目　次

目　次 2
表 一 覧 3
図 一 覧 4
略号一覧 5
1 まとめ 8
1.1 吸収 8
1.2 分布 8
1.3 代謝 9
1.4 排泄 10
1.5 薬物間相互作用 10
2 分析法 11
2.1 放射性同位体標識 LNP023 11
2.2 放射能分析法 11
2.3 LNP023 の定量法 11
2.4 代謝物の特性評価及び構造解析 12
3 吸収 13
3.1 吸収及びバイオアベイラビリティ 13
3.2 LNP023 の薬物動態 13
3.3 不純物 A*の吸収 17
3.4 トキシコキネティクス 17
3.4.1 ラットにおけるトキシコキネティクス 17
3.4.2 イヌにおけるトキシコキネティクス 17
3.4.3 サルにおけるトキシコキネティクス 18
3.4.4 生殖発生毒性試験におけるトキシコキネティクス 18
3.4.5 がん原性試験におけるトキシコキネティクス 19
3.4.6 光毒性試験におけるトキシコキネティクス 19
3.4.7 不純物の毒性試験におけるトキシコキネティクス 19
4 分布 20
4.1 LNP023 の血液／血漿分布及び血漿蛋白結合 20
4.2 ラットにおける組織分布 21
5 代謝（動物種間の比較） 22
5.1 *In vitro* 代謝 23
5.1.1 肝細胞及び細胞分画における *in vitro* 代謝 23
5.1.2 LNP023 の酸化的代謝に関与する酵素の同定 24
5.1.3 LNP023 のグルクロン酸抱合代謝に関与する酵素の同定 25
5.1.4 構造アラート及び共有結合 26

＊新薬承認情報提供時に置き換えた

5.2 *In vivo* 代謝 26
5.2.1 ラットにおける *in vivo* 代謝 26
5.2.2 イヌにおける *in vivo* 代謝 27
5.2.3 動物におけるヒト循環血漿中代謝物の網羅性 28
5.2.4 ヒト代謝物の薬理活性 28
6 排泄 28
6.1 尿及び糞中排泄 28
6.2 胆汁中排泄 29
7 薬物動態学的薬物間相互作用 30
7.1 酵素阻害 30
7.1.1 CYP 酵素の可逆的阻害 30
7.1.2 CYP 酵素の時間依存的阻害 30
7.1.3 UGT 酵素の阻害 31
7.2 酵素誘導 31
7.3 膜透過性及び肝細胞系における輸送 32
7.4 トランスポーターの基質となる可能性 33
7.4.1 排出トランスポーターの基質となる可能性 33
7.4.2 取り込みトランスポーターの基質となる可能性 33
7.5 トランスポーター阻害 34
7.5.1 排出トランスポーター阻害 34
7.5.2 取り込みトランスポーター阻害 35
8 その他の薬物動態試験 36
9 考察及び結論 37
10 参考文献 39
11 図表 39

表一覧

Table 3-1 動物に ^{14}C-LNP023 を単回静脈内及び経口投与したときの血漿中総放射能の PK パラメータ（平均値） 14
Table 3-2 動物に ^{14}C-LNP023 又は LNP023 を単回静脈内投与したときの血漿中 LNP023 の PK パラメータ（平均値） 15
Table 3-3 動物に ^{14}C-LNP023 又は LNP023 を単回経口投与したときの血漿中 LNP023 の PK パラメータ（平均値） 16
Table 4-1 LNP023 の血液／血漿分布及び血漿蛋白結合 21
Table 6-1 ラットに ^{14}C-LNP023 を単回静脈内及び経口投与したときの ^{14}C-LNP023 由来放射能及び LNP023 の排泄 29
Table 11-1 LNP023 を単回投与した PK／ADME 試験一覧 40

Table 11-2 動物及びヒトにおける LNP023 の代謝物 41

図 一 覧

Figure 2-1 ^{14}C 標識 LNP023 の化学構造及び標識部位 11
Figure 5-1 ラット，イヌ，及びヒトにおける LNP023 の代謝 23

略号一覧

略号	省略していない表現（英）	省略していない表現（日）
-	not available	データなし
AAG	α1-acid-glycoprotein	α1-酸性糖蛋白質
ADME	absorption, distribution, metabolism, and excretion	吸収・分布・代謝・排泄
AMP	adenine monophosphate	アデニル酸（アデノシン一リン酸）
ATP	adenosine triphosphate	アデノシン三リン酸
AUC	area under the drug plasma (blood / tissue) concentration-time curve	血漿（血液／組織）中薬物濃度-時間曲線下面積
AUC0-t	AUC from time zero to time t	血漿中薬物濃度-時間曲線下面積（0～t 時間）
AUCinf	AUC from time zero to infinity	血漿中薬物濃度-時間曲線下面積（0～無限大時間）
AUClast	AUC from time zero to the time of the last quantifiable concentration	血漿（血液／組織）中薬物濃度-時間曲線下面積（0～最終定量可能時点）
BA	bioavailability	バイオアベイラビリティ
BCRP	breast cancer resistance protein	乳癌耐性蛋白質
bid	bis in die	1 日 2 回
BSEP	bile salt export pump	
Cb	concentration in blood	血液中濃度
Cbc	concentration in blood cells	血球中濃度
CL	clearance	クリアランス
CLb	blood clearance	血液クリアランス
CLh,b	hepatic blood clearance	肝血液クリアランス
CLint	intrinsic clearance	固有クリアランス
CLint,app	apparent intrinsic clearance	見かけの固有クリアランス
CLp	plasma clearance	血漿クリアランス
Cmax	maximum drug plasma (blood / tissue) concentration	最高血漿（血液／組織）中薬物濃度
Cp	concentration in plasma	血漿中濃度
CYP	cytochrome P450	チトクローム P450
eq	equivalents	当量
fb	fraction bound in plasma	血漿中結合画分
FB	complement factor B	補体 B 因子
fBC	fraction in blood cells	血球中分率
FMO	flavin monooxygenase	フラビンモノオキシゲナーゼ
fp	fraction in plasma	血漿中分率
fu	fraction unbound in plasma	血漿中非結合画分
HPLC	high performance liquid chromatography	高速液体クロマトグラフィー
HSA	human serum albumin	ヒト血清アルブミン
IC50	half maximal (50%) inhibitory concentration	50%阻害濃度
iv	intravenous(ly)	静脈内
Ki	inhibition constant (reversible inhibition)	阻害定数（可逆的阻害）

略号	省略していない表現（英）	省略していない表現（日）
KI	inhibition constant (time-dependent inhibition)	阻害定数（時間依存的阻害）
kinact	maximum inactivation rate constatnt	最大不活性化速度定数
Km,app	apparent Michaelis constant	見かけのミカエリス定数
KO	knockout	ノックアウト
LC-MS	liquid chromatography-mass spectrometry	液体クロマトグラフィー質量分析
LC-MS/MS	liquid chromatography-tandem mass spectrometry	液体クロマトグラフィータンデム質量分析
LLOQ	lower limit of quantification	定量下限
LNP023	iptacopan	イプタコパン
LOAEL	lowest observed adverse effect level	最小毒性量
LOD	limit of detection	検出限界
LSC	liquid scintillation counter	液体シンチレーションカウンター
MATE	multidrug and toxin extrusion	
MRP	multidrug resistance associated protein	多剤耐性関連蛋白質
na	not applicable	該当なし
nd	not detected	検出されず
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)	ニコチンアミドアデニンジヌクレオチドリン酸（還元型）
NOAEL	non observed adverse effect level	無毒性量
OAT	organic anion transporter	有機アニオントランスポーター
OATP	organic anion transporting polypeptide	有機アニオントランスポーターポリペプチド
OCT	organic cation transporter	有機カチオントランスポーター
Papp	apparent permeability	見かけの透過係数
PAPS	adenosine 3’-phosphate 5’-phosphosulfate	
P-gp	P-glycoprotein	P-糖蛋白
PK	pharmacokinetic(s)	薬物動態（学）
PSint,bile	intrinsic biliary clearance	胆汁中分泌の固有クリアランス
PSc	carrier-mediated membrane permeability	輸送担体を介した膜透過クリアランス
PSm	passive membrane permeability	受動的膜透過クリアランス
po	per os	経口
QWBA	quantitative whole body autoradiography	定量的全身オートラジオグラフィー
R	correlation coefficient	相関係数
PCR	Polymerase chain reaction	ポリメラーゼ連鎖反応
SD	standard deviation	標準偏差
SLC	solute-carrier	溶質輸送体
T1/2	elimination half-life	消失半減期
Tlast	time of last quantifiable concentration	最終定量可能時点
TK	toxicokinetic(s)	トキシコキネティクス
Tmax	time to reach the maximum drug plasma (blood / tissue) concentration following drug administration	最高血漿（血液／組織）中薬物濃度到達時間
UDPGA	uridine 5’-diphospho-α-D-glucuronic acid	ウリジン 5’ジホスホグルクロン酸

略号	省略していない表現（英）	省略していない表現（日）
UGT	uridine diphospho-glucuronosyltransferase	ウリジンジホスホ-グルクロノシルトランスフェラーゼ
UPLC	ultra performance liquid chromatography	超高速液体クロマトグラフィー
Vss	distribution volume at steady state	定常状態における分布容積

1 まとめ

本項では，実験動物におけるイプタコパン（LNP023）の薬物動態（PK）／吸収・分布・代謝・排泄（ADME）試験（Table 11-1）及びトキシコキネティクス（TK）試験（2.6.7.2 項），並びにヒト試料等を用いた薬物動態学的薬物間相互作用試験の成績について要約する。

マウス，ラット，及びイヌを用いて PK 試験を実施した。また，マウス，ラット，ウサギ，イヌ，及びサルを用いた毒性試験において TK 評価を実施した。さらに，毒性試験に用いた動物種及びヒト試料等を用いた *in vitro* 試験を実施し，血漿蛋白結合，血液／血漿分布，膜透過性，代謝，及び薬物間相互作用を検討した。

ADME 試験で用いたマウス（C57BL/6），ラット［Wistar Hannover（白色），Sprague Dawley（白色），Long-Evans（有色）］，及びイヌ（Beagle）は，毒性試験で用いた動物種と一致している。PK 試験の詳細は「2.6.5 薬物動態試験概要表」に記載されている。

PK 試験において，動物に LNP023 を Table 3-1，Table 3-2，及び Table 3-3 に示した用量で経口又は静脈内投与した。各試験では毒性試験と同様の投与方法を用いた。

ADME 試験は ^{14}C 標識 LNP023 及び非標識 LNP023 を用いて実施した。また，TK 試験については非標識 LNP023 を用いて実施した。

特に記載がない限り，LNP023 は塩酸塩（遊離塩基としての分子量：422.52）として投与した。すべての投与量は遊離塩基として示した。

1.1 吸収

マウス，ラット，及びイヌに ^{14}C-LNP023 又は LNP023 を単回経口投与したとき，LNP023 の最高血漿（血液）中濃度到達時間（Tmax）は 0.25～2 時間であった。LNP023 を単回経口投与したときの吸収率は，ラットで 81.8%であった。また，単回経口投与時のバイオアベイラビリティ（BA）はマウスで 46%，ラットで 40%～68%，及びイヌで 60%であった。

^{14}C-LNP023 又は LNP023 を単回静脈内投与したとき，検討した動物種における LNP023 のクリアランス（CL）は 0.0912～0.960 L/h/kg であった。マウス，ラット，及びイヌにおける血液クリアランス（CLb）は，肝血流量（Davies and Morris 1993）と比べて低かった（肝血流量の 5.45%～14.9%）。すべての動物種において，定常状態における分布容積（Vss）は 0.6～6.6 L/kg であり，血漿における消失半減期（T1/2）は約 2.9～7.5 時間であった。

単回及び反復投与毒性試験，がん原性試験，生殖発生毒性試験，及びその他の毒性試験において TK を評価した（2.6.6 毒性試験の概要文）。TK 評価により，マウス，ラット，ウサギ，イヌ，及びサルを用いたすべての試験において LNP023 の曝露が示された。

1.2 分布

In vitro で測定した LNP023 の血液／血漿中濃度比（Cb / Cp 比）及び血漿蛋白結合率は，検討したすべての動物種で濃度依存性を示した。10～10000 ng/mL の濃度範囲で，LNP023 濃度の上昇と

ともに Cb / Cp 比は，ラットで 0.994 から 3.59，イヌで 0.717 から 1.38，及びヒトで 0.600 から 2.14 に増加した。同濃度範囲で，LNP023 濃度の上昇とともに血漿中非結合画分（fu）は，マウスで 6.31%から 37.6%，ラットで 4.03%から 37.3%，イヌで 9.47%から 35.0%，及びヒトで 1.23%（100 ng/mL）から 25.4%に増加した。これらの濃度依存性は，LNP023 の標的分子である血漿中の補体 B 因子（FB）との飽和結合に関連する可能性がある。

ラットに ^{14}C-LNP023 を単回経口投与したとき，総放射能は脳を除いて広範囲の組織に分布し，ほとんどの組織で速やかに消失した。白色ラットにおいて，投与後 2 時間までに各組織中の総放射能は最高濃度（Cmax）に到達したが，そのうち総放射能 Cmax の組織／血液比が 3 倍を超えていたのは肝臓のみであった。脳内放射能濃度は血液中放射能濃度の 1.4%～2.2%であった。有色ラットにおいて，高い総放射能の曝露量を示したのは，ブドウ膜，肝臓，眼，及び小腸であった。これらの結果より，LNP023 が眼組織のメラニンに対して親和性を有すること，及びその結合が可逆的であることが示された。

1.3 代謝

マウス，ラット，イヌ，サル，及びヒト肝細胞を用いた *in vitro* 代謝試験において，代謝物プロファイルは動物種を通じて質的に類似していた。ラットを用いた ADME 試験において LNP023 の *in vivo* 代謝を検討したとき，主要な代謝経路は N-脱アルキル化（M1 生成），O-脱エチル化（M2 及び M9），C-酸化（M4，M6，M7，及び M10），水酸化（M4，M6，及び M10），及び LNP023 のカルボキシ部位のグルクロン酸抱合［M8（[redacted]），M9（[redacted]），及び M10］であった。イヌにおいても，*in vivo* で類似した代謝経路がみられた。

ヒト肝ミクロソーム及びヒト酵素発現系を用いた *in vitro* 試験において，LNP023 の酸化的代謝に関与する主要なチトクローム P450（CYP）分子種は CYP2C8 であった。また，直接的なグルクロン酸抱合代謝に関与する主要なウリジンジホスホ-グルクロノシルトランスフェラーゼ（UGT）分子種は UGT1A1 であることが示唆された。

^{14}C-LNP023 を単回投与後のラット血漿中における主要成分は未変化体であった［静脈内及び経口投与したとき，それぞれ総放射能の血漿中薬物濃度-時間曲線下面積（AUC）の 87.7%及び 91.0%］。

動物及びヒトにおける代謝物の曝露量を比較したとき，血漿中においてヒトに特異的な代謝物は検出されなかった。ヒト代謝物（M1，M2，M6，M7，及び M8 とその異性体）の曝露量に対する雌雄のラット及びイヌの曝露量比はいずれも 0.5 を超えていた。このことは，ラット及びイヌでこれらの代謝物に対する十分な曝露が得られたことを示す。

ラットの尿中における主要成分は，未変化体であった（静脈内及び経口投与量の約 2%～3%）。ラットの糞中における主要成分は未変化体（静脈内及び経口投与量のそれぞれ 19%及び 36%）及び M2（静脈内及び経口投与量の約 30%）であった。ラットにおいて，アシルグルクロン酸抱合代謝物である M8，M9，及び M10 が胆汁中に検出されたものの糞中には認められなかったことか

ら，これらの代謝物が腸内細菌によって脱抱合を受けそれぞれのアグリコンに逆変換されたと考える。

以上より，ラットでの LNP023 の消失過程において，胆汁中及び尿中への分泌／排泄とともに主に代謝が寄与していることが示された。

1.4 排泄

ラットにおける主要な排泄経路は糞であった。すなわち，^{14}C-LNP023 を単回静脈内及び経口投与したとき，投与放射能の 84.6%～94.4%が糞中に，4.79%～5.68%が尿中に排泄された。

ラットに ^{14}C-LNP023 を単回静脈内及び経口投与したときの LNP023 の尿中排泄率は投与量の 2.07%～3.24%であった。一方，LNP023 の糞中排泄率は投与量の 19.0%～36.1%であった。

胆管カニューレを挿入したラットに ^{14}C-LNP023 を単回静脈内投与したとき，投与放射能の大部分（79.4%）が胆汁中に排泄され，そのうち LNP023 としての排泄は投与量の 11.7%であった。

1.5 薬物間相互作用

肝ミクロソームを用いた *in vitro* 試験において，LNP023 は CYP1A2，CYP2A6，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1，及び CYP3A4/5 の可逆的阻害剤ではないと考えられた。LNP023 は CYP2C8 に対して時間依存的阻害作用を示したが，CYP1A2，CYP2B6，CYP2C9，CYP2C19，CYP2D6，及び CYP3A4/5 の時間依存的阻害剤ではないと考えられた。また，UGT1A1 に対しても阻害作用を示さなかった。

ヒト初代培養肝細胞において，LNP023 は CYP3A4 に対して弱い誘導作用を示したが，CYP2B6 及び CYP2C9 を誘導しなかった。また，CYP1A2 に対しても LNP023 は誘導剤ではないと考えられた。

Caco-2 細胞の単層膜を用いた *in vitro* 膜透過性試験において，^{14}C-LNP023 を用いて細胞の頂端膜から基底膜方向への見かけの透過係数（Papp）を評価したところ，0.5 µmol/L では $1.33 \pm 0.16 \times 10^{-6}$ cm/sec で受動的に透過することが明らかとなった。また，ヒト肝細胞系を用いた試験において，LNP023 の肝細胞への取り込み過程における溶質輸送体（SLC）の関与及び胆汁中分泌における能動輸送の関与が示唆された。

トランスポーター発現細胞又は反転膜小胞を用いた *in vitro* 試験により，LNP023 は排出トランスポーターである P-gp，乳癌耐性蛋白質（BCRP），及び多剤耐性関連蛋白質（MRP）2，並びに取り込みトランスポーターである有機アニオントランスポーターポリペプチド（OATP）1B1 及び OATP1B3 の基質であることが示された。一方，有機アニオントランスポーター（OAT）1，OAT2，OAT3，及び有機カチオントランスポーター（OCT）2 の基質ではなかった。

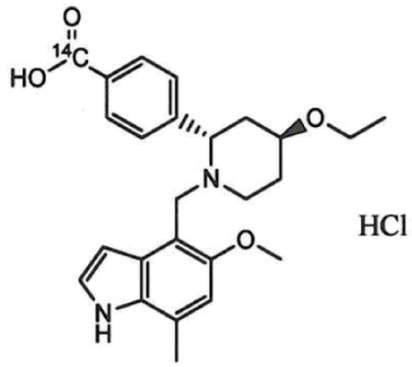
LNP023 は P-gp，OATP1B1，OATP1B3，OAT3，MATE（multidrug and toxin extrusion）1，OCT1，及び OCT2 に対して阻害作用を示した。一方，BCRP，MRP2，及び BSEP（bile salt export pump）を阻害せず，OAT1 及び MATE2-K に対しても明らかな阻害作用を示さなかった。

2 分析法

2.1 放射性同位体標識 LNP023

^{14}C-LNP023 は，合成及び精製後に塩酸塩として供給された。HPLC-放射能検出により測定した放射化学的純度は 97.6%以上であった。^{14}C-LNP023 の化学構造式を標識部位と合わせて Figure 2-1 に示す。

Figure 2-1 ^{14}C 標識 LNP023 の化学構造及び標識部位



2.2 放射能分析法

血液，血漿，胆汁，尿，糞，屍体，ケージ洗浄液，及び投与液中の放射能濃度は液体シンチレーションカウンター（LSC）により測定した。組織及び器官における放射能濃度は定量的全身オートラジオグラフィー（QWBA）により測定し，画像を放射能標準線源と比較計測することにより定量した。

2.3 LNP023 の定量法

マウス，ラット，ウサギ，及びイヌ血漿中 LNP023（遊離塩基）は，液体クロマトグラフィータンデム質量分析（LC-MS/MS）により定量した。LNP023 の LC-MS/MS 分析には，D_5-LNP023 を内部標準として用いた。

マウス血漿（DMPK R2000093 試験では 2%乳酸処理）中 LNP023 を定量するため，バリデーション済の LC-MS/MS 分析法を用いた（Table 2.6.5.2C-DMPK R1500429-01）。マウス血漿 10 µL を試料としたとき，定量下限（LLOQ）は 100 ng/mL であった。マウス血漿を−60°C 以下及び−90°C ～−50°C で長期保存したとき，LNP023 はそれぞれ少なくとも 155 日間及び 285 日間（Table 2.6.5.2G-DMPK R2000093）安定であった。

ラット血漿（2%乳酸処理）中 LNP023 を定量するため，バリデーション済の LC-MS/MS 分析法を用いた（Table 2.6.5.2A-DMPK R1500297-01）。ラット血漿 10 µL を試料としたとき，LLOQ は 100 ng/mL であった。ラット血漿を−60°C 以下及び設定温度−80°C で長期保存したとき，LNP023 はそれぞれ少なくとも 79 日間及び 238 日間（Table 2.6.5.2D-DMPK R1600441）安定であった。

ウサギ血漿（2%乳酸処理）中 LNP023 を定量するため，バリデーション済の LC-MS/MS 分析法を用いた（Table 2.6.5.2F-DMPK R1600671-01）。ウサギ血漿 10 µL を試料としたとき，LLOQ は 100 ng/mL であった。ウサギ血漿を設定温度−80°C で長期保存したとき，LNP023 は少なくとも 313 日間安定であった。一方，2%乳酸処理を行わなかったウサギ血漿を設定温度−80°C で長期保存した場合は，LNP023 は少なくとも 17 日間安定であった。

イヌ血漿（2%乳酸処理）中 LNP023 を定量するため，バリデーション済の LC-MS/MS 分析法を用いた（Table 2.6.5.2B-DMPK R1500296-01）。イヌ血漿 10 µL を試料としたとき，LLOQ は 100 ng/mL であった。イヌ血漿を−60°C 以下及び設定温度−80°C で長期保存したとき，LNP023 はそれぞれ少なくとも 78 日間及び 238 日間（Table 2.6.5.2E-DMPK R1600442 and DMPK R1600442-ad1）安定であった。

ラット血漿中不純物A*（LNP023　原薬の不純物及び分解産物）を定量するため，バリデーション済の LC-MS/MS 分析法を用いた（Table 2.6.5.2H-DMPK R2000262）。ラット血漿 20 µL を試料としたとき，LLOQ は 2.00 ng/mL であった。ラット血漿を−90°C～−50°C で長期保存したとき，不純物A*は少なくとも 139 日間安定であった。

2.4　代謝物の特性評価及び構造解析

^{14}C-LNP023 を用いたラット及びヒトの ADME 試験（「Table 2.6.5.9A-DMPK R1500361」及び CLNP023A2101 試験）で得られた血漿，尿，糞，及び胆汁試料中における代謝物濃度及び代謝物プロファイルを，放射能検出器と組み合わせた高速液体クロマトグラフィー（HPLC）により検討した。代謝物の構造解析は液体クロマトグラフィー質量分析（LC-MS）又は LC-MS/MS を用いた。

循環血中の LNP023 の代謝物は，ラット及びイヌ毒性試験で得られたプール血漿（「Table 2.6.5.9B-DMPK R1570193-metid」及び「Table 2.6.5.9C-DMPK R1570192-metid」）並びにヒト被験者から得られたプール血漿［CLNP023X2101（DMPK-BT-R1701500-metid）試験］について分析することにより検討した。AUC プール（未変化体及び代謝物の含有比が AUC における比と同一になるよう各時点の血漿を採取時点に応じた割合でプール）試料中の代謝物は LC-MS 又は LC-MS/MS を用いて同定し，ヒト試料については標準品を用いて LC-MS により半定量を行った。毒性試験で用いた動物種（ラット及びイヌ）でヒト循環血漿中代謝物に対する十分な曝露が得られているかについて評価するために，ヒト及び動物の血漿試料をマトリックスマッチング（分析時のマトリックス効果を相殺するため，一方の動物種のブランクマトリックスを等容量加えて希釈）させ超高速液体クロマトグラフィー（UPLC）-MS を用いて分析し，代謝物のピーク面積比を比較した（Gao et al. 2010）（Table 2.6.5.9D-DMPK R1809129-metid）。

3 吸収

マウス，ラット，及びイヌに ^{14}C-LNP023 又は LNP023 を単回静脈内又は経口投与したときの吸収，BA，及び PK を検討した。実施したすべての ADME 試験の一覧を Table 11-1 に示す。^{14}C-LNP023 を静脈内又は経口投与した試験はラットで実施した。血漿中総放射能の PK パラメータを Table 3-1 に示す。また，マウス，ラット，及びイヌに単回静脈内投与したときの PK パラメータを Table 3-2 に，マウス，ラット，及びイヌに単回経口投与したときの PK パラメータを Table 3-3 に示す。

3.1 吸収及びバイオアベイラビリティ

ラットに ^{14}C-LNP023（10 mg/kg）を単回経口投与したとき，血漿中総放射能の Tmax は 0.5 時間であった。吸収率は，ラットにおいて血漿中総放射能の AUC を基に見積もると 81.8%であった（Table 3-1）。

マウス，ラット，及びイヌに ^{14}C-LNP023（10 mg/kg）又は LNP023（10 又は 30 mg/kg）を単回経口投与したとき，LNP023 の Tmax は 0.25～2 時間であった。また，単回経口投与したときの BA はマウスで 46%，ラットで 40%～68%，イヌで 60%であった（Table 3-3）。

3.2 LNP023 の薬物動態

^{14}C-LNP023（2 mg/kg）又は LNP023（0.1～10 mg/kg）を単回静脈内投与したとき，LNP023 の血漿クリアランス（CLp）はすべての動物種で 0.0912～0.960 L/h/kg であった。これらの値から算出した CLb（0.101～0.619 L/h/kg）をそれぞれの動物種における肝血流量の文献値［マウス：90 mL/min/kg，ラット：55.2 mL/min/kg，及びイヌ：30.9 mL/min/kg（Davies and Morris 1993）］と比較すると，マウス（11.5%），ラット（8.88%～14.9%），イヌ（5.45%～6.47%）と低かった。すべての動物種において，T1/2 は約 2.9～7.5 時間であった。また，Vss は 0.6～6.6 L/kg であった（Table 3-2）。

^{14}C-LNP023（10 mg/kg）又は LNP023（10 又は 30 mg/kg）を単回経口投与時の LNP023 の PK パラメータを Table 3-3 に示す。ラットに単回経口投与したときの LNP023 の T1/2 は約 4～10 時間であった。

Table 3-1 動物に ^{14}C-LNP023 を単回静脈内及び経口投与したときの血漿中総放射能の PK パラメータ（平均値）

動物種 (報告書番号)	性 (n)	投与経路	用量 (mg/kg)	標識	Tmax (h) [a]	Cmax (µg eq /mL) [b]	Cmax / 用量 (µg eq/mL) / (mg/kg) [b]	AUClast (µg eq·h /mL) [b]	Tlast (h) [a]	AUClast / 用量 (µg eq·h/mL) / (mg/kg) [b]	T1/2 (h)	吸収率 (%)
ラット (DMPK R1500361)	雄 (3)	iv	2	^{14}C	0.083 [c]	2.19	1.08	3.28	24	1.61	5.21	-
ラット (DMPK R1500361)	雄 (3)	po	10	^{14}C	0.5	1.09	0.109	13.2	24	1.31	8.18	81.8 [d]

Source：Table 2.6.5.3C-DMPK R1500361

[a] 中央値

[b] µmol eq/L 単位のパラメータは，[µg eq/mL = µmol eq/L × 422.52 / 1000]の式に従って µg eq/mL 単位とした。

[c] 最初の測定時点

[d] 吸収率（%）は，静脈内投与時の用量補正した総放射能 AUCinf に対する経口投与時の用量補正した総放射能 AUCinf の比率，すなわち[(AUCinf / 用量 po) / (AUCinf / 用量 iv)] × 100 により算出した。

Table 3-2 動物に ^{14}C-LNP023 又は LNP023 を単回静脈内投与したときの血漿中 LNP023 の PK パラメータ（平均値）

動物種 (報告書番号)	性 (n)	用量 (mg/kg)	AUClast (µg·h/mL)	Tlast (h)	AUClast / 用量 (µg·h/mL) / (mg/kg)	T1/2 (h)	CLp (L/h/kg)	CLb (L/h/kg)	Vss (L/kg)
マウス (RD-2016-00013)	雄 (2)	1	0.700 [a)]	7	0.700 [a)]	7.2	0.960 [b)]	0.619 [c)]	6.6
ラット (RD-2016-00014)	雄 (2)	1	2.08 [a)]	24	2.08 [a)]	6.7	0.456 [b)]	0.294 [c)]	2.3
ラット (DMPK R1500361)	雄 (3)	2	2.95 [a)]	24 [d)]	1.45 [a)]	5.08	0.680	0.439 [c)]	3.01
ラット (DMPK R2100550-01)	雄 (3)	2	2.63	24 [d)]	1.32	2.91	0.768	0.495 [c)]	1.49
イヌ (RD-2016-00020)	雄 (3)	0.1	0.985 [a, f)]	24	9.85 [a, f)]	5.6	0.1 [e)]	0.1 [b)]	0.6
イヌ (DMPK R2101187)	雄 (3)	3.5	41.5	49 [d)]	11.0	7.12	0.0942	0.104 [c)]	0.750
イヌ (DMPK R2101187)	雄 (3)	10	124	49 [d)]	11.0	7.48	0.0912	0.101 [c)]	0.768

Source：Table 2.6.5.3A-RD-2016-00013，Table 2.6.5.3B-RD-2016-00014，Table 2.6.5.3C-DMPK R1500361，Table 2.6.5.3E-DMPK R2100550-01，Table 2.6.5.3F-RD-2016-00020，Table 2.6.5.3G-DMPK R2101187

[a)] µmol/L 単位のパラメータは，[µg/mL = µmol/L × 422.52 / 1000]の式に従って µg/mL 単位とした。

[b)] mL/min/kg 単位の CL パラメータは，L/h/kg 単位に変換した。

[c)] CLb は，CLp を Cb / Cp 比（4.1 項）で除することにより算出した。Cb / Cp 比は濃度に依存することが示されたが，マウス（ラットの値を使用）：1.55，ラット：1.55（1000 ng/mL で測定），及びイヌ：0.904（1000 ng/mL で測定）を固定値として用いた。

[d)] 中央値

[e)] CLp は，CLb に Cb / Cp 比（4.1 項）を乗ずることにより算出した。Cb / Cp 比は濃度に依存することが示されたが，イヌの値：0.904（1000 ng/mL で測定）を固定値として用いた。

[f)] 血液パラメータを実測した。これをイヌでの Cb / Cp 比の固定値 0.904（1000 ng/mL で測定）で除することにより血漿パラメータを求めた。

Table 3-3 動物に ^{14}C-LNP023 又は LNP023 を単回経口投与したときの血漿中 LNP023 の PK パラメータ（平均値）

動物種 (報告書番号)	性 (n)	用量 (mg/kg)	Tmax (h)	Cmax (µg/mL)	Cmax / 用量 (µg/mL) / (mg/kg)	AUClast (µg·h/mL)	Tlast (h)	AUClast / 用量 (µg·h/mL) / (mg/kg)	T1/2 (h)	BA (%)
マウス (RD-2016-00013)	雄 (2)	10	0.6	0.949 a)	0.0949 a)	3.21 a)	7	0.321 a)	-	46
ラット (RD-2016-00014)	雄 (2)	30	2	5.22 a)	0.174 a)	22.4 a)	7	0.747 a)	-	40
ラット (DMPK R1500361)	雄 (3)	10	0.25 b)	0.570 a)	0.0562 a)	7.14 a)	24 b)	0.701 a)	9.70	49.7
ラット (DMPK R2100550-01)	雄 (3)	10	0.5 b)	1.41	0.141	9.05	48 b)	0.905	4.46	68.3
イヌ (RD-2016-00020)	雄 (3)	10	0.8	10.1 a, c)	1.01 a, c)	59.1 a, c)	24	5.91 a, c)	-	60

Source：Table 2.6.5.3A-RD-2016-00013，Table 2.6.5.3B-RD-2016-00014，Table 2.6.5.3C-DMPK R1500361，Table 2.6.5.3E-DMPK R2100550-01，Table 2.6.5.3F-RD-2016-00020

BA：バイオアベイラビリティ

a) µmol/L 単位のパラメータは，[µg/mL = µmol/L × 422.52 / 1000]の式に従って µg/mL 単位とした。

b) 中央値

c) 血液パラメータを実測した。これをイヌでの Cb / Cp 比の固定値 0.904（1000 ng/mL で測定）で除することにより血漿パラメータを求めた。

3.3 不純物A*の吸収

不純物A*はLNP023原薬の不純物及び分解産物であり（3.2.S.3.2），ラットを用いた *in vivo* 試験により遺伝毒性について評価した（2.6.6-8.6 項）。不純物A* が体内に吸収される可能性を評価するために，雄ラットに 不純物A*を経口投与した（Table 2.6.5.3D-DMPK R2000379）。不純物Aの用量を 3，10，30，100，及び 300 mg/kg としたとき，血漿中 不純物A*の Cmax 及び総曝露量に用量比を上回る増加が認められた。また，用量比を上回る曝露量増加はラットにおけるTK 試験（3.4.7 項）においても認められた。

3.4 トキシコキネティクス

マウス，ラット，ウサギ，イヌ，及びサルを用いた毒性試験の一部として TK を評価した（2.6.6 毒性試験の概要文）。得られた曝露量データは「2.6.7 毒性試験概要表」に含まれている。

3.4.1 ラットにおけるトキシコキネティクス

ラットを用いた 4 試験：2 週間反復経口投与毒性試験（100，300，及び 1000 mg/kg/日，Table 2.6.7.6-1370303），4 週間反復経口投与毒性試験（100，300，及び 1000 mg/kg/日，Table 2.6.7.7A-1470568），13 週間反復経口投与毒性試験（50，150，及び 500 mg/kg/日，Table 2.6.7.7B-1570195），及び 26 週間反復経口投与毒性試験（50，150，及び 750 mg/kg/日，Table 2.6.7.7C-1570193）において，LNP023 を経口投与したときの TK を評価した（2.6.6-3.1 項，1370303 試験-4.4 項，1470568 試験-4.6 項，1570195 試験-4.8 項，1570193 試験-4.7 項）。

50～1000 mg/kg/日で反復経口投与したとき，Tmax は約 0.5～7 時間であった。LNP023 の曝露量は 50～1000 mg/kg/日の範囲で用量の増加に伴い増加し，曝露量の増加は用量に比例する傾向にあった。また，検討したすべての用量において，初回投与時と反復投与時で曝露量に明らかな差がなかったことから累積性がほとんどないことが示唆された。雌雄における血漿中の LNP023 曝露量は，検討したすべての用量で初回投与時及び反復投与時のいずれにおいても同様であった。

3.4.2 イヌにおけるトキシコキネティクス

イヌを用いた 4 試験：2 週間反復経口投与毒性試験（100，300，及び 1000 mg/kg/日，Table 2.6.7.6-1470220），4 週間反復経口投与毒性試験（15，50，及び 300 mg/kg/日，Table 2.6.7.7D-1470567），13 週間反復経口投与毒性試験（5，30，及び 150 mg/kg/日，Table 2.6.7.7E-1570194），及び 39 週間反復経口投与毒性試験（5，30，及び 150 mg/kg/日，Table 2.6.7.7F-1570192）において，LNP023 を経口投与したときの TK を評価した（2.6.6-3.2 項，1470220 試験-4.4 項，1470567 試験-4.7 項，1570194 試験-4.5 項，1570192 試験-4.11.6 項）。

5～1000 mg/kg/日で反復経口投与したとき，Tmax（平均値又は中央値）は 0.5～3 時間であり一部の試験で用量の増加に伴い遅延する傾向を示した。LNP023 の曝露量は 5～1000 mg/kg/日の範囲で用量の増加に伴い増加し，曝露量の増加は用量に比例する傾向にあった（1000 mg/kg/日の初

回投与時を除く）。また，5～300 mg/kg/日の用量において，初回投与時と反復投与時で曝露量に明らかな差がなかったことから累積性がほとんどないことが示唆された。雌雄における血漿中のLNP023 曝露量は，検討したすべての用量で初回投与時及び反復投与時のいずれにおいてもほぼ同様であった。

3.4.3 サルにおけるトキシコキネティクス

サルを用いた用量漸増投与試験（10，50，100，300，及び 600 mg/kg，Table 2.6.7.5-1370389）において，LNP023 を経口投与したときの TK を評価した（2.6.6-2.2 項，1370389 試験-4.3 項）。

LNP023 を投与したすべての動物で LNP023 の曝露が確認された。LNP023 の Tmax（平均値）はすべての用量群で 0.667～6 時間であった。LNP023 の曝露量は検討した用量範囲で用量に比例して増加する傾向にあった。

3.4.4 生殖発生毒性試験におけるトキシコキネティクス

生殖発生毒性試験では，ラットを用いた胚・胎児発生に関する試験（100，300，及び 1000 mg/kg/日，Table 2.6.7.13A-1670321）並びにウサギを用いた 2 試験：胚・胎児発生に関する用量設定試験（50，300，及び 600 mg/kg/日，Table 2.6.7.13B-1670322）及び胚・胎児発生に関する試験（100，250，及び 450 mg/kg/日，Table 2.6.7.13C-1670323）において，LNP023 を経口投与したときの TK を評価した（2.6.6-6.2 項，1670321 試験-4.6 項，1670322 試験-4.6 項，1670323 試験-4.5 項）。

また，幼若イヌを用いた 2 試験：8 週間反復経口投与用量設定試験（5，30，150，300，及び 600 mg/kg/日，Table 2.6.7.15A-1770671）及び 52 週間反復経口投与毒性試験（5，30，及び 150 mg/kg/日，Table 2.6.7.15B-1870009）においても，LNP023 を経口投与したときの TK を評価した（2.6.6-6.4 項，1770671 試験-4.3 項，1870009 試験-4.8 項）。

ラットの試験において，妊娠動物に LNP023 を反復経口投与したとき，母動物での LNP023 の曝露量は用量の増加に伴い増加し，曝露量の増加は用量比を下回った。高用量投与群では，消化管からの吸収が飽和することが示唆された。

ウサギの試験において，妊娠 7～20 日目の動物に LNP023 を反復経口投与したとき，いずれの用量群においても投与したすべての動物で LNP023 の曝露が確認された。妊娠 20 日目において，Tmax は投与後 1～7 時間であり，用量の増加に伴い遅延した。LNP023 の曝露量は検討したすべての用量で用量の増加に伴い増加し，曝露量の増加は用量に比例する傾向にあった。

イヌの試験において，幼若動物に LNP023 を反復経口投与したときの初回投与後及び反復投与後の，LNP023 の曝露量［血漿中濃度-時間曲線下面積（0～24 時間）（AUC0-24）］は 5～600 mg/kg（初回投与）又は 5～300 mg/kg/日（反復投与）の範囲で用量の増加に伴い増加した。血漿中曝露量の増加は初回投与時あるいは反復投与時の採血日によらず用量に比例する傾向にあった。幼若イヌに反復投与したとき，LNP023 の曝露量に明らかな性差は認められず，LNP023 の累積も認められなかった。

3.4.5 がん原性試験におけるトキシコキネティクス

マウスを用いた 2 試験：28 日間反復経口投与用量設定試験（150，500，及び 1000 mg/kg/日，Table 2.6.7.10C-1770875）及び 26 週間がん原性試験（100，300，及び 1000 mg/kg/日，Table 2.6.7.10D-1770876），並びにラットを用いた 104 週間がん原性試験（150，300，及び 750 mg/kg/日，Table 2.6.7.10A-1770877）において，LNP023 を経口投与したときの TK を評価した（2.6.6-5.2 項，2.6.6-5.1 項，1770875 試験-4.6 項，4.7 項，1770876 試験-4.5 項，1770877 試験-4.6 項）。1770875 試験及び 1770876 試験では，マウスでヒト血漿中代謝物に対する十分な曝露が得られているかについて評価するために代謝物 M9（[redacted]）についても分析した。

マウスの試験において，LNP023 の曝露量は 100～1000 mg/kg/日の範囲で用量の増加に伴い増加した。LNP023 曝露量の増加は，用量に比例する傾向にあった。雌雄における LNP023 曝露量は同様であった。150～1000 mg/kg/日の用量において，初回投与時と反復投与時で曝露量にほとんど差がなく，LNP023 の累積性はみられなかった。*In vivo* において，LNP023 は速やかに M9 に代謝された。M9 の曝露量は，100～1000 mg/kg/日の範囲で LNP023 の用量の増加に伴い増加した。M9 曝露量の増加は，用量比をやや下回った。1770875 試験及び 1770876 試験において，未変化体の曝露量に対する M9 曝露量の相対比率をそれぞれの AUC0-24h から算出すると，0.689%～1.55%であった。マウスに LNP023 を反復投与したとき，M9 の累積性は認められなかった。

ラットの試験において，LNP023 の曝露量は 150～750 mg/kg/日の範囲で用量の増加に伴い増加した。LNP023 曝露量の増加は，用量に比例する傾向にあった。LNP023 曝露量に性差は示されなかった。ラットに反復投与したとき，LNP023 の明らかな累積はみられなかった。

3.4.6 光毒性試験におけるトキシコキネティクス

マウスを用いた紫外線照射局所リンパ節アッセイ（100，300，及び 1000 mg/kg/日，Table 2.6.7.17A-1470564）において，LNP023 を経口投与したときの TK を評価した（2.6.6-8.1.2 項，1470564-Appendix 12）。

マウスに LNP023 を 3 日間反復投与したとき，Tmax は 0.5～1 時間であった。100 から 1000 mg/kg/日への用量増加に伴う血漿中 LNP023 曝露量の増加は用量比を上回った。

3.4.7 不純物の毒性試験におけるトキシコキネティクス

不純物A*は LNP023 原薬の不純物及び分解産物である。ラットを用いた 4 週間反復経口投与による *in vivo* 遺伝子突然変異試験及び小核試験（31.25，62.50，及び 125.0 mg/kg/日，Table 2.6.7.17E-2070173）において，不純物A*を投与したときの 不純物A*の TK を評価した（2070173試験-5.6 項）。

31.25～125.0 mg/kg/日で反復経口投与したとき，不純物A* の Tmax は 0.25～3 時間であった。不純物A* の曝露量は 31.25～125.0 mg/kg/日の範囲で用量の増加に伴い増加し，曝露量の増加は用量比を上回る傾向にあった。また，125.0 mg/kg/日の用量群を除いて初回投与時と反復投与時で曝露量に明らかな差がなかったことから累積性がほとんどないことが示唆された。125.0 mg/kg/

日の用量群では，反復投与28日目のAUC0-24hが初回投与日及び15日目における値の約2倍高かった。

4 分布

4.1 LNP023の血液／血漿分布及び血漿蛋白結合

*In vitro*においてLNP023のラット，イヌ，及びヒト血球への分布を検討した（Table 2.6.5.6A-DMPK R1500362）。LNP023のCb / Cp比は，すべての動物種で濃度依存性を示した（Table 4-1）。10～10000 ng/mLの濃度範囲で，LNP023濃度の上昇とともに血漿中分率（fp）の平均値は，ラットで54.3%から16.4%，イヌで71.1%から37.6%，及びヒトで93.3%から24.3%に減少した。これに対応して，各動物種におけるCb / Cp比は，ラットで0.994から3.59，イヌで0.717から1.38，及びヒトで0.600から2.14に増加した（Table 2.6.5.6A-DMPK R1500362）。

また，*in vitro*におけるLNP023の血漿蛋白結合についても，検討したすべての動物種において濃度依存性を示した（Table 4-1）。10～10000 ng/mLの濃度範囲で，LNP023濃度の上昇とともにfuは，マウス（野生型）で6.31%から37.6%，ラットで4.03%から37.3%，イヌで9.47%から35.0%，及びヒトで1.23%（100 ng/mL）から25.4%に増加した（Table 2.6.5.6B-DMPK R1600148，Table 2.6.5.6A-DMPK R1500362）。

さらに，ヒト血清アルブミン（HSA），α1-酸性糖蛋白質（AAG），及びリポ蛋白質を用いた*in vitro*試験において，結合率は検討した濃度範囲（100～10000 ng/mL）で濃度依存的でなかった。LNP023のfuは，HSAに対して71.6%±3.31%，AAGに対して90.7%±0.379%，及びリポ蛋白質に対して84.0%±0.866%であった（Table 2.6.5.6A-DMPK R1500362）。

FBノックアウト（KO）マウス由来の血漿を用いて検討したところ，野生型マウス由来の血漿中におけるLNP023のfu（平均値）が濃度依存性を示した（10～10000 ng/mLの濃度範囲で6.31%～37.6%）のに対して，KOマウス由来の血漿中におけるfuはLNP023濃度に依存しなかった（35.8%～40.8%）（Table 2.6.5.6B-DMPK R1600148）。

これらのデータから，血漿中蛋白質に対するLNP023の結合及び濃度依存性は，標的分子でありヒト血漿中で多量に存在するFB［血漿中の濃度：315±102 mg/L，平均±SD（Silva et al. 2012）］との飽和結合に関連する可能性がある。

Table 4-1 LNP023 の血液／血漿分布及び血漿蛋白結合

動物種	設定濃度 (ng/mL)	血液／血漿分布					血漿蛋白結合	
		ヘマトクリット	血漿中分率 (fp) (%)	血液／血漿中濃度比 (Cb / Cp)	血球／血漿中濃度比 (Cbc / Cp)	血球中分率 (fBC)	血漿中結合画分 (fb) (%)	血漿中非結合画分 (fu) (%)
ラット	10	0.46	54.3	0.994	0.986	0.46	96.0 a)	4.03 a)
	100	0.41	49.0	1.20	1.50	0.51	94.2	5.76
	1000	0.41	38.2	1.55	2.33	0.62	86.5	13.5
	10000	0.41	16.4	3.59	7.31	0.84	62.7	37.3
イヌ	10	0.49	71.1	0.717	0.422	0.29	90.5	9.47
	100	0.48	64.0	0.812	0.609	0.36	89.8	10.2
	1000	0.48	57.5	0.904	0.799	0.43	82.2	17.8
	10000	0.48	37.6	1.38	1.80	0.62	65.0	35.0
ヒト	10	0.44	93.3	0.600	0.0913	0.07	95.6 b)	4.35 b)
	100	0.48	80.1	0.649	0.269	0.20	98.8	1.23
	1000	0.48	52.1	0.998	0.995	0.48	93.0	7.00
	10000	0.48	24.3	2.14	3.37	0.76	74.6	25.4

Source：Table 2.6.5.6A-DMPK R1500362

血液／血漿分布のデータは n = 3 の測定値，蛋白結合のデータは n = 4 の測定値の平均を示す。

a) n = 3 の測定データの平均を示す。

b) n = 4 のうち 2 試料が検出限界（LOD）未満であったため，n = 2 の測定データの平均を示す。

LNP023 の血漿蛋白結合率の濃度依存性が検討したすべての動物種でみられ，それが主に FB との飽和結合に関連していると考えられることを考慮し，それぞれの動物種に対応する曝露量比（安全マージン）は血漿中 LNP023 濃度及び非結合型 LNP023 濃度の双方に基づいて算出された（2.6.6-Table 9-1，Table 9-2）。毒性試験に用いた動物種（ラット及びイヌ）並びにヒトにおける非結合型 LNP023 濃度を見積もるために，Emax モデルを用いた。モデルから算出した非結合型 LNP023 濃度を用いて，主要な反復投与毒性試験，がん原性試験，及び生殖発生毒性試験における無毒性量（NOAEL）／最小毒性量（LOAEL）での血漿中曝露量を，第 I 相試験での LNP023 用量（200 mg bid）を投与したときの定常状態における曝露量と比較し，曝露量比を算出した（DMPK R2200449 試験）。

4.2 ラットにおける組織分布

雄の白色（Wistar Hannover）又は有色（Long-Evans）ラットに ^{14}C-LNP023 を 10 mg/kg の用量で単回経口投与し，投与後 168 時間までの ^{14}C-LNP023 由来放射能の組織分布を QWBA により検討した（Table 2.6.5.5A-DMPK R1500361）。さらに，雌雄の有色ラットに ^{14}C-LNP023 を 10 mg/kg の用量で単回経口投与し，投与後 336 時間までの組織分布を QWBA により検討した（Table 2.6.5.5B-DMPK R1701426）。

白色ラットに経口投与したとき，総放射能は脳を除く全身にわたって分布し，すべての組織で投与後 0.25～2 時間に Tmax となった。^{14}C-LNP023 由来放射能はその後減少し，多くの組織・器官

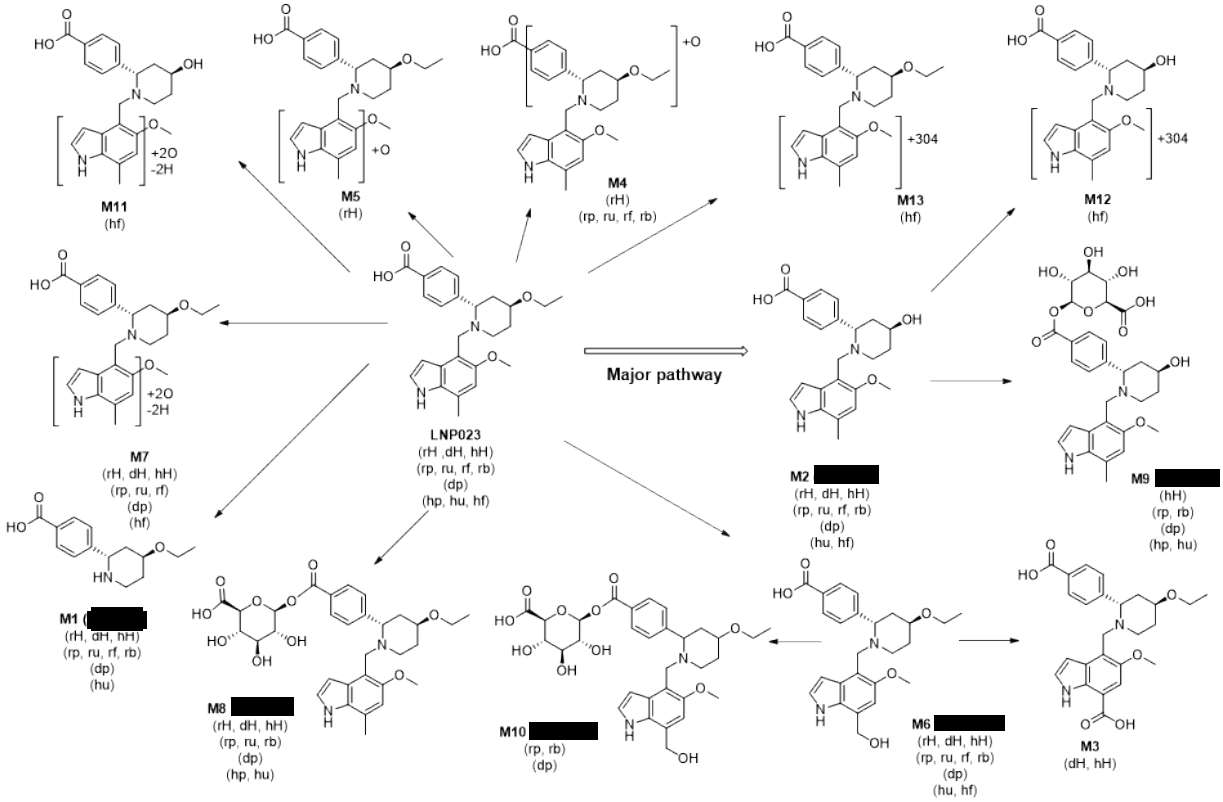
では放射能が検出されたのは投与後 8 あるいは 24 時間まで［すなわち最終定量可能時点（Tlast）］であり速やかに消失したが，例外は腎臓（Tlast：72 時間）及び肝臓（168 時間）であった。多くの組織における放射能の T1/2 は血液（4.06 時間）と同程度であったが，肝臓（40.3 時間）及び腎臓（皮質）（25.6 時間）においては血液に比べて明らかに長かった。総放射能 Cmax の組織／血液比が 3 倍を超えていたのは，肝臓のみであった。投与後 2 時間までの脳内放射能濃度は相当する血液中放射能濃度の 1.4%～2.2%であった。有色ラットにおいて，投与後 168 時間まで ^{14}C-LNP023 由来放射能はメラニンを含有する眼組織（脈絡膜及び毛様体）で検出され，その結合は少なくとも部分的に可逆的であった（Table 2.6.5.5A-DMPK R1500361）。ラットに LNP023 を静脈内投与したときの血漿中濃度から算出した Vss は中程度であり，その値は 3.01 L/kg であった（Table 3-2）。

有色ラットに LNP023 を 10 mg/kg の用量で単回経口投与したとき，総放射能は広範囲の組織に分布し，大部分の組織で雄では投与後 0.5～2 時間に，雌では投与後 0.5 時間に Tmax となった。多くの組織では投与後 24 時間までに Tlast となったが，例外は肝臓（Tlast：72 時間），有色皮膚（72 時間，雄），並びにブドウ膜及び眼（336 時間）であった。このデータは有色ラットを用いた先の試験結果（Table 2.6.5.5A-DMPK R1500361）と一致しており，LNP023 が眼組織のメラニンに対して親和性を有すること，及びメラニンとの結合が可逆的であることが示された。組織中薬物濃度-時間曲線下面積（0～最終定量可能時点）（AUClast）のデータに基づくと，雌雄ラットに共通して総放射能の曝露量が最も高かった組織はブドウ膜，肝臓，眼，及び小腸であった。測定した雌雄に特異的なすべての器官における組織内濃度は対応する血液中濃度よりも低かった。^{14}C-LNP023 は血液脳関門を越えて脳に分布することはなかった（Table 2.6.5.5B-DMPK R1701426）。

5 代謝（動物種間の比較）

マウス，ラット，イヌ，サル，及びヒト肝細胞，ヒト細胞分画，ヒト酵素発現系等を用いた *in vitro* 試験，並びにラット及びイヌにおける *in vivo* 試験において，LNP023 の代謝を検討した。LNP023 並びに *in vitro* 及び *in vivo* 試験で認められた主要代謝物の構造及び代謝物プロファイルをそれぞれ Figure 5-1 及び Table 11-2 に示す。ヒトにおける *in vivo* 代謝試験の成績は「2.7.2-3.1.3 項」に記載されている。

