terminal half-life; T_{max} = time to maximum concentration; PK = pharmacokinetics; MOV = molnupiravir; NHC = N-hydroxycytidine.
Values represent mean ± standard deviation of combined male and female dogs (n=8).
Additional Information: Plasma samples were not stabilized to prevent esterase activity during sample collection in this study.

Sec. 2.6.5.7 Pharmacokinetics: Pharmacokinetics Following a Single Dose in Monkeys

Test Article: MOV
Report Number: [Ref. 4.2.2.2: PK006MK4482]

Species/Strain	Monkey/Cynomolgus		Monkey/Cynomolgus		Monkey/Cynomolgus	
Number of animals/Gender	4/M/4/F		4/M/4/F		4/M/4/F	
Feeding condition	Fed		Fed		Fed	
Vehicle/Formulation	5% PEG 400, 0.5% Tween 80 in Saline (v/v/v)		1% methylcellulose in water (w/v)			
Route of administration	IV[a]		P.O.			
Dose (mg/kg)	100		300		1000	
Sample	Plasma		Plasma		Plasma	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Analyte	NHC	MOV	NHC	MOV	NHC	MOV
$t_{½}$ (hr)	11.1 ± 10.1	0.1 ± 0.1	4.4 ± 2.2	0.2 ± 0.3	3.2 ± 1	1.0 ± 0.5
$AUC_{0\text{-}inf}$ (µM*hr)	246.4 ± 32.6	3.7 ± 1.5	277.6 ± 125.4	0.02 ± 0.03	887.2 ± 365.3	0.41 ± 0.11
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	0.81 ± 0.11	0.01 ± 0.00	0.30 ± 0.14	0.00 ± 0.00	0.29 ± 0.12	0.0 ± 0.00
C_{max} (µM)	405.0 ± 50.1	26.3 ± 10.2	91.5 ± 63.6	0.02 ± 0.03	270.1 ± 193.6	0.2 ± 0.16
C_{max} /Dose (kg/L)	1.33 ± 0.16	0.09 ± 0.03	0.10 ± 0.07	0.00 ± 0.00	0.09 ± 0.06	0.00 ± 0.00
T_{max} (hr)	0.083	0.083	1.4 ± 0.7	0.22 ± 0.36	2.5 ± 2.3	0.88 ± 0.73

Abbreviations: F = female; M = male; PEG 400 = polyethylene glycol 400; IV = intravenous; P.O. = per oral; hr = hour; LC-MS/MS = liquid chromatography-tandem mass spectrometry; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$ /Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; T_{max} = time to maximum concentration; MOV = molnupiravir; NHC = N-hydroxycytidine.
Values represent mean ± standard deviation of combined male and female monkeys (n=8).
[a] IV bolus.
Additional Information: Plasma samples were not stabilized to prevent esterase activity during sample collection.

Sec. 2.6.5.7 Pharmacokinetics: Pharmacokinetics Following a Single Dose in Monkeys (Cont.)

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.2: PK007MK4482]

Species/Strain	Monkey/Cynomolgus		Monkey/Cynomolgus		Monkey/Cynomolgus	
Number of animals/Gender	4/M/4/F		4/M/4/F		4/M/4/F	
Feeding condition	Fasted		Fed		Fed	
Vehicle/Formulation	240 mM citrate buffer in distilled water, pH 3		240 mM citrate buffer in distilled water, pH 3		80% PEG 400 and 20% DMA (v/v)	
Test article	NHC		NHC		MOV	
Route of administration	P.O.		P.O.		P.O.	
Dose (mg/kg)	100		100		130	
Sample	Plasma		Plasma		Plasma	
Analyte	NHC		NHC		NHC	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters	M	F	M	F	M	F
$t_{½}$ (hr)	1.0	0.8	0.8	0.9	1.8	1.9
$AUC_{0\text{-}inf}$ (µM*hr)	6.1	7.9	5.9	7.3	32.0	45.1
C_{max} (µM)	3.0	3.9	3.3	3.3	8.1	12.3
T_{max} (hr)	1.5	0.63	0.8	0.9	2.0	1.3

Abbreviations: F = female; M = male; PEG 400 = polyethylene glycol 400; DMA = dimethylacetamide; P.O. = per oral; hr = hour; LC-MS/MS = liquid chromatography-tandem mass spectrometry; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; C_{max} = maximum concentration; T_{max} = time to maximum concentration; MOV = molnupiravir; NHC = N-hydroxycytidine; PK = pharmacokinetics.
Values represent means (n=4).
Additional Information: Plasma samples were not stabilized to prevent esterase activity during sample collection.

Sec. 2.6.5.8 Pharmacokinetics: Pharmacokinetics Following a Single Dose in Ferrets

Test Article: MOV

Report Number: [Ref. 4.3: 05JF0D] [Ref. 4.2.2.2: PK008MK4482]

Report number	[Ref. 4.3: 05JF0D][a]				[Ref. 4.2.2.2: PK008MK4482][b]
Species/Strain	Ferret/Mustela putorius furo				Ferret/Mustela putorius furo
Number of animals/Gender	3/F	3/F	3/F	3/F	3/M
Feeding condition	NS	NS	NS	NS	NS
Vehicle/Formulation	1% methylcellulose in water (w/v)	1% methylcellulose in water (w/v)	1% methylcellulose in water (w/v)	1% methylcellulose in water (w/v)	1% methylcellulose in water (w/v)
Route of administration	P.O.	P.O.	P.O.	P.O.	P.O.
Dose (mg/kg)	4	20	128	512	20
Sample	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	NHC	NHC	NHC	NHC	NHC
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
$t_{½}$ (hr)	8.2 ± 1.7	4.7 ± 1.3	5.1 ± 0.8	4.2 ± 0.6	ND
$AUC_{0\text{-}inf}$ (µM*hr)	13.2 ± 4.8	72.5 ± 32.1	322.4 ± 42.6	791.4 ± 390.7	ND
$AUC_{0\text{-}inf}$ /Dose (kg*hr/L)	1.1 ± 0.4	1.2 ± 0.5	0.8 ± 0.1	0.5 ± 0.3	ND
C_{max} (µM)	3.5 ± 1.5	15.4 ± 1.9	100.1 ± 22.3	209.2 ± 106.0	9.1 ± 4.2
C_{max} /Dose (kg/L)	0.3 ± 0.1	0.3 ± 0.03	0.3 ± 0.1	0.1 ± 0.1	ND
T_{max} (hr)	1.7 ± 0.6	1.8 ± 1.9	1.7 ± 0.0	2.5 ± 1.0	4[c]

Abbreviations: F = female; M = males; P.O. = per oral; hr = hour; LC-MS/MS = liquid chromatography-tandem mass spectrometry; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}inf}$ /Dose = AUC normalized to dose; C_{max} = maximum concentration; C_{max}/Dose = C_{max} normalized to dose; T_{max} = time to maximum concentration; MOV = molnupiravir; NHC = N-hydroxycytidine, ND = not determined; NS = not specified
Values represent mean ± standard deviation (n=3).
[a] MOV was not quantified in this study.
[b] MOV was administered to three ferrets orally at 20 mg/kg, blood was collected at 4 hr post-dose, MOV was not detectable.
[c] Blood samples were collected at 4 hr post-dose only.
Additional Information: Plasma samples were not stabilized to prevent esterase activity during sample collection in both of the studies.

Sec. 2.6.5.9 Pharmacokinetics: Pharmacokinetics Following Repeated Doses in Ferrets

Test Article: MOV

Report Number: [Ref. 4.3: 05JF0D] [Ref. 4.2.2.2: PK009MK4482]

Report number	[Ref. 4.3: 05JF0D]		[Ref. 4.2.2.2: PK009MK4482][c]			
Species/Strain	Ferret/Mustela putorius furo		Ferret/Mustela putorius furo			
Number of animals/Gender	3/F	3/F	2/F	3/F	3/F	3/F
Feeding condition	NS	NS	NS	NS	NS	NS
Vehicle/Formulation	1% methylcellulose in water (w/v)		1% methylcellulose in water (w/v)			
Route of administration	P.O.	P.O.	P.O.	P.O.	P.O.	P.O.
Dose (mg/kg)/Regimen	7 x 20 mg/kg [a]	1 x 20 mg/kg /QD + 6 x 7 mg/kg [b]	0.8/BID	2.3/BID	7/BID	21/BID
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	NHC	NHC	NHC	NHC	NHC	NHC
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
$t_{½}$ (hr)	6.7 ± 0.1	9.2 ± 4.5	ND	ND	ND	ND
$AUC_{0\text{-}inf}$ (µM*hr)	63.8 ± 8.3	29.2 ± 4.0	ND	ND	ND	ND
C_{max} (µM)	25.1 ± 15.2	11.0 ± 4.0	0.015 ± 0.015[d]	0.17 ± 0.08	0.28 ± 0.06	1.0 ± 0.15
T_{max} (hr)	1.5 ± 0.9	1.5 ± 0.9	8	8	8	8

Abbreviations: F = female; P.O. = per oral; BID = twice daily; QD = once daily; hr = hour; LC-MS/MS = liquid chromatography-tandem mass spectrometry; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; C_{max} = maximum concentration; T_{max} = time to maximum concentration; MOV = molnupiravir; NHC = N-hydroxycytidine; ND = not determined.; NS = not specified.
Values represent mean ± standard deviation (n=3, except as noted).
[a] MOV was administered orally to ferrets at 20 mg/kg BID for 7 doses. MOV not determined in this study.
[b] MOV was administered orally to ferrets with a single loading dose of MOV at 20 mg/kg, followed by 6 doses of 7 mg/kg BID. MOV not determined in this study.
[c] Ferrets were challenged with Influenza A/California/07/2009 virus. Following 24 hr postchallenge, MOV was administered orally BID at 0.8, 2.3, 7, or 21 mg/kg to influenza A virus infected ferrets, blood samples were collected 8 hr after the 6th dose. Plasma levels of MOV were negligible at all doses.
[d] Values represent mean ± standard error (n=2).
Additional Information: Plasma samples were not stabilized to prevent esterase activity during sample collection in both of the studies.

Sec. 2.6.5.10 Pharmacokinetics: Tissue Distribution in Mice

Test Article: MOV
Report Numbers: [Ref. 4.2.2.2: PK001MK4482]

Species/Strain	Mouse/CD-1
Number of animals/Gender	3 per time point/F
Feeding Condition	NS
Vehicle/Formulation	10% PEG 400, 2.5% Cremophor RH 40, 87.5%Water (v/v/v)
Route of Administration	P.O.
Dose (mg/kg)	64, 190, 635
Analyte	NHC, NHC-TP
Method of Analysis	LC-MS/MS
Sampling Time	0.25, 0.5, 1, 2, 3, 4, 8, 24 hr (post-dose)

PK parameters

Tissue	Dose (mg/kg)	Analyte	T_{max} (hr)	C_{max} (µg/g)[a]	$AUC_{0\text{-}last}$ (µg*hr/g)[a]	$AUC_{0\text{-}inf}$ (µg*hr/g)[a]	$t_{½}$ (hr)
Lung	64	NHC	0.5	9.6	14.2	14.2	1.2
Lung	64	NHC-TP	2	0.9	6.9	7.3	5.5
Lung	190	NHC	0.5	16.2	31.9	32.0	3.1
Lung	190	NHC-TP	2	2.1	20.6	22.8	7.0
Lung	635	NHC	0.5	54.8	105.0	105.2	1.9
Lung	635	NHC-TP	3	6.9	63.8	67.8	5.6
Brain	64	NHC	0.5	1.3	4.1	4.2	4.4
Brain	64	NHC-TP	4	0.8	7.3	7.6	5.1
Brain	190	NHC	0.5	2.6	10.6	11.0	5.5
Brain	190	NHC-TP	2	1.5	15.5	16.7	6.1
Brain	635	NHC	0.5	8.2	31.2	32.3	5.5
Brain	635	NHC-TP	4	4.0	43.5	49.5	7.4
Liver	64	NHC	0.5	3.3	3.5	ND	ND
Liver	64	NHC-TP	1	1.2	4.1	5.9	16.6
Liver	190	NHC	0.5	0.6	1.2	ND	ND
Liver	190	NHC-TP	2	0.4	6.0	11.0	20.7
Liver	635	NHC	0.5	8.7	9.3	9.3	1.1
Liver	635	NHC-TP	0.5	7.1	20.2	28.1	16.6
Spleen	64	NHC	0.5	35.2	47.3	47.4	1.4
Spleen	64	NHC-TP	2	5.7	49.2	54.6	7.1
Spleen	190	NHC	0.5	77.9	126.4	126.7	3.2
Spleen	190	NHC-TP	2	9.1	94.3	97.3	4.8
Spleen	635	NHC	0.5	165.9	320.4	322.1	3.7

Spleen	635	NHC-TP	2	24.1	297.3	310.4	5.4
Kidney	64	NHC	0.5	9.5	10.7	10.7	1.0
Kidney	64	NHC-TP	1	1.0	4.2	4.8	8.5
Kidney	190	NHC	0.5	25.6	32.0	32.0	3.7
Kidney	190	NHC-TP	1	1.9	8.1	9.6	9.9
Kidney	635	NHC	0.5	92.1	126.0	126.3	3.4
Kidney	635	NHC-TP	1	4.4	26.6	28.9	6.7
Heart	64	NHC	0.5	8.6	11.4	11.4	1.8
Heart	64	NHC-TP	1	1.1	6.5	6.6	4.3
Heart	190	NHC	0.5	15.4	28.5	28.5	2.0
Heart	190	NHC-TP	2.0	1.3	12.7	13.6	5.8
Heart	635	NHC	0.5	58.1	91.2	91.8	3.9
Heart	635	NHC-TP	3	4.0	40.0	44.5	7.0

Abbreviations: F = female; hr = hour; PEG 400 = polyethylene glycol 400; P.O. = per oral; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate, also known as EIDD-2061; LC-MS/MS = liquid chromatography-tandem mass spectrometry; $t_{½}$ = terminal half-life; $AUC_{0\text{-inf}}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-last}}$ = area under the concentration-time curve from 0 to the last quantifiable concentration; C_{max} = maximum concentration; T_{max} = time to maximum concentration; ND = not determined; NS = not specified; PK =pharmacokinetics.
Values represent means (n=3).
[a] Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.

Sec. 2.6.5.11 Pharmacokinetics: Tissue Distribution in Rats

Sec. 2.6.5.11.1 Pharmacokinetic Parameters of NHC and NHC-TP in Tissues Collected from Male Sprague Dawley Rats After a Single Oral Administration of MOV

Test Article: MOV
Report Number: [Ref. 4.2.2.3: PK012MK4482]

Species/Strain	Rat/SD					
Number of animals/Gender	3 per time point/M					
Feeding Condition	Fed					
Vehicle/Formulation	10% PEG 400, 2.5% Cremophor RH 40 in Water (v/v/v)					
Route of Administration	P.O.					
Dose (mg/kg)	320					
Analyte	NHC, NHC-TP					
Method of Analysis	LC-MS/MS					
Sampling Time	1, 2, 4, 6, 8, 24 hr (post-dose)					
PK Parameters						
Tissue	**Analyte**	**T_{max} (hr)**	**C_{max} (µg/g)[a]**	**$AUC_{0\text{-}last}$ (µg*hr/g)[a]**	**$AUC_{0\text{-}inf}$ (µg*hr/g)[a]**	**$t_{½}$ (hr)**
Lung	NHC	2	43.0	253.4	254.8	3.4
Lung	NHC-TP	4	19.5	237.8	243.3	4.3
Brain	NHC	2	9.7	96.3	99.8	5.1
Brain	NHC-TP	6	4.5	69.3	81.6	7.8
Liver	NHC	1	22.9	51.2	51.3	2.8
Liver	NHC-TP	1	1.5	20.1	22.4	7.4
Spleen	NHC	2	84.1	533.9	535.5	3.0
Spleen	NHC-TP	4	18.8	204.2	207.1	3.8
Kidney	NHC	2	44.6	218.8	219.0	2.5
Kidney	NHC-TP	2	4.5	33.6	34.0	3.9
Heart	NHC	1	48.3	228.3	228.7	2.7
Heart	NHC-TP	4	4.4	50.4	51.2	4.0

Abbreviations: M = male; PEG 400 = polyethylene glycol 400; P.O. = per oral; hr = hour; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; LC-MS/MS = liquid chromatography-tandem mass spectrometry; $t_{½}$ = terminal half-life; $AUC_{0\text{-}inf}$ = area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-}last}$ = area under the concentration-time curve from 0 to the last quantifiable concentration; C_{max} = maximum concentration; T_{max} = time to maximum concentration; SD = Sprague Dawley; PK =pharmacokinetics.
Values represent means (n=3).
[a] Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
Additional information: MOV was not observed in kidney or brain and was only observed at low levels near the limit of detection in other tissues.

Sec. 2.6.5.11 Pharmacokinetics: Tissue Distribution in Rats (Cont.)

Sec. 2.6.5.11.2 Tissue Distribution of NHC and NHC-TP in Rats After Single or Multiple Oral Administrations of MOV

Test Article: MOV
Report Numbers: [Ref. 4.2.2.3: PK067MK4482]

Species/Strain	Rat/SD					
Number of animals/Gender	2 per time point/F, 2 per time point/M					
Feeding Condition	Fed					
Vehicle/Formulation	1% methylcellulose in water (w/v)					
Route of Administration	P.O.					
Dose (mg/kg/day)	200					
Analyte	NHC, NHC-TP					
Method of Analysis	LC-MS/MS					
Sampling Time	2, 4, 24 hr (post-dose)					
Tissue	**Analyte**	**Day 1** [a]			**Day 7** [a]		
		2 hr (µg/g)	4 hr (µg/g)	24 hr (µg/g)	2 hr (µg/g)	4 hr (µg/g)	24 hr (µg/g)
Liver	NHC	2.7 ± 2.4	1.1 ± 0.8	< 0.1	2.5 ± 1.5	0.9 ± 0.8	< 0.1
Liver	NHC-TP	2.0 ± 1.6	1.2 ± 0.9	0.4 ± 0.4	1.1 ± 0.2	0.9 ± 0.5	0.2 ± 0.0
Heart	NHC	15.1 ± 5.5	29.2 ± 26.0	0.0 ± 0.0	16.7 ± 8.0	4.6 ± 4.7	0.2 ± 0.1
Heart	NHC-TP	2.2 ± 0.7	10.9 ± 12.7	< 0.1	1.4 ± 0.4	0.7 ± 0.2	0.1 ± 0.0
Brain	NHC	10.7 ± 1.6	6.7 ± 1.9	0.4 ± 0.1	9.0 ± 0.9	4.1 ± 2.0	0.3 ± 0.2
Brain	NHC-TP	1.8 ± 0.1	2.0 ± 1.0	0.4 ± 0.1	1.0 ± 0.4	0.7 ± 0.3	0.8 ± 0.5
Kidney	NHC	21.9 ± 21.1	5.5 ± 4.4	< 0.1	17.7 ± 13.1	5.6 ± 5.7	0.2 ± 0.1
Kidney	NHC-TP	1.9 ± 3.2	0.6 ± 0.5	0.1 ± 0.0	1.1 ± 0.6	0.7 ± 0.5	1.0 ± 0.3
Lung	NHC	18.2 ± 16.0	5.7 ± 3.8	0.1 ± 0.1	13.8 ± 5.3	8.3 ± 5.5	0.1 ± 0.0
Lung	NHC-TP	1.4 ± 0.6	3.4 ± 2.2	0.1 ± 0.1	1.2 ± 0.5	1.0 ± 0.4	0.3 ± 0.2
Spleen	NHC	100.2 ± 64.8	29.6 ± 17.7	0.5 ± 0.5	98.8 ± 12.9	50.0 ± 24.9	1.0 ± 0.5
Spleen	NHC-TP	3.3 ± 1.2	3.8 ± 1.1	0.2 ± 0.2	2.4 ± 0.3	1.6 ± 0.8	0.3 ± 0.1

Abbreviations: F = female; M = male, hr = hour, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; P.O. = per oral; SD = Sprague Dawley.
Values represent mean ± standard deviation of combined two male and two female rats (n = 4).
[a] Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
Additional information: MOV was administered once daily by oral gavage to male and female rats for seven days at doses of 200 mg/kg/day. Liver, heart, brain, kidney, lung, and spleen samples were collected from 2 rats/sex at 2, 4, and 24 hr after the 1st and last (7th) dose. MOV was not detectable or was near the limit of quantitation in all tissues and at all time points tested.

Sec. 2.6.5.11 Pharmacokinetics: Tissue Distribution in Rats (Cont.)

Sec. 2.6.5.11.2 Tissue Distribution of NHC and NHC-TP in Rats After Single or Multiple Oral Administrations of MOV (Cont.)

