2.6.4 薬物動態試験の概要文

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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）
ADME	absorption, distribution, metabolism and excretion	吸収・分布・代謝・排泄
AUC0-t	area under the drug plasma (serum/blood) concentration-time curve (AUC) from time zero to time 't' where t is a defined time point after administration	0 時間から t 時間までの血漿（血清／血液）中薬物濃度-時間曲線下面積
AUCinf	area under the drug plasma (serum/blood) concentration-time curve from time zero to infinity	0 時間から無限大時間までの血漿（血清／血液）中薬物濃度-時間曲線下面積
AUClast	area under the drug plasma (serum/blood) concentration-time curve from time zero to the time of the last quantifiable concentration	0 時間から最終濃度測定可能時点までの血漿（血清／血液）中薬物濃度-時間曲線下面積
AUCtau,ss	area under the drug plasma (serum/blood) concentration-time curve for a dosing interval at steady state	定常状態における投与間隔内の血漿（血清／血液）中薬物濃度-時間曲線下面積
bi	bile	胆汁
Cb/Cp	blood-to-plasma ratio	血液／血漿中濃度比
Cmax	maximal drug plasma (serum/blood) concentration	最高血漿（血清／血液）中薬物濃度
CL	clearance	クリアランス
CLb	clearance from blood	血中クリアランス
CLp	clearance from plasma	血漿クリアランス
CL/F	apparent clearance	見かけのクリアランス
CYP	cytochrome P450 enzyme	チトクローム P450
F	boavailability	バイオアベイラビリティ
fe	Feces	糞
FMO3	Flavin-containing monooxygenase enzyme 3	フラビン含有モノオキシゲナーゼ 3
fp	fraction bound in plasma	血漿中結合画分
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
iv	intravenous	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/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
Ln_1	lymph nodes (mandibular)	リンパ節（下顎）
Ln_2	lymph nodes (mesenteric)	リンパ節（腸間膜）
LSC	liquid scintillation counter	液体シンチレーションカウンター
MAOA/B	monoamine oxidase A and	モノアミンオキシダーゼ A 及び B
na	not applicable	該当なし
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	有機アニオン輸送ポリペプチド

略号	省略していない表現（英）	省略していない表現（日）
OCT	organic cation transporter	有機カチオントランスポーター
PAH	*p*-aminohippuric acid	*p*-アミノ馬尿酸
PK	oharmacokinetic	薬物動態
QWBA	quantitative whole-body autoradiography	定量的全身オートラジオグラフィー
RSV	rifamycin SV	リファマイシン SV
T1/2	elimination half-life	消失半減期
TEA	tetraethylammonium	塩化テトラエチルアンモニウム
Tmax	time to reach the maximum drug plasma (serum/blood) concentration following drug administration	最高血漿（血清／血液）中薬物濃度到達時間
tr	trace	微量
ur	urine	尿
Vss	The volume of distribution at steady-state	定常状態における分布容積
Vz	distribution volume during terminal elimination phase	消失相での分布容積
Vz/F	apparent distribution volume	見かけの分布容積

1 まとめ

シポニモドのラット，ウサギ，イヌ，及びサルにおける吸収，分布，代謝並びに排泄（ADME）を，*in vivo* 及び *in vitro* 試験で検討した。標識あるいは非標識シポニモドを投与した ADME 試験及びトキシコキネティクス（TK）試験における用法・用量の一覧を Table 1-1 に，*in vitro* 試験を Table 1-2 に示す。

本項では，本化合物の名称としてシポニモドを用いる。ただし，シポニモドは社内報告書では BAF312 と記載されており，本項の一部の図表でもこれらの名称で記述している。本化合物の薬物動態（PK）試験には毒性試験と同一の動物種及び系統を用いた。

Table 1-1 シポニモドの ADME 及び TK 試験

試験の種類	種	性	投与経路	投与期間	投与量 [a)] (mg/kg)	投与剤型	放射性標識	測定対象物質	報告書番号
ADME	マウス	雄	経口	単回	8, 25	懸濁液	^{14}C	^{14}C-シポニモド 代謝物	4.2.2.2-1-DMPK R1300411-02
PK，分布	マウス	雄	経口	反復	25（1 日 1 回）	懸濁液	-	シポニモド M16 M17	4.2.2.2-7-DMPK R1500775
ADME	ラット アルビノ及び有色	雄	静脈内	単回	1 [b)]	溶液	^{14}C	^{14}C-シポニモド 代謝物	4.2.2.2-2-DMPK R0500017-02
		雄	経口	単回	3 [b)]	溶液	^{14}C	^{14}C-シポニモド 代謝物	
ADME	ラット アルビノ及び有色	雄	静脈内	単回	1	溶液	^{14}C [c)]	^{14}C-シポニモド 代謝物	4.2.2.2-3-DMPK R0900164-01
		雄	経口	単回	3, 10	溶液	^{14}C [c)]	^{14}C-シポニモド 代謝物	
乳汁移行	ラット，授乳中	雌	経口	単回	10	懸濁液	^{14}C	^{14}C-シポニモド 代謝物	4.2.2.3-5-DMPK R1300583
PK	ラット，	雄，雌	静脈内	単回	1	溶液	-	シポニモド	4.2.2.2-4-DMPK R1600437
PK/PD	カニクイザル	雄	静脈内	単回	1	溶液	-	シポニモド	4.2.2.2-5-RD-2005-00583
		雌	静脈内	単回	1	溶液	-	シポニモド	

試験の種類	種	性	投与経路	投与期間	投与量 [a)] (mg/kg)	投与剤型	放射性標識	測定対象物質	報告書番号
		雄	経口	単回	0.03, 0.1, 0.3, 1, 3	溶液	-	シポニモド	
		雌	経口	単回	0.03, 0.1, 0.3, 1, 3	溶液	-	シポニモド	
ADME	カニクイザル	雄	経口	単回	15	懸濁液	^{14}C	^{14}C-シポニモド 代謝物	4.2.2.2-6-DMPK R1000166-01
TK, 2 週間毒性試験	マウス,	雄, 雌	経口	2 週間	0, 50, 150, 300	懸濁液	-	シポニモド	4.2.3.2-1-0770648
TK, 13 週間毒性試験	マウス	雄, 雌	経口	13 週間	0, 5, 15, 35, 80	懸濁液	-	シポニモド	4.2.3.2-2-0770649
TK, 39 週間毒性試験	マウス [c]	雄	経口	1, 3, 7, 14, 28, 91, 274 日間（39 週間）	25, 75	懸濁液	-	シポニモド，M16, M17	4.2.3.7.3-2-1370782
TK, がん原性試験	マウス [c]	雄, 雌	経口	104 週間	0, 2, 8, 25	懸濁液	-	シポニモド	4.2.3.4.1-1-0870139
薬物弁別試験	ラット	雄, 雌	経口	単回	0.3, 1, 50, 150 (雄) 0.15, 0.5, 10, 50 (雌)	懸濁液	-	シポニモド	4.2.3.7.4-2-1270591
TK, 単回投与毒性	ラット	雄, 雌	静脈内	単回	0.01, 0.05, 0.4, 2.0/ 2 mL	溶液	-	シポニモド	4.2.3.7.4-3-1270590
TK, 2 週間毒性試験	ラット	雄	経口	2 週間	0, 10, 30, 100	懸濁液	-	シポニモド	4.2.3.2-3-0410113
TK, 4 週間毒性試験	ラット	雄, 雌	経口	4 週間	0, 10, 50, 200 (雄) 0, 10, 50, 200 → 100 [d)] (雌)	懸濁液	-	シポニモド	4.2.3.2-4-0670037
TK, 4 週間毒性試験	ラット	雄, 雌	経口	4 週間	0, 15, 50 (雄, 雌)	懸濁液	-	シポニモド	4.2.3.7.6-11-1670150
身体的依存試験	ラット	雄, 雌	経口	4 週間	0.3, 1.0, 50, 150 (雄) 0.15, 0.5, 10, 50 (雌)	懸濁液	-	シポニモド	4.2.3.7.4-1-1570351
TK, 26 週間毒性試験	ラット	雄, 雌	経口	26 週間	0, 15, 50, 150 (雄) 0, 5, 15, 50 (雌)	懸濁液	-	シポニモド	4.2.3.2-5-0770059
TK, 104 週間毒性試験	ラット	雄, 雌	経口	104 週間	0, 3, 10, 30 (雌) 0, 10, 30, 90 (雄)	懸濁液	-	シポニモド	4.2.3.4.1-2-0870138
TK, 92 日間探索的試験	ラット	雄	経口	1, 3, 7, 14, 28, 92 日間	90	懸濁液	-	シポニモド，M16, M17	4.2.3.7.3-3-1370783

試験の種類	種	性	投与経路	投与期間	投与量 a) (mg/kg)	投与剤型	放射性標識	測定対象物質	報告書番号
胚・胎児発生に関する試験	ラット	雌	経口	交配 2 週間前から妊娠 6 日まで	0, 1, 5, 40	懸濁液	-	シポニモド	4.2.3.5.2-1-0770653
受胎能及び着床までの初期胚発生に関する試験	ラット	雌	経口	妊娠 6～21 日	0, 0.1, 0.3, 1	懸濁液	-	シポニモド	4.2.3.5.1 -2-1070054
胚・胎児発生に関する試験	ウサギ	雌	経口	妊娠 7～21 日	0, 0.1, 1, 5	懸濁液	-	シポニモド	4.2.3.5.2-3-0770652
TK, 2 週間毒性試験	カニクイザル	雄，雌	経口	2 週間	0, 10, 30, 100	懸濁液	-	シポニモド	4.2.3.2-6-0570040
TK, 4 週間毒性試験	カニクイザル	雄，雌	経口	4 週間	0, 10, 50, 200 → 150[e)]	懸濁液	-	シポニモド	4.2.3.2-7-0670007
TK, 26 週間毒性試験	カニクイザル	雄，雌	経口	26 週間	0, 10, 50, 100	懸濁液	-	シポニモド	4.2.3.2-8-0770061
TK, 52 週間毒性試験	カニクイザル	雄，雌	経口	52 週間	0, 10, 30, 100	懸濁液	-	シポニモド	4.2.3.2-9-0770339

a) シポニモドはヘミフマル酸塩（分子量：574.64）として投与し，遊離塩基（分子量：516.61）換算した，b) 遊離塩基として投与，c) ^{14}C-標識位置 2，その他の試験はすべて標識位置 1（Figure 2-1），d) 雌の投与量を 13 日目に 200 mg/kg/日から 100 mg/kg/日へと減量した， e) 投与量を 8 日目に 200 mg/kg/日から 150 mg/kg/日へと減量した

Table 1-2 シポニモドの *in vitro* 試験

試験の種類	報告書番号
血漿蛋白結合率並びに血球移行性を検討した試験	4.2.2.3-2-DMPK R0400881-01, 4.2.2.3-3-DMPK R1300902-01, 4.2.2.3-4-DMPK R1500677-01
肝ミクロソーム及び肝細胞を用いた代謝試験	4.2.2.3-5-DMPK R1300583, 4.2.2.4-2-DMPK R0400863-01
肝細胞における肝取込みを検討した試験	4.2.2.7-1-DMPK R0700963
反応性代謝試験	4.2.2.4-3-DMPK R0900482

1.1 薬物動態

動物（マウス，ラット，ウサギ，イヌ，及びサル）の薬物動態は，^{14}C-標識したシポニモドを静脈内又は経口投与して検討した。

雄ラットに ^{14}C-シポニモド 3 mg/kg を経口投与したときの吸収率は 58%～71%であった。雄ラットにシポニモド 3 mg/kg，及び雌雄サルにシポニモド 1 mg/kg を経口投与したときの絶対的バイオアベイラビリティの平均値は，それぞれ 49%～52%及び 54%～83%であった。シポニモド

1 mg/kg を静脈内投与したときの血漿クリアランスは，雄及び雌ラットでそれぞれ 0.4～0.5 L/h/kg 及び 0.074 L/h/kg，サルで 0.098 L/h/kg であった。ラットの薬物動態には性差が認められ，雌雄間の CYP の発現量の差が影響したと考えられる（Martignoni et al. 2006）。雌ラットのクリアランスは雄に比べて小さく，雌ラットの T1/2 は 28 時間であったのに対し，雄ラットでは T1/2 は 5～6 時間であった。

雄マウスにシポニモド 8～25 mg/kg，雌雄ラットにシポニモド 3～10 mg/kg，及び雌雄サルにシポニモド 1～15 mg/kg を経口投与したときの Tmax（中央値）は 2～8 時間であった。シポニモドの累積性については，初回投与に比べ反復投与後に曝露量が増加する傾向が認められた。

1.2 分布

シポニモドの *in vitro* 血漿蛋白結合率は，マウス，ラット，ウサギ，イヌ，及びサルでいずれも 99%超であり，濃度依存性は認められなかった（マウス及びウサギの最終濃度 1～100 ng/mL，ラット，イヌ，サルの最終濃度 4 又は 40 ng/mL）。シポニモドの血中／血漿中濃度比にも濃度依存性はなく，マウスで 1.06，ラットで 1.21，ウサギで 1.11，イヌで 0.74，及びサルで 1.01 であった（マウス及びウサギの最終濃度 10～70000 ng/mL，ラット，イヌ，サルの最終濃度： 10～10000 ng/mL）。

^{14}C-シポニモド 8 mg/kg をアルビノマウスに単回経口投与したとき，シポニモド及びその代謝物は全身に分布し，涙腺，肝臓，腎臓（皮質）及びリンパ節に高い放射能が認められた。

^{14}C-シポニモドを有色及びアルビノラットに単回静脈内投与（^{14}C-シポニモド 1 mg/kg）並び単回経口投与（^{14}C-シポニモド 3 mg/kg）にしたとき，シポニモド及びその代謝物は全身に分布した。静脈内投与後 15 分で全身に広く分布し，副腎皮質に高い放射能が認められた。経口投与では，消化管への分布以外は静脈内投与後の放射能分布パターンと類似していた。有色ラットの組織内放射能分布はアルビノラットと類似しており，メラニン含有組織である眼（脈絡膜）から放射能が比較的早く消失することから，メラニンへの親和性は強くないと考える。

^{14}C-シポニモド 3 mg/kg を有色ラットに 7 日間反復経口投与したとき，脳内組織／血液の AUClast 比は小脳白質（15.1），脳梁（12.9）及び延髄（12.4）で高かった。サルにシポニモド 10～200 mg/kg/日を 2，4，26 及び 52 週間反復経口投与したとき，投与後 24 時間の脳／血漿中濃度比は，6.1～9.6（2 週間），6.6～7.6（4 週間），11.3～14.8（26 週間）及び 8.6～12.4（52 週間）であった。シポニモドは脳血液関門を通過し，高い中枢移行性を示した。

妊娠 7 日目のウサギに，シポニモド 0.1，1 及び 5 mg/kg/日で 14 日間反復経口投与（妊娠 20 日目まで）したとき，胎児への移行が認められた。1 mg/kg/日及び 5 mg/kg/日群の胎児でシポニモドが定量可能であり，胎児放射能の母体血中放射能に対する比はそれぞれ 2.6 及び 1.6 であった。胎児はシポニモドに曝露されていることが示唆された。

1.3 代謝

マウス，ラット及びサル肝のミクロソーム及び肝細胞を用いた *in vitro* 試験において，シポニモドの主な代謝経路は，1）水酸化（シクロヘキシル環の水酸化），2）グルクロン酸抱合，3）硫酸抱合と推定された。水酸化代謝物（M5，M6 及び M7）が，マウス，ラット，及びサルで検出された。

^{14}C-シポニモドをマウス（8 mg/kg），ラット（3 mg/kg），及びサル（15 mg/kg）に単回経口投与したとき，いずれも血漿中の主成分は未変化体であり，それぞれ総放射能の 38%，67%，及び 79%であった。^{14}C-シポニモドをマウス（8 mg/kg），ラット（3 mg/kg），及びサル（15 mg/kg）に単回経口投与したときの主な代謝物として，それぞれ M17 （コレステロールエステル体，総放射能の 27%），M6（水酸化代謝物，総放射能の 18%），及び M3（M5 の O-グルクロン酸抱合体，総放射能の 7%）が検出された。

マウスに ^{14}C-シポニモド 8 mg/kg を単回経口投与したとき，糞中の主代謝物は M5（投与量の 29%），尿中の主代謝物は M17（投与量の 1%）であった。ラットに ^{14}C-シポニモド 3 mg/kg を単回経口投与したとき，糞中の主代謝物は M4（投与量の 26%），尿中の主代謝物は M1 及び M8（いずれも投与量の 3%）であった。サルに ^{14}C-シポニモド 15 mg/kg を単回経口投与したとき，糞中の主代謝物は M5（投与量の 28%）であり，尿中の代謝物は 1%以下であった。

また，胆管にカニュレーション処置したラットに ^{14}C-シポニモド 1 mg/kg を静脈内投与したとき，投与 72 時間後までに投与量の 8%が未変化体として胆汁から回収された。胆汁中の主代謝物は， M4［M4a（M5 の硫酸抱合体，投与量の 17%），M4c（M6 の硫酸抱合体，投与量の 7%），及び M4b（M7 の硫酸抱合体，投与量の 3%）］であった。

1.4 排泄

^{14}C-シポニモドをマウス（8 mg/kg），ラット（3 mg/kg），及びサル（15 mg/kg）に単回経口投与したとき，総放射能の 74.9%～98.2%が糞中に排泄され，1.44%～6.95%が尿中に排泄された。ラットでは経口投与後 96 時間までに総放射能の 100%が，サルでは投与後 336 時間までに総放射能の 99.9%が排泄された。マウスでは投与後 168 時間に，総放射能の 16.1%が屍体中から回収された。

授乳中のラットに ^{14}C-シポニモド 10 mg/kg を単回経口投与したとき，シポニモド及びその代謝物はラットの乳汁中に分泌され，未変体の乳汁／血漿中未変化体濃度比は約 0.5 であった。

1.5 薬物間相互作用

ヒト *in vitro* 及び *in vivo* 薬物間相互作用試験については，「2.7.2 臨床薬理試験」を参照のこと。動物試料を用いた *in vivo* 薬物間相互作用試験は実施していない。

ラット及びサルにおいて，シポニモドは能動的肝取込みトランスポーターの基質ではなく，代謝クリアランスは 10～15 μL/min/mg であった。ラット及びサル肝細胞による肝取込みに対して，

リファマイシン SV (RSV，OATP 阻害剤），*p* - アミノ馬尿酸（PAH，OAT 基質）及び塩化テトラエチルアンモニウム（TEA，OCT 基質）のいずれも阻害作用を示さなかった。

1.6 トキシコキネティクス

マウス，ラット及びイヌにシポニモドを経口投与したときの毒性試験において，シポニモドのトキシコキネティクスを評価した。また，ラット及びウサギに経口投与したときの生殖発生毒性試験においても評価した。

雌雄マウスにシポニモド 5～80 mg/kg を単回経口投与したときの未変化体の曝露量は，雄雌ともに用量にほぼ比例して増加した。反復投与（1 日 1 回 2～13 週間）したときの曝露量はばらつきが大きく，用量比をわずかに下回った。

39 週間反復投与試験において，雄マウスにシポニモド 25 及び 75 mg/kg/日を反復投与したとき，用量増加に伴い未変化体の曝露量が増加した。75 mg/kg/日投与群において，274 日目の M17 の曝露量は投与初日に比べて 58 倍以上増加し，明らかな累積性を示した。

雌雄ラットにシポニモド 10～200 mg/kg を単回経口投与したときの未変化体の曝露量は，雄ラットの低用量（10～50 mg/kg）を除き，雌雄ラットで用量比を下回った。雄ラットにシポニモド 10 mg/kg/日又は 50 mg/kg/日を 4 週間反復投与したとき累積は認められなかったが，200 mg/kg/日投与での累積係数は 2.7 であった。雌ラットにシポニモドを投与したときの曝露量は雄ラットに比べて高く，4 週間反復投与時の曝露量は投与初日に比べて増加する傾向がみられた。

雌雄サルにシポニモド 10～150 mg/kg を単回経口投与したときの未変化体の曝露量は，雄サルの低用量（10～50 mg/kg/日）を除き，雌雄サルで用量比を下回った。シポニモド 10～200 mg/kg/日を反復投与（1 日 1 回 2～52 週間）したときの曝露量は，雄雌ともに用量にほぼ比例して増加した。曝露量はばらつきが大きく，明らかな性差は認められなかった。反復投与（2～52 週間）時の曝露量は投与初日に比べて増加する傾向にあった。

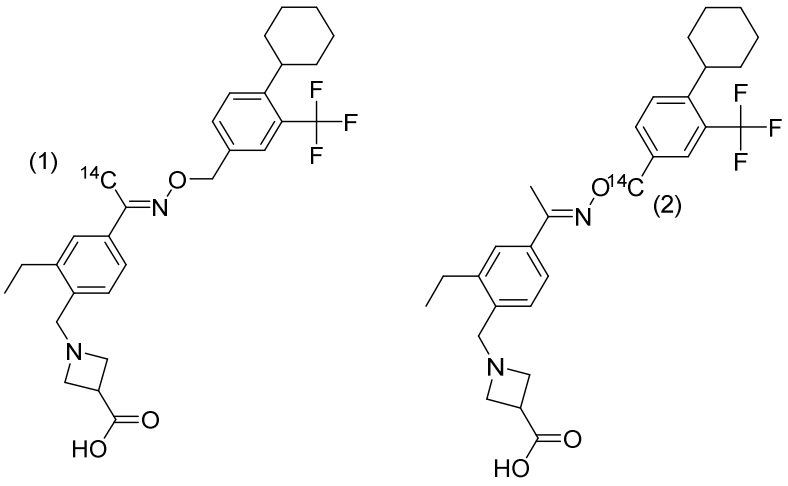
妊娠ウサギにシポニモド 0.1～5 mg/kg を 2 週間反復経口投与したときの未変化体の曝露量は，用量にほぼ比例して増加した。

2 分析法

2.1 放射性標識

シポニモドの ADME 試験（Table 1-1）では，^{14}C-シポニモドを用いた。シポニモド及び主要な代謝物 M17（LYS815）の化学構造式を標識部位と合わせてそれぞれ Figure 2-1 及び Figure 2-2 に示す。

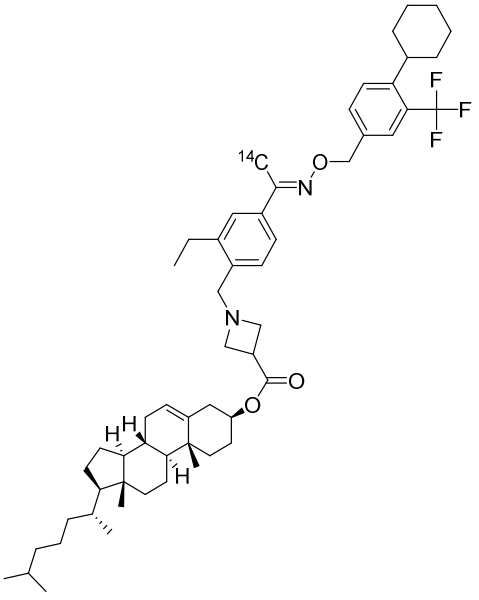
Figure 2-1 シポニモドの化学構造式及び標識部位



標識位置 1　　標識位置 2

HPLC-放射能検出により測定した放射化学的純度はいずれも 97.9%以上であった。

Figure 2-2 M17 (LYS815)の化学構造式及び標識部位



^{14}C-LYS815

HPLC-放射能検出により測定した放射化学的純度は 99.7%以上であった。

2.2 放射能の測定

全血，血漿，胆汁，乳汁，尿，糞，屍体，組織，ケージ洗液及び投与液，及びクロマト画分中の放射能は，適切なシンチレータを加え，液体シンチレーションカウンター（LSC）により直接測定した。糞中の放射能濃度は，ホモジネート試料を可溶化した後，LSC によって測定した。臓器及び組織中の放射能濃度は，定量的全身性オートラジオグラフィー（QWBA），又は臓器及び組織のホモジネート試料を可溶化した後，LSC によって測定した（2.6.5.2 項）。

2.3 シポニモド及び代謝物の定量

非臨床試験における主なシポニモド及び代謝物の濃度測定法を Table 2-1 に示す。内部標準物質として D_{11}-シポニモドを使用した。

マウス血漿，ラット血漿，脳，心臓及び肺組織ホモジネート，ウサギ血漿又は胎児ホモジネート，並びにサル血漿，心臓，肺及び脳組織中シポニモドの定量下限（LLOQ）はいずれも 5.00 ng/mL であった。

血漿中におけるシポニモドの安定性は，マウスでは-15°C 以下で少なくとも 347 日まで，ラットで-85°C 以下で少なくとも 269 日まで，ウサギで-65°C 以下で少なくとも 265 日まで，サルで-65°C 以下で少なくとも 616 日までは確認されている。

マウス及びラットを用いた毒性試験で血漿中及び組織中の代謝物 M16 及び M17 を定量した。マウス血漿中 M17 及び組織中 M17 の LLOQ はそれぞれ 10.0 ng/mL 及び 50.0 ng/mL，マウス血漿中 M16 及び組織中 M16 の LLOQ はそれぞれ 1.00 ng/mL 及び 2.00 ng/mL であった。ラット血漿中 M17 及び組織中 M17，ラット血漿中 M16 及び組織中 M16 の LLOQ はいずれも 5.00 ng/mL であった。[Table 2.6.5.2 L-DMPK R0500135a-01]，[Table 2.6.5.2 M-DMPK R1200039a]，[Table 2.6.5.2N-DMPK R0500135-01]，[Table 2.6.5.2O-DMPK R1200039-02]，[Table 2.6.5.2P-DMPK R1600622]，[Table 2.6.5.2Q-DMPK R0500135c-02]，[Table 2.6.5.2R-DMPK R0500135b-02]，[Table 2.6.7.17B− 1370782]，[Table 2.6.7.17B− 1370783]

Table 2-1 シポニモド及び代謝物（M16 及び M17）の濃度測定法

測定対象物質	測定方法	試料	試料容量 (μL)	定量下限 (LLOQ) (ng/mL)	報告書番号
シポニモド	LC-MS/MS	マウス血漿	25 又は 50	5.00	4.2.2.1-12-DMPK R0500135a-01, 4.2.2.1-13-DMPK R1200039a
シポニモド	LC-MS/MS	ラット血漿，脳，心臓，肺	50	5.00	4.2.2.1-14-DMPK R0500135-01
シポニモド	LC-MS/MS	ラット血漿	25	5.00	4.2.2.1-15-DMPK R1200039-02
シポニモド	LC-MS/MS	ラット血漿	25	5.00	4.2.2.1-16-DMPK R1600622
シポニモド	LC-MS/MS	ウサギ血漿，胎児	50	5.00	4.2.2.1-17-DMPK R0500135c-02
シポニモド	LC-MS/MS	サル血漿，脳，心臓，肺	50	5.00	4.2.2.1-18-DMPK R0500135b-02
M17	LC-MS/MS	マウス血漿／組織 a)	50/ 50	10.0/ 50.0	4.2.3.7.3-2-1370782
M16	LC-MS/MS	マウス血漿／組織 a)	50/ 50	1.00/ 2.00	4.2.3.7.3-2-1370782
M17	LC-MS/MS	ラット血漿／組織 b)	50/ 50	5.00/ 5.00	4.2.3.7.3-3-1370783
M16	LC-MS/MS	ラット血漿／組織 b)	50/ 50	5.00/ 5.00	4.2.3.7.3-3-1370783

Source： Table 2.6.5.2 L-DMPK R0500135a-01, Table 2.6.5.2 M-DMPK R1200039a, Table 2.6.5.2N-DMPK R0500135-01, Table 2.6.5.2O-DMPK R1200039-02, Table 2.6.5.2P-DMPK R1600622, Table 2.6.5.2Q-DMPK R0500135c-02, Table 2.6.5.2R-DMPK R0500135b-02, Table 2.6.7.17B− 1370782, Table 2.6.7.17B− 1370783

a) 肝臓，筋肉，腎臓，脳，皮膚，脾臓，下顎リンパ節，腸間膜リンパ節及び涙腺，b) 骨格筋，肝臓及び皮膚／皮下

2.4 代謝物の同定及び構造解析

代謝物の組成分析は，放射能検出器を接続した HPLC を用いた。*In vitro* 代謝試験はマウス，ラット及びヒトの肝ミクロソーム及び肝細胞を用いて実施した。

In vivo 代謝物は，血漿（マウス，ラット，ウサギ，イヌ，及びサル），尿（マウス，ラット，サル）及び糞（マウス，ラット，サル），ラット胆汁を用いて検討した。代謝物の構造解析は，構造既知代謝物との保持時間の比較，あるいは単離後に質量分析及び核磁気共鳴分光分析により実施した。

なお，反応性代謝物の検討には，ヒト組換え P450 酵素，モノアミンオキシダーゼ A 及び B（MAO A/B），及びフラビン含有モノオキシゲナーゼ 3（FMO）を用いた。

3 吸収

マウス，ラット，及びサルにシポニモドを経口又は静脈内投与したときの吸収，バイオアベイラビリティ及び薬物動態を検討した。

3.1 吸収及びバイオアベイラビリティ

マウス，ラット，サルに ^{14}C-シポニモドを静脈内又は経口投与したときの血漿中総放射能の PK パラメータを Table 3-1 に示す。

^{14}C-シポニモド 3～15 mg/kg を経口投与したときの総放射能濃度の Tmax は，検討したすべての動物種で 6～8 時間であった（Table 3-1）。^{14}C-シポニモド 3 mg/kg を経口投与したときの雄ラットでの吸収率は，総放射能の 57.5%～71.2%と推定された [Table 2.6.5.3B-DMPK R0500017-02] [Table 2.6.5.3C-DMPK R0900164-01]。

Table 3-1 ^{14}C-シポニモドを単回静脈内又は経口投与したときの血漿中総放射能の PK パラメータ

動物種 (試験番号)	性, n	投与経路	投与量 (mg/kg) [a]	Tmax (h)	Cmax (µgEq/mL)	Cmax/投与量 (µgEq/mL/(mg/kg))	AUClast (µgEq·h/mL)	AUClast/投与量 (µgEq·h/mL/(mg/kg))	T1/2 (h)	吸収率 (%)
マウス* (DMPK R1300411-02)	雄, 3/時点	経口	8	8	2.27	0.284	151 [d]	18.9	55.6	-
ラット (DMPK R0500017-02)	雄，3	静脈内	1	0.083 [b]	1.16 ± 0.254	1.16	3.38 ± 0.0496 [c]	3.38	6.1± 1.3	-
	雄，3	経口	3	8 [b]	0.332 ± 0.0156	0.111	5.53 ± 1.18 [c]	1.84	na	57.5*
ラット (DMPK R0900164-01) [g]	雄，3	静脈内	1	0.083 [b]	0.899 ± 0.197	0.899	3.97 ± 0.132 [c]	3.97	na	-
	雄，3	経口	3	8 [b]	0.418 ± 0.0486	0.139	8.99 ± 0.713 [c]	3.00	na	71.2 ± 5.20
サル (DMPK R1000166-01)	雄，3	経口	15	7 [b]	5.22 ± 1.20	0.348	174 ± 7.23 [e]	11.6	56.9± 22.3	-

Source：Table 2.6.5.3A-DMPK R1300411-02, Table 2.6.5.3B-DMPK R0500017-02, Table 2.6.5.3C-DMPK R0900164-01, Table 2.6.5.3F-DMPK R1000166-01

na = 該当なし，a) 投与量は遊離塩基（分子量：516.61）換算した，b) 中央値，c) AUC0-96h，d) AUC0-168h，e) AUC0-336h，g) ^{14}C-標識位置 2，他の試験は標識位置 1 (Figure 2-1)

平均値 ± 標準偏差，*：平均値より算出

ラット及びサルにシポニモド 1 mg/kg を単回静脈内投与したときのシポニモドの薬物動態パラメータを Table 3-2 に，雌雄ラットにシポニモド 1 mg/kg を静脈内投与したときの血漿中シポニモド濃度-時間推移を Figure 3-1 に示す。

ラットの薬物動態には性差が認められ，雌雄間の CYP の発現量の差が影響したと考えられる（Martignoni et al. 2006）。雌ラット（0.074 L/h/kg）のクリアランスは雄（0.4～0.5 L/h/kg）に比べて小さく，雌ラットの T1/2 は 28 時間であったのに対し，雄ラットでは T1/2 は 5～6 時間であった。

Table 3-2 シポニモドを単回静脈内投与したときの血漿中シポニモドの PK パラメータ

動物種 (試験番号)	性，n	投与量 a) (mg/kg)	AUClast (ng·h/mL)	AUClast/投与量 (ng·h/mL)/(mg/kg)	T1/2 (h)	CLp (L/h/kg)	CLb (L/h/kg)	Vss (L/kg)
ラット (DMPK R0500017-02)	雄，3	1	2880 ± 217 b)	2720	6.01 ± 1.34	0.361 ± 0.0232	-	2.15 ± 0.311
ラット (DMPK R0900164-01)	雄，3	1	2090 ± 133 c)	2072 c)	0.237 ± 0.0266 g)/ 4.79 ± 0.410 h)	0.485 ± 0.0335	0.401 ± 0.0274	2.98 ± 0.0709
ラット (DMPK R1600437)	雄，3/ 雌，3	1 1	1920 ± 220 d)/ 6520 ± 655 d)	1860/ 6390	4.78 ± 0.459/ 28.8 ± 4.86	0.530 ± 0.0621/ 0.0739 ± 0.00375	0.442 / 0.0616	2.40 ± 0.133/ 2.88 ± 0.434
サル#, f) (RD-2005-00583)	雄，2+ 雌，2	1	10600 ± 1031 e)	10600	19 ± 0.5	-	0.098 ± 0.01	2.12 ± 0.12

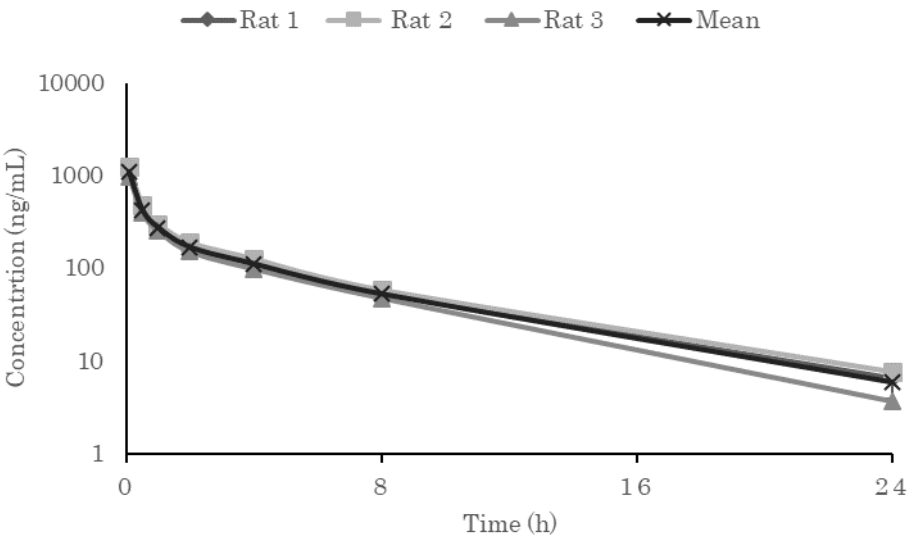
Source： Table 2.6.5.3B-DMPK R0500017-02, Table 2.6.5.3C-DMPK R0900164-01, Table 2.6.5.3D-DMPK R1600437, Table 2.6.5.3E-RD-2005-00583

a) 投与量は遊離塩基（分子量：516.61）換算した，b) AUC0-96h，c) AUCinf 及び AUCinf/投与量，d) AUC0-24h，e) AUC0-240h，f) 血中濃度，g) T1/2α，h) T1/2β

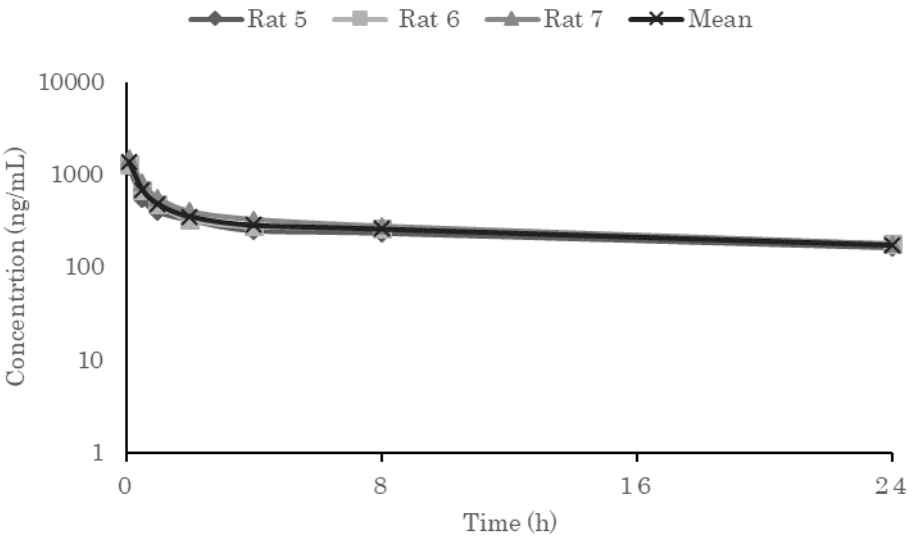
平均値 ± 標準偏差，#：平均値 ± 標準誤差

Figure 3-1　　雌雄ラットにシポニモド 1 mg/kg を単回静脈内投与したときの血漿中シポニモド濃度-時間推移

雄ラット



雌ラット



Source：4.2.2.2-4-DMPK R1600437-Table 6-2，Table 6-3

マウス，ラット，及びサルにシポニモド 1～25 mg/kg を経口投与したときのシポニモドの PK パラメータを Table 3-3 に示す。シポニモドを経口投与したときの未変化体の Tmax は，検討したすべての動物種で 2～8 時間の範囲であった（Table 3-3）。シポニモドを経口投与したときのバ

イオアベイラビリティは，雄ラットで 49%～52% [Table 2.6.5.3B-DMPK R0500017-02] [Table 2.6.5.3C-DMPK R0900164-01]，サルで 54%～83%であった [Table 2.6.5.3E- RD-2005-00583]。

雄マウスにシポニモド 25 mg/kg を 1 日 1 回 14 日間反復経口投与したとき，投与後 5 日以内に定常状態に達し，累積係数は約 1.3 であった（Table 3-3）。

Table 3-3　シポニモドを単回又は反復経口投与したときの血漿中シポニモドの PK パラメータ

動物種 (試験番号)	性，n	投与量 a) (mg/kg/日)	Tmax (h)	Cmax (ng/mL)	Cmax/ 投与量 (ng/mL)/ (mg/kg)	AUClast (ng·h/mL)	AUClast/ 投与量 (ng·h/mL) /(mg/kg)	T1/2 (h)	F (%)
マウス* (DMPK R1300411-02)	雄，3/時点	8 (SD)	8	2300	287	64060 d)	7870	27.2	-
マウス* (DMPK R1500775)	雄，2/時点	25 (SD)	6	5950	241	112000 e)	4530	-	-
		25 (MD)	6	7910	319	141000 e)	5690		
ラット (DMPK R0500017-02)	雄，3	3 (SD)	8 b)	259 ± 47.5	86.0	4050 ± 1370 f)	1340	-	48.7 ± 16.9
ラット (DMPK R0900164-01)	雄，3	3 (SD)	8 b)	235 ± 32.3	73.1	3470 ± 630 f)	1080	-	52.1 ± 9.17
ラット* (DMPK R1300583)	雌，8	10 (SD)	8	2580	266	94540 g)	9740	28.1	-
サル (DMPK R1000166-01)	雄，3	15 (SD)	7 b)	3800 ± 686	253	111000 ± 4360 h)	7400	62.1 ± 7.89	-
サル# (RD-2005-00583) c)	雄，1 雌，3	1 (SD)	2 b)	354 ± 39	354	7550 ± 845 d)	7560	18 ± 0.8	71 i)

Source： Table 2.6.5.3A-DMPK R1300411-02, Table 2.6.5.3B-DMPK R0500017-02, Table 2.6.5.3C-DMPK R0900164-01, Table 2.6.5.3E- RD-2005-00583, Table 2.6.5.3F-DMPK R1000166-01, Table 2.6.5.4A-DMPK R1500775, Table 2.6.5.7A-DMPK R1300583

a) 投与量は遊離塩基（分子量：516.61）換算した，b) 中央値，c) 血中濃度，d) AUC0-168h，e) AUC0-24h，f) AUC0-96h，g) AUC0-72h，h) AUC0-336h，i) 0.03～3 mg/kg のシポニモドを経口投与したときのバイオアベイラビリティは 54%～83%

SD：単回投与，MD：反復投与

平均値 ± 標準偏差，*：平均値より算出，#：平均値 ± 標準誤差

3.2 トキシコキネティクス

マウス，ラット，及びイヌにおける毒性試験でのトキシコキネティクス（TK）を評価した。また，ラット及びにウサギに経口投与したときの生殖発生毒性試験での TK も評価した。

マウスにシポニモド 5～80 mg/kg/日を単回投与したとき，雌雄ともに用量に比例して曝露量が増加した。シポニモド 5～300 mg/kg/日を 1 日 1 回 2～13 週間反復投与したとき，AUC0-24h 及び Cmax は用量比を下回った。マウスの曝露量に性差は認められず，1 日 1 回 13 週間反復投与後の曝露量は，単回投与後の曝露量と同程度であった（4.2.3.2-1-0770648，4.2.3.2-2-0770649）。

39 週間反復試験において，雄マウスにシポニモド 25 及び 75 mg/kg/日を反復投与したとき，用量増加に伴い未変化体の曝露量が増加した。75 mg/kg/日投与群において，274 日目の LYS815（M17）の曝露量は投与初日に比べて 58 倍以上増加し，明らかな累積性を示した。また，シポニモド 25 及び 75 mg/kg/日群において， M17 及び LYG778（M16）が検出された。

ラット 4 週間毒性試験及び 26 週間毒性試験におけるシポニモドの TK パラメータをそれぞれ Table 3-4 に示す。雄ラットにシポニモド 10～50 mg/kg を単回経口投与したとき，用量に比例して曝露量が増加する傾向にあったが，200 mg/kg/日投与では用量比を下回った。シポニモド 10 及び 50 mg/kg/日を 4 週間反復投与したとき累積は認められなかったが，200 mg/kg/日投与での累積係数は 2.7 であった。雌ラットの曝露量は単回投与及び反復投与で用量比を下回り， シポニモドを 1 日 1 回 4 週間反復投与したとき，明らかな累積が認められた。ラットにシポニモドを 4 又は 26 週間反復経口投与したとき，雌ラットの曝露量は雄ラットに比べて高かった。

ラットの雌受胎能及び着床までの初期胚発生に関する試験（4.2.3.5.1-2-1070054）及び胚・胎児発生に関する試験（4.2.3.5.2-1-0770653）において，用量の増加とともにシポニモドの曝露量は増加した。

サル 4 週間毒性試験におけるシポニモドの TK パラメータを Table 3-4 に示す。

雌雄サルにシポニモド 10～150 mg/kg を単回経口投与したときの曝露量は，雄サルの低用量（10～50 mg/kg/日）を除き，雌雄サルで用量比を下回った。シポニモド 10～200 mg/kg/日を 1 日 1 回 2～52 週間反復投与したときの曝露量は，雄雌ともに用量にほぼ比例して増加した。曝露量はばらつきが大きく，明らかな性差は認められなかった。反復投与（2～52 週間）時の曝露量は投与初日に比べて増加する傾向にあった（4.2.3.2-6-0570040，4.2.3.2-7-0670007，4.2.3.2-8-0770061，4.2.3.2-9-0770339）。

ウサギにおける胚・胎児発生に関する試験では，妊娠ウサギにシポニモド 0.1～5 mg/kg を 2 週間反復経口投与したときの曝露量は，用量にほぼ比例して増加した（4.2.3.5.2-3-0770652）。

Table 3-4 シポニモドの TK パラメータ

動物種 (n)	投与量	採血日	AUC0-24h (ng· h/mL)		Cmax (ng/mL)		Tmax (h)		資料番号／試験番号
	(mg/kg)	(日)	M	F	M	F	M	F	
マウス[a] 各時点 n=2	50	16	378000	280000	24100	16100	3.00	1.00	4.2.3.2-1 0770648
	150	16	491000	512000	28300	30600	7.00	7.00	
	300	16	965000	647000	61200	40500	7.00	7.00	
マウス[a] 各時点 n=2	5	1	33400	25200	2000	1600	6.00	3.00	4.2.3.2-2 0770649
		91	44700	40100	2730	2570	3.00	3.00	
	15	1	98900	77600	5770	4190	3.00	6.00	
		91	155000	62600	8860	3590	6.00	3.00	
	35	1	259000	180000	15100	10500	3.00	3.00	
		91	195000	139000	12200	7940	3.00	3.00	
	80	1	542000	414000	30100	23900	6.00	6.00	
		91	355000	229000	20300	14600	6.00	3.00	
ラット[a] 各時点 n=2	10	1	19500	52100	1410	2700	3.00	6.00	4.2.3.2-4 0670037
		29	21700	107000	1900	5670	3.00	3.00	
	50	1	93300	204000	6360	12100	3.00	3.00	
		29	86300	404000	6640	21500	3.00	3.00	
	200	1	134000	367000	9430	18300	3.00	3.00	
		29	365000	603000*	21600	30200*	6.00	3.00*	
ラット[b] 各時点 n=3	5	26	-	70200	-	4140	-	6.00	4.2.3.2-5 0770059
		152	-	77000	-	3820	-	6.00	
	15	26	31400	150000	2250	7830	1.00	6.00	
		152	48200	191000	3330	9680	6.00	6.00	
	50	26	93600	320000	6520	17500	3.00	6.00	
		152	106000	372000	7060	19400	6.00	6.00	
	150	26	185000	-	13500	-	6.00	-	
		152	198000	-	13300	-	6.00	-	
ラット[b] 各時点 n=3	0.1	0	-	317	-	17.4	-	7.00	4.2.3.5.1 -2 1070054
		10	-	841	-	46.4	-	3.00	
	0.3	0	-	915	-	46.1	-	7.00	
		10	-	2100	-	103	-	3.00	
	1.0	0	-	4410	-	236	-	7.00	
		10	-	9030	-	468	-	7.00	
ラット n=5	1[c]	17	-	10300 ± 660	-	498 ± 35.5	-	7.00[3.00 -7.00]	4.2.3.5.2-1 0770653
	5	17	-	57900 ± 6360	-	2870 ± 252	-	7.00[3.00 -7.00]	
	40	17	-	316000 ± 47900	-	14800 ± 1850	-	7.00[3.00 -7.00]	

動物種 (n)	投与量	採血日	AUC0-24h (ng· h/mL)		Cmax (ng/mL)		Tmax (h)		資料番号／試験番号
	(mg/kg)	(日)	M	F	M	F	M	F	
ウサギ n=5	0.1 [c]	20	-	NR	-	6.52 ± 0.755	-	3.00[3.00 -7.00]	4.2.3.5.2-3 0770652
	1	20	-	958 ± 258	-	66.4 ± 10.3	-	3.00[3.00 -7.00]	
	5	20	-	5510 ± 646	-	426 ± 48.8	-	3.00[3.00 -3.00]	
サル n=2	10 [d]	1	79400	55100	4980	3380	6.00	6.00	4.2.3.2-6 0570040
		14	116000	78000	6330	4500	6.00	3.00	
	30 [d]	1	223000	140000	15100	8290	6.00	6.00	
		14	192000	226000	13700	12600	3.00	3.00	
	100	1	341000, 513000	315000, 405000	20100, 32800	22000, 24800	6.00, 6.00	6.00, 6.00	
		14	325000, 336000	313000, 382000	16200, 18200	17500, 18200	6.00, 6.00	6.00, 3.00	
サル n=3	10	1	70500 ± 7450	78600 ± 5590	4330 ± 508	5030 ± 441	6.00[6.00 -6.00]	3.00[3.00 -6.00]	4.2.3.2-7 0670007
		28	95600 ± 4260	106000 ± 26100	5170 ± 76.4	5860 ± 1460	6.00[6.00 -6.00]	3.00[3.00 -6.00]	
	50	1	318000 ± 59900	266000 ± 12200	19900 ± 3570	16500 ± 651	6.00[6.00 -6.00]	6.00[6.00 -6.00]	
		28	475000 ± 170000	364000 ± 73700	27200 ± 9510	20500 ± 4800	6.00[6.00 -6.00]	6.00[6.00 -6.00]	
	200[#]	1	812000 ± 126000	718000 ± 334000	47400 ± 7010	40300 ± 16600	6.00[6.00 -6.00]	6.00[6.00 -6.00]	
		8	988000 ± 283000	628000 ± 125000	54700 ± 16400	37100 ± 9560	6.00[6.00 -6.00]	6.00[3.00 -6.00]	
		28	1110000[g]	1190000 ± 180000	59000[g]	66500 ± 9540	6.00, 6.00[g]	6.00[6.00 -6.00]	
サル n=6	10 [c]	1	83500 ± 14500	84100 ± 27700	4690 ± 777	4830 ± 1200	6.00[6.00 -6.00]	6.00[3.00 -6.00]	4.2.3.2-8 0770061
		22	120000 ± 29700	113000 ± 48500	6310 ± 1410	6550 ± 2280	4.50[3.00 -6.00]	3.00[3.00 -6.00]	
		148	132000 ± 44200	140000 ± 45100	6690 ± 2210	7890 ± 1880	6.00[3.00 -6.00]	3.00[3.00 -6.00]	
	50 [c]	1	254000 ± 44700	298000 ± 61200	13900 ± 3230	15900 ± 2960	6.00[6.00 -6.00]	6.00[3.00 -6.00]	
		22	369000 ± 156000	440000 ± 89200	19300 ± 7280	22800 ± 3420	4.50[3.00 -6.00]	6.00[6.00 -6.00]	
		148	580000 ± 141000[e]	619000 ± 36000	31700 ± 5540[e]	32600 ± 11400	6.00[6.00 -6.00][e]	6.00[3.00 -6.00]	
	100	1	401000 ± 93100	446000 ± 84900	21600 ± 3800	23100 ± 5480	6.00[6.00 -24.0]	6.00[6.00 -24.0]	
		22	723000 ± 449000	1180000 ± 65000	36400 ± 22000	60100 ± 19900	6.00[3.00 -6.00]	6.00[3.00 -6.00]	
		148	843000 ± 590000	1040000 ± 399000	41800 ± 28100	54800 ± 23900	4.50[0.00 -6.00]	6.00[0.50 0-24.0]	

動物種 (n)	投与量	採血日	AUC0-24h (ng· h/mL)		Cmax (ng/mL)		Tmax (h)		資料番号／試験番号
	(mg/kg)	(日)	M	F	M	F	M	F	
サル n=6	10 [c]	1	67400 ± 9160	51900 ± 20000	4040 ± 628	3200 ± 685	4.50[3.00 -6.00]	4.50[3.00 -6.00]	4.2.3.2-9 0770339
		28	92500 ± 22600	101000 ± 54400	5110 ± 1090	5800 ± 2630	4.50[3.00 -6.00]	6.00[6.00 -6.00]	
		154	94000 ± 8790	106000 ± 63100	5250 ± 717	5910 ± 3190	4.50[3.00 -6.00]	4.50[3.00 -6.00]	
		357	95400 ± 16600	124000 ± 80900	5440 ± 785	6620 ± 3170	4.50[3.00 -6.00]	3.00[3.00 -6.00]	
	30 [c]	1	198000 ± 5450	157000 ± 32300	11000 ± 772	9380 ± 1810	6.00[3.00 -6.00]	4.50[3.00 -6.00]	
		28	351000 ± 53200	242000 ± 53700	19200 ± 1490	13900 ± 2470	4.50[3.00 -6.00]	6.00[3.00 -6.00]	
		154	427000 ± 100000	278000 ± 88700	23800 ± 5700	16100± 2870	6.00[3.00 -6.00]	6.00[3.00 -6.00]	
		357	331000 ± 54000	215000 ± 54200	18000 ± 2960	12100 ± 3130	6.00[3.00 -6.00]	6.00[6.00 -6.00]	
	100	1	339000 ± 89200	293000 ± 76800	19100 ± 5640	16800 ± 4760	6.00[6.00 -6.00]	6.00[6.00 -6.00]	
		28	786000 ± 323000	761000 ± 244000	41900 ± 16400	40600 ± 11700	6.00[3.00 -6.00]	6.00[3.00 -6.00]	
		154	1020000 ± 628000[f]	750000 ± 212000	53300 ± 32300[f]	40200 ± 10600	6.00[3.00 -6.00][f]	6.00[6.00 -6.00]	
		357	884000 ± 387000[e]	565000 ± 170000	46000 ± 20300[e]	31000 ± 8560	6.00[6.00 -6.00][e]	6.00[6.00 -6.00]	

Source：4.2.3.2-1-0770648, 4.2.3.2-2-0770649, 4.2.3.2-4-0670037, 4.2.3.2-5-0770059, 4.2.3.5.1-2-1070054, 4.2.3.5.2-1-0770653, 4.2.3.5.2-3-0770652, 4.2.3.2-6-0570040, 4.2.3.2-7-0670007, 4.2.3.2-8-0770061, 4.2.3.2-9-0770339

平均値 ± 標準偏差，Tmax は中央値 [最小値 - 最大値]

*：13 日目に雌の投与量を 200 mg から 100 mg に減量，#：Day 8 から投与量を 200 mg/kg から 150 mg/kg に減量，M: 雄，F: 雌，NR: ポイント不足のため算出できず

a : 各時点（n=2）の血漿中濃度の平均値より算出，b : 各時点（n=3）の血漿中濃度の平均値より算出，c : N=4，d : N=1，e : N=3, f : N=5，g : N=2

4 分布

4.1 *In vitro* 血漿蛋白結合率及び血球への移行性

マウス，ラット，ウサギ，イヌ，及びサルの血漿に ^{14}C-シポニモドを添加（マウス及びウサギの最終濃度 1～100 ng/mL，ラット，イヌ，サルの最終濃度 4 又は 40 ng/mL）し，ゲルろ過法により血漿蛋白結合率を *in vitro* で検討した。また，血液に ^{14}C-シポニモドを添加（マウス及びウサギの最終濃度 10～70000 ng/mL，ラット，イヌ，サルの最終濃度： 10～10000 ng/mL）し，血球への移行性を検討した[Table 2.6.5.6A-DMPK R0400881-01, DMPK R1300902-01]。

シポニモドの血漿蛋白結合率は検討したすべての動物種で 99%超であり，濃度依存性は認められなかった。平均非結合型分率（fu）は，マウス，ラット，ウサギ，イヌ，及びサルで，それぞれ 0.0147 ± 0.00242%， 0.03 ± 0.01%，0.0323 ± 0.00590%，0.01 ± 0.002%，及び 0.03 ± 0.002%であった。

シポニモドの血液／血漿中濃度比（Cb/Cp）にも濃度依存性（マウス及びウサギの最終濃度 10～70000 ng/mL，ラット，イヌ，サルの最終濃度： 10～10000 ng/mL）は認められなかった。Cb/Cp 並びに平均血漿中分率（fp）は，マウス，ラット，ウサギ，イヌ，及びサルで，それぞれ 1.06，1.21，1.11，0.74，及び 1.01，並びに 60%，48%，59%，71%，及び 56%あった。

マウス及びラットの血漿に ^{14}C-LYS815 を添加（最終濃度 2～200 ng/mL）し，ゲルろ過法により血漿蛋白結合率を *in vitro* で検討した。

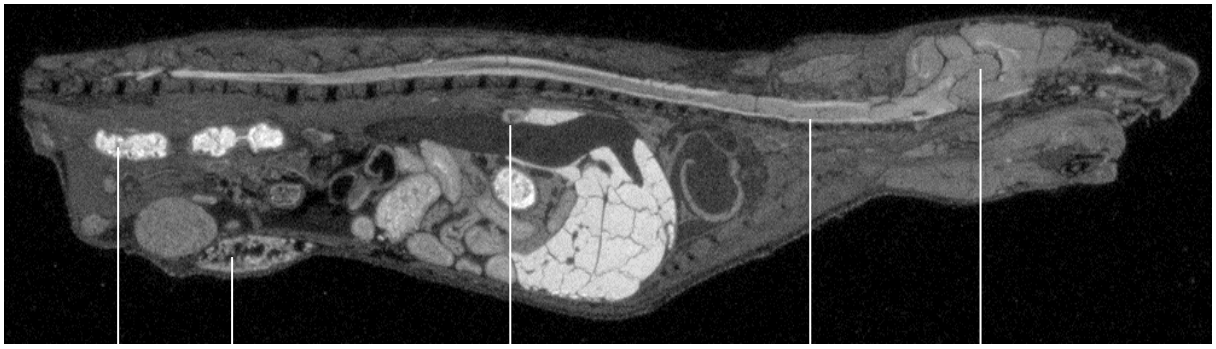
^{14}C-LYS815（M17）の血漿蛋白結合率は，マウス及びラットで>99.9%超であり，濃度依存性が認められ，最低濃度（2 mg/mL）での fu は他の濃度に比べて高かった（最大 3.5 倍）。マウス及びラットの fu はそれぞれ 0.0255 ± 0.0127%及び 0.0439 ± 0.0246%であった [Table 2.6.5.6B-DMPK R1500677-01]。

4.2 組織及び臓器分布

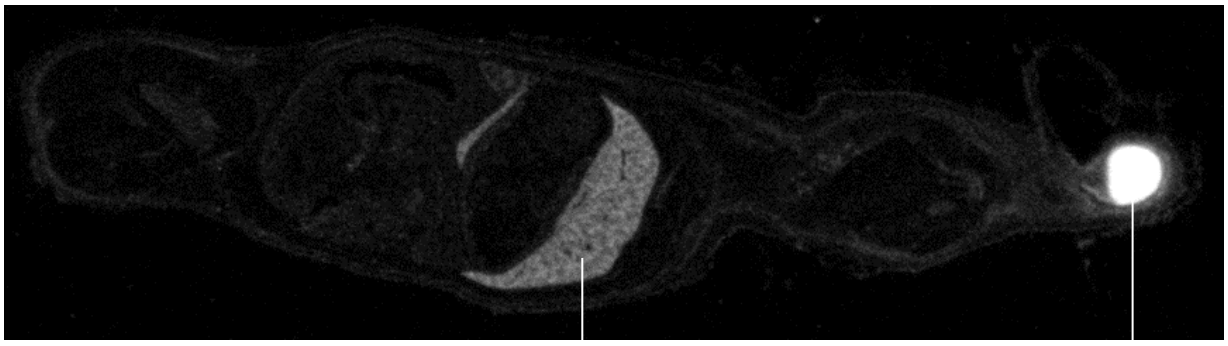
雄マウスに ^{14}C-シポニモド 8 mg/kg を単回経口投与したときの投与後 5 分，24 時間，168 及び 336 時間の臓器・組織内放射能濃度を検討した [Table 2.6.5.5A-DMPK R1300411-02]。投与後 48 時間及び 336 時間の組織中放射能分布を Figure 4-1 に示す。高い放射能濃度が認められた組織は涙腺，肝臓，腎臓（皮質及び皮髄境界部）及びリンパ節であり，336 時間でも放射能濃度が検出されたことから，多くの組織からの放射能の消失は緩徐であった。

Figure 4-1 **^{14}C-シポニモド 8 mg/kg をマウスに単回経口投与したときの投与後 48 時間及び 336 時間の組織中放射能分布**

投与後 48 時間



投与後 336 時間



Source: DMPK R1300411-02- Figure 7-3

* 涙腺中濃度は 48 時間後と 336 時間後で同程度であった。白色部位は放射能の存在を示す。

アルビノ及び有色ラットにシポニモド 1 mg/kg を単回静脈内投与したとき，投与後 15 分で全身に広く分布し，副腎皮質に高い放射能濃度が認められた（Table 4-1）。経口投与では，消化管への分布以外は静脈内投与後の放射能分布パターンと類似していた（Table 4-2）。有色ラットの組織内放射能分布はアルビノラットと類似しており，メラニン含有組織である眼（脈絡膜）から放射能が比較的早く消失することから，メラニンへの親和性は強くないと考える。

Table 4-1 アルビノ及び有色ラットにシポニモド 1 mg/kg を単回静脈内投与したときの臓器・組織内放射能濃度

臓器・組織	アルビノラット					有色ラット	
	0.25 h	2 h	4 h	24 h	168 h	24 h	168 h
副腎（皮質）	8730	3750	2810	589	114	499	123
副腎（髄質）	4290	2010	1210	257	42.6	177	T
胆汁	12800	10500	7540	63.0	n.d.	70.8	n.d.
血液	604	332	238	T	n.d.	T	n.d.
血液（LSC）	548	281	197	8.89	n.d.	47.8	n.d.
血管壁	1810	940	847	50.1	n.d.	63.0	n.d.
骨髄	1500	1040	615	75.9	n.d.	n.d.	n.d.
骨ミネラル	158	144	72.3	n.d.	n.d.	180	n.d.
脳	449	759	661	234	n.d.	n.d.	n.d.
脳（小脳溝）	273	488	426	n.m.	n.d.	120	n.d.
脳（小脳）	444	909	765	208	n.d.	120	n.d.
脳（大脳皮質）	453	837	723	185	n.d.	341	n.d.
脳（脳梁）	261	418	368	328	n.d.	193	n.d.
脳（上丘）	421	1260	708	245	n.d.	289	n.d.
脳（延髄）	440	951	790	390	n.d.	99.2	n.d.
脳（嗅球）	361	837	584	143	n.d.	211	n.d.
脳（線条体）	288	620	563	269	n.d.	206	n.d.
脳（視床）	387	868	666	207	n.d.	58.4	n.d.
脳（脳室）	75.4	333	120	55.8	n.d.	n.d.	n.d.
軟骨	T	T	T	n.d.	n.d.	148	n.d.
脈絡叢	2600	1130	646	123	n.d.	248	T
精巣上体	296	454	445	267	T	104	n.d.
眼（脈絡膜）	847	801	770	88.3	n.d.	n.d.	n.d.
眼（マイボーム腺）	n.s.	1100	1120	263	n.d.	243	n.d.
眼（硝子体）	n.d.	T	n.d.	n.d.	n.d.	n.d.	n.d.
脂肪（褐色）	2560	1870	796	138	n.d.	68.2	n.d.
脂肪（白色）	138	150	97.1	T	n.d.	T	n.d.
ハーダー腺	759	1390	1680	364	n.d.	376	n.d.
心臓	4810	2120	1280	116	n.d.	62.5	n.d.
腸壁（大腸）	796	961	759	172	n.d.	154	n.d.
腸壁（十二指腸）	1120	1650	n.m.	70.8	n.d.	n.m.	n.d.
腎臓（皮質）	3700	2390	1610	217	T	254	T
腎臓（髄質）	3150	1440	940	101	n.d.	88.9	n.d.
腎臓（腎盂）	1540	651	527	51.6	n.d.	61.5	n.d.
涙腺	2000	1890	1530	155	n.d.	105	n.d.
肝臓	6410	3100	3240	300	T	284	T
肺	1770	997	754	73.9	n.d.	58.9	n.d.