Figure 5-1　　ラット，イヌ，及びヒトにおける LNP023 の代謝



Source：DMPK R1500300，DMPK R1500361，DMPK R1570193-metid，DMPK R1570192-metid，DMPK RCLNP023A2101-metid

凡例：rH：ラット肝細胞，dH：イヌ肝細胞，hH：ヒト肝細胞，rp：ラット血漿，ru：ラット尿，rf：ラット糞，rb：ラット胆汁，dp：イヌ血漿，hp：ヒト血漿，hu：ヒト尿，hf：ヒト糞便

5.1　*In vitro* 代謝

5.1.1　肝細胞及び細胞分画における *in vitro* 代謝

^{14}C-LNP023（1 µmol/L）を肝細胞とインキュベーションしたときの固有クリアランス（CLint）は，ラット：1.58 µL/min/10^6 cells，イヌ：0.220 µL/min/10^6 cells，ヒト：0.564 µL/min/10^6 cells であった（Table 2.6.5.10A-DMPK R1500300）。このデータを用いてスケーリング手法により *in vivo* における肝血液クリアランス（CLh,b）を算出すると，CLh,b 算出値は検討したすべての動物種で低く，ラット（6.11 mL/min/kg），イヌ（1.60 mL/min/kg），及びヒト（1.34 mL/min/kg）の順に減少した。この傾向は実測値に基づく *in vivo* クリアランス（3.2 項，2.7.2-Table 2-3）と一致していた。

マウス，ラット，イヌ，サル，及びヒト肝細胞を用いて ^{14}C-LNP023 又は LNP023 とインキュベーションすることにより，*in vitro* 代謝プロファイルを検討した（Table 2.6.5.10A-DMPK R1500300，Table 2.6.5.10C-DMPK R1900278-metid）。肝細胞による *in vitro* 代謝は動物種を通じて概ね同様であり，以下の代謝反応により特徴づけられた：N-脱アルキル化（M1 生成），O-脱エ

チル化（M2 及び M9），C-酸化（M3，M4，M5，M6，及び M7），水酸化（M4，M5，及び M6），及び LNP023 のカルボキシ部位のグルクロン酸抱合（M8 及び M9）。これらの代謝物のうち M1，M2，及び M6 は，ヒト肝細胞における代謝クリアランスを左右する主要な一次酸化代謝物として同定された（Table 2.6.5.10A-DMPK R1500300）。ヒト肝細胞で同定されたすべての代謝物は，検討した少なくとも 1 種のヒト以外の動物種においても認められた。全体として，代謝物プロファイルは動物種を通じて質的に類似していた（Table 11-2）。

^{14}C-LNP023 をヒト肝細胞とインキュベーションしたときの代謝プロファイルから，生成した代謝物のおよそ 50%が酸化的代謝物であり残りの約 50%はアシルグルクロン酸抱合代謝物であることが示された。また，補酵素［ニコチンアミドアデニンジヌクレオチドリン酸（還元型）（NADPH），ウリジン 5’ジホスホグルクロン酸（UDPGA），及び／又は PAPS（adenosine 3’-phosphate 5’-phosphosulfate）］存在下で，^{14}C-LNP023 をヒト肝臓の細胞分画（肝ミクロソーム，S9，及びサイトゾル）とインキュベーションしたとき，肝ミクロソーム及び S9 では肝細胞と類似した代謝物の生成が認められたが，サイトゾルによる代謝はみられなかった（Table 2.6.5.10E-DMPK R1500516）。

NADPH 存在下で ^{14}C-LNP023（1～300 µmol/L）をプールしたヒト肝ミクロソーム（150 名のドナー由来）とインキュベーションしたときの酸化的代謝速度はミカエリス・メンテン式により表現され，見かけのミカエリス定数（Km,app）は 163.3 ± 22.6 µmol/L，Vmax は 90.3 ± 6.1 pmol/min/mg protein，*in vitro* での酸化的代謝の見かけの固有クリアランス（CLint,app）は 0.6 ± 0.1 µL/min/mg protein であった。この結果から，肝臓における CYP 酵素による LNP023 の酸化的代謝クリアランスは低いことが示された（2.6.5.10E-DMPK R1500516）。また，UDPGA の存在下で ^{14}C-LNP023（5～750 µmol/L）をプールしたヒト肝ミクロソーム（150 名のドナー由来）とインキュベーションしたときのアシルグルクロン酸抱合代謝物（M8 及びその異性体である M8c）への代謝速度はミカエリス・メンテン式により表現され，Km,app は 241 µmol/L，Vmax は 297 pmol/min/mg protein，及び CLint,app は 1.23 µL/min/mg protein であった（Table 2.6.5.10G-DMPK R1600698）。

5.1.2 LNP023 の酸化的代謝に関与する酵素の同定

プールしたヒト肝ミクロソーム（150 名のドナー由来）及びヒト酵素発現系［CYP 及びフラビンモノオキシゲナーゼ（FMO）分子種］を用いた *in vitro* 試験において，LNP023 の酸化的代謝に関与する酵素分子種の同定を行った（Table 2.6.5.10E-DMPK R1500516，Table 2.6.5.10F-DMPK R1600579）。

CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1，CYP3A4，又は FMO に対する選択的阻害剤の存在下及び非存在下で，^{14}C-LNP023（20 µmol/L）を NADPH とともにヒト肝ミクロソームとインキュベーションしたとき，CYP2C8 選択的阻害剤（montelukast）による明らかな代謝阻害効果がみられた（最高濃度の阻害剤存在下における代謝活性は 53%に低下）。

これに対して他の酵素分子種の阻害剤による効果はわずか又はほとんどみられなかった（単独での各阻害剤の存在下における代謝活性：78%～115%）。また，NADPH 存在下で ^{14}C-LNP023（5 µmol/L）をヒト酵素発現系（17 種の CYP 及び FMO3 を含む）とインキュベーションしたとき，CYP2C8（代謝率：5.4%，M1，M2，及び M6 生成）に加えて，CYP1A1（肝外酵素）（代謝率：2.4%，M6 生成），CYP2D6（代謝率：1.8%，M1 及び M6 生成），CYP2C9（代謝率：1.0%，M2 及び M6 生成），及び CYP3A4（代謝率：0.8%，M1，M2，及び M6 生成）による LNP023 の代謝がみられたが，その他の分子種（CYP1A2 を含む）による代謝物生成は認められなかった（Table 2.6.5.10E-DMPK R1500516）。同様にヒト酵素発現系（18 種の CYP 及び 3 種の FMO）と ^{14}C-LNP023（10 µmol/L）をインキュベーションした場合も，CYP1A1，CYP2C8，CYP2D6 による LNP023 の代謝がみられ，その他の CYP 分子種及び FMO における明らかな代謝活性は認められなかった（Table 2.6.5.10F-DMPK R1600579）。

さらに，^{14}C-LNP023（2～400 µmol/L）をヒト CYP2C8 発現系とインキュベーションしたときの LNP023 代謝速度はミカエリス・メンテン式により表現され，LNP023 の Km,app は 31.93 µmol/L，Vmax は 2.64 pmol/min/pmol CYP，及び CLint,app は 0.083 µL/min/pmol CYP であった。CYP2D6 については，代謝率が低く，十分な代謝速度が得られなかったため同様の速度論的解析は実施できず，LNP023 濃度 2 µmol/L での代謝速度を用いた。得られたパラメータ，試験系での非結合画分，及び肝ミクロソームにおける CYP 分子種の発現量（文献情報等による値）を用いて，肝ミクロソームにおけるそれぞれの CYP による非結合型 LNP023 の CLint を外挿し（CYP2C8：0.78 µL/min/mg protein 及び CYP2D6：0.02 µL/min/mg protein），これらの値から肝臓における酸化的代謝に対するそれぞれの寄与率を算出すると，CYP2C8：98%及び CYP2D6：2%であった（Table 2.6.5.10F-DMPK R1600579）。

以上のことから，LNP023 の酸化的代謝に関与する主要な CYP 分子種は CYP2C8 であり，寄与は少ないが CYP2D6 の関与も示唆された。また，CYP1A1 による代謝がみられたことから，*in vivo* において肝外での LNP023 代謝が生じる可能性が考えられる。

5.1.3 LNP023 のグルクロン酸抱合代謝に関与する酵素の同定

ヒト酵素発現系（UGT 分子種）及びヒト肝ミクロソームを用いた *in vitro* 試験において，LNP023 の直接的グルクロン酸抱合代謝に関与する酵素分子種の同定を行った（Table 2.6.5.10H-DMPK R1600140，Table 2.6.5.10G-DMPK R1600698）。

UDPGA 存在下で ^{14}C-LNP023（5 µmol/L）をヒト UGT 発現系（13 種）と 1 時間インキュベーションしたとき，アシルグルクロン酸抱合代謝物である M8（[redacted]）は UGT1A1（代謝率：約 2%），UGT1A8（腸に発現する肝外酵素）（代謝率：約 2%），及び UGT1A3（代謝率：約 0.5%）により生成した（Table 2.6.5.10H-DMPK R1600140）。

また，^{14}C-LNP023（10 µmol/L）を UDPGA の存在下で 16 名の単独ドナー由来及びプールした肝ミクロソームとインキュベーションし，アシルグルクロン酸抱合代謝物（M8 及び M8c）への

代謝速度とミクロソーム中の UGT 特異的マーカー酵素活性（UGT1A1，UGT1A4，UGT1A6，UGT1A9，及び UGT2B7）との相関を検討した。その結果，代謝速度と UGT1A1 特異的マーカーとの間で相関がみられた（相関係数 R：0.83）のに対して，その他の UGT 分子種については R 値が低く（0.47 以下）相関がみられなかった（Table 2.6.5.10G-DMPK R1600698）。

以上のことから，LNP023 の直接的なグルクロン酸抱合代謝に関与する主要な UGT 分子種は UGT1A1 であることが示唆された。ただし，その他の肝臓あるいは肝外組織に発現する UGT 分子種が代謝に関与する可能性は除外できない。

5.1.4 構造アラート及び共有結合

構造的視点から，アシルグルクロン酸抱合代謝物である M8，M9，及び M10 は反応性を有する可能性が考えられる。代謝物が *in vivo* において反応性を示す可能性を探るため，リン酸緩衝液中における安定性を評価したところ，緩衝液中でのアシルグルクロン酸抱合代謝物 M8（[redacted]）及び M9（[redacted]）の T1/2 はそれぞれ約 1.6 及び 2.0 時間であった（Table 2.6.5.10D-DMPK R1500191，Table 2.6.5.10B-DMPK R1900261-metid）。また，ヒト肝ミクロソーム及びヒト肝細胞において LNP023 の代謝活性化により反応性代謝物が生成する可能性を評価した（Table 2.6.5.10I-DMPK R1500514）。共有結合による蛋白質付加体の形成に基づいて，反応性代謝物の生成量を評価したところ，両試験系で観察された反応性代謝物の生成量は，代謝活性化に対して規定された閾値を下回っていた。したがって，LNP023 では *in vivo* において懸念となるような代謝活性化が生じる可能性は低いと考える。

5.2 *In vivo* 代謝

ラットを用いた ADME 試験において，*in vivo* における ^{14}C-LNP023 の代謝を検討した（Table 2.6.5.9A-DMPK R1500361）。マウスでは代謝に特化した試験を実施しなかったが，2 つのがん原性試験（1770875 試験及び 1770876 試験，3.4.5 項）でアシルグルクロン酸抱合体である M9（[redacted]）を測定した。また，ヒト及び毒性試験で用いた動物種（ラット及びイヌ）に LNP023 を反復投与したときの定常状態で得られたプール血漿中の代謝物を分析し，代謝物の曝露量比を算出した（Table 2.6.5.9D-DMPK R1809129-metid，5.2.3 項）。

5.2.1 ラットにおける *in vivo* 代謝

ラットにおいて，血漿，尿，及び糞について得られた LNP023 の代謝物プロファイルは投与経路によらず質的に類似していた（Table 2.6.5.9A-DMPK R1500361）。ラットに ^{14}C-LNP023 を投与したときの *in vivo* 代謝は以下の代謝反応により特徴づけられた：N-脱アルキル化（M1 生成），O-脱エチル化（M2 及び M9），C-酸化（M4，M6，M7，及び M10），水酸化（M4，M6，及び M10），及び LNP023 のカルボキシ部位のグルクロン酸抱合（M8，M9，及び M10）（Figure 5-1，DMPK R1500361）。

雄ラットに ^{14}C-LNP023 を単回静脈内投与（2 mg/kg）及び単回経口投与（10 mg/kg）したとき（Table 2.6.5.9A-DMPK R1500361），血漿中における主要成分は未変化体であった（それぞれ総放射能 AUC の 87.7%及び 91.0%）。循環血漿中に代謝物である M1，M2，M4，及び M8 も検出されたが，総放射能の全身曝露量に対する割合はいずれも 2.4%未満であった。

尿中における主要成分は未変化体（投与量の約 2%～3%）及び M1（投与量の約 1%～2%）であった。尿中に検出された他の代謝物（M2，M4，M6，M7，及び M8）はいずれも投与量の 1%未満であった。

ラットの糞中における主要成分は未変化体（静脈内及び経口投与量のそれぞれ 19%及び 36%）及び M2（静脈内及び経口投与量のそれぞれ約 30%）であった。静脈内及び経口投与したとき，糞中に検出された他の第 1 相代謝物は M1，M4，及び M6 であり，それぞれ投与量の 10%未満であった。

胆管カニューレを挿入したラットに静脈内投与（2 mg/kg）したとき，胆汁中には投与量の 11.7%が未変化体として排泄された。胆汁中の主要成分は M2 及び M8（それぞれ投与量の約 20%）であり，次いで M1，M9，M4，M10，及び M6（それぞれ投与量の約 3%～6%）であった。アシルグルクロン酸抱合代謝物である M8，M9，及び M10 が胆汁中で検出されたものの糞中には認められなかった。したがって，これらの代謝物が腸内細菌によって脱抱合を受けそれぞれのアグリコンに逆変換されたと考える（Table 2.6.5.9A-DMPK R1500361，DMPK R1500361）。

以上より，LNP023 は尿中及び胆汁中への分泌／排泄とともに，主に代謝によって消失することが示された。

なお，LNP023 を 750 mg/kg/日の用量で反復経口投与したラット毒性試験においても，血漿中代謝物を LC-MS により分析した（Table 2.6.5.9B-DMPK R1570193-metid）。その結果，反復投与 154 日目において，AUC0-24h プール試料中に LNP023 とともに 7 つの代謝物（M8 は異性体を含む）が検出された。これらの代謝物は，ラット血漿又は排泄物中で同定された代謝物（Table 2.6.5.9A-DMPK R1500361）に対応するものであった（Table 11-2）。

5.2.2 イヌにおける *in vivo* 代謝

LNP023 を 150 mg/kg/日の用量で反復経口投与したイヌ毒性試験において，血漿中代謝物を LC-MS により分析した（Table 2.6.5.9C-DMPK R1570192-metid）。その結果，反復投与 273 日目において，AUC0-24h プール試料中に LNP023 とともに 7 つの代謝物（M8 は異性体を含む）が検出された。これらの代謝物は，*in vitro* 試験（Table 2.6.5.10A-DMPK R1500300）又はラット *in vivo* 試験（Table 2.6.5.9A-DMPK R1500361）で同定された代謝物に対応するものであった（Table 11-2）。代謝経路は，N-脱アルキル化，O-脱アルキル化，酸化，脱水素反応，グルクロン酸抱合，及びそれらの組み合わせを含んでいた。

5.2.3 動物におけるヒト循環血漿中代謝物の網羅性

毒性試験で用いた動物種（ラット及びイヌ）でヒト血漿中代謝物に対する十分な曝露が得られているかについて，ヒトに LNP023 を 200 mg bid，反復経口投与（14 日目）したときの定常状態における代謝物の曝露量をラット（750 mg/kg/日，反復経口投与 154 日目）及びイヌ（150 mg/kg/日，反復経口投与 273 日目）での曝露量と比較することにより評価した（Table 11-2，Table 2.6.5.9D-DMPK R1809129-metid）。その結果，血漿中においてヒトに特異的な代謝物は検出されなかった。ヒト代謝物（M1，M2，M6，M7，及び M8 とその異性体）の曝露量に対する雌雄のラット及びイヌの曝露量比はいずれも 0.5 を超えていた。このことは，ラット及びイヌでこれらの代謝物に対する十分な曝露が得られたことを示す。一方，アシルグルクロン酸抱合代謝物である M9 の曝露量については，ヒトに対するラット及びイヌにおける曝露量比が 0.5 未満であり，動物での曝露量はヒトに比べて低かった。また，ヒト及びラットに ^{14}C-LNP023 をそれぞれ 100 mg 及び 10 mg/kg の用量で経口投与した ADME 試験における代謝物の曝露量を比較したところ，上述の試験での代謝物プロファイル並びに代謝物 M8 及び M9 の曝露量関係（ラット＜ヒト）と類似した結果が示された（Table 11-2，DMPK RCLNP023A2101-metid）。M9 は ^{14}C-LNP023 を 2 mg/kg 静脈内投与後のラット胆汁中に認められ，投与量の 2.98%を占めた（Table 2.6.5.9A-DMPK R1500361）。この結果は，M9 の全身曝露レベルは低いものの，ラット肝臓が M9 に曝露されたことを示している。

M9 の曝露は，マウス 26 週間がん原性試験（Table 2.6.7.10D-1770876）においても確認された。このマウス試験での定常状態における曝露量（LNP023 を 1000 mg/kg/日反復投与 148 日目における AUC0-24h）及びヒトでの曝露量（200 mg bid 反復投与 30 日目における AUC0-12h）（DMPK R2201049-metID）に基づくと，ヒトに対するマウスの曝露量比は 0.398 であった。

5.2.4 ヒト代謝物の薬理活性

ヒト血漿中で検出された 2 つの代謝物である M8 及び M9 並びにヒト便中で最も多く認められた代謝物である M2 について薬理活性を評価したところ，それらの活性の強さは未変化体と比べて 1/150～1/27 であった（RD-2020-00095，2.7.2-2.1.2 項）。

6 排泄

6.1 尿及び糞中排泄

ラットに ^{14}C-LNP023 を単回投与したときの尿及び糞中排泄データを Table 6-1 に示す。

ラットにおける主要な排泄経路は，静脈内及び経口投与のいずれにおいても糞であった。静脈内（2 mg/kg）及び経口投与（10 mg/kg）したとき，投与した総放射能量の 84.6%～94.4%が糞中に，4.79%～5.68%が尿中に排泄された。投与放射能は，静脈内及び経口投与後 168 時間までにほぼ完全に回収された（それぞれ 92.4%及び 97.9%～100%）（Table 2.6.5.13A-DMPK R1500361，Table 2.6.5.13B-DMPK R1701426）。

ラットに ^{14}C-LNP023 を単回静脈内及び経口投与したときの LNP023 の尿中排泄率は投与量の 2.07%～3.24%であった。また，LNP023 の糞中排泄率は投与量の 19.0%～36.1%であった（Table 2.6.5.13A-DMPK R1500361）。

Table 6-1 ラットに ^{14}C-LNP023 を単回静脈内及び経口投与したときの ^{14}C-LNP023 由来放射能及び LNP023 の排泄

動物種	ラット a)	ラット a)	ラット b)
性 (n)	雄 (3)	雄 (3)	雄 (3)
投与経路	iv	po	po
用量 (mg/kg)	2	10	10
放射性標識	^{14}C	^{14}C	^{14}C
尿中排泄率			
放射能 (投与量に対する%)	5.68	5.09	4.79
採取期間 (h)	0-168	0-168	0-168
LNP023 (投与量に対する%)	3.24	2.07	-
採取期間 (h)	0-72	0-72	-
糞中排泄			
放射能 (投与量に対する%)	84.6	94.4	92.8
採取期間 (h)	0-168	0-168	0-168
LNP023 (投与量に対する%)	19.0	36.1	-
採取期間 (h)	0-72	0-72	-
放射能の回収率 (%，168 h)	92.4	100	97.9

Source：Table 2.6.5.13A-DMPK R1500361，Table 2.6.5.13B-DMPK R1701426

-：データなし

数値は平均値を示す。

a) DMPK R1500361［回収率は，表中の値に加えて静脈内投与では動物屍体（168 時間）：投与量の 0.580%，ケージ洗浄液（168 時間）：1.44%，経口投与では動物屍体（168 時間）：0.0771%，ケージ洗浄液（168 時間）：0.560%を含む］

b) DMPK R1701426［回収率は，表中の値に加えて動物屍体（168 時間）：投与量の 0.0955%，ケージリンス（0～144 時間，24 時間おきに採取した合計）：0.163%，ケージ洗浄液（168 時間）：0.0149%，ケージ拭き取り（168 時間）：0.0284%を含む］

6.2 胆汁中排泄

胆管カニューレを挿入した雄ラットに ^{14}C-LNP023（2 mg/kg）を単回静脈内投与したとき，投与放射能の大部分（79.4%）が胆汁中に排泄され，尿中への排泄はわずか（13.0%）であった。このとき，糞中への排泄は投与放射能の 4.21%であった（Table 2.6.5.14-DMPK R1500361）。胆管カニューレを挿入したラットにおいて，投与量の 3.51%が LNP023 として糞中に排泄された（Table 2.6.5.9A-DMPK R1500361）。また，胆汁中への LNP023 としての排泄が投与量の 11.7%であったことから，ラットにおいて未変化体の主要な消失経路は胆汁中排泄ではないことが示唆された（Table 2.6.5.9A-DMPK R1500361）。

7 薬物動態学的薬物間相互作用

薬物動態学的薬物間相互作用の可能性を評価するため，ヒト試料，ヒト酵素発現系，及びヒトトランスポーター発現系を用いた *in vitro* 試験により，酵素阻害／誘導，LNP023 の膜透過性及び肝細胞系における輸送，並びにトランスポーターの基質となる可能性及び阻害の可能性を検討した。それらの試験成績を以下に示す。なお，ヒトにおける *in vivo* 薬物動態学的薬物間相互作用に関する試験成績については，2.7.2-3.4.2 項を参照のこと。

7.1 酵素阻害

ヒト肝ミクロソーム（UGT についてはヒト酵素発現系も使用）を用いた *in vitro* 試験において，LNP023 が CYP 及び UGT 酵素活性を阻害する可能性を検討した（Table 2.6.5.12B-DMPK R1500321，Table 2.6.5.12A-DMPK R1300641，Table 2.6.5.12E-DMPK R1900589，Table 2.6.5.12C-DMPK R1500936）。

7.1.1 CYP 酵素の可逆的阻害

NADPH の存在下で，LNP023（0～100 µmol/L）を CYP 分子種に選択的なプローブ基質とともにプールしたヒト肝ミクロソーム（150 名のドナー由来）とインキュベーションし，CYP1A2，CYP2A6，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1，及び CYP3A4/5 の酵素活性を可逆的に阻害する可能性を検討した（Table 2.6.5.12B-DMPK R1500321）。CYP 酵素活性は，プローブ基質の代謝物を LC-MS/MS を用いて測定した。その結果，LNP023 は最高濃度においても，検討したすべての CYP 分子種に対して可逆的な阻害作用をほとんど又は全く示さなかった。したがって，50%阻害濃度（IC50）は，いずれの CYP についても 100 µmol/L 超であった（Table 2.6.5.12B-DMPK R1500321）。

結論として，LNP023 は CYP1A2，CYP2A6，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1，及び CYP3A4/5 の可逆的阻害剤ではないと考えられた。

7.1.2 CYP 酵素の時間依存的阻害

プールしたヒト肝ミクロソーム（150 名のドナー由来）を用いて LNP023 が CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，及び CYP3A4/5 の酵素活性を時間依存的に阻害する可能性を検討した（Table 2.6.5.12A-DMPK R1300641，Table 2.6.5.12E-DMPK R1900589）。NADPH の存在下で，LNP023（CYP2C8：0～500 µmol/L，他の CYP 分子種：0～100 µmol/L）を肝ミクロソームとプレインキュベーション（0～48 分間）した後，CYP 分子種に選択的なプローブ基質及び NADPH を含む緩衝液で 20 倍希釈してインキュベーションを行い，残存する酵素活性を LC-MS/MS で測定した。その結果，LNP023 は最高濃度においても，CYP1A2，CYP2B6，CYP2C9，CYP2C19，CYP2D6，及び CYP3A4/5 に対して時間依存的な阻害作用を示さなかった。一方，時間依存的阻害作用は CYP2C8 のみでみられた［LNP023 の阻害定数（時間依存的阻害）KI：

179 ± 32 µmol/L，最大不活性化速度定数 kinact：0.0702 ± 0.0051 min^{-1}］（Table 2.6.5.12A-DMPK R1300641，Table 2.6.5.12E-DMPK R1900589）。

結論として，LNP023 は CYP2C8 に対して時間依存的阻害作用を示した。一方，CYP1A2，CYP2B6，CYP2C9，CYP2C19，CYP2D6，及び CYP3A4/5 の時間依存的阻害剤ではないと考えられた。

7.1.3 UGT 酵素の阻害

UDPGA 存在下で LNP023（0～100 µmol/L）を UGT 分子種のプローブ基質（β-estradiol）とともにプールしたヒト肝ミクロソーム（150 名のドナー由来）又はヒト UGT1A1 発現系とインキュベーションし，UGT1A1 酵素活性を阻害する可能性を検討した（Table 2.6.5.12C-DMPK R1500936）。酵素活性は，UGT1A1 に選択的な反応によるプローブ基質の代謝物（β-estradiol 3-glucuronide）を LC-MS/MS を用いて測定した。その結果，肝ミクロソーム及びヒト酵素発現系の両試験系において，LNP023 は最高濃度においても UGT1A1 酵素活性に対して阻害作用を示さなかった。したがって，IC50 は 100 µmol/L 超であった（Table 2.6.5.12C-DMPK R1500936）。

結論として，LNP023 は UGT1A1 の阻害剤ではないと考えられた。

7.2 酵素誘導

LNP023 をヒト初代培養肝細胞と 72 時間インキュベーションし，*in vitro* において CYP1A2，CYP2B6，CYP2C9，及び CYP3A4 の mRNA 発現量及び酵素活性を誘導する可能性を検討した（Table 2.6.5.12D-DMPK R1600704）。酵素誘導評価のためのインキュベーション後に，LNP023 による mRNA の誘導作用は逆転写反応及びリアルタイム PCR（ポリメラーゼ連鎖反応）法を用いて測定し，また CYP 酵素活性は CYP 分子種に選択的なプローブ基質を反応させてその代謝物を LC-MS/MS を用いて測定し，溶媒対照に対する相対値として評価した。本試験系における CYP 誘導作用の陽性対照として，オメプラゾール（CYP1A2），フェノバルビタール（CYP2B6），並びにリファンピシン（CYP2C9 及び CYP3A4）を用いた。その結果，肝細胞（3 名の個別ドナー由来）を LNP023（0.1～100 µmol/L）で処理したとき，CYP1A2，CYP2B6，及び CYP2C9 の mRNA 発現量，並びに CYP2B6，CYP2C9，及び CYP3A4 の酵素活性に対して，有意な誘導作用（定義：濃度依存的かつ溶媒対照に比べて 2 倍以上に増加した場合）はみられなかった。CYP1A2 の酵素活性については，ドナー番号 3 において検討した LNP023 の最高濃度（100 µmol/L）で 2.4 倍に増加したものの，同条件での mRNA 発現量増加は 1.31 倍であった。また，CYP3A4 の mRNA 発現量については，3 名のドナーすべてで 2 倍以上（それぞれ 2.75，2.49，及び 2.52 倍）となったが，誘導作用がみられたのは最高濃度のみであり，その誘導作用は陽性対照（リファンピシン）による誘導作用の 20%未満であった。誘導作用がみられたのが 1 濃度のみであったため，酵素誘導作用のパラメータは算出できなかった（Table 2.6.5.12D-DMPK R1600704）。

結論として，ヒト初代培養肝細胞において LNP023 は CYP3A4 に対して弱い誘導作用を示したが，CYP2B6 及び CYP2C9 を誘導しなかった。また，CYP1A2 に対しても LNP023 は誘導剤ではないと考えられた。

7.3 膜透過性及び肝細胞系における輸送

Caco-2 細胞の単層膜を用いた *in vitro* 試験（Table 2.6.5.16A-DMPK R1500730）において ^{14}C-LNP023 の膜透過性を評価したところ，検討した LNP023 濃度範囲（0.5～400 µmol/L）において，Caco-2 細胞の頂端膜から基底膜方向への Papp（0.5 µmol/L で $1.33 \pm 0.16 \times 10^{-6}$ cm/sec）は基底膜から頂端膜方向への Papp（0.5 µmol/L で $5.39 \pm 0.21 \times 10^{-6}$ cm/sec）に比べて明らかに低く，排出比［（基底膜から頂端膜方向）／（頂端膜から基底膜方向）の Papp 比］が 4 以上であったことから，頂端膜方向への排出トランスポーターを介した輸送の関与が示唆された。トランスポーターによる輸送能の見かけの Km 値は 438 µmol/L 超であった（Table 2.6.5.16A-DMPK R1500730）。排出トランスポーター選択的阻害剤（LY335979）の効果から，LNP023 は P-gp の基質であることが示唆された。以上より，LNP023 は Caco-2 単層膜を低～中程度の速度で受動的に透過することが明らかとなった。また，得られた膜透過のパラメータを用いて算出したとき，排出トランスポーターによる輸送能の存在下及び非存在下におけるヒトでの吸収率の推定値はそれぞれ 47%及び 61%であり，トランスポーターを阻害したときの吸収率増加は少ないと予測された（Table 2.6.5.16A-DMPK R1500730）。

ヒト肝細胞を用いた *in vitro* 試験（Table 2.6.5.16G-DMPK R1500336）において ^{14}C-LNP023（0.5 及び 130 µmol/L）の細胞への取り込みを検討した。37°C での LNP023 の取り込みは 4°C 下に比べて高く，また高濃度において取り込みクリアランスが低下した。このことから，LNP023 は受動的透過により肝細胞に取り込まれるとともに，少なくとも 1 種類の取り込み輸送系（SLC）も関与することが示唆された（受動的膜透過クリアランス PSm：4.3 µL/min/10^6 cells，輸送担体を介した膜透過クリアランス PSc の最大値：3.4 µL/min/10^6 cells）。トランスポーター選択的阻害剤（リファマイシン SV／アトルバスタチン）の効果から，LNP023 は OATP の基質であることが示唆された（Table 2.6.5.16G-DMPK R1500336）。

サンドイッチ培養したヒト肝細胞を用いた *in vitro* 試験（Table 2.6.5.16J-DMPK R1700975）において ^{14}C-LNP023（1～100 µmol/L）の肝胆移行動態を検討した。Ca^{2+}存在下（細胞及び胆汁ポケットへの蓄積量の合計を示す）でインキュベーションしたときの細胞可溶化物試料への放射能の蓄積は Ca^{2+}非存在下（細胞への蓄積量を示す）に比べて明らかに高かった。このことは，細胞間密着の完全性が維持される条件である Ca^{2+}存在下でインキュベーションすることで，隣接する肝細胞間に形成された胆汁ポケットに LNP023 が蓄積されることを示唆する。胆汁ポケットへの LNP023 の分泌は，検討した LNP023 濃度範囲で明らかな飽和を示さなかった。胆汁中分泌（肝細胞内から胆汁ポケットへの蓄積）の固有クリアランス（PSint,bile）は 0.383 µL/min/mg protein

（LNP023 の各濃度における値の平均）であった（Table 2.6.5.16J-DMPK R1700975）。以上の成績から， LNP023 は能動輸送を介して胆汁中に分泌されることが示唆された。

7.4 トランスポーターの基質となる可能性

Caco-2 細胞の膜透過試験での LNP023 の排出過程及び肝細胞系での LNP023 輸送過程に，排出トランスポーター及び取り込みトランスポーターが関与する可能性が示唆された（7.3 項）。本項では，LNP023 が種々のヒト排出トランスポーター及び取り込みトランスポーターの基質となる可能性について検討した *in vitro* 試験（Table 2.6.5.16K-DMPK R1800300，Table 2.6.5.16L-DMPK R2000245）の成績について要約する。

7.4.1 排出トランスポーターの基質となる可能性

ヒト P-gp 又は BCRP を発現させた MDCKII 細胞の単層膜を用いて，^{14}C-LNP023（0.5～800 µmol/L）の双方向（頂端膜から基底膜方向及び基底膜から頂端膜方向）への輸送を評価した。検討した LNP023 濃度範囲において，いずれの発現細胞でも基底膜から頂端膜方向への Papp が高く，排出比の最大値は P-gp 発現細胞で 7.86（400 µmol/L），BCRP 発現細胞で 5.98（10 µmol/L）であった。さらに，P-gp 及び BCRP 特異的阻害剤（P-gp：LY335979，BCRP：Ko143）を適用したときの排出比がともに 1 付近まで低下したことから，LNP023 の能動輸送における P-gp 及び BCRP の寄与が示された。検討した LNP023 濃度範囲で輸送の飽和はみられなかった（Table 2.6.5.16K-DMPK R1800300）。

また，ヒト MRP2 を過剰発現させた細胞から調製した反転膜小胞を用い，アデノシン三リン酸（ATP）存在下で ^{14}C-LNP023（0.5～400 µmol/L）とインキュベーションしたとき，膜小胞への LNP023 の蓄積量は比較対照のアデニル酸（AMP）存在下に比べて 2 倍超であったことから，MRP2 発現膜小胞における LNP023 の能動的（ATP 依存的）な蓄積が示された。検討した LNP023 濃度範囲で輸送の飽和はみられなかった（Table 2.6.5.16K-DMPK R1800300）。

以上のことから，LNP023 は排出トランスポーターである P-gp，BCRP，及び MRP2 の基質であることが示された。

7.4.2 取り込みトランスポーターの基質となる可能性

ヒト OATP1B1 又は OATP1B3 を過剰発現させた HEK293 細胞への ^{14}C-LNP023（0.25～400 µmol/L）の取り込みを検討したところ，トランスポーター特異的な細胞への蓄積（トランスポーター発現細胞と対照細胞との蓄積量の差）がみられた。蓄積比（蓄積量の発現細胞／対照細胞比）は，OATP1B1 で 2.80（1 µmol/L），OATP1B3 では 4.39（0.25 µmol/L）であった。また，いずれの発現細胞においても OATP の典型阻害剤（リファンピシン）により蓄積比が 1 付近に低下したことから，両トランスポーターの関与が示唆された。OATP1B1 の取り込みにおける CLint は LNP023 濃度によらずほぼ一定（平均値：11.1 µL/min/mg protein）であったが，OATP1B3 では濃度増加に伴い減少した（34.9 µL/min/mg protein から 23.0 µL/min/mg protein）。Km 値は

OATP1B1 ：82.5 ± 43.6 µmol/L，OATP1B3 ：17.1 ± 4.73 µmol/L であった（Table 2.6.5.16K-DMPK R1800300）。

ヒト OAT1（HEK293 細胞及び CHO-K1 細胞），OAT2（HEK293 細胞），OAT3（HEK293 細胞及び MDCKII 細胞），又は OCT2（CHO-K1 細胞）を発現させた細胞系を用い，LNP023（OAT1 及び OAT3 の試験：0.1～400 µmol/L，OAT2 及び OCT2 の試験：0.1～300 µmol/L）の細胞への取り込みを，OAT 又は OCT の典型阻害剤（OAT：プロベネシド，インドメタシン，OCT：ベラパミル）の存在下又は非存在下で検討した。その結果，OAT1，OAT2，OAT3，及び OCT2 発現細胞への LNP023 の蓄積量は，検討した濃度範囲において対照細胞と同様であった（トランスポーター特異的な細胞への取り込み指標である蓄積比が 2 倍未満）。また，典型阻害剤が細胞への蓄積に影響しなかったことから，OAT1，OAT2，OAT3，及び OCT2 を介した LNP023 の能動的蓄積は認められなかった（Table 2.6.5.16L-DMPK R2000245）。

以上のことから，LNP023 は取り込みトランスポーターである OATP1B3 及び寄与は少ないものの OATP1B1 の基質であることが示された。一方，OAT1，OAT2，OAT3，及び OCT2 の基質ではなかった。

7.5 トランスポーター阻害

LNP023 が種々のヒト排出トランスポーター及び取り込みトランスポーターを阻害する可能性について *in vitro* で検討した（Table 2.6.5.16I-DMPK R1500533，Table 2.6.5.16B-DMPK R1600716，Table 2.6.5.16D-DMPK R1500958 ， Table 2.6.5.16E-DMPK R1709014 ， Table 2.6.5.16H-DMPK R1500532，Table 2.6.5.16C-DMPK R1600717，Table 2.6.5.16F-DMPK R2000210）。以下に，それらの試験成績について要約する。

7.5.1 排出トランスポーター阻害

ヒト P-gp（LLC-PK1 細胞）又は BCRP（MDCKII 細胞）を過剰発現させた細胞系，並びに P-gp 又は BCRP を過剰発現させた HEK293 細胞から調製した反転膜小胞（ATP 存在下）を用いて，トランスポーター選択的な放射性標識プローブ基質（細胞系の試験では P-gp：^{3}H-ジゴキシン，BCRP ： ^{14}C-2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-2，膜小胞の試験では P-gp ： ^{3}H-N-methyl-quinidine，BCRP：^{3}H-estrone-3-sulfate）とともに LNP023（0.1～400 µmol/L）とインキュベーションし，LNP023 がそれぞれのトランスポーターによる輸送（細胞系：細胞からの排出，膜小胞：小胞内への蓄積）を阻害する可能性を検討した。陽性対照は P-gp：シクロスポリン A 又は PSC833，並びに BCRP：Ko143 又は Ko134 を用いた。細胞及び膜小胞内に蓄積したプローブ基質は LSC により測定した（Table 2.6.5.16I-DMPK R1500533，Table 2.6.5.16B-DMPK R1600716）。その結果，細胞系を用いた試験では，検討した濃度範囲において LNP023 は P-gp 及び BCRP のいずれに対しても阻害作用を示さなかった。なお，P-gp 発現細胞において LNP023 濃度依存的にプローブ基質（digoxin）の取り込みクリアランスが低下し，LNP023 が機序不明の能動的取り込みを阻害した可能性が考えられた（Table 2.6.5.16I-DMPK R1500533）。また，膜小胞を用いた試験

においても，LNP023 は検討した濃度範囲において BCRP を介した膜小胞へのプローブ基質の蓄積を阻害しなかった。一方，P-gp を介した輸送に対しては濃度依存的な阻害［LNP023 の最高濃度：400 µmol/L で最大 80%の阻害率（陽性対照である PSC833 の阻害効果に対する相対割合）］を示した。P-gp に対する IC50 は 37.0 µmol/L，Ki 値（競合阻害を仮定）は 27.7 µmol/L であった。したがって，LNP023 は P-gp に対して阻害作用を有することが示された。一方，BCRP に対しては阻害作用を示さなかった（Table 2.6.5.16B-DMPK R1600716）。

ヒト MRP2 を過剰発現させた Sf9 細胞又は BSEP を過剰発現させた HEK293 細胞から調製した反転膜小胞を用いて，ATP 存在下でトランスポーター選択的な放射性標識プローブ基質（MRP2：^{3}H-estradiol-17β-D-glucuronide，BSEP：Taurocholic acid, [^{3}H(G)]）とともに LNP023（MRP2 の試験：0.1～260 µmol/L，BSEP の試験：0.1～400 µmol/L）とインキュベーションし，LNP023 がそれぞれのトランスポーターによる輸送（小胞内への蓄積）を阻害する可能性を検討した。陽性対照は MRP2：MK571 及び BSEP：シクロスポリン A を用いた。膜小胞内に蓄積したプローブ基質は LSC により測定した。その結果，LNP023 は検討した濃度範囲において MRP2 及び BSEP のいずれによる膜小胞へのプローブ基質の蓄積も阻害しなかった。したがって，LNP023 は MRP2 及び BSEP に対して阻害作用を示さなかった（Table 2.6.5.16D-DMPK R1500958，Table 2.6.5.16E-DMPK R1709014）。

7.5.2 取り込みトランスポーター阻害

ヒト OATP1B1 又は OATP1B3 を過剰発現させた HEK293 細胞を用いて，OATP1B1 及び OATP1B3 に選択的な放射性標識プローブ基質（^{3}H-estradiol-17β-D-glucuronide）とともに LNP023（0.1～400 µmol/L）とインキュベーションし，LNP023 がそれぞれのトランスポーターによる輸送（細胞内への蓄積）を阻害する可能性を検討した。陽性対照はともにリファマイシン SV／アトルバスタチン（mix）を用いた。細胞内に蓄積したプローブ基質は LSC により測定した。その結果，LNP023 は OATP1B1 及び OATP1B3 を介した輸送に対して濃度依存的な阻害（LNP023 の最高濃度：400 µmol/L でそれぞれ最大 100.2%及び 45.3%の阻害率）を示した。阻害パラメータは，OATP1B1 で IC50：25.6 ± 1.8 µmol/L，Ki 値：24.9 ± 1.7 µmol/L，また OATP1B3 では IC50：400 µmol/L 超，Ki 値：392.7 µmol/L 超であった。したがって，LNP023 は OATP1B1 及び OATP1B3 に対して阻害作用を有することが示された（Table 2.6.5.16H-DMPK R1500532）。

ヒト OAT1 又は OAT3 を安定発現している HEK293 細胞，あるいは MATE1 又は MATE2-K を安定発現している MDCKII 細胞を用いて，トランスポーター選択的な放射性標識プローブ基質（OAT1：aminohippuric acid p-[glycyl-2-^{3}H]，OAT3：^{3}H-estrone-3-sulfate，MATE1 及び MATE2-K：metformin hydrochloride, [biguanidine-^{14}C]）とともに LNP023（0.1～400 µmol/L）とインキュベーションし，LNP023 がそれぞれのトランスポーターによる輸送（細胞内への蓄積）を阻害する可能性を検討した。陽性対照は OAT1：ベンズブロマロン，OAT3：プロベネシド，並びに MATE1 及び MATE2-K：ピリメタミンを用いた。細胞内に蓄積したプローブ基質は LSC により測定した。

その結果，LNP023は検討した濃度範囲でOAT1及びMATE2-Kを介したプローブ基質の細胞内への蓄積を阻害しなかった。一方，LNP023はOAT3及びMATE1を介した輸送に対して濃度依存的な阻害（LNP023の最高濃度：400 µmol/Lでそれぞれ最大72%及び47%の阻害率）を示した。阻害パラメータは，OAT3でIC50：178 µmol/L，Ki値：173 µmol/L，またMATE1ではIC50：424 µmol/L，Ki値：400 µmol/Lであった。したがって，LNP023はOAT3及びMATE1に対して阻害作用を有することが示された。一方，OAT1及びMATE2-Kに対して阻害作用を示さなかった（Table 2.6.5.16E-DMPK R1709014）。

ヒトOCT1又はOCT2を安定発現しているHEK293細胞を用いて，OCT1及びOCT2に選択的な放射性標識プローブ基質（metformin hydrochloride, [biguanidine-^{14}C]）とLNP023（0.1～400 µmol/L）をインキュベーションし，LNP023がそれぞれのトランスポーターによる輸送（細胞内への蓄積）を阻害する可能性を検討した。陽性対照はともにベラパミルを用いた。細胞内に蓄積したプローブ基質はLSCにより測定した。その結果，LNP023はOCT1及びOCT2を介した輸送に対して濃度依存的な阻害（LNP023の最高濃度：400 µmol/Lでそれぞれ最大65%及び58%の阻害率）を示した。阻害パラメータは，OCT1でIC50：322 µmol/L，Ki値：321 µmol/L（競合阻害を仮定），またOCT2ではIC50：361 µmol/L，Ki値：359 µmol/L（競合阻害を仮定）であった。したがって，LNP023はOCT1及びOCT2に対して阻害作用を有することが示された（Table 2.6.5.16C-DMPK R1600717）。

さらに，取り込みトランスポーターOAT1，OAT3，MATE1，MATE2-K，OCT1，及びOCT2については，上述と同じ発現細胞系を用い，トランスポーター選択的な放射性標識プローブ基質（OAT1に対してTenofovir, [adenine-2,8-^{3}H]を用いた以外は上述に同じ）を加える前にLNP023（0.1～400 µmol/L）と30分間プレインキュベーションした場合のLNP023の阻害作用について検討した。陽性対照はOAT1でプロベネシドを用いた以外は上述と同じ典型阻害剤を用いた。その結果，LNP023はトランスポーターを介した輸送に対して，LNP023の最高濃度：400 µmol/Lにおいて最大でOAT1：20.2%，OAT3：74.8%，MATE1：55.7%，MATE2-K：20.5%，OCT1：48.9%，及びOCT2：35.8%の阻害率を示した。これらのうち，OAT3，MATE1，及びOCT1については濃度依存的な阻害がみられた。阻害パラメータは，OAT3でIC50：188.2 µmol/L及びKi値：183.3 µmol/L，MATE1でIC50：347.2 µmol/L及びKi値：327.1 µmol/L，OCT1でIC50：398.9 µmol/L及びKi値：396.6 µmol/Lであった。なお，OAT1，MATE2-K，及びOCT2ではIC50を算出しなかった。したがって，LNP023はOAT3，MATE1，及びOCT1に対して阻害作用を有することが示された。また，OAT1，MATE2-K，及びOCT2に対しても弱い阻害の可能性が考えられた（Table 2.6.5.16F-DMPK R2000210）。

8 その他の薬物動態試験

その他の薬物動態試験は実施しなかった。

9 考察及び結論

LNP023 の PK 特性について，種々の動物種を用いて評価した。単回経口投与時の LNP023 の BA はマウス，ラット，及びイヌで 40%～68%であった。吸収率はラットで 81.8%と高く，ヒトでの 70.6%以上との推定値（2.7.2-3.1.1 項）と類似していた。このことは，LNP023 の吸収過程において排出トランスポーターによる影響が少ないことを示唆しており，Caco-2 を用いた膜透過性試験でのトランスポーター阻害時の吸収率予測はこれを裏付けている（7.3 項）。^{14}C-LNP023 又は LNP023 を単回経口投与したときの LNP023 の Tmax は検討した動物種において 0.25～2 時間であり，ヒト（CLNP023A2101 試験，CLNP023X2101 試験）での約 1～1.5 時間と同程度であった。検討した全動物種を通じて，LNP023 の CL は 0.0912～0.960 L/h/kg と低く Vss は 0.6～6.6 L/kg であり，動物に単回静脈内投与時の循環血漿からの T1/2 は約 2.9～7.5 時間であった。ラットに単回経口投与したときの LNP023 の T1/2 は約 4～10 時間であり，ヒト（CLNP023A2101 試験，CLNP023X2101 試験）での約 13～18 時間よりも短かった。

LNP023 の特徴的な性質として濃度依存的な血漿蛋白結合がみられたが，これは特にヒトにおいて循環血中で高レベルに発現している FB（薬理学的標的分子）との飽和結合に関連する可能性が考えられる。fu 値が濃度依存的であるため肝取り込みや腎排泄が影響を受ける可能性があり，その結果として非線形 PK や用量比を下回る用量-曝露量関係がヒトにおける PK 試験で観察されたと考えられる（2.7.2-3.1.5 項）。これに対して，FB との結合飽和が生じるような毒性用量では，動物種によらず用量に比例する LNP023 曝露量の増加傾向が認められた。

ラットに ^{14}C-LNP023 を経口投与したとき，総放射能は脳を除いて広範囲の組織に分布し，多くの組織から速やかに消失した。有色ラットでは，眼組織のメラニンに対する親和性がみられた。メラニン含有組織における放射能の Tlast は長かったが，メラニンとの結合は可逆的であった。ラットにおいて LNP023 の脳への移行は低い又は移行しなかった。この理由として，LNP023 の受動的膜透過性は低～中程度であるが（Table 2.6.5.16A-DMPK R1500730，7.3 項），LNP023 は P-gp の基質であるため（7.4 項），P-gp により排出を受けた可能性が考えられる。

マウス，ラット，イヌ，サル，及びヒト肝細胞を用いた *in vitro* 代謝試験において，代謝物プロファイルは動物種を通じて質的に類似していた。ラット *in vivo* 試験において LNP023 は胆汁中及び尿中への直接的な分泌／排泄とともに主に代謝により消失し，ヒトにおいても同様と考えられた（2.7.2-3.1.4 項）。ラットにおいて，N-脱アルキル化，O-脱エチル化，C-酸化，及び水酸化を含む酸化的代謝反応，並びにグルクロン酸抱合が主な代謝経路であった。イヌ及びヒト（DMPK RCLNP023A2101-metid，DMPK-BT-R1701500-metid）においても同様の代謝経路が確認され，代謝プロファイルは類似していた（Table 11-2）。ヒトの循環血中における主要成分は未変化体であり，2 つのアシルグルクロン酸抱合代謝物（M8 及び M9），さらに酸化代謝物が続いた（DMPK RCLNP023A2101-metid，DMPK-BT-R1701500-metid）。これらの代謝物は薬理学的に活性を持たないと考えられ，実際に，M8，M9，及び M2 はいずれも薬理活性への寄与はほとんどないことが確認された（2.7.2-2.1.2 項，5.2.4 項）。動物及びヒトにおける代謝物の曝露量を比較

したとき，血漿中においてヒトに特異的な代謝物は検出されなかった（Table 11-2）。ラット及びイヌにおいて，M9 を除きヒト血漿中代謝物に対する十分な曝露が得られた。ただし，M9 がラット胆汁中に認められたことは，ラット肝臓がこの代謝物に曝露されたことを示している。また，ヒト肝ミクロソーム及びヒト肝細胞を用いた *in vitro* 試験系において観察された，共有結合による蛋白質付加体の形成に基づいて評価した反応性代謝物の生成量は，代謝活性化に対して規定された閾値を下回っていた。したがって，LNP023 では *in vivo* において懸念となるような代謝活性化が生じる可能性は低いと考える。

ヒト肝ミクロソーム及びヒト酵素発現系を用いた *in vitro* 試験において，LNP023 の酸化的代謝に関与する主要な CYP 分子種は CYP2C8 であった。また，LNP023 の直接的なグルクロン酸抱合代謝に関与する主要な UGT 分子種は UGT1A1 であることが示唆された。

肝ミクロソームを用いた *in vitro* 試験において，LNP023 は CYP1A2，CYP2A6，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1，及び CYP3A4/5 の可逆的阻害剤ではないと考えられた。LNP023 は CYP2C8 に対して時間依存的阻害作用を示した（KI：179 µmol/L，kinact：0.0702 min^{-1}）。一方，LNP023 は CYP1A2，CYP2B6，CYP2C9，CYP2C19，CYP2D6，及び CYP3A4/5 の時間依存的阻害剤ではないと考えられた。また，UGT1A1 に対しても阻害作用を示さなかった。

ヒト初代培養肝細胞において LNP023 は CYP3A4 に対して弱い誘導作用を示したが，CYP2B6 及び CYP2C9 を誘導しなかった。また，CYP1A2 に対しても LNP023 は誘導剤ではないと考えられた。

トランスポーター発現細胞又は反転膜小胞を用いた *in vitro* 試験により，LNP023 は排出トランスポーターである P-gp，BCRP，及び MRP2，並びに取り込みトランスポーターである OATP1B1 及び OATP1B3 の基質であることが示された。一方，LNP023 は OAT1，OAT2，OAT3，及び OCT2 の基質ではなかった。上記に関連して，Caco-2 細胞の単層膜を用いた *in vitro* 膜透過性試験において，排出トランスポーターを阻害したときのヒトでの吸収率増加は少ない（排出トランスポーター輸送能の存在下及び非存在下における吸収率の推定値はそれぞれ 47%及び 61%）と予測された（7.3 項）。

トランスポーター発現細胞又は反転膜小胞を用いた *in vitro* 試験において，LNP023 は P-gp（Ki 値：27.7 µmol/L），OATP1B1（Ki 値：24.9 µmol/L），OATP1B3（Ki 値：392.7 µmol/L 超），OAT3（プレインキュベーションしたときの Ki 値：183.3 µmol/L），MATE1（プレインキュベーションしたときの Ki 値：327.1 µmol/L），OCT1（プレインキュベーションしたときの Ki 値：396.6 µmol/L），及び OCT2（Ki 値：359 µmol/L）に対して阻害作用を示した。一方，LNP023 は BCRP，MRP2，及び BSEP を阻害せず，OAT1 及び MATE2-K に対しても明らかな阻害作用を示さなかった。

10 参考文献

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11 図表

Table 11-1 LNP023 を単回投与した PK／ADME 試験一覧

動物種	系統	性	投与経路	用量 (mg/kg)	投与剤形	被験物質	分析対象物質	マトリックス	報告書番号
マウス	C57BL/6	雄	静脈内	1	溶液	LNP023	LNP023	血漿	RD-2016-00013
			経口	10	懸濁液	LNP023	LNP023	血漿	
ラット	Wistar Hannover	雄	静脈内	1	溶液	LNP023	LNP023	血漿	RD-2016-00014
			経口	30	懸濁液	LNP023	LNP023	血漿	
ラット	Wistar Hannover	雄	静脈内	2	溶液	^{14}C-LNP023·HCl	^{14}C, LNP023, 代謝物	血漿，血液，尿，糞，胆汁，消化管，屍体，ケージ洗浄液	DMPK R1500361
			経口	10	懸濁液	^{14}C-LNP023·HCl	^{14}C, LNP023, 代謝物	血漿，血液，尿，糞，組織，屍体，ケージ洗浄液	
	Long-Evans	雄	経口	10	懸濁液	^{14}C-LNP023·HCl	^{14}C	血液，組織	
ラット	Long-Evans	雄雌	経口	10	溶液	^{14}C-LNP023·HCl	^{14}C	血漿，血液，組織	DMPK R1701426
		雄						尿，糞，屍体，ケージリンス，ケージ洗浄液，ケージ拭き取り	
ラット	Wistar Hannover	雄	経口	3, 10, 30, 100, 300	懸濁液	不純物A*	不純物A*	血漿	DMPK R2000379
ラット	Sprague Dawley	雄	静脈内	2	溶液	LNP023·HCl	LNP023	血漿	DMPK R2100550-01
			経口	10	溶液	LNP023·HCl	LNP023	血漿	
			回腸内	10	溶液	LNP023·HCl	LNP023	血漿	
イヌ	Beagle	雄	静脈内	0.1	溶液	LNP023·HCl	LNP023	血液	RD-2016-00020
			経口	10	溶液	LNP023·HCl	LNP023		
イヌ	Beagle	雄	静脈内	3.5	溶液	LNP023·HCl	LNP023	血漿，血液	DMPK R2101187
				10	溶液	LNP023·HCl	LNP023		

