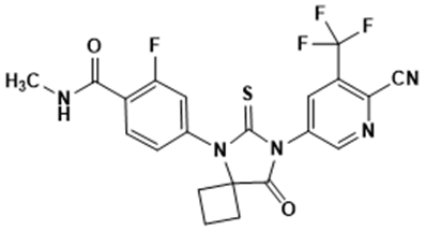
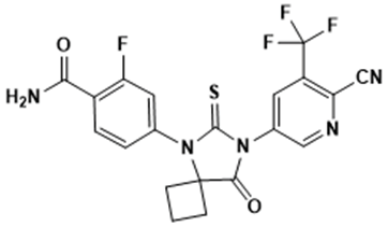
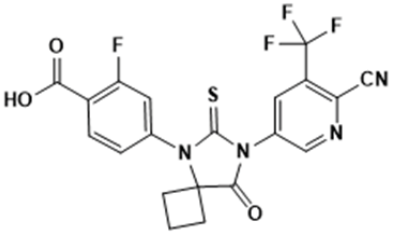
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略号一覧表

略号又は略称	化学名又は一般名	構造式	由来
アパルタミド（JNJ-56021927, ARN-509）	4-{7-[6-Cyano-5-(trifluoromethyl)pyridin-3-yl]-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-5-yl}-2-fluoro-*N*-methylbenzamide		主薬
JNJ-56142060（ARN000308, ARN-308）	*N*-脱メチル体（M3）		代謝物
JNJ-56142021（ARN000066, ARN-66）	アミド加水分解体（カルボン酸）（M4）		代謝物

略号又は略称	名称及び内容
$Ae_{0-\infty}$	0 時間から無限大時間までの累積排泄率（cumulative excretion ratio from time zero to infinite time）
AGP	α_1-酸性糖タンパク質（α_1-acid glycoprotein）
AUC	血漿中濃度－時間曲線下面積（area under the plasma concentration-time curve）
AUC_{0-24h}	0 時間から 24 時間までの血漿中濃度－時間曲線下面積（area under the plasma concentration-time curve from time zero to 24 hours）
AUC_{0-72h}	0 時間から 72 時間までの血漿中濃度－時間曲線下面積（area under the plasma concentration-time curve from time zero to 72 hours）
AUC_{0-528h}	0 時間から 528 時間までの血漿中濃度－時間曲線下面積（area under the plasma concentration-time curve from time zero to 528 hours）
$AUC_{0-\infty}$	0 時間から無限大時間までの血漿中濃度－時間曲線下面積（area under the plasma concentration-time curve from time zero to infinite time）
AUC_{last}	0 時間から最終定量可能時間までの血漿中（組織内）濃度－時間曲線下面積［area under the plasma (or tissue) concentration-time curve from time zero to the last quantifiable time］
BCRP	乳癌耐性タンパク質（breast cancer resistance protein）
BNPP	ビスニトロフェニルリン酸（bis-nitrophenyl phosphate）
Caco-2	human colon adenocarcinoma-2

略号又は略称	名称及び内容
CHO	チャイニーズハムスター卵巣（Chinese hamster ovary）
CL_p	全身血漿クリアランス（total plasma clearance）
C_{max}	最高血漿中濃度（maximum plasma concentration）
CSF	脳脊髄液（cerebral spinal fluid）
CYP	チトクローム P450（cytochrome P450）
GLP	医薬品の安全性に関する非臨床試験の実施の基準 （Good Laboratory Practice）
HSA	ヒト血清アルブミン（human serum albumin）
IC_{50}	50%阻害濃度（half-maximal inhibitory concentration）
LC-MS/MS	液体クロマトグラフィーータンデム質量分析 （liquid chromatography-tandem mass spectrometry）
LLC-PK	Lilly Laboratories cell-porcine kidney
MATE	多剤・毒性化合物排出タンパク質（multidrug and toxin extrusion protein）
MDCKII	Madin-Darby canine kidney strain II
MDR	多剤耐性タンパク質（multidrug-resistance protein）
NADPH	ニコチンアミドアデニンジヌクレオチドリン酸（還元型） ［nicotinamide adenine dinucleotide phosphate (reduced form)］
OAT	有機アニオントランスポーター（organic anion transporter）
OATP	有機アニオン輸送ポリペプチド（organic anion-transporting polypeptide）
OCT	有機カチオントランスポーター（organic cation transporter）
P_{app}	見掛けの透過係数（apparent pearmeability）
PEG	ポリエチレングリコール（polyethylene glycol）
P-gp	P-糖タンパク質（P-glycoprotein）
$t_{1/2}$	消失半減期（elimination half-life）
TK	トキシコキネティクス（toxicokinetics）
t_{max}	最高血漿中濃度到達時間（time to reach the maximum plasma concentration）
VitE-TPGS	水溶性ビタミン E（D-α-トコフェロール・ポリエチレングリコール・コハク酸エステル） ［water-soluble vitamin E (D-α-tocopherol polyethylene glycol succinate conjugate)］
V_{ss}	定常状態における分布容積（volume of distribution at steady state）

2.6.4 薬物動態試験の概要文

2.6.4.1 まとめ

アパルタミド（JNJ-56021927，ARN-509）は，去勢抵抗性前立腺癌患者の治療のために開発された，アンドロゲン受容体の強力かつ特異的な阻害薬である。本項では，*in vitro* 及び *in vivo* 試験系で得られたアパルタミドの吸収，分布，代謝，排泄及び薬物動態学的薬物相互作用の可能性に関する検討結果について要約する。アパルタミドの *in vivo* 試験は，マウス，ラット及びイヌで実施した。これらの動物種は薬理及び毒性試験で用いられたものと同じであり，系統も同じものを使用した。

2.6.4.1.1 吸収

CD-1 マウス，Sprague-Dawley ラット及びビーグル犬を用いて，アパルタミド並びに代謝物 M3（JNJ-56142060，アパルタミドの *N*-脱メチル体）及び M4（JNJ-56142021，アパルタミド又は M3 のアミド加水分解体）の薬物動態を評価するための単回及び反復投与試験を実施した。反復投与後の薬物動態については，ラット及びイヌを用いた反復投与毒性試験で実施されたトキシコキネティクス（TK）の結果からも得られた。アパルタミドのほとんどの薬物動態及び TK 試験で使用された経口製剤は，Acconon MC8-2 EP/NF，Capmul MCM EP，ポリエチレングリコール（PEG）400 USP 及び水溶性ビタミン E（D-α-トコフェロール・ポリエチレングリコール・コハク酸エステル：VitE-TPGS NF）（10：45：30：15% w/w/w/w）から成る脂質性エマルジョンであり，以後「脂質ベースのエマルジョン」と表記する。この製剤は，[redacted]である。

マウス，ラット及びイヌにおいて，アパルタミドは中程度の分布容積（2.1～2.8 L/kg）を有する低クリアランス（0.5～3.9 mL/min/kg）分子であった。アパルタミドの消失半減期（$t_{1/2}$）は，マウス（20 時間）及びラット（7 時間）と比較してイヌでより長かった（69 時間）。経口投与したときのバイオアベイラビリティは，マウスで高く（10 mg/kg の用量で 93%），イヌではほぼ 100%であった（10 mg/kg）。なお，ラットのバイオアベイラビリティは評価していない。

すべての動物種において，アパルタミド単回投与後の曝露量は，おおむね用量に比例した。アパルタミドの最高血漿中濃度（C_{max}）は，おおむね投与 2～12 時間後に認められ，最高血漿中濃度到達時間（t_{max}）は用量に依存しなかった。代謝物（M3 及び M4）はおおむね投与 24 時間後までに C_{max} に達した。経口投与時の活性代謝物 M3 の曝露量は不活性代謝物である M4 の約 2 倍であった。

反復投与したとき，低用量側ではアパルタミドの曝露量はおおむね用量に比例して増加したが，高用量側では曝露量は増加しなかったか，又は用量比例を下回って増加した。一方，代謝物（M3 及び M4）の曝露量は用量の増加とともに増加し，しばしば用量比例を上回った。ラット及びイヌにおける反復投与時のアパルタミドの累積比はそれぞれ 2～3 及び 5～8 であった。

2.6.4.1.2 分布

10 μg/mL の濃度において，^{14}C-アパルタミド及び ^{14}C-JNJ-56142060（^{14}C-M3）の血漿中非結合型分率はマウス，ラット及びイヌで同程度であったが（アパルタミド：6.3～8.2%，M3：8.2～9.4%），ヒトではやや低かった（アパルタミド：4.2%，M3：5.1%）。これらの動物種及びヒトにおける血漿タンパク結合率は，アパルタミド及び M3 の濃度（0.1～30 μg/mL）に依存しなかった。アパルタミド及び M3 の精製ヒト血清アルブミン（HSA）への結合率（それぞれ 93.6 及び 93.2%）は，ヒト血漿タンパク結合率（それぞれ 95.8 及び 94.9%）と同程度で，精製ヒト α_1-酸性糖タンパク質（AGP）への結合率（約 20%）よりも高かった。

動物及びヒトにおける ^{14}C-アパルタミド及び ^{14}C-M3 の血球移行を *in vitro* で評価した。アパルタミド及び M3 の各血液中画分（血漿水分画分，血漿タンパク結合画分又は血球結合画分）での存在割合を算出したところ，すべての種（マウス，ラット，イヌ，ウサギ及びヒト）において，^{14}C-アパルタミドの分布は ^{14}C-M3 と類似し，血液中の ^{14}C-アパルタミド及び ^{14}C-M3 は，血漿タンパク結合画分に最も多く分布し（0.500～0.685），かなりの割合が血球結合画分にも分布した（0.285～0.424）。

全身オートラジオグラフィーにより，白色及び有色の雄ラットに ^{14}C-アパルタミドを単回経口投与したときの組織分布を評価した。放射能は広範な組織に分布し，ほとんどの組織において投与 4～12 時間後に最高濃度を示すことが明らかとなった。放射能は，腹部及び褐色脂肪，肝臓，腎皮質及び髄質，副腎皮質，膵臓並びにハーダー腺において最も高い濃度を示し，水晶体，脳及び骨表面において最も濃度が低かった。微量の放射能が皮膚に残存したが，有色皮膚内の濃度は白色皮膚と同程度であり，ブドウ膜中に放射能の残存が認められなかったことから，薬物由来物質のメラニンへの結合はほとんどないと考えられる。

