TABLE OF CONTENTS

TABLE OF CONTENTS 1
LIST OF TABLES 3
LIST OF ABBREVIATIONS, ACRONYMS AND DEFINITION OF TERMS 4
1 OVERVIEW OF THE NONCLINICAL TESTING STRATEGY 8
2 PHARMACOLOGY 10
2.1 Primary Pharmacodynamics 10
2.1.1 Primary Pharmacodynamics In Vitro 10
2.1.2 Primary Pharmacodynamics In Vivo 11
2.2 Secondary Pharmacodynamics 11
2.3 Safety Pharmacology 12
2.4 Pharmacodynamic Drug Interactions 12
3 PHARMACOKINETICS 13
3.1 Analytical Methods and Validation Reports 13
3.2 Absorption 13
3.3 Distribution 13
3.4 Metabolism 14
3.5 Excretion 14
3.6 Pharmacokinetic Drug Interactions 15
3.6.1 Drug Metabolizing Enzymes and/or Transporters Involved in the Elimination of MOV and/or NHC 15
3.6.2 Inhibition of Drug Metabolizing Enzymes and Transporters 15
3.6.3 Induction of Cytochrome P450 Enzymes 15
3.7 Other Pharmacokinetic Studies 16
4 TOXICOLOGY 16
4.1 Single-Dose Toxicity 16
4.2 Repeat-Dose Toxicity 16
4.2.1 Mice 16
4.2.2 Rats 17
4.2.3 Dogs 18
4.3 Genotoxicity 19
4.4 Carcinogenicity 20
4.5 Reproductive and Developmental Toxicity 20
4.5.1 Fertility and early embryonic development 20
4.5.2 Embryo-fetal development 20
4.5.3 Prenatal and postnatal development, including maternal function 21

[redacted]

C Confidential

4.5.4 Studies in which the offspring (juvenile animals) are dosed and/or further evaluated....21
4.6 Local Tolerance....21
4.7 Other Toxicity Studies....22
4.7.1 Antigenicity/Immunogenicity....22
4.7.2 Immunotoxicity....22
4.7.3 Mechanistic studies....23
4.7.4 Dependence....23
4.7.5 Metabolites....23
4.7.6 Impurities....23
4.7.7 Other....24
4.7.7.1 Photoreactivity Assessment of MOV and NHC....24
4.7.7.2 Other Studies....24
5 INTEGRATED OVERVIEW AND CONCLUSIONS....24
6 LIST OF LITERATURE REFERENCES....31

[redacted]

LIST OF TABLES

Table 2.4: 1 Impurity and Dose Multiples 24
Table 2.4: 2 NHC Systemic Exposure Multiples 27

[redacted]

C Confidential

LIST OF ABBREVIATIONS, ACRONYMS AND DEFINITION OF TERMS

This list serves as the first use of an abbreviation, acronym, or term.

Abbreviation/Acronym	Definition
3TC	Lamivudine
ADME	Absorption, Distribution, Metabolism and Elimination
ALI	Air-liquid interface
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
$AUC_{0\text{-}24\ hr}$	Area under the curve from zero to 24 hours
B.1-G	SARS-CoV-2 Wuhan isolate
B.1.1.529	SARS-CoV-2 Omicron variant
B.1.1.7	SARS-CoV-2 Alpha variant
B.1.351	SARS-CoV-2 Beta variant
B.1.617.2	SARS-CoV-2 Delta variant
B.1.621	SARS-CoV-2 Mu variant
BCRP	Breast Cancer Resistance Protein Efflux Transporter
BDC	Bile Duct-Cannulated
BID	Twice a Day
BQL	Below the Quantifiable Limit
C.37	SARS-CoV-2 Lambda variant
CC_{50}	Half-maximal cytotoxicity concentration
CES	Carboxylesterase
CHKV	Chikungunya virus
C_{max}	Maximum concentration
CNS	Central nervous system
CNT	Concentrative Nucleoside Transporter
COVID-19	Coronavirus disease 2019
COX-2	Human cyclooxygenase 2
CTP	Cytidine Triphosphate
CYP	Cytochrome P450
DDIs	Drug-drug interaction(s)
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic acid

[redacted]

Abbreviation/Acronym	Definition
dNTP	Deoxynucleoside triphosphate
DRF	Dose range-finding
EBOV	Ebola virus
EC_{50}	Half-maximal effective concentration
EEEV	Eastern equine encephalitis virus
EMA	European Medicines Agency
ENT	Equilibrative Nucleoside Transporter
EFD	Embryofetal developmental
EIDD	Emory Institute for Drug Development
F	Female
FDA	Food and Drug Administration
FTC	Emtricitabine
GD	Gestation day
GI	Gastrointestinal
GLP	Good Laboratory Practice
HBEC	Human bronchial epithelial cell
hr	Hour
IAV	Influenza A virus
IBV	Influenza B virus
IC_{50}	Half-Maximal Inhibitory Concentration
IP	Intraperitoneal
IV	Intravenous
ICH	International Council on Harmonisation
IV	Intravenous
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LD	Lactation day
LOQ	Limit of quantitation
LLOQ	Lower limit of quantitation
LoM	Lung only Mice
M	Male
mARC	Mitochondrial Amidoxime Reducing Component
MATE	Multidrug and Toxin Extrusion Protein
MDR1	Multidrug Resistance Protein 1
MERS	Middle east respiratory syndrome
MERS-CoV	MERS-associated coronavirus

[redacted]

Abbreviation/Acronym	Definition
MHV	Mouse hepatitis virus, a model coronavirus
MOV	Molnupiravir (MK-4482, EIDD-2801)
mRNA	Messenger Ribonucleic Acid
MRP2	Multidrug Resistance-Associated Protein-2
MTD	Maximum tolerated dose
NHC	N-hydroxycytidine (EIDD-1931)
NHC-DP	N-hydroxycytidine-5′diphosphate
NHC-MP	N-hydroxycytidine-5′monophosphate
NHC-TP	N-hydroxycytidine-5′triphosphate (EIDD-2061)
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NSP	Nonstructural protein
OAT	Organic Anion Transporter
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter
P.1	SARS-CoV-2 Gamma variant
PBMC	Peripheral blood mononuclear cell
P-gp	P-glycoprotein
PK	Pharmacokinetic(s)
PMDA	Pharmaceuticals and Medical Devices Agency
P.O.	Per Oral
OECD	Organisation for Economic Co-operation and Development
Q12H	Every 12 hours
RBC	Red blood cells
RdRp	RNA-dependent RNA polymerase
RET	Reticulocytes
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SARS	Severe acute respiratory syndrome
SARS-CoV-1	SARS-associated coronavirus-1
SARS-CoV-2	SARS-associated coronavirus-2
SD	Sprague Dawley
$t_{½}$	Apparent Terminal Half-Life
TP	Triphosphate
ULOQ	Upper limit of quantitation

[redacted]

C Confidential

Abbreviation/Acronym	Definition
UTP	Uridine Triphosphate
VEEV	Venezuelan equine encephalitis virus
WA1	SARS-CoV-2, Isolate USA-WA1/2020
WOCBP	Women of child bearing potential
WT	Wild-type

[redacted]

C Confidential

1 OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

Molnupiravir (MOV; MK-4482) is the 5′-isobutyrate prodrug of the broadly active, antiviral ribonucleoside analog N-hydroxycytidine (NHC) developed for the oral treatment of adult patients with COVID-19.

MOV is hydrolyzed by esterases either during or after absorption to deliver NHC into systemic circulation. Once distributed inside cells, NHC is phosphorylated to its corresponding triphosphate anabolite (NHC-TP) and acts as a competitive alternative substrate for virally encoded RdRp. Owing to the ability of the N4-hydroxycytosine base of NHC to tautomerize, NHC-TP can pair with either guanosine or adenosine, and consequently can substitute for either CTP or UTP, respectively [Ref. 4.3: 05JF06, 06G33J, 06G338]. This results in an accumulation of mutations in the viral genome which inhibits replication.

The error catastrophe mechanism of action for MOV/NHC has been validated for MERS-CoV, VEEV, MHV and IAV, which, when grown in the presence of NHC, have significantly increased levels of mutations [Ref. 4.3: 05JF05, 05JF0D, 05JF0F]. Multi-log decreases in infectious virus yields were observed after treatment with NHC. As a consequence of this mechanism of action, the generation of drug-resistant escape mutants is considered unlikely. Data generated to date regarding the activity of NHC against viruses resistant to other nucleoside analogs showed that NHC was active against coronavirus resistant to remdesivir in cell culture assays [Sec. 2.6.2: 2.1.4], [Sec. 2.6.2: 2.1.5], [Ref. 4.3: 05K8L0]. NHC was also effective in vitro against SARS-CoV-2 variants of concern [Sec. 2.6.2: 2.1.3] [Sec. 2.6.2: 2.1.4].

MOV and NHC inhibit replication of viral pathogens from multiple RNA virus families, including pathogenic coronavirus such as SARS-CoV-2. Antiviral activity has been verified in animal models of coronavirus virus infection.

MOV, an oral direct-acting antiviral agent, will address the critical need for different therapeutic agents to treat COVID-19. MOV is administered orally enabling patients to initiate therapy in an outpatient setting. This flexibility in outpatient management would help to limit direct patient interactions with health care staff and decrease transmission risk, which is significant due to the highly contagious nature of SARS-CoV-2. In vitro, NHC has a high genetic barrier to the development of resistance in nonclinical models and retains activity against SARS-CoV-2 and MHV (a related coronavirus) harbouring remdesivir resistance-associated mutations in the viral polymerase.

The recommended dose of MOV in adults is 800 mg Q12H for 5 days. Nonclinical exposure multiples of NHC to humans were based on population pharmacokinetics analysis in adult patients with COVID-19 from Part 1 of P001 and P002 clinical trials, where a 800 mg Q12H MOV dose resulted in systemic mean exposures ($AUC_{0\text{-}24hr}$) of 75.6 µM*hr and C_{max} of 10.8 µM. MOV $AUC_{0\text{-}24hr}$ exposure multiples were not calculated given that MOV is rapidly hydrolyzed to NHC and is generally undetectable in human plasma (LOQ of 0.0152 µM). Of note, MOV and NHC C_{max} achieved in the nonclinical studies were much higher than the clinical C_{max} of 0.026 µM and 10.8 µM, respectively, in COVID-19 patients at the clinical dose of 800 mg Q12H. There was no NHC binding to plasma proteins observed at any

concentration or in any species tested and therefore plasma protein binding was not factored into the fold-margin estimates relative to C_{max}.