Source：Table 2.6.5.3A-RD-2016-00013，Table 2.6.5.3B-RD-2016-00014，Table 2.6.5.3C-DMPK R1500361，Table 2.6.5.5B-DMPK R1701426，Table 2.6.5.3D-DMPK R2000379，Table 2.6.5.3E-DMPK R2100550-01，Table 2.6.5.3F-RD-2016-00020，Table 2.6.5.3G-DMPK R2101187

＊新薬承認情報提供時に置き換えた

Table 11-2 動物及びヒトにおける LNP023 の代謝物

代謝物	*In vitro*					*In vivo*							
	肝細胞					全身曝露			曝露量比		排泄物全量		
	マウス b)	ラット a)	イヌ a)	サル b)	ヒト a)	ラット c)	イヌ d)	ヒト e)	ラット：ヒト f) (雄／雌)	イヌ：ヒト g) (雄／雌)	ラット h) 尿	ラット h) 糞	ラット h) 胆汁
フロントピーク	-	0.237	nd	-	0.0672	- / -	-	- / -	-	-	-	-	-
P3.1	-	-	-	-	-	- / -	-	- / -	-	-	-	-	-
M1 ([redacted])	+	1.80	0.712	+	0.720	1.63/ +	+	- / +	3.57 / 2.02	6.86 / 6.29	1.28	nd	2.63
M2 ([redacted])	++	4.49	0.244	++	1.37	1.39/ +	+	- / +	1.06 / 0.664	0.907 / 0.735	0.221	0.0876	20.2
M3	++	nd	0.0488	+	0.365	- / -	-	- / nd	-	-	-	-	-
M4	nd	1.69	nd	nd	nd	0.338 / -	-	- / nd	-	-	0.333	nd	5.42
M5	+	0.207	nd	+	nd	- / -	-	- / nd	-	-	-	-	-
M6 ([redacted])	++	1.42	1.52	++	0.854	- / +	+	- / +	2.68 / 2.34	13.9 / 14.4	0.234	0.0763	5.85
M7	-	0.454	0.185	-	0.154	- / +	+	- / +	6.61 / 5.62	10.9 / 6.22	0.107	nd	nd
M8 ([redacted]) 及び異性体	++	1.08	0.576	++	1.85	1.34 / +	+	8.05 / +	0.688 / 1.41	2.72 / 1.40	0.268	nd	20.4
M9 ([redacted])	+	nd	nd	+	0.547	- /+	+	5.17 / +	0.0223 / 0.0211	0.0159 / 0.0101	nd	nd	2.98
M10 ([redacted])	-	-	-	-	-	- / +	+	- / nd	-	-	nd	nd	3.12
M11	-	-	-	-	-	- / -	-	- / -	-	-	-	-	-

代謝物	*In vitro*					*In vivo*							
	肝細胞					全身曝露			曝露量比		排泄物全量		
	マウス [b]	ラット [a]	イヌ [a]	サル [b]	ヒト [a]	ラット [c]	イヌ [d]	ヒト [e]	ラット：ヒト [f]	イヌ：ヒト [g]	ラット [h]		
									（雄／雌）	（雄／雌）	尿	糞	胆汁
M12	-	-	-	-	-	- / -	-	- / -	-	-	-	-	-
M13	-	-	-	-	-	- / -	-	- / -	-	-	-	-	-
P6.7	-	nd	nd	-	nd	- / -	-	- / -	-	-	-	-	-
P14.6	-	0.789	1.07	-	1.02	- / -	-	- / -	-	-	-	-	-
LNP023	+++	86.4	93.1	+++	88.7	91.0 / +	+	83.0 / +	3.71 / 5.19	12.0 / 12.9	8.04	3.51	11.7
その他	-	-	0.0976	-	0.383	0.129 / -	-	-	-	-	0.683 + 0.729	0.0623 + 0.151	3.00 + 3.55
検出ピーク合計	na	98.6	97.6	na	96.0	95.9 / na	na	96.2 / na	na	na	11.9	3.89	78.9
沈殿物又は試料処理中でのロス	na	1.40	2.40	na	4.00	4.12 / na	na	3.78 / na	na	na	1.13	0.316	0.556
総計	na	100	100	na	100	100 / na	na	100 / na	na	na	13.0	4.21	79.4

-：データなし，na：該当なし，nd：検出されず

[a] 肝細胞と ^{14}C-LNP023（10 µmol/L）をインキュベーションしたときの代謝物及び LNP023 の存在比率（総放射能に対する%として表示）（Table 2.6.5.10A-DMPK R1500300）

[b] 肝細胞と LNP023（10 µmol/L）をインキュベーションしたときの代謝物及び LNP023 の存在比率（LC-MS/MS におけるピーク面積の相対値として表示。+：インキュベーション開始 0 時間における LNP023 のピーク面積に対する当該成分の割合が< 1%，++：当該成分の割合が 1%～10%，+++：当該成分の割合が> 10%）（Table 2.6.5.10C-DMPK R1900278-metid）

[c] ラットに ^{14}C-LNP023（10 mg/kg）を単回経口投与したときの血漿中代謝物及び LNP023 の存在比率（総放射能の全身曝露量に対する%として表示）（Table 2.6.5.9A-DMPK R1500361） / LNP023（750 mg/kg/日）を 154 日間反復経口投与したときの血漿中代謝物及び LNP023 の有無（+：当該成分が検出された場合の表示）（Table 2.6.5.9B-DMPK R1570193-metid）

[d] イヌに LNP023（150 mg/kg/日）を 273 日間反復経口投与したときの血漿中代謝物及び LNP023 の有無（+：当該成分が検出された場合の表示）（Table 2.6.5.9C-DMPK R1570192-metid）

[e] ヒトに ^{14}C-LNP023（100 mg）を単回経口投与したときの血漿中代謝物及び LNP023 の存在比率（総放射能の全身曝露量に対する%として表示）（DMPK RCLNP023A2101-metid） / LNP023（200 mg bid）を 14 日間反復経口投与したときの血漿中代謝物及び LNP023 の有無（+：当該成分が検出された場合の表示）（DMPK-BT-R1701500-metid）

[f] ヒト（LNP023 を 200 mg bid，反復経口投与 14 日目）及びラット（LNP023 を 750 mg/kg/日，反復経口投与 154 日目）での定常状態における代謝物の曝露量の比較（代謝物のピーク面積比）（Table 2.6.5.9D-DMPK R1809129-metid）

[g] ヒト（LNP023 を 200 mg bid，反復経口投与 14 日目）及びイヌ（LNP023 を 150 mg/kg/日，反復経口投与 273 日目）での定常状態における代謝物の曝露量の比較（代謝物のピーク面積比）（Table 2.6.5.9D-DMPK R1809129-metid）

[h] 胆管カニューレを挿入したラットに ^{14}C-LNP023（2 mg/kg）を単回静脈内投与したときの排泄物（プール）中における代謝物及び LNP023 の存在比率（投与量に対する%として表示）（Table 2.6.5.9A-DMPK R1500361）

目　次

目　次 2
略号一覧 5
用語の定義一覧 7
2.6.5.1　薬物動態試験：一覧 8
2.6.5.2　薬物動態試験：分析方法 15
2.6.5.2A　ラット血漿中 LNP023 分析法（DMPK R1500297-01） 15
2.6.5.2B　イヌ血漿中 LNP023 分析法（DMPK R1500296-01） 17
2.6.5.2C　マウス血漿中 LNP023 分析法（DMPK R1500429-01） 19
2.6.5.2D　ラット血漿中 LNP023 分析法（DMPK R1600441） 21
2.6.5.2E　イヌ血漿中 LNP023 分析法（DMPK R1600442 and DMPK R1600442-ad1） 23
2.6.5.2F　ウサギ血漿中 LNP023 分析法（DMPK R1600671-01） 25
2.6.5.2G　マウス血漿中 LNP023 分析法（DMPK R2000093） 27
2.6.5.2H　ラット血漿中 不純物A* 分析法（DMPK R2000262） 29
2.6.5.3　薬物動態試験：単回投与後の吸収 31
2.6.5.3A　マウスに単回静脈内及び経口投与後の薬物動態試験（RD-2016-00013） 31
2.6.5.3B　ラットに単回静脈内及び経口投与後の薬物動態試験（RD-2016-00014） 33
2.6.5.3C　ラットに単回静脈内及び経口投与後の薬物動態試験（DMPK R1500361） 34
2.6.5.3D　ラットに 不純物A* を単回経口投与後の薬物動態試験（DMPK R2000379） 36
2.6.5.3E　ラットに単回静脈内，経口，及び回腸内投与後の薬物動態試験（DMPK R2100550-01） 38
2.6.5.3F　イヌに単回静脈内及び経口投与後の薬物動態試験（RD-2016-00020） 40
2.6.5.3G　イヌに単回静脈内投与後の薬物動態試験（DMPK R2101187） 41
2.6.5.4　薬物動態試験：反復投与後の吸収 42
2.6.5.5　薬物動態試験：分布 43
2.6.5.5A　ラットに単回経口投与後の組織分布（DMPK R1500361） 43
2.6.5.5B　有色ラットに単回経口投与後の組織分布（DMPK R1701426） 49
2.6.5.6　薬物動態試験：蛋白結合 59
2.6.5.6A　*In vitro* 血漿蛋白結合及び血液への分布（DMPK R1500362） 59
2.6.5.6B　ヒト及びマウスにおける *in vitro* 血漿蛋白結合（DMPK R1600148） 64
2.6.5.6C　ラット，ウサギ，及びイヌにおける *ex vivo* 血漿蛋白結合（DMPK R1701166） 66
2.6.5.7　薬物動態試験：妊娠動物における試験 71
2.6.5.8　薬物動態試験：その他の分布試験 72
2.6.5.9　薬物動態試験：*in vivo* における代謝 73
2.6.5.9A　ラットに単回静脈内及び経口投与後の *in vivo* 代謝（DMPK R1500361） 73
2.6.5.9B　ラットに反復経口投与後の *in vivo* 代謝（DMPK R1570193-metid） 77
2.6.5.9C　イヌに反復経口投与後の *in vivo* 代謝（DMPK R1570192-metid） 78

*** 新薬承認情報提供時に置き換えた**

2.6.5.9D ヒト循環血漿中代謝物のラット，イヌ，及びヒト間における曝露量の比較（DMPK R1809129-metid） 80
2.6.5.10 薬物動態試験：*in vitro* における代謝 82
2.6.5.10A ラット，イヌ，及びヒト肝細胞における *in vitro* 代謝（DMPK R1500300） 82
2.6.5.10B [redacted]（M9）の *in vitro* における安定性（DMPK R1900261-metid） 85
2.6.5.10C マウス，ラット，サル，及びヒト肝細胞における *in vitro* 代謝（DMPK R1900278-metid） 86
2.6.5.10D [redacted]（M8）の *in vitro* における安定性（DMPK R1500191） 87
2.6.5.10E 酸化的代謝に関与する CYP 分子種同定試験（DMPK R1500516） 89
2.6.5.10F 酸化的代謝に関与する CYP 分子種同定試験（DMPK R1600579） 93
2.6.5.10G グルクロン酸抱合に関与する UGT 分子種同定試験（DMPK R1600698） 94
2.6.5.10H グルクロン酸抱合に関与する UGT 分子種同定試験（DMPK R1600140） 97
2.6.5.10I 反応性代謝物の生成（DMPK R1500514） 98
2.6.5.11 薬物動態試験：推定代謝経路 100
2.6.5.12 薬物動態試験：薬物代謝酵素の誘導／阻害 101
2.6.5.12A ヒト肝ミクロソームにおける CYP 時間依存的阻害試験（DMPK R1300641） 101
2.6.5.12B ヒト肝ミクロソームにおける CYP 阻害試験（DMPK R1500321） 105
2.6.5.12C ヒト肝ミクロソームにおける UGT1A1 阻害試験（DMPK R1500936） 106
2.6.5.12D CYP 誘導試験（DMPK R1600704） 107
2.6.5.12E CYP 時間依存的阻害試験（DMPK R1900589） 112
2.6.5.13 薬物動態試験：累積排泄 113
2.6.5.13A ラットに単回静脈内及び経口投与後の排泄（DMPK R1500361） 113
2.6.5.13B 有色ラットに単回経口投与後の排泄（DMPK R1701426） 115
2.6.5.14 薬物動態試験：胆汁中排泄 117
2.6.5.14A ラットに単回静脈内投与後の胆汁中排泄（DMPK R1500361） 117
2.6.5.15 薬物動態試験：薬物相互作用 118
2.6.5.16 薬物動態試験：その他 119
2.6.5.16A Caco-2 細胞を用いた膜透過性試験（DMPK R1500730） 119
2.6.5.16B BCRP, MDR1 親和性評価（DMPK R1600716） 120
2.6.5.16C OCT1, OCT2 親和性評価（DMPK R1600717） 122
2.6.5.16D MRP2 阻害評価（DMPK R1500958） 124
2.6.5.16E ABC 及び SLC トランスポーター阻害評価（DMPK R1709014） 125
2.6.5.16F SLC トランスポーター親和性評価（DMPK R2000210） 128
2.6.5.16G 凍結肝細胞を用いた取り込み試験（DMPK R1500336） 132
2.6.5.16H LNP023 による取り込みトランスポーター阻害試験（DMPK R1500532） 133
2.6.5.16I LNP023 による BCRP 及び P-gp 阻害試験（DMPK R1500533） 134
2.6.5.16J 培養ヒト肝細胞を用いた肝胆道系動態評価（DMPK R1700975） 137

2.6.5.16K 排出及び取り込みトランスポーターフェノタイピング（DMPK R1800300） 139
2.6.5.16L 取り込みトランスポーターフェノタイピング（DMPK R2000245） 145
2.6.5.16M ヒトに 1 日 2 回経口投与後の in vivo 代謝（DMPK BT-R1701500-metid） 158

略号一覧

略号	省略していない表現（英）	省略していない表現（日）
-	not available	データなし
AAG	α1-acid-glycoprotein	α1-酸性糖蛋白質
ABC	ATP-binding cassette	ATP 結合カセット
AMP	adenine monophosphate	アデニル酸（アデノシン一リン酸）
ATP	adenosine triphosphate	アデノシン三リン酸
AUC0-t	area under the drug plasma (blood / tissue) concentration-time curve (AUC) from time zero to time t	血漿（血液／組織）中薬物濃度-時間曲線下面積（0～t 時間）
AUCinf	AUC from time zero to infinity	血漿中薬物濃度-時間曲線下面積（0～無限大時間）
AUClast	AUC from time zero to the time of the last quantifiable concentration	血漿（血液／組織）中薬物濃度-時間曲線下面積（0～最終定量可能時点）
BCRP	breast cancer resistance protein	乳癌耐性蛋白質
bid	bis in die	1 日 2 回
BLOD	below limit of detection	検出限界未満
BLQ	below the limit of quantitation	定量限界未満
Cb	concentration in blood	血液中濃度
Cbc	concentration in blood cells	血球中濃度
CHO	chinese hamstar ovary	チャイニーズハムスター卵巣
CL	clearance	クリアランス
CLb	blood clearance	血液クリアランス
CLh,b	hapatic blood clearance	肝血液クリアランス
CLint	intrinsic clearance	固有クリアランス
CLp	plasma clearance	血漿クリアランス
Cmax	maximum drug plasma (blood / tissue) concentration	最高血漿（血液／組織）中薬物濃度
CM junction	corticomedullar junction	皮髄境界部
Cp	concentration in plasma	血漿中濃度
CYP	cytochrome P450	チトクローム P450
eq	equivalents	当量
E3S	estrone-3-sulfate	
ESI	electrospray ionization	エレクトロスプレーイオン化
fb	fraction bound in plasma	血漿中結合画分
FMO	flavin monooxygenase	フランビンモノオキシゲナーゼ
fp	fraction in plasma	血漿中分率
fu	fraction unbound in plasma	血漿中非結合画分
GI tract	gastrointestinal tract	消化管
GSH	glutathione, reduced form	還元型グルタチオン
HDL	high density lipoprotein	高比重リポ蛋白
HEK	human embryonic kidney	ヒト胎児腎臓
HLM	human liver microsomes	ヒト肝ミクロソーム

略号	省略していない表現（英）	省略していない表現（日）
HPLC	high performance liquid chromatography	高速液体クロマトグラフィー
HSA	human serum albumin	ヒト血清アルブミン
iv (or i.v.)	intravenous(ly)	静脈内
LC-MS	liquid chromatography-mass spectrometry	液体クロマトグラフィー質量分析
LC-MS(n)	liquid chromatography-data dependent multiple-stage mass spectrometry to the n^{th} power	液体クロマトグラフィー多段階質量分析
LC-MS/MS	liquid chromatography-tandem mass spectrometry	液体クロマトグラフィータンデム質量分析
LDL	low density lipoprotein	低比重リポ蛋白
LOD	limit of detection	検出限界
LLC-PK1 cells	porcine kidney cell line	ブタ腎由来細胞
LLOQ	lower limit of quantification	定量下限
LNP023	iptacopan	イプタコパン
LOQ	limit of quantification	定量限界
LSC	liquid scintillation counter	液体シンチレーションカウンター
MATE	multidrug and toxin extrusion	
MDCKII cells	mardin-darby canine kidney cells	イヌ腎由来細胞
MRM	multiple reaction monitoring	多重反応モニタリング
MRP	multidrug resistance associated protein	多剤耐性関連蛋白質
MS	mass spectrometry	質量分析
na	not applicable	該当なし
nc	not calculated	算出せず
nd	not detected	検出されず
OAT	organic anion transporter	有機アニオントランスポーター
OATP	organic anion transporting polypeptide	有機アニオントランスポーターポリペプチド
OCT	organic cation transporter	有機カチオントランスポーター
PAPS	adenosin 3’-phosphate 5’-phosphosulfate	
PBS	phosphate buffer solution	リン酸緩衝液
PEG	Polyethylene glycol	ポリエチレングリコール
PK	pharmacokinetic(s)	薬物動態（学）
po (or p.o.)	per os	経口
QWBA	quantitative whole body autoradiography	定量的全身オートラジオグラフィー
SD	standard deviation	標準偏差
SLC	solute-carrier	溶質輸送体
T1/2	elimination half-life	消失半減期
T/B ratio	tissue over blood ratio	組織／血液比
Tlast	time of last quantifiable concentration	最終定量可能時点
TK	toxicokinetic(s)	トキシコキネティクス
Tmax	Time to reach the maximum drug plasma (blood / tissue) concentration following drug administration	最高血漿（血液／組織）中薬物濃度到達時間
Tris	tris(hydroxymethyl)aminomethane	

略号	省略していない表現（英）	省略していない表現（日）
UDPGA	uridine 5’-diphospho-α-D-glucuronic acid	ウリジン 5’ジホスホグルクロン酸
UGT	uridine diphospho-glucuronosyltransferase	ウリジンジホスホ-グルクロノシルトランスフェラーゼ
ULOQ	upper limit of quantification	定量上限
UPLC	ultra performance liquid chromatography	超高速液体クロマトグラフィー
VLDL	very low density lipoprotein	超低比重リポ蛋白
VitETPGS	vitamin E d-α-tocopheryl polyethylene glycol succinate	
Vss	distribution volume at steady state	定常状態における分布容積

用語の定義一覧

用語	定義
LNP023·HCl	LNP023 hydrochloride salt
LNP023	LNP023 free base

2.6.5.1 薬物動態試験：一覧

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration or Sample(s)	Duration of dosing	Analyte(s), Dose(s) or Concentrations	GLP compliance	Testing facility	Report Number	Location in CTD
Analytical Methods and Validation Reports 2.6.5.2								
Analytical Methods and Validation 2.6.5.2A	Rat plasma	spiked samples	na	LNP023, 100-100000 ng/mL	not required	Novartis	[DMPK R1500297-01]	4.2.2.1-1
Analytical Methods and Validation 2.6.5.2B	Dog plasma	spiked samples	na	LNP023, 100-100000 ng/mL	not required	Novartis	[DMPK R1500296-01]	4.2.2.1-2
Analytical Methods and Validation 2.6.5.2C	Mouse plasma	spiked samples	na	LNP023, 100-100000 ng/mL	not required	Novartis	[DMPK R1500429-01]	4.2.2.1-3
Analytical Methods and Validation 2.6.5.2D	Rat plasma	spiked samples	na	LNP023, 0.100-100 µg/mL	not required	[redacted]	[DMPK R1600441]	4.2.2.1-4
Analytical Methods and Validation 2.6.5.2E	Dog plasma	spiked samples	na	LNP023, 0.100-100 µg/mL	not required	[redacted]	[DMPK R1600442] & [DMPK R1600442-ad1]	4.2.2.1-5
Analytical Methods and validation 2.6.5.2F	Rabbit plasma	spiked samples	na	LNP023, 0.100-100 µg/mL	not required	[redacted]	[DMPK R1600671-01]	4.2.2.1-6
Analytical Methods and Validation 2.6.5.2G	Mouse plasma	spiked samples	na	LNP023, 0.100-100 µg/mL	not required	[redacted]	[DMPK R2000093]	4.2.2.1-7
Analytical Methods and Validation 2.6.5.2H	Rat plasma	spiked samples	na	不純物A* (LNP023 impurity), 2.00-4000 ng/mL	not required	[redacted]	[DMPK R2000262]	4.2.2.1-8
Pharmacokinetics: Absorption after a Single Dose 2.6.5.3								
Absorption after a Single Dose 2.6.5.3A	Mouse, C57BL/6, male, fed	i.v. and p.o.	single	LNP023, i.v.: 1 mg/kg, p.o.: 10 mg/kg	not required	Novartis	[RD-2016-00013]	4.2.2.2-1

*** 新薬承認情報提供時に置き換えた**

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration or Sample(s)	Duration of dosing	Analyte(s), Dose(s) or Concentrations	GLP compliance	Testing facility	Report Number	Location in CTD
Absorption after a Single Dose 2.6.5.3B	Rat, Wistar Hannover, male, fed (i.v.), fasted (p.o.)	i.v. and p.o.	single	LNP023, i.v.: 1 mg/kg, p.o.: 30 mg/kg	not required	Novartis	[RD-2016-00014]	4.2.2.2-2
Absorption after a Single Dose 2.6.5.3C	Rat, Wistar Hannover, male, fed	i.v. and p.o.	single	^{14}C-LNP023·HCl, i.v.: 2 mg/kg, p.o.: 10 mg/kg	not required	Novartis	[DMPK R1500361]	4.2.2.2-3
Absorption after a Single Dose 2.6.5.3D	Rat, Wistar Hannover, male, fed	p.o.	single	不純物A* p.o.: 3, 10, 30, 100, 300 mg/kg	not required	[redacted]	[DMPK R2000379]	4.2.2.2-4
Absorption after a Single Dose 2.6.5.3E	Rat, Sprague Dawley, male, fed (i.v.), fasted (p.o., intra-ileal)	i.v., p.o., and intra-ileal	single	LNP023·HCl, i.v.: 2 mg/kg, p.o.: 10 mg/kg, intra-ileal: 10 mg/kg	not required	Novartis	[DMPK R2100550-01]	4.2.2.2-5
Absorption after a Single Dose 2.6.5.3F	Dog, Beagle, male, fasted	i.v. and p.o.	single	LNP023·HCl, i.v.: 0.1 mg/kg, p.o.: 10 mg/kg	not required	Novartis	[RD-2016-00020]	4.2.2.2-6
Absorption after a Single Dose 2.6.5.3G	Dog, Beagle, male, fasted	i.v.	single	LNP023·HCl, i.v. infusion: 3.5, 10 mg/kg	not required	[redacted]	[DMPK R2101187]	4.2.2.2-7
Pharmacokinetics: Absorption after Repeated Doses 2.6.5.4								
No studies conducted under this section								
Pharmacokinetics: Organ Distribution 2.6.5.5								
Organ Distribution 2.6.5.5A	Rat, Wistar Hannover (albino), male, fed Rat, Long-Evans, (pigmented), male, fed	p.o.	single	^{14}C-LNP023·HCl, p.o.: 10 mg/kg	not required	Novartis	[DMPK R1500361]	4.2.2.2-3
Organ Distribution 2.6.5.5B	Rat, Long-Evans (pigmented), male and female, fed	p.o.	single	^{14}C-LNP023·HCl, p.o.: 10 mg/kg	not required	[redacted]	[DMPK R1701426]	4.2.2.3-1

*** 新薬承認情報提供時に置き換えた**

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration or Sample(s)	Duration of dosing	Analyte(s), Dose(s) or Concentrations	GLP compliance	Testing facility	Report Number	Location in CTD
Plasma Protein Binding 2.6.5.6								
Plasma Protein Binding and Blood Distribution 2.6.5.6A	Blood and plasma from male, rat (Wistar Hannover), dog (Beagle), and human, HSA, AAG, Mixture solution of HDL, LDL, and VLDL lipoproteins	spiked samples *in vitro*	na	Plasma protein binding: ^{14}C-LNP023·HCl, 10-10000 ng/mL Binding to HSA, AAG, lipoproteins: ^{14}C-LNP023·HCl, 100, 1000, 10000 ng/mL	not required	Novartis	[DMPK R1500362]	4.2.2.3-2
Plasma Protein Binding and Blood Distribution 2.6.5.6B	Plasma from female wildtype mouse (C57BL/6JBom) and knockout mouse (C57BL/6JBom not expressing factor B), plasma from male human	spiked samples *in vitro*	na	^{14}C-LNP023·HCl, 10-10000 ng/mL	not required	Novartis	[DMPK R1600148]	4.2.2.3-3
Plasma Protein Binding and Blood Distribution 2.6.5.6C	Plasma from male and female rat (Wistar Hannover), female rabbits (New Zealand White), male and female dogs (Beagle)	*ex vivo*: post-dose animal plasma sample, *in vitro*: spiked samples	na	*Ex vivo*: LNP023 ·HCl, rat: 50-500 mg/kg, rabbit: 50-600 mg/kg, dog: 5-150 mg/kg *In vitro*: LNP023 ·HCl, 100-1000 ng/mL	not required	[redacted]	[DMPK R1701166]	4.2.2.3-5
Study in Pregnant or Nursing Animals 2.6.5.7								
No studies conducted under this section								
Other Distribution Study 2.6.5.8								
No studies conducted under this section								

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration or Sample(s)	Duration of dosing	Analyte(s), Dose(s) or Concentrations	GLP compliance	Testing facility	Report Number	Location in CTD
Metabolism *In Vivo* 2.6.5.9								
Metabolism *In Vivo* 2.6.5.9A	Rat, Wistar Hannover, male, fed	i.v. and p.o.	single	^{14}C-LNP023·HCl, i.v.: 2 mg/kg, p.o.: 10 mg/kg	not required	Novartis	[DMPK R1500361]	4.2.2.2-3
Metabolism *In Vivo* 2.6.5.9B	Rat, Wistar Hannover, male / female, fed	p.o.	multiple	LNP023·HCl, 750 mg/kg/day	not required	Novartis	[DMPK R1570193-metid]	4.2.2.4-1
Metabolism *In Vivo* 2.6.5.9C	Dog, Beagle, male / female, fasted	p.o.	multiple	LNP023·HCl, 150 mg/kg/day	not required	Novartis	[DMPK R1570192-metid]	4.2.2.4-2
Comparison of Exposure Ratios 2.6.5.9D	Rat (Wistar Hannover), Dog (Beagle), Human	p.o.	multiple	LNP023·HCl, Rat: 750 mg/kg/day Dog: 150 mg/kg/day Human: 200 mg bid	not required	Novartis	[DMPK R1809129-metid]	4.2.2.4-3
Metabolism *In Vitro* 2.6.5.10								
Metabolism *In Vitro* 2.6.5.10A	Hepatocytes from rat (Sprague-Dawley), dog (Beagle), and human	*in vitro*	na	^{14}C-LNP023·HCl	not required	Novartis	[DMPK R1500300]	4.2.2.4-4
Metabolite Stability *In Vitro* 2.6.5.10B	Phosphate buffer	*in vitro*	na	[redacted] (M9)	not required	[redacted]	[DMPK R1900261-metid]	4.2.2.4-5
Metabolism *In Vitro* 2.6.5.10C	Hepatocytes from mouse (CD-1), rat (Wistar Hannover), monkey (cynomolgus), and human	*in vitro*	na	LNP023	not required	Novartis	[DMPK R1900278-metid]	4.2.2.4-6
Metabolite Stability *In Vitro* 2.6.5.10D	Phosphate buffer, rat urine, human blood, plasma, and urine	*in vitro*	na	[redacted] (M8)	not required	Novartis	[DMPK R1500191]	4.2.2.4-7

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration or Sample(s)	Duration of dosing	Analyte(s), Dose(s) or Concentrations	GLP compliance	Testing facility	Report Number	Location in CTD
Metabolism *In Vitro* 2.6.5.10E	Human liver microsomes, S9, cytosol and hepatocytes	*in vitro*	na	^{14}C-LNP023·HCl	not required	Novartis	[DMPK R1500516]	4.2.2.4-8
Metabolism *In Vitro* 2.6.5.10F	Human liver microsomes	*in vitro*	na	^{14}C-LNP023	not required	Novartis	[DMPK R1600579]	4.2.2.4-9
Metabolism *In Vitro* 2.6.5.10G	Human liver microsomes	*in vitro*	na	^{14}C-LNP023	not required	Novartis	[DMPK R1600698]	4.2.2.4-10
Metabolism *In Vitro* 2.6.5.10H	Human liver microsomes	*in vitro*	na	^{14}C-LNP023·HCl	not required	Novartis	[DMPK R1600140]	4.2.2.4-11
Metabolism *In Vitro* 2.6.5.10I	Human liver microsomes and hepatocytes	*in vitro*	na	^{14}C-LNP023·HCl	not required	Novartis	[DMPK R1500514]	4.2.2.4-12
Possible Metabolic Pathways 2.6.5.11								
Please see 2.6.5.9A and 2.6.5.10A for proposed metabolic pathways.								
Induction/Inhibition of Drug-Metabolizing Enzymes 2.6.5.12								
Inhibition 2.6.5.12A	Human liver microsomes	*in vitro*	na	LNP023	not required	Novartis	[DMPK R1300641]	4.2.2.4-13
Inhibition 2.6.5.12B	Human liver microsomes	*in vitro*	na	^{14}C-LNP023·HCl, LNP023·HCl	not required	Novartis	[DMPK R1500321]	4.2.2.4-14
Inhibition 2.6.5.12C	Human liver microsomes	*in vitro*	na	LNP023·HCl	not required	Novartis	[DMPK R1500936]	4.2.2.4-15
Induction 2.6.5.12D	Human hepatocytes	*in vitro*	na	LNP023·HCl	not required	[redacted]	[DMPK R1600704]	4.2.2.4-16
Inhibition 2.6.5.12E	Human liver microsomes	*in vitro*	na	LNP023·HCl	not required	Novartis	[DMPK R1900589]	4.2.2.4-17

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration or Sample(s)	Duration of dosing	Analyte(s), Dose(s) or Concentrations	GLP compliance	Testing facility	Report Number	Location in CTD
Excretion 2.6.5.13								
Excretion 2.6.5.13A	Rat, Wistar Hannover, male, fed	i.v. and p.o.	single	^{14}C-LNP023·HCl, i.v.: 2 mg/kg, p.o.: 10 mg/kg	not required	Novartis	[DMPK R1500361]	4.2.2.2-3
Excretion 2.6.5.13B	Rat, Long-Evans, male, fed	p.o.	single	^{14}C-LNP023·HCl, p.o.: 10 mg/kg	not required	[redacted]	[DMPK R1701426]	4.2.2.3-1
Excretion into Bile 2.6.5.14								
Excretion into Bile	Bile duct-cannulated rat, Wistar Hannover, male, fed	i.v.	single	^{14}C-LNP023·HCl, i.v.: 2 mg/kg	not required	Novartis	[DMPK R1500361]	4.2.2.2-3
Drug-Drug Interactions 2.6.5.15								
No studies conducted under this section								
Other 2.6.5.16								
Permeability 2.6.5.16A	Caco-2 cell monolayers	*in vitro*	na	^{14}C-LNP023·HCl, LNP023·HCl	not required	Novartis	[DMPK R1500730]	4.2.2.7-1
Transporter 2.6.5.16B	Inside out membrane vesicles	*in vitro*	na	LNP023·HCl	not required	[redacted]	[DMPK R1600716]	4.2.2.7-2
Transporter 2.6.5.16C	Human embryonic kidney (HEK) cells	*in vitro*	na	LNP023·HCl	not required	[redacted]	[DMPK R1600717]	4.2.2.7-3
Transporter 2.6.5.16D	Sf9 cells	*in vitro*	na	^{14}C-LNP023·HCl, LNP023·HCl	not required	Novartis	[DMPK R1500958]	4.2.2.7-4
Transporter 2.6.5.16E	HEK cells, Madin-Darby canine kidney (MDCKII) cells	*in vitro*	na	LNP023·HCl	not required	[redacted]	[DMPK R1709014]	4.2.2.7-5
Transporter 2.6.5.16F	HEK cells, MDCKII cells	*in vitro*	na	LNP023·HCl	not required	[redacted]	[DMPK R2000210]	4.2.2.7-6
Transporter 2.6.5.16G	Huma hepatocytes	*in vitro*	na	^{14}C-LNP023·HCl, LNP023·HCl	not required	Novartis	[DMPK R1500336]	4.2.2.7-7
Transporter 2.6.5.16H	HEK cells	*in vitro*	na	^{14}C-LNP023·HCl, LNP023·HCl	not required	Novartis	[DMPK R1500532]	4.2.2.7-8

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration or Sample(s)	Duration of dosing	Analyte(s), Dose(s) or Concentrations	GLP compliance	Testing facility	Report Number	Location in CTD
Transporter 2.6.5.16I	MDCKII cells, porcine kidney (LLC-PK1) cells	*in vitro*	na	^{14}C-LNP023·HCl, LNP023·HCl	not required	Novartis	[DMPK R1500533]	4.2.2.7-9
Transporter 2.6.5.16J	Human hepatocytes	*in vitro*	na	^{14}C-LNP023·HCl, LNP023·HCl	not required	Novartis	[DMPK R1700975]	4.2.2.7-10
Transporter 2.6.5.16K	MDCKII cells	*in vitro*	na	^{14}C-LNP023·HCl, LNP023·HCl	not required	[redacted]	[DMPK R1800300]	4.2.2.7-11
Transporter 2.6.5.16L	HEK cells, MDCKII cells, Chinese hamstar ovary (CHO) cells	*in vitro*	na	LNP023·HCl	not required	[redacted]	[DMPK R2000245]	4.2.2.7-12
Other 2.6.5.16M	Human	p.o.	multiple	LNP023·HCl, 200 mg bid	not required	Novartis	[DMPK-BT-R1701500-metid]	4.2.2.7-13

na: not applicable

2.6.5.2 薬物動態試験：分析方法

2.6.5.2A ラット血漿中 LNP023 分析法（DMPK R1500297-01）

Study title:	Quantitative determination of LNP023 in rat plasma by LC-MS/MS	Report no. DMPK R1500297-01
Location in CTD:	4.2.2.1-1	
GLP compliance:	Not required	
Method:	Protein precipitation followed by the analysis of the sample extract by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring (MRM) mode using electrospray ionization (ESI) in positive ion mode.	
Instrument:	Sciex API 4000 and Shimadzu LC-20AD XR pumps	
Analyte(s):	LNP023 (iptacopan)	
Reference compound:	LNP023 HCl (LNP023-AAA.002)	
Internal standard:	D_5-LNP023 ([M+5]LNP023)	
Sample preparation:	10 µL plasma (blanks, calibration standards, QCs or unknowns) was added to designated tubes in a 96-well plate format. A 25 µL aliquot of D_5-LNP023 internal standard working solution (25000 ng/mL in 10% acetonitrile in water, v/v) was added to each tube except for the tubes containing blanks, for which a 25 µL aliquot of 10% acetonitrile in water (v/v) was added. After vortex-mixing for approximately 30 seconds, a 500 µL aliquot of acetonitrile was added to each tube. This was followed by vortex-mixing for approximately 5 minutes. The sample plate was centrifuged at 3000 × g for 5 minutes at approximately 10°C. 25 µL supernatant was transferred into a clean 96-well plate and mixed with 225 µL of water. After mixing properly, 5 µL of the diluted sample extract was injected onto the LC-MS/MS system.	
Results:	Validation in plasma (treated with ~2% lactic acid)	
Selectivity	Interference in blank and zero (blank + internal standard only) samples was ≤ 20% of the analytical response at the LLOQ for LNP023. Interference in blank and ULOQ without D_5-LNP023 samples was ≤ 5% of analytical response for D_5-LNP023 at the working concentration. Low QCs spiked in two different blank rat plasma lots were within ± 15% of nominal concentration.	
Stability	Stable in methanol at 10.0 mg/mL for 165 days at ≤ −15°C Stable in methanol at 10.0 mg/mL for 24 hours at room temperature Stable in rat plasma (treated with 2% lactic acid) for 24 hours at room temperature Stable in rat plasma (treated with 2% lactic acid) after 4 freeze-thaw cycles with storage at ≤ −60°C Stable in rat plasma (treated with 2% lactic acid) for 79 days at ≤ −60°C Stable in untreated rat blood for 1 hour at 2 to 8°C Stable in post-preparative rat plasma sample extracts for 7.5 days at 5°C	
Intra-day accuracy and precision	Bias between −6.7% and 14.0% (including the LLOQ) Precision between 1.1% and 8.3% (including the LLOQ)	
Inter-day accuracy and precision	Bias between −4.3% and 7.0% (including the LLOQ) Precision between 3.1% and 8.6% (including the LLOQ)	

LOQ	LLOQ: 100 ng/mL; ULOQ: 100000 ng/mL using 10 µL of plasma
Recovery	Mean recovery of LNP023 at 3 concentration levels: 121% (101% to 148%). Precision ≤ 20% at each of the low (300 ng/mL), mid (25000 ng/mL), and high (75000 ng/mL) concentration level Mean recovery of D_5-LNP023 at the working concentration level: 116% (102% to 134%). Precision ≤ 20% D_5-LNP023 normalized recovery for LNP023 at 3 concentration levels: 104% (99.5% to 111%). Precision ≤ 20%
Matrix factor	Mean matrix factor for LNP023 at 3 concentration levels: 0.915 (0.875 to 0.957). Precision ≤ 15% Mean matrix factor for D_5-LNP023 at the working concentration level: 0.907 (0.856 to 0.952). Precision ≤ 15% Mean D_5-LNP023 normalized matrix factor for LNP023 at 3 concentration levels: 1.01 (1.00 to 1.03). Precision ≤ 15%
Carryover	LNP023: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ D_5-LNP023: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards and 50% at each calibration standard level from the nominal values at a minimum of 6 different levels
Dilutions	Bias within ± 15% of the nominal concentration (5 times of ULOQ) for samples diluted up to 20-fold. Precision ≤ 15%

2.6.5.2B イヌ血漿中 LNP023 分析法（DMPK R1500296-01）

Study title:	Quantitative determination of LNP023 in dog plasma by LC-MS/MS	Report no. DMPK R1500296-01
Location in CTD:	4.2.2.1-2	
GLP compliance:	Not required	
Method:	Protein precipitation followed by the analysis of the sample extract by LC-MS/MS in MRM mode using ESI in positive ion mode.	
Instrument:	Sciex API 4000 and Shimadzu LC-20AD XR pumps	
Analyte(s):	LNP023 (iptacopan)	
Reference compound:	LNP023 HCl (LNP023-AAA.002)	
Internal standard:	D_5-LNP023 ([M+5]LNP023)	
Sample preparation:	10 µL plasma (blanks, calibration standards, QCs or unknowns) was added to designated tubes in a 96-well plate format. A 25 µL aliquot of D_5-LNP023 internal standard working solution (25000 ng/mL in 10% acetonitrile in water, v/v) was added to each tube except for the tubes containing blanks, for which a 25 µL aliquot of 10% acetonitrile in water (v/v) was added. After vortex-mixing for approximately 30 seconds, a 500 µL aliquot of acetonitrile was added to each tube. This was followed by vortex-mixing for approximately 5 minutes. The sample plate was centrifuged at 3000 × g for 5 minutes at approximately 10°C. 25 µL supernatant was transferred into a clean 96-well plate and mixed with 225 µL of water. After mixing properly, 10 µL of the diluted sample extract was injected onto the LC-MS/MS system.	
Results:	Validation in plasma (treated with ~2% lactic acid)	
Selectivity	Interference in blank and zero (blank + internal standard only) samples was ≤ 20% of the analytical response at the LLOQ for LNP023. Interference in blank and ULOQ without D_5-LNP023 samples was ≤ 5% of analytical response for D_5-LNP023 at the working concentration. Low QCs were spiked in two different dog plasma blank lots with measured value within ± 15% of nominal concentration for one lot and outside ± 15% of nominal concentration for the other lot [a)].	
Stability	Stable in dog plasma (treated with ~2% lactic acid) for 24 hours at room temperature Stable in dog plasma (treated with ~2% lactic acid) after 4 freeze-thaw cycles with storage at ≤ −60°C Stable in dog plasma (treated with ~2% lactic acid) for 78 days at ≤ −60°C Stable in untreated dog blood for 1 hour at 2-8°C Stable in post-preparative dog plasma sample extracts for 7.5 days at 5°C	
Intra-day accuracy and precision	Bias between −5.2% and 9.3% (including the LLOQ) Precision between 0.7% and 7.1% (including the LLOQ)	
Inter-day accuracy and precision	Bias between −2.4% and 5.2% (including the LLOQ) Precision between 3.7% and 6.6% (including the LLOQ)	
LOQ	LLOQ: 100 ng/mL; ULOQ: 100,000 ng/mL using 10 µL of plasma	
Recovery	Mean recovery of LNP023 at 3 concentration levels: 150% (128% to 184%). Precision ≤ 20% at each of the low (300 ng/mL), mid (25000 ng/mL), and high (75000 ng/mL) concentration level Mean recovery of D_5-LNP023 at the working concentration level: 136% (125% to 152%). Precision ≤ 20% D_5-LNP023 normalized recovery for LNP023 at 3 concentration levels: 110% (102% to 122%). Precision ≤ 20%	

Matrix factor	Mean matrix factor for LNP023 at 3 concentration levels: 0.871 (0.831 to 0.895). Precision ≤ 15% Mean matrix factor for D_5-LNP023 at the working concentration level: 0.884 (0.871 to 0.907). Precision ≤ 15% Mean D_5-LNP023 normalized matrix factor for LNP023 at 3 concentration levels: 0.987 (0.954 to 1.02). Precision ≤ 15%
Carryover	LNP023: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ D_5-LNP023: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards and 50% at each calibration standard level from the nominal values at a minimum of 6 different levels
Dilutions	Bias within ± 15% of the nominal concentration (5 times of ULOQ) for samples diluted up to 20-fold. Precision ≤ 15%

[a)] Refer to Special issue in DMPK R1500296-01.

2.6.5.2C マウス血漿中 LNP023 分析法（DMPK R1500429-01）

Study title:	Quantitative determination of LNP023 in mouse plasma by LC-MS/MS	Report no. DMPK R1500429-01
Location in CTD:	4.2.2.1-3	
GLP compliance:	Not required	
Method:	Protein precipitation followed by the analysis of the sample extract by LC-MS/MS in MRM mode using ESI in positive ion mode.	
Instrument:	Sciex API 4000 and Shimadzu LC-20AD XR pumps	
Analyte(s):	LNP023 (iptacopan)	
Reference compound:	LNP023 HCl (LNP023-AAA.002)	
Internal standard:	D_5-LNP023 ([M+5]LNP023)	
Sample preparation:	10 µL plasma (blanks, calibration standards, QCs or unknowns) was added to designated tubes in a 96-well plate format. A 25 µL aliquot of D_5-LNP023 internal standard working solution (25000 ng/mL in 10% acetonitrile in water, v/v) was added to each tube except for the tubes containing blanks, for which a 25 µL aliquot of 10% acetonitrile in water (v/v) was added. After vortex-mixing for approximately 30 seconds, a 500 µL aliquot of acetonitrile was added to each tube. This was followed by vortex-mixing for approximately 5 minutes. The sample plate was centrifuged at 3000 × g for 5 minutes at approximately 10°C. 25 µL supernatant was transferred into a clean 96-well plate and mixed with 225 µL of water. After mixing properly, 10 µL of the diluted sample extract was injected onto the LC-MS/MS system.	
Results:	Validation in plasma	
Selectivity	Interference in blank and zero (blank + internal standard only) samples was ≤ 20% of the analytical response at the LLOQ for LNP023. Interference in blank and ULOQ without D_5-LNP023 samples was ≤ 5% of analytical response for D_5-LNP023 at the working concentration. Low QCs spiked in two different blank mouse plasma lots were within ± 15% of nominal concentration.	
Stability	Stable in methanol at 10.0 mg/mL for 165 days at ≤ −15°C (DMPK R1500297-01) Stable in methanol at 10.0 mg/mL for 24 hours at room temperature (DMPK R1500297-01) Stable in mouse plasma for 24 hours at room temperature Stable in mouse plasma after 4 freeze-thaw cycles with storage at ≤ −60°C Stable in mouse plasma for 155 days at ≤ −60°C Stable in untreated mouse blood for 1 hour at 2-8°C Stable in post-preparative mouse plasma sample extracts for 7.5 days at 5°C	
Intra-day accuracy and precision	Bias between −5.6% and 10.0% (including the LLOQ) Precision between 1.8% and 9.6% (including the LLOQ)	
Inter-day accuracy and precision	Bias between −3.6% and 8.0% (including the LLOQ) Precision between 3.9% and 7.9% (including the LLOQ)	
LOQ	LLOQ: 100 ng/mL; ULOQ: 100000 ng/mL using 10 µL of plasma	
Recovery	Mean recovery of LNP023 at 3 concentration levels: 133% (118% to 150%). Precision ≤ 20% Mean recovery of D_5-LNP023 at the working concentration level: 120% (105% to 137%). Precision ≤ 20% D_5-LNP023 normalized recovery for LNP023 at 3 concentration levels: 111% (110% to 112%). Precision ≤ 20%	

Matrix factor	Mean matrix factor for LNP023 at 3 concentration levels: 0.976 (0.905 to 1.04). Precision ≤ 15% Mean matrix factor for D_5-LNP023 at the working concentration level: 1.00 (0.938 to 1.06). Precision ≤ 15% Mean D_5-LNP023 normalized matrix factor for LNP023 at 3 concentration levels: 0.973 (0.965 to 0.986). Precision ≤ 15%
Carryover	LNP023: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ D_5-LNP023: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards and 50% at each calibration standard level from the nominal values at a minimum of 6 different levels
Dilutions	Bias within ± 15% of the nominal concentration (5 times of ULOQ) for samples diluted up to 20-fold. Precision ≤ 15%

2.6.5.2D ラット血漿中 LNP023 分析法（DMPK R1600441）

Study title:	Validation of a method for the determination of LNP023 in rat plasma using liquid chromatography with tandem mass spectrometric detection	Report no. DMPK R1600441
Location in CTD:	4.2.2.1-4	
GLP compliance:	Not required	
Method:	Protein precipitation followed by the analysis of the sample extract by LC-MS/MS in MRM mode using ESI in positive ion mode.	
Instrument:	Sciex API 4000 and Waters Acquity UPLC Auxiliary pumps	
Analyte(s):	LNP023 (iptacopan)	
Reference compound:	LNP023 HCl (LNP023-AAA.004)	
Internal standard:	D_5-LNP023 ([M+5]LNP023)	
Sample preparation:	10 µL plasma (blanks, calibration standards, QCs or unknowns) was added to designated wells in a 96-well plate. A 25 µL aliquot of D_5-LNP023 internal standard working solution (25000 ng/mL in 10% acetonitrile in water, v/v) was added to each well except for the wells containing blanks, for which a 25 µL aliquot of 10% acetonitrile in water (v/v) was added. After mixing for approximately 5 minutes, a 500 µL aliquot of acetonitrile was added to each well. This was followed by vortex-mixing for approximately 5 minutes. The sample plate was centrifuged at 3000 × g for approximately 5 minutes at 10°C. 25 µL supernatant was transferred into a clean 96-well plate and mixed with 225 µL of water. After vortex-mixing for approximately 5 minutes, 3 µL of the diluted sample extract was injected onto the LC-MS/MS system.	
Results:	Validation in plasma (treated with 2% lactic acid)	
Selectivity	Interference in blank and zero (blank + internal standard only) samples was ≤ 20% of the analytical response at the LLOQ for LNP023 except for 4 runs 1, 2, 3, and 9 (20.5%-26.1%) [a]. Interference in blank and ULOQ without D_5-LNP023 samples was ≤ 5% of analytical response for D_5-LNP023 at the working concentration. LLOQ QCs spiked in six individual rat plasma (treated with 2% lactic acid) lots with each lot tested in three replicates had mean concentration within ± 20% bias of the nominal concentration for each lot, and the overall mean value within ± 20% bias of the nominal concentration and precision ≤ 20%.	
Assessment in haemolysed plasma	Low QC and high QC in 2% haemolysed plasma (treated with 2% lactic acid): precision values were ≤ 15.0% and mean bias values were within ± 15.0% of the nominal concentration for each QC level.	
Stability	LNP023 was stable in methanol at 10.0 mg/mL for 35 days at nominal −20°C. LNP023 was stable in methanol at 10.0 mg/mL for 24 hours at room temperature. LNP023 was stable in rat plasma (treated with 2% lactic acid) for 16 hours at room temperature. LNP023 was stable in rat plasma (treated with 2% lactic acid) after 4 freeze-thaw cycles with storage at nominal −20°C and −80°C. LNP023 was stable in rat plasma (treated with 2% lactic acid) for 109 days at nominal −20°C and 238 days at nominal −80°C. LNP023 was stable in untreated rat blood for 2 hours at room temperature and 2 hours on wet ice. LNP023 was stable in post-preparative rat plasma sample extracts for 98 hours at 2 to 8°C (Processed sample stability) and 87 hours at 2 to 8°C (Processed sample viability).	
Intra-day accuracy and precision	Bias between −14.1% and −0.1% (including the LLOQ) Precision between 1.4% and 11.1% (including the LLOQ)	

Inter-day accuracy and precision	Bias between −9.8% and −5.7% (including the LLOQ) Precision between 4.2% and 12.1% (including the LLOQ)
LOQ	LLOQ: 0.1 µg/mL; ULOQ: 100 µg/mL using 10 µL of plasma
Recovery	Mean recovery of LNP023 at 3 concentration levels: 73.6% (72.7% to 74.3%); repeated as 77.8% (75.4% to 79.5%). Precision ≤ 15% Mean recovery of D_5-LNP023 at the working concentration level: 93.7%; repeated as 93.2%. Precision ≤ 15%
Matrix factor	Mean D_5-LNP023 normalized matrix factor for LNP023 at low and high QC levels: 1.04 and 1.00. Precision ≤ 15% Mean matrix factor for D_5-LNP023 at the working concentration level: 1.02 to 1.03. Precision ≤ 15%
Carryover	LNP023: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ D_5-LNP023: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards from the nominal values at a minimum of 6 different levels
Dilutions	Bias within ± 15% of the nominal concentration (10 times of ULOQ) for samples diluted up to 20-fold. Precision ≤ 15%

[a)] Refer to Section 5.1 Interference Peaks in DMPK R1600441.

2.6.5.2E イヌ血漿中 LNP023 分析法（DMPK R1600442 and DMPK R1600442-ad1）

Study title:	Validation of a method for the determination of LNP023 in dog plasma using liquid chromatography with tandem mass spectrometric detection	Report no. DMPK R1600442 & DMPK R1600442-ad1
Location in CTD:	4.2.2.1-5	
GLP compliance:	Not required	
Method:	Protein precipitation followed by the analysis of the sample extract by LC-MS/MS in MRM mode using ESI in positive ion mode.	
Instrument:	Sciex API 4000 and Waters Acquity UPLC Auxiliary pumps	
Analyte(s):	LNP023 (iptacopan)	
Reference compound:	LNP023 HCl (LNP023-AAA.004 and LNP023-AAA.006)	
Internal standard:	D_5-LNP023 ([M+5]LNP023)	
Sample preparation:	10 µL plasma (blanks, calibration standards, QCs or unknowns) was added to designated wells in a 96-well plate. A 25 µL aliquot of D_5-LNP023 internal standard working solution (25000 ng/mL in 10% acetonitrile in water, v/v) was added to each well except for the wells containing blanks, for which a 25 µL aliquot of 10% acetonitrile in water (v/v) was added. After mixing for approximately 5 minutes, a 500 µL aliquot of acetonitrile was added to each well. This was followed by vortex-mixing for approximately 5 minutes. The sample plate was centrifuged at 3000 × g for approximately 5 minutes at 10°C. 25 µL supernatant was transferred into a clean 96-well plate and mixed with 225 µL of water. After vortex-mixing for approximately 5 minutes, 3 µL of the diluted sample extract was injected onto the LC-MS/MS system.	
Results:	Validation in plasma (treated with 2% lactic acid) (DMPK R1600442)	
Selectivity	Interference in blank and zero samples was ≤ 20% of the analytical response at the LLOQ for LNP023 except for run 1 (22.0%) [a)]. Interference in blank and ULOQ without D_5-LNP023 samples was ≤ 5% of analytical response for D_5-LNP023 at the working concentration. LLOQ QCs spiked in six individual dog plasma (treated with 2% lactic acid) lots with each lot tested in three replicates had mean concentration within ± 20% bias of the nominal concentration for each lot, and the overall mean value within ± 20% bias of the nominal concentration and precision ≤ 20%.	
Assessment in haemolysed plasma	Low QC and high QC in 2% haemolysed plasma (treated with 2% lactic acid): precision values were ≤ 15.0% and mean bias values were within ± 15.0% of the nominal concentration for each QC level.	
Stability	LNP023 was stable in methanol at 10.0 mg/mL for 35 days at nominal −20°C (DMPK R1600441). LNP023 was stable in methanol at 10.0 mg/mL for 24 hours at room temperature (DMPK R1600441). LNP023 was stable in dog plasma (treated with 2% lactic acid) for 16 hours at room temperature. LNP023 was stable in dog plasma (treated with 2% lactic acid) after 4 freeze-thaw cycles with storage at nominal −20°C and −80°C. LNP023 was stable in dog plasma (treated with 2% lactic acid) for 100 days at nominal −20°C and 238 days at nominal −80°C. LNP023 was stable in untreated dog blood for 2 hours at room temperature and 2 hours on wet ice. LNP023 was stable in post-preparative dog plasma sample extracts for 103 hours at 2 to 8°C (processed sample stability) and 87 hours at 2 to 8°C (Processed sample viability).	
Intra-day accuracy and precision	Bias between −15.0% and 8.0% (including the LLOQ) Precision between 1.4% and 10.8% (including the LLOQ)	

Inter-day accuracy and precision	Bias between −12.1% and −5.7% (including the LLOQ) Precision between 5.7% and 10.1% (including the LLOQ)
LOQ	LLOQ: 0.1 µg/mL; ULOQ: 100 µg/mL using 10 µL of plasma
Recovery	Mean recovery of LNP023 at 3 concentration levels: 76.3% (75.6% to 77.2%); repeated as 85.5% (79.7% to 89.0%). Precision ≤ 15% Mean recovery of D_5-LNP023 at the working concentration level: 93.5%; repeated as 96.2%. Precision ≤ 15%
Matrix factor	Mean D_5-LNP023 normalized matrix factor for LNP023 at low and high QC level: 1.01 and 1.00. Precision ≤ 15% Mean matrix factor for D_5-LNP023 at the working concentration level: 1.02 to 1.03. Precision ≤ 15%
Carryover	LNP023: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ except for run 11, in which the response in the second blank sample following injection of the ULOQ > 20% (29.4%) of the response observed for the LLOQ. The review of absolute carryover confirmed that the carryover was not significant. D_5-LNP023: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards from the nominal values at a minimum of 6 different levels
Dilutions	Bias within ± 15% of the nominal concentration (10 times of ULOQ) for samples diluted up to 20-fold. Precision ≤ 15%
Results:	Partial validation in plasma (D_5-LNP023 working solution at 2.50 µg/mL) (DMPK R1600442-ad1)
Selectivity	Interference in blank and ULOQ without D_5-LNP023 samples was ≤ 5% of analytical response for D_5-LNP023 at the working concentration.
Stability	D_5-LNP023 was stable in 10% acetonitrile in water (v/v) at 25000 ng/mL for 25 hours at room temperature (DMPK R1600671-01).
Intra-day accuracy and precision	Bias between −8.9% and 0.8% (including the LLOQ) Precision between 4.3% and 6.1% (including the LLOQ)
Carryover	LNP023: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ D_5-LNP023: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards from the nominal values at a minimum of 6 different levels

[a)] Refer to Section 5.1 Interference Peaks in DMPK R1600442.