いくつかの薬物動態試験において，アパルタミド及び M3 の脳内濃度を測定した。雄マウスでは，脳内濃度は用量に比例し，最高濃度はおおむね投与 16 時間後に認められた。投与 24 時間後のアパルタミドの脳／血漿中濃度比は，M3 の約 1.5 倍であった。アパルタミドをイヌに反復投与して同様の検討を行ったところ，雌イヌの試験では，アパルタミド及び M3 の脳内濃度は用量比例を上回って増加した。雄イヌの試験では，アパルタミド，M3 及び M4 の脳内濃度は用量にほぼ比例して増加した。一方，脳脊髄液（CSF）中において，アパルタミド濃度は用量にほぼ比例して増加し，M3 濃度は用量比例を下回って増加し，M4 濃度は高用量群で用量比例を上回って増加した。

2.6.4.1.3 代謝

無処置及び胆管カニュレーションした雄ラット（50 mg/kg），雄イヌ（10 mg/kg）並びにヒト被験者（240 mg）に ^{14}C-アパルタミドを単回経口投与し，定量的な代謝試験を実施した。アパルタミド及び主要代謝物の曝露量から，毒性試験に用いる動物種としてラット及びイヌを選択したことの適切性が確認された。

ラット血漿では，投与 12 及び 24 時間後の試料中放射能の大部分は，アパルタミド（それぞれの時点で 65 及び 53%）及び M4（12 及び 15%）由来であった。いずれの時点でも，その他に試料中放射能の 10%を超える代謝物は検出されなかった。イヌの試験では，アパルタミド及び M3 が循環血漿中の主要な成分であった。0 時間から 528 時間までの血漿中濃度－時間曲線下面積

（$AUC_{0\text{-}528h}$）に基づくと，未変化体，M3 及び M4 の存在量は，血漿中放射能のそれぞれ 41，51 及び 4%であった。ヒト被験者では，0 時間から最終定量可能時間までの血漿中濃度－時間曲線下面積（AUC_{last}）で評価したときの主要な血漿中成分は，未変化体（175 μg·h/mL）及び M3（169 μg·h/mL）であり，いずれも M4（11.1 μg·h/mL）よりも 15 倍以上高かった。すべてのイヌ試料について，各採血時点での未変化体，M3 及び M4 の濃度の合計は，血漿中放射能濃度の 90%以上に相当し，また，ヒトにおけるアパルタミド，M3 及び M4 の AUC_{last} の合計は，血漿中放射能の値（413 μg eq·h/mL）に近かった。これらの結果から，イヌ及びヒトでは血漿中放射能に占める他の代謝物の割合は極めて限定的と考えられる。

アパルタミドは，ラット，イヌ及びヒトにおいて広範に代謝された。M4 は，尿（ラット，イヌ及びヒト），糞（ラット及びイヌ）並びに胆汁（ラット）中に最も多く認められた代謝物である。したがって，*N*-メチルベンズアミド基のアミドへの *N*-脱メチル化（M3）及びそれに続くカルボン酸へのアミド加水分解（M4），並びに *N*-メチルベンズアミド基の M4 への直接的アミド加水分解がアパルタミドの主要な代謝クリアランス経路であると考えられる。イヌ及びヒトでは，アパルタミドのジアゾスピロ環の開環及び置換ピリジニル基の脱離による M19 の生成，並びにそれに続く *N*-脱メチル化による M20 の生成も比較的重要な代謝経路であった。実際に，イヌ及びヒトでは尿中に，それぞれ 6 及び 7%に相当する M20 が認められ，M4 以外のすべての代謝物より多かった。アパルタミドから直接生成する他の代謝物として，酸化的脱硫化体である M2（JNJ-56142047），システイン縮合体である M9，グリシル-システイン縮合体である M10 及びニトリル加水分解体である M13 が認められた。これらの代謝物はその後の *N*-脱メチル化によってアミドに代謝され，さらにアミド加水分解によって 2-フルオロカルボン酸に代謝される。ラットで同定された代謝物は少なく，それらは M2/M5，M3/M4 及び M9/M17 のように親／誘導体の関係であった。

アパルタミドをラット，イヌ及びヒトの肝 S9 画分又はミクロソームとともにインキュベーションしたとき，アパルタミドの代謝速度は遅かった。ヒト肝細胞を用いたリレー法により，アパルタミド及び M3 を代謝する酵素を検討したところ，M3 は主にチトクローム P450（CYP）2C8 を介して生成し，それより若干程度は小さいが CYP3A4 を介しても生成した。また，M4 の生成は，カルボキシエステラーゼ阻害薬であるビスニトロフェニルリン酸（BNPP）によって完全に阻害された。さらに，M3 を用いた別のリレー試験においても，M4 への代謝は BNPP によって完全に阻害された。これらの結果から，M4 の生成にはエステラーゼ（おそらくカルボキシエステラーゼ）のみが関与することが示された。

2.6.4.1.4 排泄

放射能の尿糞中排泄を，ラットで 168 時間，イヌで 360 時間及びヒトで 71 日間にわたって測定した。放射能の尿及び糞中への累積排泄率（投与量に対する%）は，それぞれ，ラットで 34 及び 58%，イヌで 42 及び 40%，ヒトで 65 及び 24%であった。胆管カニュレーションしたラットにおいて，放射能は投与 72 時間後までに投与量の 50%が胆汁中に排泄され，尿及び糞中にはそれぞれ 30 及び 11%が排泄された。

2.6.4.1.5　薬物動態学的薬物相互作用の可能性

アパルタミド及び M3 は，30 μmol/L までの濃度で，ヒト培養肝細胞中 CYP2B6 及び CYP3A4 を誘導した。*In vitro* では，アパルタミドは CYP2C8 及び CYP2B6 の中程度の阻害薬であり，50%阻害濃度（IC_{50}）はそれぞれ 13.9 及び 36 μmol/L であった。アパルタミドは CYP2C9，CYP2C19 及び CYP3A4 の弱い阻害薬であり，IC_{50} は 54～67 μmol/L の範囲であった。M3 は CYP2B6，CYP2C8 及び CYP2C9 の中程度の阻害薬であり，IC_{50} は 38～49 μmol/L の範囲であった。

トランスポーターを発現させた細胞株を用いた *in vitro* 試験の結果から，アパルタミド及び M3 は多剤耐性タンパク質（MDR）1［P-糖タンパク質（P-gp）］の基質であったが，乳癌耐性タンパク質（BCRP），有機アニオン輸送ポリペプチド（OATP）1B1 及び OATP1B3 の基質ではなかった。アパルタミド及び M3 は，MDR1（P-gp），BCRP，OATP1B1 及び OATP1B3 の弱い阻害薬であり，30 μmol/L までの濃度での最大阻害率は 56%であった。アパルタミド及び M3 は，有機カチオントランスポーター（OCT）2，有機アニオントランスポーター（OAT）3，多剤・毒性化合物排出タンパク質（MATE）-1 の中程度の阻害薬であった（IC_{50} の範囲は，アパルタミド：12～27 μmol/L，M3：4.8～17.6 μmol/L）。アパルタミド及び M3 の MATE-2K に対する IC_{50} は，それぞれ 38 μmol/L 及び 50 μmol/L 超であった。アパルタミド及び M3 の OAT1 に対する阻害作用は認められなかった。

In vitro における代謝酵素のプロファイル，CYP 誘導／阻害及びトランスポーターに関するこれらの試験結果の臨床的意義については，【2.4】及び【2.7.2】において考察している。

2.6.4.2 分析法

2.6.4.2.1 アパルタミド及び代謝物

各試験における試料中のアパルタミド及び代謝物の分析は，液体クロマトグラフィーータンデム質量分析（LC-MS/MS）法によって行われた。各試験に用いられた分析法の概略を以下に示す。

医薬品の安全性に関する非臨床試験の実施の基準（GLP）を適用する試験をサポートするために使用された，アパルタミド並びに代謝物 M3 及び M4 の血漿中濃度定量法は，米国食品医薬品局（Bioanalytical Method Validation，2001 年 5 月）及び欧州医薬品庁（Guideline on Bioanalytical Method Validation，2011 年 7 月）のガイドラインに従ってバリデートされ，ガイドラインで指定されたすべての基準を満たした。定量範囲，安定性及びこの分析法が使用された GLP 適用試験等のバリデーション情報の概略を表 2.6.4-1 に示す。

GLP 非適用の試験でのアパルタミド，M3 及び M4 の血漿中及び組織内濃度の定量には，あらかじめ基準を設定した LC-MS/MS 法を使用した。定量範囲はそれぞれの報告書に記載されており，各試験での定量下限値は対応する薬物動態試験概要表【2.6.5 参照】に記載した。

2.6.4.2.2 バリデートされた LC-MS/MS 法

【評価資料 4.2.2.1.1，4.2.2.1.2，4.2.2.1.3，4.2.2.1.4，4.2.2.1.5，4.2.2.1.6，4.2.3.2.2，4.2.3.2.8】

ラット及びイヌの血漿中，並びに human Ether-à-go-go-Related Gene（hERG）試験のタイロード緩衝液中のアパルタミド及び代謝物濃度定量法バリデーションの概略を表 2.6.4-1 に示す。

表 2.6.4-1 各種条件下での血漿及びタイロード緩衝液中のアパルタミド及び代謝物濃度定量法バリデーションの概略

Testing Facility	Analytes[a]	Species	Anticoagulant (Acidifier)	Validated Range (µg/mL)	Stability Time		Freeze/Thaw Cycles	Study No. (Study Reference)	Supported GLP Studies
					Melting ice (hours)	LTS at -70°C (days)			
[redacted]	Apalutamide	Rat	EDTA (HCl)	0.100 to 50.0	12	88	4	DCN 01928LX[b] (4.2.3.2.2)	VUM00004
	Apalutamide	Dog	EDTA (HCl)	0.100 to 50.0	12	83	4	DCN 01938PY[b] (4.2.3.2.8)	VUM00005
[redacted]	Apalutamide JNJ-56142060 & JNJ-56142021	Rat	EDTA (CA)	0.0250 to 25.0 0.0100 to 10.0	6 (RT)	35	4	TSLR11-076 (4.2.2.1.1)	TX-509-1001
	Apalutamide JNJ-56142060 & JNJ-56142021	Dog	EDTA (CA)	0.0250 to 25.0 0.0100 to 10.0	6 (RT)	83	4	TSLR11-077 (4.2.2.1.2)	TX-509-1002
[redacted]	Apalutamide & JNJ-56142060	Rat	EDTA	0.0250 to 25.0	74	189 (49 days at -20°C)	5	BA10608 (4.2.2.1.3)	TOX10838, TOX10897, TOX11086
		Rat[c]	EDTA	0.0250 to 25.0	27/27[d]	304 (119 days at -20°C)/ 51[d]	2/3[d]	BA11005 (4.2.2.1.5)	TOX10942
		Dog	EDTA	0.0250 to 25.0	74	177 (70 days at -20°C)	5	BA10610 (4.2.2.1.4)	TOX10895, TOX10839, TOX10811
Janssen R&D, Beerse	Apalutamide & JNJ-56142060 (in Tyrode's solution)	-	-	0.0125 to 25.0 µmol/L	7 (RT)	78 at -20°C	Not tested	BA10615 (4.2.2.1.6)	TOX10776

[a] JNJ-56142060 = M3 metabolite; JNJ-56142021 = M4 metabolite
[b] Bioanalysis method validation report is included in GLP study report
[c] Capillary microsampling method: rat plasma was diluted with 10 volumes 5% BSA solution before sample analysis, validation range is given for plasma
[d] First value for plasma, second for BSA-diluted plasma

- not applicable; BSA = bovine serum albumin; CA = citric acid; EDTA = ethylenediaminetetraacetic acid; HCl = hydrochloric acid; LTS = long-term stability; RT = room temperature.