臓器・組織	アルビノラット					有色ラット	
	0.25 h	2 h	4 h	24 h	168 h	24 h	168 h
リンパ節	517	956	687	303	n.d.	155	n.d.
筋肉	723	883	703	48.3	n.d.	T	n.d.
膵臓	2710	2520	1590	129	n.d.	84.2	n.d.
陰茎	420	480	383	82.7	n.d.	59.4	n.d.
松果体	2090	1960	1380	134	n.d.	88.9	n.d.
脳下垂体	3170	1900	1170	236	n.d.	186	n.d.
包皮腺	827	780	1760	754	n.d.	589	n.d.
唾液腺	2430	2170	1440	133	n.d.	65.1	n.d.
精嚢(contour)	703	1140	889	97.6	n.d.	T	n.d.
精嚢	402	821	599	97.6	n.d.	T	n.d.
皮膚	405	573	420	88.3	T	53.2	n.d.
脊髄	369	573	558	356	T	277	T
脊髄（灰白質）	563	976	672	245	n.d.	174	n.d.
脊髄（白質）	268	393	274	458	n.d.	292	n.d.
脊髄神経	186	394	353	422	n.d.	223	n.d.
脾臓	2410	1370	842	109	n.d.	82.7	n.d.
腺胃	2180	2620	1850	160	n.d.	85.8	n.d.
前胃	940	651	677	65.6	n.d.	T	n.d.
精巣	136	217	315	239	64.6	212	61.0
胸腺	492	821	723	121	n.d.	125	n.d.
甲状腺	4100	2040	1130	108	n.d.	57.9	n.d.
舌	2340	1810	1160	105	n.d.	49.4	n.d.
歯髄	1510	1060	599	52.2	n.d.	T	n.d.

Source: Table 2.6.5.5C- DMPK R0900164-01

放射能濃度（ngEq/mL or ngEq/g）， n.d.: <LOD (13.6 ngEq/mL or ngEq/g); T (traces) : LOD と LOQ (40.9 ngEq/mL or ngEq/g)の間

Table 4-2 アルビノ及び有色ラットにシポニモド 3 mg/kg を単回経口投与したときの臓器・組織内放射能濃度

臓器・組織	有色ラット							アルビノラット
	0.25h	2h	8h	24h	72h	96h	168h	168h
副腎（皮質）	n.d.	2380	3150	568	424	243	186	160
副腎（髄質）	n.d.	1030	1700	139	108	T	T	T
胆汁	n.d.	6720	12900	1240	n.d.	n.d.	n.d.	n.d.
血液	T	310	362	T	n.d.	n.d.	n.d.	n.d.
血管壁	n.d.	1030	1110	119	n.d.	n.d.	n.d.	n.d.
骨髄	n.d.	620	930	114	n.d.	n.d.	n.d.	n.d.
骨ミネラル	n.d.	56.8	98.2	T	n.d.	n.d.	n.d.	n.d.
脳（mean）	T	165	568	568	T	T	n.d.	n.d.
脳（小脳白質）	n.d.	165	930	258	181	n.d.	n.d.	n.d.
脳（小脳）	n.d.	160	827	269	T	n.d.	n.d.	n.d.
脳（大脳皮質）	n.d.	145	930	393	T	n.d.	n.d.	n.d.
脳（硬膜）	n.d.	145	1080	362	82.7	n.d.	n.d.	n.d.
脳（下丘）	n.d.	134	878	336	T	n.d.	n.d.	n.d.
脳（中隔核）	n.d.	150	775	336	T	n.d.	n.d.	n.d.
脳（嗅球）	n.d.	165	672	201	T	n.d.	n.d.	n.d.
脳（小脳片葉）	n.d.	145	775	377	T	n.d.	n.d.	n.d.
脳（上丘）	n.d.	150	878	372	T	n.d.	n.d.	n.d.
褐色脂肪	T	1340	2270	186	T	n.d.	n.d.	n.d.
軟骨	n.m.	341	517	103	T	T	n.d.	n.d.
脈絡叢	n.d.	723	775	124	n.d.	n.d.	n.d.	n.d.
精巣上体	T	114	429	325	62.0	T	T	67.2
眼（脈絡膜）	n.d.	315	878	336	67.2	n.d.	n.d.	n.d.
眼（レンズ）	n.d.	T	T	T	T	n.d.	n.d.	n.d.
眼（硝子体）	n.d.	T	T	T	T	n.d.	n.d.	n.d.
毛 (毛包)	1030	723	672	145	160	n.d.	n.d.	n.d.
毛（触毛）	n.m.	294	511	56.8	n.d.	n.d.	n.d.	n.d.
ハーダー腺	n.d.	351	1910	620	T	n.d.	n.d.	n.d.
心臓	T	1550	1910	150	n.d.	n.d.	n.d.	n.d.
腸壁（大腸）	n.m.	372	1290	620	n.d.	n.d.	n.d.	n.d.
腸壁（十二指腸）	n.m.	723	1450	325	n.d.	n.d.	n.d.	n.d.
腎臓（腎皮質髄質移行部）	67.2	1340	2530	243	T	T	T	T
腎臓（皮質）	62.0	1600	2940	362	103	82.7	T	T
腎臓（髄質）	72.3	1340	1700	119	T	T	T	T
腎臓（腎盂）	227	672	775	119	T	n.d.	n.d.	n.d.
涙腺	n.d.	356	2010	620	T	n.d.	n.d.	n.d.

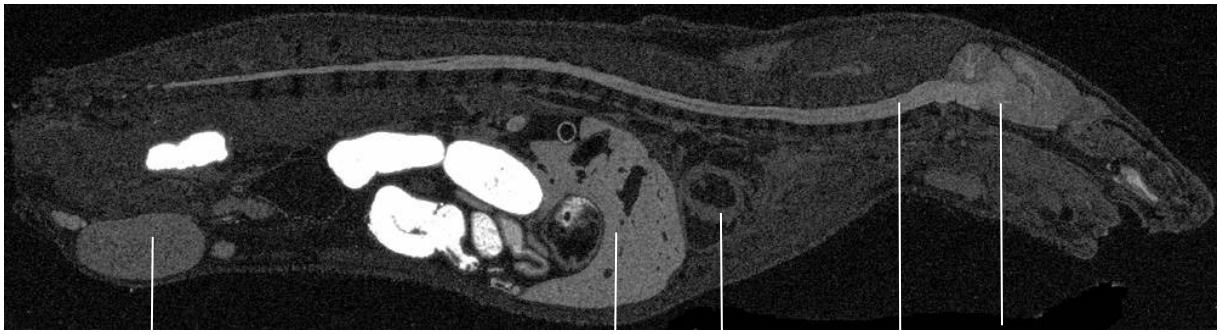
臓器・組織	有色ラット							アルビノラット
	0.25h	2h	8h	24h	72h	96h	168h	168h
肝臓	150	3670	6550	723	124	77.5	T	T
肺	T	827	1190	93.0	n.d.	n.d.	n.d.	n.d.
リンパ節	93.0	377	982	108	n.d.	n.d.	n.d.	n.d.
口腔粘膜	n.d.	305	620	196	T	T	n.d.	n.d.
筋肉	T	222	930	62.0	n.d.	n.d.	n.d.	n.d.
鼻甲骨	T	222	449	134	77.5	T	n.d.	n.d.
神経	n.d.	77.5	517	377	253	165	98.2	72.3
膵臓	n.m.	1340	2530	191	n.d.	n.d.	n.d.	n.d.
陰茎（海綿体）	n.d.	486	842	87.8	n.d.	n.d.	n.d.	n.d.
松果体	n.d.	723	1650	207	n.d.	n.d.	n.d.	n.d.
脳下垂体	n.d.	930	1700	181	n.d.	n.d.	n.d.	n.d.
包皮腺	T	160	527	119	T	T	T	n.d.
唾液腺	T	930	1860	155	n.d.	n.d.	n.d.	n.d.
精嚢	n.d.	T	82.7	T	n.d.	n.d.	n.d.	n.d.
精嚢 (contour)	n.d.	227	775	134	n.d.	n.d.	n.d.	n.d.
皮膚	134	191	455	51.7	T	T	n.d.	T
皮下組織	n.m.	176	460	98.2	82.7	98.2	62.0	n.d.
脊髄（灰白質）	n.d.	170	982	393	T	T	n.d.	n.d.
脊髄（白質）	n.d.	67.2	310	403	160	139	67.2	56.8
脊髄（mean）	n.d.	114	620	393	124	67.2	56.8	T
脾臓	n.m.	1080	1290	124	T	n.d.	T	n.d.
赤脾髄	n.m.	1190	1340	n.m.	n.m.	n.m.	n.d.	n.d.
白脾髄	n.m.	331	672	n.d.	n.d.	n.d.	n.d.	n.d.
腺胃	n.m.	2480	2690	191	n.d.	n.d.	n.d.	n.d.
前胃	n.m.	827	455	124	T	n.d.	n.d.	n.d.
精巣	T	77.5	362	315	150	134	87.8	72.3
胸腺	T	315	982	145	T	T	n.d.	n.d.
甲状腺	n.d.	1240	1860	160	n.d.	n.d.	n.d.	n.d.
舌	n.d.	930	1500	124	T	n.d.	n.d.	n.d.
歯（エナメル質）	n.d.	289	568	82.7	n.d.	n.d.	n.d.	n.d.
歯髄	n.d.	486	827	87.8	n.d.	n.d.	n.d.	n.d.
膀胱	217	517	2120	n.d.	n.d.	n.d.	n.d.	n.d.
白色脂肪	n.m.	212	129	T	n.d.	n.d.	n.d.	n.d.

Source: Table 2.6.5.5B- DMPK R0500017-02

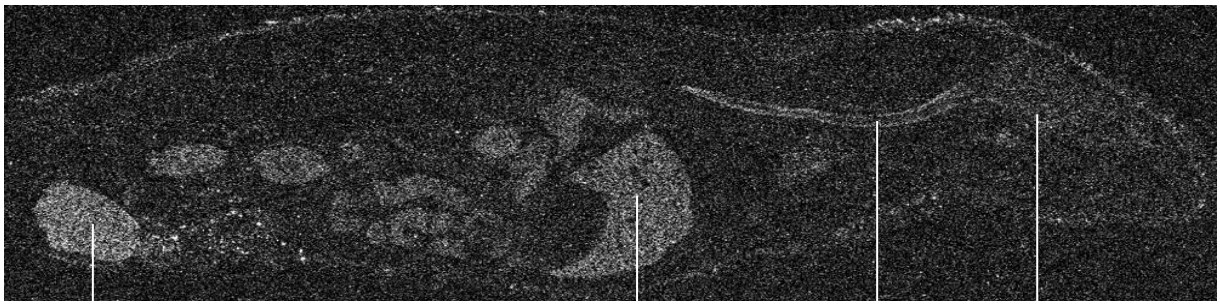
放射能濃度（ngEq/mL or ngEq/g），n.d.: <LOD (17.6 ngEq/mL or ngEq/g); T (traces) : LOD と LOQ (51.7 ngEq/mL or ngEq/g)の間

Figure 4-2 **^{14}C-シポニモド 3 mg/kg をラットに単回経口投与したときの投与後 24 時間及び 168 時間の組織中放射能分布**

投与後 24 時間



投与後 168 時間



白色部位は放射能の存在を示す

Source: 4.2.2.2-3-DMPK R0900164-01-Figure 7-5

マウス，ラット及びサルにおけるシポニモドの血漿中未変化体濃度及び組織／血漿中濃度比を Table 4-3 に示す。

マウスにシポニモド 25～75 mg/kg/日を 39 週間反復経口投与したとき，肝臓，筋肉，腎臓，脳，皮膚，脾臓，涙腺，及びリンパ節中のシポニモド濃度は，血漿中濃度に比べて 3.2～27 倍高かった。

ラットにシポニモド 10～200 mg/kg/日を 4 週間及び 26 週間反復経口投与したとき，最終投与後 24 時間の未変化体の肺／血漿中濃度比は 4.4～8.0 であり，性差は認められなかった。未変化体の心臓／血漿中濃度比は 4.8～5.9 であった。

サルにシポニモド 10～200 mg/kg/日を 2，4，26 及び 52 週間反復経口投与したとき，未変化体の肺／血漿中濃度比は 1.7～4.7 であり，性差は認められなかった。未変化体の心臓／血漿中濃度比は 2.2～4.0 であった。未変化体の投与後 24 時間の脳／血漿中濃度比は，6.1～9.6（2 週間），6.6～7.6（4 週間），11.3～14.8（26 週間）及び 8.6～12.4（52 週間）であった。

^{14}C-シポニモド 3 mg/kg/日を有色ラットに 7 日間反復経口投与したとき，未変化体の脳内組織／血液の AUClast 比は小脳白質（15.1），脳梁（12.9）及び延髄（12.4）であった [2.6.5.5D-DMPK R1200177]。

シポニモドは脳血液関門を通過し，高い中枢移行性を示した。

Table 4-3　　シポニモドを単回又は反復経口投与したときの血漿中未変化体濃度及び組織／血漿中濃度比

動物種 (投与期間) 報告書番号	投与量 (mg/kg)	採取 (日)	24 時間後又は剖検時の血漿中濃度(ng/mL)		組織／血漿中濃度比（24 時間後又は剖検時）													
					脳		心臓		肺		肝臓	腎	骨格筋	皮膚*	脾臓	Ln_1	Ln_2	涙腺
			雄	雌	雄	雌	雄	雌	雄	雌	雄	雄	雄	雄	雄	雄	雄	雄
マウス (2 週間) 4.2.3.7.3-4-0900680	10	14	—	2890	—	—	—	—	—	—	—	—	—	0.1[e]	—	—	—	—
マウス (2 週間) 4.2.2.2-7-DMPK R1500775	25	1	3550	—	—	—	—	—	—	—	7		—	1.3	—	—	—	—
	25	14	3230	—	—	—	—	—	—	—	9		—	1.3	—	—	—	—
マウス (39 週間) 4.2.3.7.3-2-1370782	25	91	13700	—	—	—	—	—	—	—	10	—	5.0	—	—	—	—	—
	25	274	6970	—	8.3	—	—	—	—	—	17	13	8.5	3.5	9.4	12	19	8.6
	75	91	27700	—	—	—	—	—	—	—	18	—	4.6	—	—	—	—	—
	75	274	18400	—	11.7	—	—	—	—	—	27	15	5.9	3.2	10	13	15	10
ラット (1 週間) 4.2.2.3-6-DMPK R1300375	3	1	36.8	—	16.3	—	—	—	—	—	—	—	—	—	—	—	—	—
ラット (4 週間) 4.2.3.2-4-0670037	10	29	131	3110	10.8	6.0	5.4	5.6	4.4	5.6	—	—	—	—	—	—	—	—
	50	29	1220	11100	10.8	5.3	5.3	4.8	5.6	4.8	—	—	—	—	—	—	—	—
	200/100[a]	29	8010	18300	10.0	7.8	5.9	5.9	6.2	5.6	—	—	—	—	—	—	—	—

動物種 (投与期間) 報告書番号	投与量 (mg/kg)	採取 (日)	24 時間後又は剖検時の血漿中濃度(ng/mL)		組織／血漿中濃度比（24 時間後又は剖検時）													
					脳		心臓		肺		肝臓	腎	骨格筋	皮膚*	脾臓	Ln_1	Ln_2	涙腺
			雄	雌	雄	雌	雄	雌	雄	雌	雄	雄	雄	雄	雄	雄	雄	雄
ラット (4 週間) 4.2.3.7.3-3-1370783[b]	90	28	1000	—	—	—	—	—	—	—	58	—	2.3	1.2	—	—	—	—
ラット (26 週間) 4.2.3.2-5-0770059	15 / 5[e]	183	334	2220	14	6	—	—	6.0	6.0	—	—	—	—	—	—	—	—
	50 / 15[e]	183	1300	5070	13	8	—	—	6.0	7.0	—	—	—	—	—	—	—	—
	150 / 50[e]	183	3030	11600	16	9	—	—	8.0	7.0	—	—	—	—	—	—	—	—
サル (2 週間) 4.2.3.2-6-0570040	10	14	3000	2080	6.1	6.9	—	—	3.0	2.4	8.5 7.0[e]	—	—	—	—	—	—	—
	30	14	3290	5720	8.6	9.6	—	—	2.2	2.6	6.9 9.4[e]	—	—	—	—	—	—	—
	100	14	10750[f]	10930[f]	7.7[f]	8.6[f]	—	—	3.3[f]	3.4[f]	9.3 9.5[e]	—	—	—	—	—	—	—
サル (4 週間) 4.2.3.2-7-0670007	10	28	2670	2800	7.2	7.1	4.0	2.9	3.5	1.8	—	—	—	—	—	—	—	—
	50	28	16400	8590	6.6	7.6	3.2	2.6	3.0	2.7	—	—	—	—	—	—	—	—
	200/150[c]		37800	32800	6.9	7.2	2.4	2.2	3.0	2.2	—	—	—	—	—	—	—	—
サル (26 週間) 4.2.3.2-8-0770061	10	182	2700	2520	11.8	12.9	—	—	4.7	4.0	—	—	—	—	—	—	—	—
	50	182	11000	18700	14.8	11.3	—	—	4.6	3.4	—	—	—	—	—	—	—	—
	100	182	24400	19400	12.5	14.1	—	—	4.4	4.2	—	—	—	—	—	—	—	—
サル (52 週間) 4.2.3.2-9-0770339	10	357/366[d]	2460	3750	9.6	9.5	—	—	2.4	1.9	—	—	—	—	—	—	—	—
	30	357/366[d]	9560	5790	8.6	9.4	—	—	1.7	1.8	—	—	—	—	—	—	—	—
	100	357/366[d]	26800	16500	11.1	12.4	—	—	1.8	3.6	—	—	—	—	—	—	—	—

Source：4.2.3.7.3-4-0900680，4.2.2.2-7-DMPK R1500775，4.2.3.7.3-2-1370782，4.2.2.3-6-DMPK R1300375，4.2.3.2-4-0670037，4.2.3.7.3-3-1370783，4.2.3.2-5-0770059，4.2.3.2-6-0570040，4.2.3.2-7-0670007，4.2.3.2-8-0770061，4.2.3.2-9-0770339

-- = 該当なし，* 皮膚（皮下を含む），Ln_1= 下顎リンパ節，Ln_2 = 腸間膜リンパ節
a: 13 日目に雌の投与量を 100 mg/kg に減量した，b: 探索的試験，c: 8 日目に投与量を 150 mg/kg に減量した，d: 血漿試料は 357 日目，組織試料は 366 日目に採取した，e: 雌，f: サル 2 例の平均組織中濃度／平均血漿中濃度比

4.3 胎盤通過

ウサギを用いた胚・胎児発生に関する試験（4.2.3.5.2-3-0770652）でシポニモドの胎盤通過を検討した。妊娠ウサギに 0.1，1 又は 5 mg/kg/日シポニモドを 2 週間（妊娠 7 日から 20 日まで）経口投与したとき，シポニモドの曝露量は用量に比例して増加した。1 mg/kg/日及び 5 mg/kg/日群の胎児（同腹児をプール）でシポニモドが定量可能であり，胎児／血漿中濃度比はそれぞれ 2.6 及び 1.6 であった。

5 代謝（動物種間の比較）

マウス，ラット，イヌ，サル及びヒト肝ミクロソーム，並びにマウス，ラット及びヒト肝細胞を用いてシポニモドの *in vitro* 代謝を検討した。また，マウス，ラット，サル，及びヒトでの *in vivo* 代謝についても検討した。

5.1 *In vitro* 代謝

^{14}C-シポニモドとマウス，ラット，サル，及びヒト肝ミクロソーム，並びにマウス，ラット，及びヒト肝細胞とインキュベートしたときの同定した代謝物を Table 5-1 に，*in vitro* の推定代謝経路を Figure 5-1 に示す。

^{14}C-シポニモドとマウス，ラット，サル，及びヒト肝ミクロソームをインキュベートしたとき，*in vitro* の主代謝経路はシクロヘキシル環の水酸化であり，代謝物として M5（LNL931），M5 の O-グルクロン酸抱合体の M3（LNL925），M6（LNL940）及び M7（CIN640）が同定された。

^{14}C-シポニモドとマウス，ラット及びヒト肝細胞をインキュベートしたとき， M5，M3，M6 及び M7 が同定された。ラット及びヒト肝細胞とのインキュベーション時には，水酸化体の硫酸抱合体（M4）が検出された。M4 は，M5，M7 及び M6 のそれぞれの硫酸抱合体の M4a（MBR808），M4b（MGH346）及び M4c（MDJ100）から構成される。

^{14}C-シポニモド 1 µmol/L を肝ミクロソームとともにインキュベートしたときの固有クリアランスは，マウス，ラット，イヌ，サル，及びヒトでそれぞれ 6 µL/min/mg，188 µL/min/mg，5 µL/min/mg 未満，80 µL/min/mg，及び 31 µL/min/mg であった[Table 2.6.5.10A-DMPK R0400863-01]。

^{14}C-シポニモドをラット，サル及びヒト肝ミクロソーム及び肝細胞とともにインキュベートし，反応性代謝物の生成を検討した[Table 2.6.5.10A-DMPK R0400863-01] [Table 2.6.5.10B-DMPK R0900482]。^{14}C-シポニモド 5 µmol/L とラット及びヒト肝ミクロソームをインキュベートしたとき，微量の反応性代謝物が検出され，^{14}C-シポニモド 5 µmol/L とラット及びサル肝細胞をインキュベートしたとき，低～中程度の反応性代謝物が検出された（4.2.2.4-2-DMPK R0400863-01）。ヒト肝細胞をインキュベートしたときラット及びサルよりも高い濃度の反応性代謝物が検出されたが（4.2.2.4-2-DMPK R0400863-01），ヒトに申請維持用量（2 mg）を投与したとき，定常状態

のシポニモド蛋白非結合型の Ch,in,u（肝臓での非結合型濃度）は 0.001 µmol/L であり，Cmax,ss,u は 0.0006 µmol/L である（2.7.2-2.1.4.1 項）ことを考慮すると，臨床的に影響はないと考えられた。また，^{14}C-シポニモド 5 µmol/L とヒト組換え P450 酵素，FMO3 及び MAO A/B をインキュベートしたとき，反応性代謝物は微量であった（4.2.2.4-3-DMPK R0900482）。

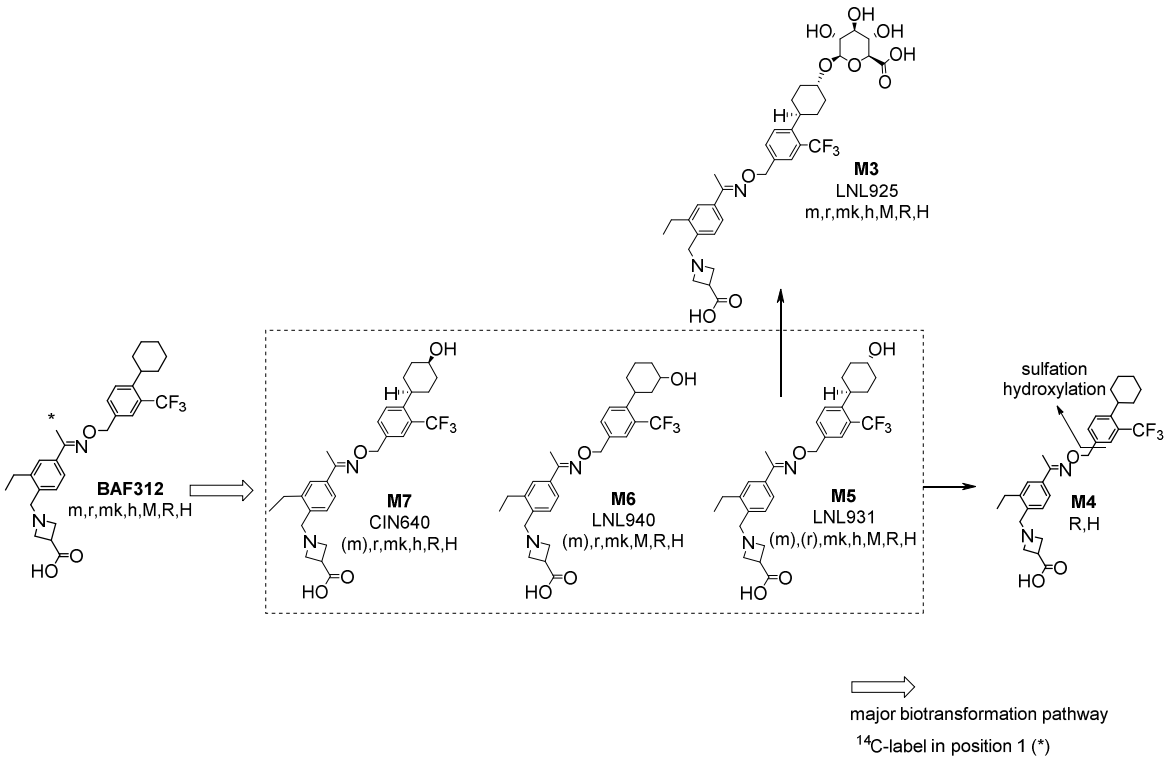
以上のことから，反応性代謝物の生成によって肝障害が誘発される可能性は非常に低いと考えられる。

Table 5-1 *In vitro* シポニモドの代謝物

	肝ミクロソーム				肝細胞		
	マウス	ラット	サル	ヒト	マウス	ラット	ヒト
M1 (BYC413)	tr	tr	-	tr*	tr*	tr*	tr*
M2 (BYD952)	tr	tr	-	tr*	tr*	tr*	tr*
M3 (LNL925)	x	xx	xx	xx	x	x	xx
M4 (Σ_{M4a-c})	-	-	-	-	-	x	xx
M5 (LNL931)	tr	x	x	x	x	x	x
M6 (LNL940)	x	xx	x	-	x	xx	x
M7 (CIN640)	tr	x	xx	x	-	x	xx
シポニモド	**xx**	**xx**	**xx**	**xx**	**xx**	**xx**	**xx**

Source: Table 2.6.5.10A- DMPK R0400863-01
肝ミクロソーム: シポニモド 5 µmol/L，60 分間インキュベーション，肝細胞: シポニモド 5 µmol/L，3 時間インキュベーション
- = 検出せず，tr = 微量，x = 検出，xx = 主成分，*定量できなかったため保持時間のみで判定

Figure 5-1 *In vitro* 推定代謝経路



Source： Table 2.6.5.11A
肝ミクロソームで検出：m＝マウス，r＝ラット，mk＝サル，h＝ヒト。肝細胞中で検出：M＝マウス，R＝ラット，H＝ヒト。括弧内は微量であることを示す。

5.2 *In vivo* 代謝

5.2.1 血漿中代謝物

^{14}C-シポニモドをマウス，ラット，及びサルに静脈内又は経口投与したとき，同定した *in vivo* の代謝物を Table 5-2 に，*in vivo* の推定代謝経路を Figure 5-2 に示す。

いずれの動物種も血漿中の主成分は未変化体であった。マウスに ^{14}C-シポニモド 8～25 mg/kg を経口投与したときの主要な代謝物は M17（LYS815）であった。ラットに ^{14}C-シポニモド 1 mg/kg を静脈内及び 3 mg/kg を経口投与したときの主要な代謝物は M6（LNL940），胆管カニュレーション処置したラットに ^{14}C-シポニモド 1 mg/kg を静脈内及び 10 mg/kg を経口投与したときの主要な代謝物は M11 であった。サルに ^{14}C-シポニモド 15 mg/kg を経口投与したときの主要な代謝物は M3（LNL925）であった。

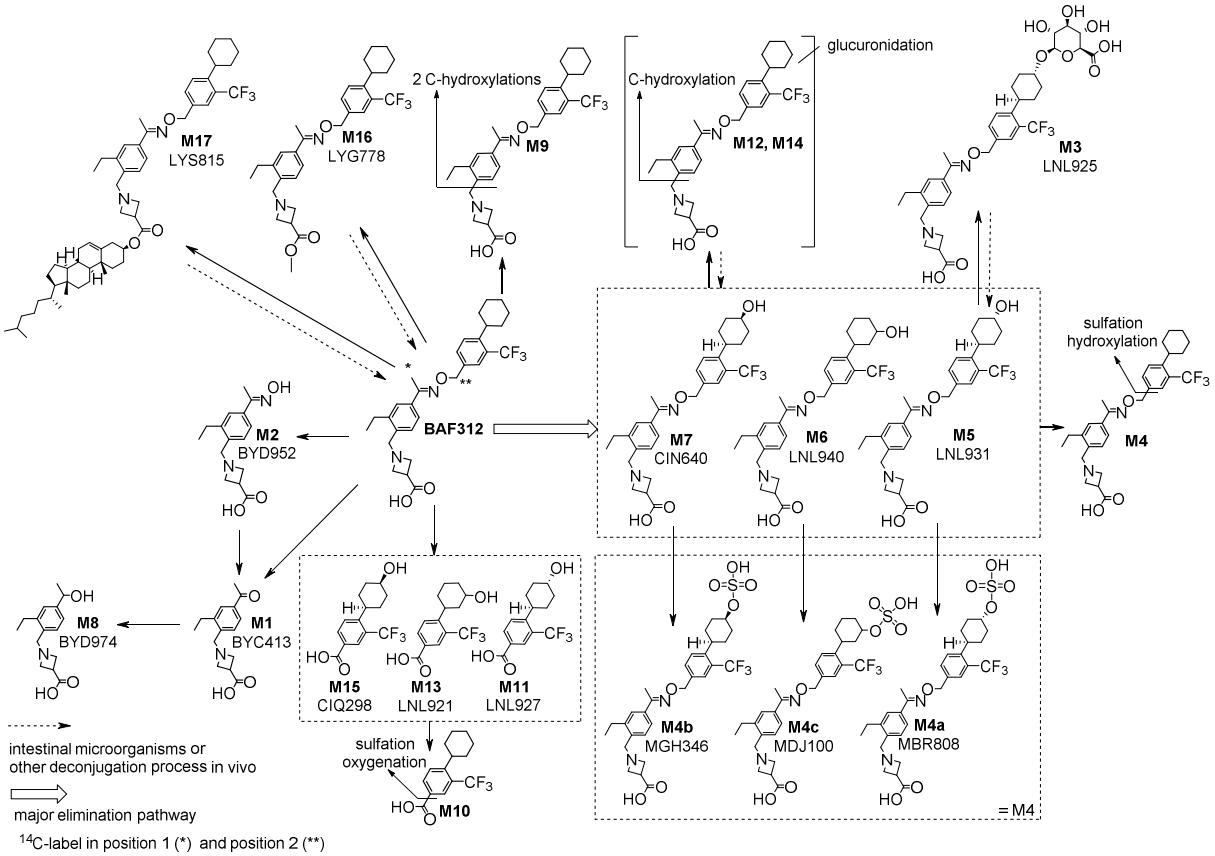
なお，授乳ラットに ^{14}C-シポニモド 10 mg/kg を経口投与したとき，総放射能の 95.3%が未変化体であり，いずれの代謝物も 3%以下であった[Table 2.6.5.9D-DMPK R1300583]。

Table 5-2 *in vivo* シポニモドの代謝物の比率

	マウス	ラット		ラット*		サル
	経口	経口	静脈内	経口	静脈内	経口
M1 (BYC413)	-	2/3	3/4	-	-	-
M2 (BYD952)	-	1/1	2/4	-	-	-
M3 (LNL925)	-	-	-	-	-	7/9
M5 (LNL931)	-	2/3	2/2	1/2	0/0	1/1
M6 (LNL940)	-	18/27	16/24	12/26	13/20	1/2
M7 (CIN640)	-	0/0	0/0	0/0	-	1/2
M8 (BYD974)	-	2/3	1/2	-	-	-
M9	-	-	0/0	-	-	-
M11 (LNL927)	-	-	-	23/51	7/11	-
M13 (LNL921)	-	-	-	6/14	5/8	-
M16 (LYG778)	12/31	-	-	-	-	-
M17 (LYS815)	27/72	-	-	-	-	-
シポニモド	**38**	**67**	**66**	**44**	**63**	**79**
その他	5/14	4/6	3/4	6/13	3/4	3/3
未回収	18/47	5/7	7/11	8/19	10/15	7/9
合計	100	100	100	100	100	100

Source: Table 2.6.5.9A-DMPK R1300411-02，Table 2.6.5.9B-DMPK R0500017-02，Table 2.6.5.9C-DMPK R0900164-01，Table 2.6.5.9E-DMPK R1000166-01
%，総放射能／未変化体，*: ^{14}C-標識位置 2 (Figure 2-1)，-＝検出せず

Figure 5-2　　シポニモドの *in vivo* 推定代謝経路



Source： Table 2.6.5.11B

5.2.1.1　M17

マウス及びラットにシポニモドを経口投与したときの M17 の PK パラメータを Table 5-3 に示す。

マウスにシポニモド 25 mg/kg を単回経口投与したとき，血漿中 M17 濃度の Tmax は投与後 24 時間であった。雄マウスにシポニモド 25 mg/kg/日を 1 日 1 回 14 日間経口投与したとき，累積係数は約 5 であった。雄マウスにシポニモド 75 mg/kg/日を 1 日 1 回 39 週間経口投与したとき，累積係数が 58 であったことから，14 日間投与では定常状態に達していなかったことが示唆された [Table 2.6.7.17B-試験 1370782]。

雄ラットにシポニモド 90 mg/kg/日を反復経口投与したとき，M17 の Tmax は，1 日目で投与後 24 時間，28 日目で投与後 3 時間，累積係数は 1.8 であった。反復経口投与したときの血漿中 M17/シポニモドの AUC0-24h 比は，マウス（2.45）と比べラット（0.0207）で低かった。

なお，ヒトに申請維持用量（2 mg）を投与したときの M17 の AUC0-24h は 1012 nmol·h/L（A2116 試験より換算）であり，マウス及びラットの AUC0-24h はそれぞれヒトの 660 倍及び 4.55 倍であると考えられる。

Table 5-3 シポニモドを反復経口投与したときの M17 の PK パラメータ（平均値）

動物種 (試験番号)	性，n	投与量 (mg/kg/日)	Tmax (h)	Cmax (nmol/L)[a)]	AUC0-24h (nmol·h/L)[a)]	Cmax 比 M17/シポニモド比 [b)]	AUC 比 M17/シポニモド比 [b)]
マウス (4.2.2.2-7-DMPK R1500775)	雄，2/時点	25 (Day 1)	24	10700	125000	0.932	0.578
	雄，2/時点	25 (Day 14) [c)]	24	30300	668000 [d)]	1.98	2.45
ラット (4.2.3.7.3-3-1370783)	雄，2/時点	90 (Day 1)	24	162	2520	0.00726	0.00779
	雄，2/時点	90 (Day 28) [e)]	3	233	4600 [d)]	0.0141	0.0207

Source：Table 2.6.5.4A-DMPK R1500775, Table 2.6.7.17B− 1370783

a) M17 の分子量：885.25，b) モル換算したシポニモドの Cmax 又は AUC 値を用いて算出，c) 14 日間，d) AUCtau，e) 28 日間

5.2.1.2 M3

雄サルに ^{14}C-シポニモド 15 mg/kg を単回経口投与したとき，M3 の Tmax は投与後 7 時間であった。Cmax は 559 nmol/L，AUC0-144h は 23800 nmol·h/mL であり，血漿中 M3 は総放射能濃度の約 7%であった[Table 2.6.5.9E-DMPK R1000166-01]。

なお，雄サルにシポニモド 10 mg/kg を 52 週間反復経口投与したとき，サルの M3 の AUC0-24h は，雄で 17359 nmol·h/L，雌で 22562 nmol·h/L であった（4.2.2.2-6-DMPK R1000166-01 の M3 比率：9.4%より換算）。ヒトに申請維持用量（2 mg）を投与したときの M3 の AUC0-24h は 358 nmol·h/L（A2118 試験より換算）であることから，サルの AUC0-24h はヒトの 48.5～63.0 倍であると考えられる。

5.2.2 組織中代謝物

マウス及びラットにおける代謝物の組織／血漿中濃度比を Table 5-4 に示す。

マウスにシポニモド 25 mg/kg/日を 2 週間反復経口投与したとき，肝臓中の未変化体及び M17 濃度は血漿中濃度に比べて 9 倍及び 2 倍高かった。マウスにシポニモド 25 mg/kg/日又は 75 mg/kg/日を 39 週間反復経口投与したとき，肝臓及び腎臓の M17 の曝露量は未変化体よりも高かった。高濃度の M17 存在下では分析中に M16 が生成する可能性があり，2 週間反復経口投与でみられなかった M16 が生成された可能性が示唆された。

雄ラットにシポニモド 90 mg/kg/日を 4 週間反復経口投与したとき，肝臓中の未変化体及び M17 濃度は血漿中濃度に比べて 58 倍及び 1.9 倍高かった。

Table 5-4　　シポニモドを単回又は反復経口投与したときの血漿中代謝物濃度及び組織／血漿中濃度比

動物種 (投与期間) 報告書番号	定量対象	投与量 (mg/kg)	採取 (日)	24 時間後又は剖検時の血漿中濃度 (ng/mL)	組織／血漿中濃度比（24 時間後又は剖検時）								
					脳	肝臓	腎	骨格筋	皮膚*	脾臓	Ln_1	Ln_2	涙腺
				雄	雄	雄	雄	雄	雄	雄	雄	雄	雄
マウス (2 週間) DMPK R1500775	M17	25	1	9500	--	<1	--	<1	<1	--	--	--	--
	M16	25	1	<LOQ	--	nc	--	nc	nc	--	--	--	--
	M17	25	14	26800	--	2	--	<1	<1	--	--	--	--
	M16	25	14	<LOQ	--	nc	--	nc	nc	--	--	--	--
	シポニモド	25	1	3550	--	7	--	2.0	1.3	--	--	--	--
		25	14	3230	--	9	--	2.4	1.3	--	--	--	--
マウス (39 週間) 1370782	M17	25	91	5300	--	1077	--	1.1	--	--	--	--	--
	M16	25	91	nr	--	nc	--	nc	--	--	--	--	--
	M17	25	274	40600	0.23	213	40	<1	<1	nc[h]	3.5	3.2	nc
	M16	25	274	8.04	0.36	78	126	1.9	5.6	55	28	84	255
	M17	75	91	7870	--	2618	--	5.3	--	--	--	--	--
	M16	75	91	nr	--	nc	--	nc	--	--	--	--	--
	M17	75	274	74400	0.46	422	29	<1	<1	nc	2.1	2.4	nc
	M16	75	274	18.4	<1	26	20	1.0	3.5	8.4	10	41	57
	シポニモド	25	91	13700	--	10	--	5.0	--	--	--	--	--
		25	274	6970	--	17	13	8.5	3.5	9.4	12	19	8.6
		75	91	27700	--	18	--	4.6	--	--	--	--	--
		75	274	18400	--	27	15	5.9	3.2	10	13	15	10
ラット (4 週間) 1370783[a]	M17	90	28	147	--	1.9	--	nc	<1	--	--	--	--
	M16	90	28	< 5	--	nc	--	nc	nc	--	--	--	--
	シポニモド	90	28	1000	--	58	--	2.3	1.2	--	--	--	--

Source： 4.2.2.2-7-DMPK R1500775，4.2.3.7.3-2-1370782，4.2.3.7.3-3-1370783,

-- = 該当なし，nr : 報告なし, nc: 算出せず,* 皮膚（皮下を含む），Ln_1 = 下顎リンパ節，Ln_2 = 腸間膜リンパ節

a:探索的試験

5.2.3 排泄物中及び胆汁中代謝物

マウス，ラット，及びサルに，^{14}C-シポニモドを単回投与したときの排泄物中及び胆汁中代謝物を Table 5-5 に示す。

尿中の代謝物はいずれの動物種でも微量であった。マウスに ^{14}C-シポニモド 8 mg/kg を経口投与したとき，尿中からは M1，M2，M8，及び M17 が検出された。ラットに ^{14}C-シポニモド 1 mg/kg を静脈内投与又は 3 mg/kg を経口投与したとき，尿中からは M1，M2，M8，M10，M11 及び M13 が検出された。サルに ^{14}C-シポニモド 15 mg/kg を経口投与したとき，尿中からは M1，M2，M3 及び M8 が検出された。

マウスに ^{14}C-シポニモド 8 mg/kg を経口投与したとき，糞中からは M4a-c，M5，M6，M7 が検出された。ラットに ^{14}C-シポニモド 1 mg/kg を静脈内投与又は 3 mg/kg を経口投与したとき，糞中からは M1，M3，M4（M4a-c），M5，M6，M7， M8，M9，M10，M11，M13，及び M14 が検出された。サルに ^{14}C-シポニモド 15 mg/kg を経口投与したとき，糞中からは M4a-c，M5，M6，M7，及び M8 が検出された。

胆管にカニュレーション処置したラットに ^{14}C-シポニモド 1 mg/kg を静脈内投与したとき，投与 72 時間後までに投与量の 8%が未変化体として回収された。胆汁中からはグルクロン酸抱合体（M3 及び M14）が検出されたが，糞中では検出できなかった。対応するアグリコン（M5 及び M6）が検出されたことから，腸内細菌叢によるグルクロン酸抱合体の脱抱合が示唆された。

Table 5-5 シポニモドを単回投与したときの排泄物中及び胆汁中代謝物

	排泄量（投与量%）														
動物種	マウス		ラット				ラット*				ラット a)			サル	
採取時間	0-168h		0-48h				0-96h				0-48h			0-144h	
投与経路	経口		経口		静脈内		経口		静脈内		静脈内			経口	
生体試料	ur	fe	ur	fe	ur	fe	ur	fe	ur	fe	ur	bi	fe	ur	fe
M1 (BYC413)	0	-	3	7	3	8	-	-	-	-	-	-	-	0	5
M2 (BYD952)	0	-	1	-	2	-	-	-	-	-	-	-	-	0	-
M3 (LNL925)	-	-	-	-	-	-	-	1	-	1	0	11	-	0	-
M4 (Σ_{M4a-c})	-	-	-	26	-	29	-	-	-	-	0	-	-	-	-
M4a (MBR808)	-	1	-	-	-	-	-	10	-	17	-	17	-	-	10
M4b (MGH346)	-	0	-	-	-	-	-	3	-	5	-	3	-	-	15
M4c (MDJ100)	-	5	-	-	-	-	-	4	-	6	-	7	-	-	3
M5 (LNL931)	-	29	-	5	-	7	-	14	-	12	0	3	0	-	28
M6 (LNL940)	-	8	-	25	-	27	-	27	-	28	0	7	2	-	8
M7 (CIN640)	-	1	-	1	-	1	-	2	-	1	0	-	0	-	8
M8 (BYD974)	0	-	3	3	2	3	-	-	-	-	-	-	-	0	1
M9	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
M10	-	-	-	-	-	-	0	2	1	2	0	-	-	-	-
M11 (LNL927)	-	-	-	-	-	-	0	1	0	1	0	-	-	-	-
M13 (LNL921)	-	-	-	-	-	-	0	5	0	4	0	-	-	-	-
M14	-	-	-	-	-	-	-	1	-	0	0	10	-	-	-
M16 (LYG778)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
M17 (LYS815)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
シポニモド	**0**	**22**	**-**	**13**	**-**	**3**	**-**	**9**	**-**	**3**	**0**	**8**	**3**	**-**	**9**
その他	0	3	0	3	0	7	0	13	1	12	1	6	0	0	7
未回収	0	5	0	1	0	0	0	0	0	2	-	-	-	0	2
合計	2	75	6	85	6	88	1	93	3	94	2	72	5	2	96

Source: Table 2.6.5.9A-DMPK R1300411-02, Table 2.6.5.9B-DMPK R0500017-02, Table 2.6.5.9C-DMPK R0900164-01, Table 2.6.5.9E-DMPK R1000166-01

*: ^{14}C-標識位置 2 (Figure 2-1)，0: 1%以下，-: 検出せず，a) 胆管カニュレーション処置ラット，ur : 尿，bi : 胆汁，fe : 糞

6 排泄

6.1 尿及び糞中排泄

マウス，ラット，及びサルに ^{14}C-シポニモドを単回投与したときの尿及び糞中排泄率を Table 6-1 に示す。主な排泄経路は糞中排泄であり，いずれの動物種においても未変化体はほとんど尿中排泄されなかった。

ラットに ^{14}C-シポニモド 1 mg/kg を静脈内投与したとき，投与した総放射能の 91.0%～95.8%が糞中に回収され，尿中排泄は 3.35%～6.58%であった。経口投与（3 mg/kg）でも同様に，総放射能の 92.0%～94.7%が糞中に排泄され，1.44%～6.95%が尿中に排泄された。

ラットに ^{14}C-シポニモド 3 mg/kg 及びサルに ^{14}C-シポニモド 15 mg/kg を経口投与したとき，総放射能の 80%以上がそれぞれ投与後 48 及び 72 時間以内に排泄された。マウスに ^{14}C-シポニモド 8 mg/kg を経口投与したとき，168 時間後の試験終了時点で総放射能の 16.1%が屍体中に回収された。

Table 6-1　　マウス，ラット及びサルにシポニモドを単回投与したときの放射能排泄率

動物種	マウス	ラット	ラット	ラット [a)]	ラット [a)]	サル
性別，n	雄，3	雄，3	雄，3	雄，3	雄，3	雄，3
投与経路	経口	静脈内	経口	静脈内	経口	経口
投与量（mg /kg）	8 [b)]	1	3	1 [b)]	3 [b)]	15 [b)]
尿中排泄率						
放射能（投与量%）	2.01	6.58	6.95	3.35	1.44	1.71
採取時間 (h)	0-168	0-96	0-96	0-168	0-168	0-336
未変化体（投与量%）	0.04	nd	nd	nd	nd	nd
採取時間 (h)	0-168	0-48	0-48	0-96	0-96	0-144
糞中排泄率						
放射能（投与量%）	74.9	91.0	92.0	95.8	94.7	98.2
採取時間 (h)	0-168	0-96	0-96	0-168	0-168	0-336
未変化体（投与量%）	21.5	2.9	13.0	3.13	9.41	9.36
採取時間 (h)	0-168	0-48	0-48	0-96	0-96	0-144

Source： Table 2.6.5.13A-DMPK R1300411-02， Table 2.6.5.13B-DMPK R0500017-02，Table 2.6.5.13C-DMPK R900164-01，Table 2.6.5.13D-DMPK R1000166-01

nd = 検出されず，a) ^{14}C-標識位置 2 (Figure 2-1)，b) シポニモドはヘミフマル酸塩（分子量：574.64）として投与し，遊離塩基（分子量：516.61）換算した

6.2　　胆汁中排泄

胆管カニュレーション処置したラットに ^{14}C-シポニモドを静脈内投与及び経口投与したときの排泄率を Table 6-2 に示す。胆管カニュレーション処置したラットに ^{14}C-シポニモド 1 mg/kg を静脈内投与したとき，投与した総放射能の 71.7%が胆汁中に回収され，尿中排泄は 1.88%であった。糞中に放射能の 4.50%が排泄されたことから，腸管内に直接排泄されることが示唆された。また，^{14}C-シポニモド 10 mg/kg を経口投与したとき，投与した総放射能の 56.9%が胆汁中に回収され，尿中排泄は 1.35%，糞中排泄は 25.6%であった。シポニモド及びその代謝物の排泄には，胆汁中排泄の寄与が大きいことが示唆された。

Table 6-2 胆管カニュレーション処置したラットに ^{14}C-シポニモドを静脈内投与及び経口投与したときの排泄率

動物種	ラット	ラット
性別，n	雄，2[a)]	雄，4
投与経路	静脈内	経口
投与量（mg 塩基/kg）[b)]	1	10
尿中排泄率		
放射能（投与量%）	1.88	1.35
採取時間 (h)	0-48	0-48
未変化体（投与量%）	tr	na
採取時間 (h)	0-48	na
糞中排泄率		
放射能（投与量%）	4.50	25.6
採取時間 (h)	0-48	0-48
未変化体（投与量%）	2.69	na
採取時間 (h)	0-48	na
胆汁中排泄率		
放射能（投与量%）	71.7	56.9
採取時間 (h)	0-48	0-48
未変化体（投与量%）	8.16	na
採取時間 (h)	0-48	na

Source： Table 2.6.5.14A-DMPK R0900164-01

na = 該当なし，tr = 微量 (0.00338)

a) 胆汁排出用カニューレの閉塞又は腹腔内への胆汁の流入により，ラット 2 例を投与前に屠殺した。

b) シポニモドはヘミフマル酸塩（分子量：574.64）として投与し，遊離塩基（分子量：516.61）換算した

6.3 乳汁中移行

授乳中のラットに ^{14}C-シポニモド 10 mg/kg を単回経口投与したときの乳汁中へのシポニモド及びその代謝物移行を検討した [Table 2.6.5.7A- DMPK R1300583]。

総放射能は乳汁中に分泌され，投与後 8 時間に Cmax に達し，血漿中及び乳汁中の放射能濃度はそれぞれ 2.31 µgEq/mL 及び 1.15 µgEq/mL であった。血漿中及び乳汁中の総放射能の AUClast はそれぞれ 89.4 µgEq·h/mL 及び 50.5 µgEq·h/mL であった。未変化体も投与後 8 時間に Cmax に達し，血漿中及び乳汁中未変化体濃度は，それぞれ 2.58 µg/mL 及び 1.05 µg/mL であった。血漿中及び乳汁中の未変化体の AUClast はそれぞれ 94.5 µg·h/mL 及び 46.5 µg·h/mL であり，乳汁中／血漿中未変化体濃度比は約 0.5 であった。シポニモド及びその代謝物は乳汁中に移行すると考える。

7 薬物間相互作用

ラット及びサル肝細胞に，^{14}C-シポニモド 0.5～50 µmol/L 及び RSV（10 µmol/L），PAH（3000 µmol/L）又は TEA（3000 µmol/L）を添加し，肝取込みを検討した。ラット及びサルにお

いて，代謝クリアランスはそれぞれ 10.2 ± 2.8 µL/min/mg 及び 15.2 ± 1.5 µL/min/mg であり，シポニモドは能動的肝取込みトランスポーターの基質ではなかった。ラット及びサル肝細胞による肝取込みに対して，RSV（OATP 阻害剤），PAH（OAT 基質）及び TEA（OCT 基質）のいずれも阻害作用を示さなかった [Table 2.6.5.16A-DMPK R0700963]。

8 考察及び結論

各種動物を用いてシポニモドの薬物動態を，*in vivo* 試験及び *in vitro* 試験において検討した。

シポニモドを経口投与したときのバイオアベイラビリティの平均値はサルで 54%～83%，ラット 49%～52%であった。いずれの動物種でも Tmax は 2～8 時間であり，ラット及びサルのクリアランスは小さく，種差が認められた。ラットの薬物動態には性差が認められ，雌雄間の CYP の発現量の差が影響したと考えられる。雌ラットのクリアランスは雄に比べて小さく，雌ラットの T1/2 は 28 時間であったのに対し，雄ラットでは T1/2 は 5～6 時間であった。

シポニモドの血漿蛋白結合率はいずれの動物種でも 99%超であり，濃度依存性は認められなかった。シポニモドの血中／血漿中濃度比は 0.74～1.21 であった。

有色ラットの組織内放射能分布はアルビノラットと類似しており，メラニン含有組織である眼（脈絡膜）から放射能が比較的早く消失することから，メラニンへの親和性は強くないと考える。妊娠ウサギにおいて，シポニモドは胎盤を通過し胎児へ移行した。シポニモド及びその代謝物はラットの乳汁中に分泌され，乳汁中／血漿中未変化体濃度比は約 0.5 であった。

マウス，ラット，及びサルにシポニモドを経口投与したとき，血漿中には主として未変化体が検出された。水酸化が主要な代謝経路であり，水酸化体はグルクロン酸抱合又は硫酸抱合を受けた。その他に，主要な代謝物としてコレステロールエステル体が検出された。

^{14}C-シポニモドをマウス，ラット，及びサルに単回経口投与したとき，総放射能の 74.9%～98.2%が糞中に排泄され，1.44%～6.95%が尿中に排泄された。

9 文献

[Martignoni M, Groothuis G, de Kanter R (2006)] Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol. Expert Opin Drug Metab Toxicol. 2(6):875-94.