Primary pharmacology studies demonstrating the antiviral activity of MOV against SARS-CoV-2, including variants of concern and remdesivir resistance-associated variants, and other RNA viruses were conducted in vitro and in mouse, Syrian hamster, and ferret models of viral infection.

Studies involving two-drug combinations were conducted to determine the potential effect of drug combinations on the antiviral activity against SARS-CoV-2. Additional investigations were performed to analyze whether NHC produces mitochondrial toxicity. MOV and NHC off target activities against a panel of enzymes, receptors, or ion channels tested were evaluated.

In addition, potential effects of MOV and/or NHC on cardiovascular, neurological, and respiratory function were assessed in several in vivo and in vitro safety pharmacology models, consistent with ICH S7A and S7B guidelines. An overview of the pharmacology studies conducted with MOV or NHC is presented in [Sec. 2.6.3: 1].

A nonclinical pharmacokinetic program was carried out to evaluate the ADME properties of MOV and the nucleoside NHC. Pharmacokinetic studies were conducted in mice, rats, dogs, monkeys and ferrets, as well as the distribution and exposure of NHC-TP in tissues. Interspecies comparison of metabolism, excretion and plasma protein binding was also conducted. In addition, MOV and NHC were evaluated as a substrate, inhibitor, or inducer of various metabolizing enzymes and transporters. An overview of the pharmacokinetic studies conducted with MOV or NHC is presented in [Sec. 2.6.5: 1].

MOV was evaluated in a comprehensive toxicology program, including genotoxicity assays (including Ames assays, in vitro and in vivo micronucleus assays, an in vivo Pig-a assay in rats, and an in vivo mutation assay in Big Blue® transgenic rats), tolerability/DRF studies in mice, rats, and dogs, repeat-dose toxicity studies of up to 3 months in rats and 1 month in mice and dogs, fertility studies in male and female rats, embryo-fetal developmental toxicity studies in rats and rabbits, and a pre- and post-natal developmental toxicity study in rats. Additional non pivotal short-term tolerability and/or TK studies were conducted in mice, rats, rabbits, and monkeys. An overview of the nonclinical toxicity studies is presented in [Sec. 2.6.7: 1].

Both the rat and dog are accepted animal models for the toxicological assessment of drugs as per current regulatory guidance (i.e., ICH M3(R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals). MOV has been shown to have acceptable in vitro and in vivo metabolism profiles and acceptable in vivo PK profiles in both rat and dog with formation of the NHC metabolite and NHC-TP anabolite in each species. The pharmacological target of NHC-TP is the exogenous viral RdRp.

All pivotal nonclinical studies were conducted in accordance with the principles of GLP. Safety pharmacology studies were conducted at [redacted]

[redacted] Canada; [redacted] USA; Merck Research Laboratories, West Point, Pennsylvania USA; or [redacted] USA. Toxicity studies were conducted at [redacted] USA [redacted] USA [redacted] USA [redacted] USA [redacted] USA [redacted] USA [redacted] USA; Merck Research Laboratories, West Point, Pennsylvania USA; or [redacted] Canada. The USA and Canada are members of OECD and therefore part of the OECD Mutual Acceptance of Data system. GLP Compliance statements are included in all pertaining study reports. All pivotal studies were supported by toxicokinetic evaluation of MOV and NHC within the study or from studies conducted under the same conditions utilizing identical doses administered using the same formulation [Sec. 2.6.7: 2]; [Sec. 2.6.7: 3]. Test article batches used in the GLP nonclinical toxicity studies are provided in [Sec. 2.6.7: 4]. MOV impurities and degradation products were qualified according to ICH Q3A(R2) and ICH Q3B(R2) guidelines [Sec. 2.4: 4.7.6].

The nonclinical studies conducted with MOV are discussed below. Overall, the nonclinical safety profile supports the safe use of MOV for the treatment of COVID-19 in adult patients.

2 PHARMACOLOGY

2.1 Primary Pharmacodynamics

An overview of the primary pharmacology studies conducted with MOV or NHC is presented in [Sec. 2.6.2: 2.1] and [Sec. 2.6.2: 2.2] and [Sec. 2.6.3: 1].

2.1.1 Primary Pharmacodynamics In Vitro

In vitro, NHC has demonstrated specific, dose-dependent inhibition of viral replication with sub- to low-micromolar EC_{50} values against SARS-CoV-2, SARS-CoV-1, and MERS-CoV, in multiple cell types, including Vero E6, Huh-7, Calu-3 lung epithelial cells, and A549-ACE2 cells.

NHC was equally effective in vitro against SARS-CoV-2 variants of concern B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), B.1.1.529 (Omicron), BA.1 (Omicron), and BA.1.1 (Omicron), variant of interest C.37 (Lambda), and variant being monitored B.1.621 (Mu) compared with the WA1 isolate in a cytopathic effect protection assay in Vero E6 cells. Based on its mechanism of action, NHC is expected to be active against newly emergent spike variants as well. MSD plans to continue to evaluate NHC's activity as novel variants of concern are identified.

NHC had comparable activity (EC_{50} values <1.6-fold) against SARS-CoV-2 replicons with remdesivir resistance-associated amino acid substitutions in NSP12 (polymerase) and against MOV treatment-emergent NSP12 and NSP14 (exonuclease) variants in a reporter replicon assay. NHC was efficiently taken up by cell lines, human, dog, and rat PBMCs, and HBEC ALI cultures, and phosphorylated to mono-, di-, and tri-phosphate forms. NHC was [redacted]

phosphorylated to NHC-TP in a dose-dependent manner in human, dog, and rat PBMCs. NHC was rapidly catabolized in PBMCs and cell lines after 24 hours of treatment. NHC-TP was rapidly metabolized in HBEC ALI cultures by 24 hours after removal of NHC, with NHC-TP concentrations higher than for NHC-DP or NHC-MP. NHC-TP was detectable at least 24 hours after treatment with 10 µM NHC in HBEC ALI cultures.

The antiviral activity of NHC did not correlate with cellular cytotoxicity concentrations as observed CC_{50} values were generally greater than the highest concentration evaluated (i.e., selectivity index [SI] values >4 in most studies).

Prolonged in vitro exposure (14 days) of CEM and HepG2 cells with MOV did not result in mitochondrial toxicity or dysfunction.

NHC also demonstrated in vitro antiviral activity against IAV H1N1, IAV H3N2, IAV H5N1, IAV H7N9, IBV, VEEV, EEEV, CHKV, and EBOV in cell culture assays.

2.1.2 Primary Pharmacodynamics In Vivo

Primary pharmacology in vivo studies demonstrating the antiviral activity of MOV and NHC against SARS-CoV-2 and other RNA viruses were conducted in mouse, ferret, Syrian hamster, and guinea pig models of viral infection.

MOV (500 mg/kg) significantly reduced infectious SARS-CoV-2 levels in lung tissue from LoM when administered 12 hours prior, 24 hours post, or 48 hours post direct infection of human lung tissue, followed by twice daily dosing thereafter [Ref. 4.3: 05R3M8]. In a ferret model, treatment with twice daily (5 mg/kg) MOV reduced SARS-CoV-2 infectious viral titers in nasal secretions from infected ferrets and suppressed all viral transmission to the untreated direct contacts, despite prolonged direct proximity between source and contact animals [Ref. 4.3: 05NRF7]. In a Syrian hamster model of SARS-CoV-2 infection and disease, MOV prophylactic or therapeutic treatment showed decreased viral RNA titers and infectious virus from lungs several days post infection [Ref. 4.3: 05R3LW].

In a Syrian hamster model of SARS-CoV-2 infection and disease using either the B.1-G (Wuhan strain), B.1.1.7 (Alpha), or B.1.351 (Beta) variants of SARS-CoV-2, treatment with MOV resulted in statistically significant reductions in viral RNA in lungs and in infectious virus lung titers regardless of variant [Ref. 4.3: 06FGX5].

MOV or NHC also showed antiviral activity in models of SARS-CoV-1, MERS-CoV, IAV H1N1, VEEV, and EBOV [Ref. 4.3: 05K8L0], [Ref. 4.3: 05JF09], [Ref. 4.3: 05JF0D], [Ref. 4.3: 05JM4T], [Ref. 4.3: 06CK43], [Sec. 2.6.3: 1].

2.2 Secondary Pharmacodynamics

Neither MOV nor NHC exhibited off-target activities of clinical concern [Sec 2.6.2: 3].

NHC was tested in an assay for inhibition potential against a panel of ion channels, including Cav1.2 (CYL5051QU), HCN4 (CYL5012HT), hERG (CYL5038), KCNQ1/minK

(CYL5007QU), Kir2.1 (CYL5032QU), Kv1.5 (CYL5018QU), Kv4.3/KChlP2 (CYL5069QU), and Nav1.5 (Cardiac Protocol) (CYL5004QU). There was no observed inhibition of function of any ion channel tested with NHC at 10 µM in this study.

In a second larger study, neither MOV nor NHC exhibited ≥50% inhibition at 10 µM against 107 of the 108 enzymes, receptors, or ion channels tested. MOV and NHC inhibited COX-2 by 62% and 52%, respectively, at 10 µM, with estimated IC_{50} values of 6.33 µM and 15.1 µM, respectively. NHC IC_{50} was 5.7- to 47-fold above the NHC EC_{50} for antiviral activity against SARS-CoV-2 in various cell lines in vitro (EC_{50} = 0.32 to 2.66 µM) and is also above the clinical C_{max} (10.8 µM) at the human dose of 800 mg Q12H. MOV IC_{50} is 243-fold above the MOV clinical C_{max} (0.026 µM) at the 800 mg Q12H dose.

NHC-TP had calculated IC_{50} values >1000 µM under physiological concentrations of dNTPs for human DNA polymerases α, β, and γ.

2.3 Safety Pharmacology

Safety pharmacology studies consistent with ICH S7A and S7B guidelines were performed to determine the cardiovascular, respiratory, and neurobehavioral effects in well-characterized nonclinical safety pharmacology models. MOV was devoid of effects on CNS, respiratory, or cardiovascular functions in well-characterized safety pharmacology models [Sec. 2.6.3: 4].