Test Article: MOV
Report Numbers: [Ref. 4.2.2.3: PK067MK4482]

Species/Strain	Rat/SD					
Number of animals/Gender	2 per time point/F, 2 per time point/M					
Feeding Condition	Fed					
Vehicle/Formulation	1% methylcellulose in water (w/v)					
Route of Administration	P.O.					
Dose (mg/kg/day)	700					
Analyte	NHC, NHC-TP					
Method of Analysis	LC-MS/MS					
Sampling Time	2, 4, 24 hr (post-dose)					
Tissue	**Analyte**	**Day 1** [a]			**Day 7** [a]		
		2 hr (µg/g)	4 hr (µg/g)	24 hr (µg/g)	2 hr (µg/g)	4 hr (µg/g)	24 hr (µg/g)
Liver	NHC	11.3 ± 9.6	3.4 ± 1.1	0.3 ± 0.4	32.2 ± 36.1	10.6 ± 12.9	0.8 ± 0.9
Liver	NHC-TP	9.7 ± 8.4	3.9 ± 2.6	1.6 ± 1.7	31.7 ± 48.3	5.1 ± 3.5	1.3 ± 1.2
Heart	NHC	42.4 ± 4.9	13.5 ± 11.9	2.1 ± 2.1	69.5 ± 44.2	27.3 ± 23.5	4.0 ± 2.6
Heart	NHC-TP	11.7 ± 6.0	4.2 ± 3.8	0.6 ± 0.6	6.5 ± 1.2	8.2 ± 2.0	1.1 ± 1.1
Brain	NHC	33.0 ± 7.1	18.0 ± 4.5	3.1 ± 3.3	18.3 ± 9.2	16.2 ± 6.5	3.3 ± 2.1
Brain	NHC-TP	5.4 ± 1.0	7.3 ± 3.8	1.4 ± 1.0	2.5 ± 0.6	3.0 ± 1.4	3.5 ± 3.6
Kidney	NHC	102.5 ± 63.1	25.9 ± 18.2	2.2 ± 3.2	105.5 ± 99.9	49.1 ± 50.0	2.7 ± 2.7
Kidney	NHC-TP	11.0 ± 8.3	4.1 ± 5.0	0.2 ± 0.2	5.5 ± 2.3	2.5 ± 1.0	2.0 ± 2.1
Lung	NHC	67.7 ± 19.7	25.0 ± 13.3	2.3 ± 2.6	60.5 ± 41.4	29.6 ± 23.1	3.8 ± 3.8
Lung	NHC-TP	7.3 ± 3.4	26.5 ± 39.9	0.6 ± 0.5	6.2 ± 0.6	7.5 ± 2.8	3.1 ± 4.4
Spleen	NHC	272.3 ± 53.6	112.9 ± 45.7	16.2 ± 21.5	201.4 ± 70.0	159.5 ± 102.1	30.2 ± 17.5
Spleen	NHC-TP	12.0 ± 4.3	17.7 ± 8.3	0.7 ± 0.5	11.1 ± 2.1	4.2 ± 0.7	6.7 ± 9.9

Abbreviations: F = female; M = male, hr = hour, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; P.O. = per oral; SD = Sprague Dawley.
Values represent mean ± standard deviation of combined two male and two female rats (n = 4).
[a] Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
Additional information: MOV was administered once daily by oral gavage to male and female rats for seven days at doses of 700 mg/kg/day. Liver, heart, brain, kidney, lung, and spleen samples were collected from 2 rats/sex at 2, 4, and 24 hr after the 1st and last (7th) dose. On Day1, MOV was quantifiable in all tissues and ranged from about 0.02 µg/g in Liver, Heart, and Kidney to ≤1.1 µg/g levels in Brain and Spleen; on Day7, levels of MOV was below or close to lower limit of quantitation levels in all tissues at all time points.

Sec. 2.6.5.11 Pharmacokinetics: Tissue Distribution in Rats (Cont.)

Sec. 2.6.5.11.2 Tissue Distribution of NHC and NHC-TP in Rats After Single or Multiple Oral Administrations of MOV (Cont.)

Test Article: MOV
Report Numbers: [Ref. 4.2.2.3: PK067MK4482]

Species/Strain	Rat/SD						
Number of animals/Gender	2 per time point/F, 2 per time point/M						
Feeding Condition	Fed						
Vehicle/Formulation	1% methylcellulose in water (w/v)						
Route of Administration	P.O.						
Dose (mg/kg/day)	2000						
Analyte	MOV, NHC, NHC-TP						
Method of Analysis	LC-MS/MS						
Sampling Time	2, 4, 24 hr (post-dose)						
Tissue	**Analyte**	**Day 1**[a]			**Day 7**[a]		
		2 hr (µg/g)	4 hr (µg/g)	24 hr (µg/g)	2 hr (µg/g)[b]	4 hr (µg/g)	24 hr (µg/g)
Liver	NHC	30.2 ± 25.5	6.8 ± 2.6	1.4 ± 1.8	109.2 ± 19.6	83.3 ± 92.7	6.0 ± 9.4
Liver	NHC-TP	39.5 ± 43.5	7.3 ± 2.1	3.3 ± 2.2	164.0 ± 23.4	91.7 ± 85.2	7.9 ± 10.1
Liver	MOV	0.4 ± 0.4	0.4 ± 0.6	< 0.1	0 ± 0	0.4 ± 0.7	< 0.1
Heart	NHC	68.3 ± 27.7	60.9 ± 30.5	18.0 ± 14.9	174.2 ± 5.4	164.8 ± 41.3	17.4 ± 11.7
Heart	NHC-TP	27.4 ± 18.0	25.5 ± 18.7	6.1 ± 5.3	19.2 ± 3.3	46.1 ± 12.5	5.4 ± 3.5
Heart	MOV	0.7 ± 1.4	0.5 ± 0.5	< 0.1	< 0.1	0.2 ± 0.1	< 0.1
Brain	NHC	39.1 ± 15.6	34.7 ± 5.4	11.1 ± 10.6	30.0 ± 2.9	35.7 ± 8.4	9.7 ± 8.3
Brain	NHC-TP	7.8 ± 2.2	15.0 ± 1.0	5.1 ± 2.0	8.9 ± 2.0	10.4 ± 1.6	4.4 ± 2.7
Brain	MOV	0.7 ± 1.4	0.9 ± 1.2	< 0.1	< 0.1	0.4 ± 0.2	0.1 ± 0.1
Kidney	NHC	197.5 ± 145.2	40.5 ± 29.4	18.3 ± 22.1	364.6 ± 44.4	245.2 ± 114.2	14.9 ± 14.7
Kidney	NHC-TP	74.2 ± 60.4	24.6 ± 15.8	5.0 ± 6.3	49.2 ± 7.2	49.0 ± 18.8	4.7 ± 2.7
Kidney	MOV	0.4 ± 0.6	0.3 ± 0.7	0 ± 0	0.1 ± 0.1	0.3 ± 0.3	< 0.1
Lung	NHC	99.1 ± 51.9	77.1 ± 51.0	15.0 ± 15.3	152.2 ± 4.0	167.2 ± 83.8	19.4 ± 20.4
Lung	NHC-TP	100.6 ± 52.2	39.8 ± 25.8	12.2 ± 9.8	55.9 ± 32.9	49.5 ± 33.8	3.4 ± 1.7
Lung	MOV	1.2 ± 1.9	7.6 ± 13.8	< 0.1	< 0.1	2.0 ± 3.7	0.2 ± 0.3
Spleen	NHC	366.5 ± 107.0	183.1 ± 40.9	62.6 ± 69.7	443.1 ± 5.7	448.5 ± 133.5	75.1 ± 29.9
Spleen	NHC-TP	61.4 ± 20.0	39.8 ± 11.2	10.8 ± 6.9	15.0 ± 7.7	35.8 ± 44.7	4.3 ± 0.9
Spleen	MOV	2.0 ± 2.7	3.3 ± 4.0	0.3 ± 0.3	< 0.1	3.9 ± 6.4	0.6 ± 1.2

Abbreviations: F = female; M = male, hr = hour, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; P.O. = per oral; SD = Sprague Dawley.
Values represent mean ± standard deviation of combined two male and two female rats (n = 4, except as noted).
[a] Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: MOV = 329.31; NHC = 259.22; NHC-TP = 499.15.
[b] Values represent mean ± standard error (n=2 of male rats).
Additional information: MOV was administered once daily by oral gavage to male and female rats for seven days at doses of 2000 mg/kg/day. Liver, heart, brain, kidney, lung, and spleen samples were collected from 2 rats/sex at 2, 4, and 24 hr after the 1st and last (7th) dose.

Sec. 2.6.5.11 Pharmacokinetics: Tissue Distribution in Rats (Cont.)

Sec. 2.6.5.11.3 Accumulation of MOV and Its Metabolites in Rat Bone Marrow After a Single Oral Administration of MOV

Test Article: MOV
Report Numbers: [Ref. 4.2.2.3: PK073MK4482]

Species/Strain		Rat/SD		
Number of animals/Gender		3 per time point/M		
Feeding Condition		Fed		
Vehicle/Formulation		10% PEG 400, 2.5% Cremophor RH 40 in Water (v/v/v)		
Route of Administration		P.O.		
Dose (mg/kg)		50, 150, 500		
Analyte		MOV, NHC, NHC-TP		
Method of Analysis		LC-MS/MS		
Sampling Time		3, 24 hr (post-dose)		
Time Points (hr)	**Dose Level (mg/kg)**	**Concentrations in Bone Marrow (µg/g)**		
		MOV	NHC	NHC-TP
3	50	BQL	2.85 ± 0.66	1.92 ± 0.38
	150	BQL	7.57 ± 1.37	4.71 ± 1.15
	500	BQL	36.39 ± 9.75	15.12 ± 4.86
24	50	BQL	BQL	0.09 ± 0.04
	150	BQL	BQL	0.15 ± 0.03
	500	BQL	0.25 ± 0.06	0.50 ± 0.13

Abbreviations: M = male, SD = Sprague Dawley; P.O. = per oral; PEG 400 = Polyethylene glycol 400; hr = hour, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; BQL = below quantitation limit.
Values represent mean ± standard deviation (n=3).
Additional information: MOV was administered to rats by oral gavage at 50 mg/kg, 150 mg/kg and 500 mg/kg doses, three male animals per dose per timepoint. Bone marrow tissue was collected from rats at 3 and 24 hr post-dose.

Sec. 2.6.5.12 Pharmacokinetics: Tissue Distribution in Dogs

Sec. 2.6.5.12.1 Tissue Distribution of NHC and NHC-TP in Dogs After a Single Oral Administration of MOV

Test Article: MOV
Report Number: [Ref. 4.2.2.3: PK068MK4482]

Species/Strain	Dog/Beagle		
Number of animals/Gender	2 per time point/M		
Feeding Condition	Fed		
Vehicle/Formulation	1% methylcellulose in water (w/v)		
Route of Administration	P.O.		
Dose (mg/kg)	100		
Analyte	MOV, NHC, NHC-TP		
Method of Analysis	LC-MS/MS		
Sampling Time	3, 24 hr (post-dose)		
Tissue	**Analyte**	3 hr (µg/g)[a]	24 hr (µg/g)[a]
Brain-Olfactory bulb	NHC	3.7 ± 0.1	0.6 ± 0.3
Brain-Olfactory bulb	NHC-TP	0.3 ± 0.3	0.4 ± 0.3
Brain-Olfactory bulb	MOV	BQL	BQL
Brain-Limbic system	NHC	1.1 ± 0.6	0.3 ± 0.2
Brain-Limbic system	NHC-TP	0.1 ± 0.0	0.1 ± 0.1
Brain-Limbic system	MOV	BQL	BQL
Spleen	NHC	12.8 ± 1.4	0.9 ± 0.0
Spleen	NHC-TP	31.1 ± 4.2	18.8 ± 5.3
Spleen	MOV	BQL	BQL
Lung	NHC	19.8 ± 3.8	3.2 ± 1.5
Lung	NHC-TP	2.4 ± 2.8	2.5 ± 1.3
Lung	MOV	BQL	BQL
Kidney	NHC	38.0 ± 8.4	0.5 ± 0.2
Kidney	NHC-TP	2.1 ± 0.2	0.3 ± 0.0
Kidney	MOV	0.3 ± 0.1	BQL
Liver	NHC	15.0 ± 4.9	0.2 ± 0.0
Liver	NHC-TP	4.4 ± 0.8	0.4 ± 0.1
Liver	MOV	BQL	BQL
Heart	NHC	15.0 ± 1.0	0.4 ± 0.1
Heart	NHC-TP	5.9 ± 0.6	1.4 ± 0.3
Heart	MOV	BQL	BQL

Abbreviations: M = male; P.O. = per oral; hr = hour; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; BQL = below quantitation limit.
Values represent mean ± standard deviation (n=2).
[a] Data were recalculated by multiplying molar unit concentrations from study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.157; MOV = 329.31.
Additional information: Following a single oral administration of MOV to dogs at 100 mg/kg, MOV, was observed only in kidney 3 hr post-dose at a relatively low level. NHC-TP was observed in all tissues sampled, with the highest exposure in spleen and the lowest in brain.

Sec. 2.6.5.12 Pharmacokinetics: Tissue Distribution in Dogs (Cont.)

Sec. 2.6.5.12.2 Tissue Distribution of NHC and NHC-TP in Dogs After Repeated Oral Administrations of MOV

Test Article: MOV
Report Number: [Ref. 4.2.2.3: PK066MK4482]

Species/Strain	Dog/Beagle			
Number of animals/Gender	2 per time point/F, 2 per time point/M			
Feeding Condition	Fed			
Vehicle/Formulation	1% methylcellulose in water (w/v)			
Route of Administration	P.O.			
Dose (mg/kg/day)	100, 300, 1000			
Analyte	NHC, NHC-TP			
Method of Analysis	LC-MS/MS			
Sampling Time	24-30 hr (post-dose)			
Tissue	Analyte	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
		24 hr (µg/g)[a]	27-28 hr (µg/g)[b]	28-30 hr (µg/g)[c]
Brain (Limbic system)	NHC	0.8 ± 0.3	1.1 ± 0.3	3.1 ± 0.8
Brain (Limbic system)	NHC-TP	1.0 ± 0.3	2.1 ± 1.4	5.7 ± 2.5
Brain (Olfactory Bulb)	NHC	1.7 ± 1.0	2.0 ± 0.2	5.1 ± 5.1
Brain (Olfactory Bulb)	NHC-TP	4.1 ± 6.0	2.2 ± 1.3	6.6 ± 2.6
Spleen	NHC	2.2 ± 0.4	8.5 ± 6.1	13.9 ± 6 9
Spleen	NHC-TP	101.2 ± 12.6	175.4 ± 61.0	177.5 ± 61.6
Lung	NHC	7.9 ± 6.8	28.7 ± 30.2	25.4 ± 24.1
Lung	NHC-TP	14.4 ± 13.1	108.2 ± 58.2	96.7 ± 46.5
Kidney	NHC	1.0 ± 0.6	2.6 ± 2.1	2.7 ± 0.9
Kidney	NHC-TP	4.9 ± 2.3	34.0 ± 42.5	20.8 ± 18.2
Liver	NHC	1.7 ± 1.3	1.0 ± 0.8	1.9 ± 0.9
Liver	NHC-TP	13.9 ± 7.3	14.4 ± 10.2	35.2 ± 4 3
Heart	NHC	1.9 ± 0.9	2.4 ± 2.4	3.6 ± 2.7
Heart	NHC-TP	8.0 ± 1.5	10.5 ± 7.8	24.1 ± 14.0

Abbreviations: F = female; M = male; P.O. = per oral; hr = hour, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Values represent mean ± standard deviation (n = 4, two male and two female dogs).
Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
[a] MOV was administered once daily by oral gavage to male and female dogs for seven days at doses of 100 mg/kg/day. Brain (olfactory bulb), brain (limbic system), spleen, lung, kidney, liver, and heart tissues were collected 24 hr after the 7th oral dose at 100 mg/kg/day at the end of the study.
[b] Tissues were collected at 27-28 hr after the 6th oral dose at 300 mg/kg/day (unscheduled termination due to toxicity).
[c] Tissues were collected at 28-30 hr after the 3rd oral dose at 1000 mg/kg/day (unscheduled termination due to toxicity).
Additional information: Following oral administration of MOV once daily for seven days at the doses of 100, 300, and 1000 mg/kg/day, MOV exposure was negligible in all tissues. NHC-TP was observed in all tissue samples, with the highest level in spleen and lung, and lowest in brain.

Sec. 2.6.5.13 Pharmacokinetics: Tissue Distribution in Monkeys

Test Article: MOV
Report Number: [Ref. 4.2.2.3: PK013MK4482]

Species/Strain	Monkey/Cynomolgus macaques			
Number of animals/Gender	2 per time point/F, 2 per time point/M			
Feeding Condition	Fed			
Vehicle/Formulation	5% PEG 400 and 0.5% Tween 80 in saline (v/v/v)			
Route of Administration	IV			
Dose (mg/kg)	100			
Analyte	NHC, NHC-TP			
Method of Analysis	LC-MS/MS			
Sampling Time	2 and 12 hr (post-dose)			
Tissue	NHC (µg/g)		NHC-TP (µg/g)	
	2 hr	12 hr	2 hr	12 hr
Brain (Olfactory Bulb)	2.7 ± 1.5	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
Brain (Limbic System)	2.5 ± 0.8	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Spleen	22.6 ± 5.5	3.7 ± 1.9	7.3 ± 3.7	11.3 ± 14.0
Lung	10.8 ± 5.5	6.6 ± 9.1	0.6 ± 0.1	0.9 ± 0.9
Kidney	1.0 ± 0.4	0.0 ± 0.0	0.5 ± 0.1	0.4 ± 0.3
Liver	2.6 ± 0.9	0.2 ± 0.3	0.6 ± 0.3	0.7 ± 0.6
Heart	1.1 ± 0.4	0.1 ± 0.0	0.5 ± 0.3	0.3 ± 0.1

Abbreviations: F = female; M = male; IV = intravenous; PEG 400 = polyethylene glycol 400; hr = hour, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate.
Values represent mean ± standard deviation (n = 4, two male and two female monkeys).
Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
Additional information: MOV was not detected in all tissues tested.

Sec. 2.6.5.14 Pharmacokinetics: Tissue Distribution in Ferrets

Test Article: MOV
Report Number: [Ref. 4.2.2.2: PK008MK4482] [Ref. 4.2.2.3: PK014MK4482]

Report number	[Ref. 4.2.2.2: PK008MK4482]		[Ref. 4.2.2.3: PK014MK4482]					
Species/Strain	Ferret/Mustela putorius furo		Ferret/Mustela putorius furo					
Number of animals/Gender	3 /M		6/F					
Feeding Condition	NS		NS					
Vehicle/Formulation	1% methylcellulose in water (w/v)		1% methylcellulose in water (w/v)					
Route of Administration	P.O.		P.O.					
Dose (mg/kg)	20		128					
Analyte	NHC, NHC-TP		NHC, NHC-TP					
Method of Analysis	LC-MS/MS		LC-MS/MS					
Sampling Time	4 hr (post-dose)		3, 10, 12 hr (post-dose)					
Tissue (µg/g)	NHC	NHC-TP	NHC	NHC	NHC	NHC-TP	NHC-TP	NHC-TP
	4 hr[a]	4 hr[a]	3 hr[a]	10 hr[b]	12 hr[c]	3 hr[a]	10 hr[b]	12 hr[c]
Lung	2.6 ± 0.5	7.0 ± 0.7	23.0 ± 7.1	6.7	2.8 ± 0.2	4.4 ± 1.1	2.9	1.6 ± 0.4
Heart	1.5 ± 0.5	1.4 ± 0.2	19.1 ± 9.5	0.4	0.3 ± 0.0	8.6 ± 2.1	2.7	1.3 ± 0.2
Liver	0.5 ± 0.3	3.5 ± 3.2	14.9 ± 10.1	0.5	0.3 ± 0.0	6.4 ± 6.5	0.9	0.4 ± 0.1
Spleen	4.2 ± 1.4	10.8 ± 2.1	16.2 ± 6.9	4.6	1.0 ± 0.1	39.6 ± 14.8	16.4	12.3 ± 1.1
Kidney	1.1 ± 0.9	0.7 ± 0.1	24.8 ± 13.8	1.6	0.5 ± 0.0	4.0 ± 2.3	0.6	0.6 ± 0.0
Brain	0.5 ± 0.1	0.5 ± 0.1	3.9 ± 2.0	0.6	0.2 ± 0.1	2.1 ± 1.1	0.1	0.1 ± 0.0

Abbreviations: F = female; M = male; P.O. = per oral; hr = hour, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; NS = not specified
[a] Values represent mean ± standard deviation (n = 3).
[b] One animal was sacrificed 10 hr post dose.
[c] Values represent mean ± standard error (n = 2).
Data were recalculated by multiplying molar unit concentrations from the study report by molecular weight of analyte. Molecular weight: NHC = 259.22; NHC-TP = 499.15.
Additional information: Following a single oral dose of 20 mg/kg MOV to ferrets, MOV was not detected in any tissue except the kidney where it was low (13 ng/g).

Sec. 2.6.5.15 Pharmacokinetics: *In Vitro* Plasma Protein Binding of NHC

Test Article: NHC
Report Number: [Ref. 4.2.2.3: PK011MK4482]

Test System	*In vitro*		
Species/Matrix	Mouse, rat, dog, monkey, and human plasma; human alpha-1 acid glycoprotein and albumin.		
Method	Equilibrium dialysis		
Analytes	NHC		
Assay	LC-MS/MS		
Matrix	**Unbound Fraction of NHC**		
	2 µM	20 µM	100 µM
CD-1 mouse plasma	1.28 ± 0.09	1.06 ± 0.09	1.10 ± 0.16
Sprague Dawley rat plasma	1.02 ± 0.08	1.07 ± 0.08	0.999 ± 0.104
Beagle dog plasma	1.00 ± 0.16	0.996 ± 0.027	1.17 ± 0.18
Cynomolgus monkey plasma	1.11 ± 0.22	1.16 ± 0.14	1.17 ± 0.08
Human plasma	1.06 ± 0.12	1.01 ± 0.11	1.13 ± 0.20
Human alpha-1 acid glycoprotein	1.03 ± 0.11	1.10 ± 0.11	1.05 ± 0.09
Human Serum Albumin	1.02 ± 0.16	1.27 ± 0.39	1.15 ± 0.17

Abbreviations: LC-MS/MS = liquid chromatography-tandem mass spectrometry; NHC = N-hydroxycytidine;
Values represent mean ± standard deviation (n = 3).