2.6.5 薬物動態試験概要表

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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）
ADME	Absorption, distribution, metabolism and excretion	吸収・分布・代謝・排泄
AUC0-t	area under the drug plasma (serum/blood) concentration-time curve (time 0 to t)	血漿（血清/血液）中薬物濃度-時間曲線下面積（時間 0～t）
AUCinf	area under the drug plasma (serum/blood) concentration-time curve (time 0 to infinity)	血漿（血清/血液）中薬物濃度-時間曲線下面積（0～無限大）
AUClast	area under the drug plasma (serum/blood) concentration-time curve (time 0 to the last measurable concentration sampling time)	血漿（血清/血液）中薬物濃度-時間曲線下面積（0～最終定量可能時点）
bdc rat	bile duct-cannulated rat	胆管カニュレーションしたラット
C0	extrapolated starting concentration	外挿により求めた投与開始時の濃度
Cb	concentration in blood	血液中濃度
Cb/Cp	concentration ratio blood/plasma	血液/血漿中濃度比
CL	clearance	クリアランス
CLh,b	hepatic clearance	肝クリアランス
CLint	intrinsic clearance	固有クリアランス
CoA	certificate of analysis	試験成績書
Cmax	maximal drug plasma (serum/blood) concentration	最高血漿（血清／血液）中薬物濃度
Cp	concentration in plasma	血漿中濃度
D	isotope of hydrogen with mass number 2, it is also represented by 2H	2H (D) 水素の標識核種
DMF	dimethylformamide	ジメチルホルムアミド
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
FMO3	flavin-containing monooxygenase enzyme 3	フラビン含有モノオキシゲナーゼ 3
fu	fraction unbound in plasma	血漿中非結合画分
GLP	good laboratory practice	医薬品の安全性に関する非臨床試験の実施の基準
HPLC	high performance liquid chromatography	高速液体クロマトグラフィー
HPLC-MS/MS	high performance liquid chromatography coupled with tandem mass spectrometry	高速液体クロマトグラフィータンデム質量分析
HPLC-RA	high performance liquid chromatography including radioactivity detection	放射能検出器を接続した高速液体クロマトグラフィー
HPLC-UV	high performance liquid chromatography coupled to ultraviolet absorbance detection	紫外吸光度検出器を接続した高速液体クロマトグラフィー
ISTD	internal standard	内部標準
i.v.	intravenous	静脈内
LC-MS	liquid chromatography coupled with mass spectrometry	液体クロマトグラフィー質量分析
LC-MS/MS	liquid chromatography coupled with 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

略号	省略していない表現（英）	省略していない表現（日）
LOD	limit of detection	検出限界
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
M^0	exact mass	精密質量
MAOA/B	monoamine oxidase A and B	モノアミンオキシターゼ A 及び B
MeOH	methanol	メタノール
MRT	mean residence time	平均滞留時間
MS	mass spectrometry	質量分析法
M{n}	numbered metabolite (M1, M2, …)	代謝物
NA	not applicable	該当なし
n.a.	not available	利用不可
n.d.	not detected	検出されず
n.m.	not measured	測定されず
n.s.	no sample	検体なし
NMR	nuclear magnetic resonance	核磁気共鳴
OAT	organic anion transporter	有機アニオントランスポーター
OATP	organic anion transporting polypeptide	有機アニオン輸送ポリペプチド
OCT	organic cation transporter	有機カチオントランスポーター
PAH	*p*-aminohippuric acid	p-アミノ馬尿酸
PK	pharmacokinetics	薬物動態（学）
p.d.	post dose	投与後
p.o.	oral(ly)	経口投与
QC	quality control	品質管理
QWBA	quantitative whole body autoradiography	定量的全身オートラジオグラフィー
RA	radioactivity	放射能
RRLC	rapid resolution liquid chromatography	ラピッドレゾリューション液体クロマトグラフィー
RSE	Radio Synthesis Europe	－
RSV	rifamycin SV	リファマイシン XV
r.t.	room temperature	室温
SD	standard deviation	標準偏差
SRM	selected reaction monitoring	選択反応モニタリング
SSE	Stable Synthesis Europe	－
T1/2	elimination half life	消失半減期
TEA	tetraethylammonium	塩化テトラエチルアンモニウム
Tmax	time to reach the maximum drug plasma (serum/blood) concentration following drug administration	最高血漿（血清／血液）中薬物濃度到達時間
ULOQ	Upper limit of quantification	定量上限
UPLC	Ultra-performance liquid chromatography	超高速高分離液体クロマトグラフィー
USE	Unlabeled Synthesis Europe	－
Vss	distribution volume at steady state	定常状態における分布容積

2.6.5.1 薬物動態試験：一覧

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Compliance	Testing Facility	Study Number	Location in CTD
Analytical Methods and validation 2.6.5.2								
Analytical Methods and validation 2.6.5.2A	Synthesis and release analysis	SSE 087-1, E-15132-074-12	NA	D_{11}-BAF312	not required	Novartis	DMPK R0500098-01-01 (SSE 087-1)	4.2.2.1-1
Analytical Methods and validation 2.6.5.2B	Synthesis and release analysis	SSE 087-2, FK1-150512-A	NA	D_{11}-BAF312	not required	Novartis	DMPK R1200161 (SSE 087-2)	4.2.2.1-2
Analytical Methods and validation 2.6.5.2C	Synthesis and release analysis	RSE 280-1, E-15132-059-32 RSE 280-2, E-15132-061-06	NA	[^{14}C]BAF312	not required	Novartis	DMPK R0400858-01-01 (RSE-280 1+2)	4.2.2.1-3
Analytical Methods and validation 2.6.5.2D	Synthesis and release analysis	RSE 280-4, E-30585-33	NA	[^{14}C]BAF312	not required	Novartis	DMPK R0400858-02 (RSE 280-4)	4.2.2.1-4
Analytical Methods and validation 2.6.5.2E	Synthesis and release analysis	RSE 280-7, E-33614-58-18	NA	[^{14}C]BAF312	not required	Novartis	DMPK R0400858-04 (RSE 280-7)	4.2.2.1-5
Analytical Methods and validation 2.6.5.2F	Synthesis and release analysis	RSE 280-9, E-19105-24-26	NA	[^{14}C]BAF312	not required	Novartis	DMPK R0600841a (RSE 280-9)	4.2.2.1-6
Analytical Methods and validation 2.6.5.2G	Synthesis and release analysis	RSE 280-13, E-17435-026-05	NA	[^{14}C]BAF312	not required	Novartis	DMPK R0900379a (RSE 280-13)	4.2.2.1-7

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Compliance	Testing Facility	Study Number	Location in CTD
Analytical Methods and validation 2.6.5.2H	Synthesis and release analysis	RSE 280-30, RR1-151013-A	NA	[^{14}C]BAF312	not required	Novartis	DMPK R1300493 (RSE 280-30)	4.2.2.1-8
Analytical Methods and validation 2.6.5.2I	Synthesis and release analysis	RSE 425-1, E-17225-066-38 RSE 425-2, E-17225-070-38	NA	[^{14}C]BAF312	not required	Novartis	DMPK R0800721a (RSE 425-1+2)	4.2.2.1-9
Analytical Methods and validation 2.6.5.2J	Synthesis and release analysis	SSE 286-1, RB1-120215-A	NA	D_7-LYS815	not required	Novartis	DMPK R1500011 (SSE 286-1)	4.2.2.1-10
Analytical Methods and validation 2.6.5.2K	Synthesis and release analysis	RSE 679-1, RR1-021015-B	NA	[^{14}C]LYS815	not required	Novartis	DMPK R1500578 (RSE679-1)	4.2.2.1-11
Analytical Methods and validation 2.6.5.2L	Plasma of mouse	spiked samples	NA	BAF312, D_{11}-BAF312, 5-10000 ng/mL	not required	Novartis	DMPK R0500135a-01	4.2.2.1-12
Analytical Methods and validation 2.6.5.2M	Plasma of mouse	spiked samples	NA	BAF312, D_{11}-BAF312, 5-5000 ng/mL	not required	[redacted]	DMPK R1200039a	4.2.2.1-13
Analytical Methods and validation 2.6.5.2N	Plasma, heart, lung and brain tissues of rat	spiked samples	NA	BAF312, D_{11}-BAF312, 5-10000 ng/mL	not required	Novartis	DMPK R0500135-01	4.2.2.1-14
Analytical Methods and validation 2.6.5.2O	Plasma of rat	spiked samples	NA	BAF312, D_{11}-BAF312, 5-5000 ng/mL	not required	[redacted]	DMPK R1200039-02	4.2.2.1-15

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Compliance	Testing Facility	Study Number	Location in CTD
Analytical Methods and validation 2.6.5.2P	Plasma of rat	Spiked samples	NA	BAF312, D_{11}-BAF312, 5-5000 ng/mL	not required	[redacted]	DMPK R1600622	4.2.2.1-16
Analytical Methods and validation 2.6.5.2Q	Plasma and fetus homogenate of rabbit	spiked samples	NA	BAF312, D_{11}-BAF312, 5-10000 ng/mL	not required	Novartis	DMPK R0500135c-02	4.2.2.1-17
Analytical Methods and validation 2.6.5.2R	Plasma, heart, lung and brain tissues of monkey	spiked samples	NA	BAF312, D_{11}-BAF312, 5-10000 ng/mL	not required	Novartis	DMPK R0500135b-02	4.2.2.1-18
Analytical Methods and validation 2.6.5.2S	Synthesis and release analysis	NA	NA	LNL925, MBR808, MGH346, MDJ100, LNL931, LNL940, CIN640,LNL927, LNL921	not required	Novartis	RD-2015-00908	4.2.2.1-19
Analytical Methods and validation 2.6.5.2T	Synthesis and release analysis	USE 030-2, PB1-090414-A	NA	LYG778	not required	Novartis	DMPK R1500911 (USE 030-2)	4.2.2.1-20
Analytical Methods and validation 2.6.5.2U	Synthesis and release analysis	USE 030-5, KFOUROLL1-1	NA	LYG778	not required	Novartis	DMPK R1500889 (USE 030-5)	4.2.2.1-21
Analytical Methods and validation 2.6.5.2V	Synthesis and release analysis	USE 031-1, RR1-140114-A	NA	LYS815	not required	Novartis	DMPK R1400006-01 (USE 031-1)	4.2.2.1-22
Analytical Methods and validation 2.6.5.2W	Synthesis and release analysis	USE 031-2 RR1-150514-A	NA	LYS815	not required	Novartis	DMPK R1400437 (USE 031-2)	4.2.2.1-23

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Comp-liance	Testing Facility	Study Number	Location in CTD
Analytical Methods and validation 2.6.5.2X	Synthesis and release analysis	USE 038-2, MPAUROLL2-1	NA	LYS815	not required	Novartis	DMPK R1500888 (USE 038-2)	4.2.2.1-24
Pharmacokinetics: Absorption after a Single Dose 2.6.5.3								
Absorption after a Single Dose 2.6.5.3A	Mouse, CD-1, male, water and food ad libitum	p.o.	single	BAF312, hemifumarate, 8 mg/kg (free base)	not required	Novartis	DMPK R1300411-02 DMPK R1300411a-01	4.2.2.2-1
Absorption after a Single Dose 2.6.5.3B	Rat, Han Wistar, male, water and food ad libitum	i.v. and p.o.	single	BAF312 free base, i.v.: 1 mg/kg, p.o.: 3 mg/kg	not required	Novartis	DMPK R0500017-02	4.2.2.2-2
Absorption after a Single Dose 2.6.5.3C	Rat, Hannover Wistar, male, water and food ad libitum	i.v. and p.o.	single	BAF312 hemifumarate, i.v.: 1 mg/kg (free base), p.o.: 3 mg/kg (free base)	not required	Novartis	DMPK R0900164-01 DMPK R0900164a	4.2.2.2-3
Absorption after a Single Dose 2.6.5.3D	Rats Hannover Wistar, male and female, water and food ad libitum	i.v.	single	BAF312, hemifumarate, 1 mg/kg (free base)	not required	Novartis	DMPK R1600437 DMPK R1600437a	4.2.2.2-4
Absorption after a Single Dose 2.6.5.3E	Monkey, cynomolgus, male and female	i.v. and p.o.	single	BAF312 free base, i.v.: 1 mg/kg, p.o.: 0.03, 0.1, 0.3, 1, 3 mg/kg	not required	Novartis	RD-2005-00583	4.2.2.2-5
Absorption after a Single Dose 2.6.5.3F	Monkey, cynomolgus, male, water and food ad libitum	p.o.	single	BAF312, hemifumarate, 15 mg/kg (free base)	not required	Novartis	DMPK R1000166-01 DMPK R1000166a	4.2.2.2-6

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Compliance	Testing Facility	Study Number	Location in CTD
Pharmacokinetics: Absorption after Repeated Doses 2.6.5.4								
Absorption after Repeated Doses 2.6.5.4A	Mouse, CD-1, male, water and food ad libitum	p.o.	single/ multiple	BAF312, hemifumarate, 25 mg/kg (free base)	not required	Novartis	DMPK R1500775 DMPK R1500775a	4.2.2.2-7
Pharmacokinetics: Organ Distribution 2.6.5.5								
Organ distribution 2.6.5.5A	Mouse, CD-1, male, water and food ad libitum	p.o.	single	BAF312 hemifumarate, 8 and 25 mg/kg (free base)	not required	Novartis	DMPK R1300411-02	4.2.2.2-1
Organ distribution 2.6.5.5B	Rat, Hannover Wistar, albino, Long Evans, pigmented, male, water and food ad libitum	i.v. and p.o.	single	BAF312 free base, i.v.: 1 mg/kg, p.o.: 3 mg/kg	not required	Novartis	DMPK R0500017-02	4.2.2.2-2
Organ distribution 2.6.5.5C	Rat, Hannover Wistar albino, Long Evans, pigmented, male, water and food ad libitum	i.v. and p.o.	single	BAF312 hemifumarate, i.v.: 1 mg/kg (free base), p.o.: 3 mg/kg (free base)	not required	Novartis	DMPK R0900164-01	4.2.2.2-3
Organ distribution 2.6.5.5D	Rat, Long Evans, pigmented, male, water and food ad libitum	p.o.	7 days	BAF312 hemifumarate, 3 mg/kg/day (free base)	not required	Novartis	DMPK R1200177	4.2.2.3-1

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Compliance	Testing Facility	Study Number	Location in CTD
Plasma Protein Binding 2.6.5.6								
Plasma protein binding 2.6.5.6A	Mouse (CD-1), male rat (Hannover Wistar), male rabbit (New Zealand), female dog (Beagle), monkey (cynomolgus), human	spiked blood and plasma samples *in vitro*	NA	[^{14}C]BAF312 and BAF312	not required	Novartis	DMPK R0400881-01, DMPK R1300902-01	4.2.2.3-2, 4.2.2.3-3
Plasma protein binding 2.6.5.6B	Mouse (CD-1), male, rat (Hanover Wistar), male, human, male	spiked plasma samples *in vitro*	NA	[^{14}C]LYS815, 2, 10, 200 ng/mL	not required	Novartis	DMPK R1500677-01 DMPK R1500677a	4.2.2.3-4
Study in Pregnant or Nursing Animals 2.6.5.7								
Absorption in nursing animals; 2.6.5.7A	Rat (Hannover Wistar), female, water and food ad libitum	p.o., Plasma, milk	single	BAF312 hemifumarate, 10 mg/kg (free base)	not required	Novartis	DMPK R1300583 DMPK R1300583a	4.2.2.3-5
Other Distribution Study 2.6.5.8								
Brain disposition in rat; 2.6.5.8A	Rat (Hannover Wistar) albino, male, water and food ad libitum	p.o., Plasma, brain	7 days	BAF312, 3 mg/kg/day	not required	Novartis	DMPK R1300375 DMPK R1300375a	4.2.2.3-6
Metabolism *In vivo* 2.6.5.9								
Metabolism *in vivo* 2.6.5.9A	Mouse, CD-1, male, food and water ad libitum	p.o.	single	[^{14}C]BAF312 hemifumarate, 8, 25 mg/kg (free base)	not required	Novartis	DMPK R1300411-02	4.2.2.2-1

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Comp-liance	Testing Facility	Study Number	Location in CTD
Metabolism *in vivo* 2.6.5.9B	Rat, Hannover Wistar, male, food and water ad libitum	i.v. and p.o.	single	[^{14}C]BAF312 hemifumarate, i.v.: 1 mg/kg (free base); p.o.: 3 mg/kg (free base)	not required	Novartis	DMPK R0500017-02	4.2.2.2-2
Metabolism *in vivo* 2.6.5.9C	Rat/Rat (bile duct cannulated), Hannover Wistar, male, food and water ad libitum	i.v. and p.o.	single	[^{14}C]BAF312 hemifumarate, i.v.: 1 mg/kg (free base); p.o.: 3 mg/kg (free base)	not required	Novartis	DMPK R0900164-01 DMPK R1000456	4.2.2.2-3, 4.2.2.4-1
Metabolism *in vivo* 2.6.5.9D	Rat, Hannover Wista), female, water and food ad libitum	p.o., Plasma, milk	single	BAF312 hemifumarate, 10 mg/kg (free base)	not required	Novartis	DMPK R1300583 DMPK R1300583a	4.2.2.3-5
Metabolism *in vivo* 2.6.5.9E	Monkey, cynomolgus, male, food and water ad libitum	p.o.	single	[^{14}C]BAF312 hemifumarate, 15 mg/kg (free base)	not required	Novartis	DMPK R1000166-01 DMPK R1000456	4.2.2.2-6, 4.2.2.4-1
Metabolism *In Vitro* 2.6.5.10								
Metabolism *in vitro* 2.6.5.10A	Microsomes from mouse, rat, dog, monkey, human and hepatocytes from rat and human; measurement of protein adduct level	*in vitro*	NA	[^{14}C]BAF312 hemifumarate, 5 µM (free base)	not required	Novartis	DMPK R0400863-01	4.2.2.4-2
Metabolism *in vitro* 2.6.5.10B	human liver microsomes, human hepatocytes, recombinant human enzymes (CYP's, MAO, FAO)	*in vitro*	NA	[^{14}C]BAF312 hemifumarate, 5 µM (free base) (microsomes and enzymes); 10 µM (free base) (hepatocytes)	not required	Novartis	DMPK R0900482	4.2.2.4-3

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Compliance	Testing Facility	Study Number	Location in CTD
Possible Metabolic Pathways 2.6.5.11								
2.6.5.11A Pharmacokinetics: Possible metabolic pathways *in vitro* in mouse, rat, dog, monkey and human								
2.6.5.11B Pharmacokinetics: Possible metabolic pathways *in vivo* in mouse, rat, monkey and human								
2.6.5.11C Pharmacokinetics: Possible metabolic pathways *in vivo* in mouse								
2.6.5.11D Pharmacokinetics: Possible metabolic pathways *in vivo* in rat								
2.6.5.11E Pharmacokinetics: Possible metabolic pathways *in vivo* in monkey								
2.6.5.11F Pharmacokinetics: Possible metabolic pathways *in vivo* in human								
Induction / Inhibition of Drug-Metabolizing Enzymes 2.6.5.12								
No studies under this section								
Excretion 2.6.5.13								
Excretion 2.6.5.13A	Mouse, CD-1, male, food and water ad libitum	p.o.	single	[^{14}C]BAF312 hemifumarate, 8 mg/kg (free base)	not required	Novartis	DMPK R1300411-02	4.2.2.2-1
Excretion 2.6.5.13B	Rat, Hannover Wistar, male, food and water ad libitum	i.v. and p.o.	single	[^{14}C]BAF312, i.v.: 1 mg/kg; p.o.: 3 mg/kg	not required	Novartis	DMPK R0500017-02	4.2.2.2-2
Excretion 2.6.5.13C	Rat, Hannover Wistar, male, food and water ad libitum	i.v. and p.o.	single	[^{14}C]BAF312 hemifumarate, i.v.: 1 mg/kg (free base); p.o.: 3 mg/kg (free base)	not required	Novartis	DMPK R0900164-01	4.2.2.2-3
Excretion 2.6.5.13D	Monkey, cynomolgus, male, food and water ad libitum	p.o.	single	[^{14}C]BAF312 hemifumarate, 15 mg/kg (free base)	not required	Novartis	DMPK R1000166-01	4.2.2.2-6

Type of study	Test system(s), Species, Strain, Sex, Feeding	Administration, Sample(s), Batch(es) [a]	Duration of dosing	Analyte(s), Dose(s), mg/kg [b,c,d] Concentrations	GLP Compliance	Testing Facility	Study Number	Location in CTD
Excretion into Bile 2.6.5.14								
Excretion into bile 2.6.5.14A	Rat, Hanover Wistar, male, food and water ad libitum	i.v. and p.o.	single	[^{14}C]BAF312 hemifumarate, i.v.: 1 mg/kg (free base); p.o.: 10 mg/kg (free base)	not required	Novartis	DMPK R0900164-01	4.2.2.2-3
Drug-Drug Interactions 2.6.5.15								
No studies under this section								
Other 2.6.5.16								
Hepatic uptake 2.6.5.16A	hepatocytes from rat, monkey and human	NA	NA	[^{14}C]BAF312, 0.5, 1, 10 and 50 µM	not required	Novartis	DMPK R0700963	4.2.2.7-1

a: Tables summarize analytical and synthetic information from new synthesized and resynthesized materials. (Re-) purified materials, which were analyzed, released for the respective studies are documented in Certificates of Analysis (CoA) and referenced under "Additional Information" of their parent batch.

b: The assignment of the hemifumarate as a co-crystal is described in NCO.

c: To obtain concentrations in molar units, divide the concentrations in ng by 516.61 g/mol.

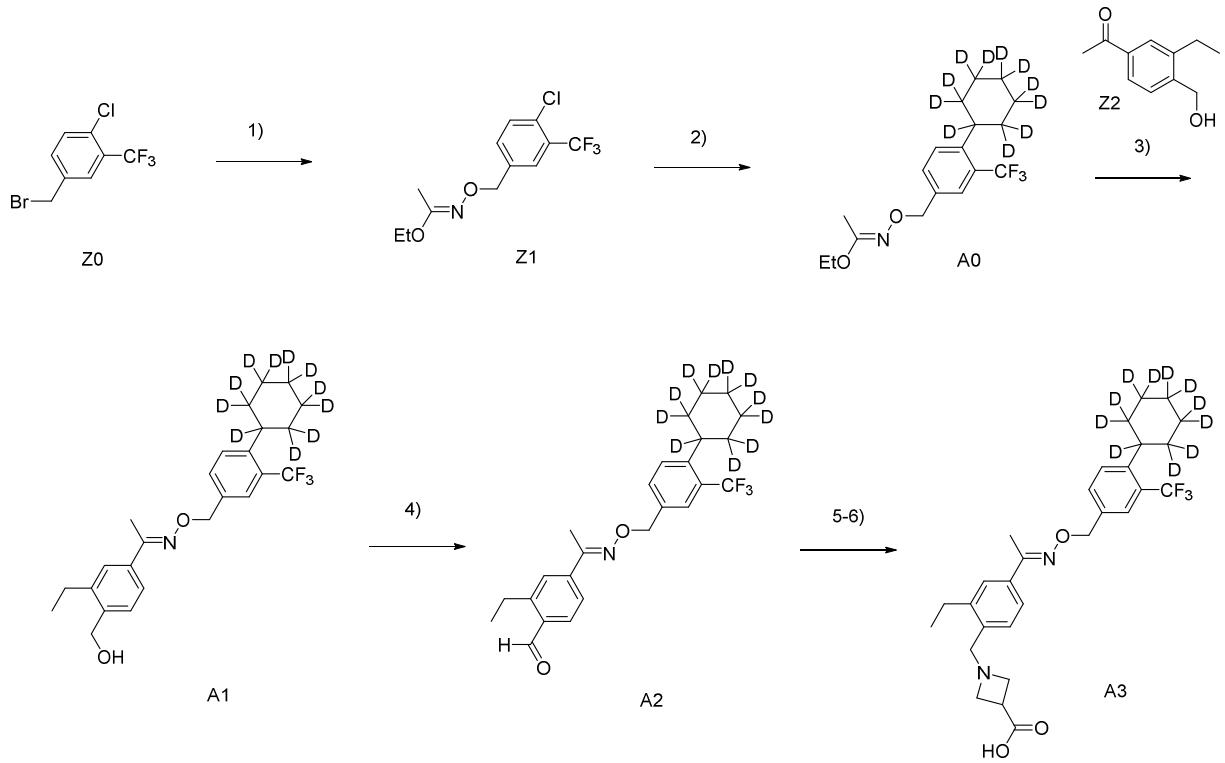
d: The majority of the preclinical PK studies using radiolabeled siponimod were performed with the ^{14}C-label in position 1. In order to investigate the cleavage/hydrolysis of the oxime ether moiety, ^{14}C-siponimod with the ^{14}C-label in position 2 (radiolabel on the benzyl-oxyamino part of the molecule) was used *in vivo* in an absorption, distribution, metabolism and excretion (ADME) study in rats (DMPK R0900164-01) and *in vitro* for the assessment of covalent protein binding (DMPK R0900482).

2.6.5.2 薬物動態試験：分析方法

2.6.5.2A Analytical Methods and Validation Reports (DMPK R0500098-01-01 (SSE 087-1))

Study title:	[D_{11}]BAF312 Synthesis and release analysis	Study no. DMPK R0500098-01-01 (SSE 087-1)
Location in CTD:	4.2.2.1-1	
GLP compliance:	not required	
Test article:	D_{11}-BAF312	
Salt form:	no	
Formula (Weight)	$C_{29}{}^{2}H_{11}H_{24}F_3N_2O_3$ (527.6 g/mol)	
Isotope Label:	Deuterium	
Synthesis Batch No.	SSE 087-1	
Preparation Batch No.	E-15132-074-12	
Analytical Methods (Identity, Mass and Chemical Purity):	HPLC-UV, ^{1}H-NMR spectroscopy, MS spectroscopy	
Analytical Results:		
-Degree of Labeling:	$M^0 + 11$	
-Mass Purity:	>99% (i.e. < 1% M^0)	
-Chemical Purity:	95.3% (HPLC-UV)	
Release restriction:	n.a.	

Synthesis Scheme

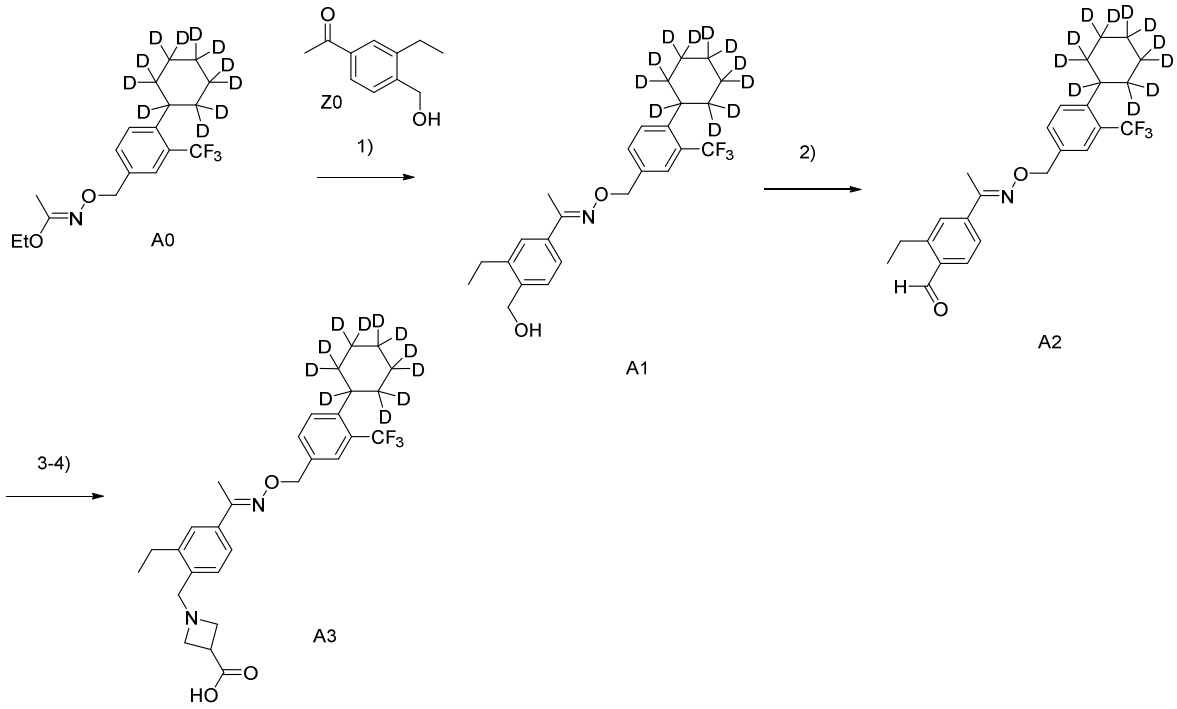


Reaction conditions: 1a) Ethyl N-hydroxyacetimidate, NaH, DMF, r.t.; 1b) Z0, DMF; 2a) $C_6D_{11}Br$, Mg, ether; 2b) $ZnCl_2$, NMP, 80°C; 2c) Z1, $Pd(P^tBu_3)_2$ 5%, NMP, 120°C; 3) conc.aq HCl, MeOH, Et_3N, pH= 4-6, r.t,; 4) MnO_2, dioxane, reflux; 5) 3-azetidine carboxylic acid, MeOH, Et_3N; 6) $NaBH_3CN$, MeOH, r.t.;

Synthesis Information	Ethyl N-hydroxyacetimidate was alkylated with benzylbromide derivative Z0 to obtain Z1. Negishi-coupling of Z1 with deuterium labeled magnesium cyclohexylbromide gave intermediate A0. Coupling of Z2 to A0 provided oxime derivative A1, which was then oxidized to the aldehyde A2 with manganese oxide. Reductive amination by reacting A2 with 3-azetidine carboxylic acid, followed by reduction with sodium cyanoborohydride and final purification by crystallization from toluene-heptane gave pure [D_{11}]NVP-BAF312 (A3).
Additional information:	no

2.6.5.2B Analytical Methods and Validation Reports (DMPK R1200161 (SSE 087-2))

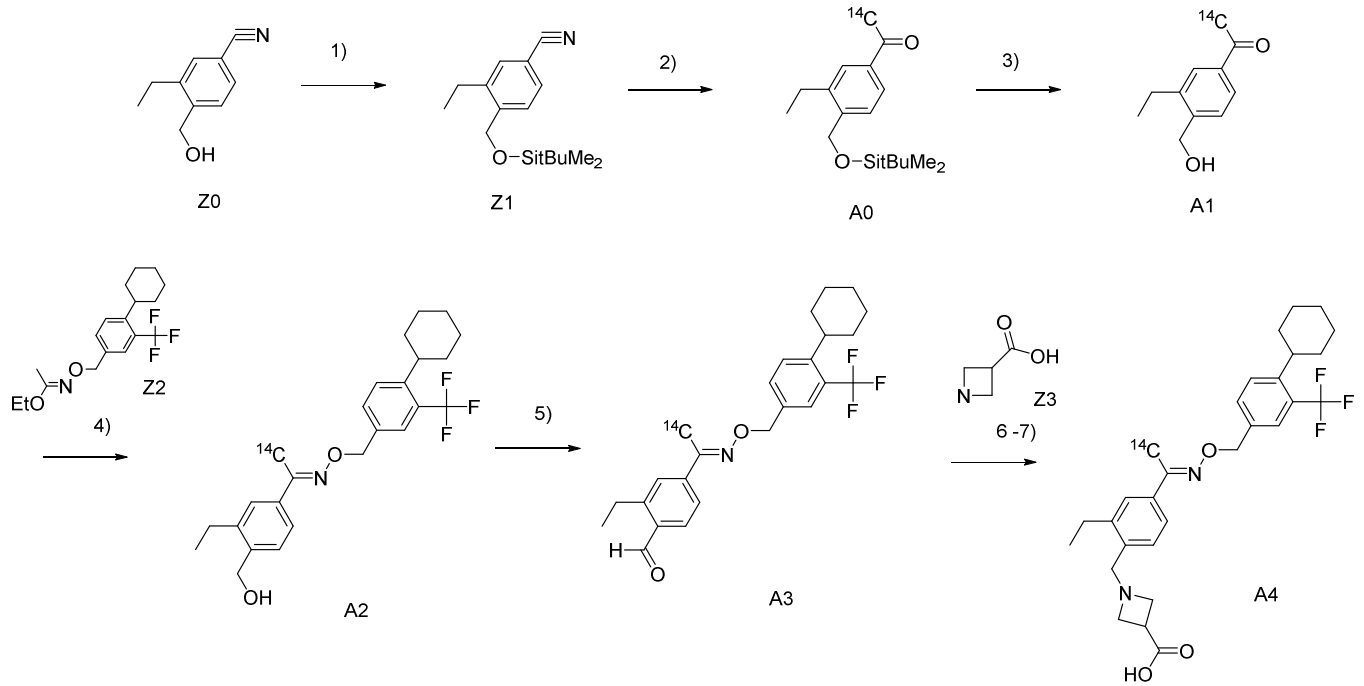
Study title:	[D_{11}]BAF312 Synthesis and release analysis	Study no. DMPK R1200161 (SSE 087-2)
Location in CTD:	4.2.2.1-2	
GLP compliance:	not required	
Test article:	D_{11}-BAF312	
Salt form:	no	
Formula (Weight)	$C_{29}{}^{2}H_{11}H_{24}F_3N_2O_3$ (527.6 g/mol)	
Stable Isotope Label:	Deuterium	
Synthesis Batch No.	SSE 087-2	
Preparation Batch No.	FK1-150512-A	
Analytical Methods (Identity, Mass and Chemical Purity):	HPLC-UV, ^{1}H-NMR spectroscopy, MS spectroscopy	
Analytical Results:		
-Degree of Labeling:	M^0 + 11	
-Mass Purity:	>99% (i.e. < 1% M^0)	
-Chemical Purity:	99.4%	
Release restriction:	[D_{11}]NVP-BAF312 (Batch SSE 087-2) was released for analytical and technical studies.	

Synthesis Scheme	 **Reaction conditions:** 1) HCl, MeOH, Et_3N, pH= 4-6, r.t.; 2) MnO_2, dioxane, reflux; 3) 3-azetidine carboxylic acid, MeOH, Et_3N; 4) $NaBH_3CN$, MeOH, r.t.;
Synthesis Information	Coupling of labeled A0 (prepared by *Alsachim*, external supplier) to the ketone derivative Z0 provided oxime derivative A1, which was then oxidized to aldehyde A2 with manganese oxide. Reductive amination process by reacting A2 with 3-azetidine carboxylic acid, followed by reduction with sodium cyanoborohydride and final HPLC preparative purification afforded [D_{11}]NVP-BAF312 (A3).
Additional information:	no

2.6.5.2C Analytical Methods and Validation Reports (DMPK R0400858-01-01 (RSE 280-1+2))

Study title:	[^{14}C]BAF312 Synthesis and release analysis (RSE280-1)	Study no. DMPK R0400858-01-01 (RSE 280-1+2)
Location in CTD:	4.2.2.1-3	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	no	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ (516.61 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE 280-1	
Preparation Batch No.	E-15132-059-32	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, ^{1}H-NMR spectroscopy, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	2.078 GBq/mmol; 4.023 MBq/mg	
-Radiochemical Purity:	99.7%	
-Chemical Purity:	99.5% (HPLC-UV)	
Release restriction:	[^{14}C]NVP-BAF312 (batch RSE 280-1) was released for technical and animal studies..	

Synthesis Scheme:



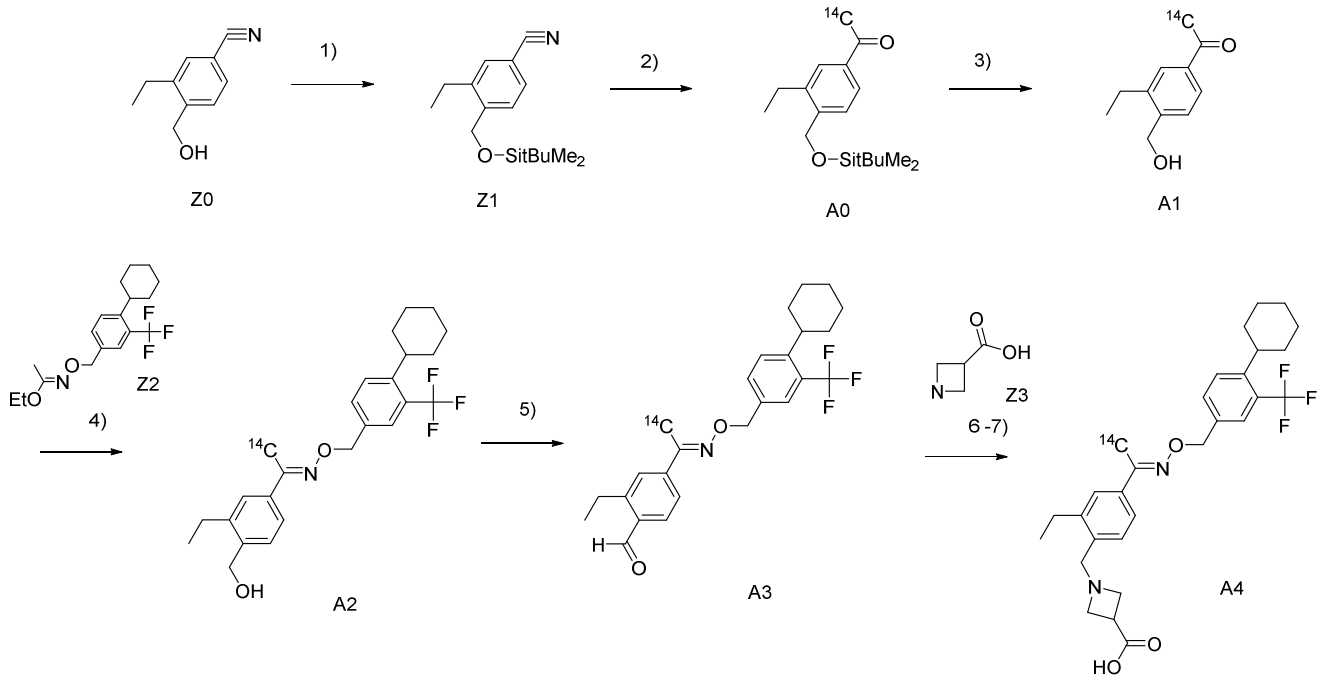
Reaction conditions: 1) ^{t}BuMe$_2$SiCl, Et$_3$N, THF; 2a) ^{14}CH$_3$I, nBuLi, THF; 2b) Z0, CuI 3%, 0°C, THF; 3) n-Bu$_4$NF, 1M in THF, r.t.; 4) conc. HClaq. MeOH, Et$_3$N, pH= 4 - 6, r.t.; 5) MnO$_2$, dioxane, reflux; 6) Z3, MeOH, Et$_3$N; 7) NaBH$_3$CN, MeOH, r.t.;

Synthesis Information:	Benzylalcohol derivative Z0 was protected to obtain Z1. Addition of labeled [^{14}C] methyl lithium, previously prepared from C-14 methyl iodide, to the cyanide Z1, catalyzed by cuprous iodide gave ketone A0. Removal of the silyl group with tetrabutylamonium fluoride gave alcohol derivative A1. Coupling of Z2 to A1 provided the oxime derivative A2, which was then oxidized to the aldehyde A3 with manganese dioxide. Reductive amination by reacting A3 with 3-azetidine carboxylic acid Z3, followed by reduction with sodium cyanoborohydride and final purification by preparative HPLC and crystallization from toluene-heptane gave pure [^{14}C]NVP-BAF312 (A4).
Additional information:	no

Analytical Methods and Validation Reports (DMPK R0400858-01-01 (RSE 280-1+2))

Study title:	[^{14}C]BAF312 Synthesis and release analysis (RSE-02)	Study no.DMPK R0400858-01-01 (RSE 280-1+2)
Location in CTD:	4.2.2.1-3	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	no	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ (516.61 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE 280-2	
Preparation Batch No.	E-15132-061-06	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, ^{1}H-NMR spectroscopy, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	2.029 GBq/mmol; 3.928 MBq/mg	
-Radiochemical Purity:	99.2%	
-Chemical Purity:	99.4%	
Release restriction:	[^{14}C]NVP-BAF312 (batch RSE 280-2) was released for technical and animal studies..	

Synthesis Scheme:



Reaction conditions: 1) $^{t}BuMe_2SiCl$, Et_3N, THF; 2a) $^{14}CH_3I$, nBuLi, THF; 2b) Z0, CuI 3%, 0°C, THF; 3) nBu_4NF, 1M in THF, r.t.; 4) conc. HClaq. MeOH, Et_3N, pH= 4 - 6, r.t.; 5) MnO_2, dioxane, reflux; 6) Z3, MeOH, Et_3N; 7) $NaBH_3CN$, MeOH, r.t.;

Synthesis Information:	Benzylalcohol derivative Z0 was protected to obtain Z1. Addition of labeled [^{14}C] methyl lithium, previously prepared from C-14 methyl iodide, to the cyanide Z1, catalyzed by cuprous iodide gave ketone A0. Removal of the silyl group with tetrabutylamonium fluoride gave alcohol derivative A1. Coupling of Z2 to A1 provided the oxime derivative A2, which was then oxidized to the aldehyde A3 with manganese dioxide. Reductive amination by reacting A3 with 3-azetidine carboxylic acid Z3, followed by reduction with cyanoborohydride and final purification by preparative HPLC and crystallization from toluene-heptane gave pure [^{14}C]BAF312 (A4)**.**
Additional information:	no

2.6.5.2D Analytical Methods and Validation Reports (DMPK R0400858-02 (RSE 280-4))

Study title:	[^{14}C]BAF312 Purification and release analysis	Study no. DMPK R0400858-02 (RSE 280-4)
Location in CTD:	4.2.2.1-4	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	no	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ (516.61 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE 280-4	
Preparation Batch No.	E-30585-33	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	2.179 GBq/mmol; 4.218 MBq/mg	
-Radiochemical Purity:	98.5%	
-Chemical Purity:	99.2% (HPLC-UV)	
Release restriction:	[^{14}C]NVP-BAF312, batch RSE 280-4, was released for technical and animal studies.	
Synthesis Scheme:	n.a.	
Synthesis Information:	n.a.	
Additional information:	produced by HPLC-purification of [^{14}C]NVP-BAF312 (RSE280-02)	

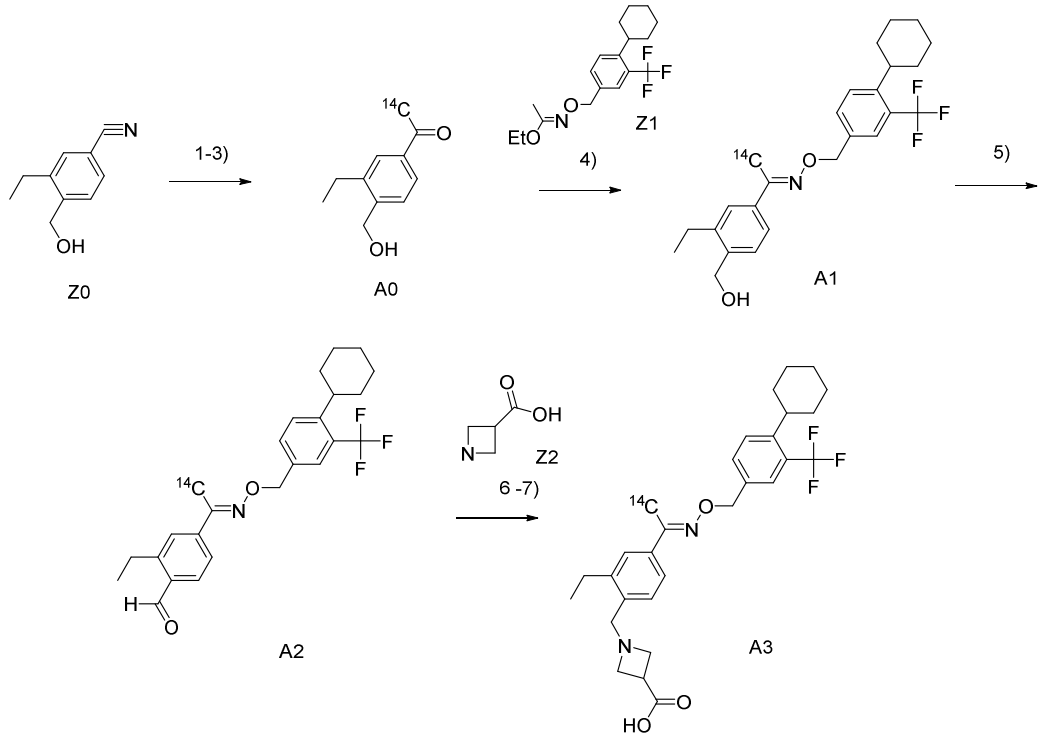
2.6.5.2E Analytical Methods and Validation Reports (DMPK R0400858-04 (RSE 280-7))

Study title:	[^{14}C]BAF312 Purification and release analysis	Study no. DMPK R0400858-04 (RSE 280-7)
Location in CTD:	4.2.2.1-5	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	no	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ (516.61 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE 280-7	
Preparation Batch No.	E-33614-58-18	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	2.09 GBq/mmol; 4.05 MBq/mg	
-Radiochemical Purity:	99.5%	
-Chemical Purity:	98.7% (HPLC-UV)	
Release restriction:	[^{14}C]NVP-BAF312 (batch RSE 280-7) was released for technical and animal studies.	
Synthesis Scheme:	n.a.	
Synthesis Information:	n.a.	
Additional information:	a) produced by HPLC-purification of [^{14}C]NVP-BAF312 (RSE280-05) and [^{14}C]NVP-BAF312 (RSE280-06) Supplementary information related to other studies using [^{14}C] BAF312: The parent batch of [^{14}C] NVP-BAF312 (RSE280-7) was purified to produce [^{14}C] NVP-BAF312 (RSE280-8). All specifications (salt form, analytical data, release decisions) are given in a Certificate of Analysis (CoA) archived together with the respective study documentation.	

2.6.5.2F Analytical Methods and Validation Reports (DMPK R0600841a (RSE 280-9))

Study title:	[^{14}C]BAF312 Synthesis and release analysis	Study no. DMPK R0600841a (RSE 280-9)
Location in CTD:	4.2.2.1-6	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	no	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ (516.61 g/mol)	
Radioisotope Label:	Carbon-14	
Radioisotope Batch No.	RSE 280-9	
Preparation Batch No.	E-19105-24-26	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, ^{1}H-NMR spectroscopy, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	2.073 GBq/mmol; 4.013 MBq/mg	
-Radiochemical Purity	99.4%	
-Chemical Purity:	97.3% (HPLC-UV)	
Release restriction:	[^{14}C]NVP-BAF312 (batch RSE 280-9) was released for technical and animal studies.	

Synthesis Scheme:



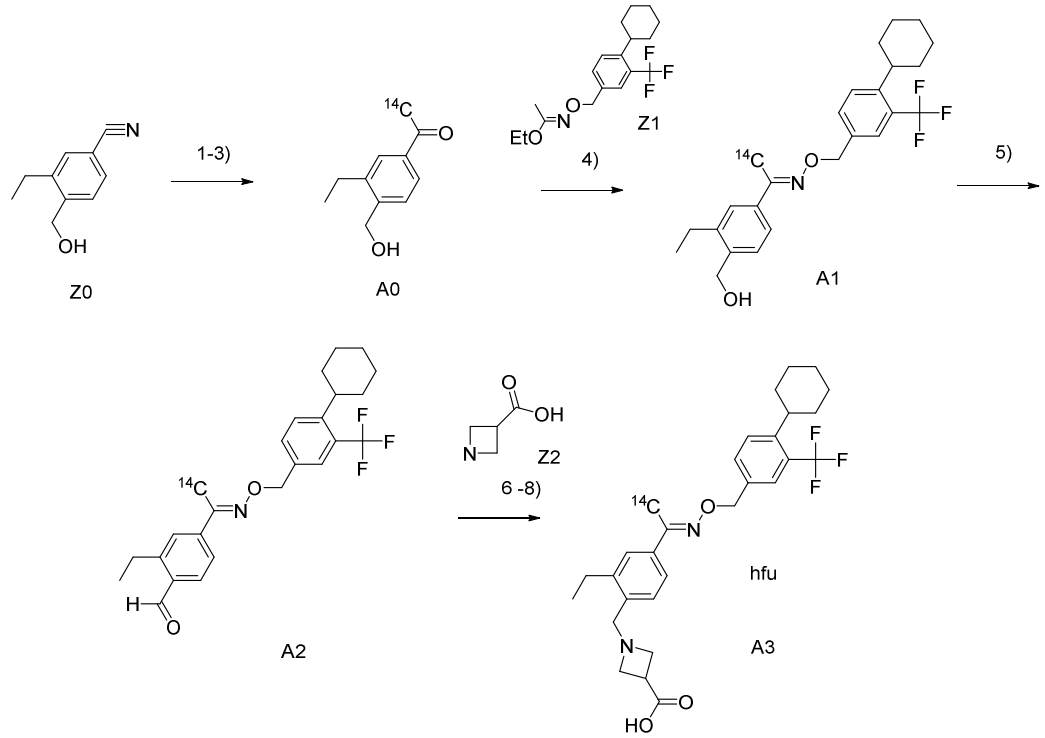
Reaction conditions: 1) ^{t}BuMe$_2$SiCl**,** Et$_3$N, THF**;** 2a) ^{14}CH$_3$I, nBuLi, THF; 2b) CuI 3%, 0°C, THF; 3) nBu$_4$NF, 1M in THF, r.t.; 4) Z1, conc. HClaq. MeOH, Et$_3$N, pH= 4-6, r.t.; 5) MnO$_2$, heptane; 6) Z2, MeOH, NaBH$_3$CN, r.t.; 7) Fumaric acid, EtOH; HPLC-purification;

Synthesis Information:	The benzylic alcohol Z0 was protected with tert-butyldimethylsilylchloride and the nitrile group was further reacted with labeled [^{14}C] methyl lithium, which was previously prepared from C-14 methyl iodide to give after removal of the silyl group the ketone A0. Coupling of Z1 to A0 provided the oxime A1, which was then oxidized to the aldehyde A2 with manganese dioxide. Reductive amination by reacting A2 with 3-azetidine carboxylic acid Z2, followed by reduction with sodium cyanoborohydride and purification of the free base yielded [^{14}C]NVP-BAF312 (A3) by preparative HPLC.
Additional information:	no

2.6.5.2G Analytical Methods and Validation Reports (DMPK R0900379a (RSE 280-13))

Study title:	[^{14}C]BAF312 Synthesis and release analysis	Study no. DMPK R0900379a (RSE 280-13)
Location in CTD:	4.2.2.1-7	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	hemifumarate	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ and 0.5 $C_4H_4O_4$ (574.6 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE 280-13	
Preparation Batch No.	E-17435-026-05	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, ^{1}H-NMR spectroscopy, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	2.046 GBq/mmol; 3.560 MBq/mg	
-Radiochemical Purity:	98.6%	
-Chemical Purity:	99.5% (HPLC-UV)	
Release restriction:	[^{14}C]NVP-BAF312 (batch RSE 280-13) was released for as precursor for preparation of radiolabeled drug substance for human ADME studies and for technical and animal studies.	