There were no neuropharmacological and body temperature effects in male rats administered single oral doses of MOV up to 500 mg/kg (TT #[redacted]-7812, [Sec. 2.6.3: 4]) [NHC C_{max} exposure 16-fold the NHC clinical C_{max} value of 10.8 µM at the 800 mg Q12H human dose (extrapolated from TK data for males from the 28-day toxicity study)]. In the in vitro hERG assays (TT #[redacted]-7807 and TT #[redacted]-9400, [Sec. 2.6.3: 4]), the IC_{50} value of MOV was estimated to be greater than 30 µM (>1000-fold above the MOV clinical C_{max} [0.026 µM] at the 800 mg Q12H dose) and the IC_{50} value of NHC was greater than 300 µM (28-fold above the NHC clinical C_{max} of 10.8 µM at the 800 mg Q12H dose). There were no hemodynamic, ECG, and body temperature changes in conscious, adult, telemetered beagle dogs administered single oral doses of MOV up to 50 mg/kg (TT #[redacted]-7811 and TT#[redacted]-5603, [Sec. 2.6.3: 4]) (5-fold the NHC C_{max} value of 10.8 µM at the 800 mg Q12H human dose, extrapolated from the TK data for males in the 28-day toxicity study in dogs). There were no changes in respiratory rate, tidal volume, or minute volume in conscious rats administered single oral doses of MOV up to 500 mg/kg (TT #[redacted]-7813, [Sec. 2.6.3: 4]) [NHC exposure 16-fold above the NHC C_{max} value of 10.8 µM at the 800 mg Q12H human dose, extrapolated from TK data for males from the 28-day toxicity study in rats]. The MOV C_{max} exposure margins at the NOEL for the in vivo safety pharmacology studies were ≥500 mg/kg in rats (23-fold higher than the human MOV C_{max} of 0.026 µM) or ≥50 mg/kg in dogs (322-fold higher than the human MOV C_{max} of 0.026 µM), extrapolated from the TK data for males in the respective 28-day studies in rats or dogs.

2.4 Pharmacodynamic Drug Interactions

NHC was evaluated alone and in combination across a range of concentrations with 3TC, abacavir, FTC, hydroxychloroquine, nelfinavir, remdesivir, ribavirin, sofosbuvir, or [redacted]

tenofovir. Antiviral activity against SARS-CoV-2 was determined for each compound alone and in combination by measuring the reduction of the SARS-CoV-2 cytopathic effect on infected Vero cells. NHC, nelfinavir, and remdesivir when tested alone demonstrated antiviral activity with EC_{50} values of 1 µM, 0.7 µM, and 1.7 µM, respectively. No cytotoxicity was observed for any compound tested (CC_{50} >20 µM) with the exception of nelfinavir, which was cytotoxic at high concentrations (CC_{50} = 11 µM). Neither synergy nor antagonism was observed for antiviral activity in vitro against SARS-CoV-2 for NHC in combination with 3TC, abacavir, FTC, hydroxychloroquine, nelfinavir, remdesivir, ribavirin, sofosbuvir, or tenofovir [Sec. 2.6.2: 5].

3 PHARMACOKINETICS

The MOV and NHC nonclinical ADME studies are listed in the Pharmacokinetics Overview Table [Sec. 2.6.5: 1].

3.1 Analytical Methods and Validation Reports

For pharmacokinetic and toxicokinetic studies, 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 [Sec. 2.6.5: 2]. For GLP toxicology studies, the bioanalytical methods were validated in accordance with FDA Guidance for Industry for the quantitation of MOV and NHC in plasma for nonclinical species. The analytical method validation reports are summarized in [Sec. 2.6.5: 3].

The total radioactivity in plasma, urine, feces and bile samples was determined by LSC. MOV and its metabolites in liver microsomes, hepatocytes, plasma, urine, and feces 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.

3.2 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 (only very low levels of MOV detected in plasma). The bioavailability of NHC after an oral dose of MOV in rats and dogs was 52% and ≥77%, respectively, and near complete absorption of [^{14}C]MOV-related radioactivity (>90%) was observed in rat and dog mass balance studies. Oral dosing studies with MOV have been completed in mouse, rat, dog, ferret, and monkey [Sec. 2.6.5: 4] [Sec. 2.6.5: 5] [Sec. 2.6.5: 6] [Sec. 2.6.5: 7] [Sec. 2.6.5: 8] [Sec. 2.6.5: 9].

3.3 Distribution

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). MOV, NHC, and NHC-TP were quantified in major tissues (lung, spleen, kidney, liver, heart and brain) from mice, rats, dogs, monkeys and ferrets following single or multiple oral doses of MOV (exposures in bone marrow were also assessed in rats). In general, MOV was either not detected or was near the detection limit in all tissues analyzed. NHC and NHC-TP were observed in all tissues and their exposures were generally dose dependent. [Sec. 2.6.5: 10] [Sec. 2.6.5: 11] [Sec. 2.6.5: 12] [Sec. 2.6.5: 13] [Sec. 2.6.5: 14].

The unbound fraction of NHC was approximately 1 in CD-1 mouse, SD rat, beagle dog, cynomolgus monkey and human plasma, as well as when tested with human alpha1-acid glycoprotein and human serum albumin [Sec. 2.6.5: 15]. The plasma binding of MOV was not assessed as it is unstable in plasma, but it is presumed similar to NHC.

3.4 Metabolism

[^{14}C]MOV was extensively metabolized in hepatocytes, and its metabolic profiles were qualitatively similar in rat, dog, monkey, and human hepatocytes. Hydrolysis to NHC was the major route of metabolism, followed by formation of the endogenous pyrimidines uridine and/or cytidine (NHC differs from cytidine by only one hydroxyl) [Sec. 2.6.5: 17.7].

The recovery of MOV-related radioactivity in excreta from male BDC Wistar Han rats and intact beagle dogs was low, and in rats, the majority of the dose was ultimately recovered from the carcasses. Metabolite profiling analysis of excreta showed that other than NHC, the majority of radioactivity was associated with endogenous pyrimidines. In rats, a small amount of both methylated and glucuronidated NHC were also observed in urine (each $\leq$1% of the total dose). The retention of radioactivity in rats and dogs suggests that the majority of the dose was ultimately metabolized to endogenous pyrimidine metabolites, similar to what was observed in vitro (uridine, cytidine, etc.), which then mix with the endogenous pyrimidine pool. The only metabolites of [^{14}C]MOV observed in rat or dog plasma were NHC and endogenous pyrimidine derivatives [Sec. 2.6.5: 16].

In human plasma and urine following oral administration of MOV, NHC, and the nucleosides cytidine and/or uridine were detected by LC/MS/MS. All exhibited approximately dose-dependent increases in exposures, indicating that some amount of the endogenous pyrimidines were derived from the oral dose of MOV. 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 [Sec. 2.6.5: 16]. As was determined in animals, the only major metabolites anticipated to circulate in human plasma besides NHC are endogenous pyrimidine derivatives.

3.5 Excretion

The recovery of [^{14}C]MOV-related radioactivity in excreta from BDC rats (72 hr) and intact dogs (96 hr) was low (<13%) indicating that the majority of the dose was retained in the body. Elimination of [^{14}C]NHC in urine was <1% of the dose in rats and 8% of the dose in

C Confidential

dogs. In rats, 54% of the dose was ultimately recovered from the carcasses (overall recovery 67%). The low recovery in rats and dog excreta was anticipated given a major route of metabolism of [^{14}C]MOV in vitro was the ultimate formation of uridine and/or cytidine, which in vivo would mix with the endogenous nucleoside pools and remain in the body. In addition, some release of [^{14}C]-labeled CO_2 is also anticipated to have occurred in these studies since [^{14}C]uracil and/or [^{14}C]3-ureidopropionic acid where also observed and are known to be further metabolized to release CO_2 [Sec. 2.6.5: 19].

3.6 Pharmacokinetic Drug Interactions

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

Near complete hydrolysis of MOV to NHC occurs during absorption/first pass, with the high capacity esterases CES1 and CES2 involved. 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. Preclinical in vitro and in vivo metabolism studies suggest the ultimate route of elimination of MOV/NHC-related material is primarily metabolism to endogenous pyrimidine nucleosides (uridine and/or cytidine). 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 [Ref. 4.3: 06CBPM], and cytidine deaminase readily converts NHC to uridine [Sec. 2.6.5: 20.4]. In vitro, NHC was found to be a substrate of the human nucleoside transporters CNT1, CNT2, CNT3, and ENT2 while MOV was a comparatively weak substrate of CNT1, and neither MOV nor NHC were substrates of human MDR1 P-gp or BCRP [Sec. 2.6.5: 30]. These data are consistent with published data that NHC is a substrate of both ENT1 and ENT2 transporters [Ref. 4.3: 07WZTW]. Based on these data, other drugs are not anticipated to affect the tissue levels of NHC-TP resulting from an oral dose of MOV.

3.6.2 Inhibition of Drug Metabolizing Enzymes and Transporters

In vitro studies demonstrated that neither MOV nor NHC are inhibitors of major human CYPs (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) or major human drug transporters (OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, MATE2K, MRP2, MDR1 P-gp and BCRP. Therefore, the overall risk of MOV or NHC to cause CYP- or transporter-mediated DDIs due to inhibition is low at clinically relevant concentrations [Sec. 2.6.5: 21] [Sec. 2.6.5: 28] [Sec. 2.6.5: 31] [Sec. 2.6.5: 35].

3.6.3 Induction of Cytochrome P450 Enzymes

In vitro studies demonstrated that neither MOV nor NHC are inducers of major human CYPs (1A2, 2B6, and 3A4). Therefore, the potential for MOV or NHC to cause DDIs due to the induction of CYPs is low at clinically relevant concentrations [Sec. 2.6.5: 29].

3.7 Other Pharmacokinetic Studies

All pertinent nonclinical pharmacokinetic studies are included in Modules 4.2.2.1 through 4.2.2.6 and provide adequate data for regulatory assessment. No additional nonclinical pharmacokinetic studies were conducted, therefore no study reports are included in Module 4.2.2.7.