Sec. 2.6.5.16 Pharmacokinetics: Metabolism *In Vivo*

Test Article: MOV

Report Number: [Ref. 4.2.2.5: PK083MK4482] [Ref. 4.2.2.4: PK086MK4482] [Ref. 4.2.2.4: PK087MK4482]

Report Number	[Ref. 4.2.2.5: PK083MK4482]		[Ref. 4.2.2.5: PK083MK4482]		[Ref. 4.2.2.4: PK086MK4482]		[Ref. 4.2.2.4: PK087MK4482]			
Species/Strain	Rat[a]/WH		Dog[b]/Beagle		Human[c]		Human[d]			
Gender/Number of animals/subjects	M/4 (BDC)	M/3 (Intact)	M/3 (Intact)		M/5, F/1	M/6	M/2, F/4		M/3, F/3	
Feeding conditions	Fasted	Fasted	Fed		Fasted	Fasted	Fasted		Fasted	
Vehicle / Formulation	1% Methylcellulose in water (w/v)	1% Methylcellulose in water (w/v)	1% Methylcellulose in water (w/v)		Capsule[e]	Capsule[e]	Capsule[e]		Capsule[e]	
Method of Administration	P.O.	P.O.	P.O.		P.O.	P.O.	P.O.		P.O.	
Dose	30 mg/kg	30 mg/kg	30 mg/kg		100 mg BID	800 mg BID	400 mg BID		800 mg BID	
Radionuclide	^{14}C	^{14}C	^{14}C		NA	NA	NA		NA	
Specific Activity (µCi/mg)	176.5	176.5	176.5		NA	NA	NA		NA	
Radioactivity of Dose Solution (µCi/mg)	2.5	30	0.17		NA	NA	NA		NA	
Biological Samples	Urine, feces	Plasma	Plasma, urine		Urine		Plasma			
Analyte(s)	[^{14}C]MOV and metabolites				MOV and metabolites		MOV and metabolites			
Assays	LC-MS/MS with off-line radiometric analysis fraction of LC column eluents				LC-MS/MS		LC-MS/MS			
Biological Matrix	Presence of MOV and Metabolites in Plasma and Excreta[f]				Peak area/IS ratio[g]		Peak area/IS ratio[g]			
	Urine	Feces	Plasma	Urine	Plasma	Urine	Urine	Plasma		Plasma	
Sample Pooling Interval (hr)	0-48	0-24	0-24	0-24	2	0-12 (Day 1)	0-12 (Day 1)	0-12 (Day 1)	0-12 (Day 11)	0-12 (Day 1)	0-12 (Day 11)
MOV	-	-	-	-	+	-	-	-	-	-	-
NHC	0.4	-	3	8	96	0.034	0.62	0.128	0.152	0.265	0.285
3-ureidopropionic acid	1.8	0.5	14	0.4	-	-	-	-	-	-	-
Uracil	1.4	4.6	6	-	-	-	-	-	-	-	-
Cytidine	0.5	-	53	-	4	Trace	0.042	-	-	-	-
Uridine[h]	-	-	21	-	-	0.0085	0.053	0.189	0.236	0.307	0.398
M1 (NHC-Glu)	0.4	-	-	-	-	0.0049	0.064	Trace	Trace	Trace	Trace
M2 (NHC-methylation)	0.5	-	-	-	-	-	-	-	-	-	-
M3 (NHC-glucose)	-	-	-	-	-	-	-	Trace	Trace	Trace	Trace
Unknown1	-	1.4	-	-	-	-	-	-	-	-	-
Unknown2	-	-	-	0.4	-	-	-	-	-	-	-

Abbreviations: BDC = bile duct-cannulated; BID = twice daily; M = male; F = female; hr = hour; P.O. = per oral; WH = Wistar Han; Glu = glucuronide; IS = internal standard; LC-MS/MS = liquid chromatography- tandem mass spectrometry; LC = liquid chromatography; MOV = molnupiravir; NHC = N-hydroxycytidine; NA = not applicable; AUC = area under the concentration-time curve; + = detected by mass spectrometry only; - = not detected.

[a] Rat bile samples were not analyzed due to low radioactivity recovered in samples.
[b] Dog fecal samples were not analyzed due to low radioactivity recovered in samples.
[c] Metabolite profiling was conducted in pooled urine (0-12 hr, Day 1) samples from healthy subjects treated with multiple oral doses at 100 mg BID or 800 mg BID (P004).
[d] Metabolite profiling was conducted in AUC pooled plasma samples (0-12 hr, Day 1 and Day 11) from healthy subjects treated with multiple oral doses at 400 mg BID or 800 mg BID for 10.5 days (P012).
[e] The capsule formulation consisted of standard pharmaceutical excipients and included hydroxy-propyl cellulose, microcrystalline cellulose, croscarmellose sodium, and magnesium stearate.
[f] Values represent percentage relative to the total integrated radioactivity in the plasma or percentages relative to the administered dose in urine and feces.
[g] All estimations of metabolites were semi-quantitative and assumed that matrix effect was the same for the metabolites in the samples from the two dose groups in each matrix.
[h] The pear area/IS ratio of uridine in pre-dose human plasma samples from average of two dose groups was 0.158.

Sec. 2.6.5.17 Pharmacokinetics: Metabolism *In Vitro*

Sec. 2.6.5.17.1 Stability of MOV in Plasma and Liver Microsomes

Test Article: MOV

Study System	*In vitro*			
Species/Matrix	Mouse, Rat, dog, monkey, and human plasma and liver microsomes			
Method	MOV (1 µM) was incubated with liver microsomes (0.5 mg/mL) and plasma from mouse, rat, dog monkey and human at 37°C for up to 2 hr.			
Analyte	MOV			
Assays	LC-MS/MS			
Species	**Plasma**		**Liver microsomes**	
	$t_{½}$ (hr)	**Report Number**	**$t_{1/2}$ (hr)**	**Report Number**
Mouse	0.017	[Ref. 4.2.2.4: PK031MK4482]	0.033	[Ref. 4.2.2.4: PK032MK4482]
Rat	0.033	[Ref. 4.2.2.4: PK036MK4482]	0.083	[Ref. 4.2.2.4: PK037MK4482]
Dog	3.2	[Ref. 4.2.2.4: PK036MK4482]	0.017	[Ref. 4.2.2.4: PK037MK4482]
Monkey	0.40	[Ref. 4.2.2.4: PK031MK4482]	0.017	[Ref. 4.2.2.4: PK032MK4482]
Human	1.05	[Ref. 4.2.2.4: PK031MK4482]	1.22	[Ref. 4.2.2.4: PK032MK4482]

Abbreviations: LC-MS/MS = liquid chromatography- tandem mass spectrometry; hr = hour; MOV = molnupiravir; $t_{½}$ = terminal half-life.
Values represent means (n = 3).
Additional information: $t_{½}$ was calculated by plotting the natural logarithm of % parent vs. time and obtaining the slope of the line. Assuming first-order kinetics, the elimination rate constant, k, is the negative (-) of the slope. $t_{½}$ =- 0.693/ (slope).

Sec. 2.6.5.17 Pharmacokinetics: Metabolism *In Vitro* (Cont.)

Sec. 2.6.5.17.2 Metabolic Stability of MOV in Simulated Gastric and Intestinal Fluids and in Phosphate Buffered Saline

Test Article: MOV

Report Number: [Ref. 4.2.2.4: PK033MK4482] [Ref. 4.2.2.4: PK035MK4482]

Study System	*In vitro*		
Species/Matrix	SGF, SIF and PBS buffer with FBS		
Method	MOV (50 µg/mL) was incubated in SGF and SIF 37°C for up to 2 hr. MOV (1 µM) was incubated in PBS buffer containing 10% of FBS at 37°C for up to 4 hr.		
Analyte	MOV		
Assays	LC-MS/MS		
Matrix	**Concentration Tested**	**t½ (hr)**	**Report No.**
SGF	50 µg/mL	>24	[Ref. 4.2.2.4: PK033MK4482]
SIF	50 µg/mL	>24	[Ref. 4.2.2.4: PK033MK4482]
PBS +10% FBS	1 µM	>24	[Ref. 4.2.2.4: PK035MK4482]

Abbreviations: PBS = phosphate buffered saline; FBS = fetal bovine serum; SGF = simulated gastric fluids; SIF = simulated intestinal fluids; LC-MS/MS = liquid chromatography–tandem mass spectrometry; hr = hour; MOV = molnupiravir; t½ = terminal half-life.
Values represent means (n = 3).
Additional information: t½ was calculated by plotting the natural logarithm of % parent vs. time and obtaining the slope of the line. Assuming first-order kinetics, the elimination rate constant, k, is the negative (-) of the slope. t½ =- 0.693/ (slope).

Sec. 2.6.5.17 Pharmacokinetics: Metabolism *In Vitro* (Cont.)

Sec. 2.6.5.17.3 Stability of NHC in Plasma, Blood, Liver S9, Liver and Intestinal Microsomes

Test Article: NHC

Study System	*In vitro*									
Species/ Matrix	Rat, dog, monkey, and human plasma; mouse , monkey, and human blood; mouse, rat, monkey, and human liver microsomes; rat, dog, monkey, and human liver S9; mouse, monkey and human intestinal microsome.									
Method	NHC (4 µM) was incubated with rat, dog, and human plasma for up to 4 hr, and monkey plasma for up to 24 hr at 37°C. $^{13}C_5$-NHC ($^{13}C_5$-labeled version of NHC, 4.24 µM) was incubated with mouse, monkey, and human blood for up to 24 hr at 37°C. NHC (4 µM) was incubated with mouse, rat, monkey, and human liver microsomes (0.5 mg/mL) in the presence of UDPGA and NADPH regenerating system for up to 2 hr at 37°C. NHC (4 µM) was incubated with rat, dog, monkey, and human liver S9 (1 mg/mL) in the presence of UDPGA and NADPH regenerating reagent for up to 2 hr at 37°C. $^{13}C_5$-NHC (3 µM) was incubated with mouse, monkey and human intestinal microsomes (0.5 mg/mL) for up to 4 hr at 37ºC.									
Analyte	NHC									
Assays	LC-MS/MS									
Species	**Plasma**		**Whole Blood**		**Liver Microsomes**		**Liver S9**		**Intestinal Microsomes**	
	t½ (hr)	**Report Number**	**t½ (hr)**	**Report Number**	**t½ (hr)**	**Report Number**	**t½ (hr)**	**Report Number**	**t½ (hr)**	**Report Number**
Mouse	-	-	>24	[Ref. 4.2.2.4: PK029MK4482]	>24	[Ref. 4.2.2.4: PK021MK4482]	-	-	>24	[Ref. 4.2.2.4: PK030MK4482]
Rat	17	[Ref. 4.2.2.4: PK015MK4482]	-	-	>24	[Ref. 4.2.2.4: PK020MK4482]	>24	[Ref. 4 2 2.4: PK017MK4482]	-	-
Dog	>24	[Ref. 4.2.2.4: PK015MK4482]	-	-	-	-	>24	[Ref. 4 2 2.4: PK018MK4482]	-	-
Monkey	6.5	[Ref. 4.2.2.4: PK024MK4482]	3	[Ref. 4.2.2.4: PK034MK4482]	>24	[Ref. 4.2.2.4: PK022MK4482]	>24	[Ref. 4 2 2.4: PK023MK4482]	>24	[Ref. 4.2.2.4: PK030MK4482]
Human	10	[Ref. 4.2.2.4: PK015MK4482]	7	[Ref. 4.2.2.4: PK028MK4482]	>24	[Ref. 4.2.2.4: PK019MK4482]	20	[Ref. 4 2 2.4: PK016MK4482]	>24	[Ref. 4.2.2.4: PK030MK4482]

Abbreviations: NHC = N-hydroxycytidine; NADPH = Nicotinamide adenine dinucleotide phosphate reduced form; UDPGA = uridine 5'-diphosphoglucuronicacid; LC-MS/MS = liquid chromatography- tandem mass spectrometry; hr = hour; t½ = terminal half-life; - = not available.
Values represent means (n = 3).
Additional information: t½ was calculated by plotting the natural logarithm of % parent vs. time and obtaining the slope of the line. Assuming first-order kinetics, the elimination rate constant, k, is the negative (-) of the slope. t½ =- 0.693/(slope).

Sec. 2.6.5.17 Pharmacokinetics: Metabolism *In Vitro* (Cont.)

Sec. 2.6.5.17.4 MOV and NHC Uptake and Anabolism to NHC-TP in Tissue Culture Cell Lines (Accumulation Phase)

Test Article: MOV and NHC

Study System	*In vitro*					
Species/Matrix	Tissue culture cell lines: A549, BEAS-2B, CEM, HepG2, Huh7, PC-3, Vero.					
Method	NHC or MOV were incubated with tissue culture cell lines at selected concentrations at 37°C/5%CO_2 for up to 24 hr. At the desired time points, cells were washed two times with DPBS and extracted with 70% methanol or 70% acetonitrile in water. Intracellular concentrations of MOV, NHC and NHC-TP were determined by LC-MS/MS.					
Analyte	MOV, NHC, NHC-TP					
Assays	LC-MS/MS					
Cell Line	**Substrate tested**	**Concentration tested (µM)**	**Analyte**	**C_{max} (pmol/10^6 cells)**	**T_{max} (hr)**	**Report Number**
A549	NHC	1	NHC	67.0 ± 5.5	6	[Ref. 4.2.2.4: PK056MK4482][a]
			NHC-TP	186.8 ± 27.6	24	
		10	NHC	571.9 ± 354.1	6	
			NHC-TP	1,866.5 ± 75.0	24	
		100	NHC	13,215.9 ± 4,067.1	6	
			NHC-TP	9,766.6 ± 410.0	6	
BEAS-2B	NHC	20	NHC	36.6 ± 8.2	16	[Ref. 4.2.2.4: PK046MK4482][b]
			NHC-TP	2,062.6 ± 221.8	24	
CEM	NHC	1	NHC	BQL	NA	[Ref. 4.2.2.4: PK058MK4482][c]
			NHC-TP	18.8 ± 8.2	3	
		10	NHC	BQL	NA	
			NHC-TP	158.4 ± 14.5	1	
		100	NHC	9.5 ± 1.9	24	
			NHC-TP	951.6 ±249.2	3	
CEM	MOV	20	MOV	5.4 ± 1.2	4	[Ref. 4.2.2.4: PK052MK4482][c]
			NHC	0.3 ± 0.05	3	
			NHC-TP	171.3 ± 7.0	24	
HepG2	NHC	20	NHC	24.1 ± 1.0	6	[Ref. 4.2.2.4: PK051MK4482][b]
			NHC-TP	732.7 ± 2.9	6	
HepG2	MOV	20	MOV	18.9 ± 2.2	3	[Ref. 4.2.2.4: PK050MK4482][b]
			NHC	13.4 ± 0.6	16	
			NHC-TP	470.3 ± 14.1	24	

Huh7	NHC	20	NHC	133.9 ± 115.5	3	[Ref. 4.2.2.4: PK054MK4482][b]
			NHC-TP	2,623.5 ± 487.0	6	
Huh7	MOV	20	MOV	77.5 ± 73.8	2	[Ref. 4.2.2.4: PK053MK4482][b]
			NHC	29.0 ± 1.7	24	
			NHC-TP	1,113.3 ± 44.1	24	
PC-3	NHC	10	NHC	33.3 ± 7.7	6	[Ref. 4.2.2.4: PK055MK4482][b]
			NHC-TP	819.5 ± 16.8	6	
Vero	NHC	1	NHC	27.1 ± 11.4	1	[Ref. 4.2.2.4: PK057MK4482][a]
			NHC-TP	240.2 ± 90.9	24	
		10	NHC	248.2 ± 51.6	6	
			NHC-TP	1,629.4 ± 221.0	24	
		100	NHC	3,774.1 ± 1,312.1	24	
			NHC-TP	5,105.3 ± 933.3	6	

Abbreviations: A549 = human adenocarcinomic alveolar basal epithelial cell line; BEAS-2B = human bronchial epithelial cell Line; CEM = T lymphoblastoid cell line; HepG2 = human liver cancer cell line; Huh7 = human hepatoma cell line; PC-3 = human prostatic cancer cell line; Vero = monkey kidney epithelial cell line; BQL = below the quantifiable limit; C_{max} = maximum concentration; DPBS = Dulbecco's Phosphate-Buffered Saline; LC-MS/MS = liquid chromatography-tandem mass spectrometry; hr = hour; MOV = molnupiravir; T_{max} = time of maximum concentration; NA = not applicable; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; LLOQ = lower limit of quantitation; ULOQ = upper limit of quantitation..
Values represent mean or mean ± standard deviation (n=3 replicates).
[a] Incubation was conducted in 0.2 x 10^6 cells/mL.
[b] Incubation was conducted in 0.35 x 10^6 cells/mL.
[c] Incubation was conducted in 4 x 10^6 cells/mL.
Additional Information: Intracellular concentration of MOV, NHC and NHC-TP in tissue culture cell lines were measured by LC-MS/MS using a qualified bioanalytical method [Ref. 4.2.2.1: 05JRCV]. The LLOQ was 1 ng/mL and the ULOQ was 1,000 ng/mL for MOV, NHC and NHC-TP.

Sec. 2.6.5.17 Pharmacokinetics: Metabolism *In Vitro* (Cont.)

Sec. 2.6.5.17.5 NHC Uptake and Anabolism to NHC-TP in Primary Cells (Accumulation Phase)

Test Article: NHC

Study System	*In vitro*				
Species/Matrix	Primary cells: human astrocytes, hBTEC, mouse, monkey, and human hepatocytes				
Method	NHC or $^{13}C_5$-NHC (20 µM) were incubated with primary cells at 37°C/5%CO_2. At the desired time points, cells were washed two times with DPBS and extracted with 70% methanol or 70% acetonitrile in water. Intracellular concentrations of NHC and its anabolites in the pyrimidine nucleotide phosphorylation pathway were determined by LC-MS/MS.				
Analyte	NHC, NHC-TP, CTP, UTP, cytidine, uridine				
Assays	LC-MS/MS				
Cell Type	**Substrate tested**	**Analyte**	**C_{max} (pmol/10^6 cells)**	**T_{max} (hr)**	**Report Number**
Normal Human Astrocytes[a]	$^{13}C_5$-NHC	$^{13}C_5$-NHC	74.4 ± 23.3	6	[Ref. 4.2.2.4: PK045MK4482]
		$^{13}C_5$-Uridine	BQL	NA	
		$^{13}C_5$-Cytidine	BQL	NA	
		$^{13}C_5$-UTP	9.4 ± 0.3	6	
		$^{13}C_5$-CTP	21.6 ± 1.0	6	
		$^{13}C_5$-NHC-TP	1,673.3 ± 63.8	6	
Primary hBTEC[b]	NHC	NHC	45.3 ± 7.9	4	[Ref. 4.2.2.4: PK047MK4482]
		NHC-TP	2,072.1 ± 327.4	6	
Primary hBTEC[c]	NHC	NHC	92.7 ± 15.0	4	[Ref. 4.2.2.4: PK048MK4482]
		NHC-TP	4,632.9 ± 285.2	4	
Primary Human Hepatocytes[d]	NHC	NHC	20.4 ± 0.9	24	[Ref. 4.2.2.4: PK025MK4482][j]
		NHC-TP	276 ± 72	4	
Primary Human Hepatocytes[e]	$^{13}C_5$-NHC	$^{13}C_5$-NHC	84.5 ± 35.9	1	[Ref. 4.2.2.4: PK043MK4482]
		$^{13}C_5$-Uridine	BQL	NA	
		$^{13}C_5$-Cytidine	BQL	NA	
		$^{13}C_5$ -UTP	106.4 ± 7.1	6	
		$^{13}C_5$-CTP	105.6 ± 7.1	6	
		$^{13}C_5$-NHC-TP	118.4 ± 4.1	6	
Primary Monkey Hepatocytes[f]	NHC	NHC	18.9 ± 8.7	2	[Ref. 4.2.2.4: PK027MK4482][j]
		NHC-TP	267 ± 36	1	

Primary Monkey Hepatocytes[g]	$^{13}C_5$-NHC	$^{13}C_5$-NHC	26.9 ± 4.5	1	[Ref. 4.2.2.4: PK041MK4482]
		$^{13}C_5$-Uridine	BQL	NA	
		$^{13}C_5$-Cytidine	BQL	NA	
		$^{13}C_5$-UTP	54.9 ± 0.6	6	
		$^{13}C_5$-CTP	655.7 ± 22.1	6	
		$^{13}C_5$-NHC-TP	578.2 ± 7.5	3	
Primary Mouse Hepatocytes[h]	NHC	NHC	129 ± 3.0	24	[Ref. 4.2.2.4: PK026MK4482][j]
		NHC-TP	33 ± 11.7	2	
Primary Mouse Hepatocytes[i]	$^{13}C_5$-NHC	$^{13}C_5$-NHC	20.5 ± 4.0	6	[Ref. 4.2.2.4: PK039MK4482]
		$^{13}C_5$-Uridine	BQL	NA	
		$^{13}C_5$-Cytidine	2.7 ± 0.7	6	
		$^{13}C_5$-UTP	90.2 ± 13.4	3	
		$^{13}C_5$-CTP	706.5 ± 58.7	3	
		$^{13}C_5$-NHC-TP	17.5 ± 2.7	6	

Abbreviations: BQL = below the quantifiable limit; C_{max} = maximum concentration; DPBS = Dulbecco's Phosphate-Buffered Saline; hBTEC = Human Bronchial/Tracheal Epithelial Cells; hr = hour; LC-MS/MS = liquid chromatography-tandem mass spectrometry; T_{max} = time of maximum concentration; NA = not applicable; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate; CTP = cytidine triphosphate; UTP = uridine triphosphate; LLOQ = lower limit of quantitation; ULOQ = upper limit of quantitation. Values represent mean or mean ± standard deviation (n=3 replicates).