2.6.5.2F ウサギ血漿中 LNP023 分析法（DMPK R1600671-01）

Study title:	Validation of a method for the determination of LNP023 in rabbit plasma using liquid chromatography with tandem mass spectrometric detection	Report no. DMPK R1600671-01
Location in CTD:	4.2.2.1-6	
GLP compliance:	Not required	
Method:	Protein precipitation followed by the analysis of the sample extract by LC-MS/MS in MRM mode using ESI in positive ion mode.	
Instrument:	Sciex API 4000 and Waters Acquity UPLC Auxiliary pumps	
Analyte(s):	LNP023 (iptacopan)	
Reference compound:	LNP023 HCl (LNP023-AAA.004 and LNP023-AAA.006)	
Internal standard:	D_5-LNP023 ([M+5]LNP023)	
Sample preparation:	10 µL plasma (blanks, calibration standards, QCs or unknowns) was added to designated wells in a 96-well plate. A 25 µL aliquot of D_5-LNP023 internal standard working solution (25000 ng/mL in 10% acetonitrile in water, v/v) was added to each well except for the wells containing blanks, for which a 25 µL aliquot of 10% acetonitrile in water (v/v) was added. After mixing for approximately 5 minutes, a 500 µL aliquot of acetonitrile was added to each well. This was followed by vortex-mixing for approximately 5 minutes. The sample plate was centrifuged at 3000 × g for approximately 5 minutes at 10°C. 25 µL supernatant was transferred into a clean 96-well plate and mixed with 225 µL of water. After vortex-mixing for approximately 5 minutes, 3 to 5 µL of the diluted sample extract was injected onto the LC-MS/MS system.	
Results:	Validation in plasma (treated with 2% lactic acid)	
Selectivity	Interference in blank and zero samples was ≤ 20% of the analytical response at the LLOQ for LNP023. Interference in blank and ULOQ samples was ≤ 5% of analytical response for D_5-LNP023 at the working concentration. LLOQ QCs spiked in six individual rabbit plasma (treated with 2% lactic acid) lots with each lot tested in a singlet had the overall mean value within ± 20% bias of the nominal concentration and precision ≤ 20%.	
Assessment in haemolysed plasma	Low QC and high QC in 2% haemolysed plasma (treated with 2% lactic acid): precision values were ≤ 15.0% and mean bias values were within ± 15.0% of the nominal concentration for each QC level.	

Stability	LNP023 was stable in methanol at 10.0 mg/mL for 35 days at nominal −20°C (DMPK R1600441). LNP023 was stable in methanol at 10.0 mg/mL for 24 hours at room temperature (DMPK R1600441). D_5-LNP023 was stable in 10% acetonitrile in water (v/v) at 25000 ng/mL for 25 hours at room temperature. LNP023 was stable in rabbit plasma (treated with 2% lactic acid) for 12 hours at room temperature. LNP023 was stable in rabbit plasma (treated with 2% lactic acid) after 3 freeze-thaw cycles with storage at nominal −20°C and 4 freeze-thaw cycles with storage at nominal −80°C. LNP023 was stable in rabbit plasma (treated with 2% lactic acid) for 33 days at nominal −20°C and 313 days at nominal −80°C. LNP023 was stable in rabbit plasma (without lactic acid treatment) for 17 days at nominal −80°C following two freeze-thaw cycles at nominal −80°C and 160 minutes at room temperature. LNP023 was stable in untreated rabbit blood for 7 hours at room temperature and 2 hours on wet ice. LNP023 was stable in post-preparative rabbit plasma sample extracts for 95 hours at 2 to 8°C (processed sample stability) and 161 hours at 2 to 8°C (Processed sample viability).
Intra-day accuracy and precision	Bias between −9.1% and 3.3% (except for the LLOQ: between −18.8% and 6.0%) Precision between 3.2% and 7.9% (including the LLOQ)
Inter-day accuracy and precision	Bias between −4.9% and −1.7% (including the LLOQ) Precision between 5.4% and 13.0% (including the LLOQ)
LOQ	LLOQ: 0.1 µg/mL; ULOQ: 100 µg/mL using 10 µL of plasma
Recovery	Mean recovery of LNP023 at 3 concentration levels: 94.6% (90.4% to 99.6%). Precision ≤ 15% Mean recovery of D_5-LNP023 at the working concentration level: 90.6%. Precision ≤ 15%
Matrix factor	Mean D_5-LNP023 normalized matrix factor for LNP023 at low and high QC level: 1.0 and 1.0. Precision ≤ 15% Mean matrix factor for D_5-LNP023 at the working concentration level: 1.0 and 1.0
Carryover	LNP023: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ except for run 12, in which the response in the second blank sample following injection of the ULOQ > 20% (44.7%) of the response observed for the LLOQ. The review of absolute carryover confirmed that the carryover was not significant. D_5-LNP023: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards from the nominal values at a minimum of 6 different levels
Dilutions	Bias within ± 15% of the nominal concentration (10 times of ULOQ) for samples diluted up to 20-fold. Precision ≤ 15%

2.6.5.2G マウス血漿中 LNP023 分析法（DMPK R2000093）

Study title:	Validation of a method for the determination of LNP023 in mouse plasma using liquid chromatography with tandem mass spectrometric detection	Report no. DMPK R2000093
Location in CTD:	4.2.2.1-7	
GLP compliance:	Not required	
Method:	Protein precipitation followed by the analysis of the sample extract by LC-MS/MS in MRM mode using ESI in positive ion mode.	
Instrument:	Sciex API 4000 and Waters Acquity UPLC Auxiliary pumps	
Analyte(s):	LNP023 (iptacopan)	
Reference compound:	LNP023 HCl (LNP023-AAA.008)	
Internal standard:	D_5-LNP023 ([M+5]LNP023)	
Sample preparation:	Sample process was conducted on wet ice. 10 µL plasma (blanks, calibration standards, QCs or unknowns) was added to designated wells in a 96-well plate. A 25 µL aliquot of D_5-LNP023 internal standard working solution (2500 ng/mL in 10% acetonitrile in water, v/v) was added to each well except for the wells containing blanks, for which a 25 µL aliquot of 10% acetonitrile in water (v/v) was added. After mixing for approximately 5 minutes, a 500 µL aliquot of acetonitrile was added to each well. This was followed by vortex-mixing for approximately 5 minutes. The sample plate was centrifuged at 3000 × g for approximately 5 minutes at 10°C. 25 µL supernatant was transferred into a clean 96-well plate and mixed with 250 µL of 0.1% formic acid in water. After vortex-mixing for approximately 5 minutes, 3 µL of the diluted sample extract was injected onto the LC-MS/MS system.	
Results:	Validation in plasma (treated with 2% lactic acid)	
Selectivity	Interference in blank and zero samples was ≤ 20% of the analytical response at the LLOQ for LNP023. Interference in blank and ULOQ samples was ≤ 5% of analytical response for D_5-LNP023 at the working concentration. LLOQ QCs spiked in six individual mouse plasma (treated with 2% lactic acid) lots with each lot tested in a singlet had the overall mean value within ± 20% bias of the nominal concentration and precision ≤ 20%. LLOQ QCs spiked in six individual transgenic mouse plasma (treated with 2% lactic acid) lots with each lot tested in a singlet had the overall mean value within ± 20% bias of the nominal concentration and precision ≤ 20%.	
Assessment in haemolysed plasma	Low QC and high QC in 2% haemolysed plasma (treated with 2% lactic acid): precision values were ≤ 15.0% and mean bias values were within ± 15.0% of the nominal concentration for each QC level.	

Stability	LNP023 was stable in methanol at 10.0 mg/mL for 35 days at nominal −20°C (DMPK R1600441). LNP023 was stable in methanol at 10.0 mg/mL for 24 hours at room temperature (DMPK R1600441). D_5-LNP023 was stable in 10% acetonitrile in water (v/v) at 2.50 µg/mL for 7 hours at room temperature (internal standard working solution). LNP023 was stable in mouse plasma (treated with 2% lactic acid) for 8 hours on wet ice. LNP023 was stable in mouse plasma (treated with 2% lactic acid) after 4 freeze-thaw cycles with storage at −90°C to −50°C. LNP023 was stable in mouse plasma (treated with 2% lactic acid) for 285 days at −90°C to −50°C. LNP023 was stable in untreated mouse blood for 2 hours at room temperature or 2 hours on wet ice. LNP023 was stable in post-preparative mouse plasma sample extracts for 147 hours at 2 to 8°C (processed sample stability) and 164 hours at 2 to 8°C (Processed sample viability).
Intra-day accuracy and precision	Bias between −10.8% and 4.0% (including the LLOQ) Precision between 2.6% and 7.8% (except for the LLOQ: between 3.4% and 19.6%)
Inter-day accuracy and precision	Bias between −9.3% and 0.3% (including the LLOQ) Precision between 3.5% and 12.7% (including the LLOQ)
LOQ	LLOQ: 0.1 µg/mL; ULOQ: 100 µg/mL using 10 µL of plasma
Recovery	Mean recovery of LNP023 at 3 concentration levels: 88.9% (85.4% to 90.8%). Precision ≤ 15% Mean recovery of D_5-LNP023 at the working concentration level: 96.7%. Precision ≤ 15%
Matrix factor	Mean D_5-LNP023 normalized matrix factor for LNP023 at low and high QC level: 1.01and 1.02. Precision ≤ 15% Mean matrix factor for D_5-LNP023 at the working concentration level: 0.99 to 1.01
Carryover	LNP023: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ D_5-LNP023: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards from the nominal values at a minimum of 6 different levels
Dilutions	Bias within ± 15% of the nominal concentration (8-times of ULOQ) for samples diluted up to 20-fold. Precision ≤ 15%

2.6.5.2H ラット血漿中 不純物A*分析法（DMPK R2000262）

Study title:	Validation of a method for the determination of 不純物A* in rat plasma using liquid chromatography with tandem mass spectrometric detection	Report no. DMPK R2000262
Location in CTD:	4.2.2.1-8	
GLP compliance:	Not required	
Method:	Protein precipitation followed by the analysis of the sample extract by LC-MS/MS in MRM mode using ESI in positive ion mode.	
Instrument:	Sciex API 6500 and Waters Acquity UPLC Auxiliary pumps	
Analyte(s):	不純物A*(LNP023 impurity)	
Reference compound:	不純物A*-NXA.001	
Internal standard:	^{13}C, D_3-不純物A*	
Sample preparation:	20 µL plasma (blanks, calibration standards, QCs or unknowns) was added to designated wells in a 96-well plate. A 25 µL aliquot of ^{13}C, D_3-不純物A* internal standard working solution (100 ng/mL in 30% acetonitrile in water, v/v) was added to each well except for the wells containing blanks, for which a 25 µL aliquot of 30% acetonitrile in water (v/v) was added. After vortex-mixing for 1 minute, a 250 µL aliquot of acetonitrile was added to each well. This was followed by vortex-mixing for 5 minutes. The sample plate was centrifuged at 3000 × g for approximately 5 minutes at room temperature. 50 µL supernatant was transferred into a clean 96-well plate and mixed with 350 µL of reconstitution solution [(10 mM ammonium formate:formic acid, 100:0.2): acetonitrile, 85:15]. After vortex-mixing for 5 minutes, 3 µL of the diluted sample extract was injected onto the LC-MS/MS system.	
Results:	Validation in plasma	
Selectivity	Interference in blank and zero samples was ≤ 20% of the analytical response at the LLOQ for 不純物A*. Interference in blank and ULOQ samples was ≤ 5% of analytical response for ^{13}C, D_3-不純物A* at the working concentration. LLOQ QCs spiked in six individual rat plasma lots with each lot tested in a singlet had the overall mean value within ± 20% bias of the nominal concentration and precision ≤ 20%.	
Assessment in haemolysed plasma	Low QC and high QC in 2% haemolysed plasma: precision values were ≤ 15.0% and mean bias values were within ± 15.0% of the nominal concentration for each QC level.	

Stability	不純物A* was stable in Acetonitrile:DMSO (50:50) at 1.00 mg/mL for 42 days at 2 to 8°C. 不純物A* was stable in Acetonitrile:DMSO (50:50) at 80.0 µg/mL for 19 days at 2 to 8°C. 不純物A* was stable in Acetonitrile:DMSO (50:50) at 1.00 mg/mL for 19 hours at room temperature. 不純物A* was stable in Acetonitrile:DMSO (50:50) at 80.0 µg/mL for 19 hours at room temperature. ^{13}C, D_3-不純物A* was stable in 30% acetonitrile in water (v/v) at 100 ng/mL for 19 hours at room temperature. 不純物A* was stable in rat plasma for 24 hours at room temperature. 不純物A*was stable in rat plasma after 4 freeze-thaw cycles with storage at −90°C to −50°C and −30°C to −10°C. 不純物A* was stable in rat plasma for 139 days at −90°C to −50°C. 不純物A* was stable in rat blood for 2 hours at room temperature or 2 hours on wet ice. 不純物A* was stable in post-preparative rat plasma sample extracts for 96 hours at 2 to 8°C (processed sample stability) and 74 hours at 2 to 8°C (Processed sample viability).
Intra-day accuracy and precision	Bias between −9.0% and 4.5% (including the LLOQ) Precision between 0.7% and 8.8% (including the LLOQ)
Inter-day accuracy and precision	Bias between −3.4% and 2.0% (including the LLOQ) Precision between 2.3% and 8.4% (including the LLOQ)
LOQ	LLOQ: 2.00 ng/mL; ULOQ: 4000 ng/mL using 20 µL of plasma
Recovery	Mean recovery of 不純物A* at 3 concentration levels: 84.5% (83.2 to 86.5%). Precision ≤ 15% Mean recovery of ^{13}C, D_3-不純物A* at the working concentration level: 89.8%. Precision ≤ 15%
Matrix factor	Mean ^{13}C, D_3-不純物A* normalized matrix factor for 不純物A* at low and high QC level: 1.02 and 1.00. Precision ≤ 15% Mean matrix factor for ^{13}C, D_3-不純物A* at the working concentration level: 0.99 to 1.03
Carryover	不純物A*: response from injection of blank sample following injection of the ULOQ ≤ 20% of the response observed for the LLOQ except for runs 1, 9, and 13, in which the response in the blank sample following injection of the ULOQ > 20% (22.1% to 101.5%) of the response observed for the LLOQ. The review of contribution from carryover confirmed that the carryover was not significant and did not affect the integrity of the data generated from these runs. A note was added to the Analytical Method to ensure run injections in subsequent sample analysis studies are protected by additional run blanks to negate any possible effects from carryover on run injections. ^{13}C, D_3-不純物A*: response from injection of blank sample following injection of the ULOQ ≤ 5% of the response observed for the working concentration
Calibration	Deviation of ± 15% (± 20% at the LLOQ) for 75% of the calibration standards from the nominal values at a minimum of 6 different levels
Dilutions	Bias within ± 15% of the nominal concentration (12.5-times of ULOQ) for samples diluted up to 20-fold. Precision ≤ 15%

2.6.5.3 薬物動態試験：単回投与後の吸収

2.6.5.3A マウスに単回静脈内及び経口投与後の薬物動態試験（RD-2016-00013）

Study title:	Pharmacokinetics of Factor B inhibitor NVP-LNP023 following single intravenous and oral administration in C57BL/6 mice	Report no. RD-2016-00013
Location in CTD:	4.2.2.2-1	
GLP compliance:	Not required	
Test article(s):	LNP023	
Species / Strain:	Mouse / C57BL/6	
Gender (M / F) / Number of animals:	Male / 4	
Feeding condition:	Fed	
Vehicle / Formulation:	i.v.: solution in 10% propylene glycol:25% (20% Solutol):PBS, p.o.: suspension in 0.5% methyl cellulose:0.5% Tween 80	
Method of Administration:	i.v.: single dose bolus, p.o.: single dose oral gavage	
Dose (mg/kg):	i.v.: 1 mg/kg, p.o.: 10 mg/kg	
Sample matrix:	Plasma	
Sampling schedule:	Blood / plasma: serial collection at 0.083 (i.v. only), 0.25 (p.o. only), 0.5, 1, 2, 4, 7 h post-dose	
Analyte(s):	LNP023	
Assay:	LNP023 in plasma: LC-MS/MS	

PK parameters	i.v. 1 mg/kg	p.o. 10 mg/kg
Tmax (h)	-	0.6
Tlast (h)	7	7
Cmax (µg/mL)	-	0.9486
AUClast (µg·h/mL)	0.7001	3.213
AUCinf (µg·h/mL)	1.029	4.5962
T1/2 (h)	7.2	-
V_{SS} (L/kg)	6.6	-
CL (mL/min/kg)	16	-
Bioavailability (%)	-	46 [a)]

-: not available

Data are mean, n = 2. Molar concentration data were converted to weight concentrations by the equation [µg/mL = µmol/L × 422.52 / 1000].

[a)] Value of bioavailability is related to the dose normalized AUCinf of LNP023 determined after intravenous administration [(AUCinf/dose p.o.) / (AUCinf/dose i.v) × 100].

2.6.5.3B ラットに単回静脈内及び経口投与後の薬物動態試験（RD-2016-00014）

Study title:	Pharmacokinetics of Factor B inhibitor NVP-LNP023 following single intravenous and oral administration in Wistar Han rats	Report no. RD-2016-00014
Location in CTD:	4.2.2.2-2	
GLP compliance:	Not required	
Test article(s):	LNP023	
Species / Strain:	Rat / Wistar Hannover	
Gender (M / F) / Number of animals:	Male / 4	
Feeding condition:	i.v.: fed, p.o.:fasted	
Vehicle / Formulation:	i.v.: solution in 2 equivalents 1N HCl, 30% PEG300, 50% (20% Crem EL), PBS, pH 7, p.o.: suspension in 2 equivalents 1N HCl, 30% PEG300, 50% (20% Crem EL), PBS, pH 7	
Method of Administration:	i.v.: single dose bolus, p.o.: single dose oral gavage	
Dose (mg/kg):	i.v.: 1 mg/kg, p.o.: 30 mg/kg	
Sample matrix:	Plasma	
Sampling schedule:	Blood / plasma: serial collection at 0 (p.o. only), 0.083 (i.v. only), 0.25, 0.5, 1, 2, 4, 7, 24 h post-dose	
Analyte(s):	LNP023	
Assay:	LNP023 in plasma: LC-MS/MS	

PK parameters	i.v. 1 mg/kg	p.o. 30 mg/kg
Tmax (h)	-	2
Tlast (h)	24	7
Cmax (µg/mL)	-	5.2236
AUClast (µg·h/mL)	2.082	22.403
AUCinf (µg·h/mL)	2.195	26.184
T1/2 (h)	6.7	-
V_{SS} (L/kg)	2.3	-
CL (mL/min/kg)	7.6	-
Bioavailability (%)	-	40 [a)]

-: not available

Data are mean, n = 2. Molar concentration data were converted to weight concentrations by the equation [µg/mL = µmol/L × 422.52 / 1000].

[a)] Value of bioavailability is related to the dose normalized AUCinf of LNP023 determined after intravenous administration [(AUCinf/dose p.o.) / (AUCinf/dose i.v) × 100].

2.6.5.3C ラットに単回静脈内及び経口投与後の薬物動態試験（DMPK R1500361）

Study title:	Absorption, distribution, metabolism, and excretion of LNP023 in rats after oral (10 mg/kg) and intravenous (2 mg/kg) administration of [^{14}C]LNP023 hydrochloride	Report no. DMPK R1500361
Location in CTD:	4.2.2.2-3	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Species / Strain:	Rat / Wistar Hannover	
Gender (M / F) / Number of animals:	Male / 6	
Feeding condition:	Fed	
Vehicle / Formulation:	i.v.: solution in 5% ethanol:15% propylene glycol:80% aqueous NaCl solution (0.9% w/v) (w/w/w), p.o.: suspension in 0.5% aqueous methylcellulose solution containing 0.5% Tween 80 (w/w)	
Method of Administration:	i.v.: single dose bolus, p.o.: single dose oral gavage	
Dose (mg/kg):	i.v.: 2 mg/kg, p.o.: 10 mg/kg	
Sample matrix:	Plasma	
Radionucleotide:	^{14}C	
Specific radioactivity:	1.50 and 3.00 MBq/mg (different batches)	
Sampling schedule:	Blood / plasma: serial collection at 0.083 (i.v. only), 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72 h post-dose	
Analyte(s):	^{14}C, LNP023	
Assay:	^{14}C in plasma: liquid scintillation counter (LSC), LNP023 in plasma: LC-MS/MS	

PK parameters	i.v. 2 mg/kg	p.o. 10 mg/kg
Actual dose (mg/kg)	2.04 ± 0.0361	10.1 ± 0.890
Total radiolabeled components in plasma		
Tmax (h) [a)]	0.083 [0.083-0.083]	0.5 [0.25-2]
Tlast (h) [a)]	24 [24-48]	24 [24-72]
Cmax (µg eq/mL)	2.19 ± 0.0718	1.09 ± 0.186
Cmax/dose [(µg eq/mL)/(mg/kg)]	1.08 ± 0.0537	0.109 ± 0.0215
AUClast (µg eq·h/mL)	3.28 ± 0.257	13.2 ± 1.48
AUClast/dose [(µg eq·h/mL)/(mg/kg)]	1.61 ± 0.110	1.31 ± 0.123

PK parameters	i.v. 2 mg/kg	p.o. 10 mg/kg
AUCinf (µg eq·h/mL)	3.37 ± 0.295	13.7 ± 1.56
AUCinf/dose [(µg eq·h/mL)/(mg/kg)]	1.65 ± 0.128	1.35 ± 0.127
T1/2 (h)	5.21 ± 0.779	8.18 ± 6.25
Fraction absorbed (%)	-	81.8 [b)]
LNP023 in plasma [c)]		
Tmax (h) [a)]	0.083 [0.083-0.083]	0.25 [0.25-4]
Tlast (h) [a)]	24 [24-48]	24 [24-72]
Cmax (µg/mL)	1.97 ± 0.129	0.570 ± 0.0570
Cmax/dose [(µg/mL)/(mg/kg)]	0.963 ± 0.0625	0.0562 ± 0.00473
AUClast (µg·h/mL)	2.95 ± 0.259	7.14 ± 0.608
AUClast/dose [(µg·h/mL)/(mg/kg)]	1.45 ± 0.113	0.701 ± 0.0213
AUCinf (µg·h/mL)	3.02 ± 0.282	7.48 ± 0.600
AUCinf/dose [(µg·h/mL)/(mg/kg)]	1.48 ± 0.125	0.735 ± 0.0240
T1/2 (h)	5.08 ± 1.44	9.70 ± 8.01
Vss (L/kg)	3.01 ± 0.925	-
CLp (L/h/kg)	0.680 ± 0.0607	-
CLb (L/h/kg)	0.439 [d)]	-
Bioavailability (%)	-	49.7 [e)]

-: not available, CLb: blood clearance, CLp: plasma clearance

Data are mean ± SD, n = 3. Molar concentration data were converted to weight concentrations by the equation [µg (or µg eq)/mL = µmol (or µmol eq)/L × 422.52 / 1000].

[a)] Median [range]

[b)] Value of fraction absorbed is related to the dose normalized AUCinf of total radiolabeled components determined after intravenous administration [(AUCinf/dose p.o.) / (AUCinf/dose i.v.) × 100].

[c)] Stabilized plasma refers to the stabilization of the acyl glucuronide metabolite, so back-conversion to LNP023 during sample preparation/analysis is minimized.

[d)] Blood-plasma concentration ratio (Cb / Cp) from (study DMPK R1500362) was used for the calculation. As this ratio was shown to be concentration dependent a fixed value of Cb / Cp = 1.55 (tested at 1000 ng/mL) was used.

[e)] Value of bioavailability is related to the dose normalized AUCinf of LNP023 determined after intravenous administration [(AUCinf/dose p.o.) / (AUCinf/dose i.v) × 100].

2.6.5.3D　ラットに 不純物A* を単回経口投与後の薬物動態試験（DMPK R2000379）

Study title:	Pharmacokinetics of 不純物A* following oral administration in the rat	Report no. DMPK R2000379
Location in CTD:	4.2.2.2-4	
GLP compliance:	Not required	
Test article(s):	不純物A* (LNP023 impurity)	
Species / Strain:	Rat / Wistar	
Gender (M / F) / Number of animals:	Hannover Male / 12	
Feeding condition:	Fed	
Vehicle / Formulation:	p.o.: suspension in 0.5% methyl cellulose:10% VitETPGS:water for 3 and 10 mg/kg dose groups, suspension in 0.5% methyl cellulose:0.1% Tween 80:water for 30, 100, and 300 mg/kg dose groups	
Method of Administration:	p.o.: single dose oral gavage	
Dose (mg/kg):	p.o.: 3, 10, 30, 100, 300 mg/kg	
Sample matrix:	Plasma	
Sampling schedule:	Blood / plasma: serial collection at 0.25, 1, 3, 7, 10, 24, 36, 48, 72 h post-dose	
Analyte(s):	不純物A*	
Assay:	不純物A* in plasma: LC-MS/MS	

PK parameters	p.o. 3 mg/kg [a)]	p.o. 10 mg/kg [a)]	p.o. 30 mg/kg	p.o. 100 mg/kg	p.o. 300 mg/kg
Formulation	Suspension	Suspension	Suspension	Suspension	Suspension
Actual Dose (mg/kg)	2.97	10.0	30.3 ± 0.260	101 ± 0.541	305 ± 4.09
Cmax (ng/mL)	5.67	18.2	187 ± 240	234 ± 210	2960 ± 1850
Cmax/dose [(ng/mL)/(mg/kg)]	1.91	1.82	6.14 ± 7.86	2.31 ± 2.06	9.75 ± 6.16
Tmax [b)] (h)	0.25	0.25	0.25 [0.25-0.25]	1.0 [1.0-1.0]	7.0 [3.0-10]
Tlast [b)] (h)	3.0	7.0	10 [10-24]	24 [24-24]	72 [48-72]
AUClast (ng·h/mL)	6.03	21.8	198 ± 187	683 ± 478	24600 ± 7830
AUClast/dose [(ng·h/mL)/(mg/kg)]	2.03	2.17	6.51 ± 6.14	6.76 ± 4.69	80.9 ± 26.3
AUCinf (ng·h/mL)	na	22.3	200 ± 187	685 ± 477	na
AUCinf/dose [(ng·h/mL)/(mg/kg)]	na	2.22	6.58 ± 6.13	6.77 ± 4.68	na
T1/2 (h)	na	1.32	2.85 ± 1.11	3.43 ± 0.735	na

na: not applicable

Data are mean ± SD, n = 3.

[a)] 3 mg/kg and 10 mg/kg dose groups consisted of one animal each.

[b)] Median [range]

2.6.5.3E ラットに単回静脈内，経口，及び回腸内投与後の薬物動態試験（DMPK R2100550-01）

Study title:	Pharmacokinetics of LNP023 after a single intravenous, oral or intra-ileal dose administration to male Sprague Dawley rats	Report no. DMPK R2100550-01
Location in CTD:	4.2.2.2-5	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Species / Strain:	Rat / Sprague Dawley	
Gender (M / F) / Number of animals:	Male / 12	
Feeding condition:	Fed (i.v.), fasted (p.o., intra-ileal)	
Vehicle / Formulation:	i.v.: solution in 10% ethanol:15% propylene glycol:75% saline (v/v/v), p.o., intra-ileal: solution in 0.5% methyl cellulose (400cP):0.5% Tween 80:99% water (w/w/v)	
Method of Administration:	i.v.: single dose bolus, p.o.: single dose oral gavage, single dose intra-ileal	
Dose (mg/kg):	i.v.: 2 mg/kg, p.o.: 10 mg/kg, intra-ileal: 10 mg/kg	
Sample matrix:	Plasma	
Sampling schedule:	Blood / plasma: serial collection at 0.083 (i.v. only), 0.25, 0.5, 1, 2, 4, 7, 24, 48 h post-dose	
Analyte(s):	LNP023	
Assay:	LNP023 in plasma: LC-MS/MS	

PK parameters	i.v. 2 mg/kg [a)]	p.o. 10 mg/kg [a)]	Intra-ileal 10 mg/kg [b)]
CL (L/h/kg)	0.768 ± 0.149	-	-
Vss (L/kg)	1.49 ± 0.142	-	-
Tmax (h) [c)]	-	0.5 [0.5-0.5]	0.25 [0.25-2.0]
Cmax (ng/mL)	-	1410 ± 185	630 ± 232
Cmax/dose [(ng/mL)/(mg/kg)]	-	141 ± 18.5	63.0 ± 23.2
Tlast (h) [c)]	24 [7.0-24]	48 [24-48]	48 [48-48]
AUClast (ng·h/mL)	2630 ± 526	9050 ± 1390	4650 ± 1480
AUClast/dose [(ng·h/mL)/(mg/kg)]	1320 ± 263	905 ± 139	465 ± 148
AUCinf (ng·h/mL)	2670 ± 486	9070 ± 1380	4740 ± 1640
AUCinf/dose [(ng·h/mL)/(mg/kg)]	1330 ± 243	907 ± 138	474 ± 164
T1/2 (h)	2.91 ± 0.960	4.46 ± 1.16	6.40 ± 0.935
Bioavailability (%)	-	68.3 ± 10.6 [d)]	35.7 ± 12.4 [d)]

-: not available

Data are mean ± SD.

[a] n = 3

[b] n = 6

[c] Median [range]

[d] Value of bioavailability is related to the dose normalized AUCinf of LNP023 determined after intravenous administration [(AUCinf/dose p.o.) / (AUCinf/dose i.v) × 100].

2.6.5.3F　イヌに単回静脈内及び経口投与後の薬物動態試験（RD-2016-00020）

Study title:	NVP-LNP023-AA pharmacokinetic profiles in male Beagle dogs after single intravenous or oral administration	Report no. RD-2016-00020
Location in CTD:	4.2.2.2-6	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Species / Strain:	Dog / Beagle	
Gender (M / F) / Number of animals:	Male / 4 (2 animals were used both for i.v. and p.o. dosing performed at different occasions, and the remaining animals either for i.v. or p.o. dosing)	
Feeding condition:	Fasted	
Vehicle / Formulation:	i.v.: solution in 10% 1-methyl-2-pyrrolidone:90% PEG 200, p.o.: solution in 0.5% methyl cellulose:0.5% Tween 80:99% Water	
Method of Administration:	i.v.: single dose bolus, p.o.: single dose oral gavage	
Dose (mg/kg):	i.v.: 0.1 mg/kg; p.o.: 10 mg/kg	
Sample matrix:	Blood	
Sampling schedule:	Blood: serial collection at pre-dose, 0.083 (i.v. only), 0.25, 0.5, 1, 2, 4, 7, 24 h post-dose	
Analyte(s):	LNP023	
Assay:	LNP023 in blood: LC-MS/MS	

PK parameters	i.v. 0.1 mg/kg	p.o. 10 mg/kg
Tmax (h)	-	0.8 ± 0.3
Cmax/dose [(µg/mL)/(mg/kg)]	-	0.9122 ± 0.126
AUC0-24h/dose [(µg·h/mL)/(mg/kg)]	8.902 ± 0.9422	5.3466 ± 0.4546
T1/2 (h)	5.6 ± 0.5	-
V_{SS} (L/kg)	0.6 ± 0.1	-
CL (mL/min/kg)	2 ± 0	-
Bioavailability (%)	-	60 [a)]

-: not available

Data are mean ± SD, n = 3. Molar concentration data were converted to weight concentrations by the equation [µg/mL = µmol/L × 422.52 / 1000].

[a)] Value of bioavailability is related to the dose normalized AUC0-24h of LNP023 determined after intravenous administration [(AUC0-24h/dose p.o.) / (AUC0-24h/dose i.v) × 100].

2.6.5.3G　イヌに単回静脈内投与後の薬物動態試験（DMPK R2101187）

Study title:	Pharmacokinetic assessment of LNP023 following a single iv infusion administration to male Beagle dogs	Report no. DMPK R2101187
Location in CTD:	4.2.2.2-7	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Species / Strain:	Dog / Beagle	
Gender (M / F) / Number of animals:	Male / 3 (animals were used both for i.v. dosing at 3.5 and 10 mg/kg performed at different occasions)	
Feeding condition:	Fasted	
Vehicle / Formulation:	i.v.: solution in 10 mM Tris buffer, pH 8.0 with NaOH for pH adjustment	
Method of Administration:	i.v.: single dose infusion over 1 h	
Dose (mg/kg):	i.v.: 3.5 mg/kg, 10 mg/kg	
Sample matrix:	Plasma	
Sampling schedule:	Blood / plasma: serial collection at pre-dose, 0.25, 0.5, 0.75, 0.95, 1.0833, 1.25, 1.5, 2, 3, 5, 8, 13, 25, 49 h post-dose	
Analyte(s):	LNP023	
Assay:	LNP023 in plasma: LC-MS/MS	

PK parameters	3.5 mg/kg	10 mg/kg
Actual dose (mg/kg)	3.79 ± 0.0751	11.3 ± 0.0577
Tmax (h) [a)]	0.95 [0.95-1.1]	0.95 [0.95-1.1]
Cmax (ng/mL)	6350 ± 715	21500 ± 971
Cmax/dose [(ng/mL)/(mg/kg)]	1670 ± 153	1900 ± 76.4
Tlast (h) [a)]	49 [49-49]	49 [49-49]
AUClast (ng·h/mL)	41500 ± 10600	124000 ± 11400
AUClast/dose [(ng·h/mL)/(mg/kg)]	11000 ± 2750	11000 ± 958
AUC0-24h (ng·h/mL)	37100 ± 6330	110000 ± 15100
AUC0-24h/dose [(ng·h/mL)/(mg/kg)]	9780 ± 1620	9740 ± 1300
CL (L/h/kg)	0.0942 ± 0.0230	0.0912 ± 0.00867
V_{SS} (L/kg)	0.750 ± 0.106	0.768 ± 0.157
T1/2 (h)	7.12 ± 3.19	7.48 ± 0.284

Data are mean ± SD, n = 3.
[a)] Median [range]

2.6.5.4 薬物動態試験：反復投与後の吸収

No pharmacokinetic studies conducted under this section.

Toxicokinetic (TK) measurements were performed in repeated dose, carcinogenicity, reproductive, and other toxicity studies (2.6.6 Toxicology Written Summary).

TK results are summarized in 2.6.7 Toxicology Tabulated Summary.

2.6.5.5 薬物動態試験：分布

2.6.5.5A ラットに単回経口投与後の組織分布（DMPK R1500361）

Study title :	Absorption, distribution, metabolism, and excretion of LNP023 in rats after oral (10 mg/kg) and intravenous (2 mg/kg) administration of [^{14}C]LNP023 hydrochloride	Report no. DMPK R1500361
Location in CTD:	4.2.2.2-3	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Species / Strain:	Rat / Wistar Hannover (albino) and Long-Evans (pigmented)	
Gender (M / F) / number of animals:	Male / 8	
Feeding condition:	Fed	
Vehicle / formulation:	p.o.: suspension in 0.5% aqueous methylcellulose solution containing 0.5% Tween 80 (w/w)	
Method of administration:	p.o.: single dose oral gavage	
Dose (mg/kg):	p.o.: 10 mg/kg	
Radionuclide:	^{14}C	
Specific activity:	1.00 MBq/mg (2 batches)	
Analyte(s) / assay:	Tissues and blood: radioactivity measured by QWBA, blood: radioactivity measured by LSC	
Sampling time:	Whole body carcass (tissues) and blood: 0.25, 2, 8, 24, 72, 168 h post-dose in Wistar Hannover, and 24, 168 h post-dose in Long-Evans	

Tissue concentrations (µg eq/g)

	Albino rats						Pigmented rats	
Time (h)	0.25	2	8	24	72	168	24	168
Blood (QWBA)	2.41	1.39	0.287	0.0281	0	0	0.114	0
Blood (LSC)	4.06	2.17	0.322	0.0400	0	0	0.148	0
Adrenal (cortex)	2.06	1.01	0.191	0	0	0	0.102	0
Adrenal (medulla)	1.89	0.972	0.166	0	0	0	0.102	0
Blood vessel wall	1.96	0.980	0.260	0	0	0	0	0
Bone marrow	1.23	0.600	0.0575	0	0	0	0.143	0
Bone mineral	0.0828	0.179	trace	0	0	0	0	0
Brain	0.0333	0.0299	-	0	0	0	0	0

	Tissue concentrations (µg eq/g)							
				Albino rats			Pigmented rats	
Time (h)	0.25	2	8	24	72	168	24	168
Cartilage	0.0465	0.272	0.0731	0	0	0	0	0
Choroid plexus	0.976	0.828	0.134	0	0	0	0	0
Epididymis	0.363	0.482	0.0799	0	0	0	0	0
Esophagus	1.21	0.587	0.117	0	0	0	0	0
Eye (choroid)	1.11	0.727	0.106	0	0	0	1.56	1.05
Eye (ciliary body)	0.790	0.409	0.175	0	0	0	2.76	2.24
Eye (lens)	trace	trace	trace	0	0	0	0	trace
Eye (vitreous body)	trace	0.0558	trace	0	0	0	0	0
Fat (brown)	1.50	0.900	0.201	0	0	0	0.0832	0
Fat (white)	0.143	0.127	0	0	0	0	0	0
Hair (follicle)	0.693	0.452	0.0706	0	0	0	0	0
Hair (tactile)	0.900	0.723	0.206	0	0	0	0	0
Hairfollicle (pigm.)	0	0	0	0	0	0	0.858	0
Hairfollicle (tactile pigm.)	0	0	0	0	0	0	0.680	0
Harderian gland	0.820	0.515	0.100	0	0	0	0.0723	0
Intestinal wall (colon)	0.799	0.439	0.790	0	0	0	0.790	0
Intestinal wall (small int.)	1.15	0.841	0.187	0.0452	0	0	0.226	0
Kidney (CM junction)	4.52	2.73	0.604	0.183	0.0887	trace	0.321	trace
Kidney (cortex)	4.65	2.27	0.570	0.211	0.0858	trace	0.365	0.0333
Kidney (medulla)	2.64	1.67	0.532	0.0382	0.0431	0	0.128	0
Lacrimal gland	1.34	0.807	0.119	trace	0	0	0.0554	0
Liver	15.0	8.03	2.14	0.634	0.202	0.0499	0.731	0.0344
Lung	2.33	1.25	0.242	0.0281	0	0	0.101	0
Lymph nodes (submandibular)	0.723	0.684	0.0862	0	0	0	0	0
Meninges	0.389	0.289	0.0714	0	0	0	0	0
Muscle	0.532	0.520	0.0507	0	0	0	trace	0
Myocard	2.08	1.13	0.226	-	0	0	0	0

	Tissue concentrations (µg eq/g)							
			Albino rats				Pigmented rats	
Time (h)	0.25	2	8	24	72	168	24	168
Pancreas	1.24	0.883	0.111	0.0276	0	0	0.104	0
Penis	0.908	0.532	0.233	0.0579	0	0	0.146	0
Pineal body	1.32	0.651	0.136	0	0	0	0.0786	0
Pituitary gland	1.52	0.853	0.0731	0	0	0	0.128	0
Preputial gland	0.963	0.613	0.0930	0	0	0	0	0
Salivary gland (submaxillary)	1.62	0.989	0.137	0	0	0	0.0592	0
Seminal vesicle wall	0.583	0.486	0	0	0	0	0	0
Seminal vesicular fluid	trace	0.258	0.240	0	0	0	0	0
Skin	0.520	0.431	0.0799	0	0	0	0	0
Skin (subcutis)	0.608	0.439	0.0849	0	0	0	0	0
Skin pigmented	0	0	0	0	0	0	0.263	0
Spinal cord	0.0431	0.0334	trace	0	0	0	0	0
Spleen	1.44	0.815	0.120	0	0	0	0.0579	0
Stomach (glandular)	2.61	1.69	0.106	0	0	0	0.0625	0
Stomach (non-glandular)	6.17	4.27	0.150	0	0	0	trace	0
Testis	0.107	0.318	0.0634	trace	0	0	trace	0
Thymus	0.621	0.461	0.0680	0	0	0	trace	0
Thyroid gland	2.16	1.10	0.125	0	0	0	0.0837	0
Tongue	1.36	0.744	0.125	0	0	0	0.0934	0
Tongue (mucous gland)	1.42	0.773	0.126	0	0	0	0.0634	0
Tooth (pulp)	1.16	0.731	0.131	0	0	0	0	0
Whole rat (with GI tract)	3.59	4.18	5.32	1.38	trace	0	3.31	0
Whole rat (without GI tract)	1.74	1.16	0.272	0.0824	trace	0	0.128	0
LOD QWBA	0.0136	0.0132	0.0167	0.0133	0.0187	0.0169	0.0243	0.0133
LLOQ QWBA	0.0273	0.0264	0.0335	0.0266	0.0375	0.0338	0.0486	0.0266

-: not available

Concentrations below LOD are reported as 0. Concentrations between LOD and LLOQ are reported as trace.

LOD (LSC): 0.00853 µg eq/g

Molar concentration data were converted to weight concentrations by the equation [µg eq/g = nmol eq/g × 422.52 / 1000].

	PK parameters in albino rats							
	Tmax (h)	Cmax (µg eq/g)	Cmax T / B ratio [a)]	Tlast (h)	AUClast (µg eq·h/g)	AUClast T / B ratio [a)]	T1/2 (h)	Range T1/2 (h)
Blood (QWBA)	0.25	2.41	1.00	24	11.2	1.00	4.06	2-24
Blood (LSC)	0.25	4.06	1.69	24	16.4	1.46	4.05	2-24
Adrenal (cortex)	0.25	2.06	0.856	8	6.55	0.586	2.31	0.25-8
Adrenal (medulla)	0.25	1.89	0.786	8	6.17	0.552	2.25	0.25-8
Blood vessel wall	0.25	1.96	0.814	8	6.55	0.586	2.77	0.25-8
Bone marrow	0.25	1.23	0.511	8	3.73	0.333	1.76	0.25-8
Bone mineral	2	0.179	0.0744	2	0.240	0.0214	nc	nc
Brain	0.25	0.0333	0.0138	2	0.0596	0.00533	nc	nc
Cartilage	2	0.272	0.113	8	1.32	0.118	nc	nc
Choroid plexus	0.25	0.976	0.405	8	4.61	0.412	2.59	0.25-8
Epididymis	2	0.482	0.200	8	2.47	0.221	nc	nc
Esophagus	0.25	1.21	0.502	8	3.84	0.343	2.37	0.25-8
Eye (choroid)	0.25	1.11	0.461	8	4.27	0.382	2.26	0.25-8
Eye (cil body)	0.25	0.790	0.328	8	2.90	0.260	2.37	0.25-8
Eye (vitreous body)	2	0.0558	0.0232	2	0.0558	0.00499	nc	nc
Fat (brown)	0.25	1.50	0.625	8	5.58	0.499	2.70	0.25-8
Fat (white)	0.25	0.143	0.0593	2	0.254	0.0227	nc	nc
Hair (follicle)	0.25	0.693	0.288	8	2.66	0.238	2.32	0.25-8
Hair (tactile)	0.25	0.900	0.374	8	4.31	0.386	3.55	0.25-8
Harderian gland	0.25	0.820	0.340	8	3.12	0.279	2.55	0.25-8
Intestinal wall (colon)	0.25	0.799	0.332	8	4.86	0.435	nc	nc
Intestinal wall (small int.)	0.25	1.15	0.479	24	6.84	0.612	5.60	2-24
Kidney (CM junction)	0.25	4.52	1.88	72	29.7	2.66	nc	nc
Kidney (cortex)	0.25	4.65	1.93	72	28.5	2.55	25.6	8-72

	PK parameters in albino rats							
	Tmax (h)	Cmax (µg eq/g)	Cmax T / B ratio [a)]	Tlast (h)	AUClast (µg eq·h/g)	AUClast T / B ratio [a)]	T1/2 (h)	Range T1/2 (h)
Kidney (medulla)	0.25	2.64	1.10	72	17.2	1.54	nc	nc
Lacrimal gland	0.25	1.34	0.558	8	4.82	0.431	2.20	0.25-8
Liver	0.25	15.0	6.25	168	107	9.57	40.3	24-168
Lung	0.25	2.33	0.967	24	10.1	0.900	4.21	2-24
Lymph nodes (submandibular)	0.25	0.723	0.300	8	3.63	0.325	2.37	0.25-8
Meninges	0.25	0.389	0.162	8	1.72	0.154	3.12	0.25-8
Muscle	0.25	0.532	0.221	8	2.70	0.242	2.13	0.25-8
Myocard	0.25	2.08	0.865	8	7.14	0.639	2.46	0.25-8
Pancreas	0.25	1.24	0.516	24	6.08	0.544	nc	nc
Penis	0.25	0.908	0.377	24	6.00	0.537	7.07	2-24
Pineal body	0.25	1.32	0.547	8	4.27	0.382	2.44	0.25-8
Pituitary gland	0.25	1.52	0.630	8	5.03	0.450	1.75	0.25-8
Preputial gland	0.25	0.963	0.400	8	3.62	0.324	2.27	0.25-8
Salivary gland (submaxillary)	0.25	1.62	0.674	8	5.87	0.526	2.15	0.25-8
Seminal vesicle wall	0.25	0.583	0.242	2	1.01	0.0904	nc	nc
Seminal vesicular fluid	2	0.258	0.107	8	1.75	0.157	nc	nc
Skin	0.25	0.520	0.216	8	2.43	0.217	2.75	0.25-8
Skin (subcutis)	0.25	0.608	0.253	8	2.56	0.229	2.67	0.25-8
Spinal cord	0.25	0.0431	0.0179	2	0.0723	0.00646	nc	nc
Spleen	0.25	1.44	0.598	8	4.94	0.442	2.16	0.25-8
Stomach (glandular)	0.25	2.61	1.08	8	9.46	0.847	1.63	0.25-8
Stomach (non glandular)	0.25	6.17	2.56	8	23.2	2.07	1.39	0.25-8
Testis	2	0.318	0.132	8	1.53	0.137	nc	nc
Thymus	0.25	0.621	0.258	8	2.61	0.234	2.36	0.25-8
Thyroid gland	0.25	2.16	0.896	8	6.80	0.609	1.89	0.25-8
Tongue	0.25	1.36	0.563	8	4.61	0.412	2.27	0.25-8

	PK parameters in albino rats							
	Tmax (h)	Cmax (µg eq/g)	Cmax T / B ratio [a)]	Tlast (h)	AUClast (µg eq·h/g)	AUClast T / B ratio [a)]	T1/2 (h)	Range T1/2 (h)
Tongue (mucous gland)	0.25	1.42	0.588	8	4.77	0.427	2.24	0.25-8
Tooth (pulp)	0.25	1.16	0.481	8	4.39	0.393	2.45	0.25-8

nc: not calculated (due to limited data set available)

Molar concentration data were converted to weight concentrations by the equation [µg eq/g = nmol eq/g × 422.52 / 1000].

[a)] T / B ratio: Tissue over blood ratio

2.6.5.5B 有色ラットに単回経口投与後の組織分布（DMPK R1701426）

Study title:	Pharmacokinetics, Absorption, Distribution and Excretion of [^{14}C]LNP023 following oral administration to male and female Long Evans rats	Report no. DMPK R1701426
Location in CTD:	4.2.2.3-1	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Species / Strain:	Rat / Long-Evans (pigmented)	
Gender (M / F) / number of animals:	Male / 7, Female / 7	
Feeding condition:	Fed	
Vehicle / formulation:	p.o.: solution in 0.5% aqueous methylcellulose solution containing 0.5% Tween 80 (w/w)	
Method of administration:	p.o.: single dose oral gavage	
Dose (mg/kg):	p.o.: 10 mg/kg	
Radionuclide:	^{14}C	
Specific activity:	1.04 MBq/mg	
Analyte(s) / assay:	Tissues: radioactivity measured by QWBA, blood and plasma: radioactivity measured by LSC	
Sampling time:	Whole body carcass (tissues) and blood / plasma: 0.5, 2, 8, 24, 72, 168, 336 h post-dose	

Tissue concentrations (µg eq/g) in Long-Evans male rats

Time (h)	0.5	2	8	24	72	168	336
Adrenal gland(s)	0.942 a)	0.908 a)	0.182 a)	BLQ a)	nd	nd	nd
Arterial wall	1.02	1.17	0.323	nd	nd	nd	nd
Bile	45.6	26.6	6.17	nd	nd	nd	nd
Blood (LSC)	2.00	1.51	0.482	BLQ	BLQ	BLQ	BLQ
Blood (QWBA)	0.811	0.879	0.286	nd	nd	nd	nd
Bone	0.040	0.0482	BLQ	nd	nd	nd	nd
Bone marrow	0.554	0.469	0.106	nd	nd	nd	nd
Brain (whole)	BLQ	BLQ	nd	nd	nd	nd	nd
Brain cerebellum	BLQ	BLQ	nd	nd	nd	nd	nd
Brain cerebrum	BLQ	BLQ	nd	nd	nd	nd	nd

Tissue concentrations (µg eq/g) in Long-Evans male rats							
Time (h)	0.5	2	8	24	72	168	336
Brain medulla	BLQ	BLQ	nd	nd	nd	nd	nd
Brain olfactory lobe	BLQ	BLQ	nd	nd	nd	nd	nd
Bulbo-urethral gland	0.706	0.642	0.195	nd	nd	nd	nd
Cecum	0.592	0.499	0.452	0.136	BLQ	nd	nd
Diaphragm	0.621	0.684	0.158	nd	nd	nd	nd
Epididymis	0.339	0.427 a)	0.121	nd	nd	nd	nd
Esophagus	0.634	2.26	0.261	nd	nd	nd	nd
Exorbital lacrimal gland	0.663	0.668	0.192	nd	nd	nd	nd
Eye lens	BLQ	BLQ	BLQ	nd	nd	nd	nd
Eye uveal tract	0.477	1.72	3.11	1.31	0.832	0.790	0.431
Eye(s)	0.101	0.383	0.613	0.252	0.191	0.138	0.0782
Fat (abdominal)	BLQ	BLQ	nd	nd	nd	nd	nd
Fat (brown)	0.427	0.448	0.136	nd	nd	nd	nd
Harderian gland	0.301	0.439	0.0980	nd	nd	nd	nd
Intervertebral ligaments	0.934	1.53	0.162	nd	nd	nd	nd
Intra-orbital lacrimal gland	0.604	0.592	0.125	nd	nd	nd	nd
Kidney cortex	2.59	2.45	0.697	0.0849	BLQ	nd	nd
Kidney medulla	1.40	1.66	0.444	BLQ	BLQ	nd	nd
Kidney(s)	2.09	2.26	0.604	0.0706	BLQ	nd	nd
Large intestine	0.435	0.477	0.137	0.126	BLQ	nd	nd
Liver	12.0	11.2	2.21	0.439	0.129	BLQ	nd
Lung(s)	0.731	0.701	0.226	nd	nd	nd	nd
Lymph node(s)	0.477	0.422 a)	0.123	nd	nd	nd	nd
Mammary gland(s)	0.115	BLQ	BLQ	nd	nd	nd	nd
Muscle	0.365	0.411	0.0748	nd	nd	nd	nd
Myocardium	0.942	0.849	0.242	nd	nd	nd	nd
Nasal turbinates	0.251	0.583	0.0989	nd	nd	nd	nd
Oral mucosa	0.395	0.558	0.166	nd	nd	nd	nd

Tissue concentrations (µg eq/g) in Long-Evans male rats							
Time (h)	0.5	2	8	24	72	168	336
Pancreas	0.710	0.532	0.254	nd	nd	nd	nd
Pituitary gland	0.786	0.769	0.167	nd	nd	nd	nd
Plasma (LSC)	1.31	1.34	0.511	BLQ	BLQ	BLQ	BLQ
Preputial gland	0.321	0.183 [a)]	0.0773 [a)]	nd	nd	nd	nd
Prostate gland	0.312	0.326	0.116	nd	nd	nd	nd
Salivary gland(s)	0.824	0.930	0.238	nd	nd	nd	nd
Seminal vesicle(s)	0.0528	0.189	0.280	nd	nd	nd	nd
Skin (nonpigmented)	0.360	0.368	0.0917	BLQ	nd	nd	nd
Skin (pigmented)	0.369	0.404	0.159	0.0697	0.0444	nd	nd
Small intestine	0.625	4.39	1.08	0.183	BLQ	nd	nd
Spinal cord	BLQ	BLQ	nd	nd	nd	nd	nd
Spleen	0.748	0.714	0.159	nd	nd	nd	nd
Stomach	1.21	0.545	0.230	nd	nd	nd	nd
Testis(es)	0.116	0.284	0.0862	nd	nd	nd	nd
Thymus	0.385	0.398	0.0684	nd	nd	nd	nd
Thyroid	0.706	0.824	0.246	BLQ	nd	nd	nd

BLQ: below the limit of quantitation (LSC: < 0.0330 µg eq/g, QWBA: < 0.0393 µg eq/g)

nd: not detected (sample shape not discernible from background or surrounding tissue)

Molar concentration data were converted to weight concentrations by the equation [µg eq/g = nmol eq/g × 422.52 / 1000].