4 TOXICOLOGY

MOV was evaluated in a comprehensive nonclinical toxicology program, including genotoxicity assays (including Ames assays, in vitro and in vivo micronucleus assays, an in vivo Pig-a assay in rats, and an in vivo mutation assay in Big Blue® transgenic rats), tolerability/DRF studies in mice, rats, and dogs, repeat-dose toxicity studies of up to 3 months in rats and 1 month in mice and dogs, fertility studies in male and female rats, embryo-fetal developmental toxicity studies in rats and rabbits, and a pre- and post-natal developmental toxicity study in rats. Additional non pivotal short-term tolerability and/or TK studies were conducted in mice, rats, rabbits, and monkeys [Sec. 2.6.7: 1].

4.1 Single-Dose Toxicity

Single-dose toxicity was evaluated as part of DRF studies (Phase A) in mice, rats, and dogs [Sec. 2.6.6: 3]. The results of single-dose toxicity assessments are included with the study reports for TT #[redacted]-7850, TT #[redacted]-7825, TT #[redacted]-7826 which can be found in Module 4.2.3.2 (no stand-alone single-dose toxicity reports are included in Module 4.2.3.1). No mortality was noted during Phase A of the DRF studies in mice, rats, and dogs. In CD1 mice given single oral doses of NHC up to 2000 mg/kg (TT #[redacted]-7850 – Phase A), there were decreases in food consumption in both sexes at 1500 mg/kg, body weight loss in females at doses ≥1500 mg/kg, and decreased body weight gain in males at 2000 mg/kg during the first few days post-treatment. In rats given single doses of MOV at doses up to 2000 mg/kg, MOV-related changes included mean body weight loss and decreases in food consumption for males and females at 2000 mg/kg during the first few days post-treatment (TT #[redacted]-7825 – Phase A). In dogs given single doses of MOV up to 2000 mg/kg, minimal, transient GI effects, including emesis and soft feces occurred at ≥300 mg/kg and body weight loss in all animals occurred by Day 3, along with decreases in food consumption (TT #[redacted]-7826 – Phase A).

4.2 Repeat-Dose Toxicity

4.2.1 Mice

In Phase B of a 7-day dose range-finding study in CD1 mice, the MTD was the highest dose tested, 1000 mg/kg/day (Day 7 NHC AUC_{0-24} = 142.35 µM*hr; C_{max} = 51.31 µM) (TT #[redacted]-7850, [Sec. 2.6.7: 6]).

In a 28-day toxicity study of MOV in WT CByB6F1 Tg(HRAS)2Jic mice (TT #[redacted] 9037, [Sec. 2.6.7: 7A]), there were decreases in mean body weight gain in males at all doses, 100 (-28%), 300 (-24%), 700 (-56%), and 2000 (-77%) mg/kg/day compared to controls and

decreases in mean body weight gain in females at 700 (-30%) and 2000 (-30%) mg/kg/day. Therefore, the NOAEL was 300 mg/kg/day in both sexes (NHC AUC_{0-24hr} exposure 1-fold the clinical NHC exposure at 800 mg Q12H, extrapolated from the 7-day oral gavage TK study in WT CByB6F1 Tg(HRAS)2Jic mice; TT #[redacted]-9011, [Sec. 2.6.7: 17C]) , based on the adverse decreases in body weight gain (which reached at least -30%) at doses ≥700 mg/kg/day.

4.2.2 Rats

In Phase B of a 7-day dose-range-finding study in rats, the MOV dose of 2000 mg/kg/day (61/91 (F/M)-fold the NHC exposure at the 800 mg Q12H human dose) was associated with clinical signs of toxicity (soiling, piloerection, unkempt appearance, and evidence of dehydration), statistically significantly ($p<0.01$) decreased body weights in males (-15% compared to controls), and changes in hematology parameters, including decreased RET counts (-89%), and white blood cell and absolute lymphocyte counts (-52%) compared to controls (TT #[redacted]-7825, [Sec. 2.6.7: 6]). High-dose males had clinical chemistry changes that included slightly (generally ≤2 fold compared to controls) increased liver enzymes (ALT and AST). High-dose females showed only mild elevations of ALT, calcium, glucose, and inorganic phosphorus. The improved tolerability in females compared to males at 2000 mg/kg/day is likely attributable to 1.4- to 3.4-fold lower exposures in females compared to males.

MOV was well tolerated when administered to rats at doses of 50, 150, or 500 mg/kg/day for 28 days (TT #[redacted]-7814, [Sec. 2.6.7: 7B]). The only test article-related finding noted was increased liver weights (12-17% in females and males, respectively relative to brain weight) at the end of the treatment phase for the 500 mg/kg/day males and females. Lymphocyte counts were within expected values for SD rats at 500 mg/kg/day. At the end of the treatment and recovery phases, no changes were noted on evaluation of bone marrow (including myeloid:erythroid ratios) at 500 mg/kg/day. Therefore, the NOAEL was ≥500 mg/kg/day (AUC_{last} = ~319 [female]/590 [male] µM*hr, 4.2-fold [female] and 7.8-fold [male] the NHC exposure at the 800 mg Q12H human dose).

In the 3-month toxicity study in rats, decreases in mean body weight gain occurred at all doses in males, 150 (-13%), 500 (-25%) and 1000 (-55%) mg/kg/day, and at 500 (-8%) and 1000 (-9%) mg/kg/day in females, compared to concurrent controls (TT #[redacted] 1014, [Sec. 2.6.7: 7C]). The decreases in body weight gain correlated with very slight to slight decreases in weekly mean food consumption in these groups. There were no changes in body weight and food consumption in low-dose females (200 mg/kg/day). Growth plate findings were observed in the bones of males at ≥500 mg/kg/day (5.4-fold the clinical NHC exposure at the 800 mg Q12H dose) and in females at 1000 mg/kg/day (9.3-fold the clinical NHC exposure at the 800 mg Q12H dose). These findings consisted of an increase in the thickness of the growth plate/physis in the femur and tibia, leading to decreased osteogenesis with variably decreased trabecular bone in the metaphysis in males at 1000 mg/kg/day and thickening of the growth cartilage of the epiphysis of long bones and patella in males and females at 1000 mg/kg/day. Minimal thickening of growth cartilage of the epiphysis was observed in males at 500 mg/kg/day. There were no changes observed in remaining tissues in

the femorotibial joint, including articular surfaces, ligaments, synovial tissue, other soft tissue, and the cortical bone. Additional cartilage findings observed only in the 3-month toxicity study in rats included a minimal to mild cytoplasmic alteration in chondrocytes of the tracheal cartilage in males only at ≥ 500 mg/kg/day. This change was not associated with degenerative or inflammatory changes, did not impact the overall structure or integrity of the cartilage, did not impact airway size or diameter, and was consequently non-adverse. There were no growth plate or cartilage findings at 150 mg/kg/day in males (0.7-fold the clinical NHC exposure at 800 mg Q12H) and at 500 mg/kg/day in females (3.3-fold the clinical NHC exposure at 800 mg Q12H). Overall, the NOAELs were 150 mg/kg/day (AUC_{0-24hr} of 52.2 µM*hr) in males (0.7-fold the clinical NHC AUC_{0-24hr} at the 800 mg Q12H dose) and 500 mg/kg/day (AUC_{0-24hr} of 250 µM*hr) in females (3.3-fold the clinical NHC AUC_{0-24hr} at the 800 mg Q12H dose), based on adverse growth plate findings in in males (in addition to the notable decreased body weight gain) at ≥500 mg/kg/day and in females at 1000 mg/kg/day.

4.2.3 Dogs

In Phase B of the 7-day dose range-finding study in dogs (TT #[redacted]-7826, [Sec. 2.6.7: 6]), doses of 300 and 1000 mg/kg/day exceeded the MTD. These dose groups were early terminated due to clinical signs of toxicity (including recumbency, pale mucous membranes, ulceration in the oral cavity) and notable body weight losses. Progressive, dose-responsive mean body weight loss, compared to pretreatment weights, typically occurred by Day 4 in dogs administered ≥300 mg/kg/day and by Day 5 in the 100 mg/kg/day groups with correlative decreases in food consumption. In general, treatment-related clinical signs during Phase B (including decreased activity, emesis, diarrhea, and partial eyelid closure) were observed in males administered ≥300 mg/kg/day and in all MOV female groups. Hematological changes were limited to marked decreases in absolute lymphocyte counts in dogs administered ≥300 mg/kg/day [likely secondary to glucocorticoid response (stress) associated with severity of clinical signs at this non-tolerated dose]. Dose-responsive changes in the clinical chemistry parameters, relative to standard reference ranges, occurred at doses ≥300 mg/kg/day, and included increases in AST (approximately 30-fold at 1000 mg/kg/day, and 2-fold at 300 mg/kg/day) as well as increased ALP, ALT (up to 5-fold at 1000 mg/kg/day only), total cholesterol, and triglycerides, with decreased potassium (K), chloride (Cl), and calcium (Ca). Macroscopic observations occurred in all MOV-treated groups and were primarily associated with the GI tract (including the mouth, esophagus, and large and small intestines). These observations included the presence of red and/or darkened foci, discolored mucosa (dark, red, and/or green), and thickened mucosa. Dose-responsive, organ weight changes were noted most often in the reproductive organs as well as in the liver, spleen, and thymus of animals administered ≥300 mg/kg/day.

In the 28-day toxicity study in dogs (TT [redacted]-7815, [Sec. 2.6.7: 7D]), the treatment phase for animals given 17 or 50 mg/kg/day was terminated early due primarily to marked body weight loss, prolonged inappetence, and/or marked hematology findings. Animals at 50 mg/kg/day received their last dose on Day 12 or 14, while animals at 17 mg/kg/day received their last dose on Day 21(males) or 22 (females). Animals from the main phase were necropsied on the day following the last dose. Recovery necropsies for animals dosed with 50 mg/kg/day

(2/sex/group) were performed on Study Day 23/24, whereas recovery animals at 17 mg/kg/day were necropsied at the end of the 28-day recovery phase for the study. Clinical signs of toxicity were limited to the 17 and 50 mg/kg/day groups and included red or black feces, decreased activity, and red areas and/or mild hemorrhaging of the oral mucosa. Necropsy findings (terminal and recovery) seen most extensively in 50 mg/kg/day males included red discoloration of the digestive tract (from oral mucosa and esophagus to large intestine) and urinary bladder, gallbladder, or mediastinal tissue (among other tissues). Similar GI changes occurred in 1 female dosed with 50 mg/kg/day at terminal necropsy only. Discolorations (red/dark involving fewer tissues) were also present in both sexes in terminal animals dosed at 17 mg/kg/day. The discoloration was attributable to hemorrhage and was most likely the result of severe thrombocytopenia observed at ≥17 mg/kg/day in males and females (described below). With the exception of 1 high-dose male with an oral erosion/ulcer, the areas of mucosal hemorrhage had histologically normal overlying epithelium, further supporting the likelihood hemorrhages were secondary to extremely low (<14,000 × 10^3 cells/µL) platelet counts observed in animals dosed at ≥17 mg/kg/day. At recovery necropsy, some GI discoloration (reddening) was still present, predominantly in the large intestines, of animals dosed at 50 mg/kg/day, likely attributable to the short recovery period relative to other dose groups. At the recovery necropsy, no GI discoloration (reddening) was seen in animals dosed at 17 mg/kg/day.