[a] Incubation was conducted in 0.1 x 10^6 cells/mL of normal human astrocytes for up to 6 hr.

[b] Incubation was conducted in 0.35 x 10^6 cells/mL of primary hBTEC cells for up to 24 hr.

[c] Incubation was conducted in 0.225 x 10^6 cells/mL of primary hBTEC for up to 24 hr.

[d] Incubation was conducted in 1.3 x 10^6 cells/mL of human hepatocytes for up to 24 hr.

[e] Incubation was conducted in 0.01 x 10^6 cells/mL of human hepatocytes for up to 6 hr.

[f] Incubation was conducted in 0.7 x 10^6 cells/mL of monkey hepatocytes for up to 24 hr.

[g] Incubation was conducted in 0.35 x 10^6 cells/mL of monkey hepatocytes for up to 6 hr.

[h] Incubation was conducted in 0.6 x 10^6 cells/mL of mouse hepatocytes for up to 24 hr.

[I] Incubation was conducted in 0.7 x 10^6 cells/mL of mouse hepatocytes for up to 3 hr.

[j] Data were recalculated by multiplying molar unit concentrations from study reports by factor of 3 based on estimated cells volume (1 µM is equivalent to 3 pmol/10^6 cells).

Additional Information: Intracellular concentration of NHC and NHC-TP in primary cells were measured by LC-MS/MS using a qualified bioanalytical method [Ref. 4.2.2.1: 05JRCV]. The LLOQ was 1 ng/mL and the ULOQ was 1,000 ng/mL; Intracellular concentration of $^{13}C_5$-NHC and its metabolites in primary cells were measured by LC-MS/MS using a qualified bioanalytical method [Ref. 4.2.2.1: 05MQGW] [Ref. 4.2.2.1: 05MP9W]. The LLOQ was 1 ng/mL and the ULOQ was 1,000 ng/mL.

Sec. 2.6.5.17 Pharmacokinetics: Metabolism *In Vitro* (Cont.)

Sec. 2.6.5.17.6 Metabolism of NHC in Primary Cell Cultures (Washout phase)

Test Article: NHC

Study System	*In vitro*			
Species/Matrix	Primary cell cultures: human astrocytes, hBTEC, mouse, monkey, and human hepatocytes			
Method	NHC or $^{13}C_5$-NHC (20 µM) were incubated with primary cell cultures at 37°C/5%CO_2 in 24-well plates, at the desired time points, cells were washed two times with DPBS and followed by incubation of cells in culture media for selected time points. At indicated time points, cells were washed two times with DPBS and were extracted with 70% methanol. Intracellular concentrations of NHC and NHC-TP were determined by LC-MS/MS.			
Analyte	NHC, NHC-TP			
Assays	LC-MS/MS			
Cell Type	**Substrate tested**	**Analyte**	**$t_{1/2}$ (hr)[e]**	**Report Number**
Normal Human Astrocytes[a]	$^{13}C_5$-NHC	$^{13}C_5$-NHC	0.2	[Ref. 4.2.2.4: PK044MK4482]
		$^{13}C_5$-NHC-TP	4.6	
Primary hBTEC[b]	NHC	NHC	0.2	[Ref. 4.2.2.4: PK049MK4482]
		NHC-TP	4.9	
Primary Human Hepatocytes[c]	$^{13}C_5$-NHC	$^{13}C_5$-NHC	0.2	[Ref. 4.2.2.4: PK042MK4482]
		$^{13}C_5$-NHC-TP	1.1	
Primary Monkey Hepatocytes[d]	$^{13}C_5$-NHC	$^{13}C_5$-NHC	0.5	[Ref. 4.2.2.4: PK040MK4482]
		$^{13}C_5$-NHC-TP	0.7	
Primary Mouse Hepatocytes[d]	$^{13}C_5$-NHC	$^{13}C_5$-NHC	BQL[f]	[Ref. 4.2.2.4: PK038MK4482]
		$^{13}C_5$-NHC-TP	0.4	

Abbreviations: BQL = below the quantifiable limit; DPBS = Dulbecco's phosphate-buffered saline; hBTEC = Human Bronchial/Tracheal Epithelial Cells; hr = hour; LC-MS/MS = liquid chromatography–tandem mass spectrometry; NHC = N-hydroxycytidine; NHC-TP = N-hydroxycytidine-5'-triphosphate, $t_{1/2}$ = terminal half-life; LLOQ = lower limit of quantitation; ULOQ = upper limit of quantitation.
Values represent mean or mean ± standard deviation (n=3 replicates).
[a] $^{13}C_5$-NHC was incubated with human astrocytes (0.1 x 10^6 cells/well) for 4 hr, cells were washed for two times with DPBS. Cells was then incubated in culture media for up to 24 hr.
[b] NHC was incubated with hBTEC cells (0.225 x 10^6 cells/well) for 24 hr, cells were washed for two times with DPBS, followed by incubation with culture media for up to 6 hr.
[c] $^{13}C_5$-NHC was incubated with human hepatocytes (0.1 x 10^6 cells/well) for 4 hr, cells were washed for two times with DPBS, followed by incubation with culture media for up to 48 hr.
[d] $^{13}C_5$-NHC was incubated with mouse and monkey hepatocytes (0.35 x 10^6 cells/well) for 4 hr, cells were washed for two times with DPBS, followed by incubation with culture media for up to 48 hr.
[e] $t_{½}$ was estimated by plotting the natural logarithm of mean concentration of analyte vs. time and obtaining the slope of the line within linear range. Assuming first-order kinetics, the elimination rate constant, k, is the negative (-) of the slope. $t_{1/2}$ =- 0.693/ (slope).
[f] Below quantitation level at first timepoint (0.5 hr) after drug withdrawal.
Additional Information: Intracellular concentration of NHC and NHC-TP in primary cells were measured by LC-MS/MS using a qualified bioanalytical method [Ref. 4.2.2.1: 05JRCV]. The LLOQ was 1 ng/mL and the ULOQ was 1,000 ng/mL; Intracellular concentration of $^{13}C_5$-NHC and $^{13}C_5$-NHC-TP in primary cells were measured by LC-MS/MS using a qualified bioanalytical method [Ref. 4.2.2.1: 05MQGW][Ref. 4.2.2.1: 05MP9W]. The LLOQ was 1 ng/mL and the ULOQ was 1,000 ng/mL.

Sec. 2.6.5.17 Pharmacokinetics: Metabolism *In Vitro* (Cont.)

Sec. 2.6.5.17.7 Metabolism of MOV in Hepatocytes

Test Article: MOV
Report Number: [Ref. 4.2.2.4: PK082MK4482]

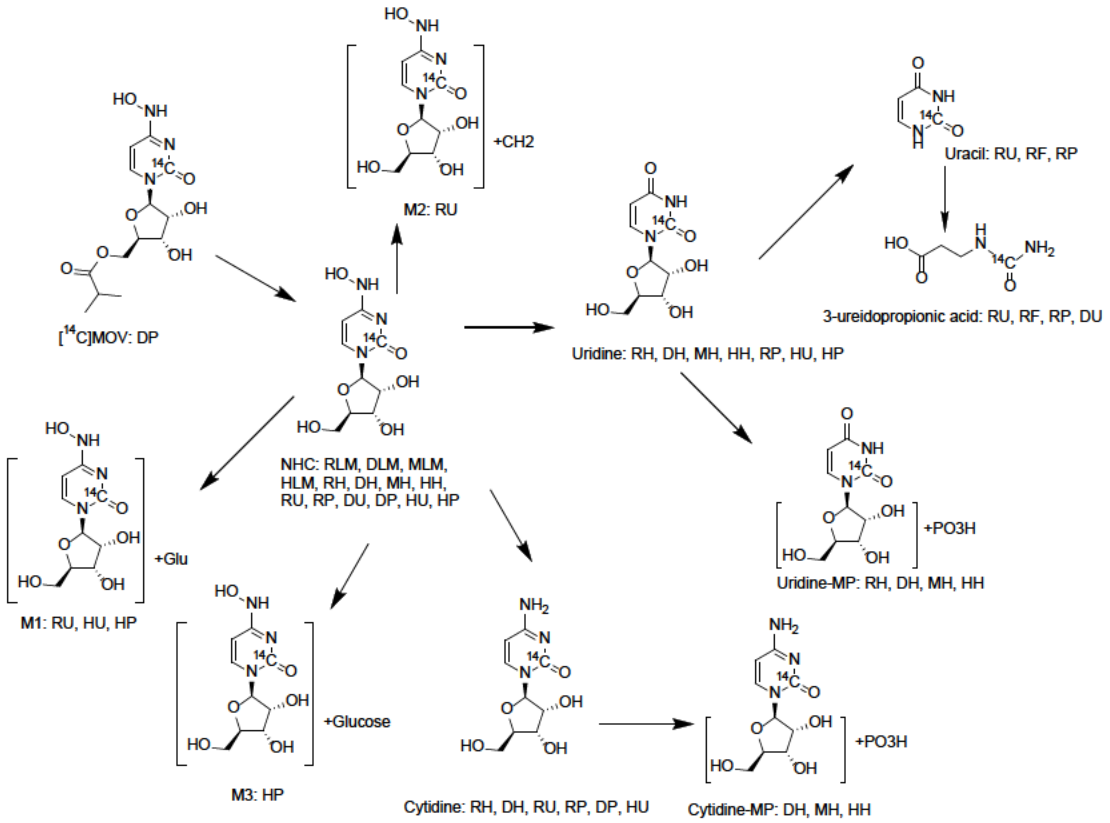
Study System	*In vitro*			
Species/Matrix	Rat, dog, monkey, and human hepatocytes			
Method for Hepatocytes	[^{14}C]MOV (10 µM) was incubated with hepatocyte suspensions (1x 10^6 cells/mL) from rat, dog, monkey, and human at 37°C for 2 hr. At the end of incubation, samples were quenched with equal amount of acetonitrile followed with centrifugation. The supernatants were analyzed by LC-MS/MS with on-line radiometric detection.			
Analyte	MOV and metabolites			
Assays	LC-MS/MS with on-line radiometric detection			
Percent of Radioactivity of Prominent Metabolites Detected in Hepatocytes				
Metabolite	Rat/SD	Dog/Beagle	Monkey/Cyno	Human
MOV	-	-	-	-
NHC	55.8	73.0	85.8	70.5
Cytidine	36.8	4.1	-	-
Uridine	3.1	2.8	6.0	26.1
Cytidine-MP	-	9.7	3.2	2.4
Uridine -MP	2.2	9.1	4.5	1.0
Unknown	-	1.3	-	-

Abbreviations: hr = hour; LC-MS/MS = liquid chromatography- tandem mass spectrometry, MOV = molnupiravir; NHC = N-hydroxycytidine; MP = monophosphate; SD = Sprague Dawley; - = Not detected.
Values represent n=1 incubation.
Additional information: Under the condition tested, NHC-triphosphate was not detected in hepatocytes from all species with the current LC-MS/MS conditions. A separate experiment with NHC-triphosphate standard has shown that NHC-triphosphate was stable in the incubation conditions. Data from *in vitro* reaction phenotyping for MOV and NHC are presented in [Sec. 2.6.5.20].

Sec. 2.6.5.18 Pharmacokinetics: Proposed Metabolic Pathways Observed *In Vitro* and *In Vivo*

Test Article: MOV

Report Number: [Ref. 4.2.2.4: PK082MK4482] [Ref. 4.2.2.5: PK083MK4482] [Ref. 4.2.2.4: PK085MK4482] [Ref. 4.2.2.4: PK086MK4482] [Ref. 4.2.2.4: PK087MK4482]



Abbreviations: MOV = molnupiravir; NHC= N-hydroxycytidine; RLM = rat liver microsomes; DLM = dog liver microsomes; MLM = monkey liver microsomes, HLM = human liver microsomes; RH = rat hepatocytes; DH = dog hepatocytes; MH = monkey hepatocytes; HH = human hepatocytes; MP = monophosphate; RU = rat urine, RF = rat feces; RP = rat plasma; DU = dog urine; DP = dog plasma; HU = human urine (unlabeled), HP = human plasma (unlabeled).

Sec. 2.6.5.19 Pharmacokinetics: Excretion in Rats and Dogs

Test Article: MOV
Report Number: [Ref. 4.2.2.5: PK083MK4482]

Species/Strain		Rat/WH (BDC)	Dog/beagle (Intact)
Gender / Number of animals		M/4	M/3
Feeding conditions		Fasted	Fed
Vehicle / Formulation		1% Methylcellulose (w/v)	1% Methylcellulose (w/v)
Method of administration		P.O.	P.O.
Dose (mg/kg)		30	30
Radionuclide		^{14}C	^{14}C
Specific activity (µCi/mg)		176.5	176.5
Radioactivity of dose solution (µCi/mg)		2.5	0.17
Analyte(s)/Assay		Total Radioactivity/Liquid Scintillation Counting	
Matrix	**Collection Time (hr)**	Percent of Dose Recovered	
Bile	Predose	0.00 ± 0.00	NA
	0-8	0.09 ± 0.02	NA
	8-24	0.05 ± 0.03	NA
	24-48	0.00 ± 0.00	NA
	48-72	0.04 ± 0.07	NA
	72-96	NS	NA
	Subtotal	0.18 ± 0.04	NA
Urine	Predose	0.00 ± 0.00	0.00 ± 0.00
	0-8	2.26 ± 0.34	4.41 ± 3.83[a]
	8-24	1.36 ± 0.12	3.56 ± 3.75[b]
	24-48	1.06 ± 0.10	0.53 ± 0.16
	48-72	0.80 ± 0.05	0.24 ± 0.06
	72-96	NS	0.18 ± 0.02
	Subtotal	5.48 ± 0.38	8.92 ± 0.28
Feces	Predose	0.00 ± 0.00	0.00 ± 0.00
	0-24	4.00 ± 3.12	0.27 ± 0.24
	24-48	1.42 ± 0.44	0.13 ± 0.09
	48-72	1.40 ± 0.18	0.05 ± 0.01
	72-96	NS	0.08 ± 0.03
	Subtotal	6.82 ±3.59	0.52 ± 0.21
Cage wash	0-72	0.22 ± 0.08	NA
	0-96	NA	0.44 ± 0.13
Carcass	72	54.26 ± 12.26	NA
Total Recovered	NA	66.96 ± 9.66	9.89 ± 0.48

Abbreviations: BDC = bile duct-cannulated; M = male; WH = Wistar Han; P.O. = per oral; NA = not applicable; NS = no sample collected; hr = hour; MOV = molnupiravir.
Values represent mean ± standard deviation.
[a] Samples were collected from 0-7 hr.
[b] Samples were collected from 7-24 hr.

Sec. 2.6.5.20 Pharmacokinetics: Identification of Enzymes Involved in the Metabolism of MOV and NHC

Sec. 2.6.5.20.1 Metabolism of MOV in Liver Microsomes

Test Article: MOV
Report Number: [Ref. 4.2.2.4: PK085MK4482]

Study System	*In vitro*
Species/Matrix	Rat, dog, monkey, and human liver microsomes
Method	[^{14}C]MOV (10 µM) was incubated with liver microsomes (0.5 mg/mL) from rat, dog, monkey, and human at 37°C for 1 hr in the presence or absence of NADPH (1 mM) with or without 0.1 mM PMSF (CES inhibitor). At the end of incubation, samples were quenched with equal amount of acetonitrile followed by centrifugation. The supernatants were analyzed by LC-MS/MS with on-line radiometric detection.
Analyte	MOV and metabolites
Assays	LC-MS/MS with on-line radiometric detection

Percent of Radioactivity of Prominent Metabolites Detected

Metabolite	Rat			Dog			Monkey			Human		
	-NADPH	+NADPH	+PMSF[a]	-NADPH	+NADPH	+PMSF[a]	-NADPH	+NADPH	+PMSF[a]	-NADPH	+NADPH	+PMSF[a]
MOV	8	-	72	2	-	78	1	3	13	39	51	90
NHC	85	86	21	87	90	16	92	90	80	57	44	6
Unknown	4	4	-	-	-	-	-	-	-	-	-	-

Abbreviations: hr = hour; CES = carboxylesterase; LC-MS/MS = liquid chromatography-tandem mass spectrometry, MOV = molnupiravir; NHC = N-hydroxycytidine; NADPH = reduced nicotinamide adenine dinucleotide phosphate; PMSF = phenylmethylsulfonyl fluoride; - = Not detected by radiometric detection.
Values represent n=1 incubation.
[a] [^{14}C]MOV was incubated with liver microsomes containing 0.1 mM PMSF (CES inhibitor) in the presence of NADPH

Sec. 2.6.5.20 Pharmacokinetics: Identification of Enzymes Involved in the Metabolism of MOV and NHC (Cont.)

Sec. 2.6.5.20.2 Hydrolysis of MOV in HIM, HIS9, HLM, HLS9 and Human Recombinant CES Isoforms

Test Article: MOV

Report Number: [Ref. 4.2.2.4: PK085MK4482]

Type of Study	CES Phenotyping					
Methods	MOV (1 µM) was incubated with pooled HLM, HLS9, HIM and HIS9 (1 mg/mL) in the presence and absence of arachidonic acid (CES1 inhibitor) and loperamide (CES2 inhibitor) at 37°C for up to 90 min. MOV (1 µM) was also incubated with human recombinant CES1 and CES2 from bactosomes at 1 mg/mL in 0.1 M of phosphate buffer at 37°C for up to 90 min. Parent disappearance and formation of NHC were monitored by LC-MS/MS.					
Analytical Method	LC-MS/MS					
Inhibitor	$CL_{int,\ unscaled}$[a] (µL/min/mg)					
	HLM	HLS9	HIM	HIS9	rCES1	rCES2
-	33.3	5.7	34.3	16.6	1.0	21.9
Loperamide (50 µM)	17.6	2.3	3.3	1.2	NA	NA
Arachidonic Acid (20 µM)	17.7	2.2	18.8	7.1	NA	NA

Abbreviations: rCES = recombinant carboxylesterase; CL_{int} = intrinsic clearance; HIM = human intestinal microsomes, HIS9 = human intestinal S9; HLM = human liver microsomes; HLS9 = human liver S9; LC-MS/MS = liquid chromatography- tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NA = not applicable;
- = no inhibitor.

[a] Unscaled CL_{int} was calculated using the parent substrate depletion approach.

Sec. 2.6.5.20 Pharmacokinetics: Identification of Enzymes Involved in the Metabolism of MOV and NHC (Cont.)

Sec. 2.6.5.20.3 Kinetic Characterization of CES1 and CES2 – Hydrolysis of MOV

Test Article: MOV
Report Number: [Ref. 4.2.2.4: PK085MK4482]

Type of Study	Enzyme Kinetic study	
Methods	MOV (5-1000 µM) was incubated with human recombinant CES1 (1 mg/mL) or CES2 (0.1 mg/mL) in 0.1 M of phosphate buffer at 37°C for 30 min. Formation of NHC were monitored by LC-MS/MS.	
Analytical Method	LC-MS/MS	
Kinetic Parameter	CES1	CES2
V_{max} (pmol/min/mg protein) [a]	>2,000	>25,000
K_m (µM)	>1,000	>1,000

Abbreviations: CES = carboxylesterase; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; V_{max} = maximal rate; K_m = michaelis constant.
[a] V_{max} values represent minimum values estimated with 1000 µM, the highest concentration tested.
Additional information: the rates of NHC formation were linear with both CES1 and CES1 at up to 1000 µM, therefore K_m is more than 1000 µM, the highest concentration tested.

Sec. 2.6.5.20 Pharmacokinetics: Identification of Enzymes Involved in the Metabolism of MOV and NHC (Cont.)

Sec. 2.6.5.20.4 Metabolism of NHC by Cytidine Deaminase

Test Article: NHC
Report Number: [Ref. 4.2.2.4: PK085MK4482]

Type of Study	Reaction phenotyping	
Methods	[^{14}C]NHC was generated by incubation of [^{14}C]MOV (10 µM) with monkey liver microsomes (0.5 mg/mL) at 37°C for 60 min. The extracted samples were further incubated with cytidine deaminase at 5 or 50 µg/mL 37°C for 20 min. Metabolite profile was analyzed by LC-MS/MS with radiometric detection.	
Analytical Method	LC-MS/MS with online radiometric detection	
Cytidine Deaminase (µg/mL)	Percent of Radioactivity of Metabolites Detected	
	NHC	Uridine
-	87	4
5	3	89
50	0	91

Abbreviations: LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; - = no enzyme.
Additional information: In the presence of cytidine deaminase (5 or 50 µg/mL), almost all of NHC (approximately 90%) was converted to uridine, no other metabolite was detected.