2.6.4.3 吸収

2.6.4.3.1 吸収

2.6.4.3.1.1 マウス

(1) 単回投与

【参考資料 4.2.2.2.1，4.2.2.2.2，4.2.2.2.3，4.2.3.4.2.1】

絶食した雄 CD-1 マウス 12 例にアパルタミドを 3 mg/kg の用量で脂質性溶液として単回静脈内投与したとき，及び絶食した雄 CD-1 マウス 9 例に 10 mg/kg の用量で懸濁液（クエン酸塩緩衝液で調製）として単回経口投与したときのアパルタミドの血漿中薬物動態を評価した（採血はいずれも 3 例／時点）【2.6.5.3A 及び 2.6.5.3B】（4.2.2.2.1 参照）。経口投与 1 時間後にアパルタミドの血漿中濃度は最高濃度に達した（C_{max}：3.51 μg/mL）。$t_{1/2}$ は，静脈内及び経口投与後で同程度であった（それぞれ 19.5 及び 17.8 時間）。静脈内投与後の定常状態における分布容積（V_{ss}）は 2.10 L/kg であり，全身血漿クリアランス（CL_p）は 1.33 mL/min/kg（肝血流量の 2%未満）であった。

また，マウスではこの他に 3 つの単回経口投与試験を実施した。最初の試験では【2.6.5.3B】（4.2.2.2.2 参照），絶食した雄 CD-1 マウス（9 例／群）にアパルタミドを 10，30 又は 100 mg/kg の用量でクエン酸塩緩衝溶液として経口投与した（採血は各群 3 例／時点）。アパルタミドの t_{max} は 2～8 時間であり，曝露量［C_{max} 及び血漿中濃度－時間曲線下面積（AUC）］は用量に比例して増加し，$t_{1/2}$ は 8.7～17.8 時間の範囲であった。

次の試験では【2.6.5.3C】（4.2.2.2.3 参照），絶食した雄 CD-1 マウス（9 例／群）にアパルタミドを 10，30 又は 100 mg/kg の用量で，脂質ベースのエマルジョン（*in vivo* 薬理試験に使用されたものと同様）として経口投与した（採血は各群 3 又は 6 例／時点）。アパルタミド及び M3（*N*-脱メチル体，JNJ-56142060）について薬物動態を評価したところ，M3 の AUC はアパルタミドの約 21～31%であった。アパルタミド及び M3 の曝露量（C_{max} 及び AUC）は，用量に比例して増加した。

3 番目の試験は，毒性試験（GLP 非適用）の単回投与期として実施し【2.6.5.3D】（4.2.3.4.2.1 参照），雌雄 CD-1 マウス（雌雄各 6 例／群）にアパルタミドを 20，100 又は 500 mg/kg の用量で脂質ベースのエマルジョンとして投与した（採血は各群 3 例／時点）。アパルタミドの t_{max} はほとんどが 3～7 時間であり，M3 の t_{max} は 24～48 時間であった。概して，アパルタミド及び M3 の曝露量（C_{max} 及び AUC）は，低用量群及び中間用量群の間では用量にほぼ比例して増加したが，高用量での溶解性が低いために中間用量群及び高用量群の間では用量比例を下回って増加した。20 及び 100 mg/kg 群ではアパルタミド及び M3 の曝露量（C_{max} 及び AUC）に性差は認められなかったが，500 mg/kg 群ではアパルタミドの曝露量は雌で高く，雌の M3 の曝露量は雄の 1/2 であった。

(2) 反復投与

【参考資料 4.2.3.4.2.1，4.2.3.4.2.2，4.2.3.4.2.3】

10 日間反復投与毒性試験（GLP 非適用）において，雌雄 CD-1 マウス（雌雄各 6 例／群）にアパルタミドを 20，50 又は 100 mg/kg/日の用量で経口投与したときのアパルタミド及び M3 の薬物動態を評価した（採血は各群雌雄各 3 例／時点）【2.6.5.4A】（4.2.3.4.2.1 参照）。アパルタミドの t_{max} は 3～7 時間，M3 の t_{max} は 3～24 時間であった。10 日間の反復投与後，M3 の血漿中濃度は投与後 0～48 時間でほぼ一定であった。反復投与後のアパルタミドの曝露量［C_{max} 及び 0 時間から 24 時間までの血漿中濃度－時間曲線下面積（$AUC_{0\text{-}24h}$）］は用量比例を下回って増加したが，M3 の曝露量は用量にほぼ比例して増加した。単回投与後と比較して，反復投与後の M3 の $AUC_{0\text{-}24h}$ は，20 及び 50 mg/kg/日群では雌雄ともに 4 倍高く，100 mg/kg/日群の雄では 2 倍高かった。100 mg/kg/日群の雌に関しては，6 例中 4 例が死亡し，10 日間反復投与後のデータが不十分であり評価できなかった。

10 日間反復投与忍容性試験（GLP 非適用）の第 1 及び 3 期において，雌雄 CD-1 マウスにアパルタミドを経口投与し，アパルタミド及び M3 の血漿中濃度を測定した【2.6.5.4B】（4.2.3.4.2.2 参照）。第 1 期ではマウスに 100 mg/kg/日の用量で投与し（採血も含め雌雄各 6 例），第 3 期では 175 又は 250 mg/kg/日の用量で投与した（雌雄各 6 例／群，採血は各群雌雄各 3 例／時点）。第 1 期の Day 0（初回投与日）では TK 試料は採取せず，100 mg/kg/日群の初回投与後のデータは得られなかった。初回（Day 0）及び 10 回目投与後（Day 9）のアパルタミドの t_{max} は 3～7 時間の範囲であり，Day 9 の t_{max} はほとんどが 3 時間であった。Day 0 ではアパルタミドの血漿中濃度は，t_{max} 以降緩徐に減少したが，Day 9 では血漿中濃度はより速やかに低下し，自己誘導が示唆された。アパルタミドの曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は初回投与後よりも反復投与後の方が小さく，初回及び反復投与後の曝露量は雌雄ともに用量比例を下回った。反復投与後のアパルタミドの曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は，高用量（175 及び 250 mg/kg/日群）では雌雄で同程度であったが，低用量（100 mg/kg/日群）では雌の方がわずかに高かった。

M3 の t_{max} は初回投与（Day 0）では 24 時間であり，反復投与（Day 9）では 3～7 時間であった。雌雄ともに，初回及び反復投与後の M3 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は用量比例を下回った。

14 日間反復投与毒性試験（GLP 非適用）において雌雄の CbyB6F1 ハイブリッドマウス（雌雄各 6 例／群）にアパルタミドを経口投与した【2.6.5.4C】（4.2.3.4.2.3 参照）。アパルタミドを 75 又は 150 mg/kg/日の用量で投与したが（採血は各群雌雄各 3 例／時点），150 mg/kg/日群は一般状態の悪化のために早期（Day 3）に投与を終了した。アパルタミド及び M3 の曝露量を，Day 1（両群）及び Day 14（75 mg/kg/日群）に評価した。150 mg/kg/日群では Day 3 の終了前に採血した。アパルタミドの t_{max} は，雌雄ともに Day 1 では 3～7 時間，Day 14 では 3 時間であった。アパルタミドの曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は Day 1 では用量にほぼ比例して増加し（比は 1.7～2.2），雌雄で同程度であった。反復投与後（Day 14）のアパルタミドの曝露量は，75 mg/kg/日群では雌雄ともに Day 1 と比較して減少し（C_{max} は雄：0.88 倍，雌：0.78 倍，$AUC_{0\text{-}24h}$ は雄：0.47 倍，雌：0.49 倍），雄の方が若干低かった。

M3 の C_{max} にはばらつきが認められ，t_{max} は 7～24 時間であった。Day 1 において，M3 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は，用量比例をわずかに上回って増加した（2.2～2.7 倍）。Day 14 の C_{max} 及び $AUC_{0\text{-}24h}$ は，雌雄ともに Day 1 と比較して増加した（C_{max} は雄：3.6 倍，雌：2.1 倍，

$AUC_{0\text{-}24h}$は雄：4.5 倍，雌：2.9 倍）。M3 の曝露量（C_{max}及び$AUC_{0\text{-}24h}$）は，Day 1 では雌雄で同程度であったが，Day 14 では雄の方がわずかに高かった。150 mg/kg/日群における Day 2 の投与 24 時間後の血漿中 M3 トラフ濃度は，Day 1 の投与 24 時間後のトラフ濃度よりも高かった（雄：3.5 倍，雌：2.1 倍）。曝露量（C_{max}及び$AUC_{0\text{-}24h}$）に基づく未変化体／代謝物比は，雌雄ともに Day 1 で 4.8～11.3 の範囲であったが，反復投与後の Day 14 では 1.0～2.7 であった。