[a)] Tissue appeared to be fat-soaked.

Tissue concentrations (µg eq/g) in Long-Evans female rats							
Time (h)	0.5	2	8	24	72	168	336
Adrenal gland(s)	1.01 [a)]	0.587 [a)]	0.184 [a)]	BLQ [a)]	nd	nd	nd
Arterial wall	1.98	1.09	0.286	nd	nd	nd	nd
Bile	61.3	16.4	3.57	nd	nd	nd	nd
Blood (LSC)	3.97	1.68	0.356	0.0382	BLQ	BLQ	BLQ
Blood (QWBA)	1.60	0.832	0.231	BLQ	nd	nd	nd

Tissue concentrations (µg eq/g) in Long-Evans female rats							
Time (h)	0.5	2	8	24	72	168	336
Bone	0.0397	BLQ	nd	nd	nd	nd	nd
Bone marrow	1.07	0.507	0.0972	nd	nd	nd	nd
Brain (whole)	0.041	BLQ	BLQ	nd	nd	nd	nd
Brain cerebellum	BLQ	BLQ	nd	nd	nd	nd	nd
Brain cerebrum	0.0837	0.039	BLQ	nd	nd	nd	nd
Brain medulla	BLQ	BLQ	nd	nd	nd	nd	nd
Brain olfactory lobe	BLQ	BLQ	nd	nd	nd	nd	nd
Cecum	0.693	1.28	2.52	0.566	- [b)]	nd	nd
Diaphragm	1.23	0.558	0.0731	nd	nd	nd	nd
Esophagus	1.10	0.976	0.739	0.040	nd	nd	nd
Exorbital lacrimal gland	1.36	0.558	0.124	nd	nd	nd	nd
Eye lens	BLQ	0.0592	nd	nd	nd	nd	nd
Eye uveal tract	1.09	1.96	2.49	2.30	0.896	0.769	0.706
Eye (s)	0.217	0.431	0.469	0.411	0.199	0.127	0.138
Fat (abdominal)	0.0439	BLQ	nd	nd	nd	nd	nd
Fat (brown)	0.676	0.300	0.0866	BLQ	nd	nd	nd
Harderian gland	0.807	0.469	0.0600	BLQ	nd	nd	nd
Intervertebral ligaments	2.02	- [b)]	0.0925	nd	nd	nd	nd
Intra-orbital lacrimal gland	1.10	0.275	0.126	BLQ	nd	nd	nd
Kidney cortex	3.76	1.59	0.410	0.0951	BLQ	nd	nd
Kidney medulla	4.15	1.39	0.243	BLQ	BLQ	nd	nd
Kidney(s)	3.91	1.52	0.363	0.0837	BLQ	nd	nd
Large intestine	0.773	0.579	0.587	0.214	nd	nd	nd
Liver	13.9	5.83	1.53	0.379	0.0921	BLQ	nd
Lung(s)	1.56	0.739	0.153	BLQ	nd	nd	nd
Lymph node(s)	0.837	0.477	0.0684	nd	nd	nd	nd
Mammary gland(s)	0.153	0.0811	BLQ	nd	nd	nd	nd
Muscle	0.625	0.351	0.0452	BLQ	nd	nd	nd

Tissue concentrations (µg eq/g) in Long-Evans female rats							
Time (h)	0.5	2	8	24	72	168	336
Myocardium	1.69	0.684	0.166	BLQ	nd	nd	nd
Nasal turbinates	0.378	0.305	0.0744	nd	nd	nd	nd
Oral mucosa	0.849	0.411	0.141	nd	nd	nd	nd
Ovary(ies)	0.672 [a)]	0.328 [a)]	0.0837 [a)]	nd	nd	nd	nd
Pancreas	1.09	0.486	0.0811	nd	nd	nd	nd
Pituitary gland	1.22	0.579	0.0849	BLQ	nd	nd	nd
Plasma (LSC)	1.90	1.20	0.339	0.0427	BLQ	BLQ	BLQ
Preputial gland	0.427 [a)]	0.142 [a)]	0.0638 [a)]	nd	nd	nd	nd
Salivary gland(s)	1.47	0.646	0.200	nd	nd	nd	nd
Skin (nonpigmented)	0.507	0.329	0.0537	BLQ	nd	nd	nd
Skin (pigmented)	0.524	0.324	0.123	0.0503	BLQ	nd	nd
Small intestine	0.892	2.25	1.12	0.250	nd	nd	nd
Spinal cord	BLQ	BLQ	nd	nd	nd	nd	nd
Spleen	1.44	0.520	0.0879	BLQ	nd	nd	nd
Stomach	0.511	0.541	0.0837	nd	nd	nd	nd
Thymus	0.756	0.378	0.0558	nd	nd	nd	nd
Thyroid	1.26	0.710	0.174	0.0439	nd	nd	nd
Uterus	1.13 [a)]	0.482 [a)]	0.123 [a)]	nd	nd	nd	nd

-: not available, BLQ: below the limit of quantitation (LSC: < 0.0330 µg eq/g, QWBA: < 0.0393 µg eq/g), nd: not detected (sample shape not discernible from background or surrounding tissue)

Molar concentration data were converted to weight concentrations by the equation [µg eq/g = nmol eq/g × 422.52 / 1000].

[a)] Tissue appeared to be fat-soaked.

[b)] Not represented (tissue not present in section)

PK parameters in Long-Evans male rats									
Matrix	Cmax	Tmax	Tlast	AUClast	T1/2	T1/2 Range		Tissue / Blood ratio	
	(µg eq/g)	(h)	(h)	(µg eq·h/g)	(h)	Lower (h)	Upper (h)	Cmax	AUClast
Adrenal gland(s)	0.942	0.50	8.00	4.90	nc	nc	nc	1.07	0.985

Matrix	PK parameters in Long-Evans male rats								
	Cmax	Tmax	Tlast	AUClast	T1/2	T1/2 Range		Tissue / Blood ratio	
	(µg eq/g)	(h)	(h)	(µg eq·h/g)	(h)	Lower (h)	Upper (h)	Cmax	AUClast
Arterial wall	1.17	2.00	8.00	6.38	nc	nc	nc	1.34	1.29
Blood (LSC)	2.00	0.50	8.00	9.08	nc	nc	nc	na	na
Blood (QWBA)	0.879	2.00	8.00	4.99	nc	nc	nc	na	na
Bone	0.0482	2.00	2.00	nc [a)]	nc	nc	nc	0.0548	nc
Bone marrow	0.554	0.50	8.00	2.63	nc	nc	nc	0.630	0.530
Brain cerebellum	na	na	na	na	na	na	na	nc	nc
Brain cerebrum	na	na	na	na	na	na	na	nc	nc
Brain medulla	na	na	na	na	na	na	na	nc	nc
Brain olfactory lobe	na	na	na	na	na	na	na	nc	nc
Bulbo-urethral gland	0.706	0.50	8.00	3.70	3.86	0.50	8.00	0.803	0.745
Cecum	0.592	0.50	24.0	8.53	11.1	2.00	24.0	0.673	1.72
Diaphragm	0.684	2.00	8.00	3.66	nc	nc	nc	0.779	0.737
Epididymis	0.427	2.00	8.00	2.30	nc	nc	nc	0.486	0.464
Esophagus	2.26	2.00	8.00	9.89	nc	nc	nc	2.57	1.99
Exorbital lacrimal gland	0.668	2.00	8.00	3.74	nc	nc	nc	0.760	0.754
Eye lens	na	na	na	na	na	na	na	nc	nc
Eye uveal tract	3.11	8.00	336	284	nc	nc	nc	3.53	57.1
Eye(s)	0.613	8.00	336	54.9	nc	nc	nc	0.697	11.1
Fat (abdominal)	na	na	na	na	na	na	na	nc	nc
Fat (brown)	0.448	2.00	8.00	2.51	nc	nc	nc	0.510	0.507
Harderian gland	0.439	2.00	8.00	2.24	nc	nc	nc	0.500	0.452
Intervertebral ligaments	1.53	2.00	8.00	7.18	nc	nc	nc	1.75	1.44
Intra-orbital lacrimal gland	0.604	0.50	8.00	3.20	nc	nc	nc	0.688	0.644
Kidney cortex	2.59	0.50	24.0	20.2	4.67	2.00	24.0	2.94	4.06
Kidney medulla	1.66	2.00	8.00	8.96	nc	nc	nc	1.89	1.81
Kidney(s)	2.26	2.00	24.0	17.8	nc	nc	nc	2.58	3.58

Matrix	PK parameters in Long-Evans male rats								
	Cmax	Tmax	Tlast	AUClast	T1/2	T1/2 Range		Tissue / Blood ratio	
	(µg eq/g)	(h)	(h)	(µg eq·h/g)	(h)	Lower (h)	Upper (h)	Cmax	AUClast
Large intestine	0.477	2.00	24.0	4.73	nc	nc	nc	0.543	0.955
Liver	12.0	0.50	72.0	95.5	nc	nc	nc	13.7	19.2
Lung(s)	0.731	0.50	8.00	4.04	nc	nc	nc	0.832	0.813
Lymph node(s)	0.477	0.50	8.00	2.43	3.70	0.50	8.00	0.543	0.489
Mammary gland(s)	0.115	0.50	0.50	nc [a)]	nc	nc	nc	0.131	nc
Muscle	0.411	2.00	8.00	2.13	nc	nc	nc	0.467	0.429
Myocardium	0.942	0.50	8.00	4.86	3.66	0.50	8.00	1.07	0.977
Nasal turbinates	0.583	2.00	8.00	2.73	nc	nc	nc	0.663	0.551
Oral mucosa	0.558	2.00	8.00	2.99	nc	nc	nc	0.635	0.601
Pancreas	0.710	0.50	8.00	3.47	nc	nc	nc	0.808	0.699
Pituitary gland	0.786	0.50	8.00	4.17	3.15	0.50	8.00	0.894	0.840
Plasma (LSC)	1.34	2.00	8.00	7.90	nc	nc	nc	na	na
Preputial gland	0.321	0.50	8.00	1.24	3.93	0.50	8.00	0.365	0.249
Prostate gland	0.326	2.00	8.00	1.88	nc	nc	nc	0.371	0.379
Salivary gland(s)	0.930	2.00	8.00	5.03	nc	nc	nc	1.06	1.01
Seminal vesicle(s)	0.280	8.00	8.00	1.60	nc	nc	nc	0.319	0.323
Skin (nonpigmented)	0.368	2.00	8.00	2.02	nc	nc	nc	0.418	0.406
Skin (pigmented)	0.404	2.00	72.0	6.93	nc	nc	nc	0.460	1.40
Small intestine	4.39	2.00	24.0	30.5	nc	nc	nc	5.00	6.13
Spinal cord	na	na	na	na	na	na	na	nc	nc
Spleen	0.748	0.50	8.00	3.90	3.16	0.50	8.00	0.851	0.786
Stomach	1.21	0.50	8.00	3.94	3.48	0.50	8.00	1.38	0.794
Testis(es)	0.284	2.00	8.00	1.44	nc	nc	nc	0.323	0.290
Thymus	0.398	2.00	8.00	2.08	nc	nc	nc	0.452	0.419
Thyroid	0.824	2.00	8.00	4.52	nc	nc	nc	0.938	0.913

na: not applicable (no measurable concentrations for the sample, etc.), nc: not calculated (due to the inability to characterize the elimination phase per SOP criteria, etc.)

Note: Brain (whole) was not reported as there were no measurable concentrations in this tissue.

Molar concentration data were converted to weight concentrations by the equation [µg eq/g = nmol eq/g × 422.52 / 1000].

[a)] Did not report due to concentration profile having less than 3 measurable concentrations.

	PK parameters in Long-Evans female rats								
Matrix	Cmax	Tmax	Tlast	AUClast	T1/2	T1/2 Range		Tissue / Blood ratio	
	(µg eq/g)	(h)	(h)	(µg eq·h/g)	(h)	Lower (h)	Upper (h)	Lower (h)	Upper (h)
Adrenal gland(s)	1.01	0.50	8.00	3.76	3.20	0.50	8.00	0.628	0.695
Arterial wall	1.98	0.50	8.00	6.93	2.80	0.50	8.00	1.24	1.28
Blood (LSC)	3.97	0.50	24.0	14.5	4.19	2.00	24.0	na	na
Blood (QWBA)	1.60	0.50	8.00	5.41	2.82	0.50	8.00	na	na
Bone	0.0397	0.50	0.50	nc [a)]	nc	nc	nc	0.0248	nc
Bone marrow	1.07	0.50	8.00	3.27	2.25	0.50	8.00	0.670	0.603
Brain cerebellum	na	na	na	na	na	na	na	nc	nc
Brain cerebrum	0.0837	0.50	2.00	nc [a)]	nc	nc	nc	0.0522	nc
Brain medulla	na	na	na	na	na	na	na	nc	nc
Brain olfactory lobe	na	na	na	na	na	na	na	nc	nc
Cecum	2.52	8.00	24.0	37.7	nc	nc	nc	1.57	6.96
Diaphragm	1.23	0.50	8.00	3.54	1.89	0.50	8.00	0.770	0.655
Esophagus	1.10	0.50	24.0	13.2	4.54	2.00	24.0	0.689	2.44
Exorbital lacrimal gland	1.36	0.50	8.00	3.83	2.31	0.50	8.00	0.852	0.707
Eye lens	0.0592	2.00	2.00	nc [a)]	nc	nc	nc	0.0369	nc
Eye uveal tract	2.49	8.00	336	335	nc	nc	nc	1.55	61.8
Eye(s)	0.469	8.00	336	63.0	nc	nc	nc	0.293	11.6
Fat (abdominal)	0.0439	0.50	0.50	nc [a)]	nc	nc	nc	0.0274	nc
Fat (brown)	0.676	0.50	8.00	2.06	2.72	0.50	8.00	0.422	0.381
Harderian gland	0.807	0.50	8.00	2.75	2.01	0.50	8.00	0.504	0.507
Intervertebral ligaments	2.02	0.50	8.00	nc [a)]	nc	nc	nc	1.26	nc
Intra-orbital lacrimal gland	1.10	0.50	8.00	2.51	nc	nc	nc	0.689	0.464

PK parameters in Long-Evans female rats

Matrix	Cmax	Tmax	Tlast	AUClast	T1/2	T1/2 Range		Tissue / Blood ratio	
	(µg eq/g)	(h)	(h)	(µg eq·h/g)	(h)	Lower (h)	Upper (h)	Lower (h)	Upper (h)
Kidney cortex	3.76	0.50	24.0	15.0	5.76	2.00	24.0	2.35	2.77
Kidney medulla	4.15	0.50	8.00	10.1	1.96	0.50	8.00	2.59	1.87
Kidney(s)	3.91	0.50	24.0	14.3	5.61	2.00	24.0	2.44	2.63
Large intestine	0.773	0.50	24.0	11.1	nc	nc	nc	0.483	2.05
Liver	13.9	0.50	72.0	66.8	17.1	8.00	72.0	8.65	12.4
Lung(s)	1.56	0.50	8.00	4.82	2.34	0.50	8.00	0.976	0.886
Lymph node(s)	0.837	0.50	8.00	2.83	2.09	0.50	8.00	0.522	0.523
Mammary gland(s)	0.153	0.50	2.00	nc [a)]	nc	nc	nc	0.0953	nc
Muscle	0.625	0.50	8.00	2.07	1.99	0.50	8.00	0.391	0.383
Myocardium	1.69	0.50	8.00	4.73	2.41	0.50	8.00	1.05	0.877
Nasal turbinates	0.378	0.50	8.00	1.75	3.12	0.50	8.00	0.236	0.322
Oral mucosa	0.849	0.50	8.00	2.81	3.13	0.50	8.00	0.530	0.519
Ovary(ies)	0.672	0.50	8.00	2.15	2.63	0.50	8.00	0.420	0.398
Pancreas	1.09	0.50	8.00	3.16	2.08	0.50	8.00	0.683	0.584
Pituitary gland	1.22	0.50	8.00	3.65	2.01	0.50	8.00	0.763	0.673
Plasma (LSC)	1.90	0.50	24.0	10.4	4.72	2.00	24.0	na	na
Preputial gland	0.427	0.50	8.00	1.15	nc	nc	nc	0.266	0.213
Salivary gland(s)	1.47	0.50	8.00	4.48	2.82	0.50	8.00	0.916	0.829
Skin (nonpigmented)	0.507	0.50	8.00	1.90	2.31	0.50	8.00	0.317	0.351
Skin (pigmented)	0.524	0.50	24.0	3.50	8.80	2.00	24.0	0.327	0.646
Small intestine	2.25	2.00	24.0	23.6	nc	nc	nc	1.41	4.36
Spinal cord	na	na	na	na	na	na	na	nc	nc
Spleen	1.44	0.50	8.00	3.65	1.97	0.50	8.00	0.900	0.675
Stomach	0.541	2.00	8.00	2.79	nc	nc	nc	0.338	0.515
Thymus	0.756	0.50	8.00	2.34	2.04	0.50	8.00	0.472	0.432
Thyroid	1.26	0.50	24.0	6.21	5.87	2.00	24.0	0.789	1.14

Matrix	PK parameters in Long-Evans female rats								
	Cmax	Tmax	Tlast	AUClast	T1/2	T1/2 Range		Tissue / Blood ratio	
	(µg eq/g)	(h)	(h)	(µg eq·h/g)	(h)	Lower (h)	Upper (h)	Lower (h)	Upper (h)
Uterus	1.13	0.50	8.00	3.30	2.51	0.50	8.00	0.704	0.610

na: not applicable (no measurable concentrations for the sample, etc.), nc: not calculated (due to the inability to characterize the elimination phase per SOP criteria, etc.)

Note: Brain (whole) was not reported as there were only one measurable concentrations in this tissue.

Molar concentration data were converted to weight concentrations by the equation [µg eq/g = nmol eq/g × 422.52 / 1000].

[a)] Did not report due to concentration profile having less than 3 measurable concentrations.

2.6.5.6 薬物動態試験：蛋白結合

2.6.5.6A *In vitro* 血漿蛋白結合及び血液への分布（DMPK R1500362）

Study title:	*In vitro* blood distribution and plasma protein binding of [^{14}C]LNP023 including stability in blood and plasma of rat, dog, and human, and binding to isolated human plasma proteins	Report no. DMPK R1500362
Location in CTD:	4.2.2.3-2	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl, LNP023·HCl	
Samples, Species (Strain), Sex:	Blood and plasma from male rat (Wistar Hannover), dog (Beagle), and human	
Study system:	Pools of fresh blood and defrosted plasma ($n \geq 3$ for all species). Anticoagulant was K_3-EDTA for all experiments. Isolated plasma proteins: human serum albumin (HSA, 40 g/L), α1-acid glycoprotein (AAG, 1 g/L), lipoproteins (HDL, LDL, and VLDL, 3.9 mg/mL, 3.6 mg/mL, and 1.3 mg/mL, respectively)	
Radionuclide:	^{14}C	
Specific activity:	5.015 MBq/mg (free base)	
Method:	Stability in plasma and blood: Incubation in plasma for 0, 0.5, 1, 3, 7, and 24 h, and in blood for 0, 0.5, 1, 3, and 7 h at 37°C *In vitro* blood distribution: Incubation in blood at 37°C for 0.5 h, and cell separation by centrifugation *In vitro* plasma protein binding: Fraction unbound (fu) investigated by equilibrium dialysis	
Analyte(s) / Assay:	^{14}C: LSC, LNP023 parent for stability: LC-MS/MS	

Stability in plasma (% of LNP023 relative to the concentration at 0 h)

Species Incubation time (h)	Rat (%)	Dog (%)	Human (%)
0	100 ± 4.60	100 ± 2.70	100 ± 0.72
0.5	103	102	96.0
1	104	99.4	101
3	96.1	102	111
7	97.8	102	100
24	95.6	105	97.1

Data are mean ± SD of 3 determinations at 0 h and single determination at all other time points.

Stability in blood (% of LNP023 relative to the concentration at 0 h)			
Species	Rat (%)	Dog (%)	Human (%)
Incubation time (h)			
0	100	100	100
0.5	87.0	102	105
1	83.6	100	79.9
3	89.3	90.0	103
7	96.0	84.7	89.4

Data are mean of 3 determinations at 0 h and single determination at all other time points.

***In vitro* blood distribution of ^{14}C-LNP23 in rat**										
Nominal concentration (ng/mL)	Blood concentration (ng eq/mL)		Plasma concentration (ng eq/mL)		fp (%)		Cb / Cp		Cbc / Cp	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	9.88	0.219	9.94	0.216	54.3	1.69	0.994	0.0309	0.986	0.0672
100	111	1.18	92.0	0.377	49.0	0.558	1.20	0.0137	1.50	0.0334
1000	933	5.97	603	5.44	38.2	0.423	1.55	0.0171	2.33	0.0417
10000	8440	106	2350	22.9	16.4	0.261	3.59	0.0571	7.31	0.139

Cb: concentration in blood, Cbc: concentration in blood cells, Cp: concentration in plasma, fp: fraction in plasma
Data are mean and SD of 3 determinations.

***In vitro* blood distribution of ^{14}C-LNP23 in dog**										
Nominal concentration (ng/mL)	Blood concentration (ng eq/mL)		Plasma concentration (ng eq/mL)		fp (%)		Cb / Cp		Cbc / Cp	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	9.41	0.244	13.1	0.0553	71.1	1.87	0.717	0.0188	0.422	0.0384
100	105	1.18	129	1.14	64.0	0.915	0.812	0.0116	0.609	0.0242
1000	898	4.41	994	12.6	57.5	0.782	0.904	0.0123	0.799	0.0256
10000	8810	157	6370	83.7	37.6	0.833	1.38	0.0306	1.80	0.0638

Cb: concentration in blood, Cbc: concentration in blood cells, Cp: concentration in plasma, fp: fraction in plasma
Data are mean and SD of 3 determinations.

***In vitro* blood distribution of ^{14}C-LNP23 in human**

Nominal concentration (ng/mL)	Blood concentration (ng eq/mL)		Plasma concentration (ng eq/mL)		fp (%)		Cb / Cp		Cbc / Cp	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	9.00	0.127	15.0	0.0469	93.3	1.35	0.600	0.00867	0.0913	0.0197
100	103	3.52	159	2.38	80.1	2.99	0.649	0.0242	0.269	0.0504
1000	895	6.51	897	17.0	52.1	1.06	0.998	0.0203	0.995	0.0423
10000	8200	8.45	3840	62.2	24.3	0.394	2.14	0.0347	3.37	0.0723

Cb: concentration in blood, Cbc: concentration in blood cells, Cp: concentration in plasma, fp: fraction in plasma
Data are mean and SD of 3 determinations.

***In vitro* plasma protein binding of ^{14}C-LNP023 in rat**

Nominal concentration (ng/mL)	Total concentration before dialysis (ng eq/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fu (%)		fb (%)
				Mean	SD	Mean
10	9.59	8.37	0.337	4.03 [a)]	0.171	96.0 [a)]
100	103	88.5	5.09	5.76	0.281	94.2
1000	993	769	104	13.5	1.28	86.5
10000	9460	5440	2030	37.3	2.45	62.7

fb: fraction bound in plasma, fu: fraction unbound in plasma
Data are mean and SD of 4 determinations.
[a)] data of 3 determinations

***In vitro* plasma protein binding of ^{14}C-LNP023 in dog**						
Nominal concentration (ng/mL)	Total concentration before dialysis (ng eq/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fu (%)		fb (%)
				Mean	SD	Mean
10	9.70	7.94	0.751	9.47	1.17	90.5
100	101	82.4	8.38	10.2	0.165	89.8
1000	1010	742	132	17.8	0.568	82.2
10000	8420	5060	1770	35.0	0.744	65.0

fb: fraction bound in plasma, fu: fraction unbound in plasma

Data are mean and SD of 4 determinations.

***In vitro* plasma protein binding of ^{14}C-LNP023 in human**						
Nominal concentration (ng/mL)	Total concentration before dialysis (ng eq/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fu (%)		fb (%)
				Mean	SD	Mean
10	10.1	9.24	0.403	4.35 [a)] [3.38, 5.33]	na	95.6 [a)] [96.6, 94.7]
100	97.2	90.2	1.11	1.23	0.158	98.8
1000	1010	847	59.3	7.00	0.280	93.0
10000	9400	5970	1520	25.4	1.37	74.6

fb: fraction bound in plasma, fu: fraction unbound in plasma, na: not applicable

Data are mean and SD of 4 determinations.

[a)] Data are mean of 2 determinations with [individual values] as two samples were below LOD (0.165 ng/mL).

***In vitro* binding of ^{14}C-LNP023 to HSA**						
Nominal concentration (ng/mL)	Total concentration before dialysis (ng eq/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fu (%)		fb (%)
				Mean	SD	Mean
100	102	47.9	32.7	68.2	2.03	31.8
1000	1020	469	337	71.9	3.30	28.1
10000	10300	5070	3790	74.8	2.21	25.2
			Mean ± SD [a)]	71.6	3.31	28.4

fb: fraction bound, fu: fraction unbound

Data are mean and SD of 4 determinations per each concentration.

[a)] Mean and SD of the fu or fb mean values at 3 concentrations.

***In vitro* binding of ^{14}C-LNP023 to AAG**						
Nominal concentration (ng/mL)	Total concentration before dialysis (ng eq/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fu (%)		fb (%)
				Mean	SD	Mean
100	103	41.4	37.7	91.0	2.59	8.95
1000	1040	403	364	90.3	1.60	9.69
10000	10700	4230	3850	90.9	1.10	9.09
			Mean ± SD [a)]	90.7	0.379	9.25

fb: fraction bound, fu: fraction unbound

Data are mean and SD of 4 determinations per each concentration.

[a)] Mean and SD of the fu or fb mean values at 3 concentrations.

***In vitro* binding of ^{14}C-LNP023 to lipoproteins (HDL, LDL, VLDL)**						
Nominal concentration (ng/mL)	Total concentration before dialysis (ng eq/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fu (%)		fb (%)
				Mean	SD	Mean
100	111	45.6	38.8	85.0	2.00	15.0
1000	1040	421	352	83.5	2.84	16.5
10000	10100	4100	3430	83.5	1.87	16.5
			Mean ± SD [a)]	84.0	0.866	16.0

fb: fraction bound, fu: fraction unbound

Data are mean and SD of 4 determinations per each concentration.

[a)] Mean and SD of the fu or fb mean values at 3 concentrations.

2.6.5.6B ヒト及びマウスにおける *in vitro* 血漿蛋白結合（DMPK R1600148）

Study title:	*In vitro* plasma protein binding of [^{14}C]LNP023 in plasma of human and mouse (wildtype and factor B knock-out type)	Report no. DMPK R1600148
Location in CTD:	4.2.2.3-3	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Samples, Species (Strain), Sex:	Plasma from female wildtype mouse (C57Bl/6JBom) and knockout mouse (C57BL/6JBom not expressing factor B), plasma from male human	
Study system:	Pools of defrosted plasma ($n \geq 3$ for all species). Anticoagulant was K_3-EDTA for all experiments.	
Radionuclide:	^{14}C	
Specific activity:	5.015 MBq/mg (free base)	
Method:	*In vitro* plasma protein binding: fu investigated by equilibrium dialysis	
Analyte(s) / Assay:	^{14}C: LSC	

***In vitro* binding of ^{14}C-LNP023 to wildtype mouse plasma**

Nominal concentration (ng/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fb (%) Mean	SD	fu (%) Mean
10	8.49	0.535	93.7	0.169	6.31
100	87.2	6.44	92.6	0.449	7.38
1000	640	137	78.5 [a)]	0.188	21.5 [a)]
10000	6360	2380	62.4 [a)]	3.05	37.6 [a)]

fb: fraction bound in plasma, fu: fraction unbound in plasma

Data are mean and SD of 4 determinations.

[a)] data of 3 determinations

***In vitro* binding of ^{14}C-LNP023 to factor B knock-out mouse plasma**					
Nominal concentration (ng/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fb (%)		fu (%)
			Mean	SD	Mean
10	5.95	2.35	60.6	0.643	39.4
100	64.2	22.9	64.2	4.77	35.8
1000	565	209	63.0	1.94	37.0
10000	5750	2340	59.2	1.30	40.8

fb: fraction bound in plasma, fu: fraction unbound in plasma
Data are mean and SD of 3 determinations.

***In vitro* binding of ^{14}C-LNP023 to human plasma**					
Nominal concentration (ng/mL)	Actual concentration in plasma after dialysis (ng eq/mL)	Actual concentration in buffer after dialysis (ng eq/mL)	fb (%)		fu (%)
			Mean	SD	Mean
10	8.60	BLOD	nc	na	nc
100	93.5	1.04	98.9	0.139	1.11
1000	880	41.3	95.3	0.603	4.70
10000	7060	1650	76.6	1.66	23.4

BLOD: below limit of detection, fb: fraction bound in plasma, fu: fraction unbound in plasma, nc: not calculated, na: not applicable
Data are mean and SD of 4 determinations.

2.6.5.6C ラット，ウサギ，及びイヌにおける *ex vivo* 血漿蛋白結合（DMPK R1701166）

Study title:	Determination of *Ex-vivo* Plasma Protein Binding of LNP023 in Rats. Rabbits, and Dogs	Report no. DMPK R1701166
Location in CTD:	4.2.2.3-5	
GLP compliance:	Not required	
Test article(s):	LNP023·HCL	
Samples, Species (Strain), Sex:	Control and post-dose plasma from male and female (*ex vivo* only) rats (Wistar Hannover), female rabbits (New Zealand White), male and female (*ex vivo* only) dogs (Beagle)	
Study system:	*In vitro* study: pools of control plasma (n ≥ 3 for all species) spiked with LNP023 with or without treatment of 2% lactic acid. Anticoagulant was K_2-EDTA.	
	Ex vivo study: pools of plasma from safety studies (pooled per time point within the same dose level for all species). Plasma samples were treated with 2% lactic acid. Anticoagulant was K_2-EDTA for all experiments.	
Method:	*In vitro* and *ex vivo* plasma protein binding: fu investigated by equilibrium dialysis	
Analyte(s) / Assay:	LNP023: LC-MS/MS	

***In vitro* protein binding of LNP023 with control rat, rabbit, and dog plasma (without treatment of lactic acid)**

Species	Nominal concentration (ng/mL)	Initial concentration (ng/mL)	fb (%)		fu (%)	Recovery (%)	
			Mean	SD [a)]	Mean	Mean	SD
Rat	1000	1020	72.4	8.92	27.6	72.0	11.4
	10000	9840	36.1	6.26	63.9	81.2	5.68
Rabbit	1000	811	11.1	11.1	88.9	78.5	7.25
	10000	9800	28.2	9.29	71.8	71.3	4.07
Dog	1000	1010	73.7	2.01	26.3	80.0	3.78
	10000	9540	41.3	8.82	58.7	80.3	7.51

fb: fraction bound in plasma, fu: fraction unbound in plasma

Data are mean and SD of 3 determinations.

[a)] Standard deviation applies to both fb (%) and fu (%).

***Ex vivo* protein binding of LNP023 in study samples (with treatment of lactic acid)**					
Species (Dose level)	Time point (h)	Initial concentration (ng/mL)	fb (%) Mean	fu (%) Mean	Recovery (%) Mean
Rat (low)	0.5	4180	50.3	49.7	96.3
	1	3490	46.2	53.8	103
	3	1890	74.2	25.8	93.1
	7	863	54.7	45.3	94.4
	24	227	58.4	41.6	86.9
Rat (intermediate)	0.5	11400	41.5	58.5	92.8
	1	9510	52.9	47.1	100
	3	4830	39.2	60.8	101
	7	1550	50.5	49.5	96.1
	24	601	56.7	43.3	97.0
Rat (high)	0.5	21600	33.6	66.4	96.5
	1	25200	52.9	47.1	90.2
	3	22600	31.3	68.7	106
	7	7530	45.2	54.8	98.8
	24	1040	44.9	55.1	100
Rabbit (low)	0.5	5410	67.8	32.2	94.4
	1	4070	28.3	71.7	103
	3	2430	25.2	74.8	114
	7	878	44.9	55.1	97.6
	24	478	38.4	61.6	97.4
Rabbit (intermediate)	0.5	7350	23.3	76.7	105
	1	10200	15.1	84.9	102
	3	13200	10.1	89.9	103
	7	8090	20.4	79.6	100
	24	873	35.4	64.6	98.6

***Ex vivo* protein binding of LNP023 in study samples (with treatment of lactic acid)**					
Species (Dose level)	Time point (h)	Initial concentration (ng/mL)	fb (%) Mean	fu (%) Mean	Recovery (%) Mean
Rabbit (high)	0.5	18200	1.28	98.7	111
	1	25400	35.4	64.6	122
	3	40600	15.6	84.4	95.3
	7	29900	35.4	64.6	131
	24	5830	21.7	78.3	105
Dog (low)	0.5	2360	38.2	61.8	109
	1	2370	34.8	65.2	106
	3	1720	42.8	57.2	102
	7	1030	49.2	50.8	95.9
	24	381	51.1	48.9	97.2
Dog (intermediate)	0.5	12300	41.8	58.2	111
	1	11800	26.5	73.5	119
	3	8660	39.2	60.8	111
	7	3870	45.0	55.0	102
	24	1250	37.9	62.1	102
Dog (high)	0.5	37800	41.5	58.5	128
	1	45500	46.2	53.8	124
	3	41400	37.1	62.9	113
	7	20200	33.3	66.7	119
	24	5680	27.5	72.5	103

fb: fraction bound in plasma, fu: fraction unbound in plasma

Data are mean of 2 determinations.

Effect of 5% CO_2 on *in vitro* plasma protein binding of LNP023 with control rat plasma

CO_2	Nominal concentration (ng/mL)	Initial concentration (ng/mL)	fb(%) Mean	fb(%) SD [a)]	fu (%) Mean	Recovery (%) Mean	Recovery (%) SD
without	100	109	91.5	3.00	8.48	82.2	5.50
	10000	7930	68.3	3.32	31.7	73.2	6.45
5%	100	109	87.3	8.05	12.7	71.8	30.8
	10000	7930	59.3	7.35	40.7	80.7	3.90

fb: fraction bound in plasma, fu: fraction unbound in plasma

Data are mean and SD of 3 determinations.

[a)] Standard deviation applies to both fb (%) and fu (%).

Effect of pH on plasma protein binding of LNP023 with control rat plasma (with or without pH adjustment after treatment with lactic acid)

pH Adjusted	Nominal concentration (ng/mL)	Initial concentration (ng/mL)	fb (%) Mean	fb (%) SD [a)]	fu (%) Mean	Recovery (%) Mean	Recovery (%) SD
Yes	100	136	84.0	1.40	16.0	67.1	9.83
	10000	8260	38.4	17.4	61.6	84.4	8.39
No	100	109	26.8	15.2	73.2	76.7	6.70
	10000	7850	56.3	15.2	43.7	77.0	7.78

fb: fraction bound in plasma, fu: fraction unbound in plasma

Data are mean and SD of 3 determinations.

[a)] Standard deviation applies to both fb (%) and fu (%).

Effect of acidification on *in vitro* plasma protein binding of LNP023 with control rat and dog plasma (without treatment of lactic acid)

Species	Nominal concentration (ng/mL)	Initial concentration (ng/mL)	fb (%) Mean	fu (%) Mean	Recovery (%) Mean
Rat	100	55.4	87.8	12.2	94.1
	1000	892	85.3	14.7	100
	4000	3460	70.8	29.2	119
	10000	9660	55.6	44.4	96.6

Effect of acidification on *in vitro* plasma protein binding of LNP023 with control rat and dog plasma (without treatment of lactic acid)

Species	Nominal concentration (ng/mL)	Initial concentration (ng/mL)	fb (%) Mean	fu (%) Mean	Recovery (%) Mean
Dog	100	97.6	89.5	10.5	97.5
	1000	991	77.6	22.4	86.2
	4000	3540	63.4	36.6	114
	10000	9000	49.8	50.2	101

fb: fraction bound in plasma, fu: fraction unbound in plasma

Data are mean of 2 determinations.

Effect of acidification on *in vitro* plasma protein binding of LNP023 with control rat and dog plasma (with treatment of lactic acid)

Species	Nominal concentration (ng/mL)	Initial concentration (ng/mL)	fb (%) Mean	fu (%) Mean	Recovery (%) Mean
Rat	100 [a)]	89.4	82.6	17.4	83.3
	100 [b)]	92.3	90.9	9.08	100
	1000 [b)]	762	54.3	45.7	124
	10000 [a)]	7990	38.5	61.5	94.7
Dog	100 [a)]	84.6	69.5	30.5	87.5
	100 [b)]	88.3	77.2	22.8	110
	1000 [b)]	885	54.7	45.3	92.2
	10000 [a)]	7710	27.6	72.4	94.4

fb: fraction bound in plasma, fu: fraction unbound in plasma

Data are mean of 2 determinations.

[a)] Data obtained in March 2018

[b)] Data obtained in February 2018

2.6.5.7 薬物動態試験：妊娠動物における試験

No studies conducted under this section.

2.6.5.8 薬物動態試験：その他の分布試験

No studies conducted under this section.

2.6.5.9 薬物動態試験：*in vivo* における代謝

2.6.5.9A ラットに単回静脈内及び経口投与後の *in vivo* 代謝（DMPK R1500361）

Study title:	Absorption, distribution, metabolism, and excretion of LNP023 in rats after oral (10 mg/kg) and intravenous (2 mg/kg) administration of [^{14}C]LNP023 hydrochloride	Report no. DMPK R1500361
Location in CTD:	4.2.2.2-3	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Species / Strain:	Rat / Wistar Hannover	
Gender (M / F) / Number of animals:	Male / 9	
Feeding condition:	Fed	
Vehicle / Formulation:	i.v.: solution in 5% ethanol:15% propylene glycol:80% aqueous NaCl solution (0.9% w/v) (w/w/w) p.o.: suspension in 0.5% aqueous methylcellulose solution containing 0.5% Tween 80 (w/w)	
Method of Administration:	i.v.: single dose bolus, p.o.: single dose oral gavage	
Dose (mg/kg):	i.v.: 2 mg/kg, p.o.: 10 mg/kg	
Sample matrix:	Plasma, urine, feces, bile	
Radionucleotide:	^{14}C	
Specific radioactivity:	1.50 and 3.00 MBq/mg (different batches)	
Sampling schedule:	Blood / plasma: serial collection at 0.25, 0.5, 1, 2, 8, 24, 48, 72 h post-dose, urine: 0-8, 8-24 h on first day then daily up to 96, 96-168 h post-dose, feces: daily up to 96, 96-168 h post-dose, bile: daily up to 48 h post-dose	
Analyte(s):	^{14}C, LNP023, metabolites	
Assay:	Total radioactivity measurements by LSC, metabolite profiling by UPLC with radiodetection. Metabolite characterization by LC-MS/MS	

LNP023 and metabolites in plasma after i.v. and p.o. dosing

Matrix	Plasma (pools of n = 3 per each time point)			
Administration	i.v.		p.o.	
Component	AUC0-8h (µmol eq·h/L)	AUC0-8h (%)	AUC0-24h (µmol eq·h/L)	AUC0-24h (%)
M1	0.143	2.36	0.452	1.63
M2	0.102	1.67	0.386	1.39
M4	0.0533	0.877	0.0935	0.338

LNP023 and metabolites in plasma after i.v. and p.o. dosing				
Matrix	Plasma (pools of n = 3 per each time point)			
Administration	i.v.		p.o.	
Component	AUC0-8h (µmol eq·h/L)	AUC0-8h (%)	AUC0-24h (µmol eq·h/L)	AUC0-24h (%)
M8	0.0458	0.755	0.371	1.34
LNP023	5.33	87.7	25.2	91.0
Sum of additional components	0.105	1.73	0.0359	0.129
Total detected	5.78	95.1	26.6	95.9
Lost during sample processing	0.296	4.88	1.14	4.12
Total radiolabeled components in original sample	6.07	100	27.7	100

LNP023 and metabolites in excreta after i.v. and p.o. dosing						
Matrix	Excreta (pools of n = 3 per each time point)					
Administration	i.v. (% of dose)			p.o (% of dose)		
Components	Urine (0-72h)	Feces (0-72h)	Total (0-72h)	Urine (0-72h)	Feces (0-72h)	Total (0-72h)
M1	1.36	2.87	4.23	2.17	1.21	3.38
M2	0.0610	31.9	32.0	0.0418	29.3	29.3
P6.2	-	-	-	0.0527	n.d.	0.0527
M4	0.0781	5.99	6.07	0.0780	6.59	6.67
M6	0.105	9.19	9.29	0.0790	6.67	6.75
M7	0.00990	nd	0.00990	nd	0.526	0.526
M8	0.0522	nd	0.0522	0.137	nd	0.137
LNP023	3.24	19.0	22.2	2.07	36.1	38.1
P14.0-17.0	0.151	5.31	5.47	0.0442	3.90	3.95
Sum of additional components	0.432	4.25	4.68	0.306	3.54	3.84
Total detected	5.50	78.5	84.0	4.97	87.7	92.7
Lost during sample processing	0.0951	5.82	5.91	0.0502	6.20	6.25

LNP023 and metabolites in excreta after i.v. and p.o. dosing						
Matrix	Excreta (pools of n = 3 per each time point)					
Administration	i.v. (% of dose)			p.o (% of dose)		
Components	Urine (0-72h)	Feces (0-72h)	Total (0-72h)	Urine (0-72h)	Feces (0-72h)	Total (0-72h)
Total radiolabeled components in original sample	5.59	84.3	89.9	5.02	93.9	99.0

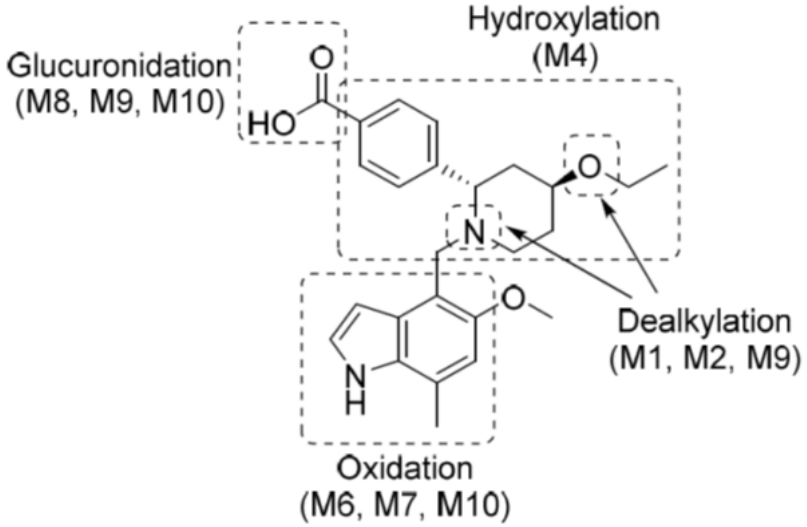
-: not available, nd: not detected

LNP023 and metabolites in excreta after i.v. dosing (bile duct-cannulated rats)				
Matrix	Excreta (pools of n = 3 per each time point)			
Administration	i.v. (% of dose)			
Components	Urine (0-48h)	Feces (0-48h)	Bile (0-48h)	Total (0-48h)
M1	1.28	nd	2.63	3.90
M9	nd	nd	2.98	2.98
M2	0.221	0.0876	20.2	20.5
M4	0.333	nd	5.42	5.75
M10	nd	nd	3.12	3.12
M6	0.234	0.0763	5.85	6.16
M7	0.107	nd	nd	0.107
M8 and isomers [a)]	0.268	nd	20.4	20.7
LNP023	8.04	3.51	11.7	23.3
P14.0-17.0	0.729	0.151	3.55	4.43
Sum of additional components	0.683	0.0623	3.00	3.74
Total detected	11.9	3.89	78.9	94.7
Lost during sample processing	1.13	0.316	0.556	2.00
Total radiolabeled components in original sample	13.0	4.21	79.4	96.7

nd: not detected

[a)] Several isomers of the acyl glucuronide M8 were detected in bile samples. As these isomers are expected to be formed by chemical decomposition of a primary acyl glucuronide metabolite (M8), possibly during/after sample collection, the quantities of all isomers of M8 were summed.

Proposed main metabolic pathway of LNP023 in rats



2.6.5.9B ラットに反復経口投与後の *in vivo* 代謝（DMPK R1570193-metid）

Study title:	LNP023: 26 Week Oral (Gavage) Administration Toxicity Study in the Rat followed by a 27 Week Recovery Period	Report no. DMPK R1570193-metid
Location in CTD:	4.2.2.4-1	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Species / Strain:	Rat, Wistar Hannover	
Gender (M / F) / Number of animals:	Male / 9, Female / 10	
Feeding condition:	Fed	
Vehicle / Formulation:	p.o.: suspension in 0.5% aqueous methylcellulose solution containing 0.5% Tween 80 (w/w)	
Method of Administration:	p.o.: multiple dose oral gavage, once daily for at least 26 weeks	
Dose (mg/kg):	750 mg/kg/day	
Sample matrix:	Plasma	
Sampling schedule:	Blood / plasma: serial collection at 0.5, 1, 3, 7, 24 h post-dose on day 154	
Analyte(s):	LNP023, metabolites	
Assay:	Metabolite identification by LC-MS	

Component in plasma AUC0-24h pool [a)]	Male rat plasma	Female rat plasma
M1	+	+
M2 ([redacted])	+	+
M6 ([redacted])	+	+
M7	+	+
M8 ([redacted])	+	+
M8d	+	+
M9 ([redacted])	+	+
M10 ([redacted])	+	+
LNP023	+	+

+: detected

[a)] Plasma AUC0-24h pool: plasma from each time point pools were pooled in a time-dependent manner to generate single samples for male and female plasma, containing LNP023 and its metabolites at the same ratios as in the AUC0-24h.

2.6.5.9C　イヌに反復経口投与後の *in vivo* 代謝（DMPK R1570192-metid）

Study title:	LNP023: 39 Week Oral (Gavage) Administration Toxicity Study in the Dog followed by a 27 Week Recovery Period	Report no. DMPK R1570192-metid
Location in CTD:	4.2.2.4-2	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Species / Strain:	Dog / Beagle	
Gender (M / F) / Number of animals:	Male / 5, Female / 6	
Feeding condition:	Fasted	
Vehicle / Formulation:	p.o.: suspension in 0.5% aqueous methylcellulose solution containing 0.5% Tween 80 (w/w)	
Method of Administration:	p.o.: multiple dose oral gavage, once daily for 273 days	
Dose (mg/kg):	150 mg/kg/day	
Sample matrix:	Plasma	
Sampling schedule:	Blood / plasma: serial collection at 0, 0.5, 1, 3, 7, 24 h post-dose on day 273	
Analyte(s):	LNP023, metabolites	
Assay:	Metabolite identification by LC-MS	

Component in plasma AUC0-24h pool [a)]	Male dog plasma	Female dog plasma
M1	+	+
M2 ([redacted])	+	+
M6 ([redacted])	+	+
M7	+	+
M8a	+	+
M8 ([redacted])	+	+
M8c	+	+
M8d	+	+
M9 ([redacted])	+	+
M10 ([redacted])	+	+
LNP023	+	+

+: detected

[a)] Plasma AUC0-24h pool: plasma from each time point pools were pooled in a time-dependent manner to generate single samples, for male and female plasma, containing LNP023 and its metabolites at the same ratios as in the AUC0-24h.

2.6.5.9D ヒト循環血漿中代謝物のラット，イヌ，及びヒト間における曝露量の比較（DMPK R1809129-metid）

Study title:	Comparison of exposure ratios, between rat, dog and human, of metabolites identified in human plasma from clinical study CLNP023X2101	Report no. DMPK R1809129-metid
Location in CTD:	4.2.2.4-3	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Species / Strain:	Rat / Wistar Hannover, Dog / Beagle, Human	
Gender (M / F) / Number of animals:	Refer to 2.6.5.9B for rat, 2.6.5.9C for dog. Male / 5, Female / 1 (CLNP023X2101) for human	
Feeding condition:	Refer to 2.6.5.9B for rat, 2.6.5.9C for dog. Under fed condition (CLNP023X2101) for human	
Vehicle / Formulation:	Refer to 2.6.5.9B for rat, 2.6.5.9C for dog. Capsule (CLNP023X2101) for human	
Method of Administration:	Oral	
Dose (mg/kg):	Rat: 750 mg/kg/day, dog: 150 mg/kg/day, human: 200 mg bid	
Sample matrix:	Plasma	
Sampling schedule:	Plasma AUC0-24h pool for animals: plasma from each time point pools were pooled in a time-dependent manner to generate single samples, for male and female plasma, containing LNP023 and its metabolites at the same ratios as in the AUC0-24h. Plasma AUC0-8h pool for human: plasma from each time point were pooled in a time-dependent manner to generate single samples, for subjects, containing LNP023 and its metabolites at the same ratios as in the AUC0-8h, and aliquats from each subject pool were combined.	
Analyte(s):	LNP023 and metabolites	
Assay:	Relative exposure measurements by UPLC-MS after matrix matching	

Rat: Ratio of LNP023 and metabolite exposures in male and female rat (Day 154, 750 mg/kg/day) versus human (Day 14, 200 mg bid)

Components	Ratio male rat / human	Ratio female rat / human
LNP023	3.71	5.19
M1	3.57	2.02
M2	1.06	0.664
M6	2.68	2.34
M7	6.61	5.62
M8	0.688	1.41
M9	0.0223	0.0211

Dog: Ratio of LNP023 and metabolite exposures in male and female dog (Day 273, 150 mg/kg/day) versus human (Day 14, 200 mg bid)

Components	Ratio male dog / human	Ratio female dog / human
LNP023	12.0	12.9
M1	6.86	6.29
M2	0.907	0.735
M6	13.9	14.4
M7	10.9	6.22
M8	2.72	1.40
M9	0.0159	0.0101

2.6.5.10 薬物動態試験：*in vitro* における代謝

2.6.5.10A ラット，イヌ，及びヒト肝細胞における *in vitro* 代謝（DMPK R1500300）

Study title:	*In vitro* metabolism of [^{14}C]LNP023 in rat, dog and human hepatocytes	Report no. DMPK R1500300
Location in CTD:	4.2.2.4-4	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Study system, Species (Strain), Sex:	Hepatocytes from male rat (Sprague Dawley), male dog (Beagle), and mixed (male / female) human	
Analytical methods:	Analysis after incubation of ^{14}C-LNP023 with hepatocytes at 37°C: metabolite profiles by UPLC with off-line radiodetection, metabolite structural characterization by LC-MS and LC-MS/MS, and recoveries of radioactivity after sample preparation by LSC	
Initial concentration:	1 µM for clearance assessment, 10 µM for metabolic profiles (nominal)	
Radionucleotide:	^{14}C	
Specific activity:	5.02 MBq/mg	
Analyte(s):	^{14}C, LNP023, metabolites	

Summary of results:

Intrinsic clearances of 1 µM ^{14}C-LNP023 in hepatocytes and predicted hepatic metabolic blood clearances

Calculated parameters	Rat (male)	Dog (male)	Human (male/female)
T1/2 (h) [a)]	7.07	52.6	20.5
Time interval for T1/2 (h)	0 - 4	0.5 - 4	0 - 4
CLint (µL/min/10^6 cells)	1.58	0.220	0.564
CLh,b (mL/min/kg)	6.11	1.60	1.34
CLh,b (% hepatic blood flow [b)])	11.1	5.18	6.48

CLint: intrinsic clearance, CLh,b: hepatic blood clearance

[a)] Half-lives were evaluated by linear extrapolation on semi-logarithmic plot.

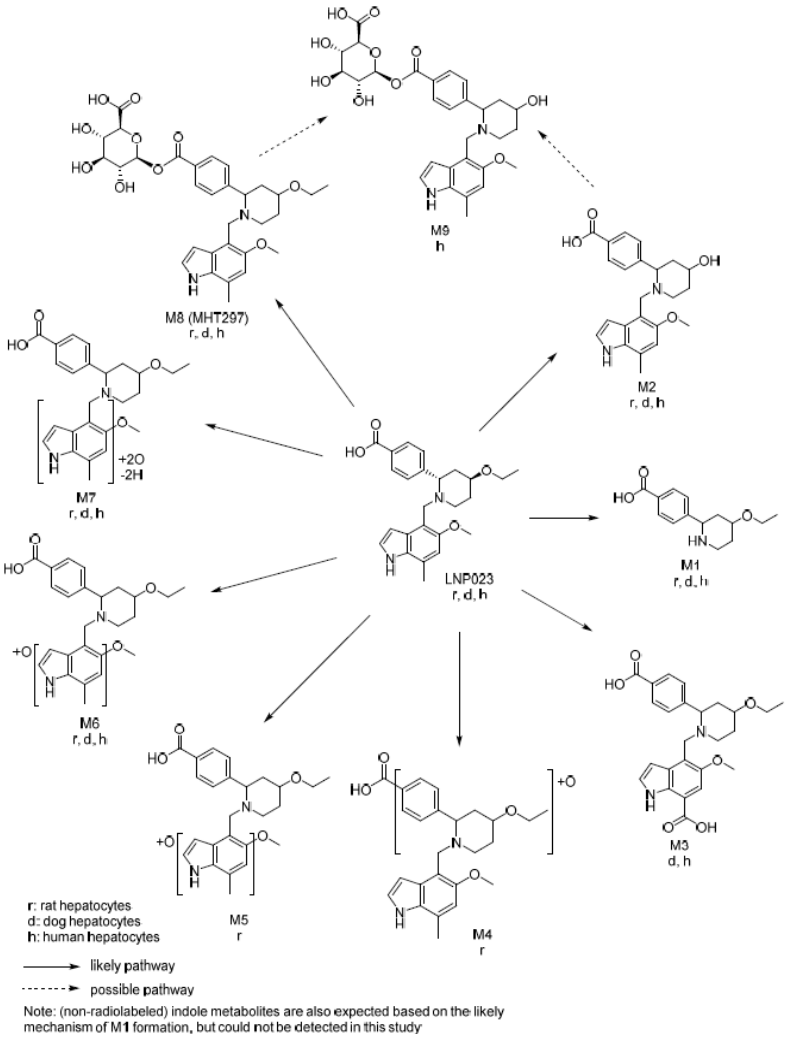
[b)] Hepatic blood flow (mL/min/kg): 55.0 (rat), 30.9 (dog), and 20.7 (human) ([Carlile DJ, Zomorodi K, Houston JB (1997)] Scaling factors to relate drug metabolic clearance in hepatic microsomes, isolated hepatocytes, and the intact liver: studies with induced livers involving diazepam. Drug Metab Dispos. 25(8):903-11, and [Davies B and Morris T (1993)] Physiological parameters in laboratory animals and humans. Pharma Res; 10(7):1093-5).