Synthesis Scheme:



Reaction conditions: 1) ^{t}BuMe$_2$SiCl**,** Et$_3$N, THF**;** 2a) ^{14}CH$_3$I, nBuLi, THF; 2b) CuI 3%, 0°C, THF; 3) nBu$_4$NF, 1M in THF, r.t.; 4). Z1, conc. HClaq. MeOH, Et$_3$N, pH= 4 - 6, r.t.; 5) MnO_2, heptane; 6) Z2, MeOH, NaBH$_3$CN, r.t.; 7. Preparative HPLC, acetonitrile:water:0.05%AcOH; 8) Fumaric acid, EtOH;

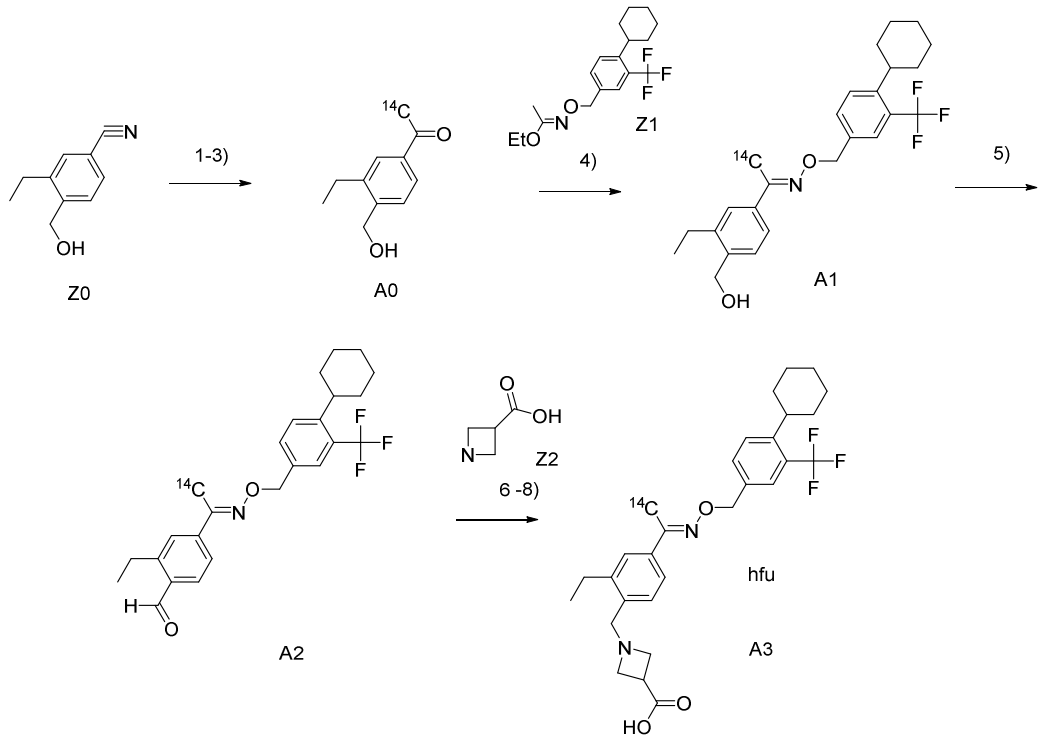
Synthesis Information:	The benzylic alcohol Z0 was protected with tert-butyldimethylsilylchloride and the cyanide group was further reacted with labeled [^{14}C] methyl lithium, which was previously prepared from C-14 methyl iodide to give after removal of the silyl group the ketone A0. Coupling of Z1 to A0 provided the oxime A1, which was then oxidized to the aldehyde intermediated A2 with manganese dioxide. Reductive amination by reacting A2 with 3-azetidine carboxylic acid Z2, followed by reduction with sodium cyanoborohydride gave crude [^{14}C]BAF312**.** The compound was purified by preparative HPLC. The free [^{14}C] NVP-BAF312 was dissolved in ethanol and treated with fumaric acid to give after crystallization the hemifumarate.

Additional information:	Supplementary information related to other studies using [^{14}C] BAF312: Batch [^{14}C] NVP-BAF312 (RSE280-13) was used to produce [^{14}C] NVP-BAF312 (RSE280-15, E-42861-105-12). Dilution to the respective specific activity and recrystallization was done under GMP-conditions. [^{14}C]NVP-BAF312 (RSE280-15, E-42861-105-12) was released for investigational use in humans. The radiochemical purity assessed by radio-HPLC and the chemical purity assessed by HPLC with UV detection was >99%. The parent batch of [^{14}C] BAF312 (RSE280-13) was (re)purified to produce [^{14}C] BAF312 RSE280-22, RSE280-25, RSE280-26, RSE280-27, RSE280-28 and RSE280-29. Batch RSE280-13 was purified by using a preparative reversed phase HPLC system to produce batch RSE280-23.The batch RSE280-23 was diluted with unlabeled BAF312 to yield the batch RSE280-24. All specifications (salt form, analytical data, and release decisions) are given in the respective Certificates of Analysis archived together with the respective study documentation.

2.6.5.2H Analytical Methods and Validation Reports (DMPK R1300493 (RSE 280-30))

Study title:	[^{14}C]BAF312 Synthesis and release analysis	Study no. DMPK R1300493 (RSE 280-30)
Location in CTD:	4.2.2.1-8	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	hemifumarate	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ and 0.5 $C_4H_4O_4$ (574.6 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE 280-30	
Preparation Batch No.	RR1-151013-A	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, ^{1}H-NMR spectroscopy, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	1.986 GBq/mmol; 3.457 MBq/mg	
-Radiochemical Purity:	98.9%	
-Chemical Purity:	98.4% (HPLC-UV)	
Release restriction:	[^{14}C]NVP-BAF312 (batch RSE 280-30) was released for analytical, technical and preclinical study purposes.	

Synthesis Scheme:



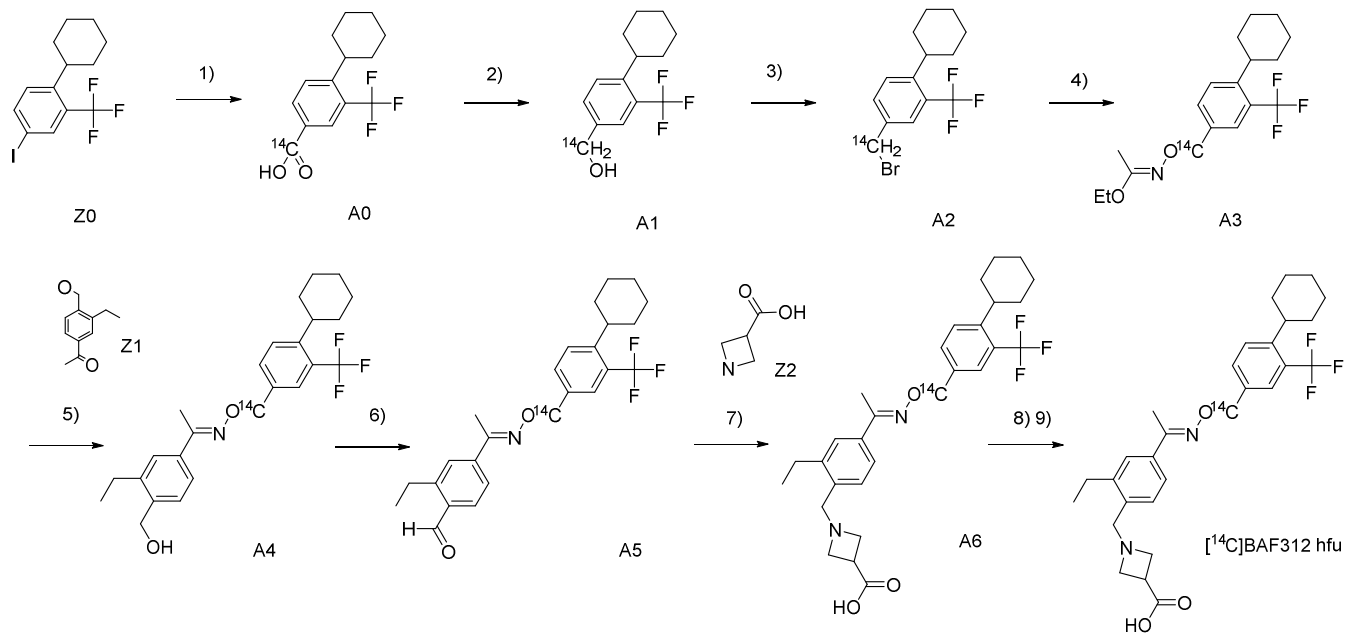
Reaction conditions*:* Steps provided by supplier Tjaden (external): 1. t-BuMe$_2$SiCl, Et$_3$N, THF; 2a. $^{14}CH_3I$, n-BuLi, THF; 2b. CuI 3%, 0°C, THF; 3. n-Bu$_4$NF, 1M in THF, r.t. (Quality: crude); Steps provided by NVS Basel Isotope Laboratories (internal): 4. Z1, conc. HClaq. MeOH, Et3N, pH= 4-6, r.t.; 5. MnO2, heptane; 6. Z2, MeOH, NaBH3CN, r.t.; 7. Preparative HPLC, acetonitrile : water : 0.05% AcOH; 8. fumaric acid, EtOH;

Synthesis Information:	Crude ketone A0 provided by Tjaden (external supplier) was coupled with Z1 to provide the oxime A1, which when oxidized to the aldehyde A2 allowed reductive amination with the 3-azetidine carboxylic acid Z2 to give crude [^{14}C]BAF312. The compound was purified by preparative HPLC. The free [^{14}C] NVP-BAF312 was dissolved in ethanol, treated with fumaric acid and recrystallized to provide its hemifumarate.
Additional information:	Supplementary information related to other studies using [^{14}C] BAF312: The parent batch of [^{14}C] NVP-BAF312 (RSE280-30) was (re)purified to produce [^{14}C] NVP-BAF312 (RSE280-31, 32, 33 and 35). All specifications (salt form, analytical data, release decisions) are given in Certificates of Analysis (CoA) archived together with the study documentation.

2.6.5.2l Analytical Methods and Validation Reports (DMPK R0800721a (RSE 425-1 +2))

Study title:	[^{14}C]BAF312 Synthesis and release analysis (RSE 425-1)	Study no. DMPK R0800721a (RSE 425-1+2)
Location in CTD:	4.2.2.1-9	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	hemifumarate	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ and 0.5 $C_4H_4O_4$ (574.6 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE 425-1	
Preparation Batch No.	E-17225-066-38	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, ^{1}H-NMR spectroscopy, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	3.599 MBq/mg	
-Radiochemical Purity:	98.1%	
-Chemical Purity:	99.5% (HPC-UV)	
Release restriction:	[^{14}C]NVP-BAF312 (batch RSE 425-1) was released for technical and animal studies.	

Synthesis Scheme:



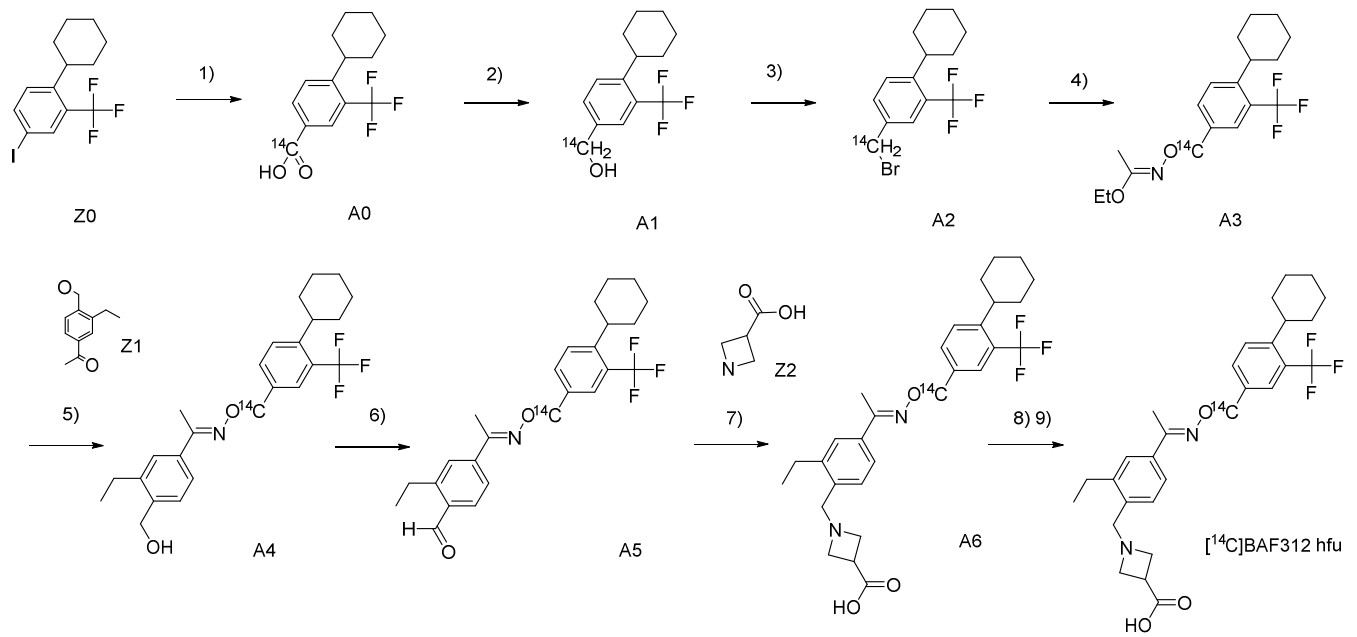
Reaction conditions: 1) nBuLi, THF, [^{14}C]CO_2, 2) BH_3.Me_2S, THF; 3) PBr_3, ether; 4) N-Hydroxy-acetimidic acid ethyl ester, NaH, DMF; 5a) Z1, HCl 37%, MeOH; 5b) Et_3N, pH= 4-6; 6) MnO_2, heptane; 7) Z2, MeOH, $NaBH_3CN$, r.t.; 8) Preparative HPLC; 9) Fumaric acid, EtOH;

Synthesis Information:	The iodide Z0 was lithiated by reaction with butyllithium and further reacted with [^{14}C] carbon dioxide. The acid A0 was reduced to the alcohol A1 with borane-dimethyl sulfide and then converted to the corresponding bromide A2 by reaction with phosphorous tribromide. The bromide A2 was transformed to the oxime A3 by addition to N-hydroxy-acetimidic acid ethyl ester and further coupled to the ketone Z1 to provide the oxime intermediate A4. Oxidation of the corresponding alcohol A4 to the aldehyde intermediate A5 by reaction with manganese dioxide and further reductive amination by reacting A5 with 3-azetidine carboxylic acid Z2, followed by reduction with sodium cyanoborohydride gave crude [^{14}C]NVP-BAF312 (A6)**.** The crude labeled compound was purified by preparative HPLC and transformed to the hemifumarate (hfu) by mixing with fumaric acid in ethanol.
Additional information:	Supplementary information related to other studies using [^{14}C] BAF312: The parent batch of [^{14}C] NVP-BAF312 (RSE425-1) was purified to produce [^{14}C] NVP-BAF312 (RSE425-3). All specifications (salt form, analytical data, release decisions) are given in Certificate of Analysis (CoA) archived together with the respective study documentation.

Analytical Methods and Validation Reports (DMPK R0800721a (RSE 425-1+2))

Study title:	[^{14}C]BAF312 Synthesis and release analysis (RSE425-2)	Study no. DMPK R0800721a (RSE 425-1+2)
Location in CTD:	4.2.2.1-9	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Salt form:	hemifumarate	
Formula (Weight) (unlabeled)	$C_{29}H_{35}F_3N_2O_3$ and 0.5 $C_4H_4O_4$ (574.6 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE 425-2	
Preparation Batch No.	E-17225-070-38	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, ^{1}H-NMR spectroscopy, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	3.424 MBq/mg	
-Radiochemical Purity:	97.9%	
-Chemical Purity:	99.2% (HPLC-UV)	
Release restriction:	[^{14}C]NVP-BAF312 (batch RSE 425-2) was released for technical and animal studies.	

Synthesis Scheme:



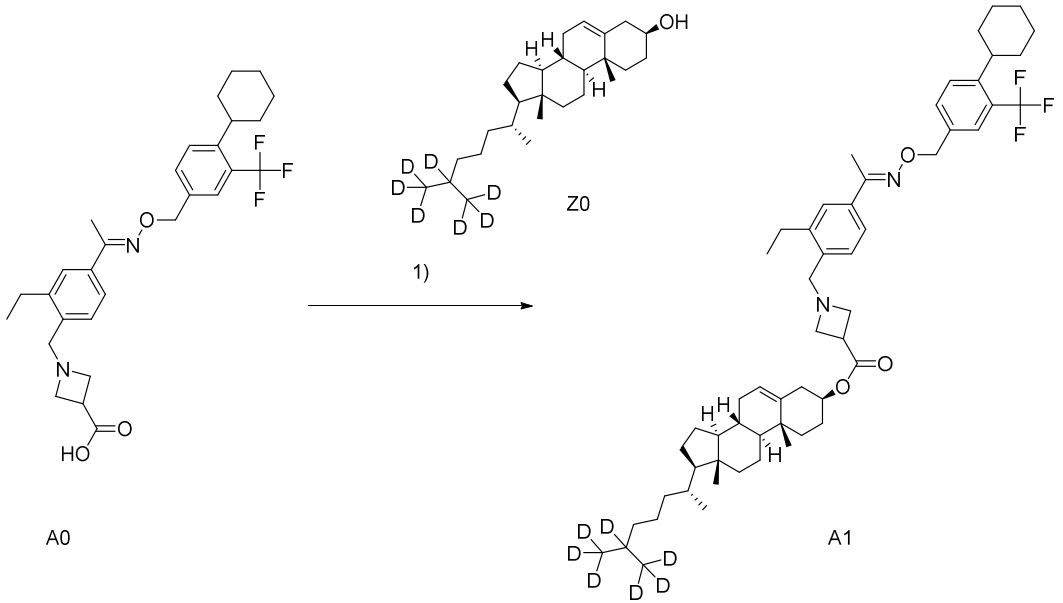
Reaction conditions: 1) nBuLi, THF, [^{14}C]CO_2, 2) BH_3.Me_2S, THF; 3) PBr_3, ether; 4) N-Hydroxy-acetimidic acid ethyl ester, NaH, DMF; 5a) Z1, HCl 37%, MeOH; 5b) Et_3N, pH= 4-6; 6) MnO_2, heptane; 7) Z2, MeOH, $NaBH_3CN$, r.t.; 8) Preparative HPLC; 9) Fumaric acid, EtOH;

Synthesis Information:	The iodide Z0 was lithiated by reaction with butyllithium and further reacted with [^{14}C] carbon dioxide. The acid A0 was reduced to the alcohol A1 with borane-dimethyl sulfide and then converted to the corresponding bromide A2 by reaction with phosphorous tribromide. The bromide A2 was transformed to the oxime A3 by addition to N-hydroxy-acetimidic acid ethyl ester and further coupled to the ketone Z1 to provide the oxime intermediate A4. Oxidation of the corresponding alcohol A4 to the aldehyde intermediate A5 by reaction with manganese dioxide and further reductive amination by reacting A5 with 3-azetidine carboxylic acid Z2, followed by reduction with sodium cyanoborohydride gave crude [^{14}C]BAF312 A6**.** The crude labeled compound was purified by preparative HPLC and transformed to the hemifumarate (hfu) by mixing with fumaric acid in ethanol.
Additional information:	no

2.6.5.2J Analytical Methods and Validation Reports (DMPK R1500011 (SSE 286-1))

Study title:	[D_7]LYS815 Synthesis and release analysis	Study no. DMPK R1500011 (SSE 286-1)
Location in CTD:	4.2.2.1-10	
GLP compliance:	not required	
Test article:	D_7-LYS815	
Salt form:	no	
Formula (Weight)	$C_{56}{}^2H_7H_{72}F_3N_2O_3$ (892.28 g/mol)	
Stable Isotope Label:	Deuterium	
Synthesis Batch No.	SSE 286-1	
Preparation Batch No.	RB1-120215-A	
Analytical Methods (Identity, Mass and Chemical Purity):	HPLC-UV, ^{1}H-NMR spectroscopy, MS spectroscopy	
Analytical Results:		
-Degree of Labeling:	$M^0 + 7$	
-Mass purity:	>99% (i.e. < 1% M^0)	
-Chemical Purity:	98.9%	
Release restriction:	Stable labeled NVP-LYS815 (batch SSE 286-1) was released for analytical and technical studies.	

Synthesis Scheme



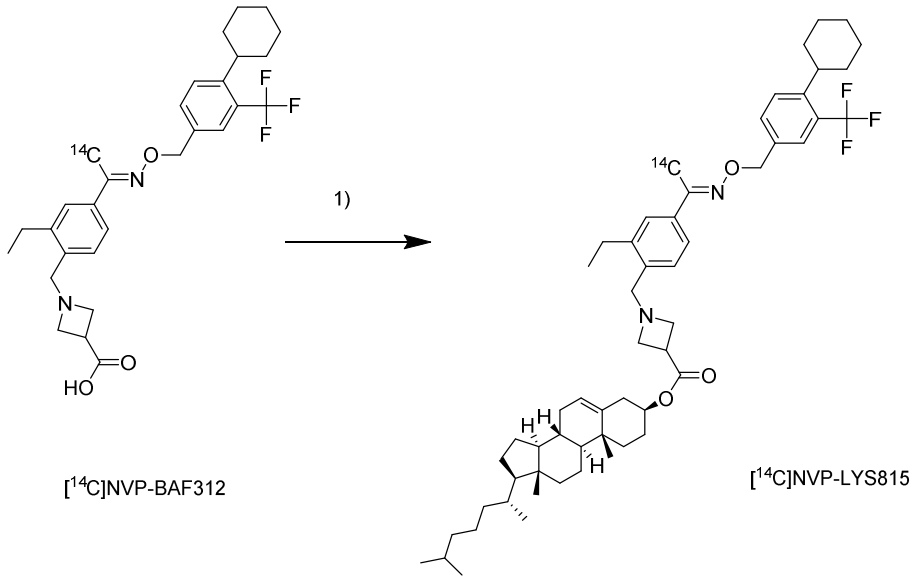
Reaction conditions: [D_7]cholesterol (CAS 83199-47-7; provider: Avanti Polar Lipids, Inc. (product number 700041P, lot CH(D7)-16)), N,N'-Diisopropylcarbodiimide (DIC), 4-dimethylamino pyridine (DMAP), CH_2Cl_2, r.t.;

Synthesis Information	N, N'-Diisopropylcarbodiimide (DIC)/ 4-dimethylamino pyridine (DMAP) mediated esterification of NVP-BAF312 (A0) with enantiomerically pure Z0 provided [D_7] NVP-LYS815 (A1).
Additional information:	no

2.6.5.2K Analytical Methods and Validation Reports (DMPK R1500578 (RSE 679-1))

Study title:	[^{14}C]LYS815 Synthesis and release analysis	Study no. DMPK R1500578 (RSE679-1)
Location in CTD:	4.2.2.1-11	
GLP compliance:	not required	
Test article:	[^{14}C]LYS815	
Salt form:	trifluoroacetate	
Formula (Weight) (unlabeled)	$C_{56}H_{79}F_3N_2O_3$ and CF_3COOH (999.27 g/mol)	
Radioisotope Label:	Carbon-14	
Synthesis Batch No.	RSE679-1	
Preparation Batch No.	RR1-021015-B	
Analytical Methods (Identity, Radiochemical and Chemical Purity, Position of Label, Specific Activity):	HPLC-UV, HPLC-RA, ^{1}H-NMR spectroscopy, MS spectroscopy, LSC	
Analytical Results:		
-Specific Activity:	2.005 GBq/mmol; 2.265 MBq/mg	
-Radiochemical Purity:	99.7%	
-Chemical Purity:	99.9% (HPLC-UC)	
Release restriction:	[^{14}C]NVP-LYS815 (batch RSE 679-1) was released for analytical, technical and preclinical study purposes.	

Synthesis Scheme:



Reaction conditions: 1) a. [^{14}C] NVP-BAF312 (RSE 280-30 ROH); CH_2Cl_2, N, N'-diisoproylcarbodiimide (DIC), 4-dimethylamino pyridine (DMAP), cholesterol; b.) preparative HPLC purification;

Synthesis Information:	Cholesterol was coupled with [^{14}C] NVP-BAF312 (RSE 280-30 ROH) using N, N'-diisoproylcarbodiimide (DIC)/ 4-dimethylamino pyridine (DMAP) mediated conditions. Subsequent HPLC purification afforded [^{14}C] NVP-LYS815.
Additional information:	no

2.6.5.2L Analytical Methods and Validation Reports (DMPK R0500135a-01)

Study title:	Quantitative determination of BAF312 in mouse plasma by LC-MS/MS. Method validation report	Study no. DMPK R0500135a-01
Location in CTD:	4.2.2.1-12	
GLP compliance:	no	
Method:	The method for a 50 µL plasma sample consists of protein precipitation in 200 µL acetonitrile containing internal standard, centrifugation, dilution of the supernatant (1:2, v/v) in 0.2% formic acid in water, injection of 20 µL into an LC-MS/MS system and measurement in SRM mode using APCI positive as the ionization method	
Instrument:	HPLC-MS/MS	
Substances analyzed:	BAF312 (Siponimod)	
Reference compounds:	BAF312	
Internal standards:	D_{11}-BAF312	
Results:	Validation in plasma	
Specificity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ	
Stability	Stable in stock solutions for 187 days at ≤-15°C and for 153 days at 2-8°C Stable in post-preparative extracts for 24h at 8°C Stable for 3 freeze-thaw cycles with storage at ≤-65°C Stable in spiked mouse plasma for 24h at room temperature	
	Stable in incurred mouse plasma for 347 days at ≤-15°C	
	D_{11}-BAF312 was stable in stock solutions for 153 days at 2-8°C	
Intra-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of mouse plasma	
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 for samples diluted up to 10-fold. Precision ≤ 15%	
Comments:	To obtain concentrations in molar units, divide the concentrations in ng by 516.61 g/mol.	
	Data available in (DMPK R0500125-01)	

2.6.5.2M Analytical Methods and Validation Reports (DMPK R1200039a)

Study title:	Validation of a method for the quantitative determination of BAF312 in mouse plasma by LC-MS/MS. Method validation report	Study no. DMPK R1200039a
Location in CTD:	4.2.2.1-13	
GLP compliance:	yes	
Method:	The method for a 25 µL plasma sample consists of protein precipitation in 50 µL acetonitrile containing internal standard, centrifugation, dilution of the supernatant (1:2, v/v) in 0.2% formic acid in water, injection of 2 µL into an LC-MS/MS system and measurement in SRM mode using ESI positive as the ionization method	
Instrument:	HPLC-MS/MS	
Substances analyzed:	BAF312 (Siponimod)	
Reference compounds:	BAF312	
Internal standards:	D_{11}-BAF312	
Results:	Validation in plasma	
Selectivity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ	
	Contribution of D_{11}-BAF312 to the signal of BAF312 ≤ 20% of analytical response at LLOQ	
	Contribution of BAF312 to the signal of D_{11}-BAF312 ≤ 5% of analytical response at the working concentration	
Stability	Stable in stock solutions for 87 days at ≤-85°C	
	Stable in post-preparative extracts for 123h at 8°C Stable for 3 freeze-thaw cycles with storage at ≤-25°C Stable in spiked mouse plasma for 6h at room temperature	
	Stable in spiked mouse plasma for 200 days at <-25°C	
	Stable in incurred mouse plasma for 182 days at ≤-25°C	
Intra-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
Inter-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 5000 ng/mL using 25 µL of mouse plasma	
Recovery	Range of recovery of BAF312: 89.4 to 96.3%. Precision ≤ 15%	
	Recovery for D_{11}-BAF312: 91.5%. Precision ≤ 15%	
Matrix effect:	Mean matrix factor at 3 concentration levels and 2 sources: 0.692 to 0.766. Precision of ≤ 15%	

	D_{11}-BAF312 normalized matrix factor at 3 concentration levels: 0.920 to 0.939. Precision of ≤ 15%
	Haemolized plasma for BAF312: variability of matrix effect ≤ 15%
Carryover	Response after injection of 5 blank samples following injection of the ULOQ <20% of the response observed for the LLOQ for BAF312
	Response after injection of one blank sample following injection of the ULOQ <5% of the response observed for the working concentration for D_{11}-BAF312
Calibration	Deviation of ±15% (±20% at the LLOQ) for 75% of the calibration standards from the nominal values at a minimum of 8 different levels
Incurred sample reanalysis	Normalized difference between first and repeat assay result within ±20% for at least two-thirds (2/3) of samples analyzed
Dilutions	Bias within ±15% of the nominal concentration for samples diluted up to 10-fold. Precision ≤ 15%
Special issues:	None
Comments:	To obtain concentrations in molar units, divide the concentrations in ng by 516.61 g/mol.

2.6.5.2N Analytical Methods and Validation Reports (DMPK R0500135-01)

Study title:	Quantitative determination of BAF312 in rat plasma and heart, lung and brain tissues by LC-MS/MS. Method validation report	Study no. DMPK R0500135-01
Location in CTD:	4.2.2.1-14	
GLP compliance:	no	
Method:	The method for a 50 µL plasma or tissue homogenate sample consists of protein precipitation in 200 µL acetonitrile containing internal standard, centrifugation, dilution of the supernatant (1:2,v/v) in 0.2% formic acid in water, injection of 20 µL into an LC-MS/MS system and measurement in SRM mode using APCI positive as the ionization method	
Instrument:	HPLC-MS/MS	
Substances analyzed:	BAF312 (Siponimod)	
Reference compounds:	BAF312	
Internal standards:	D_{11}-BAF312	
Results:	Validation in plasma	
Specificity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ	
Stability	Stable in stock solutions for 187 days at ≤-15°C and for 146 days at 2-8°C Stable in post-preparative extracts for 96h at 5°C Stable for 3 freeze-thaw cycles with storage at ≤-65°C Stable in spiked rat plasma for 96h at room temperature Stable in incurred rat plasma for 84 days at ≤-65°C D_{11}-BAF312 was stable in stock solutions for 153 days at 2-8°C	
Intra-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
Inter-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of rat plasma	
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 for samples diluted up to 2-fold. Precision ≤ 15%	
Special issues:	Only 2 QC's instead of 5 were prepared in second run of Intra-day thus overall number of replicates equal to 12 instead of 15. Overall CV% in acceptance criteria, no impact.	
Results:	Validation in brain homogenate and cross validation in heart homogenate	

Specificity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ
Intra-day accuracy and precision	Mean bias within ±30% of the nominal values Precision of ≤ 30%
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of rat brain or heart homogenate
Calibration	Deviation of ±30% for 75% of the calibration standards from the nominal values at a minimum of 6 different levels
Results:	Cross validation in lung homogenate (calibrated against Calibration standards in brain homogenate)
Specificity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ
Stability	Stable in post-preparative extracts for 72h at 5°C
Intra-day accuracy and precision	Mean bias within ±30% of the nominal values Precision of ≤ 30%
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of rat lung homogenate
Calibration	Calibration standards prepared in brain homogenate
Comments:	To obtain concentrations in molar units, divide the concentrations in ng by 516.61 g/mol.

2.6.5.2O Analytical Methods and Validation Reports (DMPK R1200039-02)

Study title:	Validation of a method for the determination of BAF312 in rat plasma samples by HPLC-MS/MS. Method validation report	Study no. DMPK R1200039-02
Location in CTD:	4.2.2.1-15	
GLP compliance:	no	
Method:	The method for a 25 µL plasma sample consists of protein precipitation in 50 µL acetonitrile containing internal standard, centrifugation, dilution of the supernatant (1:2, v/v) in 0.2% formic acid in water, injection of 2 µL into an LC-MS/MS system and measurement in SRM mode using ESI positive as the ionization method	
Instrument:	HPLC-MS/MS	
Substances analyzed:	BAF312 (Siponimod)	
Reference compounds:	BAF312	
Internal standards:	D_{11}-BAF312	
Results:	Validation in plasma	
Selectivity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ	
Stability	Stable in stock solutions for 87 days at ≤-85°C	
	Stable in stock solutions for 6.75h at room temperature	
	Stable in spiked rat plasma for 125 days at ≤-85°C and <-25°C	
	Stable in incurred rat plasma for 269 days at ≤-85°C	
Intra-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
Inter-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 5000 ng/mL using 25 µL of rat plasma	
Recovery	Range of recovery of BAF312: 98.7 to 108.4%. Precision ≤ 15%	
Matrix effect:	Mean matrix factor at 3 concentration levels and 2 sources: 0.934 to 1.038. Precision of ≤ 15%	
	D_{11}-BAF312 normalized matrix factor at 3 concentration levels: 1.006 to 1.148. Precision of ≤ 15%	
Carryover	Response after injection of 4 blank samples following injection of the ULOQ <20% of the response observed for the LLOQ for BAF312	
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 for samples diluted up to 10-fold. Precision ≤ 15%	
Special issues:	None	

Comments: To obtain concentrations in molar units, divide the concentrations in ng by 516.61 g/mol.

2.6.5.2P Analytical Methods and Validation Reports (DMPK R1600622)

Study title:	Validation of a method for the determination of BAF312 in rat plasma using liquid chromatography with tandem mass spectrometric detection	Study no. DMPK R1600622
Location in CTD:	4.2.2.1-16	
GLP compliance:	no	
Method:	The method for a 25 µL plasma sample consists of addition of 25 µL of internal standard intermediate solution followed by protein precipitation with 150 µL acetonitrile, centrifugation, evaporation of a 100 µL aliquot of the supernatant, reconstitution with 150 µL of acetonitrile: water (10:90), injection of 5 µL into an LC-MS/MS system and measurement in SRM mode using ESI positive as the ionization method.	
Instrument:	HPLC-MS/MS	
Substances analyzed:	BAF312 (Siponimod)	
Reference compounds:	BAF312	
Internal standards:	D_{11}-BAF312	
Results:	Validation in plasma	
Selectivity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ	
Stability	Stable in stock solutions for 15 days at 2-8°C	
	Stable in stock solutions for 6h at room temperature Stable in post-preparative extracts for 71h at 2-8°C	
	Stable for 4 freeze-thaw cycles with storage at ≤-20°C and ≤-80°C Stable in spiked rat plasma for 24h at room temperature	
	Stable in spiked rat plasma for 34 days at <-20°C	
	Stable in spiked rat plasma for 142 days at ≤-80°C	
	Stable in rat whole blood for 2h at room temperature and on wet ice	
Intra-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
Inter-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 5000 ng/mL using 25 µL of rat plasma	
Recovery	Range of recovery of BAF312: 98.9 to 100.1%. Precision ≤ 15%	
Matrix effect:	Mean normalized matrix factor at 2 concentration levels: 1.0. Precision of ≤ 15%	
	D_{11}-BAF312 normalized matrix factor at 2 concentration levels: 1.1. Precision of ≤ 15%	

Carryover	Response after injection of 1 blank samples following injection of the ULOQ <20% of the response observed for the LLOQ for BAF312
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 for samples diluted up to 20-fold. Precision ≤ 15%
Special issues:	None
Comments:	To obtain concentrations in molar units, divide the concentrations in ng by 516.61 g/mol.

2.6.5.2Q Analytical Methods and Validation Reports (DMPK R0500135c-02)

Study title:	Quantitative determination of BAF312 in rabbit plasma and fetus homogenate by LC-MS/MS. Method validation report	Study no. DMPK R0500135c-02
Location in CTD:	4.2.2.1-17	
GLP compliance:	no	
Method:	The method for a 50 µL plasma or fetus homogenate sample consists of protein precipitation in 200 µL acetonitrile containing internal standard, centrifugation, dilution of the supernatant (1:2, v/v) in 0.2% formic acid in water, injection of 20 µL into an LC-MS/MS system and measurement in SRM mode using APCI positive as the ionization method	
Instrument:	HPLC-MS/MS	
Substances analyzed:	BAF312 (Siponimod)	
Reference compounds:	BAF312	
Internal standards:	D_{11}-BAF312	
Results:	Validation in plasma	
Specificity *	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ	
Stability	Stable in stock solutions for 187 days at ≤-15°C and for 146 days at 2-8°C [a)] Stable in post-preparative extracts for 24h at 5°C Stable for 3 freeze-thaw cycles with storage at ≤-65°C Stable in spiked rabbit plasma for 24h at room temperature Stable in spiked rabbit plasma for 265 days at ≤-65°C D_{11}-BAF312 was stable in stock solutions for 153 days at ≤-15°C	
Intra-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)	
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of rabbit plasma	
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 for samples diluted up to 10-fold. Precision ≤ 15%	
Special issues:	* Specificity not in acceptance criteria, but no impact on study measured (blanks and zero samples in study were in acceptance criteria, and no positive control measured)	
Results:	Cross validation in fetus homogenates (calibrated against Cs in plasma)	
Specificity **	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ	

Stability	Stable in post-preparative extracts for 48h at 5°C
Intra-day accuracy and precision	Mean bias within ±30 of the nominal values Precision of ≤ 30%
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of rabbit fetus homogenate
Calibration	Calibration standards prepared in plasma
Special issues:	**No contribution of BAF312 to the signal of the ISTD assessed. Specificity not in acceptance criteria, but no impact on study measured (no positive control measured)
Comments:	To obtain concentrations in molar units, divide the concentrations in ng by 516.61 g/mol. a) Data available in DMPK R0500135-01

2.6.5.2R Analytical Methods and Validation Reports (DMPK R0500135b-02)

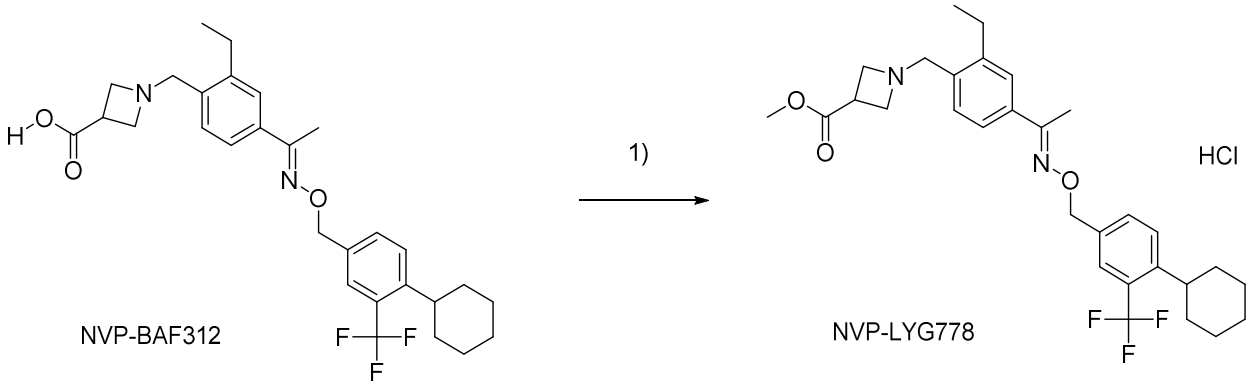
Study title:	Quantitative determination of BAF312 in monkey plasma and brain, lung and heart tissues by LC-MS/MS. Method validation report — Study no. DMPK R0500135b-02
Location in CTD:	4.2.2.1-18
GLP compliance:	no
Method:	The method for a 50 µL plasma or tissue homogenate sample consists of protein precipitation in 200 µL acetonitrile containing internal standard, centrifugation, dilution of the supernatant (1:2, v/v) in 0.2% formic acid in water, injection of 20 µL into an LC-MS/MS system and measurement in SRM mode using APCI positive as the ionization method
Instrument:	HPLC-MS/MS
Substances analyzed:	BAF312 (Siponimod)
Reference compounds:	BAF312
Internal standards:	D_{11}-BAF312
Results:	Validation in plasma
Specificity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ
Stability	Stable in stock solutions for 187 days at ≤-15°C and for 146 days at 2-8°C [a)] Stable in post-preparative extracts for 72h at 8°C Stable for 3 freeze-thaw cycles with storage at ≤-65°C Stable in spiked monkey plasma for 24h at room temperature Stable in spiked monkey plasma for 232 days at ≤-65°C Stable in incurred monkey plasma for 616 days at ≤-65°C D_{11}-BAF312 was stable in stock solutions for 153 days at 2-8°C
Intra-day accuracy and precision	Mean bias within ±15% (±20% at LLOQ) of the nominal values Precision of ≤15% (≤20% at LLOQ)
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of monkey plasma
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 for samples diluted up to 10-fold. Precision ≤ 15%
Special issues:	2 methods used: HPLC and RRLC. No contribution of BAF312 to the signal of the ISTD assessed for HPLC method. Specificity for RRLC method not in acceptance criteria, but no impact on study measured (lowest concentration measured was about 30 times above the LLOQ, and no positive control measured)
Results:	Validation in brain and lung homogenates

Specificity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ
Stability	Stable for 2 freeze-thaw cycles with storage at ≤-65°C Stable in spiked monkey brain and lung homogenates for 24h at room temperature
	Stable in spiked monkey brain and lung homogenates for 52 days at ≤-65°C
Intra-day accuracy and precision	Mean bias within ±30% of the nominal values Precision of ≤ 30%
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of monkey brain or lung homogenate
Calibration	Deviation of ±30% for 75% of the calibration standards from the nominal values at a minimum of 6 different levels
Results:	Cross validation in heart homogenate (calibrated against Calibration standards in brain homogenate)
Specificity	Interference of BAF312 ≤ 20% of analytical response at LLOQ Interference of D_{11}-BAF312 ≤ 5% of analytical response at LLOQ
Intra-day accuracy and precision	Mean bias within ±30 of the nominal values Precision of ≤ 30%
LOQ	LLOQ: 5.00 ng/mL; ULOQ: 10000 ng/mL using 50 µL of monkey heart homogenate
Calibration	Calibration standards prepared in brain homogenate
Comments:	To obtain concentrations in molar units, divide the concentrations in ng by 516.61 g/mol.
	a) Data available in DMPK R0500135-01

2.6.5.2S Analytical Methods and Validation Reports (RD-2015-00908)

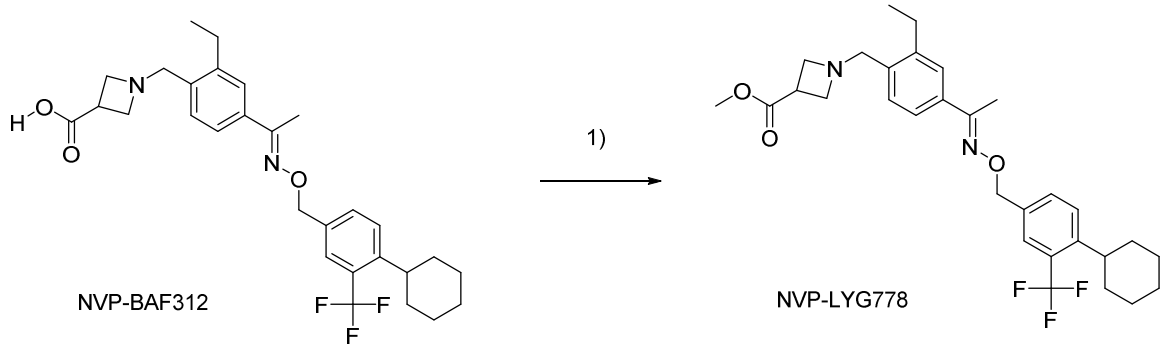
Study title:	Biocatalytic syntheses of NVP-BAF312 metabolites	Study no. RD-2015-00908
Location in CTD:	4.2.2.1-19	
GLP compliance:	not required	
Test article:	LNL931, LNL940, CIN640, LNL927, LNL921, CIQ298, LNL924, LNL925, MBR808, MDJ100, MGH346	
Salt form:	No salt or diammonium salt	
Formula (Weight) (unlabeled)	LNL931, CIN640, LNL940: $C_{29}H_{35}F_3N_2O_4$ (532.6 g/mol), LNL927, LNL921, LNL924, CIQ298: $C_{14}H_{15}F_3O_3$ (288.3 g/mol) LNL925: $C_{35}H_{43}F_3N_2O_{10}$ (708.7 g/mol) MBR808, MDJ100, MGH346: $C_{29}H_{35}F_3N_2O_7S$(612.7 g/mol)	
Analytical Methods:	HPLC-UV for purity determination Structures by HRMS (High Resolution Mass Spectrometry) and NMR (^{13}C-HSQC, ^{1}H, ROESY, COSY)	
-Chemical Purity:	LNL931 (range; 98-99.4%), LNL940 (range; 86.6 to >98%), CIN640 (range; 96.3 to >98%), LNL927 (98.3%), LNL921 (95%), CIQ298 (82%), LNL924 (91%), LNL925 (range; 98% to >98%), MBR808 (range; >98 to >99%), MDJ100 (>98%), MGH346 (>98%)	
Release restriction:	LNL931, LNL940, CIN640, LNL927, LNL921, LNL924, LNL925, CIQ298, MBR808, MDJ100 and MGH346 were released for analytical and technical study purposes.	
Synthesis Scheme:	BAF312 —1) Hydroxylation→ LNL931, CIN640, LNL940 NIO469 —1) Hydroxylation→ LNL927, LNL921, LNL924, CIQ298 LNL931 —2) Glucuronidation→ LNL925 LNL931 —3) Sulfation→ MBR808 LNL940 —3) Sulfation→ MDJ100 CIN640 —3) Sulfation→ MGH346	
Synthesis Information:	1) Incubation of BAF312 with three fungi strains ATCC20034, DSM1344 and DSM858 in medium NL148s or incubation of NIO469 with two fungi strains DSM1344 and DSM1169 in medium NL148s, 2) UDP glucuronic acid incubated with dog or rabbit liver S9 fraction, HEPES buffer, pH 8.5; 3) Dimethyl formamide, 60°C, NEt_3-SO_3	

2.6.5.2T Analytical Methods and Validation Reports (DMPK R1500911 (USE 030-2))

Study title:	LYG778 Synthesis and release analysis	Study no. DMPK R1500911 (USE 030-2)
Location in CTD:	4.2.2.1-20	
GLP compliance:	not required	
Test article:	LYG778	
Salt form:	hydrochloride	
Formula (Weight)	$C_{30}H_{37}F_3N_2O_3$ and HCl (567.09 g/mol)	
Synthesis Batch No.	USE 030-2	
Preparation Batch No.	PB1-090414-A	
Analytical Methods (Identity, UV-Purity):	HPLC-UV, ^{1}H-NMR spectroscopy, MS spectroscopy	
Analytical Results:		
-Chemical Purity:	97.5% (HPLC-UV)	
Release restriction:	NVP-LYG778 (batch USE 030-2) was released for analytical and technical studies.	
Synthesis Scheme:		
	Reaction conditions:1) a) CH_2Cl_2, oxalyl chloride, 30 min., r.t., evaporation (crude); b) methanol (absolute), 60 min., r.t., flash chromatography;	
Synthesis Information:	The intermediately formed acyl chloride (step 1a) was reacted with methanol (step 1b) and subsequently purified to obtain the final product NVP-LYG778.	

Additional information: no

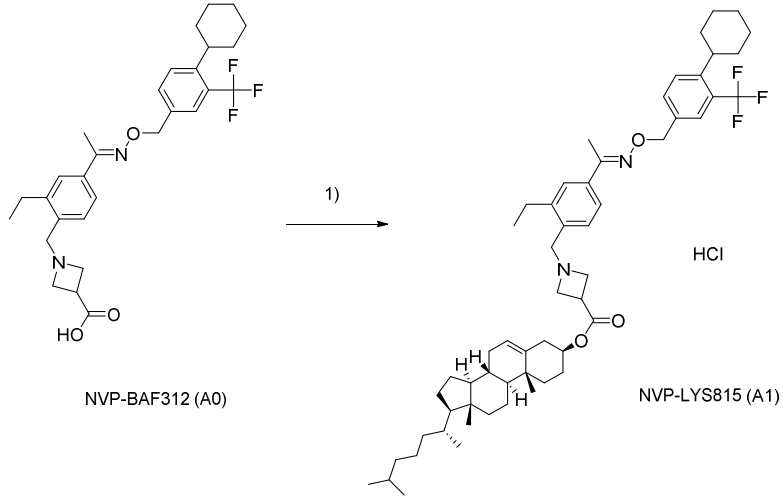
2.6.5.2U Analytical Methods and Validation Reports (DMPK R1500889 (USE 030-5))

Study title:	LYG778 Synthesis and Release analysis	Study no. DMPK R1500889 USE 030-5)
Location in CTD:	4.2.2.1-21	
GLP compliance:	not required	
Test article:	LYG778	
Salt form:	no	
Formula (Weight)	$C_{30}H_{37}F_3N_2O_3$ (530.62 g/mol)	
Synthesis Batch No.	USE 030-5	
Preparation Batch No.	KFOUROLL1-1	
Analytical Methods (Identity, UV-Purity):	HPLC-UV, ^{1}H-NMR spectroscopy , HPLC-MS spectroscopy	
Analytical Results:		
-Chemical Purity:	98.6% (HPLC-UV)	
Release restriction:	LYG778 (batch USE 030-5) was released for analytical and technical studies.	
Synthesis Scheme:		
	Reaction conditions: 1) a) NVP-BAF312, CH_2Cl_2, oxalyl chloride, 20 min, r.t.; evaporation (crude); b) methanol (absolute), 60 min. r.t.;	
Synthesis Information:	NVP-BAF312 was converted into its acid chloride (step 1a), which was subsequently reacted with methanol to afford the final product NVP-LYG778 after purification.	

2.6.5.2V Analytical Methods and Validation Reports (DMPK R1400006-01 (USE 031-1))

Study title:	LYS815 Synthesis and release analysis	Study no. DMPK R1400006-01 (USE 031-1)
Location in CTD:	4.2.2.1-22	
GLP compliance:	not required	
Test article:	LYS815	
Salt form:	hydrochloride	
Formula (Weight)	$C_{56}H_{79}F_3N_2O_3$ and HCl (921.7 g/mol)	
Synthesis Batch No.	USE 031-1	
Preparation Batch No.	RR1-140114-A	
Analytical Methods (Identity, UV-Purity):	HPLC-UV, ^{1}H-NMR spectroscopy, MS spectroscopy	
Analytical Results:		
-Chemical Purity:	95.6% (HPLC-UV)	
Release restriction:	n.a.	

Synthesis Scheme:



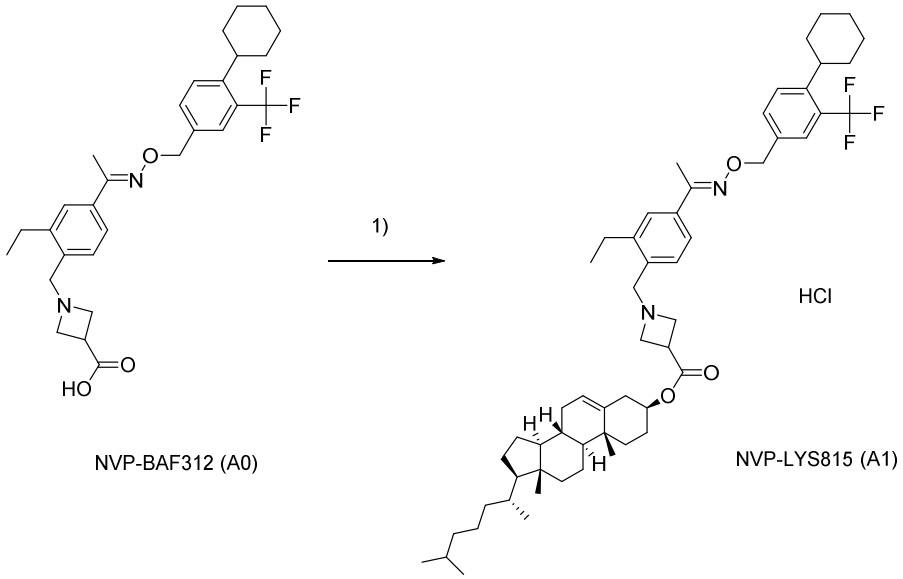
Reaction Conditions: 1a) oxalyl chloride, catalytic amount of dimethylformamide (DMF), r.t., 20 min, evaporation (crude); b) cholesterol (Aldrich

	36,279-4), CH_2Cl_2, r.t., 72 h;
Synthesis Information:	NVP-BAF312-NX (AO) was converted into its acyl chloride (step 1a) and subsequently reacted with cholesterol (step 1b). Final purification provided NVP-LYS815 (A1).
Additional information:	no

2.6.5.2W Analytical Methods and Validation Reports (DMPK R1400437 (USE 031-2))

Study title:	LYS815 Synthesis and release analysis	Study no. DMPK R1400437 (USE 031-2)
Location in CTD:	4.2.2.1-23	
GLP compliance:	not required	
Test article:	LYS815	
Salt form:	hydrochloride	
Formula (Weight)	$C_{56}H_{79}F_3N_2O_3$ HCl (921.7 g/mol)	
Synthesis Batch No.	USE 031-2	
Preparation Batch No.	RR1-150514-A	
Analytical Methods (Identity, UV-Purity):	HPLC-UV, ^{1}H-NMR spectroscopy, MS spectroscopy	
Analytical Results:		
-Chemical Purity:	98.4% (HPLC-UV)	
Release restriction:	NVP-LYS815 (batch USE 031-02) was released for analytical and technical studies.	

Synthesis Scheme:



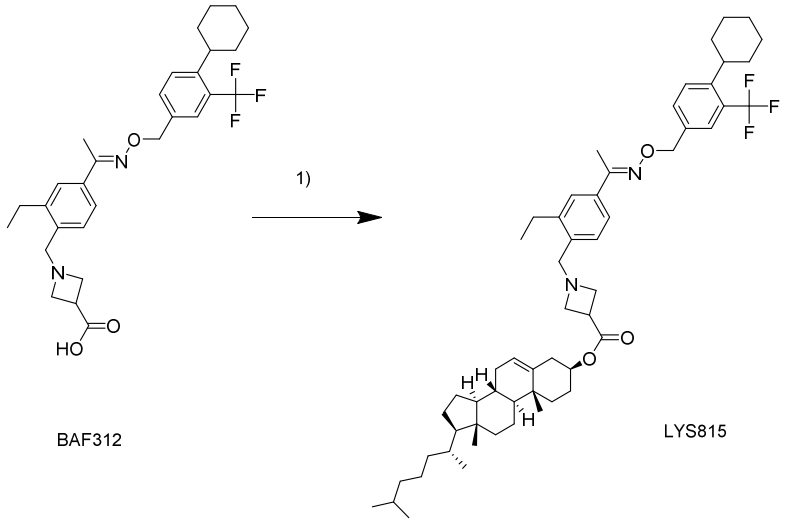
Reaction conditions: 1 a) CH_2Cl_2, oxalyl chloride, DMF, evaporation (crude); b) CH_2Cl_2, cholesterol, r.t., 72 h, purification;

Synthesis Information:	The respective acid chloride of NVP-BAF312 (A0) (step 1a) was reacted with cholesterol (step 1b) and subsequently purified to obtain the final product NVP-LYS815 (A1).
Additional information:	no

2.6.5.2X Analytical Methods and Validation Reports (DMPK R1500888 (USE038-2))

Study title:	LYS815 Synthesis and release analysis	Study no. DMPK R1500888 (USE 038-2)
Location in CTD:	4.2.2.1-24	
GLP compliance:	not required	
Test article:	LYS815	
Salt form:	no	
Formula (Weight)	$C_{56}H_{79}F_3N_2O_3$ (885.25 g/mol)	
Synthesis Batch No.	USE 038-2	
Preparation Batch No.	MPAUROLL2-1	
Analytical Methods (Identity, UV-Purity):	HPLC-UV, ^{1}H-NMR spectroscopy, MS spectroscopy	
Analytical Results:		
-Chemical Purity:	98.2% (HPLC-UV)	
Release restriction:	The unlabeled drug substance LYS815 (batch USE 038-02) was released for analytical and technical studies.	