2.6.4.3.1.2 ラット

(1) 単回投与

【参考資料 4.2.2.2.4，4.2.2.2.5，4.2.2.2.6，4.2.3.1.1，4.2.3.2.5】

静脈内投与後及び種々の経口製剤投与後の薬物動態を検討した開発初期の一連の試験において，ラットにアパルタミドを単回投与したときの薬物動態を評価した【2.6.5.3A 及び 2.6.5.3E】（4.2.2.2.4 参照）。静脈内投与試験（GLP 非適用）で，絶食した雄 Sprague-Dawley ラット（4 例）にアパルタミドを 3 mg/kg の用量で静脈内投与用脂質性溶液として投与したところ，1 例のラットが投与 2 時間後に死亡した。曝露量［0 時間から無限大時間までの血漿中濃度－時間曲線下面積（$AUC_{0\text{-}\infty}$）］は約 13 μg·h/mL，V_{ss}は 2.21 L/kg，CL_pは 3.88 mL/min/kg（肝血流量の約 7%）及び$t_{1/2}$は 7.3 時間であった。経口投与試験では，雄 Sprague-Dawley ラットに，3 製剤（2 種類の脂質性製剤及び 1 種類の水性製剤）のアパルタミドを 1000 mg/kg の用量で投与した（4 例／製剤）。経口投与後のt_{max}（7～10 時間）及び$t_{1/2}$（8.6～11.2 時間）は製剤間で類似していたが，曝露量（C_{max}及び$AUC_{0\text{-}\infty}$）は水性製剤の方が 2～4 倍高かった。

追加実施した 4 つの単回投与試験においてアパルタミド（1 試験では M3）を経口投与し，アパルタミド若しくは代謝物（又はその両者）の濃度を測定した。

アパルタミドエタノール付加物は，多くの動物試験で使用されているため，遊離塩基（JNJ-56021927-AAA）とエタノール付加物（JNJ-56021927-ZAH）を比較するブリッジング試験（GLP 非適用）を実施した【2.6.5.3F】（4.2.3.1.1 参照）。

雄 Sprague-Dawley ラットに，アパルタミドの遊離塩基又はエタノール付加物を脂質ベースの媒体中，5 及び 50 mg/kg の用量で経口投与した（各原薬でそれぞれ 3 例／群）。血漿中アパルタミド及び M3 濃度並びに薬物動態パラメータから，2 種類の原薬はほぼ同様であることが示された。アパルタミドのt_{max}は 8～13 時間であり，曝露量（C_{max}及び AUC）は用量に比例して増加した。M3 の平均t_{max}は高用量群では 12～13 時間（範囲：4～24 時間）であった。低用量群での M3 のt_{max}はデータが不十分であったため評価できなかった。

忍容性試験（GLP 非適用）の単回投与期において【2.6.5.3G】（4.2.3.2.5 参照），雌 Sprague-Dawley ラットにアパルタミドを 50，100，150 又は 250 mg/kg の用量で脂質ベースのエマルジョンとして経口投与した（3 例／群）。アパルタミドの平均t_{max}は 9.3～12 時間（範囲：8～12 時間）であり，M3 の平均t_{max}は 12～26 時間（範囲：12～31 時間）であった。アパルタミドの曝露量（C_{max}及び AUC）は 50 及び 100 mg/kg 群の間では用量にほぼ比例して増加し，100 及び 150 mg/kg 群の間では用量比例を下回って増加し，250 mg/kg 群では増加しなかった。同様の傾向が M3 についても認められた。曝露量（C_{max}及び AUC）に基づく代謝物／未変化体比は，すべての用量で 0.01～0.02 の範囲であった。

M3 の薬物動態を評価するために，絶食した雄 Sprague-Dawley ラットに M3 を単回静脈内又は経口投与した（GLP 非適用）【2.6.5.3H】（4.2.2.2.5 参照）。ラットに M3 を 3 mg/kg の用量で静脈内投与，又は 10 mg/kg の用量で経口投与し（各 4 例），M3 及び M4 の血漿中濃度を測定した。M3 を静脈内投与したときの $AUC_{0-\infty}$は 3.09 μg·h/mL，CL_pは 16.2 mL/min/kg，V_{ss}は 2.63 L/kg，$t_{1/2}$は約 5 時間であった。経口投与時の M3 の $AUC_{0-\infty}$は 8.02 μg·h/mL であった。M4 の $AUC_{0-\infty}$は，静脈内投与で 1.78 μg·h/mL，経口投与で 4.18 μg·h/mL であり，M3 の約 1/2 であった。

アパルタミド及びその代謝物の血漿中薬物動態を更に評価するために，雄 Sprague-Dawley ラットにアパルタミド又は M3 を 500，1000 及び 2000 mg/kg の用量で経口投与し（4 例／群），アパルタミド，M3 及び M4 の血漿中濃度を測定した（GLP 非適用）【2.6.5.3I】（4.2.2.2.6 参照）。アパルタミド（500～2000 mg/kg）を経口投与したとき，アパルタミド，M3 及び M4 の C_{max}（及び t_{max}）はそれぞれ 13.4～18.6 μg/mL（11～12 時間），0.700～1.41 μg/mL（18～24 時間）及び 1.49～2.33 μg/mL（14～24 時間）であった。アパルタミドを 500，1000 及び 2000 mg/kg の用量で経口投与したとき，アパルタミドの $AUC_{0-\infty}$は，それぞれ 516，521 及び 410 μg·h/mL，M3 の $AUC_{0-\infty}$はそれぞれ 42.8，34.3 及び 23.6 μg·h/mL，M4 の $AUC_{0-\infty}$はそれぞれ 73.0，64.5 及び 56.8 μg·h/mL であった。一方，M3 を 500，1000 及び 2000 mg/kg の用量で経口投与したとき，M3 の $AUC_{0-\infty}$はそれぞれ 122，102 及び 146 μg·h/mL，M4 の $AUC_{0-\infty}$はそれぞれ 71.9，65.9 及び 95.3 μg·h/mL であった。アパルタミドを投与したときの C_{max}及び $AUC_{0-\infty}$に基づく代謝物／未変化体比は，それぞれ M3 で 5.3～7.6%及び 5.8～8.3%，M4 で 10.0～12.6%及び 12.0～14.1%の範囲であった。

(2) 反復投与

【評価資料 4.2.3.2.2，4.2.3.2.3，4.2.3.2.4，4.2.3.2.6，参考資料 4.2.3.2.1，4.2.3.2.5】

14 日間反復投与毒性試験（GLP 非適用）において，雄 Sprague-Dawley ラット（4 例／群）にアパルタミドを 150，300 又は 600 mg/kg/日の用量で 1 日 1 回経口投与した（採血は各群 2 例／時点）【2.6.5.4D】（4.2.3.2.1 参照）。なお，単回投与時の 48 時間後の濃度を測定するために，Day 2 では投与しなかった。300 mg/kg/日以上の用量で死亡が認められた。高用量群の曝露量（C_{max}及び AUC）に，低用量群との明らかな差は認められなかったが，中間用量群の曝露量は一貫して最も高かった。Day 1 では，t_{max}は中間用量群で遅く（他の群で 12 時間に対して 24 時間），$t_{1/2}$は 2 倍長かったが（他の群で 12 時間に対して 25 時間），Day 14 では明確な違いは認められなかった。これらの現象の理由は不明であるが，中間用量群でより強い毒性所見が発現したことと一致しており，血漿試料の取り違えによっては説明できないと考えられる。Day 14 の AUC_{0-24h}は Day 1（$AUC_{0-\infty}$）の約 1/2 であった。この原因も不明であるが，高用量投与による薬物代謝酵素の誘導に起因している可能性がある。

14 日間の回復期間を設定した 28 日間反復投与毒性試験（GLP 適用）において，雄 Sprague-Dawley ラット（9 例／群）にアパルタミドを 50，150 又は 250 mg/kg/日の用量で経口投与したときのアパルタミドの TK を評価した（採血は各群 3 例／時点）【2.6.5.4E】（4.2.3.2.2 参照）。250 mg/kg/日群の TK 用動物はすべて，Day 3 に死亡するか又は状態悪化のために安楽死させたことから，Day 1 以外のデータは得られなかった。アパルタミドは初回投与後 24 時間にわたって血漿中に認められ，最終投与 72 時間後まで検出された。Day 28 におけるアパルタミドの t_{max}は 8～

12 時間であった。Day 1 及び 28 において，アパルタミドの曝露量（C_{max} 及び AUC）は用量増加とともに増加し，累積も認められた。低用量及び中間用量群の累積比は，それぞれ 1.19 及び 1.10 であった。

13 週間反復投与毒性試験（GLP 適用）において，雄 Sprague-Dawley ラット（9 例／群）にアパルタミドを 25，50 又は 100 mg/kg/日の用量で経口投与し，Day 1，28 及び 91 にアパルタミド，M3 及び M4 の血漿中濃度を測定した（採血は各群 3 例／時点）【2.6.5.4F】（4.2.3.2.3 参照）。アパルタミド及び代謝物の濃度は用量にほぼ比例して増加したが，100 mg/kg/日群の Day 91 における M3 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は用量比例を上回って増加した（例えば，C_{max} は 25 mg/kg/日群の 4 倍ではなく 7.45 倍）。t_{max} に明確な用量依存性は認められず，アパルタミド及び M3 の t_{max} は 8～12 時間であり，M4 の t_{max} は 6～24 時間の範囲（8 時間が最も多い）であった。アパルタミド及び代謝物はすべて累積を示し，Day 91 の $AUC_{0\text{-}24h}$ は Day 1 の 2～3 倍であった。Day 91 の $AUC_{0\text{-}24h}$ に基づく代謝物／未変化体比は M3 で 0.028～0.055，M4 で 0.077～0.101 であった。

26 週間反復投与毒性試験（GLP 適用）において，雄 Sprague-Dawley ラット（8 例／群）にアパルタミドを 25，75 又は 150 mg/kg/日の用量で経口投与し，Day 28 及び 91 の投与前（採血は各群 8 例）並びに TK パラメータ算出のため Day 182（採血は各群 4 例／時点）に，アパルタミド及び M3 の血漿中濃度を測定した【2.6.5.4G】（4.2.3.2.4 参照）。Day 182 における t_{max} は，アパルタミドで 4 時間，M3 で 12 時間であった。曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は用量の増加とともに増加したが，アパルタミド及び M3 ともに用量比例を下回った。アパルタミド及び M3 ともに，Day 182 の血漿中濃度が持続的に維持されたため，消失相は明確ではなかったが，反復投与による累積が認められた。$AUC_{0\text{-}24h}$ に基づく代謝物／未変化体比は 0.0338～0.0545 の範囲であった。