Adverse histomorphological bone marrow changes affecting all hematopoietic cell lines and causing subsequent hematological abnormalities (including total WBC count, lymphocytes, neutrophils, reticulocytes, RBCs and platelets) occurred at doses ≥17 mg/kg/day with minor, nonadverse hematological changes at the low-dose, 6 mg/kg/day. The hematologic changes were relatively mild at Day 7 and more severe hematologic changes were not apparent until after 14 to 21 days of continuous dosing. The hematopoietic changes were partially reversible following the ~1-week recovery period for the 50 mg/kg/day group, or fully reversible following the ~1-month recovery period for the 17 mg/kg/day group (with essentially normal hematology after a longer recovery period at doses ≤17 mg/kg/day). Focal hemorrhages (mostly minimal) observed following the dosing period in several animals at ≥17 mg/kg/day (most prominently in the GI tract) were considered to be secondary to severe thrombocytopenia.

In conclusion, the NOAEL and MTD was 6 mg/kg/day (AUC_{last} = 9.53 µM*hr, 0.13-fold the NHC exposure at the 800 mg Q12H human dose) primarily due to adverse bone marrow toxicity that affected all hematopoietic cell lines at ≥17 mg/kg/day (AUC_{last} of ~30 µM*hr [Day 1], 0.4-fold the NHC exposure at the 800 mg Q12H human dose). Hematologic changes were relatively mild at Day 7 with more severe changes apparent after 14 to 21 days of continuous dosing. The bone marrow changes were partially reversible following the ~1 week recovery period (50 mg/kg/day group) and fully reversible following an ~1-month recovery period (17 mg/kg/day).

4.3 Genotoxicity

The mutagenic and genotoxic potential of MOV was comprehensively assessed in standard and follow-up regulatory in vitro and in vivo assays designed to detect potential effects on

genes (ie, mutations); the overall conclusion was that MOV was not mutagenic or genotoxic in vivo. MOV and NHC induced mutations in the bacterial reverse mutation assay (TT #[redacted]-7810, [Sec. 2.6.7: 8B] and TT #[redacted]-7851, [Sec. 2.6.7: 8A]). MOV did not induce chromosome damage in in vitro or in vivo assays (TT #[redacted]-7806, [Sec. 2.6.7: 8C] and TT #[redacted]-7816, [Sec. 2.6.7: 9A]). Consistent with current regulatory guidances (ICH S2[R1], ICH M7[R1], WHO/IPCS) which recommend follow-up in vivo testing to address the biological/in vivo relevance of in vitro positive results; two robust in vivo mutagenicity studies were performed in rats to understand the in vivo relevance of in vitro positive mutagenicity assays. Both Pig-a mutagenicity (TT #[redacted]-7808, [Sec. 2.6.7: 9B]) and Big Blue® (cII Locus) transgenic (TT #[redacted]-9025, [Sec. 2.6.7: 9C]) rodent assays were conducted per recommended guidelines by international groups [Ref. 4.3: 05RB64, 07WXW2, 07WXX2] and OECD TG 488 [Ref. 4.3: 07WXW5], respectively. These assays included evaluation of standard tissues/cell populations including bone marrow (rapid proliferating tissue) and liver (slow proliferating tissue; high metabolizing capacity) for the Big Blue® (cII Locus) transgenic rodent assay, and reticulocytes and red blood cells for the Pig-a mutagenicity assay. The animals were administered MOV at doses up to the maximum tolerated dose, 500 mg/kg/day, and for longer durations (28 days) than the proposed 5-day clinical administration of MOV in humans. The negative results of these in vivo mutagenicity assays provide strong evidence that the in vitro mutagenicity is not relevant to in vivo mammalian systems. Potential reasons for lack of translation of in vitro findings to in vivo mammalian systems include differences in metabolism, pharmacokinetics, exposure, replication, and DNA repair processes within a whole animal model compared with in vitro test conditions.

4.4 Carcinogenicity

Carcinogenicity studies have not been completed and are not required to support this marketing authorization application for the treatment of COVID-19 in adults, based on the short duration of treatment. Therefore, no study reports are included in Module 4.2.3.4.

4.5 Reproductive and Developmental Toxicity

4.5.1 Fertility and early embryonic development

There were no MOV-related effects on female (TT #[redacted]-9000, [Sec. 2.6.7: 12A]) or male (TT #[redacted]-9012, [Sec. 2.6.7: 12B]) fertility, or on early embryonic development up to the highest dose tested, 500 mg/kg/day, (2.1/6.1-fold (female/male) the clinical NHC exposure at 800 mg Q12H).

4.5.2 Embryo-fetal development

In pregnant rats administered MOV during the organogenesis period (TT #[redacted]-7054, [Sec. 2.6.7: 13A] and TT #[redacted]-7000, [Sec. 2.6.7: 13B]), developmental toxicity consisted of embryolethality (post-implantation losses), malformations/teratogenicity (abnormal and/or small eye/eye socket, absent kidney and axial skeleton abnormalities) and variations (cervical ribs and trace supernumerary ribs) at 1000 mg/kg/day (7.5-fold the clinical NHC exposure at 800 mg Q12H), and reduced fetal growth at ≥500 mg/kg/day (≥2.9-fold the clinical NHC exposure at 800 mg Q12H). There was no developmental toxicity at doses up to 250 [redacted]

mg/kg/day (0.8-fold the clinical NHC exposure at 800 mg Q12H). Maternal toxicity included decreased food consumption and body weight loss, resulting in the early sacrifice of individual animals at 1000 mg/kg/day and decreased body weight gain at ≥500 mg/kg/day.

In pregnant rabbits (TT #[redacted]-7064, [Sec. 2.6.7: 13C] and TT #[redacted]-7010, [Sec. 2.6.7: 13D]) developmental toxicity was limited to reduced mean fetal body weights at 750 mg/kg/day (18-fold the clinical NHC exposure at 800 mg Q12H). There was no developmental toxicity at up to 400 mg/kg/day (6.5-fold the clinical NHC exposure at 800 mg Q12H). Maternal toxicity included decreased food consumption and body weight gain, and abnormal fecal output at ≥400 mg/kg/day.

Overall, embryolethality (post-implantation losses) and teratogenicity were limited to rats exposed to 7.5-fold the clinical NHC exposure at 800 mg Q12H during the organogenesis period. These developmental findings were not observed in rats up to 2.9-fold the clinical NHC exposure at 800 mg Q12H and rabbits up to 18-fold the clinical NHC exposure at 800 mg Q12H.

4.5.3 Prenatal and postnatal development, including maternal function

There were no MOV-related effects on growth, sexual maturation, or on neurobehavioral or reproductive function in F1 generation rats following oral administration of MOV (100, 250 or 500 mg/kg/day) to F0 females from GD 6 through LD 20 (TT #[redacted]-9010, [Sec. 2.6.7: 14]). Findings in F0 dams were limited to non-adverse transient decreases in mean body weight gain during pregnancy at ≥250 mg/kg/day. Therefore, the NOAEL for maternal toxicity in the F0 females and developmental toxicity in the F1 offspring was ≥500 mg/kg/day (1.6-fold the clinical NHC exposure at 800 mg Q12H).

4.5.4 Studies in which the offspring (juvenile animals) are dosed and/or further evaluated

Studies in juvenile animals have not been completed and are not required to support this marketing authorization application for the treatment of COVID-19 in adults. Therefore, no study reports are included in Module 4.2.3.5.4.

4.6 Local Tolerance

Local tolerance was assessed as part of the repeat-dose toxicity studies [Sec. 2.4: 4]. There was no evidence of a local effect of MOV on the gastrointestinal tract in the GLP repeat-dose toxicity studies in mice, rats or dogs. The focal hemorrhages observed in the gastrointestinal tract in the 28-day dog study were attributable to severe thrombocytopenia.

In addition, two local tolerance studies (a bovine corneal opacity and permeability test and a MatTek EpiDerm™ MTT viability assay; (TT #[redacted]-7801 and TT #[redacted]-7802, [Sec. 2.6.7: 16])), were conducted in support of the occupational safety program. MOV was classified as a mild irritant in the BCOP assay and as a very mild irritant in the MatTek EpiDerm™ assay.

[redacted]

4.7 Other Toxicity Studies

4.7.1 Antigenicity/Immunogenicity

There were no observations or changes considered to be due to potential antigenicity induced by the test article in the repeat-dose toxicity studies [Sec. 2.6.6: 3]. Therefore, no antigenicity/immunogenicity evaluations were conducted, and no study reports are included in Module 4.2.3.7.1.

4.7.2 Immunotoxicity

MOV is not associated with a cause for immunotoxicity concern and no additional immunotoxicity study was conducted. The hematopoietic/bone marrow toxicity and associated hematological findings observed in dogs at NHC AUC exposure lower than the clinical NHC AUC exposure affected all blood progenitor cell lines, including immune system progenitor cells. According to ICH S8 guideline, specific assays to determine immunotoxicity for such compounds are not valuable in drug risk assessment. It is also important to note that similar findings were not observed in rats exposed to 15-fold the clinical NHC AUC exposure for 3 months, in mice exposed to 19-fold the clinical NHC AUC exposure for 1 month, in nonhuman primates exposed to 4-fold the clinical NHC AUC exposure for 1 week, and in rabbits exposed to 29-fold the clinical NHC AUC exposure for 2 weeks, and that there were no hematologic changes indicative of bone marrow toxicity or immunosuppression in clinical trials.