Sec. 2.6.5.21 Pharmacokinetics: Reversible Inhibition of Human CYP Isoforms

Sec. 2.6.5.21.1 Reversible Inhibition of Human CYP Isoforms

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK080MK4482]

Type of Study	Reversible inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by MOV or NHC			
Methods	Individual probe substrate of CYP enzymes was incubated with pooled human liver microsomes at 0.25 mg/mL in the presence of NADPH regenerating system with MOV or NHC (0.13-100 µM) or positive control inhibitors for the required incubation time in 100 mM potassium phosphate buffer, pH 7.4.			
Analytical Method	LC-MS/MS			
CYP Isoform	Probe Reaction	IC_{50} (µM)		
		Control Inhibitor	MOV	NHC
1A2	Phenacetin *O*-Deethylation	0.0100 ± 0.0006 α-Naphthoflavone	>100	>100
2B6	Bupropion Hydroxylation	0.483 ± 0.033 Ticlopidine	>100	>100
2C8	Amodiaquine *N*-Deethylation	0.242 ± 0.014 Montelukast	>100	>100
2C9	Diclofenac 4-Hydroxylation	0.790 ± 0.061 Sulfaphenazole	>100	>100
2C19	Mephenytoin 4′-Hydroxylation	0.202 ± 0.005 (S)-(+)-N-3-Benzylnirvanol	>100	>100
2D6	Dextromethorphan *O*-Demethylation	0.084 ± 0.007 Quinidine	>100	>100
3A4	Midazolam 1′-Hydroxylation	0.021 ± 0.002 Ketoconazole	>100	>100
3A4	Testosterone 6β-Hydroxylation	0.036 ± 0.002 Ketoconazole	>100	>100

Abbreviations: CYP = cytochrome P450; NADPH = reduced nicotinamide adenine dinucleotide phosphate; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; IC_{50} = concentration required for 50% inhibition.
IC_{50} values represent means (n=3 incubations).

Sec. 2.6.5.21 Pharmacokinetics: Reversible Inhibition of Human CYP Isoforms (Cont.)

Sec. 2.6.5.21.1 Reversible Inhibition of Human CYP Isoforms (Cont.)

Test Article: MOV and NHC

Report Number: [Ref. 4.2.2.6: PK088MK4482] [Ref. 4.2.2.6: PK089MK4482]

Type of Study	Reversible inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by MOV or NHC			
Methods	Individual probe substrate of CYP enzymes was incubated with pooled human liver microsomes at 0.25 mg/mL in the presence of NADPH regenerating system with MOV or NHC (1.29-1000 µM) or positive control inhibitors for the required incubation time in 100 mM potassium phosphate buffer, pH 7.4.			
Analytical Method	LC-MS/MS			
CYP Isoform	Probe Reaction	IC_{50} (µM)		
		Control Inhibitor	MOV	NHC
1A2	Phenacetin *O*-Deethylation	0.0084 ± 0.0006 α-Naphthoflavone	>1000	>1000
2B6	Bupropion Hydroxylation	0.329 ± 0.021 Ticlopidine	>1000	>1000
2C8	Amodiaquine *N*-Deethylation	0.152 ± 0.0088 Montelukast	>1000	>1000
2C9	Diclofenac 4-Hydroxylation	0.778 ± 0.108 Sulfaphenazole	>1000	>1000
2C19	Mephenytoin 4′-Hydroxylation	0.201 ± 0.0098 (S)-(+)-N-3-Benzylnirvanol	>1000	>1000
2D6	Dextromethorphan *O*-Demethylation	0.09474 ± 0.0077 Quinidine	>1000	>1000
3A4	Midazolam 1′-Hydroxylation	0.0309 ± 0.0024 Ketoconazole	>1000	>1000
3A4	Testosterone 6β-Hydroxylation	0.0364 ± 0.0018 Ketoconazole	>1000	>1000

Abbreviations: CYP = cytochrome P450; NADPH = reduced nicotinamide adenine dinucleotide phosphate; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; IC_{50} = concentration required for 50% inhibition.
IC_{50} values represent means (n=3 incubations).
Additional information: Risk assessment of MOV or NHC as a perpetrator of CYP-Mediated Drug Interactions, based on the 2020 FDA, 2012 EMA and 2018 MHLW guidance, is provided in [Sec. 2.6.5: 22], [Sec. 2.6.5: 23], [Sec. 2.6.5: 24], [Sec. 2.6.5: 25], [Sec. 2.6.5: 26] and [Sec. 2.6.5: 27], respectively.

Sec. 2.6.5.22 Pharmacokinetics: Drug-Drug Interaction, Enzyme Inhibition by MOV (FDA)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK088MK4482]

Summary Results: Assessment of risk for *in vivo* DDI's between MOV and co-administered substrates of various enzymes based on the FDA guidance (2020)[a]								
Enzyme	**Model**	**IC_{50} (µM)**	**$K_{i,u}$[b] (µM)**	**$I_{max,u}$[c] (µM)**	**I_{gut}[d] (µM)**	**R_1[e]**	**$R_{1,gut}$[f]**	**Threshold Cutoff For Potential DDI**
Systemic								
CYP1A2	Basic	>1000	>1000	0.03	-	<1.00	-	$R_1 \geq 1.02$
CYP2B6	Basic	>1000	>1000	0.03	-	<1.00	-	$R_1 \geq 1.02$
CYP2C8	Basic	>1000	>1000	0.03	-	<1.00	-	$R_1 \geq 1.02$
CYP2C9	Basic	>1000	>1000	0.03	-	<1.00	-	$R_1 \geq 1.02$
CYP2C19	Basic	>1000	>1000	0.03	-	<1.00	-	$R_1 \geq 1.02$
CYP2D6	Basic	>1000	>1000	0.03	-	<1.00	-	$R_1 \geq 1.02$
CYP3A4/midazolam	Basic	>1000	>1000	0.03	-	<1.00	-	$R_1 \geq 1.02$
CYP3A4/testosterone	Basic	>1000	>1000	0.03	-	<1.00	-	$R_1 \geq 1.02$
Intestinal								
CYP3A4/midazolam	Basic	>1000	>1000	-	9717	-	<11	$R_{1,gut} \geq 11$
CYP3A4/testosterone	Basic	>1000	>1000	-	9717	-	<11	$R_{1,gut} \geq 11$

Abbreviations: DDI = drug-drug interaction; MOV = molnupiravir; FDA = Food and Drug Administration; CYP = cytochrome P450; IC_{50} = concentration required for 50% inhibition; $I_{max,u}$ = maximum unbound plasma concentration; I_{gut} = theoretical maximum gastrointestinal drug concentration calculated as the dose/250mL; $K_{i,u}$ = unbound inhibition constant; $f_{u,mic}$ = unbound fraction in the microsomal system; $f_{u,p}$ = unbound fraction in plasma; K_i = inhibition constant; C_{max} = maximum concentration; BID = twice daily; R_1 = ratio of intrinsic clearance values of a probe substrate for an enzymatic pathway in the absence and in the presence of the interacting drug in cases of reversible inhibition; - = not applicable.

[a] *In Vitro* Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug-Drug Interaction Studies Guidance for Industry. Issued by: U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER), Jan 2020.

[b] $K_{i,u} = IC_{50} \times f_{u,mic}$, assumed $f_{u,mic} = 1$.

[c] $I_{max,u} = C_{max} \times f_{u,p}$ where MOV C_{max} = 0.026 µM with 800 mg MOV dose BID after the final dose on Day 6 (P004) and assumed $f_{u,p} = 1$.

[d] I_{gut} = dose/250 mL = 800 mg/250 mL = 9717 µM (MW=329.31).

[e] $R_1 = 1 + (I_{max,u} / K_{i,u})$.

[f] $R_{1,gut} = 1 + (I_{gut} / K_{i,u})$.

Sec. 2.6.5.23 Pharmacokinetics: Drug-Drug Interaction, Enzyme Inhibition by MOV (EMA)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK088MK4482]

Summary Results: Assessment of risk for *in vivo* DDI's between MOV and co-administered substrates of various transporters based on the EMA guidance (2012)[a]								
Enzyme	**Model**	**IC_{50} (µM)**	**Ki[b] (µM)**	**[I][c] (µM)**	**$[I]_{gut}$ [d] (µM)**	**[I]/Ki**	**$[I]_{gut}$/Ki**	**Threshold Cutoff For Potential DDI**
Systemic								
CYP1A2	Basic	>1000	>1000	0.03	-	<0.00	-	[I]/Ki≥0.02
CYP2B6	Basic	>1000	>1000	0.03	-	<0.00	-	[I]/Ki≥0.02
CYP2C8	Basic	>1000	>1000	0.03	-	<0.00	-	[I]/Ki≥0.02
CYP2C9	Basic	>1000	>1000	0.03	-	<0.00	-	[I]/Ki≥0.02
CYP2C19	Basic	>1000	>1000	0.03	-	<0.00	-	[I]/Ki≥0.02
CYP2D6	Basic	>1000	>1000	0.03	-	<0.00	-	[I]/Ki≥0.02
CYP3A4/midazolam	Basic	>1000	>1000	0.03	-	<0.00	-	[I]/Ki≥0.02
CYP3A4/testosterone	Basic	>1000	>1000	0.03	-	<0.00	-	[I]/Ki≥0.02
Intestinal								
CYP3A4/midazolam	Basic	>1000	>1000	-	9717	-	<10	$[I]_{gut}$ /Ki≥10
CYP3A4/testosterone	Basic	>1000	>1000	-	9717	-	<10	$[I]_{gut}$/Ki≥10

Abbreviations: DDI = drug-drug interaction; MOV = molnupiravir; EMA = European Medicines Agency; IC_{50} = concentration required for 50% inhibition; $f_{u,mic}$ = unbound fraction in the microsomal system; $f_{u,p}$ = unbound fraction in plasma; [I] = maximum unbound plasma concentration; $[I]_{gut}$ = theoretical maximum gastrointestinal drug concentration calculated as the dose/250mL; BID = twice daily; Ki = inhibition constant; Cmax = maximum concentration; - = not applicable.

[a] Committee for Human Medicinal Products (CHMP). Guideline on the investigation of drug interactions. In: CPMP/EWP/560/95/Rev. 1 Corr. 2. London, England: European Medicines Agency; 2012.

[b] $Ki = IC_{50} \times f_{u,mic}$ assumed $f_{u,mic} = 1$.

[c] $[I] = Cmax \times f_{u,p}$ where MOV C_{max} = 0.026 µM with 800 mg MOV dose BID after the final dose on Day 6 (P004) and assumed $f_{u,p} = 1$.

[d] $[I]_{gut}$ = dose/250 mL = 800 mg/250 mL = 9717 µM (MW=329.31).

Sec. 2.6.5.24 Pharmacokinetics: Drug-Drug Interaction, Enzyme Inhibition by MOV (MHLW)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK088MK4482]

Summary Results: Assessment of risk for *in vivo* DDI's between MOV and co-administered substrates of various enzymes based on the MHLW guidance (2018)[a]								
Enzyme	**Model**	**IC_{50} (µM)**	**K_i[b] (µM)**	**[I][c] (µM)**	**$[I]_g$[d] (µM)**	**R[e]**	**R_g[f]**	**Threshold Cutoff For Potential DDI**
Systemic								
CYP1A2	Basic	>1000	>1000	0.03	-	<1.00	-	R ≥1.02
CYP2B6	Basic	>1000	>1000	0.03	-	<1.00	-	R ≥1.02
CYP2C8	Basic	>1000	>1000	0.03	-	<1.00	-	R ≥1.02
CYP2C9	Basic	>1000	>1000	0.03	-	<1.00	-	R≥1.02
CYP2C19	Basic	>1000	>1000	0.03	-	<1.00	-	R≥1.02
CYP2D6	Basic	>1000	>1000	0.03	-	<1.00	-	R≥1.02
CYP3A4/midazolam	Basic	>1000	>1000	0.03	-	<1.00	-	R≥1.02
CYP3A4/testosterone	Basic	>1000	>1000	0.03	-	<1.00	-	R≥1.02
Intestinal								
CYP3A4/midazolam	Basic	>1000	>1000	-	9717	-	<11	R_g≥11
CYP3A4/testosterone	Basic	>1000	>1000	-	9717	-	<11	R_g≥11

Abbreviations: DDI = drug-drug interaction; MOV = molnupiravir; MHLW = Ministry of Health, Labour and Welfare; IC_{50} = concentration required for 50% inhibition; $f_{u,mic}$ = unbound fraction in the microsomal system; $f_{u,p}$ = unbound fraction in plasma; [I] = maximum unbound plasma concentration; $[I]_g$ = theoretical maximum gastrointestinal drug concentration calculated as the dose/250mL; R = the ratio of intrinsic clearance value of the substrate under the presence and absence of the investigational drug for specific enzyme reaction; Ki = unbound inhibition constant; BID = twice daily; C_{max} = maximum concentration; - = not applicable.

[a] Guideline on drug interaction for drug development and appropriate provision of information. Issued by Ministry of Health, Labour and Welfare, Jul 2018.

[b] $K_i = IC_{50} \times f_{u,mic}$ assumed $f_{u,mic} = 1$.

[c] $[I] = C_{max} \times f_{u,p}$ where MOV C_{max} = 0.026 µM with 800 mg MOV dose BID after the final dose on Day 6 (P004) and assumed $f_{u,p} = 1$.

[d] $[I]_g$ = dose/250 mL = 800 mg/250 mL = 9717 µM (MW=329.31).

[e] $R = 1 + ([I]/K_i)$.

[f] $R_g = 1 + ([I]_g/K_i)$.

Sec. 2.6.5.25 Pharmacokinetics: Drug-Drug Interaction, Enzyme Inhibition by NHC (FDA)

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK089MK4482]

Summary Results: Assessment of risk for *in vivo* DDI's between NHC and co-administered substrates of various enzymes, based on the FDA guidance (2020)[a]								
Enzyme	**Model**	**IC_{50} (µM)**	**$K_{i,u}$[b] (µM)**	**$I_{max,u}$[c] (µM)**	**I_{gut}[d] (µM)**	**R_1[e]**	**$R_{1,gut}$[f]**	**Threshold Cutoff for Potential DDI**
Systemic								
CYP1A2	Basic	>1000	>1000	10.8	-	<1.01	-	$R_1 \geq 1.02$
CYP2B6	Basic	>1000	>1000	10.8	-	<1.01	-	$R_1 \geq 1.02$
CYP2C8	Basic	>1000	>1000	10.8	-	<1.01	-	$R_1 \geq 1.02$
CYP2C9	Basic	>1000	>1000	10.8	-	<1.01	-	$R_1 \geq 1.02$
CYP2C19	Basic	>1000	>1000	10.8	-	<1.01	-	$R_1 \geq 1.02$
CYP2D6	Basic	>1000	>1000	10.8	-	<1.01	-	$R_1 \geq 1.02$
CYP3A4/midazolam	Basic	>1000	>1000	10.8	-	<1.01	-	$R_1 \geq 1.02$
CYP3A4/testosterone	Basic	>1000	>1000	10.8	-	<1.01	-	$R_1 \geq 1.02$
Intestinal								
CYP3A4/midazolam	Basic	>1000	>1000	-	9717	-	<11	$R_{1,gut} \geq 11$
CYP3A4/testosterone	Basic	>1000	>1000	-	9717	-	<11	$R_{1,gut} \geq 11$

Abbreviations: DDI = drug-drug interaction; FDA = Food and Drug Administration; MOV = molnupiravir; NHC = N-hydroxycytidine; IC_{50} = concentration required for 50% inhibition; $f_{u,mic}$ = unbound fraction in the microsomal system; $f_{u,p}$ = unbound fraction in plasma; $I_{max,u}$ = maximum unbound plasma concentration; I_{gut} = theoretical maximum gastrointestinal drug concentration calculated as the dose/250mL; $K_{i,u}$ = unbound inhibition constant; K_i = inhibition constant; C_{max} = maximum concentration; R_1 = ratio of intrinsic clearance values of a probe substrate for an enzymatic pathway in the absence and in the presence of the interacting drug in cases of reversible inhibition; - = not applicable.

[a] *In Vitro* Drug Interaction Studies - Cytochrome P450 Enzyme - and Transporter-Mediated Drug-Drug Interaction Studies Guidance for Industry. Issued by: U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER), Jan 2020.

[b] $K_{i,u} = IC_{50} \times f_{u,mic}$ assumed $f_{u,mic} = 1.0$.

[c] $I_{max,u} = C_{max} \times f_{u\,p}$ where the NHC C_{max} = 10.8 µM based on Pop PK analysis from P002 with 800 mg dose of MOV and $f_{u\,p} = 1.0$.

[d] I_{gut} = dose/250 mL, assumed (worst case scenario) 100% conversion of MOV dose to NHC in the gut = 800 mg/250 mL = 9717 µM (MW = 329.31 for MOV).

[e] $R_1 = 1 + (I_{max,u}/K_{i,u})$.

[f] $R_{1,gut} = 1 + (I_{gut}/K_{i,u})$.

Sec. 2.6.5.26 Pharmacokinetics: Drug-Drug Interaction, Enzyme Inhibition by NHC (EMA)

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK089MK4482]

Summary Results: Assessment of risk for *in vivo* DDI's between NHC and co-administered substrates of various transporters based on the EMA guidance (2012)[a]								
Enzyme	**Model**	**IC_{50} (µM)**	**Ki[b] (µM)**	**[I][c] (µM)**	**$[I]_{gut}$ [d] (µM)**	**[I]/Ki**	**$[I]_{gut}/K_i$**	**Threshold Cutoff For Potential DDI**
Systemic								
CYP1A2	Basic	>1000	>1000	10.8	-	<0.01	-	$[I]/K_i \geq 0.02$
CYP2B6	Basic	>1000	>1000	10.8	-	<0.01	-	$[I]/K_i \geq 0.02$
CYP2C8	Basic	>1000	>1000	10.8	-	<0.01	-	$[I]/K_i \geq 0.02$
CYP2C9	Basic	>1000	>1000	10.8	-	<0.01	-	$[I]/K_i \geq 0.02$
CYP2C19	Basic	>1000	>1000	10.8	-	<0.01	-	$[I]/K_i \geq 0.02$
CYP2D6	Basic	>1000	>1000	10.8	-	<0.01	-	$[I]/K_i \geq 0.02$
CYP3A4/midazolam	Basic	>1000	>1000	10.8	-	<0.01	-	$[I]/K_i \geq 0.02$
CYP3A4/testosterone	Basic	>1000	>1000	10.8	-	<0.01	-	$[I]/K_i \geq 0.02$
Intestinal								
CYP3A4/midazolam	Basic	>1000	>1000	-	9717	-	<10	$[I]_{gut}/K_i \geq 10$
CYP3A4/testosterone	Basic	>1000	>1000	-	9717	-	<10	$[I]_{gut}/K_i \geq 10$

Abbreviations: DDI = drug-drug interaction; MOV = molnupiravir; NHC = N-hydroxycytidine; EMA = European Medicines Agency; IC_{50} = concentration required for 50% inhibition; $f_{u,mic}$ = unbound fraction in the microsomal system; $f_{u,p}$ = unbound fraction in plasma; [I] = maximum unbound plasma concentration; $[I]_{gut}$ = theoretical maximum gastrointestinal drug concentration calculated as the dose/250mL; Ki = inhibition constant; Cmax = maximum concentration; - = not applicable.

[a] Committee for Human Medicinal Products (CHMP). Guideline on the investigation of drug interactions. In: CPMP/EWP/560/95/Rev. 1 Corr. 2. London, England: European Medicines Agency; 2012.

[b] $K_i = IC_{50} \times f_{u,mic}$ assumed $f_{u,mic} = 1$

[c] $[I] = Cmax \times f_{u,p}$ where the NHC C_{max} = 10.8 µM based on Pop PK analysis from P002 with 800 mg dose of MOV and $f_{u\,p}$ = 1.0.

[d] $[I]_{gut}$ = dose/250 mL, assumed (worst case scenario) 100% conversion of MOV dose to NHC in the gut = 800 mg/250 mL = 9717 µM (MW = 329.31 for MOV).

Sec. 2.6.5.27 Pharmacokinetics: Drug-Drug Interaction, Enzyme Inhibition by NHC (MHLW)

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK089MK4482]

Summary Results: Assessment of risk for in vivo DDI's between NHC and co-administered substrates of various enzymes based on the MHLW guidance (2018)[a]								
Enzyme	**Model**	**IC_{50} (µM)**	**K_i[b] (µM)**	**[I][c] (µM)**	**$[I]_g$[d] (µM)**	**R[e]**	**R_g[f]**	**Threshold Cutoff for Potential DDI**
Systemic								
CYP1A2	Basic	>1000	>1000	10.8	-	<1.01	-	R ≥1.02
CYP2B6	Basic	>1000	>1000	10.8	-	<1.01	-	R ≥1.02
CYP2C8	Basic	>1000	>1000	10.8	-	<1.01	-	R ≥1.02
CYP2C9	Basic	>1000	>1000	10.8	-	<1.01	-	R ≥1.02
CYP2C19	Basic	>1000	>1000	10.8	-	<1.01	-	R≥1.02
CYP2D6	Basic	>1000	>1000	10.8	-	<1.01	-	R ≥1.02
CYP3A4/midazolam	Basic	>1000	>1000	10.8	-	<1.01	-	R≥1.02
CYP3A4/testosterone	Basic	>1000	>1000	10.8	-	<1.01	-	R≥1.02
Intestinal								
CYP3A4/midazolam	Basic	>1000	>1000	-	9717	-	<11	R_g≥11
CYP3A4/testosterone	Basic	>1000	>1000	-	9717	-	<11	R_g≥11

Abbreviations: DDI = drug-drug interaction; MOV = molnupiravir; NHC = N-hydroxycytidine; MHLW = Ministry of Health, Labour and Welfare; IC_{50} = concentration required for 50% inhibition; $f_{u,mic}$ = unbound fraction in the microsomal system; $f_{u,p}$ = unbound fraction in plasma; [I] = maximum unbound plasma concentration; $[I]_g$ = theoretical maximum gastrointestinal drug concentration calculated as the dose/250mL; R = the ratio of intrinsic clearance value of the substrate under the presence and absence of the investigational drug for specific enzyme reaction; K_i = unbound inhibition constant; Cmax = maximum concentration, - = not applicable.