28 日間反復投与忍容性試験（GLP 非適用）において，雌 Sprague-Dawley ラット（10 例／群）にアパルタミドを 25，50 又は 100 mg/kg/日の用量で経口投与し，2 及び 4 週間後にアパルタミド及び M3 の血漿中濃度を測定した（採血は各群 4 例／時点）【2.6.5.4H】（4.2.3.2.5 参照）。雄ラットを用いた 28 日間反復投与毒性試験において 150 及び 250 mg/kg/日群で毒性が認められたため【2.6.5.4E】（4.2.3.2.2 参照），本試験では高用量を 100 mg/kg/日に設定した。2 週間の投与により定常状態に達し，血漿中アパルタミド及び M3 濃度はほぼ一定に推移した。アパルタミドの定常状態での曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は，25 及び 50 mg/kg/日群の間では用量に比例して増加し，50 及び 100 mg/kg/日群の間では用量比例を下回って増加した。M3 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は，低用量側では用量比例を上回り，高用量側では用量にほぼ比例して増加した。反復投与後のアパルタミド及び M3 の C_{max} は単回投与と比較して高かった。一方，反復投与後のアパルタミド及び M3 の $AUC_{0\text{-}24h}$ は，50 mg/kg/日群では単回投与後の $AUC_{0\text{-}\infty}$ と同程度であり，100 mg/kg/日群では単回投与後の $AUC_{0\text{-}\infty}$ より小さかった。$AUC_{0\text{-}24h}$ に基づく代謝物／未変化体比は単回及び反復投与時で同様であり，検討したすべての用量において 0.01～0.02 の範囲であった。

26 週間反復投与毒性試験（GLP 適用）において，雌 Sprague-Dawley ラット（6 例／群）にアパルタミドを 25，50，100 又は 150 mg/kg/日の用量で経口投与した（採血は各群 3 例／時点）【2.6.5.4I】（4.2.3.2.6 参照）。血漿中アパルタミド及び M3 濃度を，Day 1，28，92 及び 182 に測定した。アパルタミドの t_{max} は，Day 1 では 8～12 時間，Day 28，92 及び 182 ではおおむね 4 時間であった。血漿中濃度が持続していたため，消失相は明確ではなかった。Day 1 の曝露量

（C_{max} 及び $AUC_{0\text{-}24h}$）は，用量に比例して増加したが，反復投与後の曝露量は 25 及び 50 mg/kg/日群では用量に比例して増加し，高用量側（50～150 mg/kg/日群）では，用量比例を下回り，投与期間が長くなると 100 及び 150 mg/kg/日群間で同程度の値になった。アパルタミドの $AUC_{0\text{-}24h}$ の累積比はすべての群で 1.3～3.3 の範囲であり，投与期間が長いほど大きかった。

M3 の t_{max} は，各測定日において 25，50 及び 100 mg/kg/日群では 12 時間，150 mg/kg/日群では 8～24 時間であった。M3 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は，おおむねすべての群で用量比例を上回って増加したが，Day 92 及び 182 の 100 及び 150 mg/kg/日群の曝露量は同程度であった。M3 の累積は投与期間が長いほど増大する傾向を示したが，150 mg/kg/日群の累積比は Day 28 以降同程度であった。Day 92 及び 182 における 100 及び 150 mg/kg/日群間の関係を除いて，代謝物／未変化体比も用量とともに増加し，$AUC_{0\text{-}24h}$ に基づく代謝物／未変化体比は，Day 28 における 150 mg/kg/日群が最大（0.0378）であった。M3 の $AUC_{0\text{-}24h}$ の累積比は 2.6～7.6 の範囲であり，アパルタミドの投与期間が長いほど大きい傾向にあった。

2.6.4.3.1.3　　イヌ

(1) 単回投与

【評価資料 4.2.2.2.9，4.2.2.2.10，4.2.2.2.11，参考資料 4.2.2.2.7，4.2.2.2.8，4.2.3.2.12】

単回静脈内投与試験（GLP 非適用）において，雄ビーグル犬（3 例）にアパルタミドを 3 mg/kg の用量で脂質性溶液として単回静脈内投与したとき，$AUC_{0\text{-}\infty}$ は 105 μg·h/mL であった【2.6.5.3A】（4.2.2.2.7 参照）。極めて低い CL_p（0.486 mL/min/kg）に起因して $t_{1/2}$ は 68.8 時間と長かった。V_{ss} は中程度（2.76 L/kg）であった。

イヌを用いた単回経口投与試験（6 試験）のうち，5 試験は製剤の最適化を目的とした開発初期の試験（GLP 非適用）であった。残る 1 試験は，雌イヌを用いた 21 日間反復経口投与毒性試験（GLP 非適用）の単回投与期として実施した【2.6.5.3O】（4.2.3.2.12 参照）。検討した製剤の中には，Acconon MC8-2 EP/NF，Capmul MCM EP，PEG 400 USP，VitE-TPGS NF（10：45：30：15% w/w/w/w）から成る脂質ベースのエマルジョンが含まれる。これは，*in vivo* の薬理試験及び初期の臨床試験で使用されたアパルタミド製剤（初期臨床試験では JNJ-56021927 軟カプセル剤，30 mg）と同じ組成である。この製剤を用いたデータについて以下に説明するが，試験によっては異なる名称を用いているため，試験ごとに使用された製剤の名称を表 2.6.4-2 に示す。その他の製剤の薬物動態に関する結果は，薬物動態試験概要表【2.6.5 参照】に記載する。

表 2.6.4-2 単回経口投与による薬物動態試験で使用された製剤（脂質ベースのエマルジョン）の名称

Study Report (Study Reference)	Name Used for the Lipid-Based Emulsion[a]
DR-[redacted]-002-DSD (4.2.2.2.7)	Formulation B
DR-[redacted]-011-DSD (4.2.2.2.8)	Formulation 7
FK10587 (4.2.2.2.9)	Formulation F
FK10634 (4.2.2.2.10)	Formulation A
FK10786 (4.2.2.2.11)	Formulations 1 and 2
TOX10780 (4.2.3.2.12)	Oral and Capsule

[a] Components of the lipid-based emulsion formulation: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, Vit E-TPGS NF (10:45:30:15 %w/w/w/w)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; VitE-TPGS = water-soluble vitamin E (D-α-tocopherol polyethylene glycol succinate conjugate).

単回経口投与試験（GLP 非適用）では，単回静脈内投与試験（GLP 非適用）と同じ雄ビーグル犬（3 例）にアパルタミド（製剤 B）を 10 mg/kg の用量で硬ゼラチンカプセル剤として投与した【2.6.5.3J】（4.2.2.2.7 参照）。経口投与後の C_{max}（5.0 μg/mL）は投与 3.3 時間後に認められ，この t_{max} はビタミン E の代わりに Tween-80 を用いた他の脂質性製剤（製剤 A）より約 70%短かった。$AUC_{0-\infty}$は 505 μg·h/mL（製剤 A の約 0.9 倍）であり，$t_{1/2}$ は 90.7 時間であった。

薬物動態試験（GLP 非適用）において，絶食した雄ビーグル犬（3 例）にアパルタミド（製剤 7）を 60 mg（6.7±0.7 mg/kg）の用量で軟カプセル剤として経口投与した【2.6.5.3K】（4.2.2.2.8 参照）。軟カプセル剤として投与したときの薬物動態パラメータは，5 種類の非晶質プロトタイプ製剤を投与したときのパラメータと明確な差は認められなかった。投与 1.7 時間後に 3.5 μg/mL の C_{max} に達し，$AUC_{0-\infty}$は 175 μg·h/mL であった。

試作製剤の薬物動態を評価するための試験（GLP 非適用）において，絶食した雄ビーグル犬（4 例）にアパルタミド（製剤 F）を 60 mg の用量で軟カプセル剤（30 mg カプセル剤 2 錠）として経口投与した【2.6.5.3L】（4.2.2.2.9 参照）。吸収は速やかであり，投与 1.3 時間後に 4.11 μg/mL の C_{max} に達した。$AUC_{0-\infty}$は 167 μg·h/mL であり，前記の試験と同程度であった。

試作製剤の薬物動態を評価するための別の試験（GLP 非適用）において，絶食した雄ビーグル犬（4 例）にアパルタミド（製剤 A）を 60 mg の用量で軟カプセル剤（30 mg カプセル剤 2 錠）として経口投与した【2.6.5.3M】（4.2.2.2.10 参照）。投与 1.8 時間後に 2.78 μg/mL の C_{max} に達し，0 時間から 168 時間までの血漿中濃度－時間曲線下面積（AUC_{0-168h}）は 144 μg·h/mL であった。

絶食した雄ビーグル犬（4 例／製剤）に異なる添加剤を有する 2 種類の製剤を 60 mg の用量で軟カプセル剤として経口投与したところ，両製剤は同様であった（GLP 非適用）【2.6.5.3N】（4.2.2.2.11 参照）。t_{max} が 24 時間であった 1 例を除いて，投与 1～3.25 時間後に 0.897～0.904 μg/mL の C_{max} に達した。これまでの試験結果と比較して $t_{1/2}$ はわずかに短く（41～59 時間），$AUC_{0-\infty}$も低かった（47.8～64.3 μg·h/mL）。

単回経口投与毒性試験（GLP 適用）の用量漸増期において，雌ビーグル犬にアパルタミドを 5, 10 又は 20 mg/kg の用量で経口胃管法にて，又はカプセル剤として投与し（各 3 例／群），アパルタミド及び M3 の血漿中濃度を測定した【2.6.5.3O】（4.2.3.2.12 参照）。血漿中アパルタミド濃度は，おおむね投与 1～4 時間後に C_{max} に達し，その後，最終採血時間である投与 72 時間後ま

でほぼ一定に推移した。C_{max}はおおむね用量の増加とともに増加した（胃管法：1.99～5.23 μg/mL，カプセル剤投与：1.93～8.02 μg/mL）。0 時間から 72 時間までの血漿中濃度－時間曲線下面積（$AUC_{0\text{-}72h}$）は用量にほぼ比例し，胃管法で 59.5～202 μg·h/mL，カプセル剤投与で 73.8～325 μg·h/mL の範囲であったが，いくつかの投与群では群内にばらつきが認められた（例えば，10 mg/kg の胃管法で 59.5～227 μg·h/mL，カプセル剤投与で 43.4～144 μg·h/mL）。それらのばらつきは投与後 1～2 時間以内に発現した嘔吐に起因している可能性があり，特に胃管法によって中間用量及び高用量を投与したイヌではその可能性が高いと考えられる。