Synthesis Scheme:



	Reaction Conditions: 1) NVP-BAF312, CH_2Cl_2, N,N'-diisopropylcarbodiimide (DIC), cholesterol, 4-dimethylamine pyridine (DMAP), 20 h, r.t.;
Synthesis Information:	N, N'-Diisopropylcarbodiimide (DIC)/ 4-dimethylamine pyridine (DMAP)-mediated coupling of cholesterol to NVP-BAF312 gave crude NVP-LYS815, which was subject to Reverse Phase (RP8)-HPLC purification.
Additional information:	no

2.6.5.3 薬物動態試験：単回投与後の吸収

2.6.5.3A Pharmacokinetics: Absorption after a Single Dose - Mouse (DMPK R1300411-02)

Study title:	Pharmacokinetics, distribution, metabolism and excretion in mice after oral administration of [^{14}C]BAF312	Study no. DMPK R1300411-02, DMPK R1300411a-01
Location in CTD:	4.2.2.2-1	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312 hemifumarate	
Species / Strain:	Mouse / CD-1	
Gender(M/F) / Number of animals:	M / 30 (3 per time point)	
Feeding condition:	Water and food ad libitum	
Vehicle / Formulation	Suspension in aqueous solution of 0.5% Hydroxypropylcellulose	
Route of dosing:	Oral (gavage) / single dose	
Dose (mg/kg):	8 (referring to free base)	
Analyte /Radionuclide:	Radioactivity / ^{14}C	
Analyte:	BAF312	
Sample times (h)	0.5, 1, 2, 4, 8, 24, 48, 72, 96, 168 h post dose	
Samples:	Plasma	
Assay:	Radioactivity: Liquid scintillation counting (LSC) BAF312: Liquid chromatography –tandem mass spectrometry (LC-MS/MS)	

PK parameters	Radioactivity in plasma	BAF312 in plasma
Actual dose (mg/kg)	8.14 ± 0.461	
Tmax (h):	8.0	8.0
Cmax (ng/mL or ngEq/mL):	2270	2300
AUClast (ng•h/mL or ngEq•h/mL)	151000	64100
AUCinf (ng•h/mL or ngEq•h/mL)	171000	64600
T1/2 (h)	55.6	27.2
T1/2 range (h)	[24-168]	[24-168]

Additional information:	Values are given as mean or mean ± SD.
Conclusion:	
	Maximum plasma concentrations of both total radiolabeled components and BAF312 were observed at 8 h post-dose, indicating a medium to slow absorption. Exposure to BAF312 in terms of AUClast represented 42% of the total radiolabeled components, indicating that the parent drug was a major component in systemic circulation.
	Apparent terminal half-lives for total radiolabeled components and the parent compound were 55.6 h and 27.2 h, respectively, thus suggesting a slow elimination as well as the presence of one or more long-lived metabolites.

2.6.5.3B Pharmacokinetics: Absorption after a Single Dose - Rat (DMPK R0500017-02)

Study title:	Absorption, distribution, metabolism and excretion in male rats after single intravenous (1 mg/kg) and peroral (3 mg/kg) administration of [^{14}C]BAF312		Study no. DMPK R0500017-02
Location in CTD:	4.2.2.2-2		
GLP compliance:	not required		
Test article:	[^{14}C]BAF312		
Species / Strain:	Rat (HAN:WIST) albino		
Gender(M/F) / Number of animals:	M / 3		
Feeding condition:	Water and food ad libitum		
Vehicle / Formulation	Intravenous and oral: Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)		
Route of dosing:	Intravenous bolus (into vena femoralis) / single dose Oral (gavage) / single dose		
Dose (mg/kg):	Intravenous: 1; oral: 3		
Analyte /Radionuclide:	Radioactivity / ^{14}C		
Analyte:	BAF312		
Sample times (h)	0 (oral only), 0.083 (intravenous only), 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96 h post dose		
Samples:	Blood, plasma		
Assay:	Radioactivity: Liquid scintillation counting (LSC) BAF312: Liquid chromatography –tandem mass spectrometry (LC-MS/MS)		

	Intravenous 1 mg/kg		Oral 3 mg/kg	
PK parameters [a]	^{14}C	BAF312	^{14}C	BAF312
Actual dose (mg/kg)	1.06 ± 0.0130	-	3.01 ± 0.0451	-
Tmax (h):	0.083	0.083	6.67 ± 2.31	6.67 ± 2.31
Cmax (ng/mL or ngEq/mL):	1160 ± 254	1160 ± 300	332 ± 15.6	259 ± 47.5
T1/2 (h):	6.1 ± 1.3	6.01 ± 1.34	n.a.	n.a.
AUC(T1-Tlast)/dose (ng•h/mL or ngEq•h/mL)/(mg/kg)	3180 ± 85.2	-	1830 ± 396	-
AUC(T0-Tlast)/dose (ng•h/mL or ngEq•h/mL)/(mg/kg)	-	2710 ± 188	-	1350 ± 467
AUC(T1-inf)/dose	-	2770 ± 181	-	n.a.

(ng•h/mL or ngEq•h/mL)/(mg/kg)				
Vss (L/kg)	-	2.15 ± 0.311	-	n.a.
CLplasma (L/h/kg)	-	0.361 ± 0.0232	-	n.a.

Additional information: Values are given as mean or mean ± SD

a: Pharmacokinetic parameters of total radioactivity (^{14}C) and BAF312 in plasma of rats after single intravenous and peroral administration

Conclusion:

The oral absorption amounted to minimum of 57.5% (based on dose-normalized ^{14}C-AUC value). The bioavailability was estimated to 48.7%.

2.6.5.3C Pharmacokinetics: Absorption after a Single Dose - Rat (DMPK R0900164-01)

Study title:	Absorption, distribution, and metabolism in male rats after single intravenous (1 mg/kg) and peroral (3 and 10 mg/kg) administration of [^{14}C]BAF312 (with radiolabel on the benzyl-oxyamino part of the molecule)	Study no. DMPK R0900164-01, DMPK R0900164a
Location in CTD:	4.2.2.2-3	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312 hemifumarate (with radiolabel on the benzyl-oxyamino part of the molecule)	
Species / Strain:	Rat (Hanover Wistar)	
Gender(M/F) / Number of animals:	M / 6 (3 for oral, 3 for intravenous)	
Feeding condition:	Water and food ad libitum	
Vehicle / Formulation	Intravenous and oral: Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)	
Route of dosing:	Intravenous bolus (into vena femoralis) / single dose Oral (gavage) / single dose	
Dose (mg/kg):	Intravenous: 1; oral: 3 (doses referring to the free base)	
Analyte /Radionuclide:	Radioactivity / ^{14}C	
Analyte:	BAF312	
Sample times (h)	0.083 (intravenous only), 0.25 (oral only), 0.5, 1, 2, 4, 8, 24, 48, 72 and 96 h post dose	
Samples:	Blood, plasma	
Assay:	Radioactivity: Liquid scintillation counting (LSC) BAF312: Liquid chromatography –tandem mass spectrometry (LC-MS/MS)	

	Intravenous 1 mg/kg		Oral 3 mg/kg	
PK parameters [b]	^{14}C in plasma	BAF312 in plasma	^{14}C in plasma	BAF312 in plasma
Actual dose (mg/kg)	1.01 ± 0.0100	-	3.21 ± 0.0306	-
Cmax (ng/mL or ngEq/mL):	899 ± 197	806 ± 81.6	418 ± 48.6	235 ± 32.3
Tmax (h):	0.083 (median)	0.083 (median)	8 (median)	8 (median)
AUClast (ng•h/mL or ngEq•h/mL)	3970 ± 132	-	8990 ± 713	3470 ± 630
AUCinf (ng•h/mL or ngEq•h/mL)	-	2090 ± 133	-	n.a.
T1/2 (h):	28.6 [b]	-	n.a.	-
T1/2alpha (h):	-	0.237 ± 0.0266	-	n.a.
T1/2beta:	-	4.79 ± 0.410	-	

Fbeta	-	89.0 ± 1.56	-	
CL (L/(h·kg))	-	0.485 ± 0.0335	-	n.a.
Vss (L/kg)	-	2.98 ± 0.0709	-	n.a.
Absorption (%)	-	-	71.2 ± 5.20	-
Bioavailability (%)	-	-	-	52.1 ± 9.17

Additional information:

a: Pharmacokinetic parameters of total radioactivity (^{14}C) and BAF312 in plasma of rats after single intravenous and peroral administration; Values are given as mean ± SD

b: value of one rat (Rat 2) which might reflect most accurately the apparent terminal elimination T1/2 (time range: 24-96 h)

Conclusion:

A moderate oral absorption of compound-related radioactivity (71.2%) and bioavailability (52.1%) of BAF312 was observed.

The plasma clearance of BAF312 was 0.485 L/ (h·kg) and the blood clearance 0.401 L/ (h·kg) which corresponded to 12.1% of the liver blood flow.

The apparent terminal half-life T1/2 of BAF312 was 4.79 h.

The volume of distribution (2.98 L/kg) at steady state for BAF312 was moderate and approx. 4.5 times the total body water.

2.6.5.3D Pharmacokinetics: Absorption after a Single Dose - Rat (DMPK R1600437)

Study title:	PK of BAF312 in male and female rats after intravenous bolus administration	Study no. DMPK R1600437, DMPK R1600437a
Location in CTD:	4.2.2.2-4	
GLP compliance:	not required	
Test article:	BAF312 hemifumarate	
Species / Strain:	Rat / Wistar Hannover	
Gender (M/F) / Number of animals per group:	M / 3, F / 3	
Feeding condition:	Water and food *ad libitum*	
Vehicle / Formulation	4.5% Cremophor EL in 5% aqueous glucose solution	
Route of dosing:	Intravenous / single dose	
Dose (mg/kg):	1 (referring to the base)	
Analyte:	BAF312	
Sample times (h)	0.083, 0.5, 1, 2, 4, 8 and 24	
Samples:	Plasma (blood collected in K_2EDTA-coated tubes)	
Assay:	LC-MS/MS with positive electrospray ionization	
Route of administration:	intravenous	
Gender	male	female
Nominal dose:	1 mg/kg (referring to base)	1 mg/kg (referring to base)
PK parameters in plasma (mean ± SD)		
Actual dose (mg/kg) [a]	1.03 ± 0.0100	1.02 ± 0.0153
Tmax (h) [b]	0.083 [0.083 – 0.083]	0.083 [0.083 – 0.083]
Cmax (ng/mL)	1100 ± 140	1370 ± 155
Tlast (h) [a]	24 [24 – 24]	24 [24 – 24]
AUClast (h·ng/mL)	1920 ± 220	6520 ± 655
CLplasma (L/(h·kg))	0.530 ± 0.0621	0.0739 ± 0.00375
CLblood (L/(h·kg)) [d]	0.442	0.0616
Vss (L/kg)	2.40 ± 0.133	2.88 ± 0.434

T1/2 (h)	4.78 ± 0.459	28.8 ± 4.86 [c]
T1/2 range	[4 - 24]	[4 - 24]

a: referring to the free base, b: median [range];
c: Observation period used to estimate T1/2 is shorter than twice the estimated T1/2 value. Therefore, the estimated T1/2 values might be biased and have to be considered with caution.
d: Based on blood/plasma concentration ratio for BAF312 of 1.2 (*in vitro*; DMPK R0400881-01), blood clearance of 0.442 L/(h·kg) was estimated

Additional information: Pharmacokinetic parameters based on mean plasma concentrations.

Conclusion:

Cmax was 1.25- fold higher in female rats compared to male rats.

AUClast was 3.40 – fold higher in female rats.

CLblood was ~7-fold higher in male than in female rats and represent 13% and 2% of the hepatic blood flow.

Vss was comparable in male and female rats (approx. 4 –fold higher than the total body water.

PK differences are most likely due to gender specific differences in CYP expression in rats, which is a species specific phenomenon.

2.6.5.3E Pharmacokinetics: Absorption after a Single Dose - Monkey (RD-2005-00583)

Study title:	NVP-BAF312 : PK/PD profiles after p.o. and i.v. administration in cynomolgus monkey			Study no. RD-2005-00583		
Location in CTD:	4.2.2.2-5					
GLP compliance:	not required					
Test article:	BAF312					
Species / Strain:	Monkey / Cynomolgus					
Gender(M/F) / Number of animals:	M / 4, F / 3 (n=4 (2M+2F) per dosing)					
Feeding condition:	Water and food *ad libitum*					
Vehicle / Formulation	Intravenous and oral: Ethanol/HCl 0.1M/glucose 5% in water					
Route of dosing:	Oral (gavage) / single dose Intravenous / single dose					
Dose (mg/kg):	0.03, 0.1, 0.3, 1, 3					
Analyte:	BAF312					
Sample times (h)	Predose, 0.25 (intravenous only), 0.5, 1, 2, 4, 7, 24, 48, 72h post dose, and every day for 7-10 days (oral only) or every day for 7 days (intravenous only).					
Samples:	Blood (collected in EDTA-coated tubes)					
Assay:	BAF312: Micro Liquid chromatography –tandem mass spectrometry (MicroLC-MS/MS)					
Route of administration:	oral					intravenous
Dose:	3 mg/kg	1 mg/kg	0.3 mg/kg	0.1 mg/kg*	0.03 mg/kg*	1 mg/kg
PK parameters in whole blood (mean ± SEM)						
T1/2 (h)	20 ± 0.3	18 ± 0.8	15 ± 1.0	14	14	19 ± 0.5
MRT (h)	24 ± 2	22 ± 2	22 ± 1.5	19	21	22 ± 1.0
Cmax (ng/mL)	731 ± 76	354 ± 39	99 ± 13	23	9	-
C0 (ng/mL)	-	-	-	-	-	1174 ± 84
Tmax (h)	4 [4-7]	2 [2-4]	3 [2-7]	4	2	-
AUC0-t (ng•h/mL)	18838 ± 2646	7546 ± 845	2650 ± 297	576	220	10561 ± 1031
AUCinf (ng•h/mL)	18864 ± 2654	7561 ± 857	2650 ± 297	576	220	10585 ± 1039
Oral BAV (AUCinf) (%)	59	71	83	54	69	-
Vss (L/kg)	-	-	-	-	-	2.12 ± 0.12

CL (L/h/kg)	-	-	-	-	-	0.098 ± 0.01
Additional information:	Tmax: Median [range], *: PK parameters of 0.1 and 0.3 mg/kg were calculated from the mean PK concentration.					

Conclusion:

BAF312 was orally bioavailable (54-83% at doses between 0.03 and 3 mg/kg) in the Cynomolgus monkey, with an acceptable elimination half-life after oral dosing (14-20 h). The results show a good dose-related increase in blood exposure, with linearity up to 3 mg/kg and low level of intra- and inter-individual variability.

2.6.5.3F Pharmacokinetics: Absorption after a Single Dose - Monkey (DMPK R1000166-01)

Study title:	Absorption, metabolism and excretion following an oral dose of [^{14}C]BAF312 in the monkey		Study no. DMPK R1000166-01, DMPK R1000166a
Location in CTD:	4.2.2.2-6		
GLP compliance:	not required		
Test article:	[^{14}C]BAF312		
Species / Strain:	Monkey / Cynomolgus		
Gender(M/F) / Number of animals:	M / 3		
Feeding condition:	Diet once daily. Nutritional enrichment (i.e., fruit) provided daily		
Vehicle / Formulation	0.5% (w/v) hydroxypropyl-cellulose (grade HF, Klucel) solution		
Route of dosing:	Oral (gavage) / single dose		
Dose (mg/kg):	15 (referring to free base)		
Analyte /Radionuclide:	Radioactivity / ^{14}C		
Analyte:	BAF312		
Sample times (h)	Predose, 0.5, 1, 2, 4, 7, 10, 24, 48, 72, 96, 144, 168, 216, 264, 336 h post dose		
Samples:	Blood, plasma		
Assay:	Radioactivity: Liquid scintillation counting (LSC) BAF312: Liquid chromatography –tandem mass spectrometry (LC-MS/MS)		
PK parameters (mean ± SD)	Radioactivity in blood	Radioactivity in plasma	BAF312 in plasma
Cmax (ngEq/mL):	4820 ± 688	5220 ± 1200	-
Cmax (ng/mL):	-	-	3800 ± 686
Tmax (h):	7.0 ± 3.0	7.0 ± 3.0	7.0 ± 3.0
AUClast (ngEq•h/mL)	138000 ± 16000	174000 ± 7230	-
AUClast (ng•h/mL)	-	-	111000 ± 4360
AUCinf (ngEq•h/mL)	147000 ± 17600	186000 ± 11500	-
AUCinf (ng•h/mL)	-	-	111000 ± 4360
T1/2 (h)	32.4 ± 7.29	56.9 ± 22.3	62.1 ± 7.89

Additional information:	To obtain concentrations in molar units, divide the concentrations in ngEq (equal to ng-eq) or ng by 516.61 g/mol.
Conclusion:	
	Tmax of plasma BAF312 was attained at 7 h post dose, indicating slow absorption. The Cmax and AUCinf were 3800 ng/mL and 111000 ng•h/mL, respectively. A long terminal elimination half-life between 57 -71 h was observed.

2.6.5.4 薬物動態試験：反復投与後の吸収

2.6.5.4A Pharmacokinetics: Absorption after Repeated Doses

Study title:	Pharmacokinetics and tissue distribution of BAF312, LYG778 and LYS815 in male mice after multiple oral administration of BAF312 hemifumarate (25 mg base/kg/day)			Study no. DMPK R1500775, DMPK R1500775a
Location in CTD:	4.2.2.2-7			
GLP compliance:	not required			
Test article:	BAF312 hemifumarate			
Species / Strain:	Mouse / CD-1			
Gender(M/F) / Number of animals:	M / 30 (2 per time point)			
Feeding condition:	Water and food ad libitum			
Vehicle / Formulation:	Suspension in aqueous solution of 0.5% Hydroxypropylcellulose			
Method of Administration:	Oral (gavage) / single and 14-daily doses			
Dose (mg/kg/day):	25 (referring to free base)			
Sample (Whole blood, plasma, etc.):	Plasma, liver, skin/subcutis, and skeletal muscle			
Analyte:	BAF312, LYG778 (M16), and LYS815 (M17)			
Assay:	LC-MS/MS			
PK parameters (means, n=2):				
	Single dose (Day 1)		Multiple dose (day 14)	
Plasma:	BAF312	LYS815	BAF312	LYS815
Tmax (h)	6	24	6	24
Cmax (ng/mL (µM))	5950 (11.5)	9500 (10.7)	7910 (15.3)	26800 (30.3)
AUC0-24h (h·ng/mL (h·µM))	112000 (217)	111000 (125)	141000 (273)	591000 (668)
AUC0-264h (h·ng/mL (h·µM))	218000 (422)	1190000 (1340)	nc	nc
Liver:				
Tmax (h)	6	96	nc	nc
Cmax (ng/g (nmol/g))	49800 (96.4)	2200 (2.49)	nc	nc
AUC0-264h (h·ng/g (h·nmol/g))	1890000 (3660)	455000 (514)	nc	nc
Skin/subcutis:				

Tmax (h)	6	nc	nc	nc
Cmax (ng/g (nmol/g))	7760 (15.0)	0	nc	nc
AUC0-264h (h·ng/g (h·nmol/g))	355000 (687)	nc	nc	nc
Skeletal muscle:				
Tmax (h)	6	144	nc	nc
Cmax (ng/g (nmol/g))	11700 (22.6)	90.3 (0.102)	nc	nc
AUC0-264h (h·ng/g (h·nmol/g))	521000 (1010)	nc	nc	nc
Accumulation in tissues:		Concentration ratio Day 14 / 1		
		BAF312	LYS815	
Liver	6 h	1.69	167	
	24 h	1.13	31.2	
Skin/subcutis	6 h	1.13	nc	
	24 h	0.887	nc	
Skeletal muscle	6 h	1.50	nc	
	24 h	1.07	nc	

Additional information:	nc: not calculated due to limited data set available No LYG778 (M16) was detected in any plasma or tissue samples investigated.
Conclusions:	After a single oral dose, BAF312 and LYS815 (M17) were measurable in plasma until 264 h post-dose. Cmax was observed at 6 h post-dose for BAF312 and at 24 h post-dose for LYS815, with an approximately 3-fold higher exposure (based on AUClast, molar units) to the metabolite LYS815 compared to the parent compound. After 14 oral daily doses, a distinct accumulation (~5-fold) was observed for LYS815 in plasma, whereas no or low accumulation (~1.3-fold) was seen for BAF312 (based on AUC0-24h). Steady state was reached on Day 5 or earlier for both the parent compound and LYS815. Regarding tissue distribution, exposure to BAF312 after a single or multiple oral doses was at least 4-fold higher compared to LYS815 in all investigated tissues, with the liver showing the highest exposure to both compounds (tissue to plasma concentration ratios of up to ~10-fold for BAF312 and ~2 for LYS815), compared to skin/subcutis and skeletal muscle (tissue to plasma concentration ratios up to ~2-fold for BAF312, ~0.02 for LYS815). After repeated dosing, mean LYS815 concentrations in liver were 30 to 170-fold higher on Day 14 compared to Day 1, whereas no or little differences were observed for BAF312 (<2-fold) in any investigated tissues.

2.6.5.5 薬物動態試験：分布

2.6.5.5A Pharmacokinetics: Organ Distribution – Mouse (DMPK R1300411-02)

Study title:	Pharmacokinetics, distribution, metabolism and excretion in mice after oral administration o	Study no. DMPK R1300411-02
Location in CTD:	4.2.2.2-1	
GLP compliance:	not required	
Test article	[^{14}C]BAF312 hemifumarate	
Species:	Mouse / CD-1	
Gender(M/F) / number of animals:	M / 46 (Group 1: 30, Group 2: 4; group 3: 3, group 4: 9)	
Feeding condition:	Water and food available *ad libitum*	
Vehicle / formulation:	Suspension in aqueous solution of 0.5% Hydroxypropylcellulose	
Method of administration:	Oral (gavage) / single dose	
Dose (mg/kg):	8 (referring to the free base) (group 1, 2 and 3) 25 (referring to free base) (group 4)	
Radionuclide:	^{14}C	
Analyte / assay (unit):	Radioactivity / Quantitative Whole Body Autoradiography and Liquid Scintillation Counting (nmol/g)	
Sampling time:	Group 1: 0.5, 1, 2, 4, 8, 24, 48, 72, 96 and 168 h post dosing Group 2: 24, 48, 168 and 336 h post dosing Group 3: 168 h post dosing Group 4: 8, 96, 168 h post dosing	
Analytical methods:	Determination of radioactivity in blood and tissue homogenates: Liquid scintillation counting (LSC) Determination of tissue distribution of radioactivity: Quantitative whole body radiography.	

Mean concentrations and pharmacokinetic parameters of total radiolabeled components and BAF312 in brain (n=3 / time point) (Group 1)

Time points (h)	Mean brain concentrations			Brain/plasma ratio		
	^{14}C (ngEq/g)		BAF312 (ng/g) [a]	^{14}C		BAF312 [a]
	Mean	SD		Mean	SD	
0.5	108	42.8	99.2	0.293	0.0387	0.261
1	1310	1420	--	0.606	0.201	--
2	1410	160	1190	0.985	0.0320	0.811
4	3320	269	--	2.08	0.327	--
8	5990	940	5060	2.70	0.770	2.20
24	6200	137	--	4.37	0.535	--
48	2410	368	1990	1.85	0.268	5.89
72	1050	130	--	1.62	0.277	--
96	502	49.0	392	0.688	0.0901	7.29
168	811	1060	145	0.997 [b]	0.718 [b]	6.91
Tmax (h)	24					
Cmax (ngEq/g)	6200					
AUClast (ngEq•h/g)	311000					
T1/2 (h)	34.9 [b]					
T1/2 (range) (h)	[48-168]					
Additional information:	a: concentrations of BAF312 in brain were derived from metabolite patterns in pooled brain samples b: excluding mouse No. 30 --: not measured					

Tissue concentrations (expressed in ngEq/mL or ngEq/g) of total radiolabeled components after single oral dose of 8 mg/kg [^{14}C]BAF312 (n=1/time point) (Group 2)

Time points:	24 h	48 h	168 h	336 h
Adrenal (cortex)	4830	3770	3220	2460
Adrenal (medulla)	2940	2300	1970	651
Bile	23000	10800	2840	330
Blood (QWBA)	1180	723	150	52.2
Blood (LSC)	1350	739	139	50.4
Blood vessel wall	1850	1320	462	280
Bone marrow	2600	1680	672	332
Bone mineral	280	186	36.9	33.3
Brain	5790	3450	303	83.2
Cartilage	134	112	39.2	22.3
Epididymis	4390	2830	474	224
Esophagus	2600	1510	579	112
Eye (choroid)	2910	1910	316	208
Eye (ciliary body)	7130	2830	806	309
Eye (lens)	134	126	73.9	58.9
Eye (vitreous body)	144	65.1	8.83	T
Fat (brown)	3500	1990	484	153
Fat (white)	405	344	121	51.4
Hair (follicle)	1540	868	376	226
Hair (tactile)	3660	1660	462	330
Harderian gland	8730	4410	532	207
Heart	4800	3000	563	261
Intestinal wall (colon)	3840	1980	563	269
Intestinal wall (small intestine)	6250	3120	806	189
Kidney (CM junction)	24500	14600	4380	847
Kidney (cortex)	28600	20600	6200	1220
Kidney (medulla)	4710	3180	630	194
Lacrimal gland	29100	28300	28200	26800

Liver	19400	12200	11900	2090
Lung	3230	2000	971	656
Lymph nodes (submandibular)	n.s.	n.s.	13000	8110
Muscle	2060	971	123	67.7
Pancreas	4940	3090	411	176
Penis	1600	1420	397	212
Pineal body	1750	909	196	148
Pituitary gland	3930	2730	465	273
Preputial gland	2430	2260	402	235
Salivary gland (submaxillary)	3570	2160	408	321
Seminal vesicle wall	976	816	141	85.2
Seminal vesicular fluid	124	58.4	6.82	0
Skin	956	661	218	137
Skin (subcutis)	1000	708	223	151
Spinal cord	5480	3990	415	101
Spinal cord (grey matter)	6200	2060	192	86.3
Spinal cord (white matter)	5420	5530	615	75.9
Spleen	2740	1820	1130	496
Spleen (red pulp)	2890	1970	1540	863
Spleen (white pulp)	2120	1510	558	228
Stomach (glandular)	7080	5730	573	264
Stomach (non glandular)	3220	2150	514	183
Testis	2760	2570	723	456
Thymus	2410	1460	315	144
Thyroid gland	5940	2650	486	212
Tongue	3490	2150	402	222
Tongue (mucous gland)	3950	2320	289	104
Tooth (pulp)	2070	987	620	708
LOD (ngEq/mL or ngEq/g)	4.65	4.55	3.26	5.89
LOQ (ngEq/mL or ngEq/g)	9.30	9.09	6.51	11.8

Additional information: Values below the LOD are reported as 0. LOD: 3.26-5.89 ngEq/mL or ngEq/g. LOQ: 6.51-11.8 ngEq/mL or ngEq/g; T: trace, between LOD and LOQ;

Pharmacokinetic parameters of total radiolabeled components after oral dose of 8 mg/kg [^{14}C]BAF312 (n=1/time point) (Group 2)

Tissues	Tmax (h)	Cmax (ngEq/mL or ngEq/g)	Cmax Tissue/blood	Tlast (h)	AUClast (ngEq•h/mL or ngEq•h/g)	AUClast Tissue/blood	T1/2 [a] (h)	T1/2 range (h)
Adrenal (cortex)	24	4830	4.1	336	1060000	9.96	468	48-336
Adrenal (medulla)	24	2940	2.5	336	573000	5.39	153	48-336
Bile	24	23000	19.5	336	1770000	16.6	57.0	48-336
Blood (QWBA)	24	1180	1	336	106000	1.00	77.5	48-336
Blood (LSC)	24	1350	1.15	336	110000	1.03	76.0	48-336
Blood vessel wall	24	1850	1.57	336	230000	2.16	132	48-336
Bone marrow	24	2600	2.21	336	308000	2.89	125	48-336
Bone mineral	24	280	0.238	336	28200	0.265	123	48-336
Brain	24	5790	4.91	336	438000	4.12	55.0	48-336
Cartilage	24	134	0.114	336	18800	0.177	127	48-336
Epididymis	24	4390	3.72	336	396000	3.72	81.2	48-336
Esophagus	24	2600	2.21	336	264000	2.48	76.4	48-336
Eye (choroid)	24	2910	2.47	336	271000	2.54	94.1	48-336
Eye (ciliary body)	24	7130	6.05	336	517000	4.86	91.8	48-336
Eye (lens)	24	134	0.114	336	27900	0.262	271	48-336
Eye (vitreous body)	24	144	0.122	168	8680	0.0816	37.5	24-168
Fat (brown)	24	3500	2.97	336	310000	2.91	79.0	48-336
Fat (white)	24	405	0.343	336	56300	0.529	107	48-336
Hair (follicle)	24	1540	1.31	336	173000	1.62	152	48-336
Hair (tactile)	24	3660	3.11	336	302000	2.84	129	48-336
Harderian gland	24	8730	7.41	336	620000	5.83	67.2	48-336
Heart	24	4800	4.08	336	434000	4.08	84.3	48-336
Intestinal wall (colon)	24	3840	3.26	336	338000	3.18	102	48-336
Intestinal wall (small intestine)	24	6250	5.31	336	506000	4.76	71.8	48-336

Kidney (CM junction)	24	24500	20.8	336	2340000	22.0	70.2	48-336
Kidney (cortex)	24	28600	24.3	336	3160000	29.7	70.7	48-336
Kidney (medulla)	24	4710	4	336	449000	4.22	72.6	48-336
Lacrimal gland	24	29100	24.7	336	9040000	85.0	3530	48-336
Liver	24	19400	16.5	336	3240000	30.5	108	48-336
Lung	24	3230	2.74	336	417000	3.92	183	48-336
Lymph nodes (submandibular)	NA	13000	11	336	2860000	26.9	n.c.	n.c.
Muscle	24	2060	1.75	336	143000	1.35	78.0	48-336
Pancreas	24	4940	4.2	336	415000	3.90	71.9	48-336
Penis	24	1600	1.36	336	215000	2.03	108	48-336
Pineal body	24	1750	1.49	336	148000	1.39	116	48-336
Pituitary gland	24	3930	3.33	336	381000	3.58	90.3	48-336
Preputial gland	24	2430	2.07	336	299000	2.81	91.7	48-336
Salivary gland (submaxillary)	24	3570	3.04	336	327000	3.07	110	48-336
Seminal vesicle wall	24	976	0.829	336	110000	1.03	92.0	48-336
Seminal vesicular fluid	24	124	0.105	168	7590	0.0714	35.7	24-168
Skin	24	956	0.811	336	114000	1.07	131	48-336
Skin (subcutis)	24	1000	0.851	336	120000	1.13	134	48-336
Spinal cord	24	5480	4.65	336	487000	4.58	55.5	48-336
Spinal cord (grey matter)	24	6200	5.26	336	332000	3.12	65.3	48-336
Spinal cord (white matter)	48	5530	4.69	336	625000	5.88	47.1	48-336
Spleen	24	2740	2.33	336	401000	3.77	153	48-336
Spleen (red pulp)	24	2890	2.46	336	506000	4.76	238	48-336
Spleen (white pulp)	24	2120	1.8	336	259000	2.43	107	48-336
Stomach (glandular)	24	7080	6.01	336	687000	6.46	67.3	48-336
Stomach (non glandular)	24	3220	2.74	336	322000	3.03	82.5	48-336
Testis	24	2760	2.35	336	394000	3.71	119	48-336
Thymus	24	2410	2.05	336	220000	2.07	88.7	48-336
Thyroid gland	24	5940	5.04	336	421000	3.96	81.4	48-336
Tongue	24	3490	2.96	336	315000	2.96	91.1	48-336

Tongue (mucous gland)	24	3950	3.35	336	313000	2.94	66.2	48-336
Tooth (pulp)	24	2070	1.75	336	269000	2.53	674	48-336
Whole mouse (with GIT)	24	3830	3.25	336	503000	4.73	96.1	48-336
Whole mouse (without GIT)	24	3540	3.01	336	491000	4.62	102	48-336

Additional information: a: All T1/2 values greater than 144 h need to be taken with caution, as the span of time used for T1/2 calculations is only 288 h.
n.c.: not calculated due to lack of declining data
NA: not applicable due to limited data

Concentrations (expressed in ngEq/mL or ngEq/g) of total radiolabeled components after single oral dose of 8 mg/kg [^{14}C]BAF312 in plasma and selected tissues (mean ± SD of n=3) at 168 h post dose (mean ± SD, n=3) (Group 3)

Tissues / organs:	Mean	SD	CV (%)
Plasma	333	102	30.7
Adrenal	8370	4100	49.0
Brain	163	22.0	13.5
Fat	124	11.9	9.62
Heart	509	40.0	7.86
Liver	6820	1530	22.4
Lung	1510	208	13.8
Kidney	9250	12500	135
Muscle	164	4.13	2.51
Skin	438	162	36.9
Testis	904	126	13.9

Additional information: none

Concentrations (expressed in ngEq/mL or ngEq/g) of total radiolabeled components after single oral dose of 25 mg/kg [^{14}C]BAF312 in plasma and selected tissues (mean ± SD of n=3) at 8, 96 and 168 h post dose (mean ± SD, n=3) (Group 4)

Tissues / organs:	Time point (h)	Mean	SD	CV (%)
Plasma	8	6250	697	11.1
	96	1300	303	23.4
	168	522	86.3	16.5

Liver	8	78000	5270	6.78
	96	42900	179	0.417
	168	19500	2610	13.4
Spleen	8	13600	3810	28.0
	96	3200	79.0	2.47
	168	2420	568	23.6
Brain	8	21700	2140	9.86
	96	1960	436	22.2
	168	625	2.98	0.478
Lacrimal gland	8	75900	6460	8.49
	96	337000	90400	26.9
	168	299000	69200	23.2
Kidney	8	88900	1660	1.87
	96	25300	2900	11.5
	168	6150	1480	24.1
Additional information:	none			
Conclusion:	At the dose of 8 mg/kg, total radiolabeled components were extravascularly distributed throughout the body at a high extent, and slowly eliminated, with concentrations still measurable at 336 h post-dose and half-lives in most tissues comparable or longer than that observed in blood. A 22- to 85-fold higher exposure (based on AUClast) compared to blood was observed for lacrimal gland, liver, kidney (cortex), lymph nodes and CM junction of the kidney, the highest exposed tissues. Brain/plasma concentration ratios of total radiolabeled components ranged between 0.293 and 4.37, suggesting a significant brain penetration of BAF312 and/or its metabolites. Unchanged BAF312 was the major component (77%) of total radiolabeled components in brain. Results obtained from mice orally dosed at 25 mg/kg confirmed, from the analysis of liver, spleen, lacrimal gland and kidney that the majority of radioactivity left in the carcass at 168 h post dose (16% of the dose) can likely be attributed to the cholesterol ester metabolite M17 (LYS815).			

2.6.5.5B Pharmacokinetics: Organ Distribution – Rat (DMPK 0500017-02)

Study title:	Absorption, distribution, metabolism and excretion in male rats after single intravenous (1 mg/kg) and peroral (3 mg/kg) administration of [^{14}C]BAF312	Study no. DMPK R0500017-02
Location in CTD:	4.2.2.2-2	
GLP compliance:	not required	
Species:	Rat (HAN:WIST) albino (Group 5 and 6), crt:(LE)BR pigmented (Group 7 and 8)	
Gender(M/F) / number of animals:	M / 11 (Group 5:1, Group 6:1, Group 7: 2, Group 8: 7)	
Feeding condition:	Water and food available *ad libitum*	
Vehicle / formulation:	Intravenous and oral: Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)	
Method of administration:	Intravenous bolus (into vena femoralis) / single dose (Group 5 and 7) Oral (gavage) / single dose (Group 6 and 8)	
Dose (mg/kg):	Intravenous: 1; oral: 3	
Radionuclide:	^{14}C	
Analyte / assay (unit):	Radioactivity / Quantitative Whole Body Autoradiography (nmol/g)	
Sampling time:	Group 5 and 6: 168 h after the last dosing Group 7: 0.083 and 168 h post dosing Group 8: 0.25, 2, 8, 24, 72, 96 and 168 h post dosing	
Analytical methods:	Determination of radioactivity in blood: Liquid scintillation counting (LSC) Determination of tissue distribution of radioactivity: QWBA; LOD: 17.6 and 7.75 ngEq/mL or ngEq/g after p.o. and i.v. dosing, respectively	

Tissue concentrations (expressed in ngEq/mL or ngEq/g) of total radiolabeled components after single intravenous dose of 1 mg/kg [^{14}C]BAF312 in albino (Group 5) and pigmented rats (Group 7)

Time points:	P 0.083 h	P 168 h	A 168 h
Tissues / organs:			
Adrenal (cortex)	8270	119	108
Adrenal (medulla)	3200	T	T
Bile	n.d.	n.d.	n.d.
Blood	1030	n.d.	n.d.
Blood vessel wall	n.d.	n.d.	n.d.

Bone marrow	1190	T	n.d.
Bone mineral	139	n.d.	n.d.
Brain (Cerebellar white matter)	181	n.d.	n.d.
Brain (Cerebellum)	449	n.d.	n.d.
Brain (Cerebral cortex)	403	n.d.	n.d.
Brain (Dura mater)	387	n.d.	n.d.
Brain (Inf. Colliculus)	620	n.d.	n.d.
Brain (Lateral septal nu)	315	n.d.	n.d.
Brain (mean)	356	T	T
Brain (Olfactory bulb)	372	n.d.	n.d.
Brain (Reticular nu)	336	n.d.	n.d.
Brain (Sup.colliculus)	418	n.d.	n.d.
Brown fat	1650	T	n.d.
Cartilage	672	n.d.	n.d.
Choroid plexus	1960	n.d.	n.d.
Epididymis	181	30.5	T
Eye (choroid)	982	n.d.	n.d.
Eye (lens)	T	n.d.	n.d.
Eye (vitreous body)	T	n.d.	n.d.
Hair (follicle)	620	n.d.	n.d.
Hair (tactile)	1140	n.d.	n.d.
Harderian gland	620	n.d.	n.d.
Heart	6200	n.d.	n.d.
Intestinal wall (colon)	568	n.d.	n.d.
Intestinal wall (duodenum)	878	n.d.	n.d.
Kidney (CM junction)	3250	n.d.	n.d.
Kidney (cortex)	3870	26.9	T
Kidney (medulla)	4390	T	T
Kidney (pelvis)	3460	n.d.	n.d.
Lachrymal gland (Lacrimal gland)	723	T	T

Liver	5680	25.8	T
Lung	3560	n.d.	n.d.
Lymph nodes	1080	n.d.	n.d.
Mouth (mucosa)	827	n.d.	n.d.
Muscle	506	T	n.d.
Nasal turbinates	305	T	T
Nerves	145	42.4	25.8
Oesophagus	672	n.d.	n.d.
Pancreas	2170	n.d.	n.d.
Penis (corpus cavernosum)	723	119	T
Pineal gland	1700	n.d.	n.d.
Pituitary gland	2170	43.4	n.d.
Preputial gland	1030	n.d.	n.d.
Salivary gland	1760	T	T
Seminal vesicle	T	n.d.	n.d.
Seminal vesicle (contour)	320	n.d.	n.d.
Skin	191	T	T
Skin (subcutis)	346	n.d.	n.d.
Spinal cord (gray)	387	T	T
Spinal cord (white)	103	34.1	T
Spinal cord (mean)	269	T	T
Spleen (mean)	2220	n.d.	T
Spleen (red pulp)	2480	n.d.	n.d.
Spleen (white pulp)	878	n.d.	n.d.
Stomach (glandular)	1700	n.d.	n.d.
Stomach (non glandular)	486	n.d.	n.d.
Testis	93.0	82.7	72.3
Thymus	723	T	T
Thyroid gland	4600	n.d.	n.d.
Tongue	1600	n.d.	n.d.

Tooth (enamel)	1290	n.d.	n.d.
Tooth (pulp)	1290	n.d.	n.d.
Urinary bladder	568	n.d.	n.d.
White fat	98.2	n.d.	n.d.
Additional information:	n.d.: <LOD (7.75 ngEq/mL or ngEq/g); T (traces) : between LOD and LOQ (22.7 ngEq/mL or ngEq/g); A : albino rat; P : pigmented rat		

Tissue concentrations (expressed in ngEq/mL or ngEq/g) of total radiolabeled components after single oral dose of 3 mg/kg [^{14}C]BAF312 in albino (Group 6) and pigmented rats (Group 8)

Time points:	P 0.25h	P 2h	P 8h	P 24h	P 72h	P 96h	P 168h	A 168h
Tissues (ngEq/mL or ngEq/g)								
Adrenal (cortex)	n.d.	2380	3150	568	424	243	186	160
Adrenal (medulla)	n.d.	1030	1700	139	108	T	T	T
Bile	n.d.	6720	12900	1240	n.d.	n.d.	n.d.	n.d.
Blood	T	310	362	T	n.d.	n.d.	n.d.	n.d.
Blood vessel wall	n.d.	1030	1110	119	n.d.	n.d.	n.d.	n.d.
Bone marrow	n.d.	620	930	114	n.d.	n.d.	n.d.	n.d.
Bone mineral	n.d.	56.8	98.2	T	n.d.	n.d.	n.d.	n.d.
Brain (Cerebellar white matter)	n.d.	165	568	568	181	n.d.	n.d.	n.d.
Brain (Cerebellum)	n.d.	165	930	258	T	n.d.	n.d.	n.d.
Brain (Cerebral cortex)	n.d.	160	827	269	T	n.d.	n.d.	n.d.
Brain (Dura mater)	n.d.	145	930	393	82.7	n.d.	n.d.	n.d.
Brain (Inf. Colliculus)	n.d.	145	1080	362	T	n.d.	n.d.	n.d.
Brain (Lateral septal nu)	n.d.	134	878	336	T	n.d.	n.d.	n.d.
Brain (mean)	T	150	775	336	T	T	n.d.	n.d.
Brain (Olfactory bulb)	n.d.	165	672	201	T	n.d.	n.d.	n.d.
Brain (Reticular nu)	n.d.	145	775	377	T	n.d.	n.d.	n.d.
Brain (Sup.colliculus)	n.d.	150	878	372	T	n.d.	n.d.	n.d.
Brown fat	T	1340	2270	186	T	n.d.	n.d.	n.d.
Cartilage	n.m.	341	517	103	T	T	n.d.	n.d.

Choroid plexus	n.d.	723	775	124	n.d.	n.d.	n.d.	n.d.
Epididymis	T	114	429	325	62.0	T	T	67.2
Eye (choroid)	n.d.	315	878	336	67.2	n.d.	n.d.	n.d.
Eye (lens)	n.d.	T	T	T	T	n.d.	n.d.	n.d.
Eye (vitreous body)	n.d.	T	T	T	T	n.d.	n.d.	n.d.
Hair (follicle)	1030	723	672	145	160	n.d.	n.d.	n.d.
Hair (tactile)	n.m.	294	511	56.8	n.d.	n.d.	n.d.	n.d.
Harderian gland	n.d.	351	1910	620	T	n.d.	n.d.	n.d.
Heart	T	1550	1910	150	n.d.	n.d.	n.d.	n.d.
Intestinal wall (colon)	n.m.	372	1290	620	n.d.	n.d.	n.d.	n.d.
Intestinal wall (duodenum)	n.m.	723	1450	325	n.d.	n.d.	n.d.	n.d.
Kidney (CM junction)	67.2	1340	2530	243	T	T	T	T
Kidney (cortex)	62.0	1600	2940	362	103	82.7	T	T
Kidney (medulla)	72.3	1340	1700	119	T	T	T	T
Kidney (pelvis)	227	672	775	119	T	n.d.	n.d.	n.d.
Lachrymal gland	n.d.	356	2010	620	T	n.d.	n.d.	n.d.
Liver	150	3670	6550	723	124	77.5	T	T
Lung	T	827	1190	93.0	n.d.	n.d.	n.d.	n.d.
Lymph nodes	93.0	377	982	108	n.d.	n.d.	n.d.	n.d.
Mouth (mucosa)	n.d.	305	620	196	T	T	n.d.	n.d.
Muscle	T	222	930	62.0	n.d.	n.d.	n.d.	n.d.
Nasal turbinates	T	222	449	134	77.5	T	n.d.	n.d.
Nerves	n.d.	77.5	517	377	253	165	98.2	72.3
Pancreas	n.m.	1340	2530	191	n.d.	n.d.	n.d.	n.d.
Penis (corpus cavernosum)	n.d.	486	842	87.8	n.d.	n.d.	n.d.	n.d.
Pineal gland	n.d.	723	1650	207	n.d.	n.d.	n.d.	n.d.
Pituitary gland	n.d.	930	1700	181	n.d.	n.d.	n.d.	n.d.
Preputial gland	T	160	527	119	T	T	T	n.d.
Salivary gland	T	930	1860	155	n.d.	n.d.	n.d.	n.d.
Seminal vesicle	n.d.	T	82.7	T	n.d.	n.d.	n.d.	n.d.

Seminal vesicle (contour)	n.d.	227	775	134	n.d.	n.d.	n.d.	n.d.
Skin	134	191	455	51.7	T	T	n.d.	T
Skin (subcutis)	n.m.	176	460	98.2	82.7	98.2	62.0	n.d.
Spinal cord (gray)	n.d.	170	982	393	T	T	n.d.	n.d.
Spinal cord (white)	n.d.	67.2	310	403	160	139	67.2	56.8
Spinal cord (mean)	n.d.	114	620	393	124	67.2	56.8	T
Spleen	n.m.	1080	1290	124	T	n.d.	T	n.d.
Spleen (red pulp)	n.m.	1190	1340	n.m.	n.m.	n.m.	n.d.	n.d.
Spleen (white pulp)	n.m.	331	672	n.d.	n.d.	n.d.	n.d.	n.d.
Stomach (glandular)	n.m.	2480	2690	191	n.d.	n.d.	n.d.	n.d.
Stomach (non glandular)	n.m.	827	455	124	T	n.d.	n.d.	n.d.
Testis	T	77.5	362	315	150	134	87.8	72.3
Thymus	T	315	982	145	T	T	n.d.	n.d.
Thyroid gland	n.d.	1240	1860	160	n.d.	n.d.	n.d.	n.d.
Tongue	n.d.	930	1500	124	T	n.d.	n.d.	n.d.
Tooth (enamel)	n.d.	289	568	82.7	n.d.	n.d.	n.d.	n.d.
Tooth (pulp)	n.d.	486	827	87.8	n.d.	n.d.	n.d.	n.d.
Urinary bladder	217	517	2120	n.d.	n.d.	n.d.	n.d.	n.d.
White fat	n.m.	212	129	T	n.d.	n.d.	n.d.	n.d.
Additional information:	n.d.: <LOD (17.6 ngEq/mL or ngEq/g); n.m.: not measured, T (traces) : between LOD and LOQ (51.7 ngEq/mL or ngEq/g); A : albino rat; P : pigmented rat							

AUCs (expressed in ngEq•h/mL or ngEq•h/g) of radioactivity after single oral dose of 3 mg/kg [^{14}C]BAF312 in albino (Group 6) and pigmented rats (Group 8)

Tissues:	AUC (ngEq•h/mL or ngEq•h/g)	AUC ratio (AUCtissue/AUCblood)
Adrenal (cortex)	95100	13.1
Adrenal (medulla)	32500	4.5
Bile	211000	29.1
Blood	7230	1.0
Blood vessel wall	17300	2.4

Bone marrow	17600	2.4
Bone mineral	3100	0.4
Brain (Cerebellar white	33100	4.6
Brain (Cerebellum)	20700	2.9
Brain (Cerebral cortex)	19600	2.7
Brain (Dura mater)	26900	3.7
Brain (Inf. Colliculus)	25800	3.6
Brain (Lateral septal nu)	22200	3.1
Brain (mean)	22200	3.1
Brain (Olfactory bulb)	16000	2.2
Brain (Reticular nu)	22700	3.1
Brain (Spinal cord gray)	25800	3.6
Brain (Spinal cord white)	31500	4.4
Brain (Spinal cord) mean	29400	4.1
Brain (Sup.colliculus)	23800	3.3
Brown fat	37700	5.2
Cartilage	12400	1.7
Choroid plexus	17000	2.4
Epididymis	19600	2.7
Eye (choroid)	24800	3.4
Eye (lens)	2580	0.4
Eye (vitreous body)	2580	0.4
Hair (follicle)	22200	3.1
Hair (tactile)	9820	1.4
Harderian gland	44400	6.1
Heart	33100	4.6
Intestinal wall (colon)	36700	5.1
Intestinal wall (duodenum)	30500	4.2
Kidney (CM junction)	43400	6.0
Kidney (cortex)	58900	8.1

Kidney (medulla)	30000	4.1
Kidney (pelvis)	17000	2.4
Lachrymal gland	44400	6.1
Liver	119000	16.5
Lung	20700	2.9
Lymph nodes	17600	2.4
Mouth (mucosa)	16000	2.2
Muscle	14500	2.0
Nasal turbinates	14500	2.0
Nerves	38700	5.4
Oesophagus	86800	12.0
Pancreas	40300	5.6
Penis (corpus cavernosum)	11900	1.6
Pineal gland	28900	4.0
Pituitary gland	29400	4.1
Preputial gland	7390	1.0
Salivary gland	30500	4.2
Seminal vesicle	3100	0.4
Seminal vesicle (contour)	15500	2.1
Skin	9820	1.4
Skin (subcutis)	18600	2.6
Spleen	24300	3.4
Spleen (red pulp)	20700	2.9
Spleen (white pulp)	10300	1.4
Stomach (glandular)	46500	6.4
Stomach (non glandular)	13900	1.9
Testis	29400	4.1
Thymus	18600	2.6
Thyroid gland	31500	4.4
Tongue	25800	3.6

Tooth (enamel)	10800	1.5
Tooth (pulp)	15000	2.1
Urinary bladder	27400	3.8
White fat	4130	0.6

Additional information:	none
Conclusion:	The highest and longest lasting radioactivity levels were found in the adrenal cortex, myelin (represented by the nerves, cerebellar white matter and white spinal cord) and testis. After i.v. and p.o. dosing, compound-related radioactive material was not taken up into the melanin-containing structures in a specific manner, but taken up rather extensively into the brain. The distribution of radioactivity in the brain was characterized by a regional specificity in the time period 8-24 h p.d. (i.e. 1080 ngEq/g in the inf. colliculus vs. 568 ngEq/g in the cerebellar white matter at 8 h p.d. and 568 ngEq/g in the cerebellar white matter vs. 201 ngEq/mL in the olfactory bulb at 24 h p.d.) and by a subsequent rapid elimination, except from the cerebellar white matter and dura mater. The highest exposures were found in the bile (211000 ngEq•h/mL), liver (119000 ngEq•h/g), adrenal cortex (95100 ngEq•h/g), oesophagus (86800 ngEq•h/g) and kidney cortex (58900 ngEq•h/g) and were approx. 29, 16, 13, 12 and 8-fold higher than that of blood (7230 ngEq•h/mL). The AUCs in all other tissues were also higher than that of blood except in the bone mineral, lens, vitreous body and seminal vesicle (40% of that of blood) and white fat (60% of that of blood).

2.6.5.5C Pharmacokinetics: Organ Distribution – Rat (DMPK 0900164-01)

Study title:	Absorption, distribution, and metabolism in male rats after single intravenous (1 mg/kg) and peroral (3 and 10 mg/kg) administration of [^{14}C]BAF312 (with radiolabel on the benzyl-oxyamino part of the molecule)	Study no. DMPK R0900164-01
Location in CTD:	4.2.2.2-3	
GLP compliance:	not required	
Test article	[^{14}C]BAF312 hemifumarate (with radiolabel on the benzyl-oxyamino part of the molecule)	
Species:	Hanover Wistar rat (albino), Long Evans rat (pigmented)	
Gender(M/F) / number of animals:	M / 14 (Hanover Wistar: 5 (1 mg/kg), 5 (3 mg/kg); Long Evans: 2 (1 mg/kg), 2 (3 mg/kg))	
Feeding condition:	Water and food available *ad libitum*	
Vehicle / formulation:	Intravenous and oral: Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)	
Method of administration:	Intravenous bolus (into vena femoralis) / single dose oral (gavage) / single dose	
Dose (mg/kg):	Intravenous: 1, oral: 3 (doses referring to the free base)	
Radionuclide:	^{14}C	
Analyte / assay (unit):	Radioactivity / Quantitative Whole Body Autoradiography (ngEq/mL or ngEq/g)	
Sampling time:	Hanover Wistar: 0.25, 2, 4, 24 and 168 h post dosing. Long Evans: 24 and 168 h post dosing	
Analytical methods:	Determination of radioactivity in blood: Liquid scintillation counting (LSC) Determination of tissue distribution of radioactivity: Quantitative whole body radiography.	

Tissue concentrations (expressed in ngEq/mL or ngEq/g) of total radiolabeled components after single intravenous dose of 1 mg/kg [^{14}C]BAF312 hemifumarate in albino and pigmented rats (n=1/time point)

Time points:	A 0.25 h	A 2 h	A 4 h	A 24 h	A168 h	P 24 h	P 168 h
Tissues / organs:							
Adrenal (cortex)	8730	3750	2810	589	114	499	123
Adrenal (medulla)	4290	2010	1210	257	42.6	177	T
Bile	12800	10500	7540	63.0	n.d.	70.8	n.d.
Blood	604	332	238	T	n.d.	T	n.d.
Blood (LSC)	548	281	197	8.89	n.d.	47.8	n.d.