[a] Guideline on drug interaction for drug development and appropriate provision of information. Issued by Ministry of Health, Labour and Welfare, Jul 2018.

[b] $K_i = IC_{50} \times f_{u,mic}$ assumed $f_{u,mic} = 1$ for NHC.

[c] $[I] = C_{max} \times f_{u,p}$ where the NHC C_{max} = 10.8 µM based on Pop PK analysis from P002 with 800 mg dose of MOV and $f_{u\,p} = 1.0$.

[d] $[I]_g$ = dose/250 mL, assumed (worst case scenario) 100% conversion of MOV dose to NHC in the gut = 800 mg/250 mL = 9717 µM (MW = 329.31 for MOV).

[e] $R = 1 + ([I]/K_i)$.

[f] $R_g = 1 + ([I]_g/K_i)$.

Sec. 2.6.5.28 Pharmacokinetics: Time-Dependent Inhibition of Human CYP Isoforms

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK079MK4482]

Type of Study	Time-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A by MOV and NHC in human liver microsomes						
Methods	Probe substrates of CYP isoforms were incubated in pooled human liver microsomes enriched with NADPH in the presence of MOV or NHC (10 and 50 µM) or the positive control inhibitors to assess the potential of TDI for CYP isoforms						
Analytical Method	LC-MS/MS						
CYP Isoform	K_{obs} (min^{-1})						
	Negative control inhibitor (methanol)	**Positive control inhibitor**		**MOV (10 µM)**	**MOV (50 µM)**	**NHC (10 µM)**	**NHC (50 µM)**
CYP1A2	0.0136	Furafyline	0.0258 (0.5 µM) 0.0909 (8 µM)	0.0145	0.0108	0.0126	0.0154
CYP2B6	0.0092	Thio-TEPA	0.0266 (9.4 µM) 0.0327 (150 µM)	0.0089	0.0094	0.0084	0.0098
CYP2C8	0.0040	Gemfibrozil glucuronide	0.0029 (3.1 µM) 0.0460 (50 µM)	0.0089	0.0094	0.0000[c]	0.0078
CYP2C9	0.0028	Tienilic Acid	0.0426 (1.3 µM) 0.1146 (20 µM)	0.0000	0.0000[c]	0.0001	0.0000[c]
CYP2C19	0.0012	S-Fluoxetine	0.0013 (16 µM) 0.0336 (250 µM)	0.0031	0.0000[c]	0.0040	0.0000[c]
CYP2D6	0.0089	Paroxetine	0.0243 (0.63 µM) 0.0968 (10 µM)	0.0119	0.0084	0.0102	0.0112
CYP3A[a]	0.0101	Erythromycin	0.0252 (3 µM) 0.0422 (48 µM)	0.0113	0.0102	0.0116	0.0137
CYP3A[b]	0.0100	Erythromycin	0.0077 (3 µM) 0.0360 (48 µM)	0.0091	0.0000[c]	0.0104	0.0106

Abbreviations: CYP = cytochrome P450; NADPH = reduced nicotinamide adenine dinucleotide phosphate; LC-MS/MS = liquid chromatography-tandem mass spectrometry; K_{obs} = observed rate constant; MOV = molnupiravir; NHC = N-hydroxycytidine; TDI = time-dependent inhibition.
K_{obs} values represent means (n=3 incubations).
[a] Midazolam was used as the probe substrate.
[b] Testosterone was used as the probe substrate.
[c] The calculated line for inactivation exhibited a positive slope; therefore, the K_{obs} value was reported as 0.
Additional Information: The enzyme activities that were used to evaluate MOV and NHC inhibition of CYPs included phenacetin *O*-deethylase (CYP1A2), efavirenz 8'-hydroxylase (CYP2B6), amodiaquine *N*-deethylase (CYP2C8), diclofenac 4'-hydroxylase (CYP2C9), (S)-mephenytoin 4'-hydroxylase (CYP2C19), bufuralol 1'-hydroxylase (CYP2D6), midazolam 1'-hydroxylase (CYP3A), and testosterone 6β-hydroxylase (CYP3A). MOV and NHC was preincubated with human liver microsomes (1 mg/mL for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP3A testosterone; 2 mg/mL for CYP2C19; 0.5 mg/mL for CYP3A midazolam) enriched with NADPH (1 mM) in 100 mM phosphate buffer at 37°C at selected time points from 0 up to 30 min. At each time point, aliquots of the reaction mixture were diluted with the same buffer containing probe substrates. The incubation was continued for an additional time for each specific isoform from 1 up to 25 min at 37°C.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms

Sec. 2.6.5.29.1 Induction of Human CYP1A2 by MOV

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK065MK4482]

Type of Study		CYP1A2 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with MOV (2, 10 and 50 µM), or the positive control inducer omeprazole (50 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot HH1086**		**Lot HH1142**		**Lot HH1144**	
Treatment	**µM**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**
		Fold change[a]					
Omeprazole	50	35.3	26.4	15.1	12.6	29.1	25.5
MOV	2	1.0	0.8	0.8	0.8	1.1	0.8
	10	0.8	0.8	0.8	0.7	0.9	0.8
	50	0.7	0.7	0.9	0.7	1.0	0.7

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, MOV did not induce CYP1A2 mRNA and enzyme activity across the three lots. Omeprazole was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms (Cont.)

Sec. 2.6.5.29.1 Induction of Human CYP1A2 by MOV (Cont.)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK071MK4482]

Type of Study		CYP1A2 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with MOV (0.1 to 20 µM), or the positive control inducer omeprazole (50 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot 312**		**Lot 336**		**Lot 399**	
Treatment	**µM**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**
		Fold change[a]					
Omeprazole	50	39.5	17.2	38.4	17.1	46.6	7.6
MOV	0.1	1.0	1.0	1.0	1.0	1.0	1.0
	0.5	0.9	0.9	1.0	1.2	1.0	0.9
	1	0.9	0.9	1.0	1.2	1.5	0.9
	5	0.9	0.9	0.8	1.0	1.0	0.9
	10	0.7	0.8	0.6	1.0	0.9	0.8
	20	0.8	0.7	0.7	1.0	0.9	0.8

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, MOV did not induce CYP1A2 mRNA and enzyme activity across the three lots. Omeprazole was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms (Cont.)

Sec. 2.6.5.29.2 Induction of Human CYP2B6 by MOV

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK065MK4482]

Type of Study		CYP2B6 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with MOV (2, 10, and 50 µM) or the positive control inducer phenobarbital (1000 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot HH1086**		**Lot HH1142**		**Lot HH1144**	
Treatment	**µM**	**CYP2B6 mRNA**	**Bupropion hydroxylation**	**CYP2B6 mRNA**	**Bupropion hydroxylation**	**CYP2B6 mRNA**	**Bupropion hydroxylation**
		Fold change[a]					
Phenobarbital	1000	15.9	9.9	21.7	12.8	15.2	6.3
MOV	2	0.9	1.0	0.9	0.8	0.8	0.8
	10	0.7	0.8	1.1	0.8	1.0	0.8
	50	0.7	0.8	0.9	0.7	0.8	0.7

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, MOV did not induce CYP2B6 mRNA and enzyme activity across the three lots. Phenobarbital was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms (Cont.)

Sec. 2.6.5.29.2 Induction of Human CYP2B6 by MOV (Cont.)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK071MK4482]

Type of Study		CYP2B6 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with MOV (0.1 to 20 µM) or the positive control inducer phenobarbital (1000 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot 312**		**Lot 336**		**Lot 399**	
Treatment	**µM**	**CYP2B6 mRNA**	**Bupropion hydroxylation**	**CYP2B6 mRNA**	**Bupropion hydroxylation**	**CYP2B6 mRNA**	**Bupropion hydroxylation**
		Fold change[a]					
Phenobarbital	1000	14.4	7.3	11.9	7.6	9.5	13.9
MOV	0.1	1.5	0.9	1.0	1.1	1.1	1.1
	0.5	1.2	1.1	0.8	1.2	0.8	1.0
	1	1.4	1.1	0.9	1.2	1.3	1.0
	5	1.2	1.0	0.7	1.1	0.9	1.0
	10	1.1	1.0	0.5	1.0	0.9	0.9
	20	1.3	1.0	0.6	1.1	0.8	0.9

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, MOV did not induce CYP2B6 mRNA and enzyme activity across the three lots. Phenobarbital was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms (Cont.)

Sec. 2.6.5.29.3 Induction of Human CYP3A4 by MOV

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK065MK4482]

Type of Study		CYP3A4 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with MOV (2, 10, 50 µM), or the positive control inducer rifampicin (20 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot HH1086**		**Lot HH1142**		**Lot HH1144**	
Treatment	**µM**	**CYP3A4 mRNA**	**Testosterone 6*β*-hydroxylation**	**CYP3A4 mRNA**	**Testosterone 6*β*-hydroxylation**	**CYP3A4 mRNA**	**Testosterone 6*β*-hydroxylation**
		Fold change[a]					
Rifampicin	20	231	9.5	238	9.4	281	13.0
MOV	2	1.3	1.0	0.9	1.0	0.8	0.9
	10	1.0	1.0	1.1	1.0	1.0	1.0
	50	1.3	0.9	1.2	0.9	1.5	1.1

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, MOV did not induce CYP3A4 mRNA and enzyme activity across the three lots. Rifampicin was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms (Cont.)

Sec. 2.6.5.29.3 Induction of Human CYP3A4 by MOV (Cont.)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK071MK4482]

Type of Study		CYP3A4 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with MOV (0.1 to 20 µM), or the positive control inducer rifampicin (10 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot 312**		**Lot 336**		**Lot 399**	
Treatment	**µM**	**CYP3A4 mRNA**	**Testosterone 6*β*-hydroxylation**	**CYP3A4 mRNA**	**Testosterone 6*β*-hydroxylation**	**CYP3A4 mRNA**	**Testosterone 6*β*-hydroxylation**
		Fold change[a]					
Rifampicin	10	23.4	11.1	27.7	16.2	27.5	10.7
MOV	0.1	1.2	1.0	1.1	1.1	1.1	1.0
	0.5	1.2	1.0	0.8	1.1	1.0	1.0
	1	1.3	1.0	1.0	0.9	1.6	0.9
	5	1.1	1.0	0.7	0.9	1.0	0.9
	10	1.0	0.9	0.6	0.7	0.8	0.8
	20	1.0	0.8	0.6	0.7	0.8	0.7

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, MOV did not induce CYP3A4 mRNA and enzyme activity across the three lots. Rifampicin was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms (Cont.)

Sec. 2.6.5.29.4 Induction of Human CYP1A2 by NHC

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK072MK4482]

Type of Study		CYP1A2 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with NHC (0.1 to 20 µM), or the positive control inducer omeprazole (50 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot 312**		**Lot 336**		**Lot 399**	
Treatment	**µM**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**	**CYP1A2 mRNA**	**Phenacetin *O*-deethylation**
		Fold change[a]					
Omeprazole	50	39.5	17.2	38.4	17.1	46.6	7.6
NHC	0.1	1.0	0.9	1.0	1.2	1.1	1.0
	0.5	1.0	0.9	1.0	1.1	1.2	1.0
	1	1.0	0.9	1.0	1.2	1.1	1.0
	5	0.8	0.8	0.8	1.0	1.0	0.9
	10	0.9	0.9	0.7	1.2	1.0	1.0
	20	0.8	0.8	0.7	0.9	1.1	1.0

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NHC = N-hydroxycytidine; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, NHC did not induce CYP1A2 mRNA and enzyme activity across the three lots. Omeprazole was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms (Cont.)

Sec. 2.6.5.29.5 Induction of Human CYP2B6 by NHC

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK072MK4482]

Type of Study		CYP2B6 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with NHC (0.1 to 20 µM), or the positive control inducer phenobarbital (1000 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot 312**		**Lot 336**		**Lot 399**	
Treatment	**µM**	**CYP2B6 mRNA**	**Bupropion hydroxylation**	**CYP2B6 mRNA**	**Bupropion hydroxylation**	**CYP2B6 mRNA**	**Bupropion hydroxylation**
		Fold change[a]					
Phenobarbital	1000	14.4	7.3	11.9	7.6	9.5	13.9
NHC	0.1	1.2	1.0	1.0	1.1	1.0	1.0
	0.5	1.1	1.0	1.1	1.1	1.0	1.1
	1	1.0	0.9	1.0	1.1	0.9	1.0
	5	1.1	1.0	0.8	1.1	0.9	1.0
	10	1.3	1.0	0.7	1.1	0.8	1.0
	20	1.2	1.1	1.0	1.0	0.9	1.0

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NHC = N-hydroxycytidine; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, NHC did not induce CYP2B6 mRNA and enzyme activity across the three lots. Phenobarbital was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.29 Pharmacokinetics: Induction of Human CYP Isoforms (Cont.)

Sec. 2.6.5.29.6 Induction of Human CYP3A4 by NHC

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK072MK4482]

Type of Study		CYP3A4 Induction					
Methods		Plated cryopreserved human hepatocytes from three donors were incubated with NHC (0.1 to 20 µM), or the positive control inducer rifampicin (10 µM) for 48 hr.					
Analytical Methods		mRNA quantitation by real time PCR and enzyme activity by LC-MS/MS					
Donor ID		**Lot 312**		**Lot 336**		**Lot 399**	
Treatment	**µM**	**CYP3A4 mRNA**	**Testosterone 6β-hydroxylation**	**CYP3A4 mRNA**	**Testosterone 6β-hydroxylation**	**CYP3A4 mRNA**	**Testosterone 6β-hydroxylation**
		Fold change[a]					
Rifampicin	10	23.4	11.1	27.7	16.2	27.5	10.7
NHC	0.1	1.4	1.0	1.0	1.0	1.1	1.2
	0.5	1.3	1.1	1.2	1.0	1.1	1.2
	1	1.3	1.1	0.9	0.8	1.1	1.1
	5	1.0	1.1	0.7	0.7	0.9	0.9
	10	1.2	0.9	0.6	0.7	0.9	0.9
	20	1.1	0.9	0.7	0.7	0.9	1.0

Abbreviations: CYP = cytochrome P450; hr = hour; mRNA = messenger ribonucleic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NHC = N-hydroxycytidine; PCR = polymerase chain reaction.
[a] Fold change represents the mean fold change of treated samples compare to vehicle control samples (n=3).
Additional Information: Under the condition tested, NHC did not induce CYP3A4 mRNA and enzyme activity across the three lots. Rifampicin was used as the positive control, confirming the functionality of the assay.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters

Sec. 2.6.5.30.1 Pharmacokinetics: *In Vitro* Permeability of MOV and NHC Across Caco-2 Cells

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.2: PK010MK4482]

Type of Study	Permeability				
Methods	*In vitro* bi-directional permeability assessment of MOV and NHC across Caco-2 cell monolayers				
Analyte	MOV and NHC				
Analytical Method	LC-MS/MS				
Compound	**Inhibitor (µM)**	**P_{app} (10^{-6} cm/s)**			**B-A/A-B efflux ratio**
		A-B	**B-A**	**Mean[a]**	
MOV (10 µM)	-	0.365	0.222	0.294	0.609
	Haloperidol (100)	0.349	0.330	0.340	0.945
	Ko134 (10)	0.284	0.253	0.269	0.891
	MK571 (20)	0.573	0.455	0.514	0.793
NHC (10 µM)	-	1.29	0.915	1.10	0.711
	Haloperidol (100)	1.76	0.820	1.29	0.465
	Ko134 (10)	0.992	0.615	0.804	0.620
	MK571 (20)	1.71	0.631	1.17	0.369
Digoxin (10 µM)	-	2.27	28.2	ND	12.4
	Haloperidol (100)	3.72	21.2	ND	5.71
Rosuvastatin (10 µM)	-	0.228	13.0	ND	56.9
	Ko134 (10)	0.769	4.63	ND	6.02
Saquinavir (10 µM)	-	0.875	17.5	ND	19.9
	MK571 (20)	0.935	8.23	ND	8.80

Abbreviations: A-B = Apical - basolateral; B-A = Basolateral - apical; Caco-2 = colorectal adenocarcinoma cells; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; P_{app}= apparent permeability; ND = not determined; - = without inhibitor; BCRP = breast cancer resistance protein; MRP2 = multi-drug resistance protein 2; Ko134 = tetracyclic fumitremorgin C; P-gp = P-glycoprotein.
[a] Average of (A-B) and (B-A) P_{app} values calculated as measure of passive permeability, in the absence of efflux transport.
Values represent means (n =3).
Additional information: Digoxin (P-gp), rosuvastatin (BCRP), and saquinavir (MRP2) were used as positive controls and were inhibited by positive control inhibitors, haloperidol (P-gp inhibitor), Ko134 (BCRP inhibitor) or MK571 (MRP2 inhibitor), confirmed the functionality of the assay.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters (Cont.)

Sec. 2.6.5.30.2 Pharmacokinetics: *In Vitro* Permeability of MOV and NHC Across LLC-PK1 Cells

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK084MK4482]

Type of Study	Permeability		
Methods	*In vitro* bi-directional permeability assessment of [^{14}C]MOV and NHC across LLC-PK1 cell monolayers		
Analyte	[^{14}C]MOV and NHC		
Analytical Method	LC-MS/MS and LSC		
Compound	**Concentration (µM)**	**B-A/A-B efflux ratio**	**Mean P_{app} (10^{-6} cm/sec)**
[^{14}C]MOV	5	1.0	2.7
NHC	5	0.9	2.9
[^{3}H]Mannitol	5	1.0	2.8
[^{3}H]Metoprolol	1	1.0	28.4
[^{3}H]Verapamil	1	1.1	29.8

Abbreviations: A-B = Apical - basolateral; B-A = Basolateral - apical; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LSC = liquid scintillation counting; LLC-PK1 = Lilly Laboratories Cell Porcine Kidney; MOV = molnupiravir; NHC = N-hydroxycytidine; P_{app}= apparent permeability.
Values represent means (n =3).
Additional information: Verapamil, metoprolol and mannitol were used as positive controls, confirming the functionality of the assay.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters (Cont.)

Sec. 2.6.5.30.3 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Human P-Glycoprotein

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK063MK4482]

Type of Study	Transporter phenotyping						
Methods	Uptake of MOV and NHC into Sf9-MDR1 vesicles						
Analytical Method	LC-MS/MS						
Compound	**Inhibitor (µM)**	**Time (min)**	**Control vesicles**		**MDR1 vesicles**		
			AMP (pmole/mg vesicle)	**ATP (pmole/mg vesicle)**	**AMP (pmole/mg vesicle)**	**ATP (pmole/mg vesicle)**	**Fold of uptake in ATP compared to AMP[d]**
[^{3}H] Methyl quinidine (0.5 µM)	-	10	15.884 ± 0.182	15.699 ± 0.377	15.970 ± 0.386	125.735 ± 0.230	12.2
	CsA (10 µM)	10	16.039 ± 0.076	16.074 ± 0.262	15.569 ± 0.414	16.073 ± 0.199	NA
MOV (5 µM)[c]	-	0	3.598 ± 0.806	4.086 ± 0.564	5.579 ± 0.242[b]	3.344 ± 0.312	0.6
	-	1	3.911 ± 0.364	3.485 ± 0.503	5.193[a]	3.562 ± 0.223	0.7
	-	2	4.068 ± 0.384	5.151 ± 0.664	4.848 ± 0.850	4.190 ± 0.285	0.9
	-	5	4.349 ± 0.129	4.731 ± 0.779	4.904 ± 0.713	5.350 ± 1.112	1.1
	-	10	5.628 ± 0.592	7.290 ± 0.945	6.092 ± 0.485	6.757 ± 2.241	1.1
	CsA (10 µM)	10	5.024 ± 0.961	6.478 ± 1.416	7.171 ± 3.356	5.157 ± 0.480	NA
[^{3}H] Methyl quinidine (0.5 µM)	-	10	21.395 ± 0.421	21.307 ± 0.206	20.903 ± 0.494	133.998 ± 0.685	6.4
	CsA (10 µM)	10	20.965 ± 0.713	20.877 ± 0.281	20.368 ± 0.088	21.171 ± 0.950	NA
NHC (5 µM)	-	0	3.160[a]	3.607 ± 0.902	4.840 ± 0.284	2.790 ± 0.156[b]	0.6
	-	1	2.100[a]	4.367 ± 1.542	3.480 ± 0.816	3.027 ± 0.591	0.9
	-	2	3.687 ± 1.194	4.473 ± 1.851	3.660 ± 0.672	3.727 ± 0.639	1.0
	-	5	3.100 ± 0.361	3.807 ± 0.316	3.467 ± 1.312	2.450 ± 0.580[b]	0.7
	-	10	4.440 ± 0.418	5.167 ± 0.901	5.093 ± 1.426	3.960 ± 1.065	0.8
	CsA (10 µM)	10	4.700 ± 1.592	5.340 ± 1.178	4.200 ± 0.424[b]	4.547 ± 1.292	NA

Abbreviations: AMP = Adenosine monophosphate; ATP = Adenosine triphosphate; MDR1 = human multidrug resistance P-glycoprotein; P-gp = P-glycoprotein; Sf9 = *Spodoptera frugiperda*; CsA = cyclosporin A; LC-MS/MS = liquid chromatography- tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NA = not applicable;
- = without inhibitor.
Values represent mean ± standard deviation (n =3 except where noted).
[a] N = 1, [b] N=2.
[c] As MOV is expected to be converted to NHC intracellularly, uptake of MOV was measured as total accumulation of MOV and NHC at each time point.
[d] Fold difference is calculated by dividing the uptake into MDR1 P-gp containing vesicles in presence of ATP by that in the presence of AMP, at specific time points.
Additional Information: Time- and ATP-dependent uptake of MOV and NHC (5 µM) into membrane vesicles containing human MDR1 or control vesicles was measured.
[^{3}H]Methyl quinidine was used as a positive control substrate and CSA (10 µM) as a positive inhibitor.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters (Cont.)