最終採血時間である投与 72 時間後まで，血漿中 M3 濃度はほぼすべてのイヌについて増加し続けた。嘔吐を考慮すると，M3 のC_{max}は用量にほぼ比例して増加し，胃管法では 0.825～2.19 μg/mL，カプセル剤投与では 0.631～2.32 μg/mL の範囲であった。また，$AUC_{0\text{-}72h}$も用量にほぼ比例して増加し，胃管法で 39.4～113 μg·h/mL，カプセル剤投与で 30.4～111 μg·h/mL の範囲であった。

胃管法においてより多くの嘔吐が認められたが，単回経口投与時の投与方法間に一貫した曝露量（C_{max}及び$AUC_{0\text{-}72h}$）の差は認められなかった。

(2) 反復投与

【評価資料 4.2.3.2.8，4.2.3.2.10，4.2.3.2.11，4.2.3.2.13，**参考資料** 4.2.3.2.7，4.2.3.2.9，4.2.3.2.12】

21 日間反復投与毒性試験（GLP 非適用）において，雄ビーグル犬に 1 日 1 回反復経口投与したときのアパルタミドの薬物動態を評価した【2.6.5.4J】（4.2.3.2.7 参照）。イヌ（2 例／群）にアパルタミドを 10，20 又は 40 mg/kg/日の用量でカプセル剤として投与した。なお，単回投与時の 48 時間後の濃度を測定するために，Day 2 では投与しなかった。薬物動態プロファイルを Day 1 及び 21 に評価し，追加のトラフ試料を Day 8 及び 15 に採取して血漿中アパルタミド濃度を測定した。高用量群では，発作のために 2 例を早期に安楽死させたため（Day 13 及び 14 に各 1 例），薬物動態は評価できなかった。Day 1 におけるアパルタミドの血漿中曝露量（C_{max}及び$AUC_{0\text{-}\infty}$）は，用量に比例して増加した。$t_{1/2}$は 50～80 時間（すなわち，投与間隔の 2～3 倍）であり，Day 21 に曝露量（C_{max}及び$AUC_{0\text{-}24h}$）の累積が認められた。

28 日間の回復期間を設定した 28 日間反復投与毒性試験（GLP 適用）において，雄ビーグル犬（5 mg/kg/日群では 5 例，10 及び 25 mg/kg/日群では 3 例の回復期動物を含めて各 8 例）にアパルタミドを 5，10 又は 25 mg/kg/日の用量でカプセル剤として経口投与した【2.6.5.4K】（4.2.3.2.8 参照）。血漿中アパルタミドの薬物動態プロファイルを評価するために Day 1 及び 28 の投与 24 時間後までの血液を採取し，追加のトラフ試料を Day 8，15 及び 22 に採取した。高用量群については，一般状態の悪化により，3 例の動物を Day 7，8 及び 9 にそれぞれ安楽死させ，残りの動物の投与を中止したため，Day 28 のデータは得られなかった。得られたデータから，おおむね投与 2～8 時間後の間にC_{max}が認められ，血漿中濃度は用量に比例して増加することが示された。t_{max}は Day 1 において 2.40～5.38 時間，Day 28 において 3.50～7.13 時間の範囲であった。トラフの血漿中濃度から Day 28 までに定常状態に達することが示された。トラフ濃度及び Day 28 に得られた濃度から，累積が示され，累積比はC_{max}で約 5，$AUC_{0\text{-}24h}$で約 6 であった。回復期間である Day 50 までにアパルタミドの血漿中濃度はすべて定量下限（0.100 μg/mL）未満となった。

上述のイヌにおける 28 日間反復投与毒性試験（GLP 適用，4.2.3.2.8 参照）の Day 28 の血漿試料を用いて，M3 の血漿中曝露を追加で評価した（GLP 非適用）【2.6.5.4L】（4.2.3.2.9 参照）。再分析した血漿中 M3 濃度から薬物動態パラメータを求め，以前に算出したアパルタミドのパラメータを用いて代謝物／未変化体比を評価した。定常状態（Day 28）の M3 濃度は Day 28 に採取したすべての血漿試料で定量可能であり，濃度も一定に維持されていた。C_{max} 及び $AUC_{0\text{-}24h}$ は用量に比例して増加し，5 mg/kg/日群でそれぞれ 4.35 μg/mL 及び 96.5 μg·h/mL，10 mg/kg/日群で 7.69 μg/mL 及び 172 μg·h/mL であった。C_{max} 及び $AUC_{0\text{-}24h}$ に基づく代謝物／未変化体比は 5 mg/kg/日群でそれぞれ 77.6 及び 85.9%であり，10 mg/kg/日群では 59.9 及び 62.0%であった。

60 日間の回復期間を設定した 13 週間反復経口投与毒性試験（GLP 適用）において，雄ビーグル犬（2.5 mg/kg/日群では 5 例，5 及び 10 mg/kg/日群では 3 例の回復期動物を含めて各 8 例）にアパルタミドを 2.5，5 又は 10 mg/kg/日の用量でカプセル剤として 91 日間経口投与した。薬物動態プロファイルを評価するために，アパルタミド，M3 及び M4 の血漿中濃度を Day 1，28 及び 91 に測定した【2.6.5.4M】（4.2.3.2.10 参照）。概して，アパルタミド及び M4 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は用量比例をわずかに上回って増加したが，M3 の曝露量は用量にほぼ比例して増加した。すべての用量で，Day 1 と比較すると，Day 91 におけるアパルタミド，M3 及び M4 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は，それぞれ 6～8 倍，10～38 倍及び 15～24 倍に増加し，累積が示された。Day 91 の $AUC_{0\text{-}24h}$ に基づく M3 及び M4 の代謝物／未変化体比は，それぞれ 0.48～0.72 及び 0.09～0.11 の範囲であり，Day 1 と比較して増加した。回復期間の試料から算出したアパルタミド，M3 及び M4 の定常状態における $t_{1/2}$ は，それぞれ 78.6，188 及び 123 時間であった。

39 週間反復経口投与毒性試験（GLP 適用）において，雄ビーグル犬（4 例／群）にアパルタミドを 2.5，5 又は 10 mg/kg/日の用量でカプセル剤として 273 日間経口投与した【2.6.5.4N】（4.2.3.2.11 参照）。Day 182（投与 26 週後）及び Day 273（投与 39 週後）に薬物動態プロファイルを評価するために，また，Day 28 及び 91 の投与 24 時間後のトラフ濃度を評価するために，アパルタミド及び M3 の血漿中濃度を測定した。Day 182 における 1 例を除いて，すべての動物で Day 182 及び 273 のアパルタミドの t_{max} は投与 2～24 時間後の間に認められた（除外した 1 例の t_{max} は Day 182 の 0 時間，すなわち前回投与 24 時間後に相当する時間であった）。2.5，5 及び 10 mg/kg/日群のアパルタミドの t_{max} は，それぞれ，Day 182 において 6.50，10.5 及び 9.00 時間，Day 273 において 13.5，12.5 及び 10.0 時間であった。アパルタミド及び M3 の血漿中濃度はともに持続したため，消失相を明確にすることはできなかった。アパルタミドの曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は，用量比例をわずか上回って増加した。トラフ濃度は，Day 28，91，182 及び 273 で同程度であり，Day 28 までに定常状態に達したことが示された。Day 182 から 273 への累積はわずかであり，$AUC_{0\text{-}24h}$ の累積比は 1.2～1.3 の範囲であった。3 例を除いて，すべての動物で M3 の t_{max} は投与 2～24 時間後の間に認められた。除外した 3 例の t_{max} は，すべて 0 時間（すなわち前回投与 24 時間後に相当する時間）であった。2.5，5 及び 10 mg/kg/日群の M3 の t_{max} は，それぞれ，Day 182 において 4.50，7.00 及び 6.00 時間，Day 273 において 18.5，15.0 及び 13.5 時間であった。M3 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は用量比例をわずかに下回って増加した。アパルタミドと同様に，M3 のトラフ濃度の結果から，Day 28 までに定常状態に達したことが示された。Day 182 から 273 への明確な累積はなく，$AUC_{0\text{-}24h}$ の累積比は約 1.1 であった。$AUC_{0\text{-}24h}$ に基づく

代謝物／未変化体比は用量の増加に伴い低下し，Day 182 において 0.505～0.962，Day 273 において 0.458～0.811 の範囲であった。

21 日間反復経口投与毒性試験（GLP 非適用）において，雌ビーグル犬（3 例／群）にアパルタミドを 5，10 又は 20 mg/kg/日の用量でカプセル剤として投与し，薬物動態プロファイルを評価するために投与 2 及び 3 週後のアパルタミド及び M3 の血漿中濃度を測定した【2.6.5.4O】（4.2.3.2.12 参照）。アパルタミド及び M3 の血漿中濃度は，投与後 24 時間の採取期間中ほぼ一定であった。累積が認められたが，血漿中濃度推移が平坦であり，AUC の外挿値が大きくなるため，確定できなかった。3 週間後，アパルタミドの C_{max} は単回投与後と比較して 2.7～5.9 倍に増加した。3 週間後の $AUC_{0\text{-}24h}$ に基づく代謝物／未変化体比は 5，10 及び 20 mg/kg/日群でそれぞれ 0.8，0.7 及び 0.5 であり，単回投与後の値（0.1～0.2 の範囲）と比較して増大した。

1 カ月の回復期間を設定した 13 週間反復経口投与毒性試験（GLP 適用）において，雌ビーグル犬（2.5 及び 5 mg/kg/日群では各 4 例，10 mg/kg/日群では 2 例の回復期動物を含めて 6 例）にアパルタミドを 2.5，5 又は 10 mg/kg/日の用量で投与期間中連日投与した【2.6.5.4P】（4.2.3.2.13 参照）。Day 28 にアパルタミド及び M3 の血漿中トラフ濃度を測定し，Day 90 の薬物動態プロファイルを評価するためにアパルタミド及び M3 の血漿中濃度を測定した。アパルタミドの曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）は用量にほぼ比例して増加した。Day 28 及び 90 の投与 24 時間後のトラフ濃度は同程度であり，Day 28 までに定常状態に達したことが示された。Day 90 における M3 の C_{max} は，ほとんどのイヌで投与 24 時間以内に到達したが，濃度が持続したため消失相は明確ではなかった。M3 の曝露量（C_{max} 及び $AUC_{0\text{-}24h}$）も用量にほぼ比例して増加した。また，アパルタミドと同様に，Day 28 までに定常状態に達した。$AUC_{0\text{-}24h}$ に対する代謝物／未変化体比は用量の増加に伴い低下し，0.562～0.832 の範囲であった。