LNP023 and metabolites in incubates with hepatocytes of different species and in a control incubate

Species	Rat	Dog	Human	Control
Component	Percent of radioactivity in incubate			
Front peak	0.237	nd	0.0672	0.580
M1	1.80	0.712	0.720	0.500
M2	4.49	0.244	1.37	0.500
M3	nd	0.0488	0.365	nd
M4	1.69	nd	nd	nd
M5	0.207	nd	nd	nd
M6	1.42	1.52	0.854	nd
M7	0.454	0.185	0.154	nd
M8 and isomers	1.08	0.576	1.85	nd
M9	nd	nd	0.547	nd
P6.7	nd	nd	nd	0.960
P14.6	0.789	1.07	1.02	0.860
LNP023	86.4	93.1	88.7	95.6
Sum of additional components	-	0.0976	0.383	0.960
Total detected by HPLC	98.6	97.6	96.0	100
Pellet	1.40	2.40	4.00	0.00
Total in incubation	100	100	100	100

-: not available, nd: not detected

Proposed *in vitro* metabolic pathways of LNP023



2.6.5.10B [redacted]（M9）の *in vitro* における安定性（DMPK R1900261-metid）

Study title:	Acyl glucuronide metabolite ([redacted]) stability in phosphate buffer pH 7.4 at 37°C	Report no. DMPK R1900261-metid
Location in CTD:	4.2.2.4-5	
GLP compliance:	Not required	
Test article(s):	[redacted] (M9)	
Study system:	100 mM phosphate buffer pH 7.4 at 37°C	
Analytical methods:	Stability (depletion) of [redacted], formation of parent compound (M2) and potential acyl glucuronide isomers in phosphate buffer at 37°C after pre-incubation for 5 min were monitored by LC-MS	
Initial concentration:	10 µM for [redacted] stability (nominal)	
Analyte(s):	[redacted], potential products including parent compound (M2) and acyl glucuronide isomers	

Parameter	[redacted] depletion
T1/2 (h)	2.01
Time range (h)	0 - 4

Key results: [redacted] was depleted and demonstrated the formation of acyl glucuronide isomers, whereas no hydrolysis to parent compound was observed. T1/2 for [redacted] was calculated to be approximately 2.0 h over the time range 0-4 h.

2.6.5.10C　マウス，ラット，サル，及びヒト肝細胞における *in vitro* 代謝（DMPK R1900278-metid）

Study title:	*In vitro* metabolism of LNP023 in mouse, rat, monkey, and human hepatocytes	Report no. DMPK R1900278-metid
Location in CTD:	4.2.2.4-6	
GLP compliance:	Not required	
Test article(s):	LNP023	
Study system, Species (Strain), Sex:	Hepatocytes from male mouse (CD-1), male rat (Wistar Hannover), male monkey (cynomolgus), and mixed (male / female) human	
Analytical methods:	Analysis after incubation of LNP023 with hepatocytes at 37°C: metabolite profiling and structural characterization by LC-MS(n)	
Nominal concentration:	10 µM for metabolic profiles	
Analyte(s):	LNP023, metabolites	

Species	Mouse	Rat	Monkey	Human
Component	Relative abundances of LNP023 and metabolite s identified in mouse, rat, monkey, and human hepatocytes at 4 h			
M1	+	+	+	+
X1	+	+	+	+
M9	+	+	+	+
X2	nd	+	+	nd
X3	+	+	+	+
X4	+	+	+	+
X12	nd	+	nd	+
M2	++	++	++	++
M5	+	nd	+	+
M4	nd	++	nd	nd
M8	++	++	++	+
M6	++	++	++	+
M3	++	+	+	+
X14	++	nd	nd	nd
LNP023	+++	+++	+++	+++

+: component was < 1% of LNP023 MS peak area at 0 h, ++: component was between 1-10% of LNP023 MS peak area at 0 h, +++: component was > 10% of LNP023 MS peak area at 0 h.

nd: not detected

2.6.5.10D [redacted]（M8）の *in vitro* における安定性（DMPK R1500191）

Study title:	Stability of acyl glucuronide [redacted] in phosphate buffer, rat urine, and human blood, plasma and urine	Report no. DMPK R1500191
Location in CTD:	4.2.2.4-7	
GLP compliance:	Not required	
Test article(s):	[redacted] (M8)	
Study system:	100 mM phosphate buffer pH 7.4 and *in vivo* matrices (human blood, plasma, urine, and rat urine)	
Analytical methods:	Stability of [redacted] in phosphate buffer at 37°C after pre-incubation for 5 min and *in vivo* matrices at ambient temperature and 4°C were monitored by LC-MS	
Initial concentration:	1 and 10 µM for [redacted] stability (nominal)	
Analyte(s):	MHT297, potential products	

Stability of [redacted] (10 µM) in phosphate buffer

T1/2 (h)	1.57

Stability of [redacted] in rat and human *in vivo* matrices

Incubation medium	Temperature (ºC)	Substrate concentration (µM)	pH/acidification	Incubation time (h)	% substrate remaining after incubation
human blood	4	1	not acidified	4	59.7
human blood	4	10	not acidified	4	58.3
human blood	ambient	1	not acidified	4	38.3
human blood	ambient	10	not acidified	4	42.1
human plasma	4	1	not acidified	4	103
human plasma	4	10	not acidified	2	93.5
human plasma	ambient	1	not acidified	4	62.7
human plasma	ambient	10	not acidified	4	79.5
human plasma	4	1	0.7% lactic acid	4	99.0
human plasma	4	10	0.7% lactic acid	4	99.0
human plasma	ambient	1	0.7% lactic acid	4	105
human plasma	ambient	10	0.7% lactic acid	4	98.7

Stability of [redacted] in rat and human *in vivo* matrices					
Incubation medium	Temperature (ºC)	Substrate concentration (µM)	pH/acidification	Incubation time (h)	% substrate remaining after incubation
human urine	4	1	pH 5.9	24	97.9
human urine	4	1	pH 7.1	24	64.0
human urine	4	1	acidified pH 2.9	24	93.1
human urine	4	1	acidified pH 2.7	24	98.2
human urine	ambient	1	pH 5.9	24	85.8
human urine	ambient	1	pH 7.1	4	38.5
human urine	ambient	1	acidified pH 2.9	24	81.4
human urine	ambient	1	acidified pH 2.7	24	70.9
rat urine	4	1	pH 6.5	24	100
rat urine	4	1	pH 8.3	4	62.2
rat urine	4	1	acidified pH 3.9	24	92.8
rat urine	4	1	acidified pH 3.3	24	108
rat urine	ambient	1	pH 6.5	24	66.3
rat urine	ambient	1	pH 8.3	4	2.20
rat urine	ambient	1	acidified pH 3.9	24	34.3
rat urine	ambient	1	acidified pH 3.3	24	92.7
Key results: T1/2 for [redacted] in phosphate buffer was measured as approximately 1.6 h. [redacted] was found to be unstable in blood, plasma, and urine under some incubation conditions. Both hydrolysis and isomerization of the acyl glucuronide was observed.					

2.6.5.10E 酸化的代謝に関与する CYP 分子種同定試験（DMPK R1500516）

Study title:	Identification of human cytochrome P450 enzymes involved in the oxidative metabolism of LNP023 *in vitro*	Report no. DMPK R1500516
Location in CTD:	4.2.2.4-8	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Study system:	Pool of human liver microsomes (HLM) (from 150 individual donors), UltraPool human liver S9 (150 donors), UltraPool human liver cytosol (150 donors), human cryopreserved hepatocytes (mixed pool), supersomes of recombinant enzymes [cytochrome P450 (CYP) and flavin monooxygenase (FMO)3]	
Analytical methods:	Total amount of metabolites formed, identification of metabolites formed, and assessment of the extent of metabolic turnover of ^{14}C-LNP023 by HPLC combined with radioactivity detection	

The involvement of individual CYP and other metabolic enzymes in the *in vitro* metabolism of LNP023 has been investigated using pooled HLM, S9 fractions, liver cytosol and human hepatocytes.

Metabolic profiles: after incubation of ^{14}C-LNP023 (1, 5 or 10 µmol/L) at 37°C with HLM, S9, cytosol, recombinant enzymes for 1 h, or hepatocytes for up to 3 h, samples were subjected to protein precipitation and analyzed by LSC and HPLC combined with radio-detection.

Enzyme kinetics: parameters (Km,app, Vmax, and CLint,app) of ^{14}C-LNP023 oxidative metabolism in HLM were determined within a nominal concentration range of 1.00-300 µmol/L using SigmaPlot.

Identification of involved enzymes: inhibitory effects of selective enzyme inhibitors on metabolism rates of ^{14}C-LNP023 (20 µmol/L) in HLM were measured to assess contribution of individual enzymes, and metabolites formed after incubation with individual recombinant enzymes were analyzed by HPLC combined with radio-detection.

Metabolites of ^{14}C-LNP023 as detected in human hepatocytes and subcellular preparations

			Hepatocytes	Liver microsomes				Liver S9	Cytosol
Peak or Metabolite	Retention time (min)	[M-H]-		Without NADPH	NADPH	UDPGA	NADPH UDPGA	NADPH UDPGA PAPS	PAPS
M1	5.9	252.2	o	-	o	o	o	o	
M9	7.5	573.2	o				o	o	
M2	8.7	395.2	o	-	o		o	o	
M6	10.1	441.2	o	-	o		o	o	
M8	10.3	601.3	o			o	+	o	
M8c	10.7	601.3	o			o	o	+	
M8d	11.2	601.3	o			o	o	+	
LNP023	12.6	423.2	++	++	++	++	++	++	++

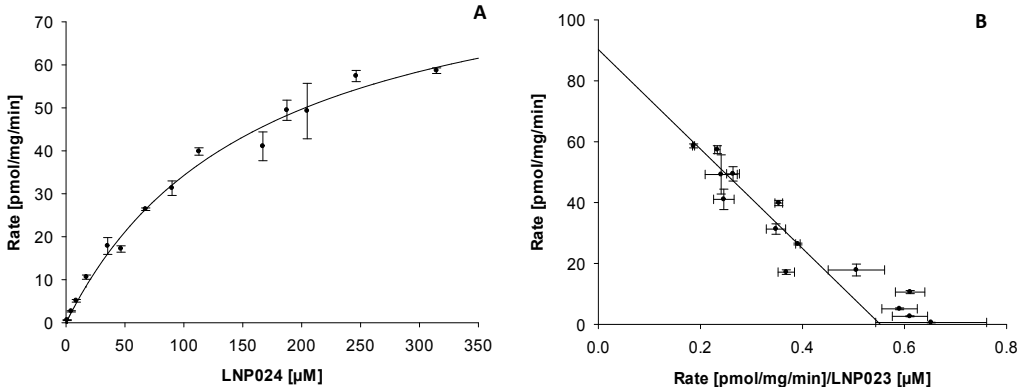
UDPGA: uridine 5'-diphospho-α-D-glucuronic acid, PAPS: adenosin 3'-phosphate 5'-phosphosulfate, -: below 0.5% of total, o: between 0.5%-3%, +: between 3%-10%, ++: above 10%, Incubation times for hepatocytes were 3 h, all others 1 h

Effect of CYP-selective inhibitors on total ^{14}C-LNP023 metabolism in HLM		
Enzyme	CYP3A4	
Inhibitor	Azamulin	
Concentration (µmol/L)	% activity	SD
0.04	104	11.4
0.08	94	6.7
0.16	111	6.0
0.31	102	15.2
0.63	103	8.5
1.25	103	6.3
2.50	98	1.5
5.00	95	6.6
Enzyme	CYP3A4	
Inhibitor	Ketoconazole	
Concentration (µmol/L)	% activity	SD
0.03	106	2.4
0.06	101	0.9
0.13	98	0.4
0.25	95	2.1
0.50	91	3.6
1.00	91	2.6
Enzyme	CYP2C8	
Inhibitor	Montelukast	
Concentration (µmol/L)	% activity	SD
0.06	92	6.0
0.13	95	5.2
0.25	89	1.6

Effect of CYP-selective inhibitors on total ^{14}C-LNP023 metabolism in HLM								
0.50			79			0.6		
1.00			71			2.6		
2.00			53			1.5		
Combinations of inhibitors								
Inhibitor mix			% activity			SD		
Montelukast (CYP2C8), Sulfaphenazole (CYP2C9), Ketoconazole (CYP3A4), Quinidine (CYP2D6)			20			3.4		
Sulfaphenazole (CYP2C9), Ketoconazole (CYP3A4), Quinidine (CYP2D6)			73			6.8		
Montelukast (CYP2C8), Sulfaphenazole (CYP2C9), Quinidine (CYP2D6)			35			7.5		
Montelukast (CYP2C8), Ketoconazole (CYP3A4)			31			1.4		
Enzyme	CYP2C9		CYP2D6		CYP1A2		CYP2B6 / 2C19	
Inhibitor	Sulfaphenazole		Quinidine		Furafylline		Ticlopidine	
	Concentration (µmol/L)	% activity	Concentration (µmol/L)	% activity	Concentration (µmol/L)	% activity	Concentration (µmol/L)	% activity
	0.5	94	0.2	115	1	101	1	101
	5.0	104	2.0	100	10	101	10	98
Enzyme	CYP2E1		FMO3		CYP2C19			
Inhibitor	Sodium diethyldithiocarbamate		Methimazole		Loratadine			
	Concentration (µmol/L)	% activity	Concentration (µmol/L)	% activity	Concentration (µmol/L)	% activity		
	10	107	16	101	0.2	104		
	100	78	160	91	2.0	111		

Enzyme kinetics of ^{14}C-LNP023 metabolism in HLM

The kinetic parameters of the *in vitro* metabolism of ^{14}C-LNP023 in pooled HLM were determined from measured turnover rates (total metabolite formation) at different concentrations (1-350 µmol/L). The obtained curve was fitted according to the Michaelis-Menten equation. Panels (A) and (B) illustrate Michaelis-Menten and Eadie-Hofstee plots (V vs. V/S), respectively.



Vmax (pmol/min/mg)	Km,app (µmol/L)	CLint,app (Vmax/ Km,app) (µL/mg/min)
90.3 ± 6.1	163.3 ± 22.6	0.6 ± 0.1

Key results: Incubations with LNP023 in HLM along with selective enzyme inhibitors incubations demonstrated that CYP2C8 plays a key role in oxidative metabolism of LNP023. A much lower contribution of CYP3A4, CYP2C9, and CYP2D6 to the metabolism of LNP023 was confirmed by using respective selective inhibitors as well as recombinant enzymes. The major oxidative metabolites M1, M2, and M6 in HLM were also formed by recombinant CYP2C8, CYP2C9, CYP2D6, CYP3A4, and CYP1A1. Other recombinant CYPs did not metabolize LNP023.

2.6.5.10F 酸化的代謝に関与する CYP 分子種同定試験（DMPK R1600579）

Study title:	Identification of human CYP enzymes involved in the oxidative metabolism of LNP023	Report no. DMPK R1600579
Location in CTD:	4.2.2.4-9	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023	
Study system:	Pool of HLM (from 150 individual donors), microsomes prepared from baculovirus-infected insect cells (BTI-TN-5B1-4) expressing human CYP and FMO enzymes	
Analytical methods:	Radiometry by LSC, Formation of metabolites by HPLC combined with radio-detection	

The incubations were carried out in 0.1 mol/L phosphate buffer, pH 7.4 at 37°C. Incubations were prepared as follows: 100 mmol/L $MgCl_2$ (5 mmol/L final concentration), substrate and human liver microsomes or recombinant human CYP or FMO enzymes were added to an appropriate volume of the buffer and pre-incubated for 3 minutes at 37°C. The reaction was started by addition of a fresh 10 mmol/L solution of NADPH (1 mmol/L final concentration). The samples were incubated at 37°C in a ThermoMixer® C with agitation. The enzymatic reactions were stopped and the protein was precipitated by addition of an equal volume of acetonitrile. After at least 30 min at −80°C the samples were centrifuged. The supernatant was withdrawn. Radiometry of supernatant aliquots after mixing with liquid scintillation counting cocktail was performed on LSC. For samples of low substrate concentration, supernatants were evaporated then mixed with acetonitrile and water to achieve a final solution. Samples were analyzed by HPLC combined with radio-detection.

CYP isoenzyme contribution to total CYP-mediated oxidative metabolism in HLM

Enzyme	Km,u [a] (µmol/L)	Vmax (1/min) or (pmol/min/pmol CYP)	ISEF [b]	ISEF·Vmax/Km,u (µL/min/pmol)	Abundance [b] (pmol P450/mg)	Recombinant CLint,u (µL/min/mg)	HLM fm,CYP
rhCYP2C8	31	2.6	0.380	0.033	24	0.78	98% [c]
rhCYP2D6	na	na	0.299	0.002 [b]	8	0.02	2%
Total CYPs (rhCYP)	na	na	na	na	na	0.80	100% [c]
Total CYPs (HLM) [d]	163	90	na	na	459	0.57	na

na: not applicable, ISEF: inter-system extrapolation factors (Proctor et al 2004), fm,CYP: CYP isoenzyme contribution to total CYP-mediated metabolism in HLM

[a] Km,app corrected using unbound fraction in microsomal incubations fumic (0.97 for rhCYP2C8 (0.31 mg/mL) and 0.97 for HLM (1.0 mg/mL)) according to the equation fumic = 0.137 × mg protein/mL^2 − 0.1681 × mg protein/mL + 1.005 (DMPK R1500321)

[b] Rowland et al 2004 and Simcyp (Version 13.1)

[c] The calculated CLint,u values were extrapolated to the respective HLM CLint,u values with the help of the hepatic abundance information.

[d] Kinetic data taken from DMPK R1500516

Key results: CYP2C8 is the major enzyme contributing to oxidative metabolism (fm,CYP2C8, 98%) followed by minor involvement of CYP2D6 (2%).

2.6.5.10G　グルクロン酸抱合に関与する UGT 分子種同定試験（DMPK R1600698）

Study title:	Identification of human UGT-glucuronosyltransferase isoenzymes involved in the direct glucuronidation of LNP023	Report no. DMPK R1600698
Location in CTD:	4.2.2.4-10	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023	
Study system:	Pool of HLM (from 150 individual donors), microsomes single donor's livers (16 different individuals)	
Analytical methods:	The formation of [redacted] (M8) and M8c by HPLC combined with radio-detection	

In pooled HLM in the presence of UDPGA, LNP023 was metabolized to two direct glucuronides being [redacted] (M8, O-glucuronidation) and to a lesser extent M8c (isomerized M8, O-glucuronidation).

Formation of both glucuronides correlated with a uridine diphospho-glucuronosyltransferase (UGT) 1A1 marker activity (17-estradiol 3-glucuronidation, R = 0.86). Therefore, it is likely that UGT1A1 is a major UGT enzyme involved in hepatic glucuronidation.

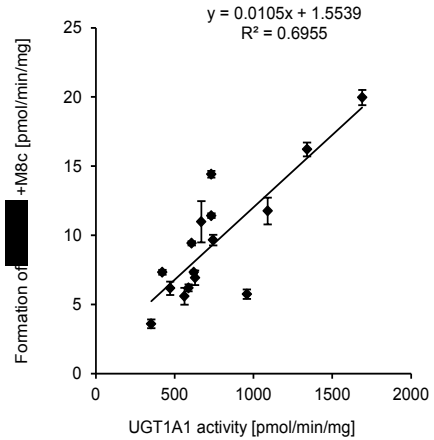
Correlation of [redacted] and M8c formation with the sample-to-sample variation in various marker enzyme activities in a bank of individual HLM

		Correlation coefficient (R) with the formation of		
Marker reaction	Enzyme	[redacted]	M8c	[redacted] + M8c
17-Estradiol 3-glucuronidation	UGT1A1	0.821	0.865	0.834
Trifluoperazine glucuronidation	UGT1A4	0.476	0.431	0.471
1-Naphthol glucuronidation	UGT1A6	0.088	0.030	0.078
Propofol glucuronidation	UGT1A9	−0.042	−0.172	−0.065
Morphine 3-glucuronidation	UGT2B7	0.120	−0.001	0.099

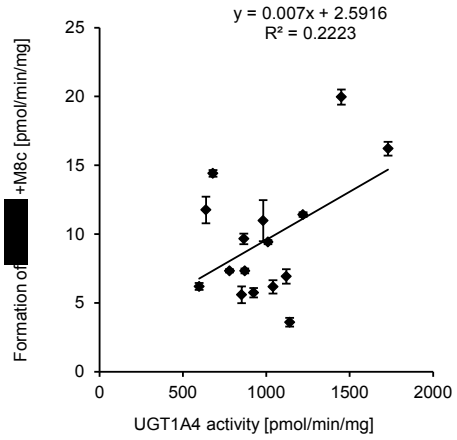
Individual human liver microsomes (n = 16) were incubated with 10 µmol/L ^{14}C-LNP023. The enzymatic rates of different metabolic pathways were correlated with known activity of enzymes in the same bank of HLM. Data shown are linear regression coefficient (R).

Correlation of [redacted] + M8c formation rate with UGT specific reactions

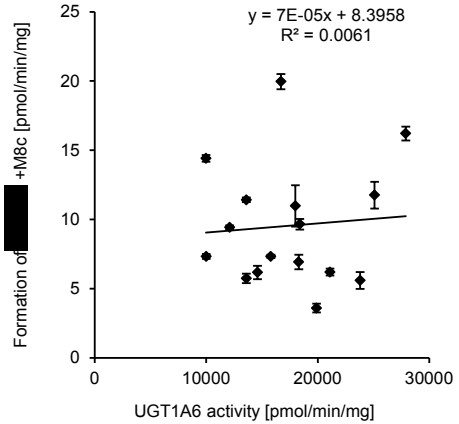
(A) UGT1A1



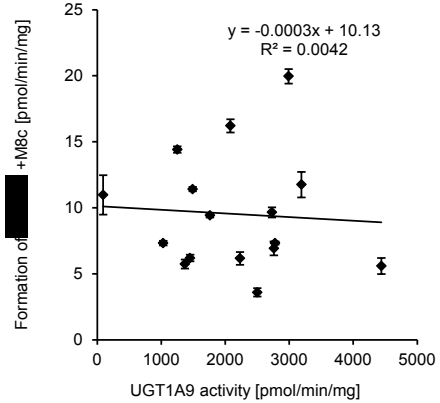
(B) UGT1A4



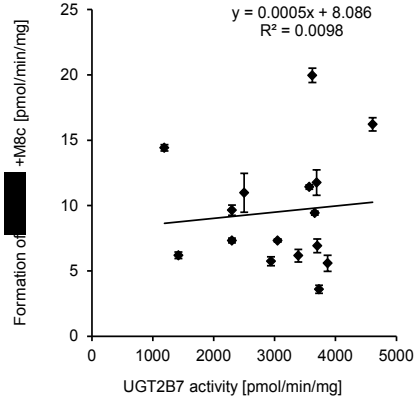
(C) UGT1A6



(D) UGT1A9



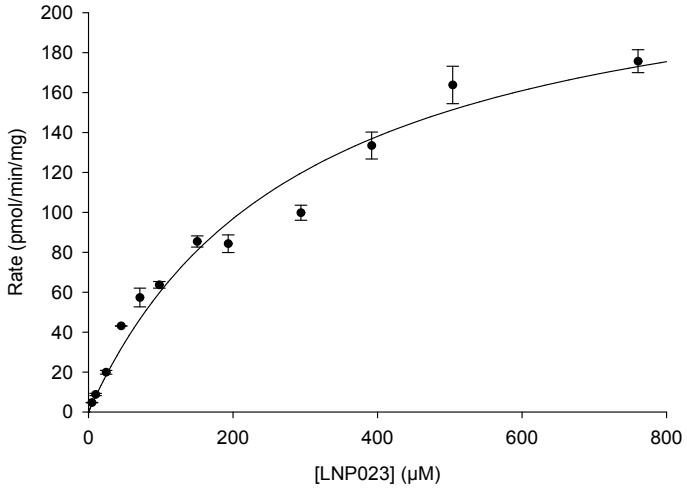
(E) UGT2B7



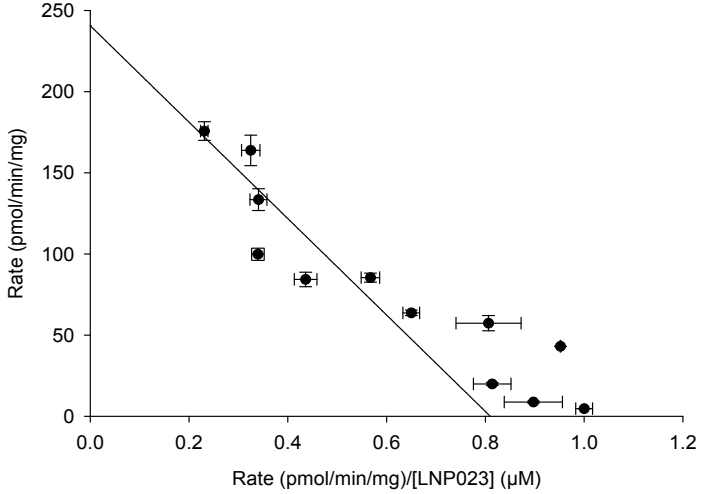
Correlation coefficient (R^2) of the metabolism rate of 10 µmol/L ^{14}C-LNP023 (means of duplicates after 45 min incubation with 1 mg/mL HLM) with marker activities from 5 UGT enzymes in a bank of 16 human liver microsomes from the Reaction Phenotyping Kit (Version 8, Cat No: H0500, XenoTech LLC, KS, USA). Sum of [redacted] and M8c formation was determined by HPLC analysis combined with radio-detection. Correlation was only observed with UGT1A1 specific reactions (17-Estradiol 3-glucuronidation) (A). Marker reactions for other enzymes (B-E): UGT1A4 (Trifluoperazine glucuronidation), UGT1A6 (1-Naphthol glucuronidation), UGT1A9 (Propofol glucuronidation), UGT2B7 (Morphine 3-glucuronidation)

Enzyme kinetics of LNP023 biotransformation in HLM

(A) Michaelis-Menten plot (Michaelis-Menten model)



(B) Eadie-Hofstee plot (Michaelis-Menten model)



Km (µmol/L)		Vmax	CLint (µL/min/mg)	
Total	Unbound [a)]	(pmol/min/mg)	Total	Unbound [a)]
240	235	296	1.23	1.26

[a)] Km,app corrected using unbound fraction in microsomal incubations fumic according to the equation;
fumic: $0.974 = 0.137 \times 1.0$ mg protein/mL$^2 - 0.1681 \times 1.0$ mg protein/mL + 1.005 (DMPK R1500321)

Key results: UGT1A1 is considered as the major enzyme involved in direct glucuronidation of LNP023 in the liver. The involvement of other UGTs in the liver or in extra-hepatic tissues cannot be excluded.

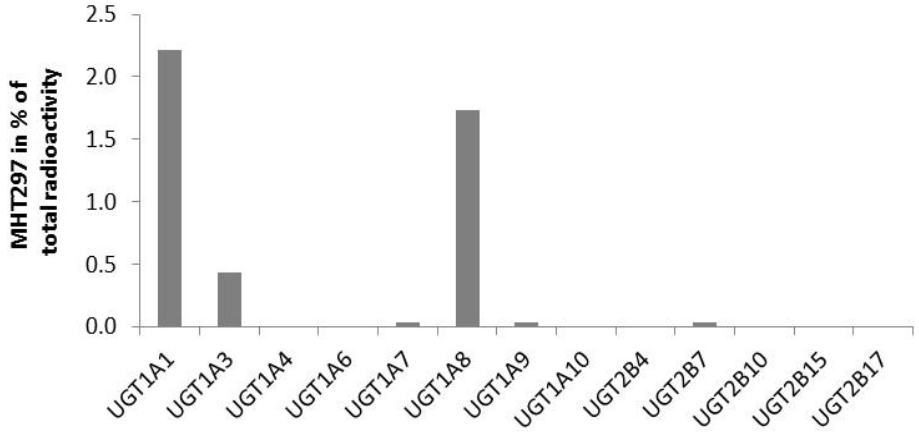
2.6.5.10H　グルクロン酸抱合に関与する UGT 分子種同定試験（DMPK R1600140）

Study title:	Identification of human uridine diphosphate glucuronosyltransferase isoenzymes involved in the direct glucuronidation of LNP023	Report no. DMPK R1600140
Location in CTD:	4.2.2.4-11	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Study system:	Microsomes prepared from baculovirus-infected insect cells (BTI-TN-5B1-4) expressing the human UGTs, insect cell membrane control preparation	
Analytical methods:	Metabolites formed from radiolabeled LNP023 by HLM or recombinant UGTs by HPLC with radio-detection, Metabolites structures by LC-MS	
Recombinant enzymes:	UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15, 2B17	

The identification of human UGTs involved in the formation of M8 was conducted by HPLC with radio-detection along with LC-MS analysis using human recombinant UGTs. ^{14}C-LNP023 was incubated at 5 µmol/L with individual recombinant human UGTs at 0.5 mg/mL total protein for one hour at 37°C. After incubation, samples were analyzed by HPLC with radio-detection for metabolite formation and by LC-MS for metabolite characterization.

HPLC with radio-detection of M8 in human recombinant UGT incubations with ^{14}C-LNP023

5 µmol/L ^{14}C-LNP023 was incubated with different UGT supersomes at 0.5 mg/mL total protein for one hour at 37°C. Proteins were removed by precipitation and samples were analyzed by HPLC with radio-detection. UGT control supersomes were used to determine the background signal, which was subtracted from detected radioactivity for M8 ([redacted]) for all UGT samples. Results are given in % of total radioactivity detected.



Key results: Formation of the acyl glucuronide M8 from LNP023 was found to be catalyzed by UGT1A1, 1A8 and to a lower extent by UGT1A3. However, the absolute contribution of the identified UGTs in human *in vivo* remains elusive due to their unknown expression levels in the liver and other organs.

2.6.5.10l 反応性代謝物の生成（DMPK R1500514）

Study title:	*In vitro* metabolism of LNP023: Assessment of reactive intermediate formation	Report no. DMPK R1500514
Location in CTD:	4.2.2.4-12	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Study system:	Pool of HLM (from 150 donors) and hepatocytes	
Analytical methods:	Covalent drug-protein adducts and total drug content present in the incubations by LSC	

The formation of reactive intermediates upon metabolic activation of ^{14}C-LNP023 was investigated using HLM, human hepatocytes as well as respective recombinant enzymes. Quantitative determination of drug-protein adduct formation was performed according to a validated method with minor modifications. The following 5-arm assay design was applied:

(i) liver microsomes – added cofactor: Glutathione, reduced form (GSH) (control)

(ii) liver microsomes – added cofactor: NADPH

(iii) liver microsomes – added cofactors: NADPH, UDPGA

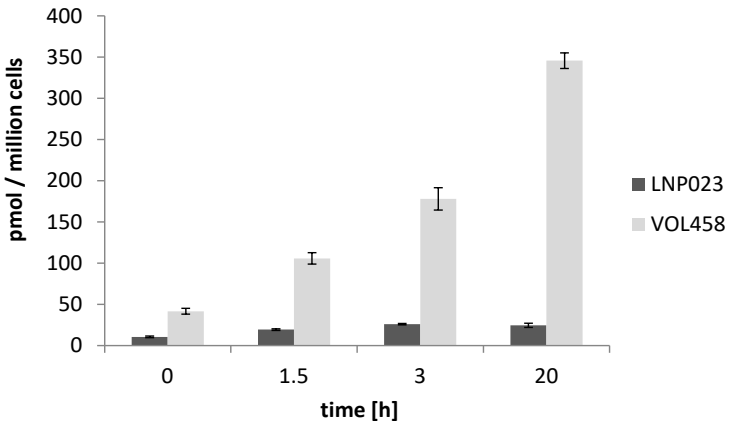
(iv) liver microsomes – added cofactors: NADPH, UDPGA, GSH

(v) hepatocytes – 0.5 × 10^6 cells per mL, kinetic experiment 0-3 h

The final concentrations of co-factors in the incubations were 1 mmol/L for NADPH, 5 mmol/L for UDPGA and 5 mmol/L for GSH.

Protein adduct formation of ^{14}C-LNP023		
(i) 1st arm:	6.2 ± 0.9 pmol/mg	HLM, GSH
(ii) 2nd arm:	17.9 ± 0.5 pmol/mg	HLM, NADPH
(iii) 3rd arm:	13.9 ± 0.3 pmol/mg	HLM, NADPH, UDPGA
(iv) 4th arm:	7.3 ± 0.5 pmol/mg	HLM, NADPH, UDPGA, GSH
(v) 5th arm:	24.4 ± 0.7 pmol/10^6 cells	Human hepatocytes

Adduct formation of LNP023 and VOL458 (Reference compound) in human hepatocytes over 20 hours



Covalent drug-protein adduct formation in human hepatocytes was measured at 0, 1, 1.5, 3, and 20 h at a substrate concentration of 10 µmol/L. While LNP023 did not form any additional adducts after 3 h, more than 300 pmol/mio cells was measured for VOL458 after 20 h.

Key results: Results indicated a low formation of reactive metabolites of LNP023 and consequently a minor formation of covalent drug-protein adducts. Glucuronidation did not have a major impact on adduct formation of LNP023 in microsomes.

2.6.5.11 薬物動態試験：推定代謝経路

Please see 2.6.5.9A and 2.6.5.10A for proposed metabolic pathways obtained from *in vivo* and *in vitro* metabolism studies, respectively.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導／阻害

2.6.5.12A ヒト肝ミクロソームにおける CYP 時間依存的阻害試験（DMPK R1300641）

Study title:	*In vitro* assessment of LNP023 as time-dependent inhibitor of major cytochrome P450 enzymes	Report no. DMPK R1300641
Location in CTD:	4.2.2.4-13	
GLP compliance:	Not required	
Test article(s):	LNP023	
Study system:	Pool of HLM (from 150 individual donors, equal gender mix)	
Probe substrate / Assay:	Phenacetin, Diclofenac, Bufuralol, Midazolam / LC-MS/MS analysis of specific metabolite formations from probe substrates by CYP	

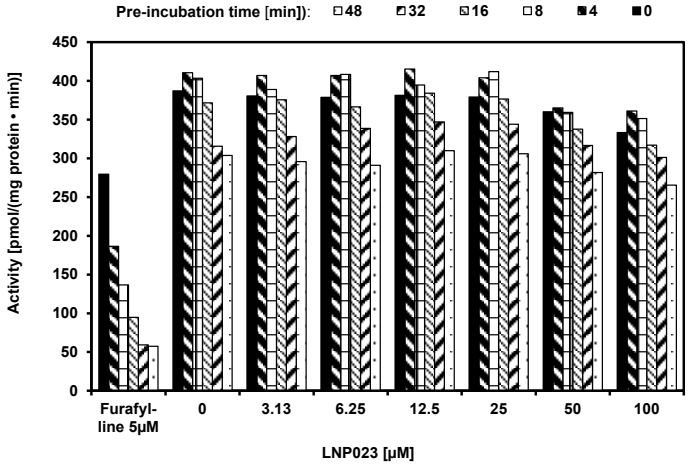
The potential of LNP023 for time-dependent (irreversible) inhibition of major human CYP enzymes was assessed in pooled human liver microsomes in the absence and presence of increasing amounts of LNP023 and with pre-incubation times up to 48 min. After the pre-incubation period, the incubations were diluted 20-fold to reduce competitive inhibition, and the remaining CYP enzymatic activities were measured using CYP-selective probe substrates. Probe substrate metabolism was assessed by LC-MS/MS analysis of specific metabolite formation.

Summary of results:

CYP enzyme	Probe reaction	Time-dependent inhibition
CYP1A2	phenacetin *O*-deethylation	not observed
CYP2C9	diclofenac 4′-hydroxylation	not observed
CYP2D6	bufuralol 1′-hydroxylation	not observed
CYP3A4/5	midazolam 1′-hydroxylation	not observed

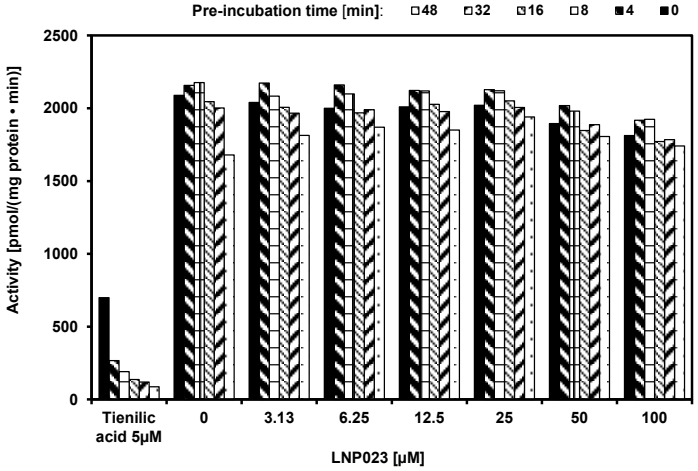
Quantitative assay for time-dependent inhibition of CYP1A2 by LNP023

Effect of pre-incubation time, LNP023 concentration and the positive control furafylline on the enzymatic activity of CYP1A2. 100 µmol/L phenacetin was used as a probe substrate for measurement of CYP1A2 activity. Values shown are the mean of duplicate determinations.



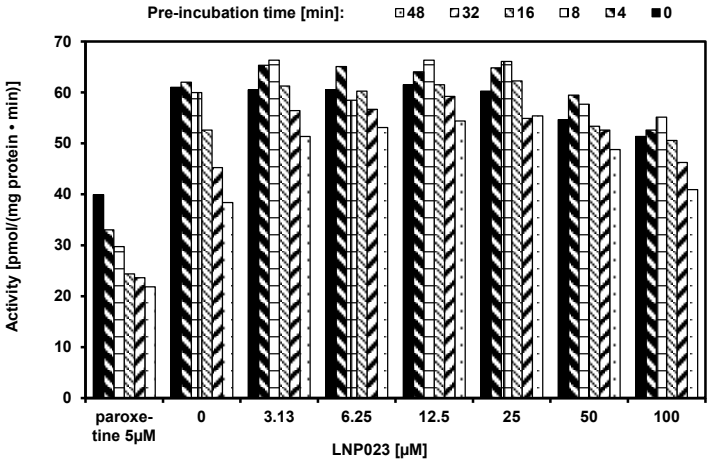
Quantitative assay for time-dependent inhibition of CYP2C9 by LNP023

Effect of pre-incubation time, LNP023 concentration and the positive control tienilic acid on the enzymatic activity of CYP2C9. 40 µmol/L diclofenac was used as a probe substrate for measurement of CYP2C9 activity. Values shown are the mean of duplicate determinations.



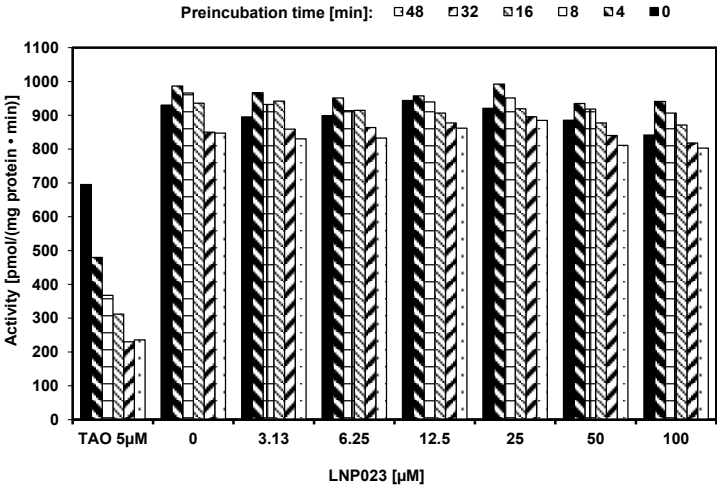
Quantitative assay for time-dependent inhibition of CYP2D6 by LNP023

Effect of pre-incubation time, LNP023 concentration and the positive control paroxetine on the enzymatic activity of CYP2D6. 50 µmol/L bufuralol was used as a probe substrate for measurement of CYP2D6 activity. Values shown are the mean of duplicate determinations.



Quantitative assay for time-dependent inhibition of CYP3A4/5 by LNP023

Effect of pre-incubation time, LNP023 concentration and the positive control troleandomycin (TAO) on the enzymatic activity of CYP3A4/5. 20 µmol/L midazolam was used as a probe substrate for measurement of CYP3A4/5 activity. Values shown are the mean of duplicate determinations.



Key results: Time-dependent inhibition by LNP023 was not observed for CYP1A2, CYP2C9, CYP2D6, and CYP3A4/5 up to a concentration of 100 µmol/L. LNP023 is not expected to act as a time-dependent inhibitor of human CYP1A2, CYP2C9, CYP2D6, or CYP3A4/5.

2.6.5.12B　ヒト肝ミクロソームにおける CYP 阻害試験（DMPK R1500321）

Study title:	*In vitro* assessment of cytochrome P450 enzyme inhibition by LNP023	Report no. DMPK R1500321
Location in CTD:	4.2.2.4-14	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl, LNP023·HCl	
Study system:	Pool of HLM (from 150 individual donors, equal gender mix)	
Probe substrate / Assay:	Phenacetin, Coumarin, Bupropion, Amodiaquine, Diclofenac, *S*-mephenytoin, Bufuralol, Chlorzoxazone, Midazolam, Testosterone / LC-MS/MS analysis of specific metabolite formations from probe substrates by CYP	

The potential of LNP023 to inhibit reversibly human CYP enzyme activity was assessed in pooled HLM by testing the effect of increasing concentrations of LNP023 (0-100 µmol/L) on the *in vitro* metabolism of several probe substrates whose metabolism is known to be selective for CYP isoenzymes. Probe substrate concentrations (1-30 µmol/L) used for these determinations were less than or equal to their reported Km values and probe substrate metabolism was assessed by LC-MS/MS analysis of specific metabolite formation.

Reversible inhibition of CYP enzymes by LNP023

CYP enzyme	Probe reaction	IC50 (µmol/L) [a]		
		Total	fumic [b]	Unbound
CYP1A2	phenacetin *O*-deethylation	> 100	0.977	> 98
CYP2A6	coumarin 7-hydroxylation	> 100	0.977	> 98
CYP2B6	bupropion hydroxylation	> 100	0.990	> 99
CYP2C8	amodiaquine *N*-deethylation	> 100	1.000	> 100
CYP2C9	diclofenac 4′-hydroxylation	> 100	0.990	> 99
CYP2C19	*S*-mephenytoin 4′-hydroxylation	> 100	0.955	> 96
CYP2D6	bufuralol 1′-hydroxylation	> 100	0.977	> 98
CYP2E1	chlorzoxazone 6-hydroxylation	> 100	0.955	> 96
CYP3A4/5	midazolam 1′-hydroxylation	> 100	0.990	> 99
CYP3A4/5	testosterone 6β-hydroxylation	> 100	0.977	> 98

[a] Concentration of inhibitor producing 50% inhibition of probe substrate metabolism

[b] Values calculated according to the equation [fumic = r,ac × F,ac／r,bc × F,bc]

r,ac: radioactivity of the LSC aliquot (supernatant) after centrifugation, r,bc: radioactivity of the LSC aliquot before centrifugation, F,ac: fraction of parent peak relative to total labeled components after centrifugation, F,bc: fraction of parent peak relative to total labeled components before centrifugation

Key results: LNP023 showed little or no reversible inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5 at LNP023 concentrations of up to 100 µmol/L.

2.6.5.12C　ヒト肝ミクロソームにおける UGT1A1 阻害試験（DMPK R1500936）

Study title:	*In vitro* assessment of UGT1A1 enzyme inhibition by LNP023	Report no. DMPK R1500936
Location in CTD:	4.2.2.4-15	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Study system:	Pool of HLM (from 150 individual donors, equal gender mix), insect cell membranes containing baculovirus-expressed recombinant human UGT	
Probe substrate / Assay:	β-estradiol / LC-MS/MS analysis of metabolite formations from probe substrates by UGT1A1	

The inhibition potential was determined by testing the effect of increasing concentrations of LNP023 (0-100 µmol/L) on the *in vitro* metabolism of the probe substrate estradiol whose 3-glucuronidation is known to be selective for UGT1A1. Probe substrate concentration (10 µmol/L) was less than or approximately equal to its reported Km value and probe substrate metabolism was assessed by LC-MS/MS analysis of metabolite formation.

Reversible inhibition of human UGT1A1

Matrix	Probe reaction	Test substance	IC50 (µmol/L) Total	fumic [a)]	Unbound
HLM	β-estradiol 3-glucuronidation	LNP023	> 100	0.977	> 98
		positive control genistein	1.3 ± 0.2	-	-
Recombinant human UGT1A1	β-estradiol 3-glucuronidation	LNP023	> 100	0.997	> 100
		positive control genistein	1.0 ± 0.0	-	-

-: not available

[a)] Calculated using the equation in DMPK R1500321

Key results: LNP023 did not show inhibitory potency for UGT1A1 in HLM or for recombinant human UGT1A1 up to a maximal concentration of 100 µmol/L. The positive control genistein inhibited UGT1A1 activity in microsomes and recombinant enzyme with IC50 values of 1.3 ± 0.2 µmol/L and 1.0 ± 0.0 µmol/L, respectively.

2.6.5.12D CYP 誘導試験（DMPK R1600704）

Study title:	Evaluation of LNP023 as inducer of drug metabolizing enzymes in human hepatocytes	Report no. DMPK R1600704
Location in CTD:	4.2.2.4-16	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Study system:	Primary human hepatocytes (three individual donors, Male / 1, Female / 2)	
Probe substrate / Assay:	Phenacetin, Bupropion, Diclofenac, Midazolam / Induction of mRNA, relative to the vehicle control by reverse transcription and real-time PCR, changes in CYP activities after the induction period by quantitative LC-MS/MS analysis of CYP-selective probe substrate metabolism	

LNP023 was examined for its potential to induce CYP enzyme CYP1A2, CYP2B6, CYP2C9 and CYP3A4 mRNA and activities in primary human hepatocytes of three individual donors after 72 h of treatment. Induction of mRNA, relative to the vehicle control, was determined by reverse transcription and real-time PCR and changes in CYP activities were assessed after the induction period by quantitative LC-MS/MS analysis of CYP-selective probe substrate metabolism. Human hepatocytes were treated with LNP023 at concentrations of up to 100 µmol/L.

CYP1A2 mRNA quantification by reverse transcription and real-time PCR, from treated and control cultured primary human hepatocytes

	Donor 1		Donor 2		Donor 3	
LNP023 (µmol/L)	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control
Control	1 ± 0.06	0 ± 0.11	1 ± 0.09	0 ± 0.32	1 ± 0.04	0 ± 0.06
0.1	0.96 ± 0.12	−0.08 ± 0.23	0.98 ± 0.31	−0.07 ± 1.12	0.78 ± 0.02	−0.34 ± 0.03
0.3	1.05 ± 0.14	0.09 ± 0.26	0.86 ± 0.11	−0.49 ± 0.38	0.87 ± 0.1	−0.2 ± 0.16
1	0.95 ± 0.03	−0.1 ± 0.06	0.81 ± 0.08	−0.68 ± 0.29	0.84 ± 0.1	−0.25 ± 0.16
3	1.1 ± 0.1	0.18 ± 0.19	0.79 ± 0.08	−0.76 ± 0.3	0.73 ± 0.23	−0.42 ± 0.36
10	1.11 ± 0.14	0.21 ± 0.27	0.98 ± 0.08	−0.07 ± 0.3	1.08 ± 0.08	0.12 ± 0.12
30	1.31 ± 0.09	0.57 ± 0.16	1.17 ± 0.07	0.6 ± 0.27	1.13 ± 0.05	0.2 ± 0.08
100	1.61 ± 0.19	1.14 ± 0.35	1.75 ± 0.27	2.72 ± 0.96	1.31 ± 0.09	0.48 ± 0.14

Data are mean ± SD

CYP1A2 activity (phenacetin O-deethylation) in cultured primary human hepatocytes after treatment for 72 h

	Donor 1		Donor 2		Donor 3	
LNP023 (µmol/L)	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control
Control	1 ± 1.	0 ± 13.7	1 ± 0.5	0 ± 14	1 ± 0.2	0 ± 1.5
0.1	0.5 ± 0.1	−6.52 ± 0.7	0.81 ± 0.3	−5.31 ± 8.5	1.18 ± 0.8	1.39 ± 6
0.3	1.07 ± 1	0.98 ± 13	1.4 ± 0.1	11.15 ± 3.4	2.22 ± 1.2	9.29 ± 8.9
1	0.98 ± 0.7	−0.32 ± 9.4	0.66 ± 0.1	−9.42 ± 2.3	0.96 ± 0.4	−0.72 ± 4
3	1.29 ± 0.5	3.79 ± 7	0.63 ± 0.1	−10.08 ± 2.5	1.99 ± 0.6	7.52 ± 4.4
10	1.73 ± 0.8	9.64 ± 10	0.55 ± 0.1	−12.25 ± 2.4	1.62 ± 0.2	4.75 ± 1.7
30	1.72 ± 0	9.41 ± 0	0.63 ± 0.1	−10.08 ± 2.5	1.66 ± 0.4	5.03 ± 2.8
100	0.53 ± 0.2	−6.18 ± 3.2	0.94 ± 0.1	−1.76 ± 2.8	2.4 ± 0.8	10.63 ± 6.3

Data are mean ± SD

CYP2B6 mRNA quantification by reverse transcription and real-time PCR, from treated and control cultured primary human hepatocytes

	Donor 1		Donor 2		Donor 3	
LNP023 (µmol/L)	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control
Control	1 ± 0.09	0 ± 1.67	1 ± 0.12	0 ± 1.75	1 ± 0.14	0 ± 1.43
0.1	0.98 ± 0.06	−0.38 ± 1.18	0.97 ± 0.09	−0.5 ± 1.36	1.11 ± 0.11	1.11 ± 1.08
0.3	1.1 ± 0.02	2.06 ± 0.4	1.05 ± 0.04	0.8 ± 0.58	1.33 ± 0.15	3.35 ± 1.49
1	1.04 ± 0.08	0.75 ± 1.62	1 ± 0.06	−0.07 ± 0.9	1.22 ± 0.07	2.24 ± 0.69
3	1.04 ± 0.06	0.81 ± 1.13	0.88 ± 0.06	−1.81 ± 0.84	1.3 ± 0.08	2.97 ± 0.79
10	1.02 ± 0.04	0.49 ± 0.75	0.84 ± 0.08	−2.37 ± 1.21	1.29 ± 0.15	2.86 ± 1.55
30	1.05 ± 0.17	0.92 ± 3.37	0.85 ± 0.16	−2.23 ± 2.4	1.34 ± 0.17	3.44 ± 1.73
100	1.12 ± 0.16	2.27 ± 3.13	1.24 ± 0.14	3.52 ± 2.1	1.34 ± 0.07	3.44 ± 0.71

Data are mean ± SD

CYP2B6 activity (OH-bupropion formation) in cultured primary human hepatocytes after treatment for 72 h

	Donor 1		Donor 2		Donor 3	
LNP023 (µmol/L)	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control
Control	1 ± 0.09	0 ± 3.3	1 ± 0.09	0 ± 4.6	1 ± 0.12	0 ± 1.6
0.1	0.95 ± 0.1	−1.75 ± 3.5	1.06 ± 0.05	3.34 ± 2.5	1.13 ± 0.04	1.73 ± 0.6
0.3	0.98 ± 0.09	−0.65 ± 3.4	0.95 ± 0.11	−2.58 ± 5.8	1.02 ± 0.1	0.3 ± 1.3
1	0.9 ± 0.02	−3.68 ± 0.6	2.02 ± 1.81	54.6 ± 96.8	1.06 ± 0.05	0.75 ± 0.7
3	1.07 ± 0.07	2.54 ± 2.6	0.87 ± 0.11	−6.93 ± 5.9	1.05 ± 0.05	0.73 ± 0.7
10	1.02 ± 0.1	0.83 ± 3.8	1 ± 0.11	0 ± 5.9	1.13 ± 0.03	1.79 ± 0.5
30	1 ± 0.12	−0.04 ± 4.3	0.88 ± 0.04	−6.66 ± 2.3	1.15 ± 0.05	2 ± 0.7
100	1.1 ± 0.05	3.77 ± 2	1.07 ± 0.01	3.49 ± 0.8	1.01 ± 0.11	0.19 ± 1.5

Data are mean ± SD

CYP2C9 mRNA quantification by reverse transcription and real-time PCR, from treated and control cultured primary human hepatocytes

	Donor 1		Donor 2		Donor 3	
LNP023 (µmol/L)	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control
Control	1 ± 0.11	0 ± 10	1 ± 0.13	0 ± 8.13	1 ± 0.09	0 ± 3.2
0.1	0.91 ± 0.24	−7.64 ± 20.92	0.82 ± 0.12	−10.99 ± 7.38	0.9 ± 0.13	−3.82 ± 4.85
0.3	0.88 ± 0.06	−10.54 ± 5.66	0.79 ± 0.16	−12.84 ± 9.81	0.91 ± 0.04	−3.16 ± 1.53
1	0.8 ± 0.12	−17.09 ± 10.4	0.73 ± 0.08	−16.26 ± 4.9	0.92 ± 0.17	−3.1 ± 6.25
3	0.75 ± 0.11	−22.15 ± 9.97	0.62 ± 0.04	−22.91 ± 2.63	0.95 ± 0.09	−1.84 ± 3.38
10	0.79 ± 0.06	−18.71 ± 4.81	0.81 ± 0.05	−11.43 ± 3.07	1 ± 0.06	0.08 ± 2.22
30	0.86 ± 0.09	−11.88 ± 8.18	0.79 ± 0.1	−12.55 ± 5.96	1.02 ± 0.06	0.64 ± 2.08
100	1.04 ± 0.19	3.37 ± 16.69	1.25 ± 0.24	14.96 ± 14.2	1.33 ± 0.21	12.11 ± 7.77

Data are mean ± SD

CYP2C9 activity (4′ OH-diclofenac formation) in cultured primary human hepatocytes after treatment for 72 h

	Donor 1		Donor 2		Donor 3	
LNP023 (µmol/L)	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control
Control	1 ± 0.2	0 ± 9	1 ± 0.1	0 ± 3.6	1 ± 0.3	0 ± 11
0.1	0.87 ± 0.3	−5.69 ± 10.9	1.04 ± 0.1	2.57 ± 6.1	1.04 ± 0	1.77 ± 1.5
0.3	1.16 ± 0.1	6.96 ± 4.1	1.34 ± 0	19.74 ± 2.3	1.28 ± 0.4	11.3 ± 14.8
1	1.27 ± 0.3	11.64 ± 12.9	1.37 ± 0.2	21.11 ± 10.3	1.34 ± 0.3	13.86 ± 12.1
3	1.03 ± 0.2	1.24 ± 9.9	1.04 ± 0.3	2.06 ± 20.1	1.3 ± 0.3	11.97 ± 12
10	1.27 ± 0.08	11.43 ± 3.6	1.86 ± 0.1	49.61 ± 8.2	1.5 ± 0.2	20.08 ± 8.9
30	1.54 ± 0.1	23.07 ± 4.6	1.04 ± 0.3	2.06 ± 20.1	1.24 ± 0.4	9.81 ± 18.2
100	1.11 ± 0.4	4.77 ± 16.3	0.55 ± 0.1	−25.8 ± 3.1	1.32 ± 0.11	12.78 ± 4.62

Data are mean ± SD

CYP3A4 mRNA quantification by reverse transcription and real-time PCR, from treated and control cultured primary human hepatocytes

	Donor 1		Donor 2		Donor 3	
LNP023 (µmol/L)	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control
Control	1 ± 0.38	0 ± 1.45	1 ± 0.07	0 ± 0.95	1 ± 0.09	0 ± 0.35
0.1	0.86 ± 0.06	−0.66 ± 0.28	0.82 ± 0.1	−2.25 ± 1.23	0.75 ± 0.22	−0.94 ± 0.84
0.3	1.03 ± 0.09	0.12 ± 0.44	0.98 ± 0.22	−0.28 ± 2.89	1.04 ± 0.18	0.15 ± 0.7
1	0.96 ± 0.17	−0.2 ± 0.78	0.76 ± 0.07	−3.12 ± 0.9	0.95 ± 0.09	−0.2 ± 0.33
3	0.94 ± 0.18	−0.27 ± 0.86	0.69 ± 0.09	−3.92 ± 1.17	0.98 ± 0.13	−0.06 ± 0.51
10	1.07 ± 0.24	0.34 ± 1.13	0.98 ± 0.16	−0.21 ± 2.09	1.37 ± 0.17	1.41 ± 0.65
30	1.94 ± 0.17	4.41 ± 0.78	1.29 ± 0.14	3.72 ± 1.74	1.98 ± 0.14	3.7 ± 0.53
100	2.75 ± 0.75	8.25 ± 3.52	2.49 ± 0.59	19.07 ± 7.62	2.52 ± 0.24	5.76 ± 0.92

Data are mean ± SD

CYP3A activity (1′ OH-midazolam formation) in cultured primary human hepatocytes after treatment for 72 h						
	Donor 1		Donor 2		Donor 3	
LNP023 (µmol/L)	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control	Fold Induction	% of Maximum of positive control
Control	1 ± 0.21	0 ± 2.67	1 ± 0.05	0 ± 2.76	1 ± 0.05	0 ± 1.1
0.1	0.96 ± 0.07	−0.56 ± 0.86	0.98 ± 0.01	−0.94 ± 0.54	1.07 ± 0.06	1.66 ± 1.45
0.3	1.02 ± 0.21	0.22 ± 2.7	1.03 ± 0.06	1.57 ± 3.4	1.03 ± 0.14	0.63 ± 3.15
1	0.99 ± 0.03	−0.11 ± 0.43	0.99 ± 0.05	−0.79 ± 2.83	1.08 ± 0.15	1.83 ± 3.44
3	0.92 ± 0.16	−0.99 ± 2.05	0.99 ± 0.04	−0.31 ± 2.49	1.11 ± 0.03	2.52 ± 0.69
10	0.97 ± 0.21	−0.42 ± 2.64	0.92 ± 0.23	−4.3 ± 12.63	1.03 ± 0.23	0.77 ± 5.27
30	0.87 ± 0.06	−1.66 ± 0.76	0.63 ± 0.03	−20.6 ± 1.72	0.73 ± 0.04	−6.18 ± 0.9
100	0.77 ± 0.09	−2.97 ± 1.16	0.52 ± 0.05	−26.72 ± 2.54	0.49 ± 0.05	−11.61 ± 1.08

Data are mean ± SD

Key results: LNP023 is not an inducer for CYP2B6, CYP2C9 but an inducer of CYP3A4 in primary cultured human hepatocytes. While > 2 fold (2.4 fold) induction of CYP1A2 was observed in hepatocytes from one of the three donors, LNP023 is not likely to be an inducer of CYP1A2 as concomitant induction of gene expression was not observed in the same donor.