Sec. 2.6.5.30.4 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Human BCRP

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK063MK4482]

Type of Study	Transporter phenotyping						
Methods	Uptake of MOV and NHC into Sf9-BCRP vesicles						
Analytical Method	LC-MS/MS						
Compound	**Inhibitor (µM)**	**Time (min)**	**Control vesicles**		**BCRP vesicles**		
			AMP (pmole/mg vesicle)	**ATP (pmole/mg vesicle)**	**AMP (pmole/mg vesicle)**	**ATP (pmole/mg vesicle)**	**Fold of uptake in ATP compared to AMP[d]**
[^{3}H]Rosuvastatin (0.125 µM)	-	10	0.819 ± 0.006	0.815 ± 0.030	0.813 ± 0.036	14.074 ± 0.139	17.3
	Ko143(10 µM)	10	0.815 ± 0.024	0.846 ± 0.031	0.819 ± 0.036	0.847 ± 0.039	NA
MOV (5 µM)[c]	-	0	4.770 ± 1.089[b]	4.520[a]	5.947 ± 1.271	6.090 ± 0.184[b]	1.0
	-	1	4.447 ± 0.851	7.527 ± 1.303	5.413 ± 1.058	5.820 ± 1.182	1.1
	-	2	5.300 ± 1.018[b]	5.953 ± 0.613	8.087 ± 1.837	5.167 ± 0.633	0.6
	-	5	6.673 ± 0.624	10.393 ± 4.694	6.300 ± 0.367	6.407 ± 0.922	1.0
	-	10	13.700[a]	10.107 ± 3.769	7.980 ± 0.296	11.710 ± 5.501[b]	1.5
	Ko143(10 µM)	10	10.307 ± 3.220	7.533 ± 1.015	7.993 ± 1.458	8.127 ± 1.457	NA
[^{3}H]Rosuvastatin (0.125 µM)	-	10	0.687 ± 0.019	0.664 ± 0.019	0.639 ± 0.019	11.855 ± 0.189	18.6
	Ko143(10 µM)	10	0.645 ± 0.030	0.660 ± 0.013	0.678 ± 0.028	0.663 ± 0.008	NA
NHC (5 µM)	-	0	BLQ	BLQ	1.996[a]	BLQ	NC
	-	1	BLQ	2.411 ± 0.607[b]	2.420 ± 0.566[b]	2.160[a]	0.9
	-	2	2.210 ± 0.099[b]	2.940 ± 0.962[b]	2.840 ± 0.566[b]	BLQ	NC
	-	5	3.033 ± 0.474	2.340 ± 0.085[b]	3.180[a]	2.230 ± 0.269[b]	0.7
	-	10	3.320 ± 0.529	2.907 ± 0.443	2.820 ± 0.227	2.620 ± 0.178	0.9
	Ko143(10 µM)	10	3.327 ± 0.947	3.253 ± 0.621	2.953 ± 0.666	2.720 ± 0.367	NA

Abbreviations: AMP = Adenosine monophosphate; ATP = Adenosine triphosphate; BCRP = breast cancer resistance protein; BLQ = below the lower limit of quantification; LC-MS/MS = liquid chromatography-tandem mass spectrometry; Ko143 = tert-butyl 3-[(3S,6S,12aS)-9-methoxy-6-(2-methylpropyl)-1,4-dioxo-1,2,3,4,6,7,12,12a-octahydropyrazino[1',2':1,6]pyrido[3,4-b]indol-3yl]propanoate; min = minute; MOV = molnupiravir; NHC = N-hydroxycytidine; Sf9 = *Spodoptera frugiperda;*
NA = not applicable; NC = not calculated; - = without inhibitor.
Values represent mean ± standard deviation (n =3 except where noted).
[a] N = 1, [b] N = 2.
[c] As MOV is expected to be converted to NHC intracellularly, uptake of MOV was measured as total accumulation of MOV and NHC at each time point.
[d] Fold difference is calculated by dividing the uptake into BCRP containing vesicles in presence of ATP by that in the presence of AMP, at specific time points.
Additional Information: Time- and ATP-dependent uptake of MOV and NHC (5 µM) into membrane vesicles containing human BCRP or control vesicles was measured. [^{3}H]Rosuvastatin was used as a positive control substrate and Ko143 (10 µM) as a positive inhibitor.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters (Cont.)

Sec. 2.6.5.30.5 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Human Uptake Transporter CNT1

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK063MK4482]

Type of Study	Transporter phenotyping				
Methods	*In vitro* uptake of MOV and NHC in MDCKII cells stably transfected with CNT1				
Analytical Method	LSC or LC-MS/MS				
Substrate	**Inhibitor**	**Time (min)**	**MDCKII cells (pmol/10^6 cells)**	**MDCKII-CNT1 cells (pmol/10^6 cells)**	**Fold difference compared to parental cells[a]**
[^{3}H]Uridine (1 µM)	-	60	0.182 ± 0.027	43.673 ± 4.126	239.8
	Gemcitabine (0.3 mM)	60	0.153 ± 0.006	15.504 ± 1.033	NA
MOV (5 µM)[b]	-	0	BLQ	BLQ	NC
	-	5	BLQ	3.384 ± 0.189	NC
	-	15	2.241 ± 0.203	5.484 ± 0.213	2.4
	-	30	3.165 ± 0.044	6.596 ± 0.634	2.1
	-	60	4.672 ± 0.556	8.025 ± 0.508	1.7
	Gemcitabine (0.3 mM)	60	4.196 ± 0.090	5.967 ± 0.495	NA
NHC (5 µM)[c]	-	0	BLQ	62.608 ± 1.062	NC
	-	5	BLQ	325.422 ± 12.873	NC
	-	15	BLQ	436.760 ± 9.760	NC
	-	30	BLQ	462.190 ± 48.791	NC
	-	60	BLQ	458.269 ± 56.796	NC
	Gemcitabine (0.3 mM)	60	BLQ	124.601 ± 10.077	NA

Abbreviations: BLQ = below limit of quantification; CNT1 = Concentrative Nucleotide Transporter 1; MDCKII = Madin-Darby Canine Kidney cells; LSC = Liquid scintillation counting; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NA = Not applicable; NC = not calculated; - = no inhibitor.
Data represent mean ± standard deviation (n=3).
[a] Fold difference was calculated by dividing the uptake in transporter transfected cells over the uptake in control cells at each time point. Fold difference was not calculated in the presence of inhibitor, as inhibitor may affect the endogenous transport in control cells.
[b] As MOV is expected to be converted to NHC intracellularly, uptake of MOV was measured as total accumulation of MOV and NHC at each time point.
[c] As NHC concentrations in all control MDCKII samples were below BLQ, the fold increase in uptake over control MDCKII cells, and the % inhibition by gemcitabine, could not be calculated.
Additional information: Gemcitabine was used as a positive control inhibitor of CNT1, confirming the functionality of the assay.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters (Cont.)

Sec. 2.6.5.30.6 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Human Uptake Transporter CNT2

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK063MK4482]

Type of Study	Transporter phenotyping				
Methods	*In vitro* uptake of MOV and NHC in MDCKII cells stably transfected with CNT2				
Analytical Method	LSC and LC-MS/MS				
Substrate	**Inhibitor**	**Time (min)**	**MDCKII cells (pmol/10^6 cells)**	**MDCKII-CNT2 cells (pmol/10^6 cells)**	**Fold difference compared to parental cells[a]**
[^{3}H]Uridine (1 µM)	-	60	0.210 ± 0.024	2.070 ± 0.062	9.9
	Adenosine (0.1 mM)	60	0.199 ± 0.010	0.358 ± 0.024	NA
MOV (5 µM)[b]	-	0	BLQ	BLQ	NC
	-	5	1.897[d]	1.427 ± 0.168[e]	0.8
	-	15	2.913 ± 0.134	2.829 ± 0.122	1.0
	-	30	4.073 ± 0.222	4.052 ± 0.037	1.0
	-	60	5.765 ± 0.398	5.454 ± 0.439	0.9
	Adenosine (0.1 mM)	60	4.807 ± 0.551[e]	4.912 ± 0.329	NA
NHC (5 µM)[c]	-	0	BLQ	BLQ	NC
	-	5	BLQ	1.011 ± 0.308	NC
	-	15	BLQ	2.363 ± 0.232	NC
	-	30	BLQ	3.704 ± 0.168[e]	NC
	-	60	0.746 ± 0.117	4.452 ± 0.237	6.0
	Adenosine (0.1 mM)	60	0.578 ± 0.070	1.091 ± 0.213	NA

Abbreviations: BLQ = below limit of quantification; CNT2 = Concentrative Nucleotide Transporter 2; MDCKII = Madin-Darby Canine Kidney cells; LSC = Liquid scintillation counting; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NA = Not applicable; NC = not calculated; - = no inhibitor.
Data represent mean ± standard deviation (n=3, except where noted).
[a] Fold difference was calculated by dividing the uptake in transporter transfected cells over the uptake in control cells at each time point. Fold difference was not calculated in the presence of inhibitor, as inhibitor may affect the endogenous transport in control cells.
[b] As MOV is expected to be converted to NHC intracellularly, uptake of MOV was measured as total accumulation of MOV and NHC at each time point.
[c] As NHC concentrations in control MDCKII cells samples at 0 - 30 minutes were BLQ, the fold increase in uptake over control MDCKII cells could not be calculated.
[d] N = 1, [e] N = 2.
Additional information: Adenosine was used as a positive control inhibitor of CNT2, confirming the functionality of the assay.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters (Cont.)

Sec. 2.6.5.30.7 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Human Uptake Transporter CNT3

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK063MK4482]

Type of Study	Transporter phenotyping				
Methods	*In vitro* uptake of MOV and NHC in MDCKII cells stably transfected with CNT3				
Analytical Method	LSC and LC-MS/MS				
Substrate	**Inhibitor**	**Time (min)**	**MDCKII cells (pmol/10^6 cells)**	**MDCKII-CNT3 cells (pmol/10^6 cells)**	**Fold difference compared to parental cells[a]**
[^{3}H]Uridine (1 µM)	-	60	0.109 ± 0.017	7.912 ± 0.316	72.7
	Adenosine (0.1 mM)	60	0.112 ± 0.009	1.027 ± 0.080	NA
MOV (5 µM)[b]	-	0	BLQ	BLQ	NC
	-	5	2.116 ± 0.129	1.854 ± 0.049	0.9
	-	15	3.459 ± 0.186	3.416 ± 0.277	1.0
	-	30	4.841 ± 0.304	4.763 ± 0.366	1.0
	-	60	6.884 ± 0.371	6.439 ± 0.332	0.9
	Adenosine (0.1 mM)	60	6.428 ± 0.822	5.657[c]	NA
[^{3}H]Uridine (1 µM)	-	60	0.280 ± 0.013	19.755 ± 0.634	70.7
	Adenosine (0.1 mM)	60	0.228 ± 0.031	1.878 ± 0.169	NA
NHC (5 µM)[d]	-	0	BLQ	BLQ	NC
	-	5	BLQ	19.391 ± 0.921	NC
	-	15	BLQ	34.919 ± 1.167	NC
	-	30	BLQ	41.512 ± 1.296	NC
	-	60	BLQ	41.477 ± 4.801	NC
	Adenosine (0.1 mM)	60	BLQ	6.859 ± 1.156	NA

Abbreviations: BLQ = below the lower limit of quantification; CNT3 = Concentrative Nucleotide Transporter 3; MDCKII = Madin-Darby Canine Kidney cells; LSC = Liquid scintillation counting; LC-MS/MS = liquid chromatography-tandem mass spectrometry; MOV = molnupiravir; NHC = N-hydroxycytidine; NA = Not applicable; NC = not calculated; - = no inhibitor.
Data represent mean ± standard deviation (n=3).
[a] Fold difference was calculated by dividing the uptake in transporter transfected cells over the uptake in control cells at each time point. Fold difference was not calculated in the presence of inhibitor, as inhibitor may affect the endogenous transport in control cells.
[b] As MOV is expected to be converted to NHC intracellularly, uptake of MOV was measured as total accumulation of MOV and NHC at each time point.
[c] N=1
[d] As NHC concentrations in all control MDCKII cells samples were BLQ, the fold increase in uptake over control MDCKII cells could not be calculated.
Additional information: Adenosine was used as a positive control inhibitor of CNT3, confirming the functionality of the assay.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters (Cont.)

Sec. 2.6.5.30.8 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Human Uptake Transporter ENT1

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK063MK4482]

Type of Study	Transporter phenotyping				
Methods	*In vitro* uptake of MOV and NHC in MDCKII cells stably transfected with ENT1				
Analytical Method	LSC and LC-MS/MS				
Substrate	**Inhibitor**	**Time (min)**	**MDCKII cells (pmol/10^6 cells)**	**MDCKII-ENT1 cells (pmol/10^6 cells)**	**Fold difference compared to parental cells[a]**
[^{3}H]Adenosine (0.1 µM)	-	60	7.143 ± 0.220	34.247 ± 3.289	4.8
	NBMPR (0.1 µM)	60	0.337 ± 0.033	9.683 ± 1.140	NA
MOV (5 µM)[b]	-	0	BLQ	BLQ	NC
	-	5	2.661 ± 0.082	3.355 ± 0.060	1.3
	-	15	3.982 ± 0.267	3.871 ± 0.269	1.0
	-	30	4.748 ± 0.230	3.717 ± 0.272	0.8
	-	60	6.612 ± 0.589	4.131 ± 0.068	0.6
	NBMPR (0.1 µM)	60	10.421 ± 1.263	4.626 ± 0.586	NA
[^{3}H]Adenosine (0.1 µM)	-	60	3.344 ± 0.241	39.241 ± 0.860	11.7
	NBMPR (0.1 µM)	60	0.132 ± 0.009	7.623 ± 0.289	NA
NHC (5 µM)	-	0	2.853 ± 0.193	2.146 ± 0.191	0.8
	-	5	6.602 ± 0.049	5.352 ± 0.163	0.8
	-	15	10.603 ± 0.738	10.517 ± 0.669	1.0
	-	30	17.288 ± 1.239	22.643 ± 0.573	1.3
	-	60	28.163 ± 0.784	50.932 ± 5.818	1.8
	NBMPR (0.1 µM)	60	3.390 ± 0.578	29.472 ± 1.613	NA

Abbreviations: BLQ = below the lower limit of quantification; ENT1 = Equilibrative Nucleoside Transporter 1; MDCKII = Madin-Darby Canine Kidney cells; LSC = Liquid scintillation counting; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NBMPR = S-(4-Nitrobenzyl)-6-thioinosine; MOV = molnupiravir; NHC = N-hydroxycytidine; NA = not applicable; NC = not calculated; - = no inhibitor.
Data represent mean ± standard deviation (n=3).
[a] Fold difference was calculated by dividing the uptake in transporter transfected cells over the uptake in control cells at each time point. Fold difference was not calculated in the presence of inhibitor, as inhibitor may affect the endogenous transport in control cells.
[b] As MOV is expected to be converted to NHC intracellularly, uptake of MOV was measured as total accumulation of MOV and NHC at each time point.
Additional information: NBMPR was used as a positive inhibitor of ENT1, confirming the functionality of the assay.

Sec. 2.6.5.30 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Drug Transporters (Cont.)

Sec. 2.6.5.30.9 Pharmacokinetics: Evaluation of MOV and NHC as a Substrate of Human Uptake Transporter ENT2

Test Article: MOV and NHC
Report Number: [Ref. 4.2.2.6: PK063MK4482]

Type of Study	Transporter phenotyping				
Methods	*In vitro* uptake of MOV and NHC in MDCKII cells stably transfected with ENT2				
Analytical Method	LSC and LC-MS/MS				
Substrate	**Inhibitor[a]**	**Time (min)**	**MDCKII cells (pmol/10^6 cells)**	**MDCKII-ENT2 cells (pmol/10^6 cells)**	**Fold difference compared to parental cells[b]**
[^{3}H]Adenosine (0.1 µM)	NBMPR (0.5 µM)	60	0.063 ± 0.011	0.268 ± 0.012	4.2
	NBMPR (10 µM)	60	0.035 ± 0.001	0.081 ± 0.002	NA
MOV (5 µM)[c]	NBMPR (0.5 µM)	0	BLQ	BLQ	NC
		5	0.844 ± 0.398[d]	0.762 ± 0.102	0.9
		15	1.619 ± 0.606	1.539 ± 0.325[d]	1.0
		30	2.277 ± 0.719	2.430 ± 0.063[d]	1.1
		60	3.232 ± 0.336	2.911 ± 0.126	0.9
	NBMPR (10 µM)	60	2.799 ± 0.219	2.937 ± 0.119	NA
NHC (5 µM)	NBMPR (0.5 µM)	0	0.608 ± 0.038	0.677 ± 0.008	1.1
		5	0.703 ± 0.099	1.021 ± 0.028[d]	1.5
		15	0.823 ± 0.078	1.199 ± 0.090[d]	1.5
		30	1.034 ± 0.139	1.749 ± 0.346	1.7
		60	1.446 ± 0.242	2.519 ± 0.502	1.7
	NBMPR (10 µM)	60	0.925 ± 0.035	1.180 ± 0.034	NA

Abbreviations: BLQ = below the lower limit of quantification; ENT2 = Equilibrative Nucleoside Transporter 2; MDCKII = Madin-Darby Canine Kidney cells; LSC = Liquid scintillation counting; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NBMPR = S-(4-Nitrobenzyl)-6-thioinosine; MOV = molnupiravir; NHC = N-hydroxycytidine; NA = Not applicable; NC = not calculated.
Data represent mean ± standard deviation (n=3, except where as noted).
[a] NBMPR at 0.5 µM was used to inhibit endogenous transport, and 10 µM NBMPR was used to inhibit both endogenous and human ENT2-mediated transport.
[b] Fold difference was calculated by dividing the uptake in transporter transfected cells over the uptake in control cells at each time point. Fold difference was not calculated in the presence of inhibitor, as inhibitor may affect the endogenous transport in control cells.
[c] As MOV is expected to be converted to NHC intracellularly, uptake of MOV was measured as total accumulation of MOV and NHC at each time point.
[d] N=2.
Additional information: NBMPR was used as a positive inhibitor of ENT2, confirming the functionality of the assay.

Sec. 2.6.5.31 Pharmacokinetics: Inhibition of Human Drug Transporters by MOV

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK064MK4482]

Type of Study	Inhibition potential on transporter proteins			
Methods	*In vitro* evaluation of MOV as an inhibitor of human OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, MATE2K, MRP2, MDR1 P-gp, and BCRP transporters			
Analytical Method	LSC			
Transporter	**Cell Lines**	**Probe Substrate**	**IC_{50} (µM)**	**Inhibition at Highest Concentration Tested**
OATP1B1	HEK293-OATP1B1	[^{3}H]Pitavastatin (0.1 µM)	>100	26% at 100 µM
OATP1B3	HEK293-OATP1B3	[^{3}H]BSP (0.1 µM)	>100	No inhibition at 100 µM
OCT1	CHO-K1-OCT1	[^{14}C]Metformin (10 µM)	>100	No inhibition at 100 µM
OCT2	HEK293-OCT2	[^{14}C]Metformin (5 µM)	>100	No inhibition at 100 µM
OAT1	MDCKII-OAT1	[^{3}H]Cidofovir (1 µM)	>100	No inhibition at 100 µM
OAT3	MDCKII-OAT3	[^{3}H]ES (1 µM)	>100	30% at 100 µM
MATE1	CHO-K1-MATE1	[^{14}C]Metformin (5 µM)	>100	No inhibition at 100 µM
MATE2K	MDCKII-MATE2K	[^{14}C]Metformin (5 µM)	>100	No inhibition at 100 µM
MRP2	Sf9-MRP2	[^{14}C]EA-SG (1 µM)	>100	No inhibition at 100 µM
MDR1 P-gp	Sf9-MDR1	[^{3}H]N-methylquinidine (0.1 µM)	>1000	19% at 1000 µM
BCRP	Sf9-BCRP	[^{3}H]Rosuvastatin (0.1 µM)	>1000	No inhibition at 1000 µM

Abbreviations: LSC = liquid scintillation counting; OATP1B1 = organic anion transporter 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; OAT1 = organic anion transporting polypeptide 1; OAT3 = organic anion transporter 3; OCT1 = organic cation transporter 1; OCT2 = organic cation transporter 2; MATE1 = multidrug and toxin extrusion protein 1; MATE2K = multidrug and toxin extrusion protein 2K; BCRP = breast cancer resistance protein; MRP2 = Multidrug resistance-associated protein 2; P-gp = P-glycoprotein; MDR1 = multidrug resistance protein; IC_{50} = concentration required for 50% inhibition; HEK293 = human embryonic kidney 293; CHO-K1 = Chinese hamster overy-K1 cells; Sf9 = *Spodoptera frugiperda;* MDCKII = Madin-Darby canine kidney cells; BSP = bromosulfophthalein; ES = estrone sulfate; EA-SG = ethacrynic acid-glutathione conjugate; MOV = molnupiravir.
Additional information: An assessment of DDI risk between MOV and co-administered substrates of these transporters, based on the 2020 FDA, 2012 EMA and 2018 MHLW guidance, is provided in [Sec. 2.6.5: 32], [Sec. 2.6.5: 33] and [Sec. 2.6.5: 34], respectively.