5 及び 10 mg/kg/日の用量で実施されたイヌの反復投与データを比較すると，雌雄の薬物動態は同様であった。

2.6.4.3.2 バイオアベイラビリティ

【参考資料 4.2.2.2.1，4.2.2.2.5，4.2.2.2.7】

アパルタミドの経口バイオアベイラビリティは，マウス（10 mg/kg）で 93.3%であり【2.6.5.3B】（4.2.2.2.1 参照），イヌ（10 mg/kg）でほぼ 100%であった【2.6.5.3J】（4.2.2.2.7 参照）。ラットでは，M3 のバイオアベイラビリティは 77.9%であったが【2.6.5.3H】（4.2.2.2.5 参照），アパルタミドのバイオアベイラビリティは得られていない。イヌで 100%を超える値が得られた原因として，採血が投与 72 時間後（すなわち，半減期と同程度の時間）までしか行われなかったため，経口投与及び静脈内投与の両方で AUC の外挿部分が大きかったことに起因している可能性がある。

2.6.4.4 分布

2.6.4.4.1 組織分布

【評価資料 4.2.2.4.2，4.2.3.2.10，参考資料 4.2.2.2.3，4.2.2.2.5，4.2.3.2.12】

定量的全身オートラジオグラフィーにより，雄の白色 Sprague-Dawley（1 例／時点，7 時点）及び有色 Lister Hooded（1 例／時点，3 時点）ラットに ^{14}C-アパルタミドを 50 mg/kg の用量で単回経口投与したときの組織分布を評価した【2.6.5.5】（4.2.2.4.2 参照）。白色ラットでは投与 1～168 時間後の範囲で，有色ラットでは投与 24～504 時間後の範囲で放射能を測定した。その結果，放射能は広範な組織に分布し，ほとんどの組織で投与 4～12 時間後に最高濃度に達することが示された。放射能濃度は，腹部及び褐色脂肪，肝臓，腎皮質及び髄質，副腎皮質，膵臓並びにハーダー腺において最も高く，水晶体，脳及び骨表面において最も低かった。

消化管組織を除いて，組織／血漿中濃度比が最も高かった部位及びその比（時間）は，皮膚：42（72 時間），肝臓：35（72 時間），ハーダー腺：25（72 時間），水晶体：20（72 時間），食道壁：17（72 時間），腹部脂肪：16（12 時間）及び甲状腺：13（72 時間）であった。また，AUC_{last} に基づく組織／血漿比は，皮膚：2.8，肝臓：8.4，ハーダー腺：4.8，水晶体：1.2，脳：0.44，骨表面：0.27 であった。脳内濃度は投与 12 時間後で最も高く（11.3 µg eq./g），投与 72 時間後には定量下限未満となった。

組織内放射能濃度は最高濃度に到達後低下し，投与 168 時間後においては，水晶体，皮膚（白色及び有色），ハーダー腺，肝臓並びに有色ラットの大腸壁及び膀胱壁を除くすべての組織で定量下限未満となった。微量の放射能が投与 504 時間後の皮膚に残存したが，有色皮膚内の放射能濃度は白色皮膚と同程度であり，ブドウ膜への放射能の残存も認められなかったことから，薬物由来物質のメラニンへの結合はほとんどないと考えられる。

いくつかの薬物動態試験において，脳内でのアパルタミド及び M3 の曝露を評価した。

雄 CD-1 マウス（3 又は 6 例／群）にアパルタミドを 10，30 又は 100 mg/kg の用量で経口投与し，投与 24 時間後までのアパルタミド及び M3 の曝露量を測定した【2.6.5.8A】（4.2.2.2.3 参照）。投与 16 及び 24 時間後の脳／血漿中濃度比を算出し，高用量群については投与 4 及び 8 時間後についても算出した。すべての用量において，投与 24 時間後の血漿中アパルタミド濃度は，10，30 及び 100 mg/kg 群の C_{max}（それぞれ 3.26，10.0 及び 28.7 µg/mL）の約 1/2 であった【2.6.5.3C 参照】。脳／血漿中濃度比は，32%（低用量群の投与 24 時間後）から 106%（高用量群の投与 4 時間後）の範囲であった。脳内及び血漿中の M3 濃度は採取期間内において時間とともに増加する傾向にあったが，脳／血漿中濃度比は，高用量群において投与 4 時間後に 78%であった以外，約 50%とほぼ一定であった。

雄 Sprague-Dawley ラット（5 例）にアパルタミドを 10 mg/kg の用量で単回経口投与し，投与 24 時間後のアパルタミドの血漿中及び脳内濃度を測定したところ，それぞれ 0.63 µg/mL 及び 0.48 µg/g であり，脳／血漿中濃度比は 76%であった【2.6.5.8A】（4.2.2.2.3 参照）。

別の試験において，雄 Sprague-Dawley ラット（4 例）にアパルタミドを 10 mg/kg の用量で単回経口投与し，投与 24 時間後の M3 の血漿中及び脳内濃度を測定したところ，それぞれ 0.083 µg/mL 及び 0.046 µg/g であり，脳／血漿中濃度比は 53%であった【2.6.5.8B】（4.2.2.2.5 参照）。

雌ビーグル犬（3 例／群）にアパルタミドを 5，10 又は 20 mg/kg/日の用量で 3 週間反復経口投与し，3 週間後の脳内及び血漿中のアパルタミド及び M3 濃度を測定した【2.6.5.8C】（4.2.3.2.12 参照）。脳内及び血漿中のアパルタミド及び M3 の濃度は用量比例を上回って増加した。脳内及び血漿中のアパルタミド濃度は，それぞれ 4.49～35.6 µg/g 及び 4.33～34.5 µg/mL であり，各群の脳／血漿中濃度比は約 1 であった。M3 は，用量の増加とともに脳内濃度は 4.01 から 24.1 µg/g に，血漿中濃度は 3.63 から 17.1 µg/mL に増加し，脳／血漿中濃度比は 1.1 から 1.4 にわずかに増加した。

別の試験において，雄ビーグル犬（5 例／群）にアパルタミドを 2.5，5 又は 10 mg/kg/日の用量で 13 週間反復経口投与し，Day 91 における脳，血漿及び CSF 中のアパルタミド，M3 及び M4 の定常状態濃度を測定した【2.6.5.8D】（4.2.3.2.10 参照）。アパルタミド，M3 及び M4 の濃度は，血漿中では用量比例をわずかに上回り，脳内では用量にほぼ比例して増加した。一方，CSF 中ではアパルタミドの濃度は用量にほぼ比例して増加し，M3 の濃度は用量比例を下回り，M4 の濃度は高用量群で用量比例を上回った。これらの傾向から，CSF／脳内濃度比は比較的一定であり（アパルタミド：6.20～7.87%，M3：4.60～5.24%，M4：4.60～4.89%），CSF／血漿中濃度比も比較的一定であった（アパルタミド：3.85～5.38%，M3：4.41～4.95%，M4：2.14～2.94%）。

2.6.4.4.2 タンパク結合及び血球移行

2.6.4.4.2.1 タンパク結合

【評価資料 4.2.2.3.1，4.2.2.3.2，4.2.2.3.3，4.2.2.3.4】

最初の試験では，単一濃度（10 µg/mL）の非標識のアパルタミド及び M3 を用いて血漿タンパク結合を平衡透析法で評価した【2.6.5.6A】（4.2.2.3.1 及び 4.2.2.3.2 参照）。アパルタミド及び M3 の非結合型分率は，動物種間において類似しており（アパルタミド及び M3 の非結合型分率はそれぞれ，マウス：12 及び 14%，ラット：13 及び 11%，イヌ：11 及び 9%），ヒトにおいてはより低かった（アパルタミド：4%，M3：5%）。

動物及びヒトの血液試料を用いて ^{14}C-アパルタミド及び ^{14}C-JNJ-56142060（^{14}C-M3）の血漿タンパク結合を超遠心法で評価した【2.6.5.6B】（4.2.2.3.3 参照）。10 µg/mL の濃度では，血漿中の ^{14}C-アパルタミド及び ^{14}C-M3 の非結合型分率は，それぞれ，マウスで 8.2 及び 9.4%，ラットで 7.2 及び 8.6%，イヌで 6.3 及び 8.2%，ウサギで 11.4 及び 13.2%，並びにヒトで 4.2 及び 5.1%であった。0.1～30 µg/mL の濃度範囲において，これらの動物種及びヒトにおけるアパルタミド及び M3 の血漿タンパク結合率は濃度に依存しなかった。アパルタミド及び M3 の精製 HSA への結合率（それぞれ 93.6 及び 93.2%）は，ヒト血漿タンパク結合率（それぞれ 95.8 及び 94.9%）と同程度で，精製 AGP への結合率（約 20%）よりも高かった。

雄 SCID Hairless Outbred マウスの血漿を用いてアパルタミド（10 µmol/L）の血漿タンパク結合率を平衡透析法で測定した【2.6.5.6C】（4.2.2.3.4 参照）。このマウスは，*in vivo* での効力を裏付ける試験（前立腺癌細胞の異種移植マウスモデル）で使用された系統である。37°C，6 時間インキュベーションしたときのアパルタミドの非結合型分率は 9%であった。

2.6.4.4.2.2 血球移行

【評価資料 4.2.2.3.3】

動物及びヒトの血液に ^{14}C-アパルタミド及び ^{14}C-M3 を添加し（10 μg/mL），37°C で 15 分間インキュベーションしたときの血球移行を評価した【2.6.5.6B】（4.2.2.3.3 参照）。アパルタミド及び M3 の各血液中画分（血漿水分画分，血漿タンパク結合画分又は血球結合画分）での存在割合を算出したところ，すべての種（マウス，ラット，イヌ，ウサギ及びヒト）において，^{14}C-アパルタミドの分布は ^{14}C-M3 と類似し，血液中の ^{14}C-アパルタミド及び ^{14}C-M3 は，血漿タンパク結合画分に最も多く分布し（0.500～0.685），かなりの割合が血球結合画分にも分布した（0.285～0.424）。

2.6.4.5 代謝

2.6.4.5.1 生体試料中の代謝物の化学構造及び存在量

【評価資料 4.2.2.4.2，4.2.2.4.3，4.2.2.4.4，参考資料 4.2.2.4.1，4.2.3.2.1，4.2.3.2.7，5.3.1.1.1】