Blood vessel wall	1810	940	847	50.1	n.d.	63.0	n.d.
Bone marrow	1500	1040	615	75.9	n.d.	n.d.	n.d.
Bone mineral	158	144	72.3	n.d.	n.d.	180	n.d.
Brain	449	759	661	234	n.d.	n.d.	n.d.
Brain (cerebellum fissure)	273	488	426	n.m.	n.d.	120	n.d.
Brain (cerebellum)	444	909	765	208	n.d.	120	n.d.
Brain (cerebral cortex)	453	837	723	185	n.d.	341	n.d.
Brain (corpus callosum)	261	418	368	328	n.d.	193	n.d.
Brain (inf. colliculus)	421	1260	708	245	n.d.	289	n.d.
Brain (medulla oblongata)	440	951	790	390	n.d.	99.2	n.d.
Brain (olfactory bulb)	361	837	584	143	n.d.	211	n.d.
Brain (striatum)	288	620	563	269	n.d.	206	n.d.
Brain (thalamus)	387	868	666	207	n.d.	58.4	n.d.
Brain (ventricles)	75.4	333	120	55.8	n.d.	n.d.	n.d.
Cartilage	T	T	T	n.d.	n.d.	148	n.d.
Choroid plexus	2600	1130	646	123	n.d.	248	T
Epididymis	296	454	445	267	T	104	n.d.
Eye (choroid)	847	801	770	88.3	n.d.	n.d.	n.d.
Eye (meibomian glands)	n.s.	1100	1120	263	n.d.	243	n.d.
Eye (vitreous body)	n.d.	T	n.d.	n.d.	n.d.	n.d.	n.d.
Fat (brown)	2560	1870	796	138	n.d.	68.2	n.d.
Fat (white)	138	150	97.1	T	n.d.	T	n.d.
Harderian gland	759	1390	1680	364	n.d.	376	n.d.
Heart	4810	2120	1280	116	n.d.	62.5	n.d.
Intestinal wall (colon)	796	961	759	172	n.d.	154	n.d.
Intestinal wall (small int.)	1120	1650	n.m.	70.8	n.d.	n.m.	n.d.
Kidney (cortex)	3700	2390	1610	217	T	254	T
Kidney (medulla)	3150	1440	940	101	n.d.	88.9	n.d.
Kidney (pelvis)	1540	651	527	51.6	n.d.	61.5	n.d.
Lacrimal gland	2000	1890	1530	155	n.d.	105	n.d.

Liver	6410	3100	3240	300	T	284	T
Lung	1770	997	754	73.9	n.d.	58.9	n.d.
Lymph nodes	517	956	687	303	n.d.	155	n.d.
Muscle	723	883	703	48.3	n.d.	T	n.d.
Pancreas	2710	2520	1590	129	n.d.	84.2	n.d.
Penis	420	480	383	82.7	n.d.	59.4	n.d.
Pineal body	2090	1960	1380	134	n.d.	88.9	n.d.
Pituitary gland	3170	1900	1170	236	n.d.	186	n.d.
Preputial gland	827	780	1760	754	n.d.	589	n.d.
Salivary gland	2430	2170	1440	133	n.d.	65.1	n.d.
Seminal vesicle (contour)	703	1140	889	97.6	n.d.	T	n.d.
Seminal vesicle	402	821	599	97.6	n.d.	T	n.d.
Skin	405	573	420	88.3	T	53.2	n.d.
Spinal cord	369	573	558	356	T	277	T
Spinal cord (grey matter)	563	976	672	245	n.d.	174	n.d.
Spinal cord (white matter)	268	393	274	458	n.d.	292	n.d.
Spinal nerves	186	394	353	422	n.d.	223	n.d.
Spleen	2410	1370	842	109	n.d.	82.7	n.d.
Stomach (glandular)	2180	2620	1850	160	n.d.	85.8	n.d.
Stomach (non glandular)	940	651	677	65.6	n.d.	T	n.d.
Testis	136	217	315	239	64.6	212	61.0
Thymus	492	821	723	121	n.d.	125	n.d.
Thyroid gland	4100	2040	1130	108	n.d.	57.9	n.d.
Tongue	2340	1810	1160	105	n.d.	49.4	n.d.
Tooth (pulp)	1510	1060	599	52.2	n.d.	T	n.d.

Additional information: A: albino rats; P: pigmented rats

n.d.: <LOD (13.6 ngEq/mL or ngEq/g); n.m.: not measured, time point was not considered for AUC estimations; n.s.:no sample. time point was not considered for AUC estimations; T (traces): between LOQ (40.9 ngEq/mL or ngEq/g) and LOD, set to 27.3 ngEq/mL or ngEq/g for AUC calculations

Pharmacokinetic parameters of total radiolabeled components after intravenous dose of 1 mg/kg [^{14}C]BAF312 hemifumarate in albino and pigmented rats (n=1/time point)

Tissues	Tmax (h)	Cmax (ngEq/mL or ngEq/g)	Cmax normalized [a]	Tlast (h)	AUC0-24h (ngEq•h/mL or ngEq•h/g) [c]	AUC0-24h normalized [b]
Adrenal (cortex)	0.25	8730	15.9	168	52700	15.0
Adrenal (medulla)	0.25	4290	7.84	168	23900	6.82
Blood	0.25	604	1.10	4 d	4190	1.20
Blood (LSC)	0.25	548	1.00	24	3510	1.00
Blood vessel wall	0.25	1810	3.31	24	13400	3.81
Bone marrow	0.25	1500	2.75	24	11000	3.12
Bone mineral	0.25	158	0.289	4 d	1220	0.349
Brain	2	759	1.39	24	11500	3.27
Brain (cerebellum fissure)	2	488	0.891	4 d	9610	2.74
Brain (cerebellum)	2	909	1.66	24	12700	3.61
Brain (cerebral cortex)	2	837	1.53	24	11800	3.37
Brain (corpus callosum)	2	418	0.764	24	8370	2.39
Brain (inf. colliculus)	2	1260	2.29	24	13000	3.71
Brain (medulla oblongata)	2	951	1.74	24	14800	4.23
Brain (olfactory bulb)	2	837	1.53	24	9760	2.78
Brain (striatum)	2	620	1.13	24	10300	2.95
Brain (thalamus)	2	868	1.58	24	11400	3.25
Brain (ventricles)	2	333	0.608	24	2580	0.735
Cartilage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Choroid plexus	0.25	2600	4.75	24	13100	3.73
Epididymis	2	454	0.828	24	8680	2.47
Eye (choroid)	0.25	847	1.55	24	11700	3.33
Eye (meibomian glands)	4	1120	2.05	24	17200	4.89
Eye (vitreous body)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Fat (brown)	0.25	2560	4.67	24	16200	4.61
Fat (white)	2	150	0.275	24	1760	0.502

Harderian gland	4	1680	3.08	24	25500	7.28
Heart	0.25	4810	8.78	24	24000	6.83
Intestinal wall (colon)	2	961	1.75	24	12700	3.61
Intestinal wall (small int.)	2	1650	3.01	24	21500	6.13
Kidney (cortex)	0.25	3700	6.75	24	28100	8.00
Kidney (medulla)	0.25	3150	5.75	24	17200	4.90
Kidney (pelvis)	0.25	1540	2.82	24	9090	2.59
Lacrimal gland	0.25	2000	3.66	24	24000	6.83
Liver	0.25	6410	11.7	168	50800	14.5
Lung	0.25	1770	3.24	24	12700	3.61
Lymph nodes	2	956	1.75	24	12900	3.68
Muscle	2	883	1.61	24	10600	3.02
Pancreas	0.25	2710	4.94	24	26200	7.47
Penis	2	480	0.876	24	6350	1.81
Pineal body	0.25	2090	3.82	24	22300	6.35
Pituitary gland	0.25	3170	5.79	24	22000	6.27
Preputial gland	4	1760	3.22	24	29200	8.32
Salivary gland	0.25	2430	4.44	24	23600	6.73
Seminal vesicle (contour)	2	1140	2.08	24	13600	3.87
Seminal vesicle	2	821	1.50	24	9510	2.71
Skin	2	573	1.05	24	6970	1.99
Spinal cord	2	573	1.05	24	11200	3.18
Spinal cord (grey matter)	2	976	1.78	24	12200	3.49
Spinal cord (white matter)	24	458	0.836	24	8580	2.44
Spinal nerves	24	422	0.771	24	9040	2.58
Spleen	0.25	2410	4.40	24	15300	4.37
Stomach (glandular)	2	2620	4.78	24	29000	8.28
Stomach (non glandular)	0.25	940	1.72	24	10300	2.93
Testis	4	315	0.575	24	6410	1.83
Thymus	2	821	1.50	24	11200	3.20

Thyroid gland	0.25	4100	7.48	24	21400	6.11
Tongue	0.25	2340	4.27	24	19500	5.57
Tooth (pulp)	0.25	1510	2.76	24	10600	3.03
Additional information:	a: value normalized to the Cmax value of blood (LSC); b: value normalized to the AUC0-24h value of blood (LSC); c: for estimation of AUC0-24h, all values below LOQ were set to 0 and all traces to 27.3 ngEq/mL or ngEq/g; d: value was either below LOD or a trace					

Tissue concentrations (expressed in ngEq/mL or ngEq/g) of total radiolabeled components after single oral dose of 3 mg/kg [^{14}C]BAF312 hemifumarate in albino and pigmented rats (n=1/time point)

Time points:	A 0.25 h	A 2 h	A 4 h	A 24 h	A168 h	P 24 h	P 168 h
Tissues / organs:							
Adrenal (cortex)	n.m.	2160	5270	966	584	2190	316
Adrenal (medulla)	n.m.	1100	2560	517	136	940	89.4
Bile	n.d.	n.d.	8830	3700	n.d.	2000	n.d.
Blood	n.d.	243	522	T	n.d.	178	n.d.
Blood (LSC)	32.1	214	431	94.5	T	T	377
Blood vessel wall	n.d.	506	1350	242	n.d.	527	n.d.
Bone marrow	n.d.	558	1200	169	n.d.	522	n.d.
Bone mineral	n.d.	70.3	297	T	n.d.	T	n.d.
Brain	n.d.	172	589	651	n.d.	1300	n.d.
Brain (cerebellum fissure)	n.d.	191	687	1170	n.d.	1650	n.d.
Brain (cerebellum)	n.d.	191	687	466	n.d.	1030	n.d.
Brain (cerebral cortex)	n.d.	194	646	486	n.d.	1100	n.d.
Brain (corpus callosum)	n.d.	88.9	324	796	n.d.	1380	n.d.
Brain (inf. colliculus)	n.d.	207	610	703	n.d.	1420	n.d.
Brain (medulla oblongata)	n.d.	196	594	997	n.d.	1710	n.d.
Brain (olfactory bulb)	n.d.	139	563	428	n.d.	847	n.d.
Brain (striatum)	n.d.	136	548	656	n.d.	1250	n.d.
Brain (thalamus)	n.d.	163	651	661	n.d.	1370	n.d.
Cartilage	n.d.	T	T	n.d.	n.d.	n.d.	n.d.
Choroid plexus	n.d.	573	1320	161	n.d.	505	n.d.

Epididymis	n.d.	89.4	393	697	76.5	832	79.0
Eye (choroid)	n.d.	356	816	214	n.d.	548	n.d.
Eye (lens)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Eye (meibomian glands)	n.d.	338	1270	1170	n.d.	1530	n.d.
Eye (vitreous body)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fat (brown)	n.d.	1160	2250	407	n.d.	672	n.d.
Fat (white)	n.m.	T	209	T	n.d.	T	n.d.
Harderian gland	n.d.	336	1500	1300	n.d.	2810	n.d.
Heart	T [b]	1210	2830	313	n.d.	1010	n.d.
Intestinal wall (colon)	n.d.	279	1010	252	n.d.	749	n.d.
Intestinal wall (small int.)	n.m.	739	1390	166	n.d.	n.m.	n.d.
Kidney (cortex)	n.m.	1140	3120	703	111	1530	T
Kidney (medulla)	n.m.	909	2820	245	n.d.	827	n.d.
Kidney (pelvis)	n.m.	410	992	194	n.d.	322	n.d.
Lacrimal gland	n.d.	363	1500	661	n.d.	1340	n.d.
Liver	191	2430	5530	956	69.2	1610	80.1
Lung	T [b]	723	1630	198	n.d.	478	n.d.
Lymph nodes	n.d.	447	785	245	163	537	207
Muscle	n.d.	283	961	167	n.d.	496	n.d.
Pancreas	n.m.	945	2600	475	n.d.	1070	n.d.
Penis	n.d.	172	517	241	n.d.	362	n.d.
Pineal body	n.d.	615	1760	466	T	775	n.d.
Pituitary gland	n.d.	821	1950	446	n.d.	852	n.d.
Preputial gland	n.s.	452	1570	1490	n.d.	2310	n.d.
Salivary gland	n.d.	759	2230	355	n.d.	982	n.d.
Seminal vesicle (contour)	n.d.	n.m.	935	286	n.d.	548	n.d.
Seminal vesicle	n.d.	n.m.	718	168	n.d.	313	n.d.
Skin	T [b]	184	510	149	T	317	n.d.
Spinal cord	n.d.	133	553	816	T	1630	T
Spinal cord (grey matter)	n.d.	174	796	796	T	1420	n.d.

Spinal cord (white matter)	n.d.	88.3	490	852	69.2	2010	69.7
Spinal nerves	n.d.	127	328	847	T	1970	76.5
Spleen	n.m.	765	1820	192	n.d.	604	n.d.
Stomach (glandular)	n.m.	1430	3700	435	n.d.	1290	n.d.
Stomach (non glandular)	n.m.	n.m.	1160	1010	n.d.	1910	n.d.
Testis	n.d.	81.6	262	507	116	904	277
Thymus	n.d.	211	697	282	T	589	T
Thyroid gland	n.d.	883	2500	282	n.d.	821	n.d.
Tongue	n.d.	630	1990	272	n.d.	677	n.d.
Tooth (pulp)	n.d.	398	997	153	n.d.	377	n.d.
Additional information:	A: albino; P: pigmented; T (traces): between LOQ (68.7 ngEq/mL or ngEq/g) and LOD b: set to 45.9 ngEq/mL or ngEq/g for AUC calculation; n.d.: < LOD (22.9 ngEq/mL or ngEq/g), value is set to 0 for AUC estimations; n.m.: not measured, time point was not considered for AUC estimations; n.s.: no sample, time point was not considered for AUC estimations;						

Pharmacokinetic parameters of total radiolabeled components after oral dose of 3 mg/kg [^{14}C]BAF312 hemifumarate in albino and pigmented rats (n=1/time point)

Tissues	Tmax (h)	Cmax (ngEq/mL or ngEq/g)	Cmax normalized [a]	Tlast (h)	AUC0-24h (ngEq•h/mL or ngEq•h/g) [c]	AUC0-24h Normalized [b]
Adrenal (cortex)	4	5270	12.2	168	71800	11.8
Adrenal (medulla)	4	2560	5.95	168	35500	5.83
Blood	4	522	1.21	4	n.a.	n.a.
Blood (LSC)	4	431	1.00	24	6100	1.00
Blood vessel wall	4	1350	3.13	24	18200	2.98
Bone marrow	4	1200	2.79	24	16000	2.62
Bone mineral	4	297	0.689	4	n.a.	n.a.
Brain	24	651	1.51	24	13300	2.19
Brain (cerebellum fissure)	24	1170	2.72	24	19600	3.22
Brain (cerebellum)	4	687	1.59	24	12600	2.06
Brain (cerebral cortex)	4	646	1.50	24	12300	2.03
Brain (corpus callosum)	24	796	1.85	24	11700	1.92

Brain (inf. colliculus)	24	703	1.63	24	14100	2.31
Brain (medulla oblongata)	24	997	2.31	24	16900	2.77
Brain (olfactory bulb)	4	563	1.31	24	10700	1.76
Brain (striatum)	24	656	1.52	24	12900	2.11
Brain (thalamus)	24	661	1.53	24	14100	2.31
Cartilage	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Choroid plexus	4	1320	3.06	24	17200	2.82
Epididymis	24	697	1.62	168	11500	1.88
Eye (choroid)	4	816	1.89	24	11800	1.93
Eye (lens)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Eye (meibomian glands)	4	1270	2.94	24	26200	4.31
Eye (vitreous body)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Fat (brown)	4	2250	5.23	24	31000	5.08
Fat (white)	4	209	0.486	4	n.a.	n.a.
Harderian gland	4	1500	3.49	24	30200	4.95
Heart	4	2830	6.57	24	36600	6.00
Intestinal wall (colon)	4	1010	2.35	24	14200	2.33
Intestinal wall (small int.)	4	1390	3.24	24	18500	3.03
Kidney (cortex)	4	3120	7.23	168	43600	7.14
Kidney (medulla)	4	2820	6.53	24	35200	5.78
Kidney (pelvis)	4	992	2.30	24	13700	2.25
Lacrimal gland	4	1500	3.49	24	23800	3.91
Liver	4	5530	12.8	168	74900	12.3
Lung	4	1630	3.78	24	21300	3.49
Lymph nodes	4	785	1.82	168	11900	1.96
Muscle	4	961	2.23	24	12800	2.09
Pancreas	4	2600	6.03	24	35200	5.78
Penis	4	517	1.20	24	8420	1.38
Pineal body	4	1760	4.08	24	25200	4.13
Pituitary gland	4	1950	4.52	24	27400	4.50

Preputial gland	4	1570	3.65	24	33100	5.43
Salivary gland	4	2230	5.17	24	29400	4.83
Seminal vesicle. (contour)	4	935	2.17	24	n.a.	n.a.
Seminal vesicle	4	718	1.67	24	n.a.	n.a.
Skin	4	510	1.18	24	7490	1.23
Spinal cord	24	816	1.89	24	14500	2.38
Spinal cord (grey matter)	4	796	1.85	24	17000	2.80
Spinal cord (white matter)	24	852	1.98	168	14100	2.31
Spinal nerves	24	847	1.97	24	12300	2.02
Spleen	4	1820	4.22	24	23500	3.85
Stomach (glandular)	4	3700	8.60	24	47900	7.86
Stomach (non glandular)	4	1160	2.70	24	n.a.	n.a.
Testis	24	507	1.18	168	8110	1.33
Thymus	4	697	1.62	24	10900	1.79
Thyroid gland	4	2500	5.79	24	31900	5.24
Tongue	4	1990	4.63	24	25800	4.24
Tooth (pulp)	4	997	2.31	24	13200	2.17
Additional information:	a: value normalized to the Cmax value of blood (LSC); b: value normalized to the AUC0-24h value of blood (LSC); c: for estimation of AUC0-24h, all values below LOQ were set to 0; n.a.: not available					
Conclusion:	Following i.v. or p.o. administration, total radiolabeled components were distributed into extra vascular tissues. Limited information on apparent terminal elimination T1/2 values were available, but greater values than 38 h can be expected for at least adrenal cortex, adrenal medulla, epididymis, kidney cortex, liver, lymph nodes, spinal cord, and testis. Specific uptake and retention of total radiolabeled components into melanin-containing structures (pigmented eye choroid) was not observed. Uptake into brain was detected.					

2.6.5.5D Pharmacokinetics: Organ Distribution – Rat oral (DMPK R1200177)

Study title:	Quantitative whole-body radiography in rats after multiple oral (3 mg/kg) administration of [^{14}C]BAF312	Study no. DMPK R1200177
Location in CTD:	4.2.2.3-1	
GLP compliance:	not required	
Species:	Rat (Long Evans, pigmented)	
Gender(M/F) / number of animals:	M / 4	
Feeding condition:	Water and food available *ad libitum*	
Vehicle / formulation:	Ethanol/polyethylene glycol 200 (PEG 200)/5% aqueous glucose solution (1/6/3, w/w/w)	
Method of administration:	Oral (gavage)	
Dose (mg/kg):	3 mg/kg/day (referring to the free base) for 7 days (once daily)	
Radionuclide:	^{14}C	
Analyte / assay (unit):	Radioactivity / Quantitative Whole Body Autoradiography (ngEq/mL or ngEq/g)	
Sampling time:	8, 24, 72, and 168 h after the last dosing	
Analytical methods:	Determination of radioactivity in blood: Liquid scintillation counting (LSC); LOD: 6.20 ngEq/mL, LLOQ: 14.0 ngEq/mL. Determination of tissue distribution of radioactivity: QWBA; LOD: 9.20 ngEq/mL or ngEq/g, LLOQ: 18.4 ngEq/mL or ngEq/g	

Tissue concentrations (expressed in ngEq/mL or ngEq/g) of total radiolabeled components after multiple oral doses of 3 mg/kg [^{14}C]BAF312 in pigmented rats (8, 24, 72, and 168 h after the 7th daily oral dose)

Time points:	8 h	24 h	72 h	168 h
Tissues / organs:	Rat #1	Rat #2	Rat #3	Rat #4
Adrenal (cortex)	6300	2290	1820	780
Adrenal (medulla)	2230	708	594	170
Blood	474	103	T	n.d.
Blood (LSC)	455	98.2	n.d.	n.d.
Blood vessel wall	1580	223	42.8	n.d.
Bone marrow	1500	255	52.7	22.4
Bone mineral	426	48.0	n.d.	n.d.
Brain (Cerebellum, Gray matter)	1340	749	62.0	31.9

Brain (Cerebellum, White matter)	2060	1760	208	51.7
Brain (Cerebral cortex)	1230	801	57.9	27.5
Brain (Choroid plexus)	1860	274	58.4	n.d.
Brain (Corpus callosum)	1680	1440	224	38.1
Brain (Fornix)	1680	1320	137	30.2
Brain (Inferior colliculus)	1500	889	97.1	33.1
Brain (Medulla oblongata)	1820	1510	117	41.1
Brain (Olfactory bulb)	801	573	61.5	24.6
Brain (Superior colliculus)	1520	914	77.0	34.0
Brain (Thalamus)	1260	868	66.6	35.8
Cartilage	72.3	40.2	33.5	n.d.
Epididymis	1500	1230	24.8	279
Esophagus	1110	205	59.4	T
Eye (choroid)	1800	500	96.6	83.7
Eye (ciliary body)	1590	511	172	190
Eye (lens)	35.8	40.2	30.4	29.6
Eye (vitreous body)	T	T	n.d.	n.d.
Fat (brown)	3630	1260	222	81.6
Fat (white)	223	62.0	T	T
Hair (follicle)	1020	322	83.7	45.4
Hair (tactile)	672	201	67.2	72.3
Harderian gland	3740	1920	142	79.0
Heart	3100	532	66.1	23.6
Intestinal wall (colon)	811	277	86.8	T
Intestinal wall (small intestine)	n.m.	666	225	34.5
Kidney (CM junction)	4250	1140	290	148
Kidney (cortex)	6200	1880	527	252
Kidney (medulla)	2590	446	76.5	24.7
Lacrimal gland	2960	909	141	75.9
Liver	8540	2130	461	154

Lung	1240	315	42.4	27.9
Lymph nodes (submandibular)	1550	387	105	49.5
Muscle	930	196	T	n.d.
Pancreas	3890	610	80.1	31.7
Penis	899	217	75.4	T
Pineal body	2850	718	157	72.3
Pituitary gland	2720	641	99.7	46.4
Preputial gland	7080	3990	2360	4570
Salivary gland (submax)	3070	589	74.4	T
Seminal vesicle wall	1030	354	84.2	n.d.
Seminal vesicular fluid	57.9	32.5	T	n.d.
Skin	458	126	43.7	T
Skin (subcutis)	405	96.6	33.8	T
Spinal cord (Gray matter)	1760	1030	97.6	64.1
Spinal cord	2380	1680	573	107
Spinal cord (White matter)	2260	1930	697	246
Spleen	2280	432	77.5	38.5
Spleen (red pulp)	2600	488	78.0	46.3
Spleen (white pulp)	1770	365	55.8	22.4
Stomach (glandular)	1220	682	105	23.2
Stomach (non glandular)	n.m.	3430	56.8	T
Testis	1120	1010	264	249
Thymus	1400	423	74.9	43.2
Thyroid gland	1280	661	88.3	32.4
Tongue	2250	473	53.2	24.2
Tongue (mucous gland)	2670	754	147	70.3
Tooth (pulp)	1340	312	30.5	n.d.

Additional information: n.d.: <LOD (9.20 ngEq/mL or ngEq/g [QWBA] or 6.20 ngEq/mL [LSC]); T (traces): between LOD and LOQ (18.4 ngEq/mL or ngEq/g [QWBA])

Pharmacokinetic parameters of total radiolabeled components after multiple oral doses of 3 mg/kg [^{14}C]BAF312 in selected tissues of pigmented rats (after the 7th daily oral dose)

Tissues	Tmax (h)	Cmax (ngEq/mL or ngEq/g)	Cmax*	AUClast (ngEq•h/mL or ngEq•h/g)	AUClast*	Tlast (h)	T1/2 (h)	T1/2 range (h)
Adrenal (cortex)	8	6300	13.3	318000	48.8	168	90.7	24-168
Adrenal (medulla)	8	2230	4.70	100000	15.4	168	67.3	24-168
Blood	8	474	1.00	6510	1.00	24	n.c.	NA
Blood (LSC)	8	455	0.960	6250	0.960	24	n.c.	NA
Blood vessel wall	8	1580	3.33	27100	4.16	72	13.5	8-72
Bone marrow	8	1500	3.16	31000	4.76	168	44.0	24-168
Bone mineral	8	426	0.898	5480	0.844	24	n.c.	NA
Brain (Cerebellum, Gray matter)	8	1340	2.83	46100	7.08	168	n.c.	NA
Brain (Cerebellum, White matter)	8	2060	4.35	98700	15.1	168	n.c.	NA
Brain (Cerebral cortex)	8	1230	2.60	45900	7.05	168	n.c.	NA
Brain (Choroid plexus)	8	1860	3.93	32500	5.00	72	n.c.	NA
Brain (Corpus callosum)	8	1680	3.54	84200	12.9	168	28.6	24-168
Brain (Fornix)	8	1680	3.55	73900	11.3	168	28.0	24-168
Brain (Inferior colliculus)	8	1500	3.16	55300	8.46	168	n.c.	NA
Brain (Medulla oblongata)	8	1820	3.84	80600	12.4	168	n.c.	NA
Brain (Olfactory bulb)	8	801	1.69	33600	5.17	168	n.c.	NA
Brain (Superior colliculus)	8	1520	3.21	54800	8.42	168	n.c.	NA
Brain (Thalamus)	8	1260	2.66	49500	7.60	168	n.c.	NA
Cartilage	8	72.3	0.152	2960	0.454	72	n.c.	NA
Epididymis	8	1500	3.16	72300	11.1	168	n.c.	NA
Esophagus	8	1110	2.34	21300	3.27	72	n.c.	NA
Eye (choroid)	8	1800	3.80	48600	7.47	168	n.c.	NA
Eye (ciliary body)	8	1590	3.34	56800	8.74	168	n.c.	NA
Eye (lens)	24	40.2	0.0848	5320	0.818	168	n.c.	NA
Eye (vitreous body)	NA	NA	NA	NA	NA	NA	n.c.	NA
Fat (brown)	8	3630	7.67	104000	15.9	168	39.1	24-168

Fat (white)	8	223	0.471	3180	0.488	24	n.c.	NA
Hair (follicle)	8	1020	2.15	30700	4.72	168	n.c.	NA
Hair (tactile)	8	672	1.42	22800	3.50	168	n.c.	NA
Harderian gland	8	3740	7.90	120000	18.5	168	n.c.	NA
Heart	8	3100	6.55	59900	9.24	168	n.c.	NA
Intestinal wall (colon)	8	811	1.72	20700	3.18	72	21.3	8-72
Intestinal wall (small intestine)	24	666	1.41	41900	6.45	168	33.9	24-168
Kidney (CM junction)	8	4250	8.96	116000	17.8	168	n.c.	NA
Kidney (cortex)	8	6200	13.0	184000	28.4	168	53.0	24-168
Kidney (medulla)	8	2590	5.48	52200	8.01	168	36.6	24-168
Lacrimal gland	8	2960	6.25	78500	12.0	168	n.c.	NA
Liver	8	8520	18.0	211000	32.5	168	40.2	24-168
Lung	8	1240	2.61	29300	4.50	168	n.c.	NA
Lymph nodes (submandibular)	8	1550	3.28	41000	6.29	168	51.9	24-168
Muscle	8	930	1.97	12800	1.96	24	n.c.	NA
Pancreas	8	3890	8.21	73400	11.3	168	n.c.	NA
Penis	8	899	1.90	19500	2.99	72	n.c.	NA
Pineal body	8	2850	6.00	71800	11.0	168	n.c.	NA
Pituitary gland	8	2720	5.75	62500	9.63	168	n.c.	NA
Preputial gland	8	7080	15.0	602000	92.5	168	n.c.	NA
Salivary gland (submaxillary)	8	3070	6.48	57300	8.83	72	12.7	8-72
Seminal vesicle wall	8	1030	2.18	25700	3.95	72	18.7	8-72
Seminal vesicular fluid	8	57.9	0.122	951	0.146	24	n.c.	NA
Skin	8	458	0.965	10600	1.62	72	20.8	8-72
Skin (subcutis)	8	405	0.854	8730	1.35	72	n.c.	NA
Spinal cord (Gray matter)	8	1760	3.70	64100	9.84	168	n.c.	NA
Spinal cord	8	2380	5.02	129000	19.8	168	36.8	24-168
Spinal cord (White matter)	8	2260	4.77	151000	23.2	168	50.1	24-168
Spleen	8	2280	4.82	48700	7.48	168	n.c.	NA
Spleen (red pulp)	8	2600	5.48	54800	8.39	168	n.c.	NA

Spleen (white pulp)	8	1770	3.74	38100	5.85	168	n.c.	NA
Stomach (glandular)	8	1220	2.58	45100	6.93	168	31.0	24-168
Stomach (non glandular)	24	3430	7.22	125000	19.1	72	n.c.	NA
Testis	8	1120	2.36	77000	11.8	168	n.c.	NA
Thymus	8	1400	2.95	37800	5.81	168	n.c.	NA
Thyroid gland	8	1280	2.69	44300	6.80	168	n.c.	NA
Tongue	8	2250	4.75	47100	7.24	168	n.c.	NA
Tongue (mucous gland)	8	2670	5.63	69700	10.8	168	n.c.	NA
Tooth (pulp)	8	1340	2.82	26800	4.11	72	12.2	8-72

Additional information: *: normalized to blood value, T1/2: elimination half-life, NA: not applicable, n.c.: not calculated due to the limited data set

Conclusion: Following 7 daily oral doses of nominal 3 mg/kg [^{14}C]BAF312, the distribution pattern of total radiolabeled components showed:

A specific distribution pattern in the brain and spinal cord at 8, 24, 72 and 168 h after the last dose with a higher distribution into the (spinal cord) white matter compared to the (spinal cord) grey matter.

Extravascular distribution throughout the body in most tissues; the highest levels in the adrenal (cortex), kidney (CMJ, cortex), liver, and pancreas; highest exposure in adrenal (cortex), kidney (cortex), liver, preputial gland, spinal cord (white matter), and stomach (non glandular) being 19.1-92.5 times higher than in blood.

At 168 h after the 7th dose, radioactivity was still observed in most tissues, amounting in total to ~1% of all administered seven doses.

2.6.5.6 薬物動態試験：たん白結合

2.6.5.6A Pharmacokinetics: Plasma Protein Binding (DMPK R0400881-01, DMPK R1300902-01)

Study title:	*In vitro* blood distribution and plasma protein binding of [^{14}C]BAF312 including stability in blood and plasma for rat, dog, cynomolgus monkey and human and binding to individual human plasma proteins *In vitro* blood distribution and plasma protein binding of [^{14}C]BAF312 including stability in blood and plasma of mouse and rabbit	Study no. DMPK R0400881-01 Study no. DMPK R1300902-01
Location in CTD	4.2.2.3-2, 4.2.2.3-3	
GLP compliance:	not required	
Test article(s):	[^{14}C]BAF312 and BAF312	
Species / sex:	Human, dog (Beagle), cynomolgus monkey, rat (Hanover Wistar) / male (DMPK R0400881-01); mouse (CD-1) / male, rabbit (New Zealand) / female) (DMPK R1300902-01)	
Study system:	Fresh blood and defrosted plasma from all species; pools of n = 3 (heparinized (DMPK R0400881-01) or in K_3-EDTA (DMPK R1300902-01)) Solutions of isolated human plasma in phosphate buffered saline (PBS): human serum albumin (HSA), α1-acid glycoprotein, γ-globulins, high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins VLDL) (DMPK R0400881-01)	
Radionuclide:	^{14}C	
Method:	*Stability in plasma and blood*: Incubation in plasma for 0, 2, 6 and 24 h (DMPK R0400881-01) or for 0, 0.5, 1, 3, 7, and 24 h (DMPK R1300902-01), and in blood for 1, 3, and 6 h (DMPK R0400881-01) or for 0.5, 1, 3, and 7 h (DMPK R1300902-01) at 37°C. Detection technique: liquid chromatography-tandem mass spectrometry (LC-MS/MS). *In vitro blood distribution*: Incubation at 37°C for 30 min after spiking; fraction in plasma (fp) obtained after cell separation by centrifugation (10 min, 1500 g, 37°C). *In vitro blood distribution kinetic*: 0, 0.5, 1 and 2 h (measured in dog blood at 100 ng/mL) (DMPK R0400881-01). *Temperature dependency*: 4°C, room temperature (22°C) and 37°C (measured in human blood at 10 and 1000 ng/mL) (DMPK R0400881-01) *In vitro plasma protein binding*: bound fraction (fb) investigation by equilibrium gel filtration. Detection technique for blood distribution, temperature dependency and plasma protein binding: Liquid scintillation counting (LSC).	
Analyte / Assay:	Radioactivity detection by LSC Parent compound detection by LC-MS/MS (Lower limit of quantification: 5.0 ng/mL using plasma sample (DMPK R0400881-01))	

BAF312 stability in plasma (in ng/mL; one sample per time point) (DMPK R0400881-01)

Nominal concentrations: 100 ng/mL for dog, rat, and monkey; 100 ng/mL and 1000 ng/mL for human

Species	Human	Dog	Dog (2nd set)	Rat	Monkey	Human (1000 ng/mL)
Incubation time (h)						

0	163	0.00 [a]	127	82.6	97.9	1380
2	158	0.00 [a]	128	80.6	105	1560
6	157	0.00 [a]	134	72.9	108	1570
24	148	0.00 [a]	119	81.4	108	1550

Additional information: a: value confirmed by reanalysis

BAF312 stability in plasma (in %; one sample per time point, 3 samples for time point 0 h) (DMPK R1300902-01)

Species	Mouse		Rabbit	
	Individual recovery (%) [a]	Recovery (%)	Individual recovery (%) [a]	Recovery (%)
Incubation time (h)				
0	11.9		11.4	
	12.2 (SD: 2.29)	-	10.5 (SD: 0.458)	-
	16.0		10.8	
0.5	11.9	89.2	12.4	114
1	12.0	89.7	11.6	107
3	11.6	87.2	11.7	108
7	12.4	92.9	11.6	107
24	11.0	82.3	13.4	124

Additional information: a: Individual recovery = chromatographic peak area ratios of BAF312 and [D_{11}]BAF312 (Internal Standard (ISTD)). The 0 h time point mean BAF312 / ISTD ratio was set to 100% recovery. Results of the subsequent time points were calculated relative to the mean 0 h time point and presented as recovery in %.

BAF312 stability in blood (in ng/mL; one sample per time point) (DMPK R0400881-01)

Nominal concentrations: 100 ng/mL for dog, rat, and monkey; 100 ng/mL and 1000 ng/mL for human

Species	Human	Dog	Dog (2^{nd} set)	Rat	Monkey	Human (1000 ng/mL)
Incubation time (h)						
0	n.s.	n.s.	344	n.s.	n.s.	n.s.
1	184	123	343	64.6	0.00 [a]	1430
3	182	98.8	467	58.5	114	1780
6	210	97.1	389	54.9	120	1760

Additional information: a: value confirmed by reanalysis, n.s: no sample

BAF312 stability in blood (in %); one sample per time point, 3 samples for time point 0 h) (DMPK R1300902-01)

Species	Mouse		Rabbit	
	Individual recovery (%) [a]	Recovery (%)	Individual recovery (%) [a]	Recovery (%)
Incubation time (h)				
0	11.3		10.4	
	12.0 (SD: 0.907)	-	10.3 (SD: 0.465)	-
	13.1		9.55	
0.5	11.3	92.8	9.24	91.7
1	13.4	111	10.2	101
3	12.0	98.8	10.3	102
7	11.5	94.8	9.12	90.5

Additional information: a: Individual recovery = chromatographic peak area ratios of BAF312 and [D_{11}]BAF312 (Internal Standard (ISTD)). The 0 h time point mean BAF312 / ISTD ratio was set to 100% recovery. Results of the subsequent time points were calculated relative to the mean 0 h time point and presented as recovery in %.

***In vitro* blood distribution of [^{14}C]BAF312 for rat, dog, monkey, human (DMPK R0400881-01) , mouse, and rabbit (DMPK R1300902-01)**

Species	Nominal concentration (ng/mL)	Actual concentration		Fraction in plasma (fp) (Mean ± SD) (n=3) (%)	Ratio Cb/Cp (Mean ± SD) (n=3)	Ratio Cbc/Cp (Mean ± SD) (n=3)
		Blood (ng/mL)	Plasma (ng/mL)			
Rat (H = 0.42)	10000	8993	7492	48 ± 0.2	1.2 ± 0.01	1.48 ± 0.01
	1000	954	788	48 ± 0.4	1.21 ± 0.01	1.50 ± 0.02
	100	99	81	48 ± 1	1.21 ± 0.02	1.50 ± 0.04
	10	10.1	8.3	48 ± 1	1.21 ± 0.02	1.50 ± 0.04
Dog (H = 0.48)	10000	9205	12313	70 ± 1	0.75 ± 0.01	0.47 ± 0.02
	1000	980	1313	70 ± 0.3	0.75 ± 0.004	0.47 ± 0.01
	100	100	135	71 ± 1	0.74 ± 0.01	0.45 ± 0.02
	10	9.6	13.6	73 ± 1	0.71 ± 0.01	0.40 ± 0.03
Monkey	10000	8661	8849	57 ± 1	0.98 ± 0.02	0.95 ± 0.05

(H = 0.44)	1000	977	975	56 ± 2	1.00 ± 0.03	1.00 ± 0.06
	100	99	99	56 ± 2	1.00 ± 0.03	1.01 ± 0.06
	10	10.3	9.92	54 ± 2	1.04 ± 0.04	1.09 ± 0.10
Human (H = 0.48)	10000	8866	11664	68 ± 2	0.76 ± 0.02	0.50 ± 0.05
	1000	1035	1342	67 ± 1	0.77 ± 0.01	0.52 ± 0.01
	100	99	132	69 ± 1	0.75 ± 0.01	0.49 ± 0.03
	10	11.5	14.8	67 ± 2	0.78 ± 0.03	0.54 ± 0.06
Mouse (H = 0.36)	70000	69300 ± 1260	62600 ± 2470	57.8 ± 2.51	1.11 ± 0.0482	1.30 ± 0.134
	10000	9370 ± 125	8860 ± 184	60.5 ± 1.49	1.06 ± 0.0262	1.16 ± 0.0728
	1000	937 ± 53.1	893 ± 3.11	61.0 ± 3.46	1.05 ± 0.0596	1.14 ± 0.166
	100	92.6 ± 3.62	90.6 ± 0.939	62.2 ± 2.53	1.02 ± 0.0413	1.06 ± 0.115
	10	11.8 ± 0.490	10.9 ± 0.341	59.1 ± 3.07	1.08 ± 0.0562	1.22 ± 0.156
Rabbit (H = 0.35)	70000	72600 ± 987	64200 ± 1610	57.5 ± 1.64	1.13 ± 0.0322	1.37 ± 0.0920
	10000	9410 ± 90.7	8460 ± 57.7	58.4 ± 0.690	1.11 ± 0.0131	1.32 ± 0.0374
	1000	921 ± 22.4	839 ± 12.0	59.2 ± 1.67	1.10 ± 0.0310	1.28 ± 0.0886
	100	99.4 ± 0.544	89.1 ± 2.14	58.3 ± 1.44	1.12 ± 0.0276	1.33 ± 0.0789
	10	9.36 ± 0.265	8.66 ± 0.686	60.1 ± 5.06	1.08 ± 0.0909	1.23 ± 0.262

Additional information: H: Hematocrit; Cb: Concentration in blood; Cbc: Concentration in blood cells; Cp: Concentration in plasma

***In vitro* plasma protein binding of [^{14}C]BAF312 for rat, dog, monkey, human (DMPK R0400881-01), mouse, and rabbit (DMPK R1300902-01) measured by equilibrium gel filtration**

Species	Nominal free concentration (ng/mL)	Actual free concentration (ng/mL)	Bound / mL plasma (ng)	Fraction bound (fb) (%)	fb (mean ± SD) (%)	Fraction unbound (fu) (%)	fu (mean) (%)
Dog	4	2.90	43342	99.993	99.991 ± 0.002	0.007	0.009
		2.96	30964	99.990		0.010	
		2.53	25428	99.990		0.010	
	40	9.19	187359	99.995	99.991 ± 0.003	0.005	0.009
		9.40	87681	99.989		0.011	
		7.29	80349	99.991		0.009	
		6.71	55979	99.988		0.012	

Human	4	3.21	21036	99.985	99.985 ± 0.003	0.015	0.015
		1.94	15273	99.987		0.013	
		2.25	12355	99.982		0.018	
	40	12.08	71063	99.983	99.980 ± 0.002	0.017	0.020
		8.00	40162	99.980		0.020	
		7.62	37295	99.980		0.020	
		6.03	27519	99.978		0.022	
Rat	4	3.18	15833	99.980	99.979 ± 0.001	0.020	0.021
		2.55	12835	99.980		0.020	
		2.17	9808	99.978		0.022	
	40	11.09	48565	99.977	99.970 ± 0.006	0.023	0.030
		7.61	23395	99.967		0.033	
		7.21	20691	99.965		0.035	
Cynomolgus monkey	4	3.09	12842	99.976	99.975 ± 0.002	0.024	0.025
		2.58	10388	99.975		0.025	
		2.14	7895	99.973		0.027	
	40	9.90	37202	99.973	99.971 ± 0.002	0.027	0.029
		7.15	25120	99.972		0.028	
		6.96	22652	99.969		0.031	
Mouse	1	0.212	1490	100	100 ± 0.00333	0.0142	0.0143
		0.0668	377	100		0.0177	
		0.0275	249	100		0.0110	
	10	1.61	10600	100	100 ± 0.00215	0.0151	0.0150
		0.401	2360	100		0.0170	
		0.224	1760	100		0.0127	
	100	10.4	64000	100	100 ± 0.00179	0.0162	0.0148
		2.87	18500	100		0.0155	
		1.95	15200	100		0.0128	
Rabbit	1	0.107	280	100	100 ± 0.00767	0.0381	0.0316
		0.0389	116	100		0.0335	

	0.0223	96.3	100		0.0231	
10	0.661	1740	100	100 ± 0.00497	0.0380	0.0332
	0.259	777	100		0.0334	
	0.194	690	100		0.0281	
100	44.4	11900	100	100 ± 0.00507	0.0375	0.0320
	2.12	6800	100		0.0312	
	1.79	6510	100		0.0274	

***In vitro* plasma protein binding of [^{14}C]BAF312 for rat, dog, monkey, and human measured by equilibrium gel filtration on a protein separating gel filtration column (DMPK R0400881-01)**

Species	Nominal free concentration (ng/mL)	Actual free concentration (ng/mL)	Bound / mL plasma (ng)	Fraction bound (fb) (%)	Fraction unbound (fu) (%)
Dog	4	2.2	30692	99.993	0.007
		3.2	27622	99.988	0.012
Human	4	2.8	22595	99.988	0.012
		3.4	31505	99.989	0.011
Rat	4	1.7	7998	99.978	0.022
		4.2	17879	99.976	0.024
Cynomolgus monkey	4	2.6	12801	99.980	0.020
		3.6	17955	99.980	0.020

***In vitro* protein binding of [^{14}C]BAF312 to human plasma proteins measured by equilibrium gel filtration on a protein separating gel filtration column (DMPK R0400881-01)**

Protein	Nominal free concentration (ng/mL)	Amount of protein loaded (µg)	Physiological plasma concentration (mg/mL)	Actual free concentration (ng/mL)	Bound / mL plasma	Fraction bound (fb) (%)	Fraction unbound (fu) (%)
HSA	4	2000	40	3.9	5015	99.92	0.08
				2.9	2855	99.90	0.10
AGP	4	50	1	2.9	1340	99.79	0.21

γ-globulin	4	500	12	5.2	52	90.93	9.07
HDL	4	50	3.9	5.7	12854	99.96	0.04
LDL	4	125	3.6	4.7	7068	99.93	0.07
VLDL	4	125	1.3	3.8	3594	99.89	0.11

Conclusion:	No relevant degradation of BAF312 was observed after incubation at 37°C in blood of all investigated species (rat, dog, money, human for up to 6h (DMPK R0400881-01), mouse, rabbit for up to 7 h (DMPK R1300902-01)). No instability of BAF312 was observed after incubation at 37°C in plasma of rat, dog, monkey, human up to 24 h (DMPK R0400881-01) and in plasma of mouse and rabbit up to 7 h (DMPK R1300902-01). The *in vitro* blood distribution kinetic of [^{14}C]-BAF312 for dog at 37°C and temperature dependency on the *in vitro* blood distribution of [^{14}C]-BAF312 for human at 4°C, 22°C and 37°C showed that for both species equilibrium was already reached at 30 min at 37°C (DMPK R0400881-01). In the concentration range of 10-10000 ng/mL [^{14}C]-BAF312 no concentration dependency of blood distribution was seen for all investigated species (rat, dog, human, monkey, rabbit and mouse. Blood distribution was species dependent showing the following fractions in plasma (fp): rat (48%) < monkey (56%) ≤ rabbit (59%) ≈ mouse (60%) < human (68%) ≈ dog (71%) (DMPK R0400881-01), (DMPK R1300902-01). Plasma protein binding was very high for all investigated species (>99%) with mean fraction unbound (fu) showing up to 3-fold differences between species: rabbit (0.0323 ± 0.00590%) ≈ rat (0.03 ± 0.01%) ≈ monkey (0.03 ± 0.002%) ≥ human (0.02 ± 0.003%) > dog (0.01 ± 0.002%) ≈ mouse (0.0147 ± 0.00242%) (DMPK R0400881-01), (DMPK R1300902-01). [^{14}C]-BAF312 was very highly bound to human plasma lipoproteins (HDL, LDL, VLDL) and to human serum albumin. The contribution of lipoproteins to the total binding in human plasma was larger compared to the contribution of albumin (DMPK R0400881-01).

2.6.5.6B Pharmacokinetics: Plasma Protein Binding (DMPK R1500677-01)

Study title:	*In vitro* plasma protein binding of [^{14}C]LYS815 in plasma of mouse, rat, and human	Study no. DMPK R1500677-01, DMPK R1500677a

Location in CTD	4.2.2.3-4
GLP compliance:	not required
Test article(s):	[^{14}C]LYS815
Species / sex:	Mouse (CD-1) / male, rat (Hanover Wistar) /male, human / male
Study system:	Defrosted plasma from respective species; pools of n ≥ 3. K3-EDTA was used as an anticoagulant.
Radionuclide:	^{14}C
Method:	*In vitro plasma protein binding*: unbound fraction (fu) investigation by equilibrium gel filtration. Detection technique: Liquid scintillation counting (LSC).
Analyte / Assay:	Radioactivity detection by LSC

***In vitro* plasma protein binding of [^{14}C]LYS815 for mouse, rat, and human (n = 3)**

Species	Nominal free concentration (ng/mL)	Actual concentration		Fraction bound (fb) (%)	Fraction unbound (fu) (%)	fb Mean (%)	fu Mean (%)	fb and fu SD (%)
		Free concentration (ng/mL)	Bound concentration (ng/mL)					
Mouse	2	0.105	347	99.97	0.0303	99.96	0.0409	0.00928
		0.0452	95.9	99.95	0.0471			
		0.0321	70.7	99.95	0.0454			
	10	0.0729	423	99.98	0.0173	99.98	0.0201	0.00246
		0.0608	289	99.98	0.0211			
		0.0644	295	99.98	0.0219			
	200	0.760	4330	99.98	0.0175	99.98	0.0155	0.00179
		0.346	2400	99.99	0.0144			
		0.378	2620	99.99	0.0144			
Rat	2	0.0726	119	99.94	0.0607	99.93	0.0746	0.0152

		0.0445	49.0	99.91	0.0908			
		0.0287	39.7	99.93	0.0721			
	10	0.0590	184	99.97	0.0320	99.97	0.0336	0.00136
		0.0524	151	99.97	0.0346			
		0.0540	158	99.97	0.0341			
	200	0.391	1620	99.98	0.0241	99.98	0.0237	0.00157
		0.340	1550	99.98	0.0219			
		0.343	1380	99.98	0.0250			
Human	2	0.0527	82.8	99.94	0.0636	99.92	0.0791	0.0136
		0.0320	44.6	99.92	0.0845			
		0.0337	37.8	99.91	0.0891			
	10	0.0587	165	99.96	0.0356	99.97	0.0346	0.000848
		0.0562	165	99.97	0.0341			
		0.0612	180	99.97	0.0341			
	200	0.342	1490	99.98	0.0229	99.98	0.0229	0.00117
		0.355	1640	99.98	0.0217			
		0.351	1460	99.98	0.0240			

Additional information:	[^{14}C]LYS815 was found to be stable in Dulbecco's Phosphate Buffered Saline (DPBS) at 37°C up to 24 h (latest investigated time point) [DMPK R1500677a]. This indicates that no stability issues could have influenced the plasma protein binding determination.
Conclusion:	[^{14}C]LYS815 was highly bound to plasma proteins (>99.9%) across investigated species. Within the concentration range of 2 - 200 ng/mL, a slight concentration dependency was observed at the lowest nominal free concentration (2 mg/mL) in all species, which consistently showed a higher fu (up to 3.5-fold). Mean fu for mice showed a tendency to be about 1.5 to 1.9 fold lower than for other species within the tested concentration range: fu human (0.0455 ± 0.0266%) ≈ fu rat (0.0439 ± 0.0246%) > fu mouse (0.0255 ± 0.0127%). However, given the unusually high adsorption of [^{14}C]LYS815 to the EGF system in the experiments performed (i.e. HPLC recoveries mostly <1%), a bias in the data cannot be ruled out, and therefore results reported need to be carefully evaluated and considered with caution.

2.6.5.7 薬物動態試験：妊娠動物における試験

2.6.5.7A Pharmacokinetics: Study in Pregnant or Nursing Animals (DMPK R1300583)

Study title:	Galactogenic transfer, kinetics and metabolism in milk and plasma after a single oral dose (10 mg base/kg) administration of [^{14}C]BAF312 hemifumarate in rats	Study no. DMPK R1300583, DMPK R1300583a
Location in CTD	4.2.2.3-5	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312 hemifumarate	
Species / strain:	Rat (lactating) /HAN:WIST	
Gender (M/F) / number of animals:	F / 8 (2 groups: 4 dams per group)	
Feeding condition:	Water and food available *ad libitum*	
Vehicle / formulation:	0.5% aqueous hydroxypropylcellulose solution, suspenson	
Method/ route / duration of administration:	p.o. (gavage) / single dose	
Dose (mg/kg):	10 mg/kg	
Day of dosing:	Day 11 after parturition	
Radionuclide:	^{14}C	
Analyte/assay (unit):	^{14}C in plasma and milk: Liquid scintillation counting (LSC) for plasma and milk BAF312 in plasma and milk: Liquid chromatography-mass spectrometry (LC-MS), LLOQ: 1.00 ng/mL	
Sampling time(s):	Serial collection: Group 1: 0.25, 3, 24, 72 h post dose, Group 2: predose, 1, 8, 48 h post dose	
Samples:	Plasma, milk (n=4 / timepoint)	

Pharmacokinetics in plasma and milk after single p.o. dose

Matrix	Blood	Plasma		Milk	
Parameter	Total radiolabeled components	Total radiolabeled components	BAF312 [a]	Total radiolabeled components	BAF312
Actual oral dose (mg/kg)	9.71 ± 0.138				

Cmax (ng/mL or ngEq/mL)	2220	2310	2580	1150	1050
Tmax (h)	8	8	8	8	8
Tlast (h)	72	72	72	72	72
AUClast (ng•h/mL or ngEq•h/mL)	87800	89400	94500	50500	46500
AUCinf (ng•h/mL or ngEq•h/mL)	107000	106000	113000	63500	not determined
T1/2 (h)	28.7	26.8	28.1	27.8	27.8
T1/2 (range)[b] (h)	24-72	24-72	24-72	24-72	24-72
Concentration ratio milk/plasma				0.305 – 0.745	0.090 – 0.614
AUClast milk/plasma ratio				0.565	0.492

Additional information: a: Bioanalytical data used; b: range over all time points;
The study design included determination of the metabolic profiles in plasma and milk

Conclusion: Following p.o. administration of 10 mg/kg [^{14}C]BAF312 to female rats, BAF312 reached approximately half lower concentrations in the milk compared with plasma.

In plasma and milk, the main radiolabeled component BAF312 contributed to 95.3% and 89.8% to the total ^{14}C-AUC0-72h, respectively. Other radiolabeled components were of minor importance.

In plasma, Cmax of total radiolabeled components (2310 ngEq/mL) and BAF312 (2580 ng/mL) were observed at 8 h post dose. Thereafter, the concentrations of total radiolabeled components declined with a half-life of 26.8 h, and the BAF312 concentration declined with a similar half-life of 28.1 h. The concentration of total radiolabeled components at 72 h was 18.8% and the BAF312 concentration was 18.1% compared to their respective Cmax value.

In milk, Cmax of total radiolabeled components (1150 ngEq/mL) and BAF312 (1050 ng/mL) were observed at 8 h post dose. Thereafter, the concentration of total radiolabeled components declined with an apparent half-life of 27.8 h. The concentration of total radiolabeled components at 72 h was 28.1% and the BAF312 concentration at 72 h was 27.1% compared to their respective Cmax at 8 h.

2.6.5.8 薬物動態試験：その他の分布試験

2.6.5.8A Pharmacokinetics: Other Distribution Study (DMPK R1300375)

Study title:	Brain disposition in rats after multiple oral (3 mg/kg/day) administration of BAF312	Study no. DMPK R1300375, DMPK R1300375a
Location in CTD	4.2.2.3-6	
GLP compliance:	not required	
Test article:	BAF312	
Species / Strain:	Rat / Wistar (Han:WIST), albino	
Gender (M/F) / Number of animals:	M / 10 (8 h: n=2; 24 h: n=2; 72 h: n=3; 168 h: n=3)	
Feeding condition:	Water and food available *ad libitum*	
Vehicle /formulation:	Ethanol/PEG200/5% aqueous glucose solution (1/6/3) (w/w/w)	
Dose:	3 mg/kg/day (referring to the free base) for 7 days (once daily)	
Route:	Oral (gavage)	
Sampling time(s):	8, 24, 72 and 168 h post dosing on the last dosing day	
Samples collected:	Blood, brain, cerebrospinal fluid (CSF)	
Analyte / Assay:	BAF312 / Detection of parent compound by liquid chromatogram-mass spectrometry (LC-MS) (LLOQ: plasma, Cerebrospinal fluid: 1 ng/mL, brain: 1 ng/g)	

Time point (h)	Rat No.	Plasma (ng/mL)	Plasma (mean)	Brain (ng/g)	Brain (mean)	CSF (ng/mL)	CSF (mean)
8	1	278	356	1210	1190	5.39	3.44
8	2	433		1170		1.49	
24	3	58.7	36.8	812	598	0	1.01
24	4	14.8		383		2.01	
72	5	0	0	19.9	18.1	0	0
72	6	0		15.6		0	
72	7	0		18.9		0	
168	8	0	0	0	0	0	0
168	9	0		0		No sample	

168	10	0	0	0
Additional information:	LLOQ: 1 ng/mL (only determined in plasma. Therefore LLOQ of 1 ng/g was considered for brain). Values below LLOQ were set to 0.			
Conclusion:	Following 7 daily oral doses of 3 mg/kg BAF312 to male Wistar rats, BAF312 was detected in plasma and CSF up to 24 h, and in brain up to 72 h, with Tmax reached at 8 h in all matrices. Therefore, BAF312 was proven to reach the brain. The distinctly lower concentrations of BAF312 in the CSF (considered a surrogate for the free concentration in the brain) indicated that BAF312 was mainly bound in the brain.			