With the exception of the bone marrow toxicity in dogs only, there were no direct MOV-related alterations in immune system organ weights and/or histology (e.g., changes in thymus, spleen, lymph nodes). The decreased spleen and thymus weights, and the decreased cellularity of the lymphoid tissue of the spleen, thymus and lymph node observed at 1000 mg/kg/day in male rats 3-month rat study were secondary to stress [Ref. 4.3: 07XDNW] associated with notable decreased body weight gain noted at this dose (-55% compared to controls).

There were no changes in serum globulins in any nonclinical species. There were no infections or tumors observed in the repeat-dose toxicity studies.

In conclusion, aside from the well-characterized monitorable and reversible hematopoietic/bone marrow toxicity and associated hematologic findings observed in dogs, there was no indication of immunotoxicity observed in the repeat-dose toxicity studies. As such, in accordance with ICH S8 Note for Guidance on Immunotoxicity Studies for Human Pharmaceuticals, no additional immunotoxicity studies are warranted.

In a local lymph node assay in mice (TT #[redacted]-7803, [Sec. 2.6.7: 17A]) conducted in support of the occupational safety program, MOV was considered a dermal sensitizer.

[redacted]

4.7.3 Mechanistic studies

The mechanism of action of MOV efficacy was assessed as part of the primary pharmacodynamics studies. Therefore, no additional mechanistic studies were conducted and hence, no study reports are included in Module 4.2.3.7.3.

4.7.4 Dependence

There was no indication that MOV/NHC has a pharmacologic profile (including off-target profile) consistent with drug abuse liability potential. In addition, NHC-TP levels were shown to be lower in the brain compared to other tissues (i.e. lung and spleen) in the tissue distribution studies in rats, dogs, monkeys and ferrets [Sec. 2.4: 3.3], and there was no evidence of psychoactive effects (i.e., sedative or stimulant effects) in the safety pharmacology or routine repeat-dose toxicity studies. As such, no abuse potential studies were conducted in animals, and no study reports are included in Module 4.2.3.7.4.

4.7.5 Metabolites

Based on in vitro and in vivo nonclinical metabolism data detailed below, there is no expectation of human circulating metabolites other than the well-characterized NHC and endogenous pyrimidines and/or pyrimidine anabolites/catabolites, and therefore no metabolite toxicity studies were conducted, and no study reports are included in Module 4.2.3.7.5.

In vitro in rat, dog and human hepatocytes, [^{14}C]MOV is rapidly hydrolyzed to [^{14}C]NHC, which is then metabolized to [^{14}C]cytidine and/or [^{14}C]uridine with further anabolism and catabolism of these endogenous nucleosides [Sec. 2.4: 3]. Based on metabolism studies in rats and dogs with [^{14}C]MOV, the only metabolites identified in plasma were [^{14}C]NHC and [^{14}C]cytidine, [^{14}C]uridine and their anabolites/catabolites. A human ADME study with labeled MOV is not planned given the majority of the [^{14}C]MOV dose in animals appeared to be converted to endogenous [^{14}C]pyrimidine anabolites/catabolites and therefore were retained in the body.

4.7.6 Impurities

MOV impurities and degradation products were qualified according to ICH Q3A(R2) and ICH Q3B(R2) guidelines. NHC (proposed specification: [redacted]%) is a significant metabolite of MOV in rats, dogs, and humans, and is therefore qualified per ICH Q3A(R2) and ICH Q3B(R2) guidelines. The animal/human dose multiple for the [redacted] (also known as [redacted]), qualified by virtue of its presence in the batch evaluated in the 28-day oral toxicity study in rats with a 14-day recovery period (TT #[redacted]-7814), is presented in [Table 2.4: 1]. The Maximum Qualified Level parameter was calculated for the daily clinical dose of 1600 mg (800 mg Q12Hr).

Table 2.4: 1 Impurity and Dose Multiples

Impurity	Toxicology Study Content % impurity	NOAEL[a]	Maximum Qualified Level[b,c]
[redacted]	[redacted][d]	500 mg/kg/day	[redacted]%

a NOAEL (No Observe Adverse Effect Level) from the Oral 28-Day Toxicology and Toxicokinetics Study in Rats with a 14-Day Recovery (TT#[redacted]-7814) using API batch [redacted].

b Based on a clinical dose of 1600 mg [800 mg B.I.D.].

c Qualified level = [% impurity x non-clinical dose (mg/kg/day)] / [body surface area conversion factor (i.e., 5 for rats) x maximum clinical dose (i.e., 32 mg/kg/day for 50 kg body weight and 1600 mg clinical dose)].

d [redacted].

In addition, potential and actual impurities, including identified impurities in each starting material, intermediate, and drug substance/product, have been assessed for mutagenic potential according to ICH M7(R1) guideline, as described in Quality documents [Sec. 3.2.S.3.2] (Drug Substance) and [Sec. 3.2.P.5.5] (Drug Product).

4.7.7 Other

4.7.7.1 Photoreactivity Assessment of MOV and NHC

MOV and NHC absorb light between 290 and 700 nm with a MEC > 1000 $M^{-1}cm^{-1}$ (TT #[redacted]-7814, [Sec. 2.6.7: 17B]). However, both MOV and NHC were shown to not generate ROS, including superoxide anion and singlet oxygen, at an aqueous concentration of 200 µM, and were not photoreactive. As such, MOV and NHC have low probability of being phototoxic in humans, and further phototoxicity testing is not warranted per ICH S10, Photosafety Evaluation of Pharmaceuticals guideline.

4.7.7.2 Other Studies

Other studies in rasH2 WT mice (hybrid), Sprague Dawley and Fischer 344 rats, rabbits, dogs, and rhesus monkeys were completed. These studies detailed in [Sec. 2.6.6: 8.7.2] (TT #[redacted]-9011, TT #[redacted]-1015, TT #[redacted]-7043 and TT #[redacted]-1005; [Sec. 2.6.7: 17C]) and [Sec. 2.6.6: 8.7.3] (TT#[redacted]-9027, TT #[redacted]-9047 and TT #[redacted]-7817; [Sec. 2.6.7: 17D]), were generally conducted in support and/or preparation for the pivotal studies, and their results do not impact the nonclinical toxicity profile of MOV.

5 INTEGRATED OVERVIEW AND CONCLUSIONS

The mechanism of antiviral activity of MOV is viral error catastrophe, a concept that is predicated on increasing the viral mutation rate beyond a biologically tolerable threshold, resulting in impairment of viral fitness and leading to viral extinction.

In vitro, NHC shows broad-spectrum activity against multiple viruses including coronaviruses SARS-CoV-2, SARS-CoV, and MERS-CoV. NHC was equally effective in

vitro against SARS-CoV-2 variants of concern B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), B.1.1.529 (Omicron), BA.1 (Omicron), and BA.1.1 (Omicron), variant of interest C.37 (Lambda), and variant being monitored B.1.621 (Mu) compared with the WA1 isolate in a cytopathic effect protection assay in Vero E6 cells. NHC was active against coronaviruses that have mutations which reduce susceptibility to remdesivir in cell culture assays. NHC was active against SARS-CoV-2 replicons with remdesivir resistance-associated amino acid substitutions in NSP12 (polymerase) and MOV treatment-emergent NSP12 and NSP14 (exonuclease) variants in a reporter replicon assay.

NHC was efficiently taken up by cell lines, PBMCs, and HBEC ALI cultures, and phosphorylated to mono-, di-, and tri-phosphate forms. NHC was phosphorylated to NHC-TP in a dose-dependent manner in human, dog, and rat PBMCs. The phosphorylated forms of NHC are catabolized in a few hours in cell lines and PMBCs after 24 hours of treatment with NHC. NHC-TP is detectable at least 24 hours after treatment with 10 μM NHC in HBEC ALI cultures.

The antiviral activity of NHC did not correlate with cellular cytotoxicity concentrations.

Prolonged in vitro exposure (14 days) of CEM and HepG2 cells with MOV did not result in mitochondrial toxicity or dysfunction.

Antiviral activity of MOV was also demonstrated in animal infection models of SARS-CoV-2, MERS-CoV, and influenza. MOV (500 mg/kg) significantly reduced infectious SARS-CoV-2 levels in lung tissue from LoM when administered 12 hours prior, 24 hours post, or 48 hours post direct infection of human lung tissue, followed by twice daily dosing thereafter. In a ferret model, treatment with MOV reduced SARS-CoV-2 infectious viral titers in nasal secretions from infected ferrets and suppressed all viral transmission to the untreated direct contacts, despite prolonged direct proximity between source and contact animals. In a Syrian hamster model of SARS-CoV-2 infection and disease, MOV prophylactic or therapeutic treatment showed decreased viral RNA titers and infectious virus from lungs several days post infection. In a Syrian hamster model of SARS-CoV-2 infection using either the B.1-G (Wuhan strain), B.1.1.7 (Alpha), or B.1.351 (Beta) variants of SARS-CoV-2, treatment with MOV resulted in statistically significant reductions in infectious virus lung titers regardless of variant.

Neither MOV nor NHC exhibited off-target activities of clinical concern. There was no impact on the in vitro antiviral activity of NHC against SARS-CoV-2 in two-drug combination studies with 3TC, abacavir, FTC, hydroxychloroquine, nelfinavir, remdesivir, ribavirin, sofosbuvir, or tenofovir. NHC-TP had calculated IC_{50} values >1000 μM under physiological concentrations of dNTPs for human DNA polymerases α, β, and γ.

MOV was devoid of effects on CNS, respiratory, or cardiovascular functions in well-characterized safety pharmacology models.

MOV is an ester prodrug that when orally administered in rats and dogs resulted in high bioavailability of NHC, and generally provided dose-proportional oral exposures to NHC in all preclinical species tested. NHC distributes widely into major animal tissues where it is

[redacted]

converted to the active triphosphate NHC-TP, which incorporates into viral RNA and results in viral error catastrophe and inhibition of replication. In rats and dogs, excretion of NHC in urine was a minor route of elimination, while metabolism of NHC to the endogenous pyrimidines uridine and cytidine appears to be the main route of elimination. Similar metabolism was observed in hepatocytes from all species including human. Based on *in vitro* 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.