Sec. 2.6.5.32 Pharmacokinetics: Drug-Drug Interaction, Transporter Inhibition by MOV (FDA)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK064MK4482]

Summary Results: Risk Assessment of MOV as a Perpetrator of Transporter-Mediated Drug Interactions based on the FDA guidance (2020)[a]						
Transporter[b]	**IC_{50} (µM)**	**I_{gut}[c] (µM)**	**$I_{in,max}$ (µM)**	**I_{gut}/IC_{50}**	**R[d]**	**Threshold Cutoff for Potential DDI**
OATP1B1	>100	-	1.6	-	<1.02	R ≥1.1
OATP1B3	>100	-	1.6	-	<1.02	R ≥1.1
MDR1 P-gp	>1000	9717	-	<9.7	-	I_{gut}/IC_{50} ≥10
BCRP	>1000	9717	-	<9.7	-	I_{gut}/IC_{50} ≥10

Abbreviations: FDA = Food and Drug Administration; DDI = drug-drug interaction; BID = twice daily; C_{max} = maximum plasma concentration; $f_{u,p}$ = unbound fraction in plasma; $I_{in,max}$ = estimated maximum plasma concentration at the inlet to the liver; I_{gut} = theoretical maximum gastrointestinal drug concentration calculated as the dose/250 mL; IC_{50} = concentration required for 50% inhibition; MDR1 = Multi drug resistance protein; OATP1B1 = organic anion transporting polypeptide 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; MOV = molnupiravir; - = not applicable.

[a] *In Vitro* Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter -Mediated Drug Interactions Guidance for Industry. Issued by: U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER), Jan2020.

[b] Systemic exposure of MOV after oral administration of MOV was every low (C_{max} = 0.026 µM in human treated with 800 mg BID after the final dose on Day 6 (P004)), therefore only presystemic DDIs were assessed.

[c] I_{gut} = dose/250 mL = 800 mg/250 mL = 9717 µM (MW = 329.31).

[d] $R = 1 + ((f_{u,p} \times I_{in,max})/IC_{50})$ where $I_{in,max,}$ = 1.6 µM estimated using Gastroplus model (estimated portal vein concentration was 525 ng/mL after 800 mg dose of MOV); assumed $f_{u,p}$ = 1.0.

Sec. 2.6.5.33 Pharmacokinetics: Drug-Drug Interaction, Transporter Inhibition by MOV (EMA)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK064MK4482]

Summary Results: Risk Assessment of MOV as a Perpetrator of Transporter-Mediated Drug Interactions based on the EMA guideline (2012)[a]				
Transporter[b]	**K_i[c] (µM)**	**25 × $I_{u,inlet,max}$ (µM)[d]**	**0.1 × Dose/250 mL (µM)[e]**	**Threshold Cutoff For Potential DDI**
OATP1B1	>100	40	-	$K_i \leq (25 \times I_{u,inlet,max})$
OATP1B3	>100	40	-	$K_i \leq (25 \times I_{u,inlet,max})$
OCT1[f]	>100	40	-	$K_i \leq (25 \times I_{u,inlet,max})$
MDR1 P-gp	>1000	-	971.7	$K_i \leq (0.1 \times Dose/250)$
BCRP	>1000	-	971.7	$K_i \leq (0.1 \times Dose/250)$

Abbreviations: EMA = European Medicines Agency; DDI = drug-drug interaction; IC_{50} = concentration required for 50% inhibition; BID = twice daily; Cmax = maximum plasma concentration; $f_{u,b}$ = unbound fraction in blood; $I_{u,inlet,max}$ = estimated unbound maximum concentration at the inlet to the liver; K_i = inhibition constant; K_m = Michaelis-Menten constant; MDR1 = Multi drug resistance protein; P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; OATP1B1 = organic anion transporting polypeptide 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; OCT1 = organic cation transporter 1; MOV = molnupiravir; - = not applicable.

[a] Committee for Human Medicinal Products (CHMP). Guideline on the investigation of drug interactions. In: CPMP/EWP/560/95/Rev. 1 Corr. 2. London, England: European Medicines Agency; 2012.

[b] Systemic exposure of MOV after oral administration of MOV was very low (Cmax = 0.026 µM in human treated with 800 mg BID after the final dose on Day 6 (P004)), therefore only presystemic DDIs were assessed.

[c] $K_i = IC_{50}$, given that the concentration of the probe substrate for each transporter was << K_m value.

[d] $I_{u,inlet,max}$ = 1.6 µM (assumed $f_{u,b}$ = 1.0) estimated using Gastroplus model (estimated portal vein concentration was 525 ng/mL after 800 mg dose of MOV).

[e] Calculated using the clinical dose of 800 mg, on a molar basis (MW = 329.31).

[f] OCT1 is a hepatic uptake transporter; therefore, used the same criteria as OATP1B1 and OATP1B3.

Sec. 2.6.5.34 Pharmacokinetics: Drug-Drug Interaction, Transporter Inhibition by MOV (MHLW)

Test Article: MOV
Report Number: [Ref. 4.2.2.6: PK064MK4482]

Summary Results: Risk Assessment of MOV as a Perpetrator of Transporter-Mediated Drug Interactions based on the MHLW guidance (2018)[a]						
Transporter[b]	**IC_{50} (µM)**	**[I] [c] (µM)**	**$I_{inlet,max}$ [d] (µM)**	**I/IC_{50}**	**$1+((f_{u,b}$ [e] x $I_{inlet,max})/K_i)$**	**Threshold Cutoff For Potential DDI**
OATP1B1	>100	-	1.6	-	<1.02	$1+((f_{u,b} \times I_{inlet,max})/K_i$ [f]$)\geq 1.1$
OATP1B3	>100	-	1.6	-	<1.02	$1+((f_{u,b} \times I_{inlet,max})/K_i$ [f]$)\geq 1.1$
MDR1 P-gp	>1000	9717	-	<10	-	$[I]/IC_{50}\geq 10$
BCRP	>1000	9717	-	<10	-	$[I]/IC_{50}\geq 10$

Abbreviations: MHLW = Ministry of Health, Labour and Welfare; DDI = drug-drug interaction; BID = twice daily; C_{max} = maximum plasma concentration; $f_{u,b}$ = unbound fraction in blood; [I] = theoretical maximum gastrointestinal drug concentration calculated as the dose 250 mL; IC_{50} = concentration required for 50% inhibition; $I_{inlet,max,}$ = estimated maximum concentration at the inlet to the liver; K_i = inhibition constant; K_m = Michaelis-Menten constant; MDR1 = Multi drug resistance protein; P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; OATP1B1 = organic anion transporting polypeptide 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; MOV = molnupiravir; - = not applicable.

[a] Guideline on drug interaction for drug development and appropriate provision of information. Issued by Ministry of Health, Labour and Welfare, Jul 2018.

[b] Systemic exposure of MOV after oral administration of MOV was very low (C_{max} = 0.026 µM in human treated with 800 mg BID after the final dose on Day 6 (P004)), therefore only presystemic DDIs were assessed.

[c] [I] = dose/250 mL = 800 mg/250 mL = 9717 µM (MW = 329.31).

[d] $I_{inlet,max}$ = 1.6 µM estimated using Gastroplus model (estimated portal vein concentration was 525 ng/mL after 800 mg dose of MOV).

[e] Assumed $f_{u,b}$ = 1.0.

[f] $K_i = IC_{50}$, given that the concentration of the probe substrate for each transporter was << K_m value.

Sec. 2.6.5.35 Pharmacokinetics: Inhibition of Human Drug Transporters by NHC

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK064MK4482]

Type of Study	Inhibition potential on transporter proteins			
Methods	*In vitro* evaluation of NHC as an inhibitor of human OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, MATE2K, MRP2, MDR1 P-gp, and BCRP transporters			
Analytical Method	LSC			
Transporter	**Cell Lines**	**Probe Substrate**	**IC_{50} (µM)**	**Inhibition at Highest Concentration Tested**
OATP1B1	HEK293-OATP1B1	[^{3}H]Pitavastatin (0.1 µM)	>100	15% at 100 µM
OATP1B3	HEK293-OATP1B3	[^{3}H]BSP (0.1 µM)	>100	4% at 100 µM
OCT1	CHO-K1-OCT1	[^{14}C]Metformin (10 µM)	>100	No inhibition at 100 µM
OCT2	HEK293-OCT2	[^{14}C]Metformin (5 µM)	>100	No inhibition at 100 µM
OAT1	MDCKII-OAT1	[^{3}H]Cidofovir (1 µM)	>100	No inhibition at 100 µM
OAT3	MDCKII-OAT3	[^{3}H]ES (1 µM)	>100	No inhibition at 100 µM
MATE1	CHO-K1-MATE1	[^{14}C]Metformin (5 µM)	>100	No inhibition at 100 µM
MATE2K	MDCKII-MATE2K	[^{14}C]Metformin (5 µM)	>100	No inhibition at 100 µM
MRP2	Sf9-MRP2	[^{14}C]EA-SG (1 µM)	>100	No inhibition at 100 µM
MDR1 P-gp	Sf9-MDR1	[^{3}H]N-methylquinidine (0.1 µM)	>1000	14% at 1000 µM
BCRP	Sf9-BCRP	[^{3}H]Rosuvastatin (0.1 µM)	>1000	No inhibition at 1000 µM

Abbreviations: LSC = liquid scintillation counting; OATP1B1 = organic anion transporting polypeptide 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; OAT1 = organic anion transporter 1; OAT3 = organic anion transporter 3; OCT1 = organic cation transporter 1; OCT2 = organic cation transporter 2; MATE1 = multidrug and toxin extrusion protein 1; MATE2K = multidrug and toxin extrusion protein 2K; BCRP = breast cancer resistance protein; MRP2 = Multidrug resistance-associated protein 2; P-gp = P-glycoprotein; MDR1 = multidrug resistance protein; IC_{50} = concentration required for 50% inhibition; DDI = drug-drug interaction; HEK293 = human embryonic kidney 293; CHO-K1 = Chinese hamster overy-K1 cells; Sf9 = *Spodoptera frugiperda;* MDCKII = Madin-Darby canine kidney cells; BSP = bromosulfophthalein; ES = estrone sulfate; EA-SG = ethacrynic acid-glutathione conjugate; NHC = N-hydroxycytidine.
Additional information: An assessment of DDI risk between NHC and co-administered substrates of these transporters, based on the 2020 FDA, 2012 EMA and 2018 MHLW guidance, is provided in [Sec. 2.6.5: 36], [Sec. 2.6.5: 37] and [Sec. 2.6.5: 38], respectively.

Sec. 2.6.5.36 Pharmacokinetics: Drug-Drug Interaction, Transporter Inhibition by NHC (FDA)

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK064MK4482]

Summary Results: Risk Assessment of NHC as a Perpetrator of Transporter-Mediated Drug Interactions based on the FDA guidance (2020)[a]							
Transporter	**IC_{50} (µM)**	**$I_{max,u}$[b] (µM)**	**$I_{in,max}$ (µM)**	**$I_{max,u}$ /IC_{50}**	**I_{gut}[c]/IC_{50}**	**R[d]**	**Threshold Cutoff For Potential DDI**
OATP1B1	>100	10.8	24	<0.11	-	<1.24	$I_{max,u}$ /IC_{50} ≥0.1 or R≥1.1
OATP1B3	>100	10.8	24	<0.11	-	<1.24	$I_{max,u}$ /IC_{50} ≥0.1 or R≥1.1
OCT2	>100	10.8	-	<0.11	-	-	$I_{max,u}$/IC_{50} ≥0.1
OAT1	>100	10.8	-	<0.11	-	-	$I_{max,u}$/IC_{50} ≥0.1
OAT3	>100	10.8	-	<0.11	-	-	$I_{max,u}$/IC_{50} ≥0.1
MATE1	>100	10.8	-	<0.11	-	-	$I_{max,u}$/IC_{50} ≥0.1
MATE2K	>100	10.8	-	<0.11	-	-	$I_{max,u}$/IC_{50} ≥0.1
MDR1 P-gp	>1000	10.8	-	<0.01	<10	-	$I_{max,u}$/IC_{50} ≥0.1 or I_{gut}/IC_{50}≥10
BCRP	>1000	10.8	-	<0.01	<10	-	$I_{max,u}$/IC_{50} ≥0.1 or I_{gut}/IC_{50}≥10

Abbreviations: FDA = Food and Drug Administration; DDI = drug-drug interaction; $I_{max,u}$ = unbound maximum plasma concentration; $f_{u,p}$ = unbound fraction in plasma; IC_{50} = concentration required for 50% inhibition; $I_{in,max}$ = estimated maximum concentration at the inlet to the liver; I_{gut} = theoretical maximum gastrointestinal drug concentration calculated as the dose/250mL; OATP1B1 = organic anion transporting polypeptide 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; OAT1 = organic anion transporter 1; OAT3 = organic anion transporter 3; OCT2 = organic cation transporter 2; MATE1 = multidrug and toxin extrusion protein 1; MATE2K = multidrug and toxin extrusion protein 2K; MDR1 = Multi drug resistance protein; P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; MOV = molnupiravir; NHC = N-hydroxycytidine; - = not applicable.

[a] *In Vitro* Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter -Mediated Drug Interactions Guidance for Industry. Issued by: U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER), Jan2020.

[b] $I_{max,u} = C_{max} \times f_{u\,p}$, where the NHC C_{max} = 10.8 µM based on Pop PK analysis from P002 with 800 mg dose of MOV and $f_{u\,p}$ = 1.0.

[c] I_{gut} = dose/250 mL assumed (worst case scenario) 100% conversion of MOV dose to NHC in the gut = 800 mg/250 mL = 9717 µM (MW = 329.31 for MOV).

[d] $R = 1 + ((f_{u,p} \times I_{in,max})/IC_{50})$ where $I_{in,max}$ = 24 µM estimated using Gastroplus model (estimated portal vein concentration of NHC was 6250 ng/mL in human after 800 mg dose of MOV); $f_{u,p}$ = 1.0.

Sec. 2.6.5.37 Pharmacokinetics: Drug-Drug Interaction, Transporter Inhibition by NHC (EMA)

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK064MK4482]

Summary Results: Risk Assessment of NHC as a Perpetrator of Transporter-Mediated Drug Interactions based on the EMA guideline (2012)[a]					
Transporter	**K_i[b] (µM)**	**50 × $Cmax_u$[c] (µM)**	**25 × $I_{u,inlet,max}$ (µM)[d]**	**0.1 × (Dose[e]/250mL)**	**Threshold Cutoff For Potential DDI**
OATP1B1	>100	540	600	-	$K_i \leq (50 \times Cmax_u)$ or $K_i \leq (25 \times I_{u,inlet,max})$
OATP1B3	>100	540	600	-	$K_i \leq (50 \times Cmax_u)$ or $K_i \leq (25 \times I_{u,inlet,max})$
OCT1[f]	>100	540	600	-	$K_i \leq (50 \times Cmax_u)$ or $K_i \leq (25 \times I_{u,inlet,max})$
OCT2	>100	540	-	-	$K_i \leq (50 \times Cmax_u)$
OAT1	>100	540	-	-	$K_i \leq (50 \times Cmax_u)$
OAT3	>100	540	-	-	$K_i \leq (50 \times Cmax_u)$
MATE1	>100	540	-	-	not provided
MATE2K	>100	540	-	-	not provided
MDR1 P-gp	>1000	540	-	971.7	$K_i \leq (50 \times Cmax_u)$ or $K_i \leq (0.1 \times (Dose/250mL))$
BCRP	>1000	540	-	971.7	$K_i \leq (50 \times Cmax_u)$ or $K_i \leq (0.1 \times (Dose/250mL))$

Abbreviations: EMA = European Medicines Agency; DDI = drug-drug interaction; IC_{50} = concentration required for 50% inhibition; K_i = inhibition constant; K_m = Michaelis-Menten constant; $Cmax_u$ = unbound maximum plasma concentration; $f_{u,p}$ = unbound fraction in plasma; $f_{u,b}$ = unbound fraction in blood; $I_{u,inlet,max}$ = estimated unbound maximum concentration at the inlet to the liver; OATP1B1 = organic anion transporting polypeptide 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; OCT1 = organic cation transporter 1; OAT1 = organic anion transporter 1; OAT3 = organic anion transporter 3; OCT2 = organic cation transporter 2; MATE1 = multidrug and toxin extrusion protein 1; MATE2K = multidrug and toxin extrusion protein 2K; NHC = N-hydroxycytidine; MDR1 = Multi drug resistance protein; P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; MOV = molnupiravir; - = not applicable.

[a] Committee for Human Medicinal Products (CHMP). Guideline on the investigation of drug interactions. In: CPMP/EWP/560/95/Rev. 1 Corr. 2. London, England: European Medicines Agency; 2012.

[b] $K_i = IC_{50}$, given that the concentration of the probe substrate for each transporter was << K_m value.

[c] $Cmax_u = Cmax \times f_{u,p}$, where the NHC Cmax = 10.8 µM based on Pop PK analysis from P002 with 800 mg dose of MOV and $f_{u,p} = 1.0$.

[d] $I_{u,inlet,max}$ = 24 µM (assumed $f_{u,b} = 1.0$) estimated using Gastroplus model (estimated portal vein concentration of NHC was 6250 ng/mL in human after 800 mg dose of MOV).

[e] Assumed (worst case scenario) 100% conversion of MOV dose to NHC in the gut = 800 mg/250 mL = 9717 µM (MW = 329.31 for MOV).

[f] OCT1 is a hepatic uptake transporter; therefore, used the same criteria as OATP1B1 and OATP1B3.

Sec. 2.6.5.38 Pharmacokinetics: Drug-Drug Interaction, Transporter Inhibition by NHC (MHLW)

Test Article: NHC
Report Number: [Ref. 4.2.2.6: PK064MK4482]

Summary Results: Risk Assessment of NHC as a Perpetrator of Transporter-Mediated Drug Interactions Based on the MHLW guidance (2018)[a]							
Transporter	**IC_{50} (µM)**	**C_{max} (µM)** [b]	**$I_{inlet,max}$ (µM)**[c]	**1 + (Unbound C_{max}**[d]**/K_i**[e]**)**	**1 +(($f_{u,b}$ x $I_{inlet,max}$)/K_i)**	**[I][f]/IC_{50}**	**Threshold Cutoff For Potential DDI**
OATP1B1	>100	10.8	24	<1.11	<1.24	-	1 + (Unbound C_{max}/K_i) or 1 +(($f_{u,b}$ x $I_{inlet,max}$)/K_i)≥1.1
OATP1B3	>100	10.8	24	<1.11	<1.24	-	1 + (Unbound C_{max}/K_i) or 1 +(($f_{u,b}$ x $I_{inlet,max}$)/K_i)≥1.1
OCT2	>100	10.8	-	<1.11	-	-	1 + (Unbound C_{max}/K_i) ≥1.1
OAT1	>100	10.8	-	<1.11	-	-	1 + (Unbound C_{max}/K_i) ≥1.1
OAT3	>100	10.8	-	<1.11	-	-	1 + (Unbound C_{max}/K_i) ≥1.1
MATE1	>100	10.8	-	<1.11	-	-	1 + (Unbound C_{max}/K_i) ≥1.02
MATE2K	>100	10.8	-	<1.11	-	-	1 + (Unbound C_{max}/K_i) ≥1.02
MDR1 P-gp	>1000	10.8	-	-	-	<10	[I]/IC_{50}≥10
BCRP	>1000	10.8	-	-	-	<10	[I]/IC_{50}≥10

Abbreviations: MHLW = Ministry of Health, Labour and Welfare; DDI = drug-drug interaction; C_{max} = maximum plasma concentration; $f_{u,p}$ = unbound fraction in plasma =1.0; $f_{u,b}$ = unbound fraction in blood (assumed =1.0); IC_{50} = concentration required for 50% inhibition; [I] = theoretical maximum gastrointestinal drug concentration calculated as the dose 250 mL; $I_{inlet,max}$ = estimated maximum concentration at the inlet to the liver; K_i = inhibition constant; K_m = Michaelis-Menten constant; OATP1B1 = organic anion transporting polypeptide 1B1; OATP1B3 = organic anion transporting polypeptide 1B3; OCT2 = organic cation transporter 2; OAT1 = organic anion transporter 1; OAT3 = organic anion transporter 3; MATE1 = multidrug and toxin extrusion protein 1; MATE2K = multidrug and toxin extrusion protein 2K; MDR1 = Multi drug resistance protein; P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; MOV = molnupiravir; NHC = N-hydroxycytidine; - = not applicable.

[a] Guideline on drug interaction for drug development and appropriate provision of information. Issued by Ministry of Health, Labour and Welfare, Jul 2018.

[b] C_{max} of NHC = 10.8 µM based on Pop PK analysis from P002 with 800 mg dose of MOV.

[c] $I_{inlet,max}$ = 24 µM estimated using Gastroplus model (estimated portal vein concentration of NHC was 6250 ng/mL in human after 800 mg dose of MOV).

[d] Unbound C_{max} = C_{max} x $f_{u\,p}$, $f_{u\,p}$ = 1.0.

[e] K_i = IC_{50}, given that the concentration of the probe substrate for each transporter was << K_m value.

[f] [I] = dose/250 mL assumed (worst case scenario) 100% conversion of MOV dose to NHC in the gut = 800 mg/250 mL = 9717 µM (MW = 329.31 for MOV).