2.6.5.12E CYP 時間依存的阻害試験（DMPK R1900589）

Study title:	*In vitro* assessment of time-dependent inhibition of cytochrome P450 enzymes CYP2B6, CYP2C8 and CYP2C19 by LNP023	Report no. DMPK R1900589
Location in CTD:	4.2.2.4-17	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Study system:	Pool of HLM (from 150 individual donors, equal gender mix)	
Probe substrate / Assay:	Bupropion, amodiaquine, S-mephenytoin / LC-MS/MS analysis of metabolite formations from probe substrates by CYP	

The potential for time-dependent (irreversible) inhibition of CYP enzymes by LNP023 was assessed by a kinetic time-dependent inhibition assay in pooled HLM in the absence and presence of increasing amounts of inhibitors and with pre-incubation times up to 48 min. After the pre-incubation period, the incubations were diluted 20-fold to reduce competitive inhibition, and the remaining CYP enzymatic activities were measured using saturating concentrations of CYP-selective probe substrates.

Time-dependent inhibition of CYP enzymes by LNP023

CYP enzyme	Probe reaction	Test compound	Time-dependent inhibition
CYP2B6	bupropion hydroxylation	LNP023	not observed
		positive control 2-phenyl-2-(1-piperidinyl)propane	KI = 1.96 µmol/L kinact = 0.00875 min^{-1}
CYP2C8	amodiaquine *N*-deethylation	LNP023	KI = 179 µmol/L KI,u = 171 µmol/L [a)] kinact = 0.0702 min^{-1}
		positive control gemfibrozil glucuronide	KI = 9.39 µmol/L kinact = 0.0839 min^{-1}
CYP2C19	S-mephenytoin 4′-hydroxylation	LNP023	not observed
		positive control paroxetine	KI = 1.63 µmol/L kinact = 0.0174 min^{-1}

[a)] Calculated using fumic data from DMPK R1500321

Key results: LNP023 showed time-dependent inhibition of CYP2C8 (KI,u = 171 µmol/L, kinact = 0.0702 min^{-1}). No time-dependent inhibition of CYP2B6 and CYP2C19 by LNP023 was found up to a concentration of 100 µmol/L.

LNP023 may inhibit the metabolic clearance of co-medications metabolized by CYP2C8, if sufficiently high concentrations are achieved *in vivo*. Due to time-dependent inhibition of CYP2C8, the decrease in enzyme activity may be amplified by administration of multiple doses. LNP023 is not expected to cause time-dependent inhibition of CYP2B6 and CYP2C19.

2.6.5.13 薬物動態試験：累積排泄

2.6.5.13A ラットに単回静脈内及び経口投与後の排泄（DMPK R1500361）

Study title:	Absorption, distribution, metabolism, and excretion of LNP023 in rats after oral (10 mg/kg) and intravenous (2 mg/kg) administration of [^{14}C]LNP023 hydrochloride	Report no. DMPK R1500361
Location in CTD:	4.2.2.2-3	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Species / Strain:	Rat / Wistar Hannover	
Gender (M / F) / Number of animals:	Male / 6	
Feeding condition:	Fed	
Vehicle / Formulation:	i.v.: solution in 5% ethanol:15% propylene glycol:80% aqueous NaCl solution (0.9% w/v) (w/w/w) p.o.: suspension in 0.5% aqueous methylcellulose solution containing 0.5% Tween 80 (w/w)	
Method of Administration:	i.v.: single dose bolus, p.o.: single dose oral gavage	
Dose (mg/kg):	i.v.: 2 mg/kg, p.o.: 10 mg/kg	
Sample matrix:	Urine, feces, carcass, and cage wash	
Radionucleotide:	^{14}C	
Specific radioactivity:	1.50 and 3.00 MBq/mg (different batches)	
Sampling schedule:	Urine: 0-8, 8-24 h post-dose on first day then daily up to 96, 96-168 h post-dose, feces: daily up to 96, 96-168 h post-dose, carcass and cage wash: 168 h post-dose	
Analyte(s):	^{14}C, LNP023	
Assay:	^{14}C in urine, feces, carcass, and cage wash: LSC, LNP023 in urine and feces: LC-MS/MS	

PK parameters:	i.v.	p.o
Dose (mg/kg):	2	10
Excretion (% of dose)		
Urine, radioactivity, 0-48 h	5.53 ± 1.79	4.96 ± 0.178
Urine, radioactivity, 0-168 h	5.68 ± 1.78	5.09 ± 0.193
Urine, LNP023, 0-72 h [a)]	3.24	2.07
Feces, radioactivity, 0-48 h	84.0 ± 2.51	88.7 ± 7.41

PK parameters:	i.v.	p.o
Dose (mg/kg):	2	10
Excretion (% of dose)		
Feces, radioactivity, 0-168 h	84.6 ± 2.60	94.4 ± 1.06
Feces, LNP023, 0-72 h [a)]	19.0	36.1
Carcass, radioactivity, 168 h	0.580 ± 0.0721	0.0771 ± 0.0146
Cage wash, radioactivity, 168 h	1.44 ± 1.16	0.560 ± 0.225
Recovery, radioactivity, 168 h	92.4 ± 2.00	100 ± 0.833

Data are mean ± SD, n = 3.

[a)] Data of pooled sample (n = 3)

2.6.5.13B　有色ラットに単回経口投与後の排泄（DMPK R1701426）

Study title:	Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]LNP023 following oral administration to male and female Long Evans rats	Report no. DMPK R1701426
Location in CTD:	4.2.2.3-1	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Species / Strain:	Rat / Long-Evans (pigmented)	
Gender (M / F) / Number of animals:	Male / 3	
Feeding condition:	Fed	
Vehicle / Formulation:	p.o.: solution in 0.5% aqueous methylcellulose solution containing 0.5% Tween 80 (w/w)	
Method of Administration:	p.o.: single dose oral gavage	
Dose (mg/kg):	10 mg/kg	
Sample matrix:	Urine, feces, carcass, cage rinse, cage wash, and cage wipe	
Radionucleotide:	^{14}C	
Specific radioactivity:	1.04 MBq/mg	
Sampling schedule:	Urine, feces: daily up to 168 h post-dose, cage rinse: after each 24 h excreta collections through 144 h post-dose, carcass, cage wash and cage wipe: 168 h post-dose	
Analyte(s):	^{14}C	
Assay:	^{14}C in urine, feces, cage rinse, carcass, cage wash, and cage wipe: LSC	

PK parameters:	p.o
Dose (mg/kg):	10
Excretion (% of dose)	
Urine, radioactivity, 0-24 h	4.63 ± 0.917
Urine, radioactivity, 0-168 h	4.79 ± 0.931
Feces, radioactivity, 0-24 h	85.6 ± 3.15
Feces, radioactivity, 0-168 h	92.8 ± 3.79
Carcass, radioactivity, 168 h	0.0955 ± 0.00855
Cage rinse, radioactivity, 0-144 h	0.163 ± 0.0335
Cage wash, radioactivity, 168 h	0.0149 ± 0.00238

PK parameters:	p.o
Dose (mg/kg):	10
Excretion (% of dose)	
Cage wipe, radioactivity, 168 h	0.0284 ± 0.0258
Recovery, 168 h	97.9 ± 3.74

Data are mean ± SD, n = 3.

2.6.5.14 薬物動態試験：胆汁中排泄

2.6.5.14A ラットに単回静脈内投与後の胆汁中排泄（DMPK R1500361）

Study title:	Absorption, distribution, metabolism, and excretion of LNP023 in rats after oral (10 mg/kg) and intravenous (2 mg/kg) administration of [^{14}C]LNP023 hydrochloride	Report no. DMPK R1500361
Location in CTD:	4.2.2.2-3	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl	
Species / Strain:	Bile duct-cannulated rat / Wistar Hannover	
Gender (M / F) / Number of animals:	Male / 4	
Feeding condition:	Fed	
Vehicle / Formulation:	i.v.: solution in 5% ethanol: 15% propylene glycol:80% aqueous NaCl solution (0.9% w/v) (w/w/w)	
Method of Administration:	i.v.: single dose bolus	
Dose (mg/kg):	i.v.: 2 mg/kg	
Sample matrix:	Urine, feces, bile, GI tract, carcass, and cage wash	
Radionucleotide:	^{14}C	
Specific radioactivity:	3.00 MBq/mg	
Sampling schedule:	Urine, feces, bile: daily up to 48 h (0-24, 24-48 h), GI tract, carcass, and cage wash: 48 h post-dose	
Analyte(s):	^{14}C	
Assay:	^{14}C in urine, feces, bile, GI tract, carcass, and cage wash: LSC	

Analyte / Time	Urine (% of dose)	Feces (% of dose)	Bile (% of dose)
^{14}C / 0-24 h	12.7 ± 1.02	2.86 ± 2.06	79.0 ± 3.30
^{14}C / 24-48 h	0.327 ± 0.201	1.34 ± 1.24	0.493 ± 0.382
^{14}C / Subtotal	13.0 ± 0.908	4.21 ± 1.47	79.4 ± 2.99
^{14}C / Carcass 48 h	0.589 ± 0.335		
^{14}C / Cage wash 48 h	1.80 ± 1.57		
^{14}C / GI tract 48 h	0.180 ± 0.184		
^{14}C / Subtotal	2.57 ± 2.08		
^{14}C / Recovery 48 h	99.2 ± 0.222		

Data are mean ± SD, n = 3 (data of one rat were not considered in the calculations, since the animal was euthanized after dosing).

2.6.5.15 薬物動態試験：薬物相互作用

No studies conducted in animal species under this section. For human *in vivo* pharmacokinetic drug-interaction studies please see (2.7.2-Section 3.4.2).

2.6.5.16 薬物動態試験：その他

2.6.5.16A Caco-2 細胞を用いた膜透過性試験（DMPK R1500730）

Study title:	Assessment of the intestinal transport of LNP023 using the gastrointestinal Caco-2 cell line	Report no. DMPK R1500730
Location in CTD:	4.2.2.7-1	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl, LNP023·HCl	
Study system:	Human intestinal cell line Caco-2 grown on permeable filter support	
Analyte / Assay:	^{14}C-LNP023, [^{3}H]Digoxin, [^{14}C]Mannitol, [^{3}H]Propranol / Radioactive samples by LSC	

The well-defined standard protocol for Caco-2 permeability assessment was used to measure transport rates of LNP023 (0.5-400 µmol/L) across Caco-2 cell monolayers.

Summary of results

Compound	Papp (AP-BL) [a] (10^{-6} cm/s)	Papp (BL-AP) [a] (10^{-6} cm/s)	Pm [a,b] (10^{-6} cm/s)	Pc,max [a,c,d] (10^{-6} cm/s)	Vc,max,app [c] (pmol/s/cm^2)	Km,app [c] (µmol/L)	Km [d] (µmol/L)
LNP023	1.33 ± 0.16	5.39 ± 0.21	2.35 ± 0.31	1.02 ± 0.35	> 4.5	> 438	> 247

Mean ± SD, n = 3

[a] Permeability data provided at lowest LNP023 concentration (nominal 0.5 µmol/L)

[b] Estimated nonspecific (passive) permeability according to the equation:

$$\text{Papp} = \text{Pm} \pm \text{Pc} = \text{Pm} \pm \frac{\text{Vc,max}}{\text{Km,app} + \text{S}} = \text{Pm} \pm \frac{\text{Pc,max} \cdot \text{Km,app}}{\text{Km,app} + \text{S}}$$

[c] Apparent maximal carrier-mediated (saturable) parameter according to the above equation

[d] Transporter affinity constant corrected for unbound intracellular concentration according to equations:

$$ERb = \frac{Pm}{Papp, AB} \quad Km = \frac{Km, app}{ERb, \max}$$

,

Key results: LNP023 permeates passively at a low to moderate rate through Caco-2 monolayers modulated by efflux transporter activity (likely P-gp). The predicted human absorption in presence or absence of efflux transporter activity (based on Papp and Pm data, respectively) was 47% and 61%.

2.6.5.16B　BCRP, MDR1 親和性評価（DMPK R1600716）

Study title:	*In vitro* Interaction Studies of LNP023 with the human BCRP and MDR1 Efflux (ABC) Transporters	Report no. DMPK R1600716
Location in CTD:	4.2.2.7-2	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Study system:	Inside-out membrane vesicles prepared from HEK293 cells overexpressing human ATP-binding cassette (ABC) transporters	
Probe substrate / Assay:	*N*-methyl-quinidine, Estrone-3-sulfate (E3S) / The amount of substrate (^{3}H-*N*-methyl-quinidine or ^{3}H-E3S) inside the filtered vesicle by LSC	

The purpose of this study was to provide data on the interaction of LNP023 with the human ABC (efflux) transporters, breast cancer resistance protein (BCRP) and P-gp in a vesicle-based assay.

LNP023 was incubated with membrane vesicle preparations (total protein: 50 µg/well for P-gp or 25 µg/well for BCRP) and the probe substrate. Incubations were carried out in the presence of 4 mmol/L adenosine triphosphate (ATP). LNP023 (0.1-400 µmol/L) was added to the reaction mixture in 0.75 µL of solvent (1% of the final incubation volume). Reaction mixtures were preincubated for 15 minutes at 32 ± 1°C. Reactions were initiated by the addition of 25 µL of 12 mmol/L MgATP, preincubated separately. Reactions were quenched by the addition of 200 µL of ice-cold washing buffer and immediate filtration via glass fiber filters mounted to a 96-well plate (filter plate). The filters were washed, dried and the amount of substrate inside the filtered vesicles was determined by LSC.

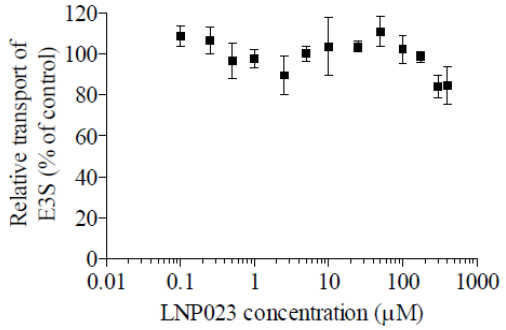
Summary of results

Transporter and assay type	Maximum inhibition at 400 µmol/L (% of control)	IC50 (µmol/L)	Ki (µmol/L)
BCRP vesicular transport	no interaction observed	no interaction observed	nc
P-gp vesicular transport	80	37.0	27.7

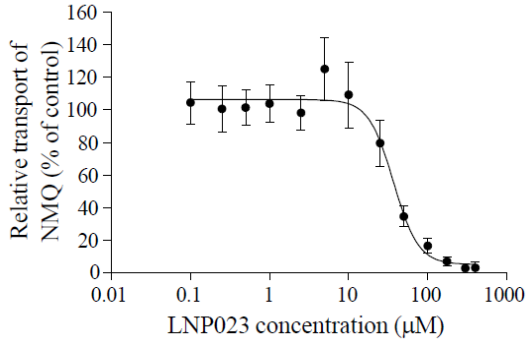
nc: not calculated

Control refers to the relative ATP dependent transport of 1 µmol/L *N*-methyl-quinidine in the presence of 1% DMSO. The Km value of *N*-methyl-quinidine on P-gp is 2.967 µmol/L. Maximum inhibition values are experimental data and not fitted values.

Inhibition of BCRP-mediated probe substrate transport by LNP023 in the vesicular transport inhibition assay



Inhibition of P-gp-mediated probe substrate transport by LNP023 in the vesicular transport inhibition assay



Key results: LNP023 is an inhibitor of P-gp (calculated IC50 37.0 µmol/L; calculated Ki: 27.7 µmol/L). As a result, LNP023 may cause an increase in the systemic exposure of P-gp substrates provided that sufficiently high LNP023 concentrations are achieved *in vivo*.

2.6.5.16C　OCT1, OCT2 親和性評価（DMPK R1600717）

Study title:	*In vitro* Interaction Studies of LNP023 with the human OCT1 and OCT2 Uptake Transporters	Report no. DMPK R1600717
Location in CTD:	4.2.2.7-3	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Study system:	HEK293 cells stably expressing the human organic cation transporter (OCT) 1, and OCT2 uptake transporter	
Probe substrate / Assay:	Metformin / Radiolabelled probe substrate (biguanidine-^{14}C) transport by LSC	

The purpose of this study was to provide data on the interaction potential of LNP023 with the human solute-carrier (SLC) (uptake) transporters: OCT1 and OCT2, expressed in stable transfected HEK293 cell lines. Uptake experiments were carried out at 37°C in hank's buffered salt solution buffer (pH 7.4) containing the probe substrate and the LNP023 (0.1-400 µmol/L) or solvent. After incubation, radiolabelled probe substrate transport was determined by measuring for LSC.

Inhibition results

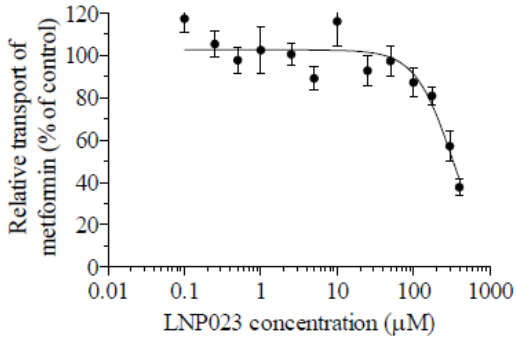
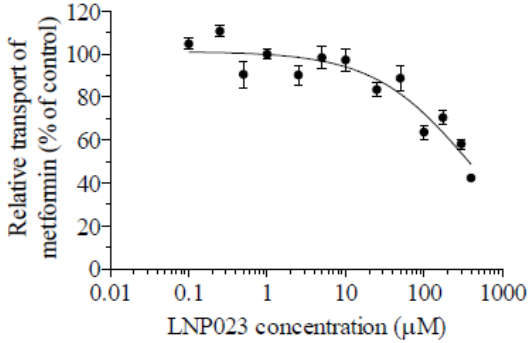
Transporter and assay type	maximum inhibition at 400 µmol/L (% of control)	IC50 (µmol/L)	Ki (µmol/L)
OCT1 uptake assay	62.2 ± 4.02	322	321
OCT2 uptake assay	57.6 ± 0.95	361	359

Mean ± SD, n = 3

Control refers to the relative transport of the probe substrate metformin in the presence of 1% DMSO (solvent).

Maximum inhibition values are normalized experimental data. Probe substrate concentrations were selected upon the results of SOLVO assay characterization. The Km values of metformin on OCT1 is 1730 µmol/L and 2803 µmol/L on OCT2.

Ki was calculated with Ki = IC50 / (1 + S / Km), where S is the substrate concentration of the probe substrate and Km is the transporter affinity of the probe substrate according to Michaelis-Menten.

Inhibition of OCT1 and OCT2-mediated probe substrate transport by LNP023 in the uptake transporter inhibition assay	
OCT1	OCT2
	
Key results: LNP023 is an inhibitor of both OCT1- and OCT2-mediated uptake transport of metformin. As a result, LNP023 may cause an increase in the systemic exposure of OCT1 or OCT2 substrates provided that sufficiently high LNP023 concentrations are achieved *in vivo*.	

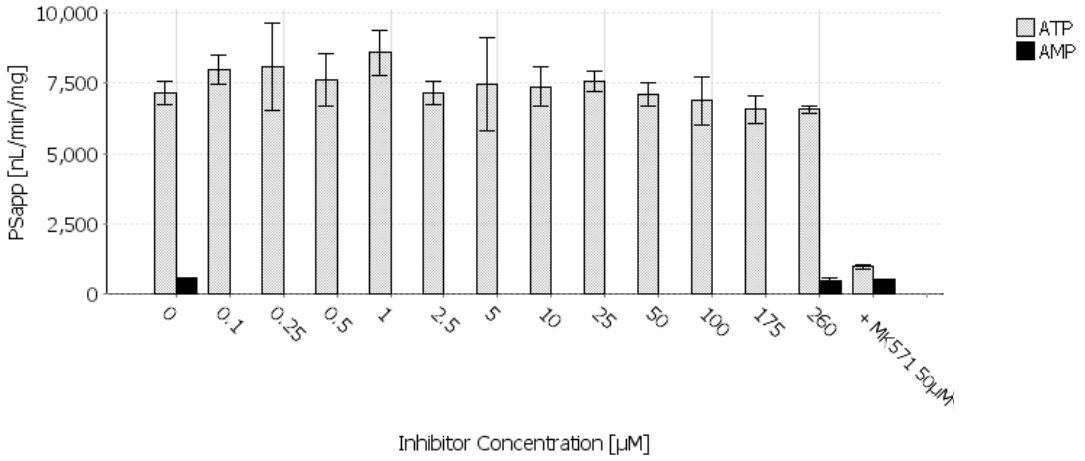
2.6.5.16D MRP2 阻害評価（DMPK R1500958）

Study title:	Assessment of efflux transporter (MRP2) inhibition by LNP023	Report no. DMPK R1500958
Location in CTD:	4.2.2.7-4	
GLP compliance:	Not required	
Test article(s):	^{14}C-LNP023·HCl, LNP023·HCl	
Study system:	(Inside-out) membrane vesicles from recombinant baculovirus infected Sf9 cells expressing human multidrug resistance associated protein (MRP) 2 protein	
Probe substrate / Assay:	Estradiol-17β-D-glucuronide ($E_2$17βG) / Radioactive samples ([^{3}H]$E_2$17βG) by LSC	

The potential of LNP023 to inhibit human ABC transporter activity was assessed using recombinant Sf9 (inside-out) vesicles overexpressing human multidrug-resistance protein 2 (MRP2, ABCC2). The inhibitory effect of LNP023 on MRP2 (up to 260 µmol/L) was assessed by determining the compound's ability to inhibit vesicular uptake the MRP2 probe substrate $E_2$17βG.

Effect of LNP023 on probe substrate uptake by Sf9 vesicles expressing human MRP2

Effect of increasing LNP023 concentrations on the apparent $E_2$17βG [nominal: 50 µmol/L] uptake into MRP2-expressing Sf9 membrane vesicles in the presence of adenine monophosphate (AMP) or ATP (black and grey bars, respectively). The data are averages of triplicate determinations and the bars represent the standard deviations.



Key results: LNP023 was shown to be no inhibitor of the transport activities of MRP2 *in vitro*. Therefore, no impact on co-medications has to be expected up to the highest concentration tested (260 µmol/L).

2.6.5.16E ABC 及び SLC トランスポーター阻害評価（DMPK R1709014）

Study title:	*In vitro* Inhibition Studies of LNP023 on the human BSEP Efflux (ABC) Transporter and the human OAT1, OAT3, MATE1 and MATE2-K Uptake (SLC) Transporters	Report no. DMPK R1709014
Location in CTD:	4.2.2.7-5	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Study system:	MDCKII or HEK293 cells stably expressing the respective uptake transporters [human multidrug and toxin extrusion (MATE) 1, MATE2-K, organic anion transporter (OAT) 1, or OAT3]	
	Inside-out membrane vesicles prepared from cells overexpressing the human BSEP ABC transporter	
Probe substrate / Assay:	Metformin, *p*-Aminohippuric acid, E3S, taurocholate / Radiolabeled probe substrate transport by LSC	

Vesicular transport inhibition assays: LNP023 (0.1-400 µmol/L) was incubated with membrane vesicle preparations and the probe substrate. Incubations were carried out in the presence of 4 mmol/L ATP. After incubation, the amount of substrate inside the filtrated vesicles was determined by LSC.

Uptake transporter inhibition assays: Uptake experiments were carried out at 37 ± 1°C in assay buffer containing the probe substrate and LNP023 (0.1-400 µmol/L) or solvent. After incubation, radiolabeled probe substrate transport was determined by measuring for LSC.

Inhibition results

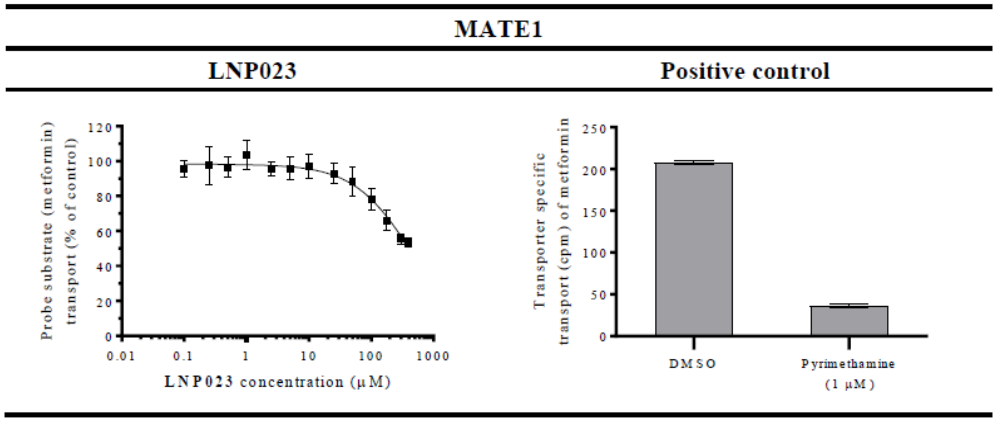
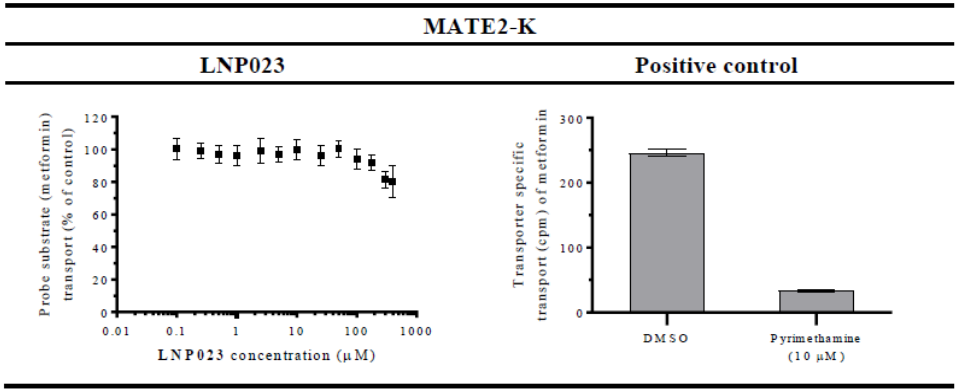
Transporter and assay type	Maximum inhibition at 400 µmol/L concentration of LNP023 (% of control [a)])	IC50 (µmol/L)	Ki (µmol/L)
BSEP vesicular transport assay	NIO	na	na
MATE1 uptake transporter assay	47	424 [b)]	400
MATE2-K uptake transporter assay	NIO	na	na
OAT1 uptake transporter assay	NIO	na	na
OAT3 uptake transporter assay	72	178	173

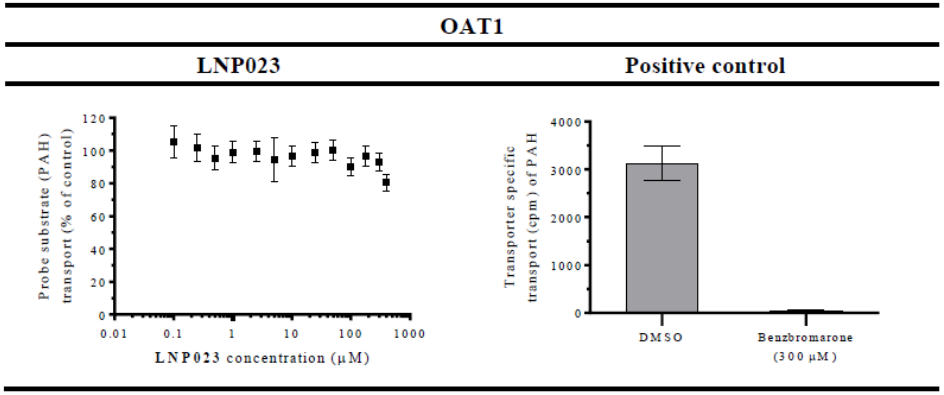
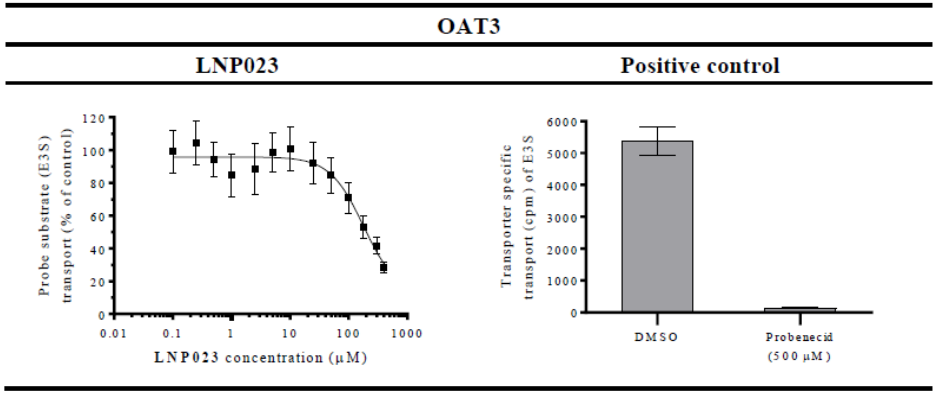
NIO: No inhibition observed, defined as inhibition < 20%, na: not applicable

[a)] Control refers to the relative transport of the probe substrate with solvent only (test article vehicle). Maximum inhibition values are normalized experimental data. Probe substrate concentrations were selected upon the results of SOLVO assay characterization. The following Km values were used in calculations: 163.6 µmol/L of metformin for MATE1 and 37.622 µmol/L of E3S for OAT3.

[b)] Bottom was constrained to 0.0; IC50 was extrapolated as it exceeded the investigated concentration range.

Ki is calculated with Ki = IC50 / (1 + S / Km), where S is the substrate concentration of the probe substrate and Km is the transporter affinity of the probe substrate according to Michaelis-Menten.

Inhibition of MATE1-mediated probe substrate (metformin) transport by LNP023 in the uptake transporter inhibition assay and the positive control	
Inhibition of MATE2-K-mediated probe substrate (metformin) transport by LNP023 in the uptake transporter inhibition assay and the positive control	

Inhibition of OAT1-mediated probe substrate (*p*-Aminohippuric acid) transport by LNP023 in the uptake transporter inhibition assay and the positive control	
Inhibition of OAT3-mediated probe substrate (E3S) transport by LNP023 in the uptake transporter inhibition assay and the positive control	
Key results: LNP023 did not inhibit the BSEP efflux (ABC), MATE2-K, and OAT1 uptake (SLC) transporters at the investigated concentrations, up to 400 µmol/L final concentration of the test article. LNP023 is an inhibitor of MATE1 (IC50 = 424 µmol/L) and OAT3 (IC50 = 178 µmol/L) uptake (SLC) transporters. The calculated K_i values were 400 µmol/L and 173 µmol/L, respectively.	

2.6.5.16F SLC トランスポーター親和性評価（DMPK R2000210）

Study title:	*In vitro* Interaction Studies of LNP023 with the human with the human MATE1, MATE2-K, OAT1, OAT3, OCT1 and OCT2 Uptake Transporters	Report no. DMPK R2000210
Location in CTD:	4.2.2.7-6	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Study system:	HEK293 and MDCKII cells stably expressing the respective uptake transporters (human MATE1, MATE2-K, OAT1, OAT3, OCT1, OCT2)	
Probe substrate / Assay:	Metformin, tenofovir, E3S / Radiolabeled probe substrate transport by LSC	

Uptake experiments were performed using HEK293 and MDCKII cells stably expressing the respective uptake transporters. Before the experiment, the cells were pre-incubated at 37°C for 30 min in assay buffer containing the respective compounds: 0.1-400 µmol/L LNP023, 1% DMSO for solvent control and the reference inhibitor. In case of blank, the wells were pre-incubated only in assay buffer. After the pre-incubation step the medium was removed. Uptake experiments were carried out at 37°C in 50 µL of assay buffers containing the probe substrate and LNP023 or solvent. After the experiment, cells were washed and lysed with 0.1 mol/L NaOH. Radiolabeled probe substrate transport was determined by measuring an aliquot from each well for LSC.

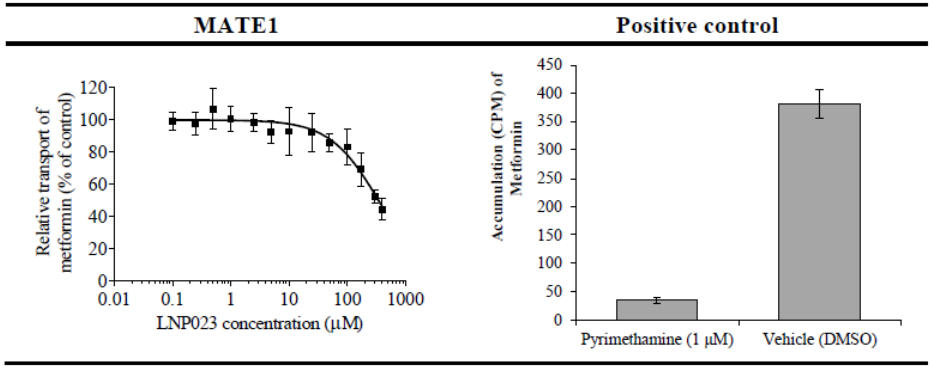
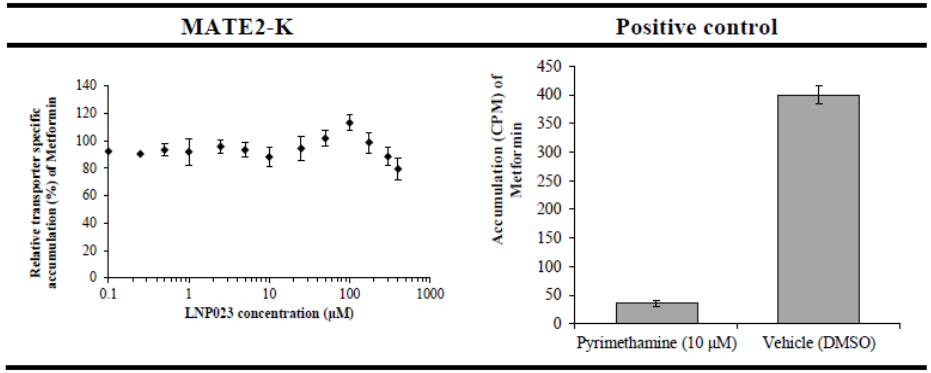
Inhibition results of LNP023

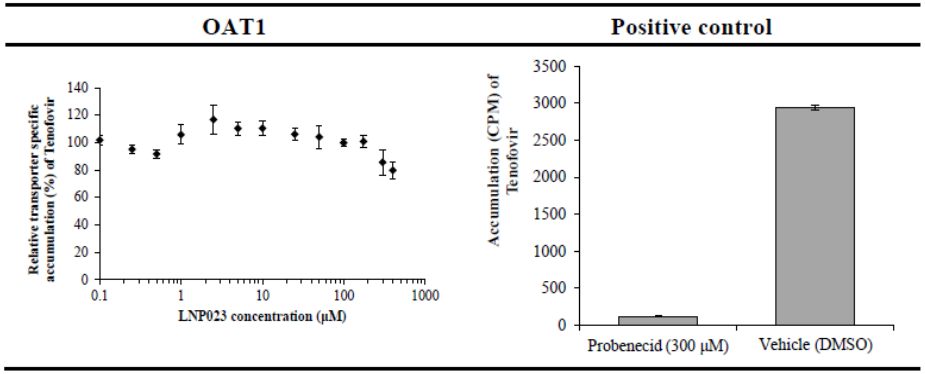
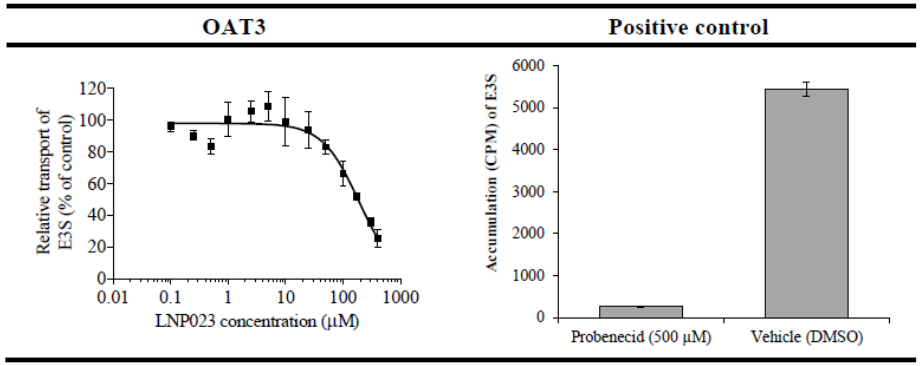
Transporter and assay type	The highest tested concentration (µmol/L)	Inhibition at the highest tested concentration (% of control [a])	IC50 (µmol/L)	Ki (µmol/L)
	Nominal			
MATE1 uptake transporter assay	400	55.7	347.2	327.1
MATE-2K uptake transporter assay		20.5	na	na
OAT1 uptake transporter assay		20.2	na	na
OAT3 uptake transporter assay		74.8	188.2	183.3
OCT1 uptake transporter assay		48.9	398.9	396.6
OCT2 uptake transporter assay		35.8	na	na

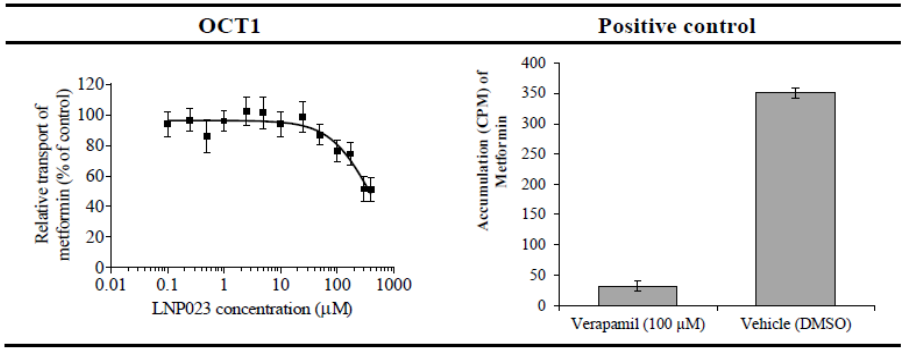
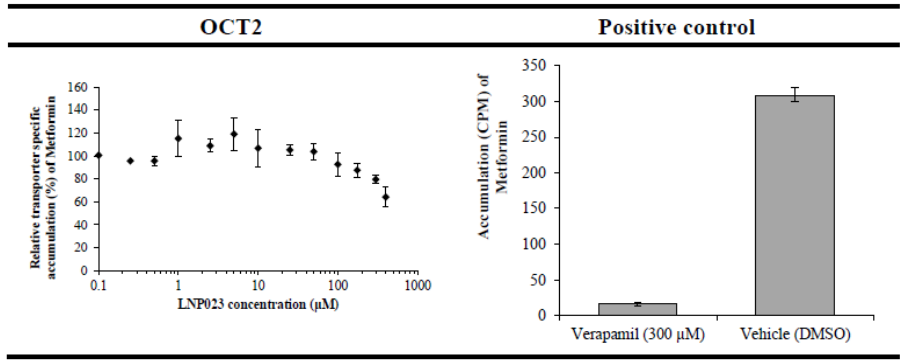
na: not applicable

[a] Control refers to the relative transport of the probe substrate tenofovir (OAT1), E3S (OAT3) or metformin (MATE1, MATE2-K, OCT1, and OCT2) with solvent only (test article vehicle). Maximum inhibition values are normalized experimental data. Probe substrate concentrations were selected upon the results of SOLVO assay characterization. The probe substrate concentration (S) tenofovir (OAT1), E3S (OAT3), and metformin (MATE1, MATE2-K, OCT1, and OCT2) is ≤ Km/3. The Km values are as follows: Km of metformin on MATE1 is 163 µmol/L, Km of metformin on MATE2-K is 1379 µmol/L, Km of metformin on OCT1 is 1730 µmol/L, Km of metformin on OCT2 is 1502 µmol/L, Km of tenofovir on OAT1 is 98.0 µmol/L, Km of E3S on OAT3 is 37.6 µmol/L.

Ki is calculated with Ki = IC50 / (1 + S / Km), where S is the substrate concentration of the probe substrate and Km is the transporter affinity of the probe substrate according to Michaelis-Menten. For OCT1 only an estimated IC50 and Ki value was calculated, since inhibition at the highest concentration did not reach 50%.

Inhibition of MATE1-mediated probe substrate (metformin) transport by LNP023 in the uptake transporter inhibition assay – IC50 determination and the positive control	
Inhibition of MATE2-K-mediated probe substrate (metformin) transport by LNP023 in the uptake transporter inhibition assay – IC50 determination and the positive control	

Inhibition of OAT1-mediated probe substrate (tenofovir) transport by LNP023 in the uptake transporter inhibition assay – IC50 determination and the positive control	
Inhibition of OAT3-mediated probe substrate (E3S) transport by LNP023 in the uptake transporter inhibition assay – IC50 determination and the positive control	

Inhibition of OCT1-mediated probe substrate (metformin) transport by LNP023 in the uptake transporter inhibition assay – IC50 determination and the positive control	
Inhibition of OCT2-mediated probe substrate (metformin) transport by LNP023 in the uptake transporter inhibition assay – IC50 determination and the positive control	

Key results: LNP023 inhibited the MATE2-K-, OAT1-, and OCT2-mediated uptake transport of metformin, tenofovir, and metformin with a maximum inhibition of 20.5%, 20.2%, and 35.8% at 400 µmol/L test article concentration. No IC50 value was calculated. LNP023 inhibited the MATE1-mediated uptake transport of metformin with a maximum inhibition of 55.7% at 400 µmol/L test article concentration. Calculated IC50 and Ki were 347.2 µmol/L and 327.1 µmol/L, respectively. LNP023 inhibited the OAT3-mediated uptake transport of E3S with a maximum inhibition of 74.8% at 400 µmol/L test article concentration. Calculated IC50 and Ki were 188.2 µmol/L and 183.3 µmol/L, respectively. LNP023 inhibited the OCT1-mediated uptake transport of metformin with a maximum inhibition of 48.9% at 400 µmol/L test article concentration. Estimated IC50 and Ki were 398.9 µmol/L and 396.6 µmol/L, respectively.

2.6.5.16G　凍結肝細胞を用いた取り込み試験（DMPK R1500336）

Study title:	Cellular uptake of LNP023 in suspensions of cryopreserved human hepatocytes	Report no. DMPK R1500336
Location in CTD:	4.2.2.7-7	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl, ^{14}C-LNP023·HCl	
Study system:	Pooled cryopreserved suspensions of isolated human hepatocytes (Celsis)	
Analytical methods:	Radioactive samples by LSC	
Inhibitor:	Rifamycin SV, Atorvastatin, *p*-Aminohippuric acid, Tetraethylammonium, Taurocholic acid, Chloroquine	

In vitro determination of the mechanism of LNP023 cellular uptake in suspensions of cryopreserved human hepatocytes. This was determined by testing the concentration- (0.5 and 130 µmol/L) and temperature- (4 and 37°C) dependency of the *in vitro* cellular uptake. The involvement of several candidate transporters OCT1, OAT2, OATPs was investigated using representative uptake transporter inhibitors. In addition the potential impact of lysosomal trapping was assessed in human hepatocytes.

Summary of results

Species	PSm [a, c)] (µL/min/10^6 cells)	PSc,max [b, c)] (µL/min/10^6 cells)	PSc,max / (PSm + PSc,max) [c)]	Inhibitor(s)	Possible transporters
Human	4.3	3.4	0.44	Rifamycin SV, Atorvastatin	OATPs

[a)] Nonspecific (passive) membrane permeability according to the equation:

$$V = PS \cdot S = PSm \cdot S + PSc \cdot S = PSm \cdot S + \frac{Vc,max \cdot S}{Km,app + S} = PSm \cdot S + \frac{PSc,max \cdot Km,app \cdot S}{Km,app + S}$$

[b)] Maximal carrier-mediated (saturable) membrane permeability according to the above equation

[c)] Parameters are rough estimations (no kinetics available)

E3S uptake by human hepatocytes

E3S (µmol/L) [a)]	Temerature (°C)	Species	PSapp (µL/10^6 cells) [b, c)]
0.0516	37	Human	285.2 ± 13.8
0.0516	4	Human	51.7 ± 4.6

[a)] Actual measured values (nominal values: 0.03 µmol/L)
[b)] Mean ± SD of n = 3 determined after 3 minutes of incubation
[c)] Vapp/S, where Vapp was calculated from the slope of the time-dependent E3S uptake [pmol/10^6 cells] between 0 and 3 minutes

Key results: Human hepatocyte uptake of LNP023 occurs, most likely, by a passive permeation process with modulation by at least one solute-carrier system. LNP023 was not trapped in hepatic lysosomes.

2.6.5.16H　LNP023 による取り込みトランスポーター阻害試験（DMPK R1500532）

Study title:	Assessment of uptake transporter (OATP1B1, OATP1B3) inhibition by LNP023	Report no. DMPK R1500532
Location in CTD:	4.2.2.7-8	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl, ^{14}C-LNP023·HCl	
Study system:	HEK293 cells stably expressing organic anion transporting polypeptide (OATP) 1B1, or OATP1B3	
Probes substrate / Assay:	$E_2$17βG / The experimental concentrations of the radiolabeled compounds by LSC	

The potential of LNP023 to inhibit human OATP1B1 and OATP1B3 activity was assessed using recombinant HEK293 cells overexpressing OATP1B1 or OATP1B3, respectively. The inhibitory effect of LNP023 up to 400 µmol/L on the major uptake transporters of the human OATP family (OATP1B1 and OATP1B3, respectively) was assessed by determining the compound's ability to inhibit cellular uptake of the probe substrate $E_2$17βG for OATP1B1 and OATP1B3. Probe substrate concentrations used for these determinations were less than or equal to their reported Km values.

Summary of results

OATP transporter	Probe substrate	IC50 [a, b] (µmol/L)	Ki [c] (µmol/L)	Max. inhibition [b] (%)
OATP1B1	$E_2$17βG	25.6 ± 1.8	24.9 ± 1.7	102.2 ± 2.8 [d]
OATP1B3	$E_2$17βG	> 400	> 392.7	45.3 ± 8.4 [e]

[a] LNP023 concentration estimated to inhibit probe substrate uptake by 50%

[b] Data used for curve fitting are mean ± SD of n = 3

[c] Calculated with Ki = IC50 / (1 + S / Km), where S is the substrate concentration of the probe substrate and Km is the transporter affinity of the probe substrate according to Michaelis-Menten

[d] By fitting function calculated maximal inhibition with respect to positive control (Rifamycin SV / Atorvastatin)

[e] Maximal observed inhibition with respect to positive control (Rifamycin SV / Atorvastatin)

Key results: LNP023 may cause an increase in the systemic exposure of co-medications whose clearance is significantly mediated by OATP1B1 or OATP1B3 transport activity provided that sufficiently high LNP023 concentrations are achieved *in vivo*.

2.6.5.16l LNP023 による BCRP 及び P-gp 阻害試験（DMPK R1500533）

Study title:	Assessment of LNP023 as an inhibitor of human breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp)	Report no. DMPK R1500533
Location in CTD:	4.2.2.7-9	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl, ^{14}C-LNP023·HCl	
Study system:	Recombinant MDCKII and LLC-PK1 cells overexpressing human BCRP and P-gp, respectively	
Probe substrate / Assay:	Digoxin, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine-2 / Specific amount of radiolabeled probe substrate inside the cells by LSC	

The potential of LNP023 to inhibit human ABC transporter activity was assessed using recombinant MDCKII and LLC-PK1 cells overexpressing human BCRP and P-gp, respectively. The inhibitory effect of LNP023 on both major efflux transporters of the human ABC family up to 400 µmol/L for P-gp and BCRP was assessed by determining the compound's ability to inhibit efflux, which leads to increased cellular accumulation of the probe substrates digoxin for P-gp, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine-2 for BCRP. Probe substrate concentrations used for these determinations were less than or equal to their reported Km values.

Effect of LNP023 on probe substrate uptake by P-gp-transporter expressing LLC-PK1 cells

LNP023 (µmol/L)	P-gp [a)]	
	PSapp [b)] (nL/min/mg protein)	Inhibition (y) [b, d)] (%)
0 (= baseline)	89.46 ± 27.12	0.00 ± 56.86
0.10	80.25 ± 22.99	−19.31 ± 48.20
0.25	91.10 ± 3.38	3.43 ± 7.09
0.50	78.20 ± 12.59	−23.61 ± 26.40
1.00	70.83 ± 15.95	−39.06 ± 33.44
2.50	51.39 ± 7.73	−79.83 ± 16.20
5.00 [e)]	55.27 ± na	−71.67 ± na
10.00 [e)]	59.57 ± na	−62.66 ± na
25.00 [e)]	68.17 ± na	−44.64 ± na
50.00	57.32 ± 4.36	−67.38 ± 9.13
100.00	36.24 ± 3.07	−111.59 ± 6.44
175.00	33.78 ± 2.46	−116.74 ± 5.15
300.00	39.10 ± 0.71	−105.58 ± 1.49
400.00	35.42 ± 2.16	−113.30 ± 4.52

Effect of LNP023 on probe substrate uptake by P-gp-transporter expressing LLC-PK1 cells		
Positive control [c]	137.16 ± 6.51	100.00 ± 13.65

na: not applicable

[a] Probe substrate: [^{3}H]digoxin [1 µmol/L]

[b] Mean ± SD, n = 3

[c] In the presence of Cyclosporine A [10 µmol/L]

[d] According to the equation: y = 100 − (100／((PSapp,p − PSapp,o)／(PSapp,p − PSapp,i)))

[e] n = 1 due to outliers

Effect of LNP023 on probe substrate uptake by BCRP-transporter expressing MDCKII cells		
LNP023 (µmol/L)	BCRP [a]	
	PSapp [b] (nL/min/mg protein)	Inhibition (y) [b, d] (%)
0 (=baseline)	356.51 ± 21.46	0.00 ± 1.30
0.10	368.54 ± 16.09	0.73 ± 0.97
0.25	359.09 ± 19.34	0.16 ± 1.17
0.50	338.47 ± 26.32	−1.09 ± 1.59
1.00	351.35 ± 25.94	−0.31 ± 1.57
2.50	310.12 ± 5.36	−2.80 ± 0.32
5.00	301.53 ± 18.04	−3.32 ± 1.09
10.00	332.45 ± 27.15	−1.45 ± 1.64
25.00	323.86 ± 22.52	−1.97 ± 1.36
50.00	304.11 ± 26.91	−3.17 ± 1.63
100.00	298.95 ± 8.93	−3.48 ± 0.54
175.00	276.62 ± 15.75	−4.83 ± 0.95
300.00	250.84 ± 22.22	−6.39 ± 1.34
400.00	215.62 ± 13.23	−8.52 ± 0.80
Positive control [c]	2011.05 ± 213.83	100.00 ± 12.92

[a] Probe substrate: [^{14}C]2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine-2 [2 µmol/L]

[b] Mean ± SD, n = 3

[c] In the presence of Ko143 [1 µmol/L]

[d)] According to the equation: y = 100 − (100／((PSapp,p − PSapp,o)／(PSapp,p − PSapp,i)))

Key results: LNP023 was shown to be no inhibitor of the transport activities of P-gp and BCRP *in vitro*. Therefore, no impact on co-medications has to be expected up to the highest concentration tested (400 µmol/L).