MOV was evaluated in a comprehensive toxicology program including genotoxicity assays (including Ames assays, in vitro and in vivo micronucleus assays, an in vivo Pig-a assay in rats, and an in vivo mutation assay in Big Blue® transgenic rats), tolerability/DRF studies in mice, rats, and dogs, repeat-dose toxicity studies of up to 3 months in rats and 1 month in mice and dogs, fertility studies in male and female rats, embryo-fetal developmental toxicity studies in rats and rabbits, and a pre- and post-natal developmental toxicity study in rats. Additional non pivotal short-term tolerability and/or TK studies were conducted in mice, rats, rabbits, and monkeys.

The MOV $AUC_{0\text{-}24hr}$ exposure multiples were not calculated given that MOV is rapidly hydrolyzed to NHC and is generally undetectable in human plasma (LLOQ of 0.0152 µM). The C_{max} at the NOAEL in rat (150 mg/kg/day) and dog (6 mg/kg/day) of 0.557 µM and 0.42 µM, respectively are much higher than the human plasma concentrations, which are generally below the LLOQ of 0.0152 µM.

NHC systemic $AUC_{0\text{-}24hr}$ exposure multiples are shown in [Table 2.4: 2].

[redacted]

C Confidential

Table 2.4: 2 NHC Systemic Exposure Multiples

Species	Study Duration or Study Type	Dose Levels (mg/kg/day)	$AUC_{0\text{-}24hr}$ (µM*hr) (F/M)[a]	Exposure Multiple Relative to 800 mg Q12H Clinical Dose[b] (F/M)
Repeat-Dose Toxicity				
Mouse (Wild-Type CByB6F1 Tg(HRAS)2 Jic)	1-Month[c] (TT #[redacted]-7825)	100	29.6	0.4
		300 (NOAEL)	76.8	1.0
		700	236	3.1
		2000	1420	19
Rat	1-Month (TT #[redacted]-7814)	50	9.7/21	0.1/0.3
		150	42/108	0.6/1.4
		500 (NOAEL)	319/590	4.2/7.8
	3-Month (TT #[redacted]-1014)	150 (M only) (NOAEL for M)	52.2	0.7
		200 (F only)	67.6	0.9
		500 (NOAEL for F)	250/408	3.3/5.4
		1000	706/1120	9.3/15
Dog	1-Month (TT #[redacted]-7815)	6 (NOAEL)	9.5	0.1
		17	29.7	0.4
		50	118.8	1.6
Reproductive and Developmental Toxicity				
Rat	Fertility (TT #[redacted]-9000, TT #[redacted]-9012)	100	14.9/45.6	0.2/0.6
		250	61.4/179	0.8/2.4
		500 (NOAEL for general toxicity and fertility - F & M)	157/463	2.1/6.1
	EFD (TT #[redacted]-7000)	100	18.4	0.2
		250 (NOAEL for maternal and developmental toxicity)	58.3	0.8
		500	217[d]	2.9
		1000[e]	570[f]	7.5
Rabbit	EFD (TT #[redacted]-7010)	125 (NOAEL for maternal toxicity)	111	1.5
		400 (NOAEL for developmental toxicity)	490	6.5
		750	1360	18

[redacted]

Confidential

Species	Study Duration or Study Type	Dose Levels (mg/kg/day)	$AUC_{0\text{-}24hr}$ (µM*hr) (F/M)[a]	Exposure Multiple Relative to 800 mg Q12H Clinical Dose[b] (F/M)
Rat	PPND (TT #[redacted]-9010)	100	9.12	0.1
		250	62.6	0.8
		500 (NOAEL for maternal and developmental toxicity)	122	1.6

AUC=area under the concentration-time curve; $AUC_{0\text{-}24hr}$=AUC from time 0 to 24 hours; F=female; GD=Gestation Day; M=male; NC=Not calculated; NHC=EIDD-1931 or N-hydroxycytidine; NOAEL=no-observed-adverse-effect level; Q12H=every 12 hours.

a Data presented as sexes combined (Wild-Type CByB6F1 Tg(HRAS)2Jic mice), F/M (repeat-dose rat and dog toxicity studies) or females only (pEFD and EFD toxicity studies).

b $AUC_{0\text{-}24hr}$ exposure multiples were calculated using clinical exposures of 75.6 µM*hr at 800 mg Q12H, based on population PK estimates of exposure in COVID-19 patients from Part 1 of P001/P002.

c Exposures for the 28-day toxicity study in Wild-Type CByB6F1 Tg(HRAS)2Jic mice (TT #[redacted]-9037) were obtained from the 7-day TK study in Wild-Type CByB6F1 Tg(HRAS)2Jic mice (TT #[redacted]-9011).

d Value is from the GLP pEFD rat study (TT #[redacted]-7054). Due to inaccurate plasma sample volumes collected 0.5, 1, 2 and 4 hours postdose at 500 mg/kg/day, no AUC0-24 hr value could be calculated for that dose group in the definitive EFD rat study (TT #[redacted]-7000).

e Dose used in the GLP pEFD rat study only (TT #[redacted]-7054). Due to maternal toxicity including mortality, the dose of 1000 mg/kg/day was not selected for the definitive EFD study; the high dose of the definitive EFD rat study (TT #[redacted]-7000) was 500 mg/kg/day.

f Value from the GLP pEFD rat study (TT #[redacted]-7054).

Below, NHC exposure multiples were calculated relative to the clinical exposure at the human dose of 800 mg Q12H, corresponding to NHC exposure of 75.6 µM*hr (based on population PK estimates of exposure in COVID-19 patients from P001/P002).

Genotoxicity/mutagenicity risk assessment: The integrated assessment of the mutagenic and genotoxic potential of MOV indicates that MOV is not mutagenic or genotoxic in in vivo mammalian systems. Based on the totality of the genotoxicity data, MOV is of low risk of genotoxicity in clinical use.

Target Organs of Toxicity: Target organs of toxicity identified in the GLP repeat-dose toxicity studies included bone marrow in dogs only, and growth plate in rats only. In addition, gastrointestinal toxicity and liver enzyme elevations were noted in the rat and dog 7-day tolerability studies at doses that exceeded the MTDs, 2000 mg/kg/day in rats and ≥300 mg/kg/day in dogs.

Bone marrow/hematopoietic findings: In the 28-day repeat-dose toxicity study in dogs (doses of 6, 17, and 50 mg/kg/day), mild dose-related hematologic findings affecting all cell lines were observed following 7 days of dosing. More severe hematologic changes were apparent after 14 to 21 days of continuous dosing leading to pancytopenia, including severe thrombocytopenia and associated hemorrhage at 17 mg/kg/day (0.4-fold the clinical NHC

[redacted]

exposure at 800 mg Q12H) and 50 mg/kg/day (2-fold the clinical NHC exposure at 800 mg Q12H). This resulted in early termination of treatment at 50 mg/kg/day after 2 weeks of dosing and at 17 mg/kg/day after 3 weeks of dosing. These hematologic changes were secondary to decreased cellularity of the bone marrow hematopoietic precursors (bone marrow depletion) observed at postmortem examinations. The hematopoietic changes were fully reversible at 17 mg/kg/day with essentially normal bone marrow and hematologic parameters following a 1-month recovery period. Animals in the 50 mg/kg/day group were euthanized approximately 10 days after treatment termination; hematopoietic changes were partially reversible during the shortened recovery period. Only minor, reversible, nonadverse hematopoietic changes were seen in animals administered 6 mg/kg/day for 28 days. The NOAEL in dogs was therefore 6 mg/kg/day (0.13-fold the NHC exposure at the 800 mg Q12H human dose).

The bone marrow/hematopoietic findings are of low risk in humans based on the following:

- Hematological changes observed after 7 days of dosing in dogs in the 28-day study were relatively mild compared with later time points and the hematopoietic and hematologic changes demonstrated reversibility upon discontinuation of treatment.
- The proposed duration for treatment of COVID-19 is 5 days, and the clinical safety database shows no hematological effects indicative of bone marrow toxicity.
- There were no hematopoietic findings in rats dosed for 3 months up to 1000 mg/kg/day (9.3/15-fold (female/male) the clinical NHC exposure at 800 mg Q12H), or in mice dosed for 1 month up to 2000 mg/kg/day (19-fold the clinical NHC exposure at 800 mg Q12H). There were no hematologic changes in female rabbits dosed for 2 weeks up to 1000 mg/kg/day (29-fold the clinical NHC exposure at 800 mg Q12H), or in rhesus monkeys dosed for 7 days up to 500 mg/kg/day (3.6-fold the clinical NHC exposure at 800 mg Q12H). Decreased hematology values, notably RETs (89%), white blood cells, and absolute lymphocyte counts (52%), were only observed at 2000 mg/kg/day in a 7-day rat DRF; (61/91-fold (female/male) the clinical NHC exposure at 800 mg Q12H).

Growth plate findings: Changes in the growth plate of bones were observed in the 3-month oral toxicity study in rats, in males at ≥500 mg/kg/day (5.4-fold the clinical NHC exposure at the 800 mg Q12H dose) and in females at 1000 mg/kg/day (9.3-fold the clinical NHC exposure at the 800 mg Q12H dose). The findings consisted of an increase in the thickness of the growth plate (physis), associated with decreased bone formation at the end of long bones in males at 1000 mg/kg/day and thickening of the growth cartilage of the epiphysis of long bones and in patella of males and females at 1000 mg/kg/day. Minimal thickening of epiphyseal growth cartilage was also observed in males at 500 mg/kg/day. There were no changes observed in remaining tissues in the femorotibial joint, including articular surfaces, ligaments, synovial tissue, other soft tissue, and the cortical bone. There were no growth plate findings in rats dosed for 1 month up to 500 mg/kg/day (4.2/7.8-fold (female/male) the clinical NHC exposure at the 800 mg Q12H dose), in dogs dosed for up to 14 days at 50 mg/kg/day (1.6-fold the clinical NHC exposure at the 800 mg Q12H dose), or in mice dosed

for 1 month at doses up to 2000 mg/kg/day (19-fold the clinical NHC exposure at 800 mg Q12H).