2.6.5.9 薬物動態試験：*in vivo* における代謝

2.6.5.9A Pharmacokinetics: Metabolism *In vivo* - Mouse (DMPK R1300411-02)

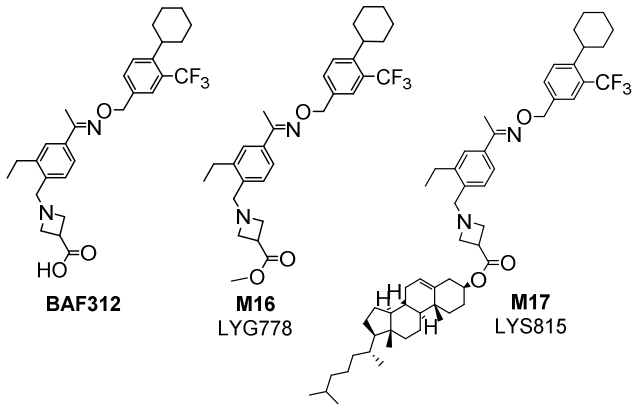
Study title:	Pharmacokinetics, distribution, metabolism and excretion in mice after oral administration of [^{14}C]BAF312	Study No. DMPK R1300411-02
Location in CTD:	4.2.2.2-1	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312 hemifumarate	
Species / Strain:	Mouse / CD-1	
Gender(M/F)	M	
Number of animals:	3 per time point	
Feeding condition:	Water and food *ad libitum*	
Vehicle / Formulation	Suspension in aqueous solution of 0.5% Hydroxypropylcellulose	
Route of dosing:	Oral (gavage) / single dose	
Dose (mg/kg):	8 (referring to free base; group 1) 25 (referring to free base; group 4), see further below	
Analyte /Radionuclide:	Radioactivity / ^{14}C	
Samples:	Plasma / Brain: 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 168 h; urine, feces (daily up to 96 h and 96-168 h)	
Assay:	Radioactivity: Liquid scintillation counting (LSC) BAF312: Liquid chromatography – mass spectrometry (LC-MS)	

[^{14}C]BAF312 and metabolites in mouse plasma and brain

	Plasma (pools of n=3 mice/time point)				Brain (pools of n=3 mice/time point)			
	Tmax	Cmax	AUC0-168 h		Tmax	Cmax	AUC0-168 h	
Metabolite	h	ngEq/mL	ngEq•h/mL	%	h	ngEq/g	ngEq•h/g	%
BAF312 (parent compound)	8	1840	60400	38.0	8	5060	238000	77.0
M16 (LYG778)	48	161	18800	11.8	-	-	-	-
M17 (LYS815)	48	381	43400	27.3	168	410	14800	4.79

P7.5	8	21.4	1390	0.874	-	-	-	-
P7.6	-	-	-	-	8	48.7	3920	1.27
P8.2	24	39.8	796	0.499	-	-	-	-
P8.5	24	41.5	832	0.522	-	-	-	-
P9.1	48	11.6	1160	0.729	-	-	-	-
P12.3	48	43.0	3740	2.35	-	-	-	-
P12.5	-	-	-	-	168	53.2	1930	0.624
Sum of others	24	20.1	399	0.251	8	69.2	3560	1.16
Total components detected	8	2000	131000	82.3	8	5170	261000	84.9
Lost during sample processing	48	207	17000	10.7	8	339	23600	7.66
Lost during HPLC	8	172	11200	7.06	8	457	23000	7.48
Total radiolabeled components in original sample	8	2270	159000	100	8	5990	308000	100

- not detected

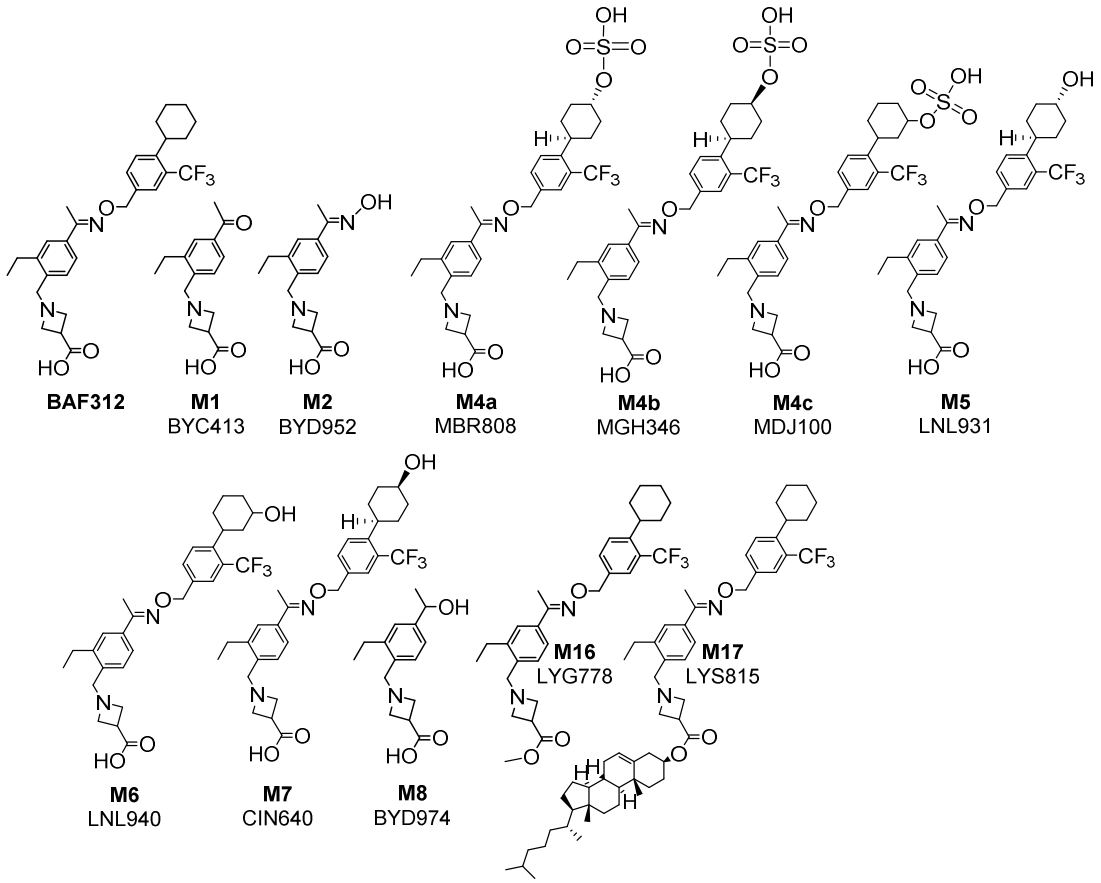


[^{14}C]BAF312 and metabolites in mouse urine and feces (amount expressed as % of dose)

Sample type	Urine supernatant S1 (extraction with ACN/H_2O) 0-168 h	Urine supernatant S2 (extraction with DMSO/ACN) 0-168 h	Feces 0-168 h

Metabolite			
BAF312 (Parent compound)	0.0372	-	21.5
M1 (BYC413)	0.183	-	-
M2 (BYD952)	0.367	-	-
M4a (MBR808)	-	-	0.995
M4b (MGH346)	-	-	0.480
M4c (MDJ100)	-	-	4.88
M5 (LNL931)	-	-	28.7
M6 (LNL940)	-	-	7.62
M7 (CIN640)	-	-	1.23
M8 (BYD974)	0.147	-	-
M16 (LYG778)	-	-	1.19
M17 (LYS815)	-	0.862	-
P0.5	-	0.024	-
P0.9	-	0.0136	-
P11.5	0.0127	-	-
P12.4	-	0.028	-
P14.5	0.00955	-	-
P15.8	-	-	0.705
P19.6	0.00907	-	-
P52.4	-	-	1.06
P55.5	-	-	0.789
Sum of others	0.0372	0.0150	0.649
Total components detected	0.803	0.943	69.8
Lost during sample processing	0.137		-
Lost during HPLC	0.0414	0.0865	5.09
Radioactivity in pool analyzed	2.01		74.9

- not detected



Conclusions:

After a single oral dose administration of 8 mg/kg [^{14}C]BAF312 to male CD-1 mice, the following conclusions were drawn:

Phase I and Phase II biotransformations of BAF312 in the mouse, based on proposed chemical structures of metabolites detected in plasma, urine and feces are summarized as follows:

Direct methylation of BAF312, forming a methyl ester conjugate M16 (LYG778)

Direct esterification of BAF312 with cholesterol, forming metabolite M17 (LYS815),

The phase I metabolic reactions involved C-hydroxylations (M5 – LNL931, M6 – LNL940, M7 -CIN640), cleavage/hydrolysis of the oxime ether (M1 – BYC413, M2 –BYD952) and further reduction (M8 (BYD974)),

One additional phase II metabolic reaction was observed (M4a, M4b and M4c) which corresponds to sulfation of hydroxylated metabolites (M5, M6, and M7).

In plasma, unchanged [^{14}C] BAF312 was the most abundant drug related component following a single oral administration of 8 mg/kg [^{14}C] BAF312 hemifumarate (dose referring to the base), representing 38.0% of total [^{14}C] AUC0-168h. In addition to the parent compound two lipophilic metabolites, a methyl ester conjugate M16 (LYG778) and a cholesterol ester conjugate M17 (LYS815) were identified as major components in the circulation and represented 11.8% and 27.3% of the [^{14}C]AUC0-168h, respectively. A number of minor unidentified components were observed (P7.5, P8.2, P8.5, P9.1 and P12.3). Each represented less than 1% of the [^{14}C] AUC0-168h, with the exception of P12.3 which represented 2.35%.

The major drug related components in feces were M5 (LNL931), BAF312, M4 (consisting of M4a, M4b, M4c) and M6 (LNL940), which together accounted for 64.2% of the administered dose Metabolites M7 (CIN640), M16 (LYG778) and unidentified peaks P15.8, P52.4 and P55.5 were all minor components, with each representing ≤1.23% of the administered dose. In urine, various [^{14}C]-labeled components were detected. BAF312, M1 (BYC413), M2 (BYD952), M8 (BYD974) and M17 (LYS815) were identified and ranged in abundance between 0.147 and 0.862% of the administered dose.

Unchanged BAF312 was the major drug related component identified in mouse brain, and accounted for 77% of the total [^{14}C] AUC0-168h after a single p.o. dose of 8 mg/kg. M17 (LYS815) was also identified, but at the last time point (168 h) only. In total, this metabolite represented 4.79% of the total [^{14}C] AUC0-168h. In Group 4 (see further below), where mice were dosed 25 mg/kg p.o., BAF312 accounted for 94.7% of total [^{14}C] AUC0-168h in brain of mice. M17 (LYS815) was again detected, in this case at 96 and 168 h post-dose, and represented 0.632% of the total [^{14}C]AUC0-168h. Given the low abundance of M17, particularly in the second dose group, a contribution from blood contamination to the AUC0-168h of this metabolite cannot be ruled out.

In a second dose group (see further below), where mice were dosed with a single p.o. dose of 25 mg/kg, the results showed BAF312 as the major component in plasma of mice, representing 66.1% of the [^{14}C] AUC0-168h. Metabolite M17 (LYS815) was also a significant metabolite, representing 23.3%. In this additional dose group, metabolite M16 was found to represent only 1.01% of the [^{14}C] AUC0-168h.

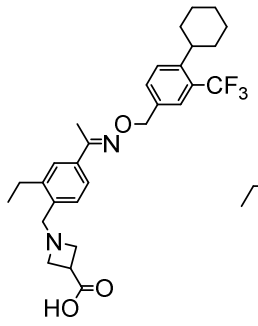
In liver, spleen, lacrimal gland and kidney, obtained from Group 4 dosed at 25 mg/kg at three time points (8, 96 and 168 h), the profiles were dominated by BAF312 and M17 (LYS815) (see further below). The percentage of [^{14}C] AUC0-168h for BAF312 ranged from 2.14% in lacrimal gland to 72.4% in the spleen, whereas for M17 (LYS815) the [^{14}C]AUC0-168h ranged from 25.5% in the spleen to 80.2% in the lacrimal gland.

Pharmacokinetics: Metabolism *In vivo* - Mouse (DMPK R1300411-02)

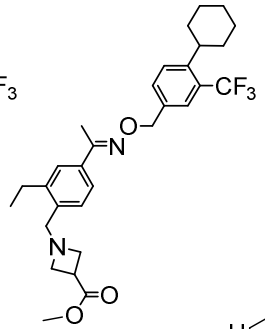
Table	Pharmacokinetics, distribution, metabolism and excretion in mice after oral administration of [^{14}C]BAF312	Study No. DMPK R1300411-02
Location in CTD:	4.2.2.2-1	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312 hemifumarate	
Species / Strain:	Mouse / CD-1	
Gender(M/F)	M	
Number of animals:	3 per time point	
Feeding condition:	Water and food *ad libitum*	
Vehicle / Formulation	Suspension in aqueous solution of 0.5% Hydroxypropylcellulose	
Route of dosing:	Oral (gavage) / single dose	
Dose (mg/kg):	25 (referring to free base; group 4)	
Analyte /Radionuclide:	Radioactivity / ^{14}C	
Samples:	Plasma, liver, brain, spleen, lacrimal gland, kidney (8, 96, 168 h)	
Assay:	Radioactivity: Liquid scintillation counting (LSC) BAF312: Liquid chromatography – mass spectrometry (LC-MS)	

Sample type	Plasma				Liver				Brain			
	Tmax	Cmax	AUC0-168 h		Tmax	Cmax	AUC0-168 h		Tmax	Cmax	AUC0-168 h	
Metabolite	h	ngEq/mL	ngEq•h/mL	%	h	ngEq/g	ngEq•h/g	%	h	ngEq/g	ngEq•h/g	%
BAF312	8	5050	280000	66.1	8	65600	3560000	45.3	8	21000	1160000	94.7
M16 (LYG778)	8	40.5	4270	1.01	-	-	-	-	-	-	-	-
M17 (LYS815)	8	827	98700	23.3	96	36200	3980000	50.5	96	93.5	7750	0.632
P7.6	-	-	-	-	-	-	-	-	8	461	26700	2.18
P12.3	96	15.4	1230	0.291	-	-	-	-	-	-	-	-
P12.6	-	-	-	-	-	-	-	-	-	-	-	-
Sum of additional components	8	125	5940	1.41	8	2060	98700	1.26	8	320	15700	1.28
Total detected	8	6040	390000	92.1	8	78000	7650000	97.1	8	21700	1210000	98.8
Lost during sample processing	96	236	33500	7.90	96	2280	227000	2.88	96	136	14900	1.22
Lost during HPLC	-	-	-	-	-	-	-	-	-	-	-	-

Total radiolabeled components in original sample	8	6250	424000	100	8	78000	7850000	100	8	21700	1220000	100
Sample type	Spleen				Lacrimal gland				Kidney			
Metabolite	Tmax h	Cmax ngEq/g	AUC0-168 h ngEq•h/g	%	Tmax h	Cmax ngEq/g	AUC0-168 h ngEq•h/g	%	Tmax h	Cmax ngEq/g	AUC0-168 h ngEq•h/g	%
BAF312	8	12500	692000	72.4	8	10700	883000	2.14	8	50900	2770000	42.6
M16 (LYG778)	-	-	-	-	-	-	-	-	-	-	-	-
M17 (LYS815)	96	2090	245000	25.5	96	276000	33100000	80.2	8	24700	2500000	38.4
P7.6	-	-	-	-	-	-	-	-	-	-	-	-
P12.3	-	-	-	-	-	-	-	-	96	461	40300	0.620
P12.6	-	-	-	-	168	9350	816000	1.97	-	-	-	-
Sum of additional components	8	261	20100	2.10	96	5420	604000	1.47	8	4950	382000	5.88
Total detected	8	12900	961000	100	96	291000	35400000	85.7	8	80600	5680000	87.5
Lost during sample processing	-	-	-	-	168	4770	364000	0.883	8	8060	811000	12.5
Lost during HPLC	-	-	-	-	96	45400	5530000	13.4	-	-	-	-
Total radiolabeled components in original sample	8	12900	961000	100	96	336000	41300000	100	8	88300	6510000	100



BAF312



M16
LYG778

M17
LYS815

Conclusions:
(See summary above)

2.6.5.9B Pharmacokinetics: Metabolism *In vivo* - Rat (DMPK R0500017-02)

Study title:	Absorption, distribution, metabolism and excretion in male rats after single intravenous (1 mg/kg) and peroral (3 mg/kg) administration of [^{14}C]BAF312	Study No. DMPK R0500017-02
Location in CTD:	4.2.2.2-2	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Species /Strain:	Rat (HAN:WIST) albino	
Gender(M/F)	M	
Number of animals:	3	
Feeding condition:	Water and food ad libitum	
Vehicle / Formulation:	Intravenous and oral: Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)	
Route of dosing:	Intravenous bolus (into vena femoralis) / single dose Oral (gavage) / single dose	
Dose (mg/kg):	Intravenous: 1; oral: 3	
Analyte/Radionuclide:	Radioactivity / ^{14}C	
Sample times (h)	Plasma: 0.08 (i.v. only), 0.25 (p.o. only), 0.5, 2, 8, 24, 48 h; Urine and feces: daily up to 96h, 24-48 h,	
Samples:	Plasma (pools of N=3 per time point); urine and feces (pools of N=3 from 0 – 48 h)	

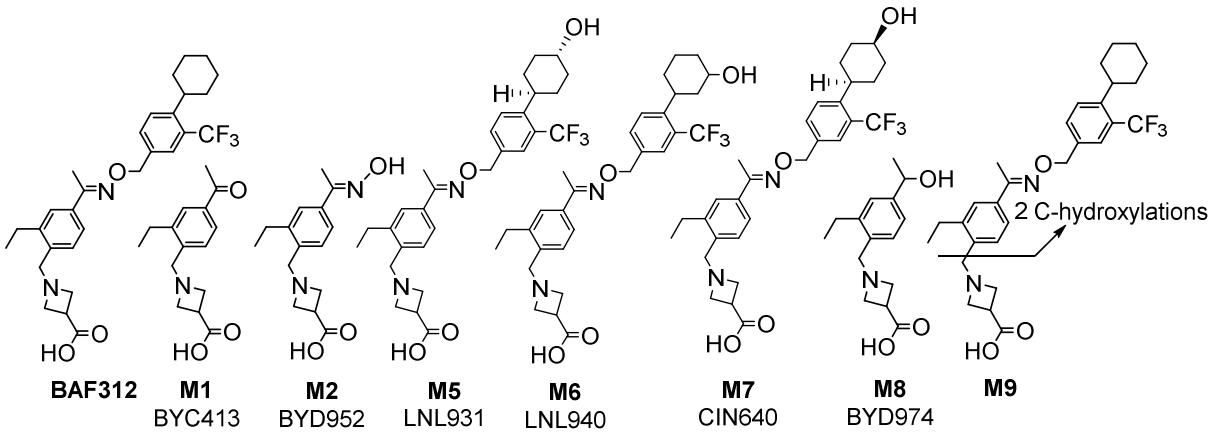
[^{14}C]BAF312 and metabolites in rat plasma [1)]

Dosing	1 mg/kg i.v.				3 mg/kg p.o.			
Metabolite	Tmax h	Cmax ngEq/mL	AUC0-48h ngEq•h/mL	%	Tmax h	Cmax ngEq/mL	AUC0-48h ngEq•h/mL	%
BAF312 (parent compound)	0.08	711	1820	65.9	4	240	3800	66.7
M1 (BYC413)	0.08	37.2	76.5	2.8	2	6.72	119	2.1
M2 (BYD952)	0.08	18.1	66.6	2.4	4	1.55	38.2	0.7
M5 (LNL931)	2	6.20	45.5	1.6	4	8.27	123	2.2
M6 (LNL940)	2	47.5	432	15.7	8	58.9	1010	17.7
M7 (CIN640)	0.08	1.55	3.62	0.1	4	1.03	10.3	0.2

M8 (BYD974)	0.08	2.07	33.6	1.2	2	3.62	102	1.8
M9	8	0.517	3.62	0.1	-	-	-	-
P53.4	4	0.517	17.0	0.6	24	0.517	11.9	0.2
P55.1	0.08	8.27	16.0	0.6	4	3.10	21.2	0.4
P56.8	2	1.55	17.0	0.6	8	3.10	80.6	1.4
Sum of others	0.08	27.9	31.5	1.1	8	6.20	95.6	1.7
Total recovered from HPLC	0.08	812	2560	92.9	4	307	5420	95.0
Lost during sample processing	0.08	25.3	207	7.1	8	12.4	310	5.0
Total radiolabeled components in sample	0.08	837	2740	100	8	320	5680	100

- not detected.

[1] Analyzed sample time points: 0.05 (i.v. only), 0.25 (p.o. only), 2, 4, 8, 24, 48 h.

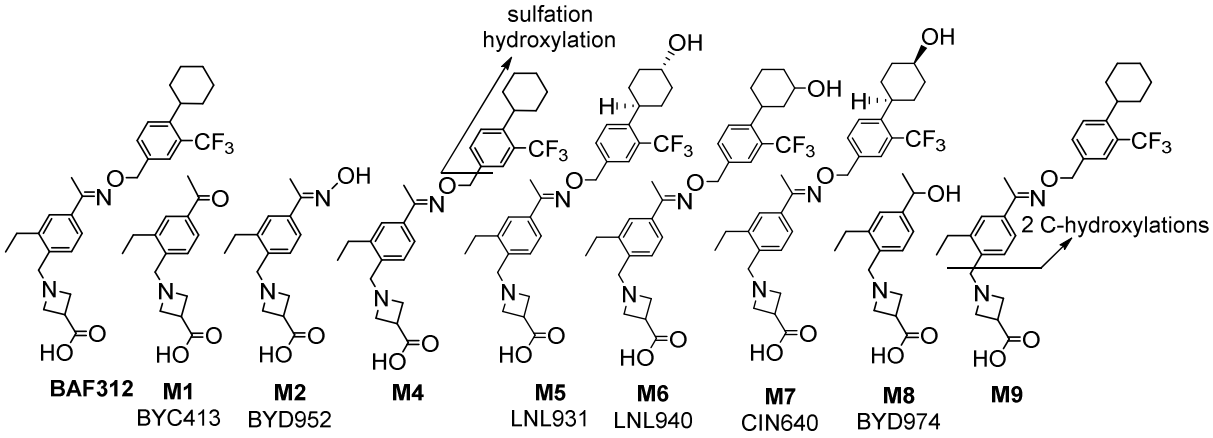


Note: The exact structures of metabolites M1, M2, M5, M6, M7 and M8 were retrospectively assigned by comparison with synthetic reference standards (Table 2.6.5.9E-DMPK R1000166-01, Table 2.6.5.2S- RD-2015-00908).

[^{14}C]BAF312 and metabolites in rat excreta (amount expressed as % of dose)		
Dosage	1 mg/kg i.v.	3 mg/kg p.o.

Metabolite	Urine (0-48 h)	Feces (0-48 h)	Urine (0-48 h)	Feces (0-48 h)
BAF312 (parent compound)	-	2.9	-	13.0
M1 (BYC413)	2.5	7.9	2.6	7.2
M2 (BYD952)	1.6	-	0.9	-
M4	-	28.7	-	26.4
M5 (LNL931)	-	7.3	-	5.2
M6 (LNL940)	-	26.9	-	24.9
M7 (CIN640)	-	1.0	-	1.0
M8 (BYD974)	2.0	3.1	2.6	2.7
M9	-	3.5	-	1.7
Sum of trace peaks	0.0	6.8	0.0	2.9
Total of peaks/recovered ^{14}C	6.1	88.1	6.1	85.3
Not recovered from sample processing	0.2	0.0	0.2	0.0
Total analyzed (pool 0-48 h)	6.3	88.1	6.3	85.3
Total excretion (time period 0-96 h)*	6.6	91.0	7.0	92.0

- : not detected. Table 2.6.5.13B-DMPK R0500017-02



Note: The exact structures of metabolites M1, M2, M5, M6, M7 and M8 were retrospectively assigned by comparison with synthetic reference standards (Table 2.6.5.9E-DMPK

R1000166-01, Table 2.6.5.2S- RD-2015-00908.

Conclusion:

Unchanged [^{14}C] BAF312 accounted for a main portion of radioactive compound in plasma extracts up to 48 h. The exposure of the rats to parent compound accounted for 65.9% (i.v.) and 66.7% (p.o.) of the plasma AUC (0-48 h). After both routes of administration, the plasma patterns of metabolites were comparable showing M6 as main metabolite based on AUC. In urine three radioactive metabolite peaks were detected, M1, M2 and M8. No unchanged [^{14}C] BAF312 was excreted with urine. Feces contained two major metabolite; M4 and M6, as well as smaller amounts of unchanged [^{14}C] BAF312. In addition several minor metabolites were detected in feces, i.e. M1, M5, M7, M8 and M9. Elimination of [^{14}C] BAF312 in rats occurred mainly by oxidative metabolism. The main metabolite M6 in plasma was formed by hydroxylation. Two further hydroxylated metabolites (M5 and M7) as well as a twice hydroxylated metabolite (M9) were observed. The metabolite M4 was formed by sulfation of a metabolite hydroxylated in the 1-cyclohexyl-4-methyl-2-trifluoromethyl-benzene moiety. In addition the metabolites M1 and M2 were attributed to cleavage/hydrolysis of the oxime ether moiety. Subsequent reduction yielded the metabolite M8.

2.6.5.9C Pharmacokinetics: Metabolism *In vivo* – Rat (DMPK R0900164-01)

Study title:	Absorption, distribution, and metabolism in male rats after single intravenous (1 mg/kg) and peroral (3 and 10 mg/kg) administration of [^{14}C]BAF312 (with radiolabel on the benzyl-oxyamino part of the molecule)	Study No. DMPK R0900164-01
Location in CTD	4.2.2.2-3	
GLP compliance	not required	
Test article	[^{14}C]BAF312 hemifumarate (with radiolabel on the benzyl-oxyamino part of the molecule)	
Species/Strain	Rat (Hannover Wistar)	
Gender(M/F)/ Number of animals	M 3 for i.v., 3 for p.o. (data from rat 11 excluded)	
Feeding condition	Water and food ad libitum	
Vehicle/Formulation	Intravenous and oral: Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)	
Route of dosing	Intravenous bolus (into vena femoralis) / single dose Oral (gavage) / single dose	
Dose (mg/kg)	Intravenous: 1; oral: 3 (doses referring to the free base)	
Analyte/Radionuclide	Radioactivity / ^{14}C	
Sample times (h)	Plasma: 0.083 (i.v. only), 0.25 (p.o. only), 2, 4, 8, 24, 48 and 96 h; Urine and feces: daily up to 96 h, 96 – 168 h	
Samples	Plasma, urine, feces	
Assay	Radioactivity: Liquid scintillation counting (LSC) Liquid chromatography – mass spectrometry (LC-MS)	

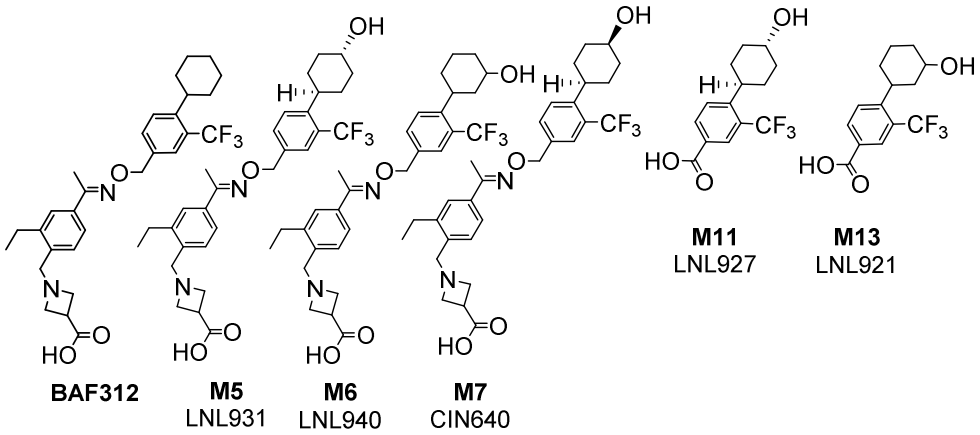
[^{14}C]BAF312 and metabolites in rat plasma [1)]

Dosing	1 mg/kg i.v.				3 mg/kg p.o.			
	(pools of n=3 per time point)				(pools of n=2 per time point)			
Metabolite	Tmax h	Cmax ngEq/mL	AUC0-48h ngEq•h/mL	%	Tmax h	Cmax ngEq/mL	AUC0-48h ngEq•h/mL	%
BAF312 (parent compound)	0.083	527	1560	63.0	4	198	3120	44.3
M5 (LNL931)	2	1.55	9.30	0.4	4	4.13	63.0	0.9
M6 (LNL940)	2	35.6	310	12.5	8	48.6	819	11.6

M7 (CIN640)	-	-	-	-	8	0.517	5.68	0.1
M11 (LNL927)	24	5.17	166	6.7	24	59.9	1600	22.7
M13 (LNL921)	48	4.13	125	5.0	24	13.9	445	6.3
P22.2	48	0.517	6.72	0.3	24	2.07	57.3	0.8
P24.4	24	0.517	11.4	0.5	24	2.58	58.9	0.8
P26.6	24	0.517	11.9	0.5	24	6.20	181	2.6
Sum of others	0.083	16.0	38.2	1.5	24	2.58	102	1.5
Total components detected	0.083	543	2240	90.4	8	265	6460	91.6
Lost during sample processing	0.083	29.4	239	9.6	8	25.8	596	8.4
Total radiolabeled components in sample	0.083	526	2480	100	8	291	7060	100

- not detected.

1) Analyzed sample time points: 0.05 (i.v. only) , 0.25 (p.o. only), 2, 4, 8, 24, 48 h



Note: The exact structures of metabolites M5, M6, M7, M11 and M13 were retrospectively assigned by comparison with synthetic reference standards (Table 2.6.5.9E-DMPK R1000166-01, Table 2.6.5.2S- RD-2015-00908)

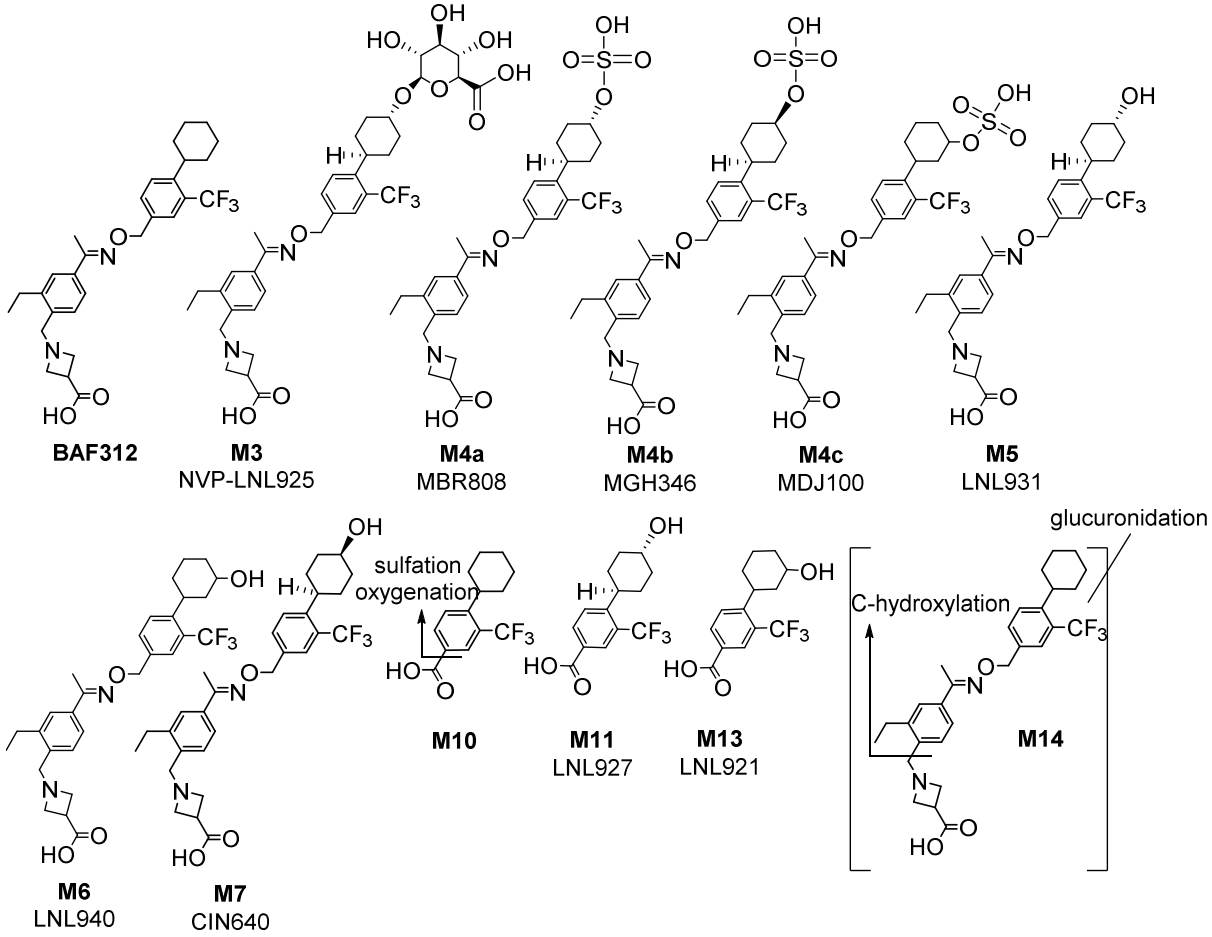
[^{14}C]BAF312 and metabolites in rat excreta (amounts expressed as % of dose)

Dosage	1 mg/kg i.v.		3 mg/kg p.o.	
Metabolite	Urine (0-96 h)	Feces (0-96 h)	Urine (0-96 h)	Feces (0-96 h)
BAF312 (parent compound)	-	3.13	-	9.41
M3 (LNL925)	-	0.890	-	0.727
M4a (MBR808)	-	16.7	-	9.55
M4b (MGH346)	-	5.14	-	2.68
M4c (MDJ100)	-	5.80	-	4.17
M5 (LNL931)	-	11.6	-	14.2
M6 (LNL940)	-	28.0	-	26.6
M7 (CIN640)	-	0.642	-	1.52
M9	-	0.707	-	0.579
M10	1.49	1.87	0.468	2.26
M11	0.224	0.679	0.160	0.883
M13	0.0417	4.02	0.028	5.09
M14	-	0.367	-	1.35
M17 (LYS715)	-	-	-	-
P17.4	0.188	-	0.0923	0.304
P19.1+P19.2	0.533 [a)]	0.358 [a)]	0.269	0.911
P20.0	0.231	-	0.105	-
P20.6	0.0402	-	0.0244	-
P22.2	0.170	0.771	0.105	2.19
P30.2	-	3.85	-	2.90
P34.8	-	0.899	-	1.39
P35.0	-	2.26	-	2.35
P35.8	-	2.51	-	1.69
Sum of additional components	0.0917	1.57	0.042	1.18
Total components detected	3.07	91.8	1.30	92.0
Lost during sample processing	0.144	2.16	0.016	0.462
Total radiolabeled components in original sample (pool 0-96 h)	3.21	93.9	1.32	92.4

Total excretion of radiolabeled components (0-168 h) *	3.35	95.8	1.44	94.7

- not detected, * Table 2.6.5.13C-DMPK R900164-01

[a)] Metabolite P19.1 and P19.2 were separated with another HPLC method in run 0900164_0032. P19.2 accounted for approx. 75% of the peak while P19.1 represented about 25%. Data are not shown, but kept on file.



Note: The exact structures of metabolites M3, M5, M6, M7, M11 and M13 were retrospectively assigned by comparison with synthetic reference standards (Table 2.6.5.9E-DMPK R1000166-01, Table 2.6.5.2S- RD-2015-00908).

Conclusion:

In plasma from intact rats, the metabolites profiles after both i.v. and p.o. administration showed BAF312 as the major component (63% and 44% of [^{14}C]-AUC0-48h). After both routes of administration, the patterns of metabolites were comparable revealing M6, M11 and M13 as main metabolites. The hydroxylated metabolite M6 accounted to 12.5% and 11.6% of the radioactivity AUC0-48h after i.v. and p.o. dosing, respectively. The cleavage products M11 and M13 amounted to 6.7% and 5.0% of [^{14}C]-AUC0-48h after i.v. dosing and to 22.7% and 6.3% after oral dosing of [^{14}C]-AUC0-48h, respectively. Minor proportions of other metabolites represented less than 3% of the radioactivity AUC0-48h each.

In feces from intact rats, the metabolites profiles after both i.v. and p.o. administration were similar and showed M4*, M5 and M6 as major components beside numerous other minor metabolites. The major metabolites M4*, M5 and M6 represented in sum 67% after i.v. dosing and 57% after p.o. dosing. The fecal excretion of BAF312 amounted only to 3.1% and 9.4% after i.v. and p.o. dosing, respectively.

In urine from intact rats, the metabolites profiles after both i.v. and p.o. administration were similar. Renal excretion of BAF312 was not observed. Main component was metabolite M10 accounting for 1.5% of the i.v. dose and 0.5% of the p.o. dose. Other minor peaks represented less than 0.5% of dose.

In bile of bile duct-cannulated rats (see table below), the major metabolites M4, M3 and M14 accounted for 26.2%, 11.2% and 9.9% of dose, respectively. The metabolites M5 and M6 were of less importance. The biliary excretion of parent drug BAF312 amounted to 8.2% of the radioactive dose. Therefore, metabolism is the major route of BAF312 elimination in the rat.

(*Additional investigations revealed that metabolite M4 was comprised of the metabolites M4a, M4b and M4c. The metabolites M4a, M4b and M4c accounted got 16.7%, 5.14% and 5.80% of the dose after i.v. administration and to 9.55%, 2.68% and 4.17% after p.o. administration, respectively.)

Pharmacokinetics: Metabolism *in vivo* – bdc rat (DMPK R0900164-01)

Study title:	Absorption, distribution, and metabolism in male rats after single intravenous (1 mg/kg) and peroral (3 and 10 mg/kg) administration of [^{14}C]BAF312 (with radiolabel on the benzyl-oxyamino part of the molecule)	Study No. DMPK R0900164-01, DMPK R1000456
Location in CTD	4.2.2.2-3	
GLP compliance	not required	
Test article	[^{14}C]BAF312 hemifumarate (with radiolabel on the benzyl-oxyamino part of the molecule)	
Species/Strain	Rat (Hanover Wistar), bile duct cannulated (bdc)	
Gender(M/F)/ Number of animals	M 2	
Feeding condition	Water and food ad libitum	
Vehicle/Formulation	Intravenous and oral: Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)	
Route of dosing	Intravenous bolus (into vena femoralis) / single dose	
Dose (mg/kg)	Intravenous: 1	
Analyte/Radionuclide	Radioactivity / ^{14}C	
Assay:	Radioactivity: Liquid scintillation counting (LSC) Liquid chromatography – mass spectrometry (LC-MS)	
Sample times (h)	0-24 h and 24-48 h	
Samples:	Urine, feces, bile	

[^{14}C]BAF312 and metabolites in bile duct cannulated rat excreta (amount expressed as % of dose)

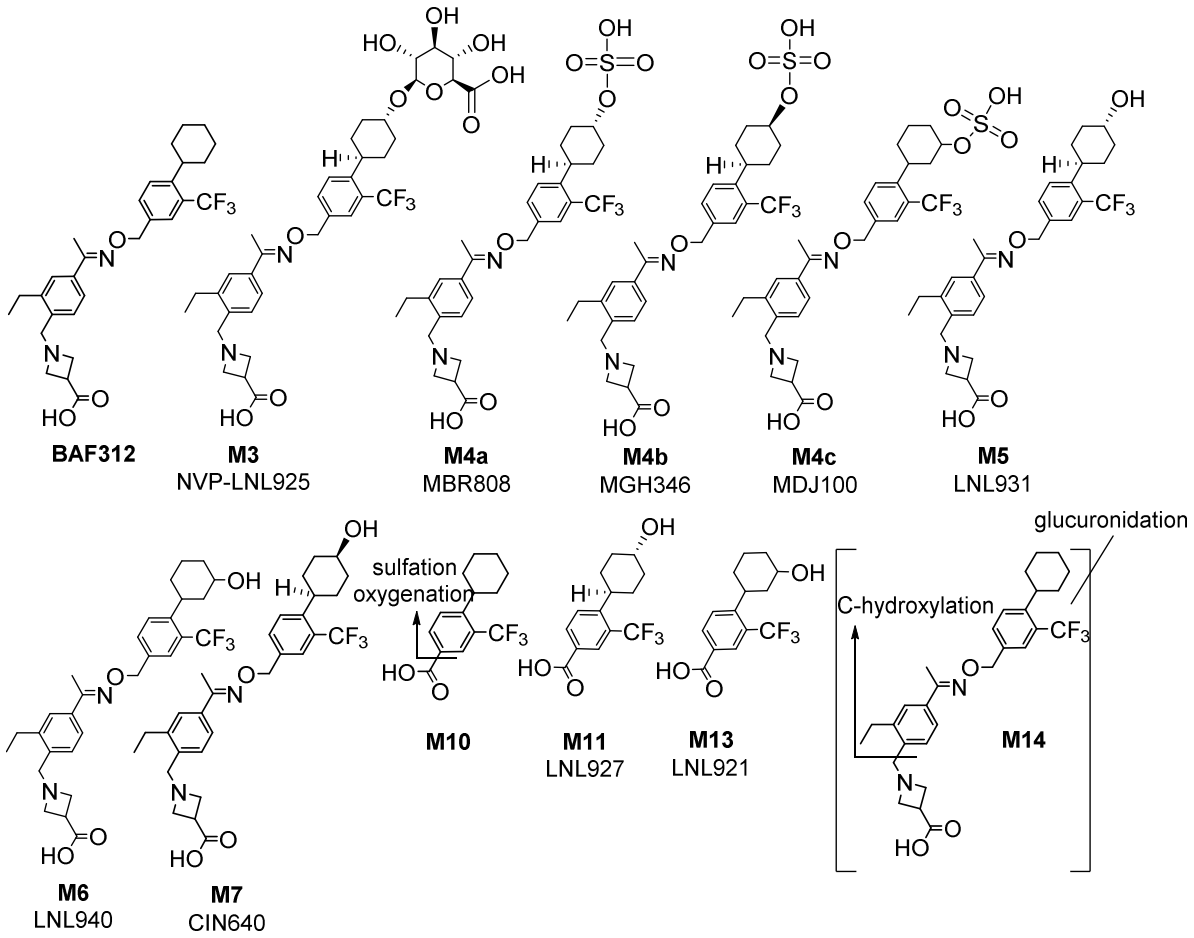
Dosage	1 mg/kg i.v.		
Metabolite	Urine (0-48 h)	Bile (0-48 h)	Feces (0-48 h)
BAF312 (parent compound)	0.00338	8.16	2.69
M3 (LNL925) [a)]	0.327	11.2	-
M4	0.124	see M4a, M4b, M4c	-
M4a (MBR808)	-	16.7	-
M4b (MGH346)	-	3.00	-

M4c (MDJ100)	-	6.54	-
M5 (LNL931) [a)]	0.0241	2.83	0.148
M6 (LNL940) [a)]	0.0111	7.05	1.61
M7 (CIN640) [a)]	0.0143	-	0.0567
M10	0.146	-	-
M11 [a)]	0.167	-	-
M13 [a)]	0.0301	-	-
M14	0.0961	9.88	-
M17 (LYS815)	na	-	na
P17.4	0.0402	-	-
P19.1+P19.2	0.223	0.982	-
P20.0	0.0506	-	-
P20.6	0.256	1.06	-
P22.2	0.167	-	-
P35.0	-	0.853	-
P35.8	-	1.35	-
P45.0	0.0555	-	-
Sum of additional components	0.144	2.13	0.000
Total components detected	1.88	71.7	4.50
Total excretion of radiolabeled components (0-48 h)	1.88	71.7	4.50

- not detected,

na not assessed

[a)] The identities of M3 with LNL925, M5 with LNL931, M6 with LNL940, M7 with CIN640, M11 with LNL927, and M13 with LNL921 were confirmed in study (DMPK R1000456).



Note: The exact structures of metabolites M3, M4a, M4b, M4c, M5, M6, M7, M11 and M13 were retrospectively assigned by comparison with synthetic reference standards (Table 2.6.5.9E-DMPK R1000166-01, Table 2.6.5.2S- RD-2015-00908).

Conclusions:

The biotransformation of BAF312 occurred essentially by the following pathways: The phase I metabolic reactions involved C-hydroxylation(s) forming the metabolites M5, M6, M7 and

M9*, cleavage/hydrolysis at the oxime ether bound and subsequent oxidations (M11, M13). Phase II reactions involved sulfation (M4**, M10) and glucuronidation (M3 and M14) of hydroxylated metabolites.

BAF312 was eliminated pre-dominantly via metabolism and only to a minor fraction via direct excretion. Independent of the route of administration, total radiolabeled components were excreted in intact rats pre-dominantly *via* feces and in bile duct-cannulated rats via bile. Total radiolabeled components were excreted completely within 168 h after dosing.

*Only observed in feces of intact animals.

**The metabolites M4a, M4b and M4c accounted to 16.7%, 3.00% and 6.54% of the dose after i.v. administration, respectively

2.6.5.9D Pharmacokinetics: Metabolism *In vivo* – Lactating rat (DMPK R1300583)

Study title:	Galactogenic transfer. Kinetics and metabolism in milk and plasma after a single oral dose (10 mg base/kg) administration of [^{14}C]BAF312 hemifumarate in rats	Study no. DMPK R1300583, DMPK R1300583a
Location in CTD	4.2.2.3-5	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312 hemifumarate	
Species / strain:	Rat (lactating) /HAN:WIST	
Gender (M/F) / number of animals:	F / 8 (2 groups: 4 dams per group)	
Feeding condition:	Water and food available *ad libitum*	
Vehicle / formulation:	0.5% aqueous hydroxypropylcellulose solution, suspension	
Route of administration:	p.o. (gavage)	
Dose (mg/kg):	10 mg/kg	
Day of dosing:	Day 11 after parturition	
Radionuclide:	^{14}C	
Analyte/assay (unit):	Liquid scintillation counting (LSC) Liquid chromatography-mass spectrometry (LC-MS)	
Analyzed samples:	Plasma and milk: 0.25, 1, 3 (no milk sample), 8, 24, 48, 72 h	

Sample type	Plasma [1)]				Plasma [2)]				Milk [1)]			
	Tmax	Cmax	AUC0-72 h		Tmax	Cmax	AUC0-72 h		Tmax	Cmax	AUC0-72 h	
Metabolite	h	ngEq/mL	ngEq•h/mL	%	h	ngEq/mL	ngEq•h/mL	%	h	ngEq/mL	ngEq•h/mL	%
BAF312	8	2200	85200	95.29	[4)]	[4)]	[4)]	[4)]	8	1050	46500	89.79
M1 (BYC413)	0.25	3.90	4.20	0.00	[4)]	[4)]	[4)]	[4)]	0.25	12.7	96.1	0.19
M2 (BYD952)	-	-	-	-	[4)]	[4)]	[4)]	[4)]	0.25	7.13	3.55	0.01
M6 (LNL940)	3	8.47	307	0.34	[4)]	[4)]	[4)]	[4)]	8	5.63	158	0.30
M8 (BYD974)	0.25	1.22	0.615	0.00	[4)]	[4)]	[4)]	[4)]	24	11.6	237	0.46
M16 (LYG778)	8	24.6	765	0.86	3	70.3	3020	3.38 [3)]	24	2.69	63.5	0.12
M17 (LYS815)	-	-	-	-	8	58.4	2020	2.26	-	-	-	-
Sum of additional components	8	11.6	584	0.65	8	2140	82700	92.57	72	5.42	184	0.35
Total detected	8	2230	86800	97.14	8	2270	87800	98.21	8	1060	47300	91.22

Lost during sample processing	-	-	-	-	8	36.9	1600	1.79	-	-	-	-
Lost during HPLC	8	74.9	2560	2.86	-	-	-	-	24	108	4550	8.78
Total radiolabeled components in original sample	8	2310	89400	100.00	8	2310	89400	100.00	8	1150	51700	100.00

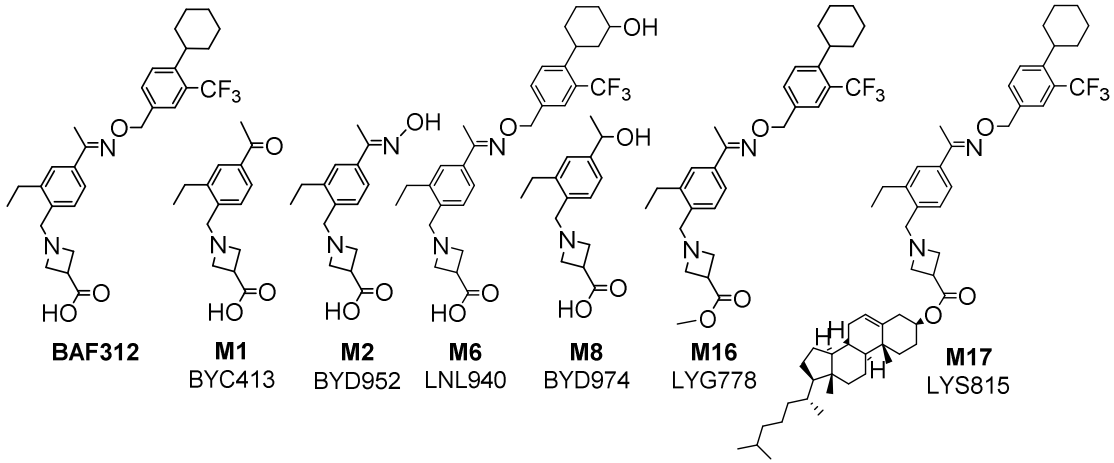
- not detected.

1) HPLC analyses.

2) UPLC analyses.

3) ~1% can be attributed to the formation during sample preparation.

4) Listed under “Sum of additional components”.



Conclusions:

Following a single oral administration of 10 mg/kg [^{14}C]BAF312 to lactating rats:

- In plasma and milk, the main radiolabeled component BAF312 contributed to 95.3% and 89.8% to the total ^{14}C-AUC0-72h, respectively.
- In plasma, Cmax of total radiolabeled components (2310 ngEq/mL) and BAF312 (2200 ngEq/mL) were observed at 8 h post dose. Thereafter, the concentrations of total radiolabeled components declined with a half-life of 26.8 h, and the BAF312 concentration declined with a similar half-life of 28.1 h. The concentration of total radiolabeled components at 72 h was 18.8% and the BAF312 concentration was 18.1% compared to their respective Cmax value.
- In milk, Cmax of total radiolabeled components (1150 ngEq/mL) and BAF312 (1050 ngEq/mL) were observed at 8 h post dose. Thereafter, the concentration of total radiolabeled components declined with an apparent half-life of 27.8 h. The concentration of total radiolabeled components at 72 h was 28.1% and the BAF312 concentration at 72 h was 27.1% compared to their respective Cmax at 8 h.

- BAF312 and its metabolites were distributed into the milk and reached lower concentrations compared to plasma with a milk/plasma ratio (AUClast) of 0.566 for total radiolabeled components, and 0.492 for BAF312. Rat pups were sucking therefore milk containing mainly BAF312 and for a very small extent metabolites of BAF312. In conclusion, following p.o. administration of 10 mg/kg [^{14}C]BAF312 to female rats, BAF312 reached approximately half lower concentrations in the milk compared with plasma. Sucking rat pups were swallowing milk containing mainly the parent compound BAF312.

2.6.5.9E Pharmacokinetics: Metabolism *In vivo* – Monkey (DMPK R1000166-01)

Study title:	Absorption, metabolism and excretion following an oral dose of [^{14}C]BAF312 in the monkey	Study No. DMPK R1000166-01, DMPK R1000456
Location in CTD	4.2.2.2-6, 4.2.2.4-1	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312 hemifumarate	
Species / strain:	Monkey / Cynomolgus	
Gender (M/F) / number of animals:	M / 3	
Feeding condition:	Water and food available *ad libitum*	
Vehicle / formulation:	0.5% (w/v) hydroxypropyl-cellulose (grade HF, Klucel) solution	
Method/ route / duration of administration:	p.o. (gavage) / single dose	
Dose (mg/kg):	15 (referring to free base)	
Radionuclide:	^{14}C	
Analyte/assay (unit):	Liquid scintillation counting (LSC), Liquid chromatography - Mass spectrometry (LC-MS)	
Analyzed samples:	Plasma 1, 4, 7, 10, 24, 72, 144 h (pools of n=3 per time point), urine and feces every 24 h until 96 h, 96 – 144 h, every 24 h until 264 h, 264 – 336 h (pools of n=3)	

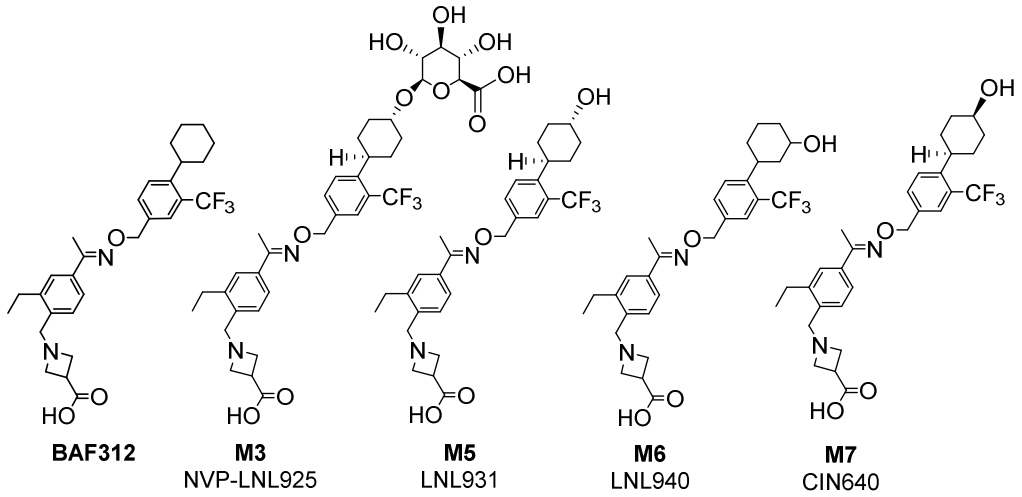
[^{14}C]BAF312 and metabolites in monkey plasma

Metabolite	Tmax h	Cmax ngEq/mL	AUC0-144h ngEq•h/mL	AUC0-144h %
BAF312 (parent compound)	4	4150	141000	79.4
M3 (LNL925)	7	289	12300	6.95
M5 (LNL931)	7	45.9	1280	0.72
M6 (LNL940)	4	64.1	2420	1.37
M7 (CIN640)	7	74.4	2550	1.44
M17 (LYS815)	-	-	-	-
P7.0	24	15.3	474	0.27
P26.6	24	14.7	456	0.26

P29.2	24	14.9	463	0.26
P32.2	10	18.3	599	0.34
P57.2	24	40.8	2600	1.47
P72.6	10	20.7	176	0.10
Total components detected	4	4590	164000	92.6
Lost during HPLC	4	367	13100	7.40
Total radiolabeled components in original sample [2)]	4	4950	177000	100

In a separate investigation, a methyl ester of BAF312 P73.0 (LYG778; M16) accounted for 1.18% of the ^{14}C-AUC0-24h. The formation of P73.0 is assumed to occur during the sample preparation (but it cannot be excluded that traces were formed metabolically).