2.6.5.16J　培養ヒト肝細胞を用いた肝胆道系動態評価（DMPK R1700975）

Study title:	Assessment of LNP023 hepatobiliary disposition *in vitro* using sandwich-cultured human hepatocytes	Report no. DMPK R1700975
Location in CTD:	4.2.2.7-10	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl, ^{14}C-LNP023·HCl	
Study system:	Human sandwich-cultured hepatocytes	
Analytical methods:	Specific activity and the measured radioactivity in hepatocyte lysates by LSC	
Substrate:	Taurocholic acid, Rosuvastatin	

For biliary excretion assay, the cells were incubated with LNP023 (1, 3, 10, 30, and 100 µmol/L) in standard buffer for 15 min at 37°C. In parallel, cells were incubated with Taurocholic acid (1 µmol/L) or rosuvastatin (0.1 µmol/L) in standard buffer for 15 min at 37°C. After the incubation period, cell lysate samples were transferred into scintillation vials, mixed with scintillation cocktail, and the radioactivity was determined using LSC.

Summary of results

CLsc [a]			PSbile [b]		
CLint,sc [a] (µL/min/mg)	Km,sc (µmol/L)	Vmax,sc (pmol/min/mg)	PSint,bile (µL/min/mg)	Km,bile (µmol/L)	Vmax,bile (pmol/min/mg)
0.121 ± 0.086	> 100	na	0.383 ± 0.280	> 22.0	na

na: not applicable

[a] Clint,sc was obtained as the average of Clapp,sc data from nominal 1-100 µmol/L

[b] Psint,bile was obtained as the average of Psbile data from nominal 1-100 µmol/L

Hepatobiliary disposition of LNP023 in sandwich-cultured human hepatocytes: Apparent parameters

S [a] (µmol/L)	Cellular accumulation in (-)buffer [b, c] (pmol/mg)	Total accumulation in (+)buffer [b, c] (pmol/mg)	Clapp,sc [b, c] (µL/min/mg)	Psapp,bile [b, c] (µL/min/mg)	BEI (%)
1.01	23.0 ± 1.1	24.7 ± 0.6	0.115 ± 0.115	0.027 ± 0.03	7.10
2.76	59.3 ± 3.2	66.4 ± 2.3	0.170 ± 0.126	0.042 ± 0.03	10.6
8.93	163 ± 9	175 ± 1	0.091 ± 0.072	0.027 ± 0.02	6.92
27.0	426 ± 11	473 ± 17	0.117 ± 0.047	0.039 ± 0.02	10.0
90.0	1221 ± 80	1372 ± 27	0.112 ± 0.072	0.044 ± 0.03	11.0

[a] Measured concentration of application solution (S). (nominal concentrations: 1, 3, 10, 30, and 100 µmol/L)

[b] Mean ± SD, n = 3

[c)] Average protein concentration was 0.499 and 0.407 mg protein/mL in (+)buffer and (-)buffer, respectively

Hepatobiliary disposition of LNP023 in sandwich-cultured human hepatocytes: Corrected parameters

S [a)] (µmol/L)	C [b, c, d)] (µmol/L)	fu,hep [e)]	Cu [c, f)] (µmol/L)	PSbile [c, d)] (µL/min/mg)
1.01	4.42 ± 0.21	0.0935	0.413 ± 0.020	0.293 ± 0.301
2.76	11.4 ± 0.6	0.0935	1.07 ± 0.06	0.452 ± 0.361
8.93	31.4 ± 1.8	0.0935	2.93 ± 0.16	0.285 ± 0.244
27.0	81.9 ± 2.1	0.0935	7.66 ± 0.20	0.414 ± 0.171
90.0	235 ± 15	0.0935	22.0 ± 1.4	0.471 ± 0.321

[a)] Measured concentration of application solution (S). (nominal concentrations: 1, 3, 10, 30, and 100 µmol/L)

[b)] Measured intracellular concentration (C)

[c)] Mean ± SD, n = 3

[d)] Average protein concentration was 0.499 and 0.407 mg protein/mL in (+)buffer and (-)buffer, respectively

[e)] estimated based on the equation with logD (pH 6.8) = 0.44: $\log(fu,hep) = -0.9161 - 0.2567 \times \log(D)$

[f)] Corrected unbound intracellular concentration (Cu) = C / fu,hep

Key results: The present data generated in sandwich-cultured human hepatocytes *in vitro* suggest that LNP023 is likely to be subject to active transport into the bile.

2.6.5.16K　排出及び取り込みトランスポーターフェノタイピング（DMPK R1800300）

Study title:	*In vitro* Transporter Phenotyping Study of LNP023 with the human BCRP, MDR1 and MRP2 Efflux (ABC) and with the human OATP1B1 and OATP1B3 uptake Transporters	Report no. DMPK R1800300
Location in CTD:	4.2.2.7-11	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl, ^{14}C-LNP023·HCl	
Study system:	BCRP or P-gp transfected MDCKII cell monolayers	
Analytical method:	Bidirectional transport of LNP023 in MDCKII-BCRP and MDCKII-P-gp cells, the amount of accumulated LNP023 retained inside the vesicles, the amount of LNP023 in the cell lysate by LSC	
Probe substrate:	$E_2$17βG	

The monolayer assays were performed using BCRP or P-gp transfected MDCKII cell monolayers. Trans-epithelial electric resistance (TEER) of each well was measured to confirm the confluency of the monolayers after the experiments. Permeability incubations were carried out in Hank's Buffered Salt Solution at 37 ± 1°C. Apical to basolateral permeability of Lucifer Yellow (LY) was assessed as a low permeability control, and antipyrine was as a high permeability compound. LY was also incubated in the presence of LNP023 at the highest testing concentration (800 µmol/L) to assess the effect of LNP023 on the monolayer integrity. LY samples were analyzed by measuring fluorescence with the following wavelengths: excitation – 430 nm, emission – 520 nm, while the antipyrine samples were analyzed with LC-MS/MS.

Bidirectional transport of LNP023 was determined through MDCKII-BCRP and MDCKII-P-gp cell monolayers. Inhibitors were added to the preincubation medium. Assay buffer containing LNP023 at twelve concentrations (0.5-800.0 µmol/L) was then added to the appropriate apical or basolateral chamber. After incubation at 37 ± 1°C, aliquots were taken from the receptor chambers to determine the amount of translocated LNP023 and controls. Samples were taken from the donor chambers before and after incubation to determine the initial concentration (C0) and recovery (R) of the test compound and the controls. Bidirectional transport of LNP023 in MDCKII-BCRP and MDCKII-P-gp cells was determined by LSC.

Bidirectional transport of LNP023 in MDCKII-BCRP and MDCKII-P-gp was also determined in the presence and absence of BCRP inhibitor Ko143 and P-gp inhibitor LY335979 individually and mixed together to confirm the specificity of the transport in MDCKII-BCRP and MDCKII-P-gp cells. The transported compound was applied at one selected concentration (0.5 µmol/L) and incubation time (120 min). The effect of the reference inhibitors was studied at one concentration (1 µmol/L Ko143, 5 µmol/L LY335979).

Summary of results

Transporter assay	Maximum ER	Substrate
BCRP monolayer assay	5.98 ± 0.48 (at 10 µmol/L)	Likely [a)]
P-gp monolayer assay	7.86 ± 0.91 (at 400 µmol/L)	Likely [a)]
Transporter assay	**Maximum fold accumulation**	**Substrate**
MRP2 vesicular transport assay	2.71 at 25 µmol/L	Likely [b)]
OATP1B1 uptake transporter assay	2.80 at 1 µmol/L	Likely (Km = 82.5 ± 43.6 µmol/L) [c)]
OATP1B3 uptake transporter assay	4.39 at 0.25 µmol/L	Likely (Km = 17.1 ± 4.73 µmol/L) [c)]

ER: efflux ratio (expressed as mean ± SD, n = 3)

[a] If the efflux ratio is > 2 - and can be inhibited by a known inhibitor of the transporter - the test article can be considered being a substrate of the respective transporter.

[b] If the ATP-dependent fold accumulation value is > 2 in transporter containing vesicles and can be inhibited by a known inhibitor of the transporter, then the test article can be considered a substrate of the transporter investigated.

[c] If the fold accumulation value is > 2 and can be inhibited by a known inhibitor of the transporter, then the test article can be considered a substrate of the transporter investigated.

Bidirectional permeability and efflux ratio values for LNP023 across MDCKII-BCRP monolayers in the absence and presence of BCRP or/and P-gp inhibitors

Compound	Concentration (µmol/L)	Papp A-B [a] (× 10^{-6} cm/s)	Papp B-A [a] (× 10^{-6} cm/s)	ER [a]
LNP023	0.5	1.48 ± 0.22	8.76 ± 0.13	5.93 ± 0.90
	1	1.46 ± 0.14	8.01 ± 0.26	5.50 ± 0.57
	2.5	1.51 ± 0.15	8.33 ± 0.54	5.53 ± 0.66
	5	1.64 ± 0.08	8.36 ± 0.31	5.11 ± 0.31
	10	1.57 ± 0.11	9.38 ± 0.38	5.98 ± 0.48
	25	1.51 ± 0.09	8.70 ± 0.78	5.77 ± 0.62
	50	1.63 ± 0.18	9.04 ± 0.19	5.54 ± 0.63
	100	1.66 ± 0.10	8.80 ± 0.53	5.29 ± 0.45
	175	1.62 ± 0.06	9.24 ± 0.25	5.69 ± 0.25
	300	1.45 ± 0.16	7.86 ± 0.29	5.42 ± 0.62
	400	1.46 ± 0.07	8.09 ± 0.18	5.55 ± 0.29
	800	1.48 ± 0.14	7.77 ± 0.10	5.23 ± 0.52
LNP023	0.5	1.48 ± 0.22	8.76 ± 0.13	5.93 ± 0.90
LNP023 + Ko143	0.5 + 1	2.43 ± 0.19	2.41 ± 0.16	0.99 ± 0.10
LNP023 + LY335979	0.5 + 5	1.96 ± 0.23	8.34 ± 0.23	4.26 ± 0.51
LNP023 + Ko143 + LY335979	0.5 + 1 + 5	2.88 ± 0.27	2.32 ± 0.11	0.81 ± 0.09

Data are mean ± SD, n = 3

[a] values calculated according the following equations:

$$P_{app} = \frac{dQ}{dT} \times \frac{1}{A \times C_0}, \quad ER = \frac{P_{app}\ B-A}{P_{app}\ A-B}$$

dQ: amount of transported test drug, dT: incubation time, A: surface of porous membrane in cm^2 (standard: 0.7), C_0: initial concentration of the compound in the donor compartment

Bidirectional permeability and efflux ratio values for LNP023 across MDCKII-P-gp monolayers in the absence and presence of P-gp or/and BCRP inhibitors				
Compound	Concentration (µmol/L)	Papp A-B [a)] (× 10^{-6} cm/s)	Papp B-A [a)] (× 10^{-6} cm/s)	ER [a)]
LNP023	0.5	1.89 ± 0.09	12.91 ± 0.51	6.83 ± 0.41
	1	2.01 ± 0.17	11.82 ± 0.14	5.89 ± 0.51
	2.5	1.89 ± 0.12	10.85 ± 0.92	5.75 ± 0.61
	5	1.94 ± 0.19	11.31 ± 0.49	5.82 ± 0.61
	10	2.14 ± 0.10	13.06 ± 0.41	6.11 ± 0.34
	25	1.87 ± 0.38	11.97 ± 0.77	6.42 ± 1.39
	50	1.81 ± 0.10	11.39 ± 0.25	6.30 ± 0.38
	100	1.91 ± 0.25	12.32 ± 0.71	6.44 ± 0.92
	175	1.61 ± 0.13	12.02 ± 0.47	7.46 ± 0.68
	300	1.69 ± 0.16	10.94 ± 0.51	6.47 ± 0.67
	400	1.39 ± 0.15	10.94 ± 0.45	7.86 ± 0.91
	800	1.51 ± 0.07	11.18 ± 0.58	7.40 ± 0.52
LNP023	0.5	1.89 ± 0.09	12.91 ± 0.51	6.83 ± 0.41
LNP023 + LY335979	0.5 + 5	3.25 ± 0.19	2.42 ± 0.13	0.75 ± 0.06
LNP023 + Ko143 + LY335979	0.5 + 1 + 5	2.38 ± 0.49	2.77 ± 0.17	1.17 ± 0.25

Data are mean ± SD, n = 3

[a)] values calculated according the following equations:

$$P_{app} = \frac{dQ}{dT} \times \frac{1}{A \times C_0} \qquad ER = \frac{P_{app}\ B-A}{P_{app}\ A-B}$$

dQ: amount of transported test drug, dT: incubation time, A: surface of porous membrane in cm^2 (standard: 0.7), C_0: initial concentration of the compound in the donor compartment

Accumulation of LNP023 in MRP2 containing vesicles measured in the vesicular transport substrate feasibility assay						
Compound	Conditions		Accumulation (pmol/mg protein)			ATP-dependent fold Accumulation [a)]
	(µmol/L)	(min)	ATP	AMP	ATP dependent [a)]	
LNP023	400		4426.88 ± 273.84	2118.58 ± 54.77	2308.30 ± 279.27	2.09
	300		3609.93 ± 217.58	1556.78 ± 179.08	2053.15 ± 281.80	2.32
	175		2164.31 ± 56.67	865.72 ± 56.67	1298.59 ± 80.15	2.50
	100		1375.12 ± 34.06	564.92 ± 71.68	810.21 ± 79.36	2.43
	50		702.37 ± 72.06	276.46 ± 32.35	425.90 ± 78.99	2.54
	25	8	370.76 ± 7.94	136.87 ± 13.08	233.89 ± 15.30	2.71
	10		140.77 ± 17.40	55.34 ± 7.51	85.42 ± 18.96	2.54
	5		71.99 ± 2.06	29.39 ± 0.57	42.60 ± 2.14	2.45
	2.5		42.17 ± 3.91	15.61 ± 2.14	26.56 ± 4.46	2.70
	1		18.05 ± 1.56	7.20 ± 0.37	10.84 ± 1.60	2.51
	0.5		7.56 ± 0.17	2.79 ± 0.23	4.77 ± 0.29	2.71

Data are mean ± SD, n = 3

[a)] values calculated according the following equations:

$$ATP - dependent\ transport = n_{ATP} - n_{AMP}$$

$$Fold\ accumulation = \frac{n_{ATP}}{n_{AMP}}$$

nATP: amount of translocated test article in the presence of 4 mmol/L ATP, in pmol/mg, nAMP: amount of translocated test article in the presence of 4 mmol/L AMP, in pmol/mg

Accumulation of LNP023 in OATP1B1 expressing and control cells measured in the uptake transporter substrate assay						
Compound	Conditions		Accumulation (pmol/mg protein)			Fold accumulation
	(µmol/L)	(min)	In HEK293-OATP1B1-LV cells	In HEK293 Mock-LV cells	Active transport	
LNP023	0.25	5	12.96 ± 1.66	5.20 ± 0.95	7.76 ± 1.91	2.49
	0.5		26.78 ± 6.11	10.51 ± 0.93	16.27 ± 6.18	2.55
	1		53.34 ± 6.60	19.06 ± 3.59	34.28 ± 7.51	2.80
	2.5		132.49 ± 3.70	53.74 ± 9.08	78.75 ± 9.81	2.47

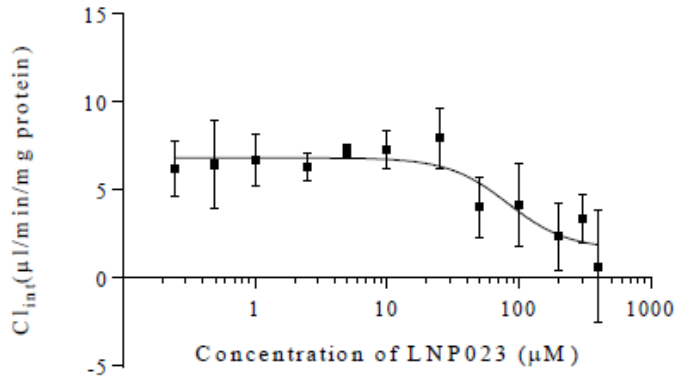
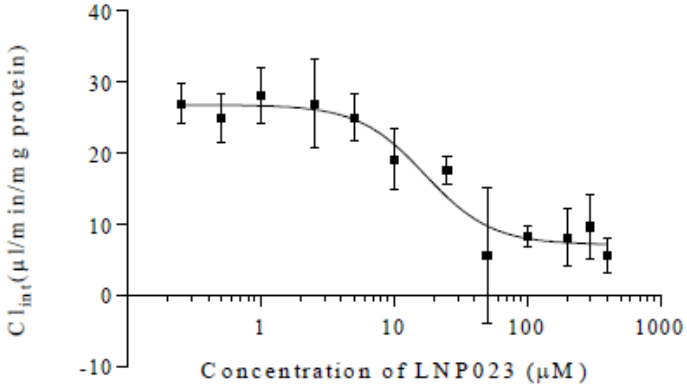
	5	303.33 ± 4.78	128.56 ± 7.75	174.77 ± 9.10	2.36
	10	617.77 ± 38.08	275.15 ± 32.91	342.62 ± 50.33	2.25
	25	1670.64 ± 195.88	748.27 ± 42.85	922.37 ± 200.51	2.23
	50	2602.07 ± 132.07	1638.17 ± 391.25	963.90 ± 412.94	1.59
	100	5225.08 ± 1075.99	3254.74 ± 298.76	1970.34 ± 1116.70	1.61
	200	8708.22 ± 1606.89	6539.67 ± 791.03	2168.55 ± 1791.04	1.33
	300	15111.16 ± 1967.03	10144.96 ± 445.98	4966.19 ± 2016.96	1.49
	400	18065.36 ± 4858.71	16794.60 ± 3968.35	1270.76 ± 6273.35	1.08

Data are mean ± SD, n = 3

Accumulation of LNP023 in OATP1B3 expressing and control cells measured in the uptake transporter substrate assay

Compound	Conditions		Accumulation (pmol/mg protein)			Fold accumulation
	(µmol/L)	(min)	In transporter expressing cells	In control cells	Active transport	
LNP023	0.25	5	43.69 ± 2.58	9.96 ± 2.59	33.73 ± 3.65	4.39
	0.5		87.67 ± 7.37	24.55 ± 4.53	63.12 ± 8.65	3.57
	1		187.48 ± 17.69	46.45 ± 8.83	141.04 ± 19.77	4.04
	2.5		452.71 ± 77.60	117.11 ± 6.74	335.60 ± 77.89	3.87
	5		894.53 ± 82.15	271.45 ± 7.77	623.08 ± 82.52	3.30
	10		1547.33 ± 191.43	593.92 ± 91.26	953.42 ± 212.07	2.61
	25		3968.10 ± 235.73	1798.75 ± 67.03	2169.34 ± 245.07	2.21
	50		6989.13 ± 1306.16	5546.92 ± 2056.78	1442.21 ± 2436.47	1.26
	100		12917.09 ± 399.68	8739.11 ± 564.04	4177.98 ± 691.29	1.48
	200		22788.34 ± 3746.34	14958.43 ± 1141.36	7829.91 ± 3916.35	1.52
	300		36968.42 ± 6779.05	22621.44 ± 702.81	14346.98 ± 6815.39	1.63
	400		44920.31 ± 2921.35	34015.64 ± 3932.70	10904.67 ± 4899.02	1.32

Data are mean ± SD, n = 3

Curve fitting of LNP023 intrinsic clearance in OATP1B1-expressing and control cells in the uptake transporter substrate assay	
Curve fitting of LNP023 intrinsic clearance in OATP1B3-expressing and control cells in the uptake transporter substrate assay	
Key results: LNP023 is a substrate of BCRP and P-gp ABC (efflux) transporters and likely a substrate of MRP2 ABC (efflux) transporter. LNP023 is a substrate of the OATP1B3 and to a lesser extent of the OATP1B1 uptake transporters.	

2.6.5.16L　取り込みトランスポーターフェノタイピング（DMPK R2000245）

Study title:	*In vitro* Transporter Phenotyping Study of LNP023 with the human OAT1, OAT2, OAT3 and OCT2 Uptake Transporters	Report no. DMPK R2000245
Location in CTD:	4.2.2.7-12	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Study system:	HEK293, MDCKII, and CHO cells stably expressing the respective uptake transporters (human OAT1, OAT2v1, OAT3, OCT2)	
Ptobe substrate / Assay:	Tenofovir, cGMP, E3S, Metformin / The amount of LNP023 in the cell lysate by LSC, the amount of protein by the BCA kit for protein determination	

Uptake experiments were performed using HEK293, MDCKII, and CHO cells stably expressing the respective uptake transporters.

The uptake of LNP023 was determined using cells overexpressing the human OAT1, OAT2, OAT3, and OCT2 uptake (SLC) transporter and control cells, at two incubation time points (5 and 10 min) and overlapping concentration range (0.1-400 µmol/L) to determine whether LNP023 was actively taken up into the cells. To confirm the interaction, the transporter-specific uptake of LNP023 was also determined in the presence of a known inhibitors of the transporters (at 0.1 µmol/L LNP023 concentration), as well.

Summary of results

Transporter assay	Maximum fold accumulation	Substrate [a)]
OAT1 uptake transporter assay (HEK293)	< 2 (1.21) at 2.5 µmol/L	No
OAT1 uptake transporter assay (HEK293)	< 2 (1.34) at 0.1 µmol/L	No
OAT1 uptake transporter assay (CHO)	< 2 (0.83 and 1.0) at 0.1 µmol/L	No
OAT2v1 uptake transporter assay (HEK293)	< 2 (1.29) at 0.1 µmol/L	No
OAT3 uptake transporter assay (HEK293)	< 2 (1.49) at 5 µmol/L	No
OAT3 uptake transporter assay (HEK293)	< 2 (1.52) at 0.1 µmol/L	No
OAT3 uptake transporter assay (MDCKII)	< 2 (1.14) at 1 and 3 µmol/L	No
OCT2 uptake transporter assay (CHO)	< 2 (0.97 and 1.09) at 0.1 µmol/L	No

[a)] If the fold accumulation value is > 2 and can be inhibited with at least 50% by a known inhibitor of the transporter the test article can be considered a substrate of the respective transporter.

Accumulation of LNP023 in OAT1 expressing and control cells in the absence and presence of specific inhibitors measured in uptake transporter substrate assay						
Compound	Conditions		Accumulation (pmol/mg protein)			Fold accumulation [a)]
	(µmol/L)	(min)	In HEK293 OAT1-LV	In HEK293 Mock-B-LV	Active transport	
LNP023	400	10	6957.29 ± 267.44	7145.82 ± 461.44	−188.53 ± 533.34	0.97
	200		3274.17 ± 132.69	3272.94 ± 129.86	1.23 ± 185.66	1.00
	100		1714.02 ± 142.14	1656.65 ± 339.67	57.37 ± 368.21	1.03
	50		796.97 ± 55.68	768.76 ± 81.41	28.22 ± 98.63	1.04
	25		348.98 ± 25.26	322.36 ± 2.28	26.62 ± 25.36	1.08
	10		117.03 ± 8.51	96.79 ± 4.17	20.24 ± 9.47	1.21
	5		47.68 ± 1.49	40.63 ± 1.43	7.05 ± 2.07	1.17
	2.5		25.23 ± 2.34	20.86 ± 1.36	4.37 ± 2.71	1.21
	1		7.99 ± 0.33	8.14 ± 0.29	−0.15 ± 0.44	0.98
	0.5		3.83 ± 0.11	3.57 ± 0.18	0.26 ± 0.21	1.07
	0.25		1.84 ± 0.03	1.86 ± 0.01	−0.03 ± 0.03	0.99
	0.1		0.88 ± 0.07	1.08 ± 0.12	−0.19 ± 0.14	0.82
LNP023	0.1	5	0.68 ± 0.03	0.51 ± 0.02	0.17 ± 0.04	1.34
LNP023 + Probenecid	0.1 + 500		1.28 ± 0.01	1.23 ± 0.06	0.05 ± 0.06	1.04
LNP023 + Ko143	0.1 + 10		1.00 ± 0.05	0.89 ± 0.03	0.10 ± 0.06	1.12
LNP023 + Probenecid + Ko143	0.1 + 500 + 10		1.36 ± 0.07	1.22 ± 0.05	0.14 ± 0.09	1.12

Data are mean ± SD, n = 3.

[a)] Values calculated according equation: $$Fold\ accumulation = \frac{UTP_{TRP}}{UTP_{CTRL}}$$

UPT_{TRP}: accumulated amount of test article or probe substrate in transfected cells normalized by protein content [pmol/mg protein]

UPT_{CTRL}: accumulated amount of test article or probe substrate in control cells normalized by protein content [pmol/mg protein]

Accumulation of LNP023 in CHO-K1-OAT1 expressing and control cells measured in uptake transporter substrate assay						
Compound	Conditions		Accumulation (pmol/mg protein)			Fold accumulation [a)]
	(µmol/L)	(min)	In CHO-K1-OAT1	In CHO-K1	Active transport	
LNP023	300	5	1764.69 ± 94.86	1895.79 ± 129.40	−131.10 ± 160.44	0.93
	100		507.56 ± 9.29	589.97 ± 45.80	−82.42 ± 46.73	0.86
	30		139.67 ± 3.79	180.81 ± 20.77	−41.14 ± 21.11	0.77
	10		43.94 ± 1.79	64.35 ± 10.01	−20.41 ± 10.17	0.68
	3		11.15 ± 0.16	15.00 ± 2.64	−3.85 ± 2.65	0.74
	1		3.46 ± 0.08	4.27 ± 0.02	−0.81 ± 0.08	0.81
	0.3		1.15 ± 0.03	1.36 ± 0.03	−0.21 ± 0.04	0.85
	0.1		0.49 ± 0.08	0.49 ± 0.02	0.00 ± 0.09	1.00
LNP023	0.1	5	0.71 ± 0.04	0.85 ± 0.17	−0.15 ± 0.18	0.83
LNP023 + Ko143	0.1 + 10		1.65 ± 0.31	2.09 ± 0.47	−0.44 ± 0.56	0.79
LNP023 + Probenecid	0.1 + 500		0.75 ± 0.04	0.78 ± 0.05	−0.03 ± 0.07	0.96
LNP023 + Probenecid + Ko143	0.1 + 500 + 10		1.15 ± 0.07	1.18 ± 0.08	−0.04 ± 0.11	0.97
LNP023 + efflux cocktail	0.1 + efflux cocktail		1.82 ± 0.35	1.98 ± 0.07	−0.17 ± 0.36	0.92

Data are mean ± SD, n = 3.

[a)] Values calculated according equation: $$Fold\ accumulation = \frac{UTP_{TRP}}{UTP_{CTRL}}$$

UPT_{TRP}: accumulated amount of test article or probe substrate in transfected cells normalized by protein content [pmol/mg protein]

UPT_{CTRL}: accumulated amount of test article or probe substrate in control cells normalized by protein content [pmol/mg protein]

Accumulation of LNP023 in HEK293-OAT2v1 expressing and control cells measured in uptake transporter substrate assay

Compound	Conditions		Accumulation (pmol/mg protein)			Fold accumulation [a)]
	(µmol/L)	(min)	In HEK293-OAT2v1-LV	In HEK293-Mock-LV	Active transport	
LNP023	300	5	3581.19 ± 106.44	4200.02 ± 199.33	−618.83 ± 225.97	0.85
	100		1074.59 ± 13.02	1348.27 ± 69.82	−273.67 ±71.02	0.80
	30		340.35 ± 9.82	384.66 ± 8.36	−44.31 ± 12.89	0.88
	10		83.26 ± 1.68	83.99 ± 3.40	−0.73 ± 3.79	0.99
	3		20.84 ± 0.71	17.93 ± 1.41	2.91 ± 1.58	1.16
	1		6.43 ± 0.10	5.29 ± 0.47	1.14 ± 0.48	1.22
	0.3		1.81 ± 0.03	1.68 ± 0.06	0.13 ± 0.06	1.08
	0.1		0.63 ± 0.05	0.59 ± 0.05	0.05 ± 0.07	1.08
LNP023	0.1	5	0.97 ± 0.03	0.75 ± 0.06	0.22 ± 0.07	1.29
LNP023 + Ko143	0.1 + 10		1.26 ± 0.04	1.23 ± 0.07	0.02 ± 0.08	1.02
LNP023 + Probenecid	0.1 + 500		0.89 ± 0.05	0.87 ± 0.02	0.02 ± 0.05	1.03
LNP023 + indomethacin	0.1 + 300		1.61 ± 0.14	1.79 ± 0.17	−0.18 ± 0.22	0.90
LNP023 + Probenecid + Ko143	0.1 + 500 + 10		1.26 ± 0.06	1.12 ± 0.07	0.15 ± 0.09	1.13
LNP023 + efflux cocktail	0.1 + efflux cocktail		1.56 ± 0.11	1.50 ± 0.06	0.06 ± 0.13	1.04
LNP023 + efflux cocktail + indomethacin	0.1 + efflux cocktail + 300		1.69 ± 0.12	1.84 ± 0.17	−0.15 ± 0.21	0.92
LNP023 + indomethacin + Ko143	0.1 + 300 +10		1.86 ± 0.04	2.03 ± 0.06	−0.16 ± 0.08	0.92

Data are mean ± SD, n = 3.

[a)] Values calculated according equation: $$Fold\ accumulation = \frac{UTP_{TRP}}{UTP_{CTRL}}$$

UPT_{TRP}: accumulated amount of test article or probe substrate in transfected cells normalized by protein content [pmol/mg protein]

UPT_{CTRL}: accumulated amount of test article or probe substrate in control cells normalized by protein content [pmol/mg protein]

Accumulation of LNP023 in OAT3 expressing and control cells in the absence and presence of specific inhibitors measured in uptake transporter substrate assay						
Compound	Conditions		Accumulation (pmol/mg protein)			Fold accumulation [a)]
	(µmol/L)	(min)	In HEK293 OAT3-LV	In HEK293 Mock-LV	Active transport	
LNP023	400	10	6834.04 ± 89.57	7976.12 ± 860.68	−1142.08 ±865.33	0.86
	200		3257.14 ± 83.97	3443.53 ± 36.93	−186.40 ± 91.73	0.95
	100		1644.42 ± 45.29	1604.82 ± 45.89	39.60 ± 64.48	1.02
	50		821.48 ± 73.20	754.35 ± 91.54	67.13 ± 117.21	1.09
	25		413.53 ± 7.05	338.37 ± 1.42	75.16 ± 7.19	1.22
	10		139.22 ± 4.23	99.78 ± 1.65	39.44 ± 4.54	1.40
	5		58.06 ± 1.50	38.89 ± 1.92	19.17 ± 2.44	1.49
	2.5		26.58 ± 0.68	18.91 ± 0.35	7.67 ± 0.77	1.41
	1		9.48 ± 0.48	6.95 ± 0.28	2.53 ± 0.55	1.36
	0.5		4.43 ± 0.36	3.81 ± 0.23	0.61 ± 0.43	1.16
	0.25		4.11 ± 0.15	3.30 ± 0.19	0.80 ± 0.24	1.24
	0.1		0.77 ± 0.04	0.58 ± 0.02	0.19 ± 0.04	1.33
LNP023	0.1	5	1.99 ± 0.13	1.31 ± 0.10	0.68 ± 0.16	1.52
LNP023 + Probenecid	0.1 + 500		2.62 ± 0.21	2.20 ± 0.03	0.42 ± 0.22	1.19
LNP023 + Ko143	0.1 + 10		2.33 ± 0.04	1.79 ± 0.13	0.54 ± 0.14	1.30
LNP023 + Probenecid + Ko143	0.1 + 500 + 10		2.78 ± 0.08	2.31 ± 0.17	0.47 ± 0.19	1.20

Data are mean ± SD, n = 3.

[a)] Values calculated according equation:

$$Fold\ accumulation = \frac{UTP_{TRP}}{UTP_{CTRL}}$$

UPT_{TRP}: accumulated amount of test article or probe substrate in transfected cells normalized by protein content [pmol/mg protein]

UPT_{CTRL}: accumulated amount of test article or probe substrate in control cells normalized by protein content [pmol/mg protein]

Accumulation of LNP023 in MDCKII-OAT3 expressing and control cells measured in uptake transporter substrate assay						
Compound	Conditions		Accumulation (pmol/mg protein)			Fold accumulation [a)]
	(µmol/L)	(min)	In MDCKII-OAT3	In MDCKII	Active transport	
LNP023	300	5	1969.85 ± 118.63	1885.81 ± 216.52	84.04 ± 246.89	1.04
	100		644.97 ± 8.53	608.06 ± 12.23	36.91 ± 14.91	1.06
	30		205.97 ± 6.54	187.44 ± 7.67	18.53 ± 10.08	1.10
	10		68.11 ± 1.74	61.59 ± 3.22	6.52 ± 3.66	1.11
	3		18.58 ± 0.10	16.30 ± 0.09	2.28 ± 0.14	1.14
	1		6.18 ± 0.34	5.44 ± 0.17	0.74 ± 0.38	1.14
	0.3		2.39 ± 0.13	2.35 ± 0.31	0.04 ± 0.33	1.02
	0.1		0.79 ± 0.02	0.73 ± 0.03	0.06 ± 0.03	1.08
LNP023	0.1	5	3.33 ± 0.10	3.16 ± 0.11	0.17 ± 0.15	1.06
LNP023 + Ko143	0.1 + 10		3.46 ± 0.13	3.41 ± 0.24	0.05 ± 0.27	1.01
LNP023 + Probenecid	0.1 + 500		2.12 ± 0.23	2.38 ± 0.12	−0.26 ± 0.26	0.89
LNP023 + Probenecid + Ko143	0.1 + 500 + 10		2.45 ± 0.36	2.75 ± 0.13	−0.30 ± 0.39	0.89
LNP023 + efflux cocktail	0.1 + efflux cocktail		3.94 ± 1.04	3.73 ± 0.16	0.21 ± 1.05	1.06

Data are mean ± SD, n = 3.

[a)] Values calculated according equation: $$Fold\ accumulation = \frac{UTP_{TRP}}{UTP_{CTRL}}$$

UPT_{TRP}: accumulated amount of test article or probe substrate in transfected cells normalized by protein content [pmol/mg protein]

UPT_{CTRL}: accumulated amount of test article or probe substrate in control cells normalized by protein content [pmol/mg protein]

Accumulation of LNP023 in CHO-OCT2 expressing and control cells measured in uptake transporter substrate assay						
Compound	Conditions		Accumulation (pmol/mg protein)			Fold accumulation [a)]
	(µmol/L)	(min)	In CHO-K1-OCT2	In CHO-K1	Active transport	
LNP023	300	5	1654.28 ±175.41	1766.76 ± 96.12	−112.48 ± 200.02	0.94
	100		479.30 ± 16.90	497.52 ± 15.45	−18.22 ± 22.90	0.96
	30		137.33 ± 7.10	159.07 ± 7.65	−21.75 ± 10.44	0.86
	10		42.28 ± 2.10	54.03 ± 1.57	−11.75 ± 2.62	0.78
	3		10.83 ± 0.36	12.09 ± 1.28	−1.25 ± 1.33	0.90
	1		3.50 ± 0.06	3.60 ± 0.31	−0.10 ± 0.31	0.97
	0.3		1.49 ± 0.04	1.71 ± 0.15	−0.21 ± 0.16	0.88
	0.1		0.55 ± 0.02	0.62 ± 0.15	−0.07 ± 0.15	0.89
LNP023	0.1	5	2.27 ± 0.13	2.08 ± 0.28	0.19 ± 0.31	1.09
LNP023 + Ko143	0.1 + 10		3.47 ± 0.16	3.84 ± 0.29	−0.38 ± 0.33	0.90
LNP023 + Verapamil	0.1 + 100		3.24 ± 0.07	3.57 ± 0.38	−0.33 ± 0.39	0.91
LNP023 + Verapamil + Ko143	0.1 + 100 + 10		3.43 ± 0.22	4.24 ± 1.06	−0.81 ± 1.09	0.81
LNP023 + efflux cocktail	0.1 + efflux cocktail		3.71 ± 0.03	6.80 ± 3.13	−3.09 ± 3.13	0.55

Data are mean ± SD, n = 3.

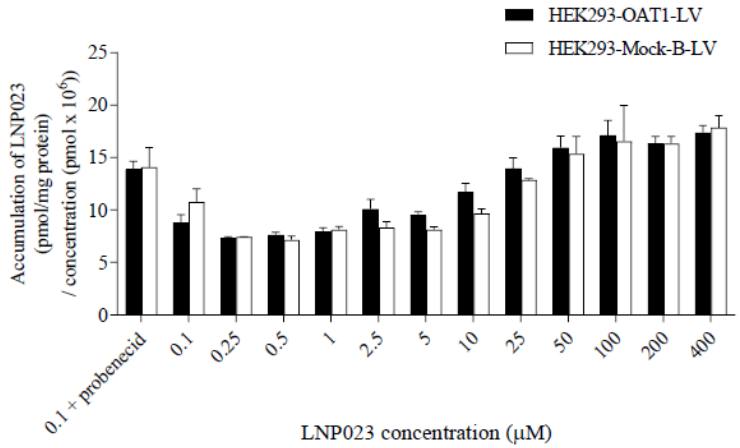
[a)] Values calculated according equation: $$Fold\ accumulation = \frac{UTP_{TRP}}{UTP_{CTRL}}$$

UPT_{TRP}: accumulated amount of test article or probe substrate in transfected cells normalized by protein content [pmol/mg protein]

UPT_{CTRL}: accumulated amount of test article or probe substrate in control cells normalized by protein content [pmol/mg protein]

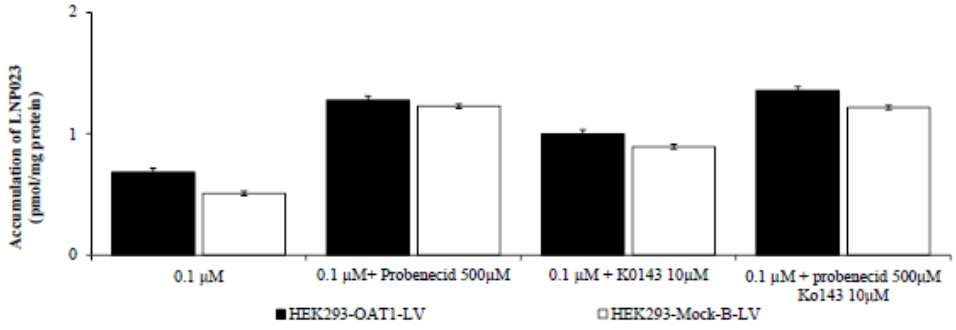
Accumulation of LNP023 in OAT1-expressing and control cells (HEK293) in the uptake transporter substrate assay in the absence and presence of the reference inhibitor of OAT1 (probenecid) at 10 minutes

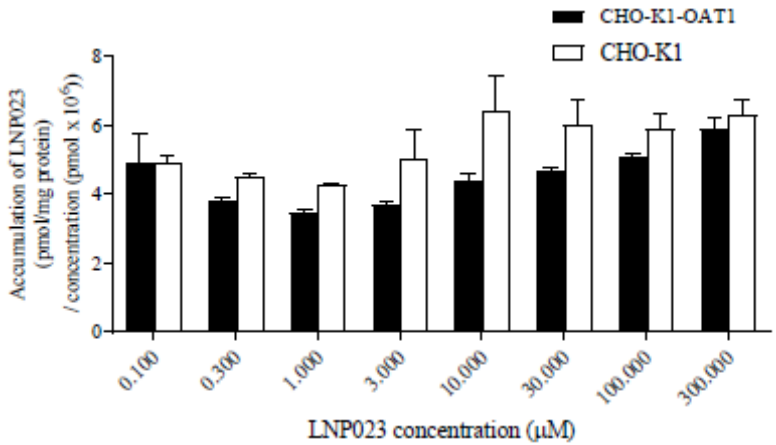
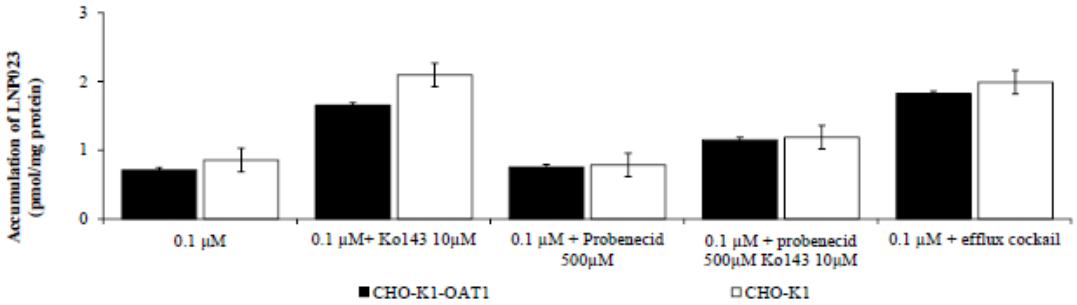
OAT1 uptake transporter substrate assay – LNP023

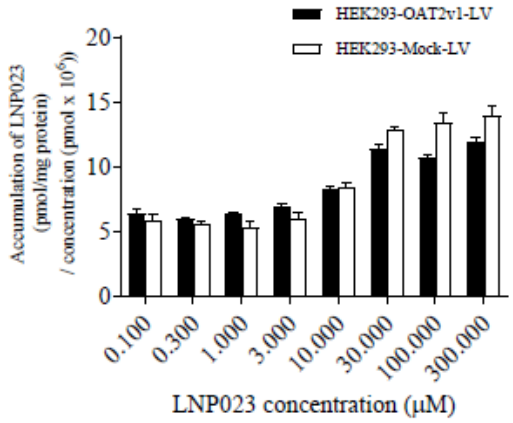
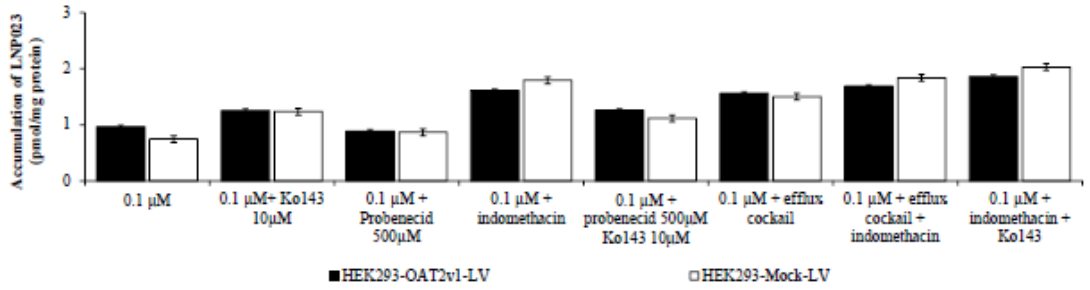


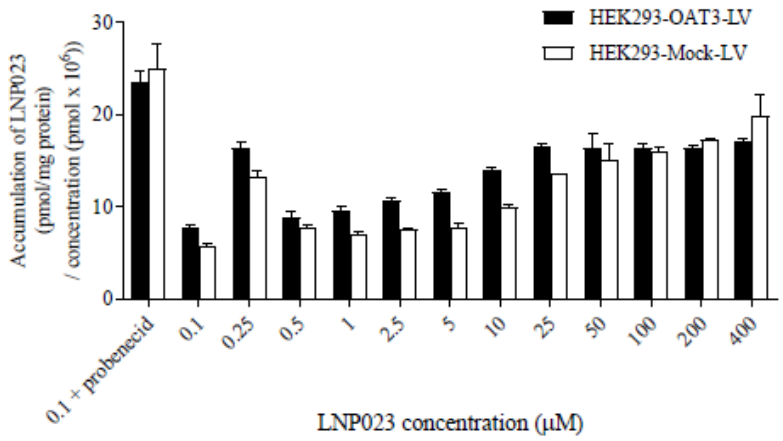
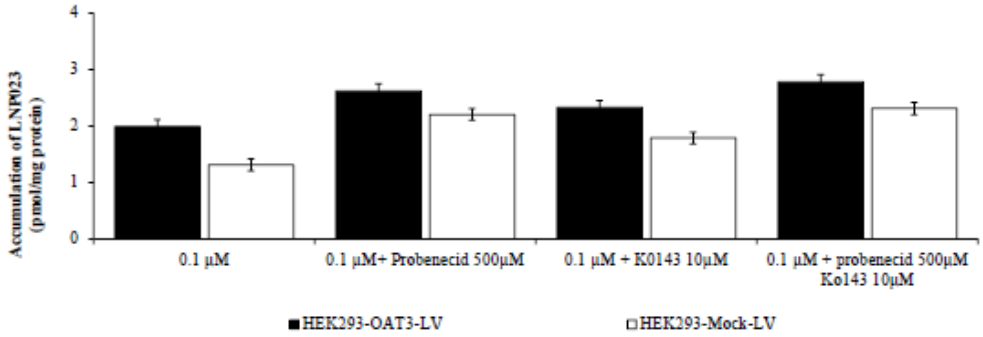
Accumulation of LNP023 in OAT1-expressing and control cells (HEK293) in the uptake transporter substrate assay in the absence and presence of the inhibitors (Ko143, probenecid) at 5 minutes

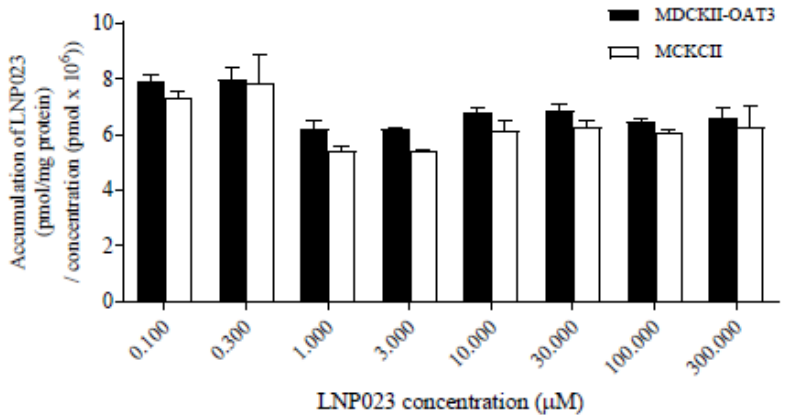
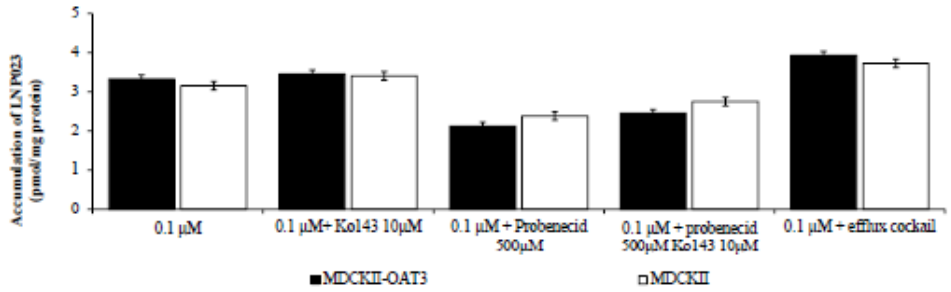
OAT1 uptake transporter substrate assay – LNP023

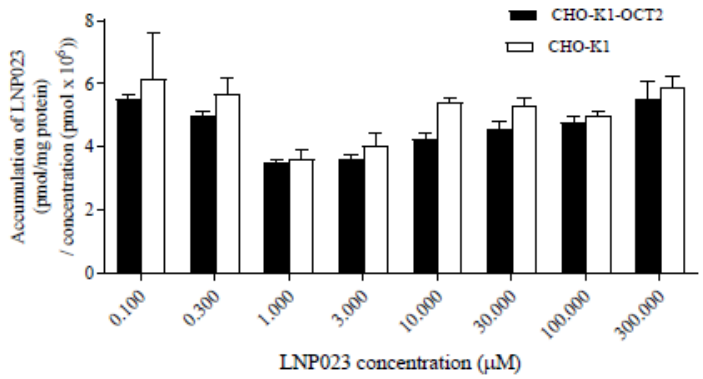
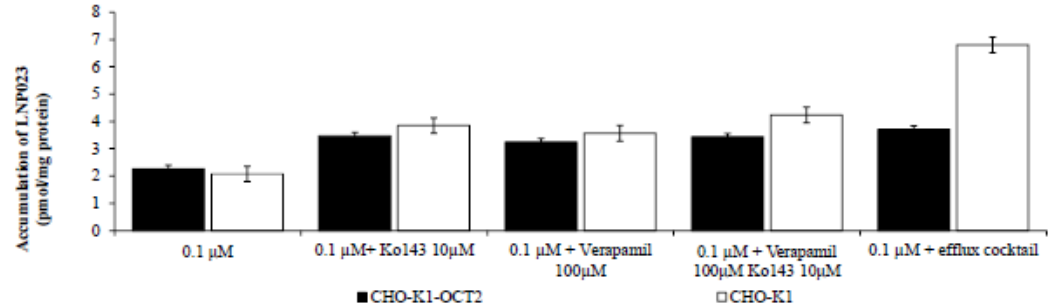


Accumulation of LNP023 in OAT1-expressing and control cells (CHO-K1) in the uptake transporter substrate assay in the absence and presence of inhibitors (Ko143, probenecid, efflux cocktail) at 5 minutes
OAT1 uptake transporter substrate assay – LNP023 
OAT1 uptake transporter substrate assay – LNP023 with specific inhibitors 

Accumulation of LNP023 in OAT2v1-expressing and control cells (HEK293) in the uptake transporter substrate assay in the absence and presence of inhibitors in the absence and presence of inhibitors (Ko143, probenecid, indomethacin, efflux cocktail) at 5 minutes
OAT2v1 uptake transporter substrate assay – LNP023 
OAT2v1 uptake transporter substrate assay – LNP023 with specific inhibitors 

Accumulation of LNP023 in OAT3-expressing and control cells (HEK293) in the uptake transporter substrate assay in the absence and presence of the reference inhibitor of OAT3 (probenecid) at 10 minutes
OAT3 uptake transporter substrate assay – LNP023 
Accumulation of LNP023 in OAT3-expressing and control cells (HEK293) in the uptake transporter substrate assay in the absence and presence of the inhibitors (Ko143, probenecid) at 5 minutes
OAT3 uptake transporter substrate assay – LNP023 

Accumulation of LNP023 in OAT3-expressing and control cells (MDCKII) in the uptake transporter substrate assay in the absence and presence of inhibitors (Ko143, probenecid, efflux cocktail) at 5 minutes	
OAT3 uptake transporter substrate assay – LNP023	
OAT3 uptake transporter substrate assay – LNP023 with specific inhibitors	

Accumulation of LNP023 in OCT2-expressing and control cells (CHO-K1) in the uptake transporter substrate assay in the absence and presence of inhibitors (Ko143, probenecid, verapamil, efflux cocktail) at 5 minutes
OCT2 uptake transporter substrate assay – LNP023 
OCT2 uptake transporter substrate assay – LNP023 with specific inhibitors 
Key results: LNP023 is not a substrate of either OAT1, OAT2, OAT3, and OCT2 uptake (SLC) transporters.

2.6.5.16M　ヒトに 1 日 2 回経口投与後の in vivo 代謝（DMPK BT-R1701500-metid）

Study title:	Exploratory metabolite assessment and semi-quantitation of metabolites of LNP023 in human plasma from clinical study CLNP023X2101 following twice daily oral doses of 200 mg	Report no. DMPK BT-R1701500-metid
Location in CTD:	4.2.2.7-13	
GLP compliance:	Not required	
Test article(s):	LNP023·HCl	
Species:	Human	
Gender(M/F) / Number of subjects:	Male / 5, Female / 1 (CLNP023X2101)	
Feeding condition:	Under fed condition (CLNP023X2101)	
Formulation:	Capsule (CLNP023X2101)	
Method of Administration:	Oral	
Dose:	200 mg bid	
Sample matrix:	Plasma	
Sampling schedule:	Day 14 plasma AUC0-8h pool: plasma from each time point were pooled in a time-dependent manner to generate single samples, for subjects, containing LNP023 and its metabolites at the same ratios as in the AUC0-8h	
Analyte(s):	LNP023 and metabolites	
Analytical methods:	Metabolites in the prepared AUC pools were identified by UPLC-MS(/MS), semi-quantified by UPLC-MS	

Metabolites identified in an AUC0-8h plasma pool, day 14 and semi-quantitative exposure estimates as % of parent following multiple 200 mg bid oral doses of LNP023

Subject id	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	LNP023
[redacted]	+	+	nd	nd	nd	+	+	30.8%	18.0%	nd	100%
[redacted]	+	+	nd	nd	nd	+	+	21.7%	17.7%	nd	100%
[redacted]	+	+	nd	nd	nd	+	+	30.9%	11.4%	nd	100%
[redacted]	+	+	nd	nd	nd	+	+	47.9%	21.8%	nd	100%
[redacted]	+	+	nd	nd	nd	+	+	30.2%	26.1%	nd	100%
[redacted]	+	+	nd	nd	nd	+	+	19.3%	16.2%	nd	100%
Mean	na	na	na	na	na	na	na	30.2%	18.5%	na	100%

nd: not detected, na: not applicable, +: detected but not semi-quantified

Key results: All six metabolites identified (M1, M2, M6, M7, M8, and M9), have been previously characterized in other DMPK studies. Metabolites M3, M4, M5, and M10 were not detected in day 14 AUC0-8h human plasma samples.