These growth plate findings are not relevant to adult humans because growth plates are no longer present in the mature skeleton of adult humans. The growth plate findings (observed in rats after 3 months of treatment, but not after 1-month of dosing) are of unclear significance in the context of short duration treatment in children. The findings were noted in rapidly growing rats (5 to 6 weeks of age at study start) that approximately doubled their body weights over the study duration. This very rapid bone growth cannot be directly translated to humans due to species-specific differences in skeletal development and bone turnover [Ref. 4.3: 05NK2R]. Additional cartilage findings observed only in the 3-month toxicity study in rats included a minimal to mild cytoplasmic alteration in chondrocytes of the tracheal cartilage in males only at ≥500 mg/kg/day. This change was not associated with degenerative or inflammatory changes, did not impact the overall structure or integrity of the cartilage, did not impact airway size or diameter, and was consequently non-adverse. There were no growth plate or cartilage findings at 150 mg/kg/day in males (0.7-fold the clinical NHC exposure at 800 mg Q12H) and at 500 mg/kg/day in females (3.3-fold the clinical NHC exposure at 800 mg Q12H).

Gastrointestinal toxicity and liver enzyme elevations: Additional findings including GI findings (e.g., stomach distention, emesis, diarrhea) and liver enzyme elevations were noted in the 7-day tolerability studies at high doses that exceeded the MTDs (2000 mg/kg/day in rats, 61/91-fold (female/male) the clinical NHC exposure at 800 mg Q12H and ≥300 mg/kg/day in dogs (19-fold the clinical NHC exposure at 800 mg Q12H). Liver enzyme elevations consisted of ≤2-fold increases in ALT and/or AST in rats at 2000 mg/kg/day, as well as approximately 2-fold increases in AST at 300 mg/kg/day in dogs, and up to 5-fold increases in ALP and ALT and 30-fold increases in AST at 1000 mg/kg/day in dogs (66-fold the clinical NHC exposure at 800 mg Q12H).

Developmental and reproductive toxicity: In the female and male rat fertility studies, there were no MOV-related effects on fertility or early embryonic development up to the highest dose tested, 500 mg/kg/day (2.1/6.1-fold (female/male) the clinical NHC exposure at 800 mg Q12H). In pregnant rats administered MOV during the organogenesis period, developmental toxicity including embryolethality (post-implantation losses) and malformations/ teratogenicity was observed at 1000 mg/kg/day (7.5-fold the clinical NHC exposure at 800 mg Q12H) and reduced fetal growth at ≥500 mg/kg/day (≥2.9-fold the clinical NHC exposure at 800 mg Q12H). There was no developmental toxicity at doses up to 250 mg/kg/day (0.8-fold the clinical NHC exposure at 800 mg Q12H). Maternal toxicity included decreased food consumption and body weight loss, resulting in the early sacrifice of individual animals at 1000 mg/kg/day and decreased body weight gain at ≥500 mg/kg/day. In pregnant rabbits, developmental toxicity was limited to reduced mean fetal body weights at 750 mg/kg/day (18-fold the clinical NHC exposure at 800 mg Q12H). There was no developmental toxicity at up to 400 mg/kg/day (6.5-fold the clinical NHC exposure at 800 mg Q12H). Maternal toxicity included decreased food consumption and body weight gain and abnormal fecal output at ≥400 mg/kg/day. In the pre- and postnatal developmental study in rats, there was no F0 maternal or F1 generation toxicity in up to the highest dose

evaluated of 500 mg/kg/day (1.6-fold the clinical NHC exposure at 800 mg Q12H). While embryolethality (post-implantation losses) and teratogenicity were limited to rats exposed to 7.5-fold the clinical NHC exposure at 800 mg Q12H during the organogenesis period, and these developmental findings were not observed in rats up to 2.9-fold the clinical NHC exposure at 800 mg Q12H and rabbits up to 18-fold the clinical NHC exposure at 800 mg Q12H, the use of MOV is conservatively not recommended during pregnancy. WOCBP should use effective contraception for the duration of treatment and for at least 4 days after the last dose.

Overall, the nonclinical safety profile supports the safe use of MOV for the treatment of COVID-19 in adult patients.

6 LIST OF LITERATURE REFERENCES

[Ref. 4.3: 05JF05] Agostini ML, Pruijssers AJ, Chappell JD, Gribble J, Lu X, Andres EL, et al. Small-molecule antiviral beta-D-N(4)-hydroxycytidine inhibits a proofreading-intact coronavirus with a high genetic barrier to resistance. J Virol. 2019 Dec;93(24):e01348-19.

[Ref. 4.3: 05JF06] Flavell RA, Sabo DL, Bandle EF, Weissmann C. Site-directed mutagenesis: generation of an extracistronic mutation in bacteriophage Qbeta RNA. J Mol Biol. 1974;89:255-72.

[Ref. 4.3: 05JF09] Toots M, Yoon JJ, Hart M, Natchus MG, Painter GR, Plemper RK. Quantitative efficacy paradigms of the influenza clinical drug candidate EIDD-2801 in the ferret model. Transl Res. 2020 Apr;218:16-28.

[Ref. 4.3: 05JF0D] Toots M, Yoon JJ, Cox RM, Hart M, Sticher ZM, Makhsous N, et al. Characterization of orally efficacious influenza drug with high resistance barrier in ferrets and human airway epithelia. Sci Transl Med. 2019 Oct 23;11:eaax5866.

[Ref. 4.3: 05JF0F] Urakova N, Kuznetsova V, Crossman DK, Sokratian A, Guthrie DB, Kolykhalov AA, et al. Beta-D-N(4)-hydroxycytidine is a potent anti-alphavirus compound that induces a high level of mutations in the viral genome. J Virol. 2018 Feb;92(3):e01965-17.

[Ref. 4.3: 05JM4T] Painter GR, Bowen RA, Bluemling GR, DeBergh J, Edpuganti V, Gruddanti PR, et al. The prophylactic and therapeutic activity of a broadly active ribonucleoside analog in a murine model of intranasal venezuelan equine encephalitis virus infection. Antiviral Res. 2019;171:104597.

[Ref. 4.3: 05K8L0] Sheahan TP, Sims AC, Zhou S, Graham RL, Pruijssers AJ, Agostini ML, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med. 2020;12, eabb5883. doi: 10.1126/scitranslmed.abb5883.

[Ref. 4.3: 05NK2R] Zoetis T, Tassinari M, Bagi C, Walthall K, Hurtt ME. Species comparison of postnatal bone growth and development. Birth Defects Research (Part B). 2003;68:86-110.

[Ref. 4.3: 05NRF7] Cox RM, Wolf JD, and Plemper RK. Therapeutically administered ribonucleoside analogue MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. Nat Microbiol (2020). https://doi.org/10.1038/s41564-020-00835-2.

[Ref. 4.3: 05R3LW] Rosenke K, Hansen F, Schwarz B, Feldmann F, Haddock E, Rosenke R, et al. Orally delivered MK-4482 inhibits SARS-CoV-2 replication in the Syrian hamster model. Preprint. Res Sq. 2020;rs.3.rs-86289. Published 2020 Oct 8. doi:10.21203/rs.3.rs-86289/v1.

[Ref. 4.3: 05R3M8] Wahl A, Gralinski LE, Johnson CE et al. SARS-CoV-2 infection is effectively treated and prevented by EIDD-2801. Nature (2021). https://doi.org/10.1038/s41586-021-03312-w.

[Ref. 4.3: 05RB64] Gollapudi BB, Lynch AM, Heflich RH, Dertinger SD, Dobrovolsky VN, Froetschl R, et al. The in vivo Pig-a assay: A report of the International Workshop on Genotoxicity Testing (IWGT) Workgroup. Mutat Res. 2015;(783):23-35.

[Ref. 4.3: 06CBPM] Krompholz N, Krischkowski C, Reichmann D, Garbe-Schonberg D, Mendel RR, Bittner F, et al. The mitochondrial Amidoxime Reducing Component (mARC) is involved in detoxification of N-hydroxylated base analogues. Chem Res Toxicol. 2012;25:2443-50.

[Ref. 4.3: 06CK43] Reynard O, Nguyen XN, Alazard-Dany N, Barateau V, Cimarelli A, Volchkov VE. Identification of a new Ribonucleoside inhibitor of Ebola virus replication. Viruses. 2015;7:6233-40. Erratum in: Viruses. 2016;8:137.

[Ref. 4.3: 06FGX5] Abdelnabi R, Foo CS, De Jonghe S, Maes P, Weynand B, Neyts J. Molnupiravir inhibits the replication of the emerging SARS-CoV-2 variants of concern (VoCs) in a hamster infection model. (Accepted Manuscript) J Infectious Diseases, 2021;jiab361, https://doi.org/10.1093/infdis/jiab361.

[Ref. 4.3: 06G338] Kabinger F, Stiller C, Schmitzová J, Dienemann C, Kokic G, Hillen HS, et al. Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis. Nat Struct Mol Biol. 2021 Aug 11.

[Ref. 4.3: 06G33J] Gordon CJ, Tchesnokov EP, Schinazi RF, Götte M. Molnupiravir promotes SARS-CoV-2 mutagenesis via the RNA template. J Biol Chem. 2021 Jul;297(1):100770.

[Ref. 4.3: 07WXW2] The in vivo erythrocyte Pig-a gene mutation assay. Part 1: Detailed review paper and performance assessment. Prepared by Heflich RH, Dertinger SD, Dobrovolsky VN, Bhalli JA, Kenyon MO, Kimoto T, et al. for the Organisation for Economic

Cooperation and Development Working Group of the National Coordinators of the Test Guidelines Programme, 20-MAY-2019.

[Ref. 4.3: 07WXW5] OECD Guidelines for the Testing of Chemicals. Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays - Test Guideline No. 488, 26-JUN-2020.

[Ref. 4.3: 07WXX2] Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides, and Biotechnology: The in vivo erythrocyte Pig-a gene mutation assay – Part 2 – Validation report. Organisation for Economic Co-operation and Development (OECD). Series on Testing and Assessment No. 316, 16-JUL-2020.

[Ref. 4.3: 07WZTW] Miller SR, McGrath ME, Zorn KM, Ekins S, Wright SH, Cherrington NJ. Remdesivir and EIDD-1931 interact with human equilibrative nucleoside transporters 1 and 2: implications for reaching SARS-CoV-2 viral sanctuary sites [manuscript]. 2021. 35 p.

[Ref. 4.3: 07XDNW] Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, et al. Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. Toxicol Pathol. 2013;41(4):560-614.

[redacted]