Radiolabeled metabolites:



[^{14}C]BAF312 and metabolites in monkey excreta (amount expressed as % of dose)

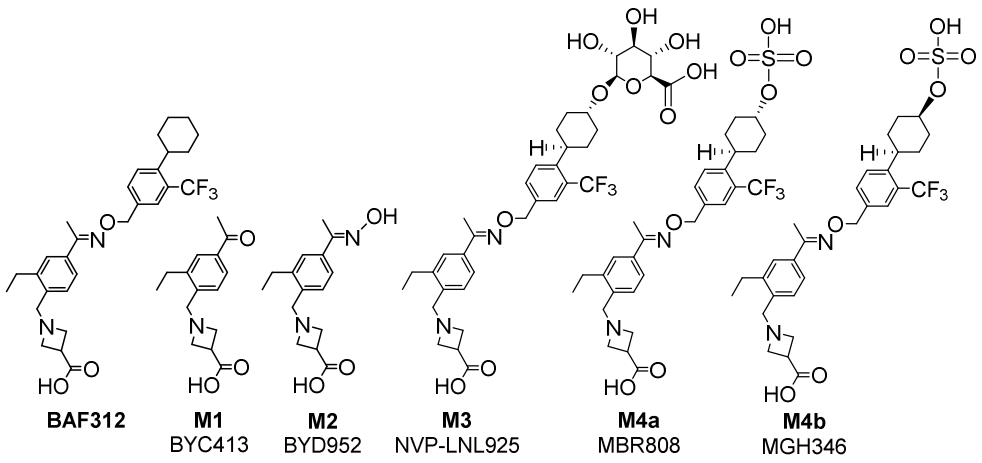
Metabolite	Urine (0-144 h) [a)]	Feces (0-144 h)
BAF312 (parent compound)	-	9.36
M1 (BYC413)	0.38	4.63

M2 (BYD952)	0.17	-
M4a (MBR808)	-	9.81
M4b (MGH346)	-	15.23
M4c (MDJ100)	-	2.86
M5 (LNL931)	-	28.31
M6 (LNL940)	-	8.25
M7 (CIN640)	-	7.63
M8 (BYD974)	0.49	1.49
P15.4	0.02	-
P17.4	0.01	-
P19.2	0.01	-
P21.8	-	0.69
P35.8	-	1.25
P52.6	-	1.05
Sum of other components	0.05	3.59
Total components detected	1.45	94.17
Lost during sample processing	0.01	1.89
Lost during HPLC	0.18	0.00
Total radiolabeled components in original sample (0-144h)	1.65	96.06
Total excretion of radiolabeled components (0-336h) *	1.71	98.2

- not detected, * Table 2.6.5.13D-DMPK R1000166-01

a) M15 (loss of ^{14}C label) was observed at <0.2% of dose using LC-MS. The identity of M15 with CIQ298 was confirmed in study [DMPK R1000456].

Radiolabeled metabolites:



Conclusions:

Pharmacokinetics: Following a 15 mg/kg oral dose in monkeys, BAF312 was slowly absorbed with mean Tmax at 7 h. Fecal excretion was the major elimination route; accounting for 98.2% of dose, and most of dose was recovered from feces within the first 7 days (~96%).

Metabolism: Phase I and Phase II biotransformation of BAF312 in the monkey – based on proposed chemical structures of metabolites detected in plasma, urine and feces – is characterized mainly by the following reactions:

- The phase I metabolic reactions involved C-hydroxylations (M5, M6 and M7), cleavage/hydrolysis of the oxime ether (M1, M2) and further reduction (M8) or oxidation (M11, M13, M15).
- Phase II reactions involved sulfation (M4a, M4b and M4c) and glucuronidation (M3) of hydroxylated metabolites.

In plasma, unchanged [^{14}C] BAF312 accounted for the main proportion of radioactive compound. The exposure of the monkeys to parent compound amounted to 79.4% of the total plasma ^{14}C-AUC0-144h and the exposure to its main metabolite M3 accounted for 6.95% after oral administration. Numerous minor metabolite peaks were detected. In feces, unchanged [^{14}C] BAF312 represents 9.36% of the radioactive dose. Six major metabolites were observed; the metabolites M1, M4a-c, M5, M6 and M7 represented in sum 76.7% of the radioactive dose. In addition few minor metabolites were detected in feces. In urine various ^{14}C-labeled metabolites were detected with metabolites M1, M2, M3 and M8 as major components amounting in sum to 1.37% of the total radioactive dose. No unchanged [^{14}C] BAF312 was excreted with urine.

Minor amounts of the non-radioactive labeled metabolites M11, M13 and M15 were detected in urine and/or plasma. Metabolite M17 (LYS815) was not detected.

2.6.5.10 薬物動態試験：*in vitro* における代謝

2.6.5.10A Pharmacokinetics: Metabolism *in vitro* (DMPK R0400863-01)

Study title:	*In vitro* biotransformation of [^{14}C]BAF312 in liver microsomes from mouse, rat, dog, cynomolgus monkey, human and in hepatocytes from rat and human; measurement of protein adduct levels	Study no. DMPK R0400863-01
Location in CTD:	4.2.2.4-2	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312	
Study system:	Commercially available cryopreserved microsomes from mouse, rat, dog, monkey and human, as well as commercially cryopreserved hepatocytes from rat and human were used as biological material. The incubations of [^{14}C] BAF312 were performed at 37°C. Enzyme reactions were stopped by the addition of 1.5 volumes of acetonitrile containing 0.02% formic acid followed by centrifugation.	
Initial concentration:	5 µmol/L; 1 µM/L for intrinsic clearance in microsomes and hepatocytes	
Analytical methods:	Radioactivity: Liquid scintillation counting (LSC) Liquid chromatography – mass spectrometry (LC-MS)	

Summary of results of assessment of intrinsic clearance in hepatocytes and liver microsomes:

Intrinsic clearance (Clint) of [^{14}C]BAF312 in hepatocytes			Intrinsic clearance (Clint) of [^{14}C]BAF312 in liver microsomes						
Calculated parameters	Unit	Human	Calculated parameters	Unit	Mouse	Rat	Dog	Monkey	Human
T1/2 [1)]	h	6.21	T1/2	min	801	24.5	n.d.	57.3	150.2
CLint [2)]	µL/min/10^6 cells	1.86	AUC(0-inf)	µM·min	1151	35	n.d.	84.9	218.6
CLint, scaled [3)]	mL/min/kg	4.73	CLint(1) [b)]	µL/min/mg LM [e)]	5.8	188.3	n.d.	80.6	30.8
CLh,b [4,5)]	mL/min/kg body weight	3.85	CLint(2) [c)]	µL/min/mg LM [e)]	5.8	188.6	n.d.	78.5	30.5
CLh,b	%	18.6	CLint [d)]	µL/min/mg LM [e)]	6	188	<5	80	31

1) Half-lives were evaluated by linear extrapolation

2) (ln2/T1/2) / 10^6 viable cells x 1000

3) (CLint / 1000) * (cells / g liver) * (g liver / kg body weight)

4) CLh = (Qh * CLint, scaled) / (Qh + CLint, scaled), Qh: hepatic blood flow; (well-stirred liver model)

5) Qh (mL/min/kg body weight): 20.7 (human)

b) Clint(1) = (0.693 / T1/2) * assay volume/(microsomal protein)

c) Clint(2) = (Dose / AUC) * assay volume/(microsomal protein)

d) Clint = [Clint(1) + Clint(2)] / 2

e) LM = microsomal protein of the liver

Summary of results of assessment of protein adduct formation:

	liver microsomes [a)]				Hepatocytes	
	1st arm LM +GSH	2nd arm LM +NADPH	3rd arm LM +NADPH +UDPGA	4th arm LM +NADPH +UDPGA +GSH		5th arm [b)] Hepatocytes
	pmol/mg	pmol/mg	pmol/mg	pmol/mg		pmol/10^6 cells
Human	16	11	11	10	Human	196
Rat	21	14	14	8	Rat	47
Monkey	n.a.	n.a.	n.a.	n.a.	Monkey	47

[a)] 60 min incubation time

[b)] 3 h incubation time

(Abbreviations: LM – liver microsomes; GSH - glutathione; n.a. – not applied; NADPH – nicotinamide adenine dinucleotide phosphate, reduced form; UDPGA - uridine 5'-diphospho-α-D-glucuronic acid)

Conclusion:

Only traces of covalent protein adducts were found in human and rat liver microsomes. Detectable but rather low to moderate levels were obtained for rat and monkey hepatocytes. However higher levels of covalent protein adducts were found for human hepatocytes. The results indicate that most of the covalent binding observed in hepatocytes is mediated by other enzymes than cytochrome P450. Although observed levels may be considered moderate to high. The relevance of these *in vitro* findings for humans *in vivo* must be discussed considering the applied dose as well as dosing regimen and the exposure at the target organ. In addition, the indication and other qualifying factors may contribute to the risk assessment.

	DMPK 0400863					DMPK 0400863	
	Incubation with liver microsomes (60 min)					Incubation with hepatocytes (3 h)	
	Species					Species	
	mouse	rat	dog	monkey	human	rat	human
BAF312	xx	xx	xx	xx	xx	xx	xx
M3 (LNL925)	x	xx	-	xx	x	xx	xx
M4	-	-	-	-	-	x	x
M5 (LNL931)	-	x	-	x	x	x	x
M6 (LNL940)	x	xx	-	x	-	xx	x
M7 (CIN640)	-	x	-	xx	x	x	xx

- not observed

x minor metabolite

xx major metabolite

	DMPK 0400863-01			DMPK 0400863-01		
	Incubation with liver microsomes (60 min)			Incubation with hepatocytes (3 h)		
	Species			Species		
	mouse	rat	human	mouse	rat	human
BAF312	xx	xx	xx	xx	xx	xx
P1 (M1*, BYC413)	tr.	tr.	tr.	tr.	tr.	tr.
P2 (M2*, BYD952)	tr.	tr.	-	tr.	tr.	tr.
M3 (LNL925)	tr.	xx	xx	x	x	xx
M4	-	-	-	-	x	xx
M5 (LNL931)	tr.	tr.	x	x	x	x
M6 (LNL940)	tr.	xx	-	x	xx	x
M7 (CIN640)	tr.	x	x	-	x	xx

- not observed

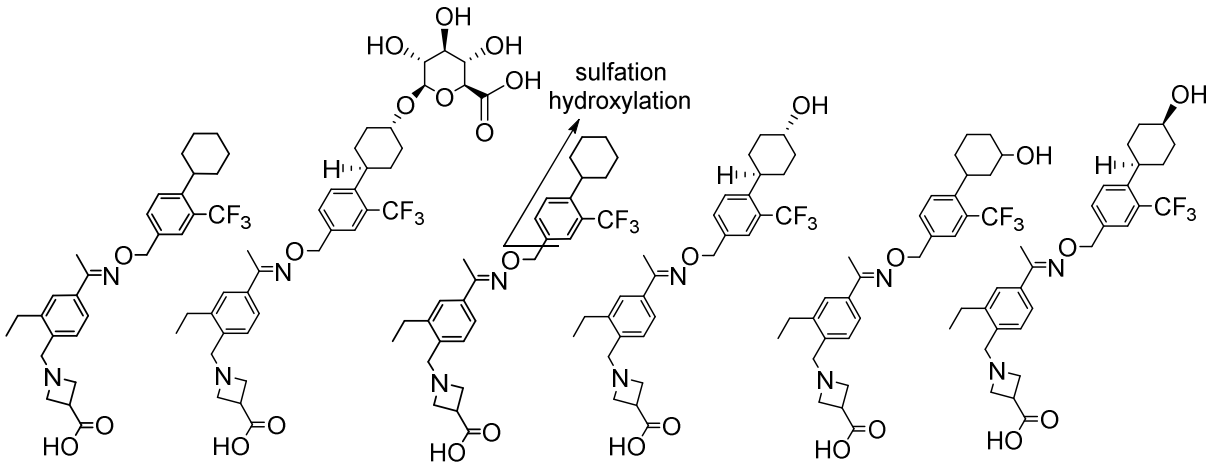
* Because of the low quantities of those trace metabolites/compounds no MS signal could be recorded. Therefore their assignments are based on the retention times only.

tr. trace

x minor metabolite

xx major metabolite

Metabolite structures:



2.6.5.10B Pharmacokinetics: Metabolism *in vitro* (DMPK R0900482)

Study title:	*In vitro* metabolism of BAF312: Assessment of reactive intermediate formation	Study no. DMPK R0900482
Location in CTD:	4.2.2.4-3	
GLP compliance:	not required	
Test article:	[^{14}C]BAF312 hemifumarate; Batch no: RSE280-13, RSE280-22 (radiolabel in position 1) RSE425-1 (with radiolabel on the benzyl-oxyamino part of the molecule, position 2)	
Study system:	Commercially available human liver microsomes (Lot 82087, BD Biosciences), as well as commercially cryopreserved human hepatocytes (Lot HU4120, Invitrogen) were used as biological material. In addition recombinant human enzymes (CYP450s, MAOA/B and FMO3) were used. The incubations of [^{14}C] BAF312 were performed at 37°C. Enzyme reactions were stopped by protein precipitation (acetonitrile) followed by centrifugation and extensive wash procedures of the obtained protein pellets.	
Initial concentration:	5 µmol/L (microsomes and recombinant enzymes and 10 µmol/L (hepatocytes)	
Analytical methods:	Radioactivity: Liquid scintillation counting (LSC), 2d-electrophoresis (SDS-PAGE) followed by autoradiography	

Summary of results of assessment of protein adduct formation:

	Human liver microsomes and recombinant enzymes [a]				Human Hepatocytes [b]
Assay design	1st arm	2nd arm	3rd arm	4th arm	5th arm
	HLM+GSH	HLM+NADPH	HLM+NADPH+UDPGA	HLM+NADPH+UDPGA+GSH	
units	pmol/mg	pmol/mg	pmol/mg	pmol/mg	pmol/10^6 cells
Protein adducts	2.9 ± 0.6	3.6 ± 0.8	3.9 ± 1.2	2.9 ± 0.9	1411 ± 208

[a] 60 min incubation time [b] 0-3 h incubation time (kinetic)

(Abbreviations: HLM – human liver microsomes; GSH - glutathione; NADPH – nicotinamide adenine dinucleotide phosphate, reduced form; UDPGA -uridine 5'-diphospho-α-D-glucuronic acid)

Protein adduct formation (pmol/mg) observed with recombinant enzymes (assay design arm 2) – [pmol adduct/nmol of enzyme]

CYP3A4 (178), CYP1A1 (339), CYP1A2 (93), CYP2A6 (214), CYP2B6 (155), CYP2C19 (92), CYP2C8 (105), CYP2C9 (47), CYP2D6 (278), CYP3A7 (174), CYP4A11 (186), CYP4F2 (88), CYP4F3B (50) FMO3 (28), MAO ab (15)

Adducted proteins detected in human hepatocytes by gel electrophoresis and autoradiography [Molecular weight in kDa]

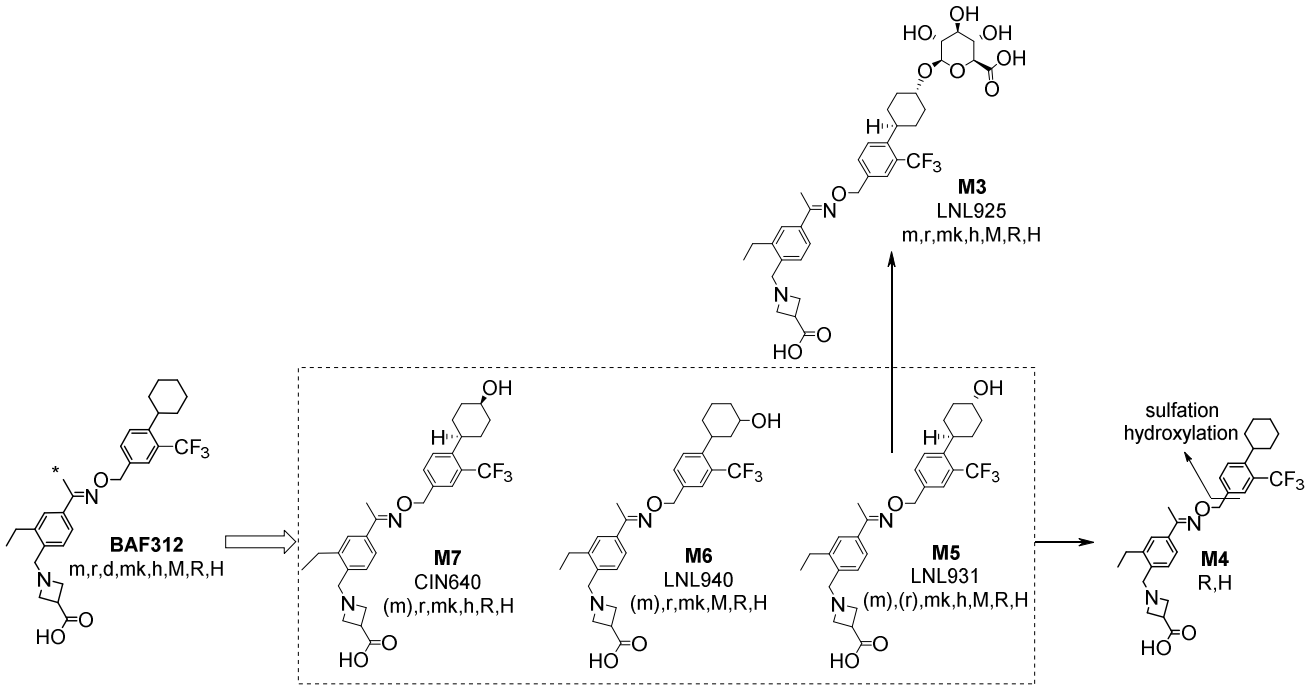
Major bands observed: 9 kDa, 12 kDa, 21 kDa, 47 kDa, 100 kDa. Proteins were not identified

Conclusion:

Formation of covalent drug-protein adducts of BAF312 detected in human hepatocytes were above the pre-defined threshold of 50 pmol/10^6 cells indicating that BAF312 is susceptible to metabolic activation. In contrast, human liver microsomes did not show a significant formation of covalent protein adducts with radiolabeled BAF312. Also incubations with individual recombinant enzymes showed only low levels of covalent binding suggesting a minor role of CYP enzymes. Analysis of potential off-target proteins in human hepatocytes showed that metabolic activation led to the labeling of few proteins from a broad range of molecular weights. None of the adducted target proteins have yet been identified. The impact of covalent protein adduct formation on the function, turnover or immunogenicity of individual proteins still remains elusive, however the low systemic exposure of BAF312 may suggest that protein adduct formation may not lead to clinically relevant safety concerns, in particular for the liver.

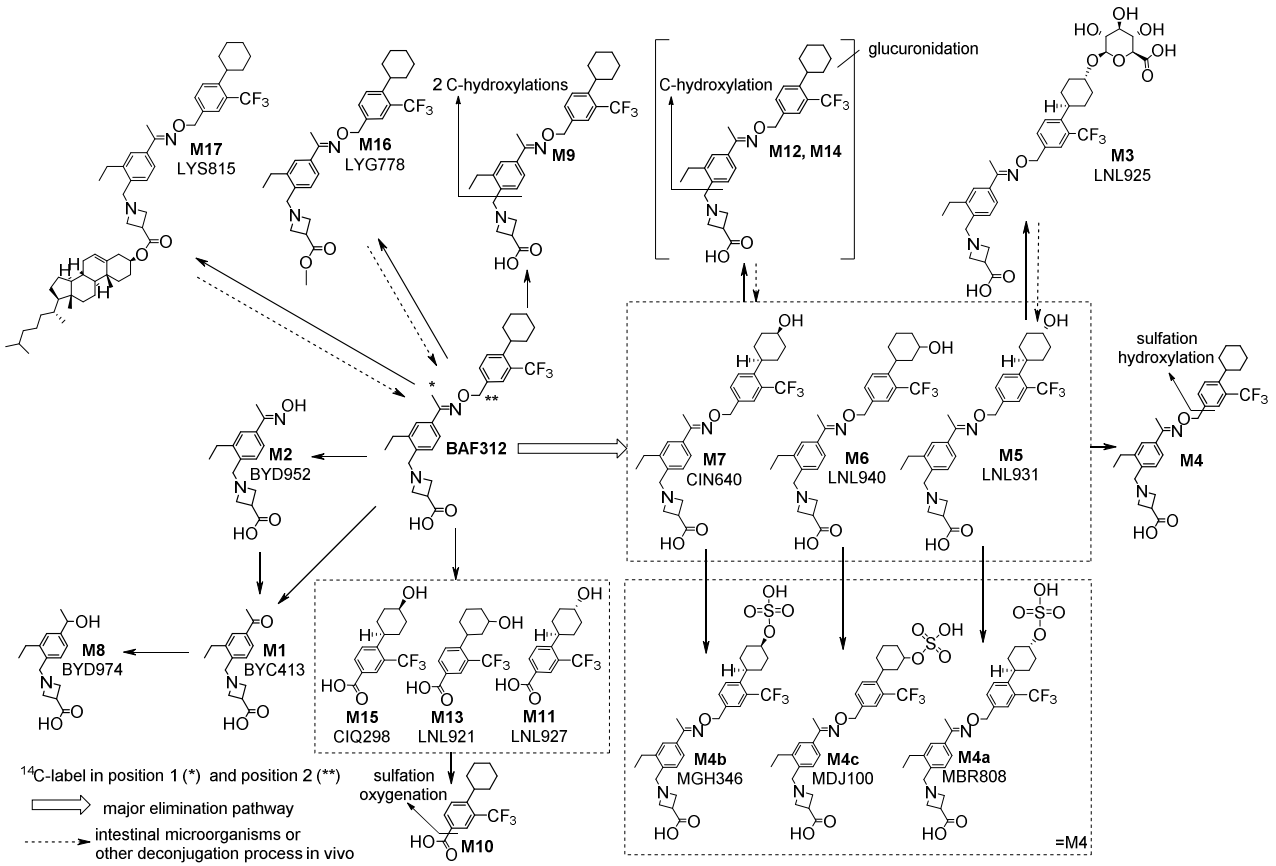
2.6.5.11 薬物動態試験：推定代謝経路

2.6.5.11A Pharmacokinetics: Metabolic pathways *in vitro* in mouse, rat, dog, monkey, and human:



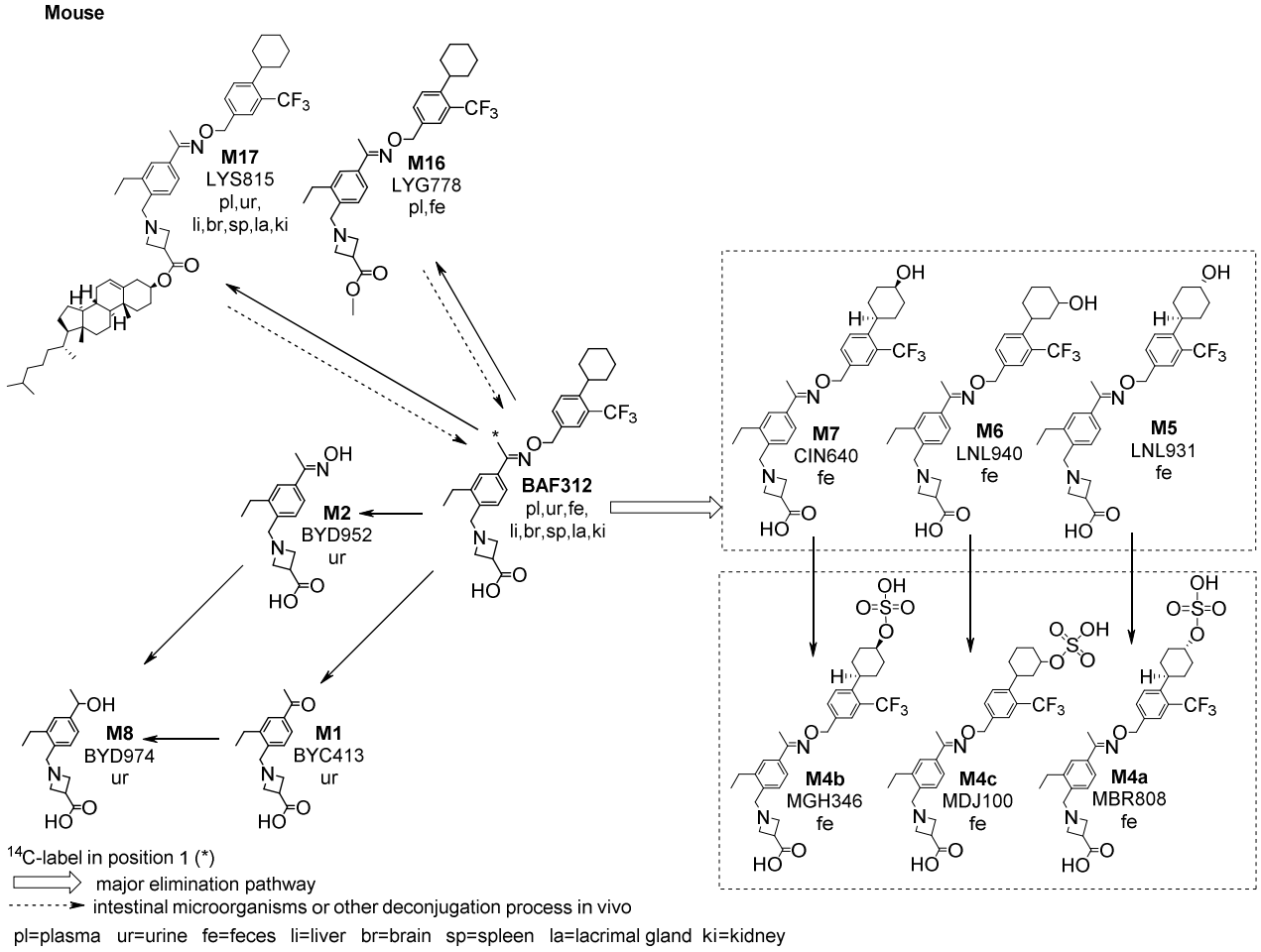
Source: Table 2.6.5.10A- DMPK R0400863-01, Table 2.6.5.2S- RD-2015-00908

2.6.5.11B Pharmacokinetics: Metabolic pathways *in vivo* in mouse, rat, monkey, and human:



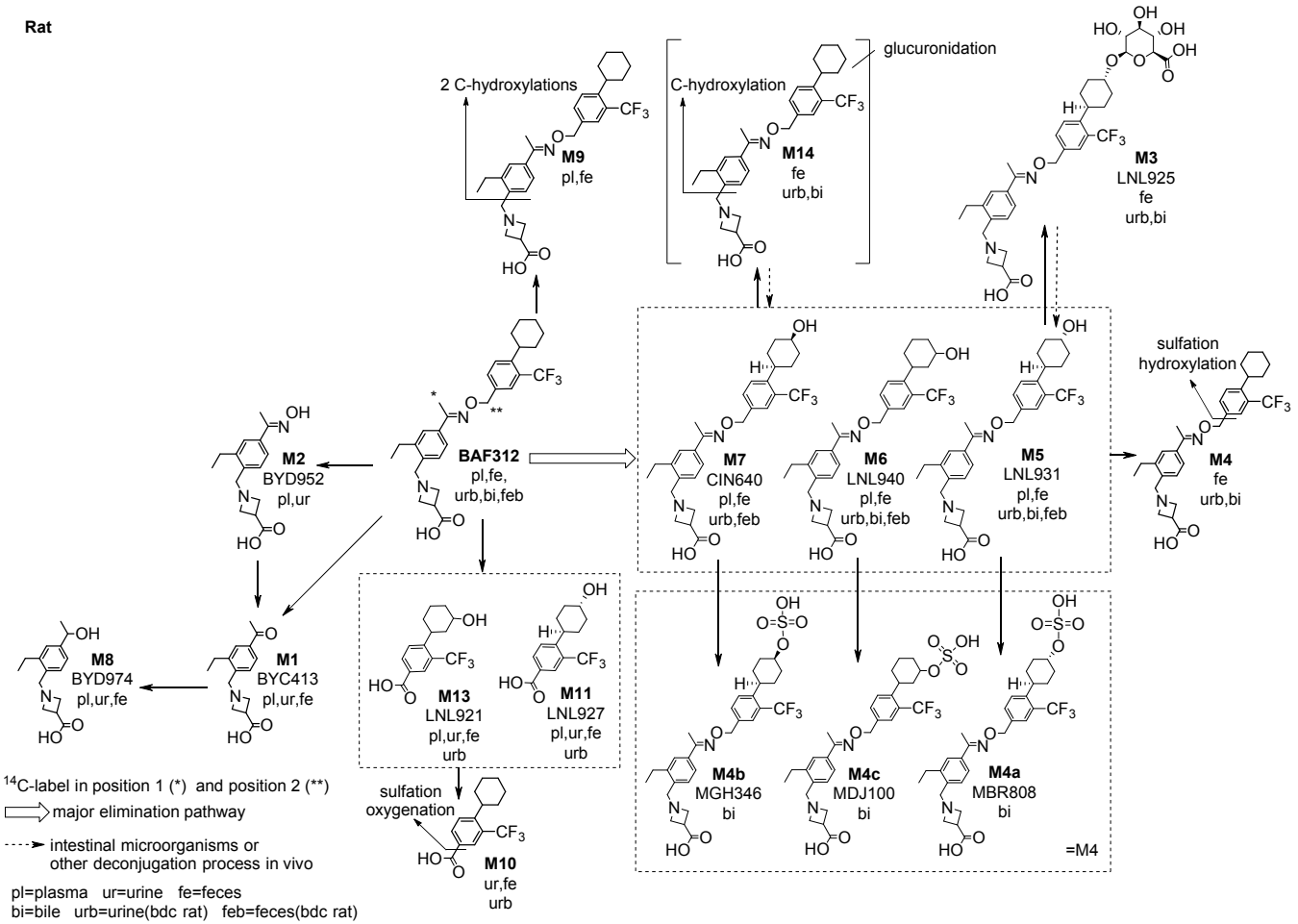
Source: Table 2.6.5.9A-DMPK R1300411-02, Table 2.6.5.9B-DMPK R0500017-02, Table 2.6.5.9C-DMPK R0900164-01, Table 2.6.5.9E-DMPK R1000166-01, CBAF312A2104, DMPK RCBAF312A2104d, Table 2.6.5.2S- RD-2015-00908

2.6.5.11C Pharmacokinetics: Metabolic pathways *in vivo* in mouse:



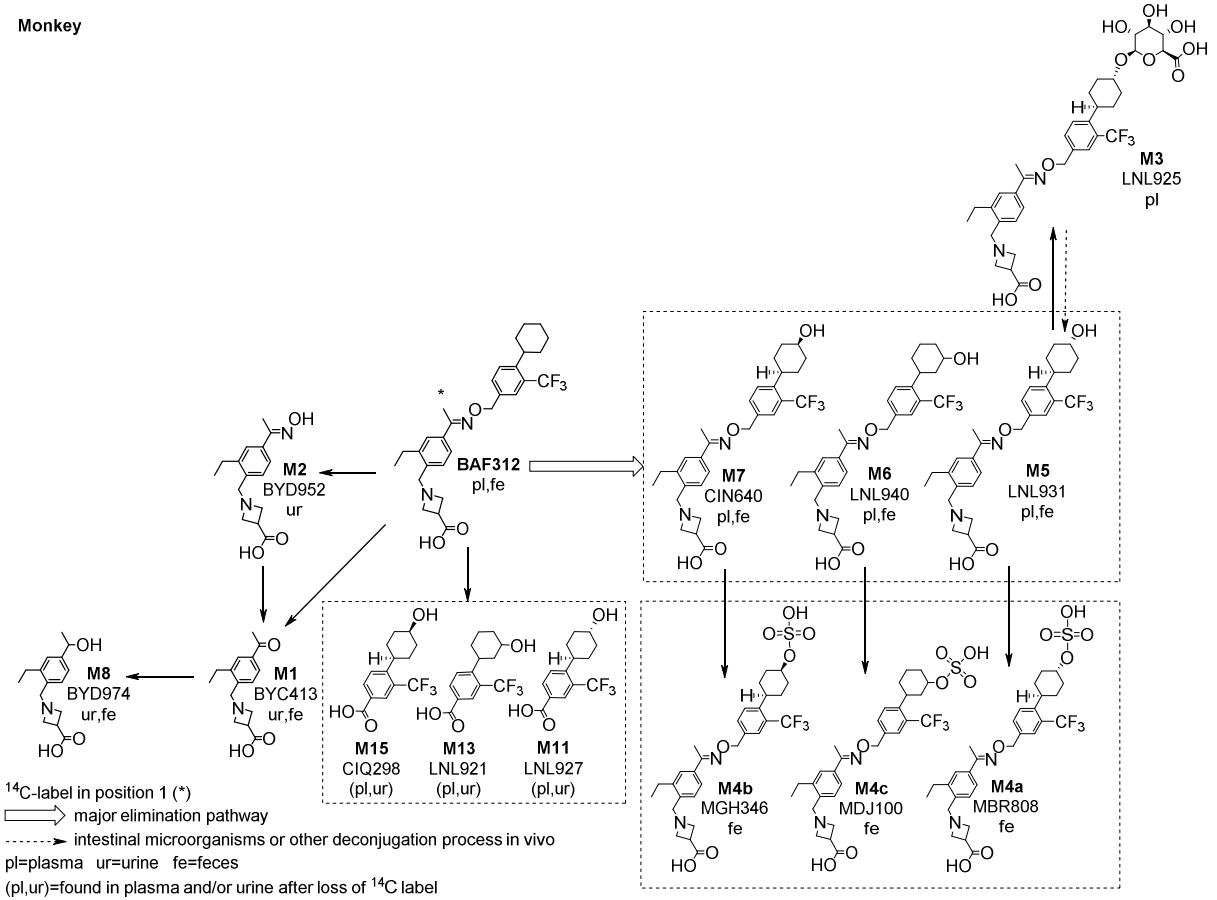
Source: Table 2.6.5.9A-DMPK R1300411-02, Table 2.6.5.2S- RD-2015-00908

2.6.5.11D Pharmacokinetics: Metabolic pathways *in vivo* in rat:



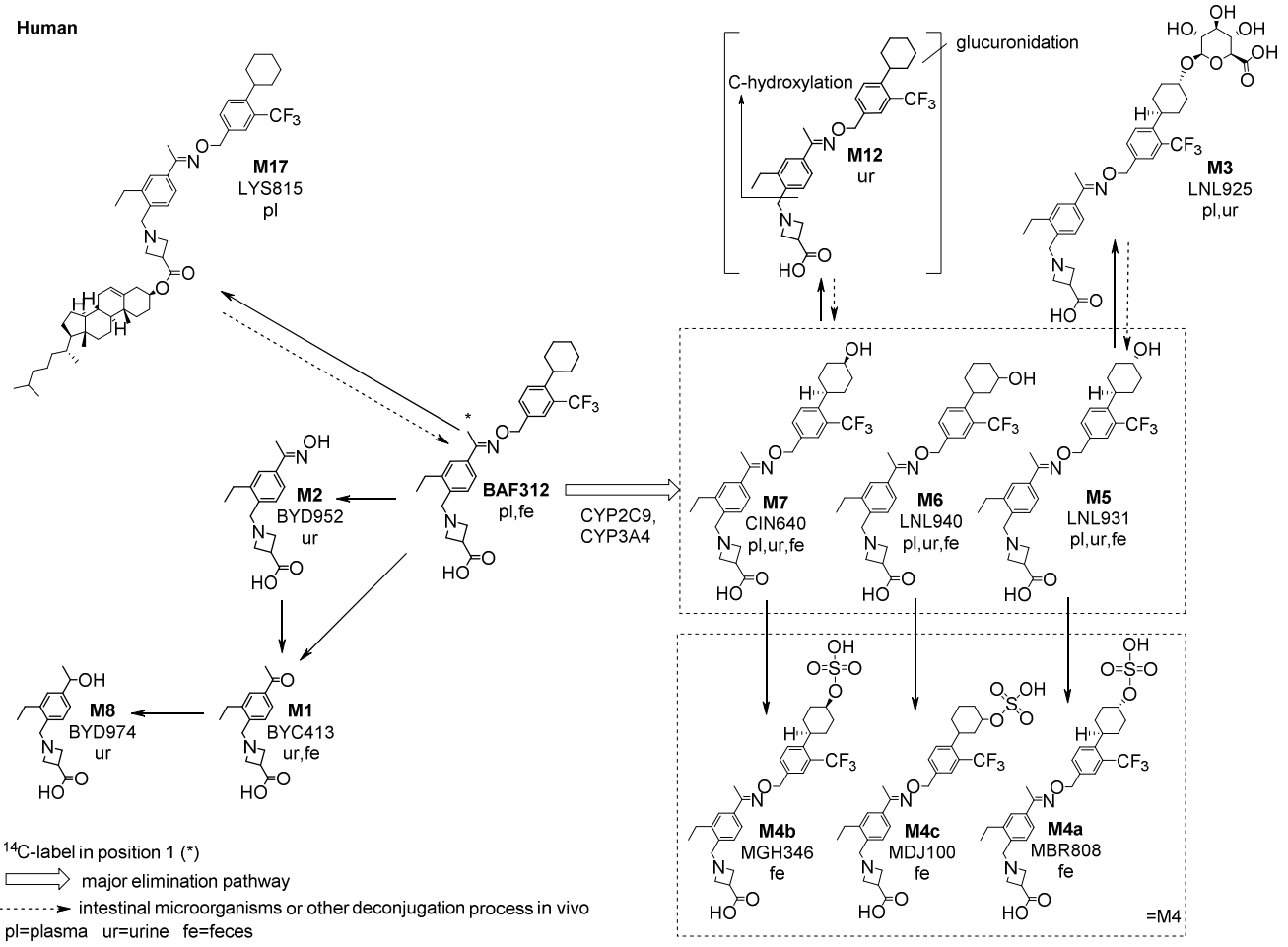
Source: Table 2.6.5.9B-DMPK R0500017-02, Table 2.6.5.9C-DMPK R0900164-01, Table 2.6.5.2S- RD-2015-00908

2.6.5.11E Pharmacokinetics: Possible metabolic pathways *in vivo* in monkey:



Source: Table 2.6.5.9E-DMPK R1000166-01, Table 2.6.5.2S- RD-2015-00908

2.6.5.11F Pharmacokinetics: Possible metabolic pathways *in vivo* in human:



Source: CBAF312A2104, DMPK RCBAF312A2104d, Table 2.6.5.2S- RD-2015-00908

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導／阻害

該当なし

2.6.5.13 薬物動態試験：累積排泄

2.6.5.13A Pharmacokinetics: Excretion – Mouse (DMPK R1300411-02)

Study title:		Pharmacokinetics, distribution, metabolism and excretion in mice after oral administration of [^{14}C]BAF312			Study no. DMPK R1300411-02
Location in CTD:		4.2.2.2-1			
GLP compliance:		not required			
Test article:		[^{14}C]BAF312 hemifumarate			
Species / Strain:		Mouse / CD-1			
Gender(M/F) / Number of animals:		M / 3			
Feeding condition:		Water and food ad libitum			
Vehicle / Formulation		Suspension in aqueous solution of 0.5% Hydroxypropylcellulose			
Route of dosing:		Oral (gavage) / single dose			
Dose (mg/kg):		8 (referring to free base)			
Analyte /Radionuclide:		Radioactivity / ^{14}C			
Samples:		Urine, feces (daily up to 96 h and 96-168 h), carcass and cage wash (168 h)			
Assay:		Radioactivity: Liquid scintillation counting (LSC)			

		8 mg/k oral dose			
Excretion route:		Urine (% of dose)	CV (%)	Feces (% of dose)	CV (%)
Interval (h)	0 – 24	0.304 ± 0.085	27.9	30.1 ± 1.08	3.58
	24 – 48	0.817 ± 0.680	83.2	24.9 ± 1.58	6.36
	48 – 72	0.343 ± 0.224	65.4	9.47 ± 1.31	13.9
	72 − 96	0.200 ± 0.090	45.2	4.45 ± 0.172	3.87
	96 − 168	0.344 ± 0.272	78.9	5.95 ± 0.418	7.02
	0 – 168 (cumulative excretion)	2.01 ± 0.612	30.5	74.9 ± 3.55	4.74
Brain	168	0.164 ± 0.216 (CV%: 132)			

Carcass	168	16.1 ± 2.96 (CV%: 18.4)
Cage wash	168	5.39 ± 1.18 (CV%: 21.8)
Total recovery		98.5 ± 1.35 (CV%: 1.37)
Additional information:		All data are expressed as mean or as mean ± SD; BAF312 (% of dose) (Table 2.6.5.9A-DMPK R1300411-02)
Conclusion:		Within 168 h after oral dosing, total radiolabeled components were predominantly excreted via feces and only to a minor extent via urine. Most of the radioactivity determined in the cage wash may also be attributed to urinary excretion. According to the cumulative fraction excreted in the consecutive 24-h periods, excretion appeared to be relatively slow. At 168 h after oral dosing, a high fraction of radioactivity was recovered in carcass, being in line with the results of the QWBA study in which significant amounts of total radiolabeled components were still detected at 336 h post-dose. Mass balance was complete.

2.6.5.13B Pharmacokinetics: Excretion – Rat (DMPK R0500017-02)

Study title:		Absorption, distribution, metabolism and excretion in male rats after single intravenous (1 mg/kg) and peroral (3 mg/kg) administration of [^{14}C]BAF312				Study no. DMPK R0500017-02	
Location in CTD:		4.2.2.2-2					
GLP compliance:		not required			not required		
Test article:		[^{14}C]BAF312			[^{14}C]BAF312		
Species / Strain:		Rat (HAN:WIST) albino			Rat (HAN:WIST) albino		
Gender(M/F) / Number of animals:		M / 3			M / 3		
Feeding condition:		Water and food ad libitum			Water and food ad libitum		
Vehicle / Formulation		Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)			Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)		
Route of dosing:		Intravenous bolus (into vena femoralis) / single dose			Oral gavage / single dose		
Dose (mg/kg):		1			3		
Analyte /Radionuclide:		Radioactivity / ^{14}C			Radioactivity / ^{14}C		
Samples:		Daily up to 96 h			Daily up to 96 h		
Assay:		Liquid scintillation counting (LSC)			Liquid scintillation counting (LSC)		
		1 mg/kg intravenous dose			3 mg/kg oral dose		
Excretion route:		Urine (% of dose)	Feces (% of dose)	Total	Urine (% of dose)	Feces (% of dose)	Total
Interval (h)	0 – 24	4.72	63.3	68.1	3.83	36.2	40.0
	24 – 48	1.53	24.8	26.3	2.50	49.1	51.6
	48 – 72	0.218	2.21	2.43	0.457	5.77	6.23
	72 − 96	0.114	0.650	0.764	0.162	0.893	1.05
	0 – 96 (cumulative excretion)	6.58 ± 0.80	91.0 ± 4.34	97.6 ± 4.31	6.95 ± 1.26	92.0 ± 2.17	98.93 ± 0.92
Carcass		not determined			not determined		
Cage wash	96	1.60			1.20		
Urine and feces	96	97.6			98.9		
Total recovery		**99.2 ± 2.41**			**100 ± 0.47**		
Additional information:		All data are expressed as mean or mean ± SD					

Conclusion:	Complete recovery of administered ^{14}C-radioactivity was achieved in excreta and cage wash within 96 h for the intravenous (99.2 ± 2.4%) and peroral route of administration (100 ± 0.47%). For both routes of administration, the major fraction of administered radioactivity was recovered in feces (intravenous: 91.0%; peroral: 92.0%) and only a minor fraction in urine (intravenous: 6.58%; peroral: 6.95%).

2.6.5.13C Pharmacokinetics: Excretion – Rat (DMPK R0900164-01)

Study title:		Absorption, distribution, and metabolism in male rats after single intravenous (1 mg/kg) and peroral (3 and 10 mg/kg) administration of [^{14}C]BAF312 (with radiolabel on the benzyl-oxyamino part of the molecule)					Study no. DMPK R0900164-01
Location in CTD:		4.2.2.2-3					
GLP compliance:		not required				not required	
Test article:		[^{14}C]BAF312 hemifumarate (with radiolabel on the benzyl-oxyamino part of the molecule)				[^{14}C]BAF312 hemifumarate (with radiolabel on the benzyl-oxyamino part of the molecule)	
Species / Strain:		Rat / Hanover Wistar				Rat / Hanover Wistar	
Gender(M/F) / Number of animals:		M / 3				M / 3	
Feeding condition:		Water and food *ad libitum*				Water and food ad libitum	
Vehicle / Formulation		Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)				Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)	
Route of dosing:		Intravenous bolus (into vena femoralis) / single dose				Oral gavage / single dose	
Dose (mg/kg):		1 (referring to free base)				3 (referring to free base)	
Analyte /Radionuclide:		Radioactivity / ^{14}C				Radioactivity / ^{14}C	
Analyte:		BAF312				BAF312	
Samples:		Daily up to 168 h				Daily up to 168 h	
Assay:		Radioactivity: Liquid scintillation counting (LSC) BAF312: HPLC-RA				Radioactivity: Liquid scintillation counting (LSC) BAF312: HPLC-RA	
		1 mg base/kg intravenous dose				3 mg base/kg oral dose	
Excretion route:		Urine (% of dose)	CV (%)	Feces (% of dose)	CV (%)	Urine (% of dose)	Feces (% of dose)
Interval (h)	0 – 24	1.43 ± 0.838	58.6	44.9 ± 20.8	46.3	0.578	28.0
	24 – 48	0.753 ± 0.529	70.3	37.6 ± 19.4	51.6	0.363	54.3
	48 – 72	0.881 ± 0.751	85.2	9.78 ± 1.88	19.2	0.292	6.61
	72 − 96	0.140 ± 0.132	94.3	1.60 ± 0.686	42.9	0.0872	3.60
	96 − 168	0.148 ± 0.126	85.1	1.89 ± 0.638	33.8	0.123	2.22
	0 – 168 (cumulative excretion)	3.35 ± 0.827	24.7	95.8 ± 2.52	2.63	1.44	94.7

BAF312	0-96	Not detectable	3.13	Not detectable	9.41
Cage wash	168	0.877 ± 0.208		0.695	
Carcass	168	0.424 ± 0.0416		0.823	
Urine	0 – 168	3.35 ± 0.827		1.44	
Feces	0 – 168	95.8 ± 2.52		94.7	
Total recovery		**100 ± 2.38 (CV%: 2.38)**		**97.6**	
Additional information:		Intravenous dosing: all data are expressed as mean ± SD; oral dosing: n=2, data are expressed as mean only.			
Conclusion:		Independent of the route of administration, total radiolabeled components were excreted in intact rats pre-dominantly via feces. Total radiolabeled components were excreted completely within 168 h after dosing indicated by a recovery of less 1% in the carcass.			

2.6.5.13D Pharmacokinetics: Excretion – Monkey (DMPK R1000166-01)

Study title:		Absorption, metabolism and excretion following an oral dose of [^{14}C]BAF312 in the monkey	Study no. DMPK R1000166-01
Location in CTD:		4.2.2.2-6	
GLP compliance:		not required	
Test article:		[^{14}C]BAF312 hemifumarate	
Species / Strain:		Monkey / Cynomolgus	
Gender(M/F) / Number of animals:		M / 3	
Feeding condition:		Water and food ad libitum	
Vehicle / Formulation		0.5% (w/v) hydroxypropyl-cellulose (grade HF, Klucel) solution	
Route of dosing:		Oral (gavage) / single dose	
Dose (mg/kg):		15 (referring to free base)	
Analyte /Radionuclide:		Radioactivity / ^{14}C	
Samples:		0 – 24, 24 – 48, 48 – 72, 72 − 96, 96-144 h, every 24 h (Day 8, Day 9, Day 10,Day 11), 264-336 h	
Assay:		Radioactivity: Liquid scintillation counting (LSC)	
		15 mg/kg oral dose	
Excretion route:		Urine (% of dose)	Feces (% of dose)
Interval (h)	0 – 24	0.559 ± 0.168	16.6 ± 14.3
	24 – 48	0.485 ± 0.202	33.4 ± 8.56
	48 – 72	0.391 ± 0.277	32.4 ± 13.2
	72 − 96	0.105 ± 0.047	7.34 ± 2.23
	96 − 168	0.109 ± 0.134	6.37 ± 3.74
	Day 8	0.024 ± 0.023	0.612 ± 0.286
	Day 9	0.013 ± 0.011	0.418 ± 0.196
	Day 10	0.017 ± 0.014	0.118 ± 0.066
	Day 11	0.014	0.232 ± 0.089
	264 − 336	0.0186	0.238 ± 0.127
	0 – 336 (cumulative excretion)	1.71 ± 0.52	98.2 ± 3.10

Total recovery (excreta and cage wash)	99.9 ± 3.11
Additional information:	All data are expressed as mean or mean ± SD; urine data also include cage wash.
Conclusion:	Fecal excretion was the major elimination route, accounting for 98.2% of dose, and most of dose was recovered from feces within the first 7 days (~96%). Total recovery of radioactivity in the excreta was complete.

2.6.5.14 薬物動態試験：胆汁中排泄

2.6.5.14A Pharmacokinetics: Excretion into Bile (DMPK R0900164-01)

Study title:		Absorption, distribution, and metabolism in male rats after single intravenous (1 mg/kg) and peroral (3 and 10 mg/kg) administration of [^{14}C]BAF312 (with radiolabel on the benzyl-oxyamino part of the molecule)								Study no. DMPK R0900164-01
Location in CTD:		4.2.2.2-3								
GLP compliance:		not required			not required					
Test article:		[^{14}C]BAF312 hemifumarate (with radiolabel on the benzyl-oxyamino part of the molecule)			[^{14}C]BAF312 hemifumarate(with radiolabel on the benzyl-oxyamino part of the molecule)					
Species / Strain:		Rat / Hanover Wistar			Rat / Hanover Wistar					
Gender(M/F) / Number of animals:		M / 2			M /3					
Feeding condition:		Water and food ad libitum			Water and food ad libitum					
Vehicle / Formulation		Ethanol/polyethylene glycol 200 (PEG200)/5% (w/v) aqueous glucose solution (1/6/3)			Aqueous 0.5% (w/v) hydroxypropylcellulose solution					
Route of dosing:		Intravenous bolus (into vena femoralis) / single dose			Oral gavage / single dose					
Dose (mg/kg):		1 (referring to free base)			10 (referring to free base)					
Analyte /Radionuclide:		Radioactivity / ^{14}C			Radioactivity / ^{14}C					
Analyte:		BAF312			BAF312					
Samples:		0 − 24 h and 24 − 48 h			0 − 24 h and 24 − 48 h					
Assay:		Radioactivity: Liquid scintillation counting (LSC) BAF312: HPLC-RA			Radioactivity: Liquid scintillation counting (LSC) BAF312: HPLC-RA					
		1 mg/kg intravenous dose			3 mg/kg oral dose					
Excretion route:		Urine (% of dose)	Feces (% of dose)	Bile (% of dose)	Urine (% of dose)	CV (%)	Feces (% of dose)	CV (%)	Bile (% of dose)	CV (%)
Interval (h)	0 – 24	1.31	1.05	55.9	0.711 ± 0.210	29.5	14.9 ± 10.6	71.1	46.1 ± 15.6	33.8
	24 – 48	0.574	3.45	15.8	0.645 ± 1.01	157	10.7 ± 10.4	97.2	10.7 ± 4.58	42.8
	0 – 48 (cumulative excretion)	1.88	4.50	71.7	1.35 ± 1.13	83.7	25.6 ± 9.59	37.5	56.9 ± 13.9	24.4
BAF312	0-48	Not	2.69	8.16	n.a.		n.a.		n.a.	

		detectable	
Cage wash	48	14.2	0.988 ± 0.733 (CV%: 74.2)
Carcass	48	0.376	8.39 ± 4.42 (CV%: 52.7)
GI tract	48	3.06	2.84 ± 1.62 (CV%: 57.0)
Urine	0 − 48	1.88	1.35 ± 1.13 (CV%: 83.7)
Feces	0 − 48	4.50	25.6 ± 9.59 (CV%: 37.5)
Bile	0 − 48	71.7	56.9 ± 13.9 (CV%: 24.4)
Total recovery		95.7	96.0 ± 0.876 (CV%: 0.913)
Additional information:		Intravenous dosing: n=2, data are expressed as mean only. Two rats had to be sacrificed before compound administration due to blockade of the effluent bile cannula or due to bile flow into the abdomen. Oral dosing: all data are expressed as mean ± SD.	
Conclusion:		Independent of the route of administration, total radiolabeled components were excreted in bile duct-cannulated rats pre-dominantly *via* bile.	

2.6.5.15 薬物動態試験：薬物相互作用

該当なし

2.6.5.16 薬物動態試験：その他

2.6.5.16A Pharmacokinetics: Other (DMPK R0700963)

Study title:	Solute-carrier mediated uptake of BAF312 into hepatocytes from rat, monkey and human	Study No. DMPK R0700963
Location in CTD	4.2.2.7-1	
GLP compliance	not required	
Test article	[^{14}C]BAF312	
Type of study	Hepatic uptake across species was assessed by measuring the time- and concentration-dependent BAF312 uptake. The involvement of several candidate transporter families (OAT, OATP, OCT) was investigated using representative uptake transporter inhibitors.	
Test system	Primary cultures of rat, monkey and human hepatocytes	
Assay	Radioactivity analysis by Liquid scintillation counting (LSC)	

Summary of results

Species	$K_{m,app}$ [a)] (μM)	CL_m [a, b)] (μL/min/mg Protein)	CL_c [a, c)] (μL/min/mg Protein)	Inhibitor(s)	Possible transporters [d)]
Rat	n.a.	10.2 ± 2.8	n.a.	not effective	n.a.
Monkey	n.a.	15.2 ± 1.5	n.a.	not effective	n.a.
Human	n.a.	10.1 ± 4.2	n.a.	not effective	n.a.

a) mean ± SD

b) Nonspecific (passive) uptake clearance

c) Carrier-mediated (saturable) uptake clearance

d) Proposed based on the inhibition profile observed in the presence of RSV (OATP family inhibitor), PAH (OAT family substrate) and TEA (OCT family substrate)

n.a. not applicable

Conclusions:

BAF312 was not a substrate of an active hepatic uptake transporter system neither in rats, monkeys nor humans. In all three species the passive diffusion component (CL_m) was similar (10-15 μL/min/mg) and relatively high. Neither RSV, PAH nor TEA showed any significant inhibitory effects on hepatic uptake by rat, monkey and human hepatocytes confirming the absence of transporter activity.