反復投与毒性試験における低用量群のラット（150 mg/kg/日の用量で 2 週間投与，4.2.3.2.1 参照）及びイヌ（10 mg/kg/日の用量で 3 週間投与，4.2.3.2.7 参照）の血漿試料を用いて定性的な代謝物プロファイルを評価した【2.6.5.9A】（4.2.2.4.1 参照）。最終投与日の投与 8 及び 12 時間後の血漿試料をプールして LC-MS/MS により分析し，総ピーク面積に対する割合（%）により代謝物の存在量を推定した。ラット及びイヌの試料中には，未変化体及び 4 種類の代謝物が存在した。その内の 3 種類の代謝物は，既知の標品との比較によって同定され，それぞれ未変化体の酸化的脱硫化体（M2：JNJ-56142047），*N*-脱メチル体である 2-フルオロベンズアミド体（M3：JNJ-56142060）及びアミド加水分解体である 2-フルオロカルボン酸体（M4：JNJ-56142021）であった。ピーク面積による推定値から，循環血漿中の主要代謝物は，ラットでは M2 及び M4，イヌでは M3 及び M4 と判断された。

無処置及び胆管カニュレーションした雄ラット（50 mg/kg，4.2.2.4.2 参照），雄ビーグル犬（10 mg/kg，4.2.2.4.3 参照）並びにヒト男性被験者（240 mg，4.2.2.4.4 参照）に ^{14}C-アパルタミドを単回経口投与し，定量的な代謝試験を実施した。

ラット（3 例／時点）の試験では，投与 12 及び 24 時間後の血漿中放射能の大部分は，未変化体（それぞれ 65 及び 53%）及び M4（それぞれ 12 及び 15%）由来であった【2.6.5.9B 参照】。いずれの時点でも，その他に試料中放射能の 10%を超える代謝物は検出されなかった。

イヌ（4 例）の試験では，未変化体及び M3 が循環血漿中成分の大部分を占める主要な成分であった【2.6.5.9C 参照】。M3 は，投与 72 時間以降は未変化体よりも多く存在した。投与 24 時間以降の M4 の濃度は M3 の濃度の約 1/10～1/20 と低かった。各採血時点での未変化体，M3 及び M4 の濃度の合計は，血漿中放射能濃度の 90%以上であり，他の代謝物の寄与はほとんどないことが示唆された。$AUC_{0\text{-}528h}$ に基づくと，未変化体，M3 及び M4 の存在量は血漿中放射能のそれぞれ 41，51 及び 4%であった。

臨床試験において，ヒト被験者（6 例）の血漿中アパルタミド，M3 及び M4 濃度を LC-MS/MS 法により測定した【2.6.5.9C】（5.3.1.1.1 参照）。各被験者には，非標識アパルタミド（30 mg カプセル剤を 8 錠）及びマイクロドーズの ^{14}C-アパルタミド［400 µg，37 kBq（1000 nCi）］を投与し，加速器質量分析法を用いて血漿中の微量放射能を測定した。AUC_{last} で評価したときの主要な血漿中成分は，未変化体（175 µg·h/mL）及び M3（169 µg·h/mL）であり，いずれも M4（11.1 µg·h/mL）よりも 15 倍以上高かった。各採血時点での未変化体，M3 及び M4 の濃度の合計は，ほとんどが血漿中放射能濃度の 90%以上に相当し，未変化体，M3 及び M4 の AUC_{last} の合計は，血漿中放射能の値（413 µg eq·h/mL）に近かった。したがって，その他の代謝物の血漿中放射能に占める割合は極めて限定的と考えられる。

M4 はラット，イヌ及びヒトの尿中の主要な代謝物であり，投与放射能に対する割合は，それぞれ 26，16 及び 31%であった【2.6.5.9D 参照】。M5（M4 の酸化的脱硫化体）は，ラット（2.8%）及びイヌ（0.16%）の尿中にのみ存在した。イヌ及びヒトの尿中では，M4 以外に投与

量の 2%を超える代謝物として M3，M19（ジアゾスピロ環の開環及び置換ピリジニル基の脱離体）並びに M20（M19 の *N*-脱メチル体）が認められた。未変化体は，イヌ（2.7%）及びヒト（1.2%）の尿中では微量な成分であった。

M4 はラット及びイヌの糞中の主要な代謝物であり，投与放射能に対する割合は，それぞれ 26 及び 19%であった（2.6.5.9D 参照）。M5（投与量の 8%）はラットの糞中で 2 番目に多い代謝物であった。糞中の微量代謝物（投与量の 2%以上）として，ラットでは M2（2%）及びシステイン縮合体 M9（2%），イヌでは M3（3%）及びそのグリシル-システイン縮合体 M12（2%），ヒトでは M3（2%）及び M4（2%）が認められた。糞中の未変化体はラット，イヌ及びヒトにおいて，それぞれ投与量の 8，5 及び 2%であった。

ラット胆汁中に排泄された主要成分は，M4，未変化体及び M9 であり，投与放射能に対する割合は，それぞれ 22，10 及び 4%であった（2.6.5.9B 参照）。

2.6.4.5.2 推定代謝経路

アパルタミドは，ラット，イヌ及びヒトにおいて広範に代謝される。*In vivo* での推定代謝経路を図 2.6.4-1（ラット及びイヌ）並びに図 2.6.4-2（ヒト）に示す。2.6.4.5.1 に示すように，M4 は尿（ラット，イヌ及びヒト），糞（ラット及びイヌ）並びにラット胆汁中で最も多く認められた代謝物であったことから，*N*-メチルベンズアミド基のアミドへの *N*-脱メチル化（M3）及びそれに続くカルボン酸へのアミド加水分解（M4），並びに *N*-メチルベンズアミド基の M4 への直接的アミド加水分解が，アパルタミドの主要な代謝クリアランス経路であると考えられる。

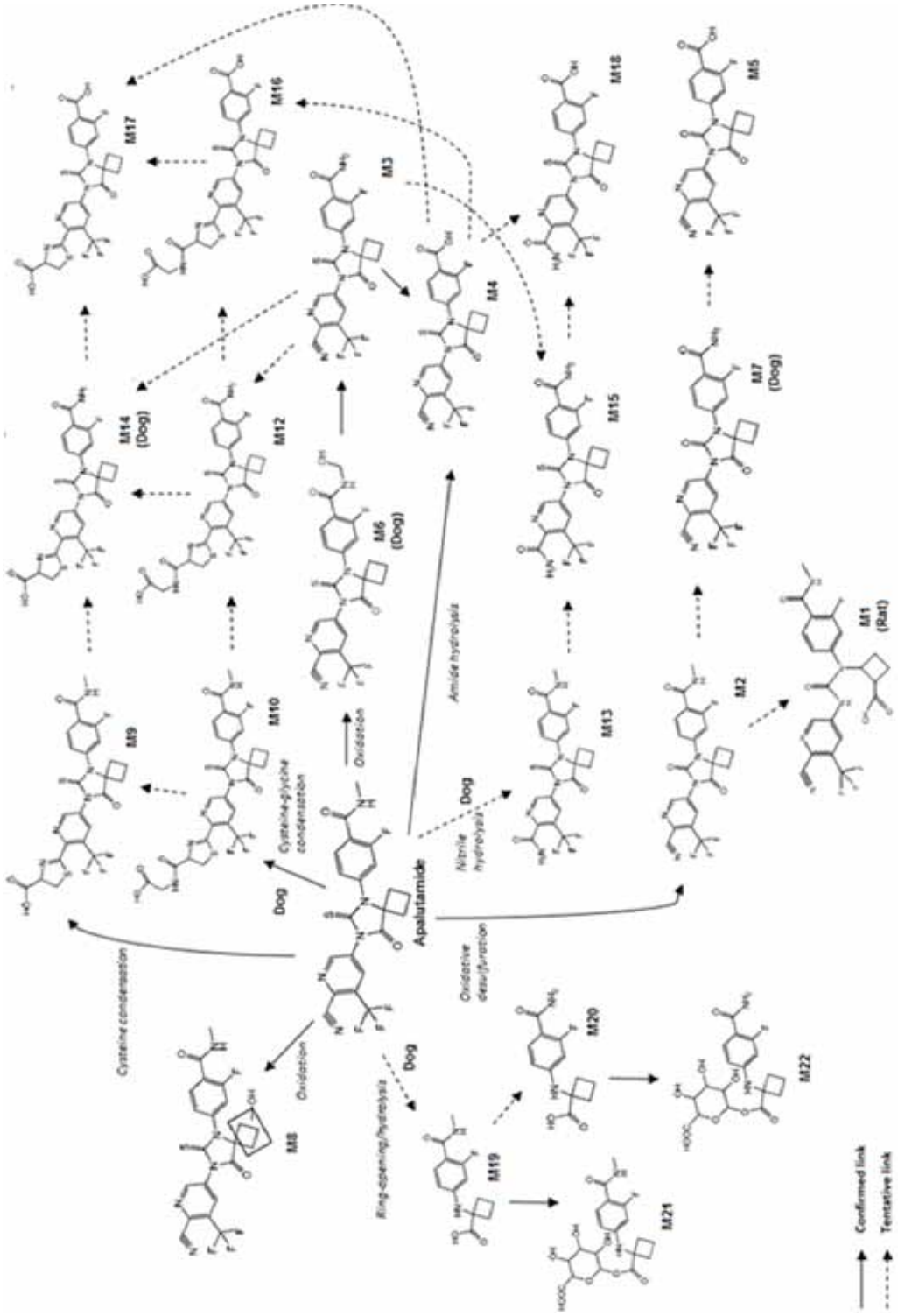
アパルタミドから M19 を生成する経路は，イヌ及びヒトにおいて比較的重要な代謝経路であると考えられる。この経路は未変化体のジアゾスピロ環の開環及び *N*-(6-シアノ-5-(トリフルオロメチル)ピリジン-3-イル)メタンチオアミド基の脱離であり，それに続く *N*-脱メチル化によって M20 が生成する。また，M19 及び M20 の *O*-グルクロン酸抱合体（それぞれ M21 及び M22）が尿中に排泄された。実際に，イヌ及びヒトでは尿中に，それぞれ投与量の 6 及び 7%に相当する M20 が認められ，M4 以外のすべての代謝物より多かった。

アパルタミドから直接生成する他の代謝物として，M2（酸化的脱硫），M8（酸化），M13（ニトリル加水分解），M9（システイン縮合）及び M10（グリシル-システイン縮合）が認められた。M8 を除くこれらの代謝物は，その後の *N*-脱メチル化によりアミドに代謝され，さらにアミド加水分解により 2-フルオロカルボン酸に代謝される。

以上の結果から，アパルタミドの代謝経路は，ラット，イヌ及びヒトの間で類似していると結論付けられた。

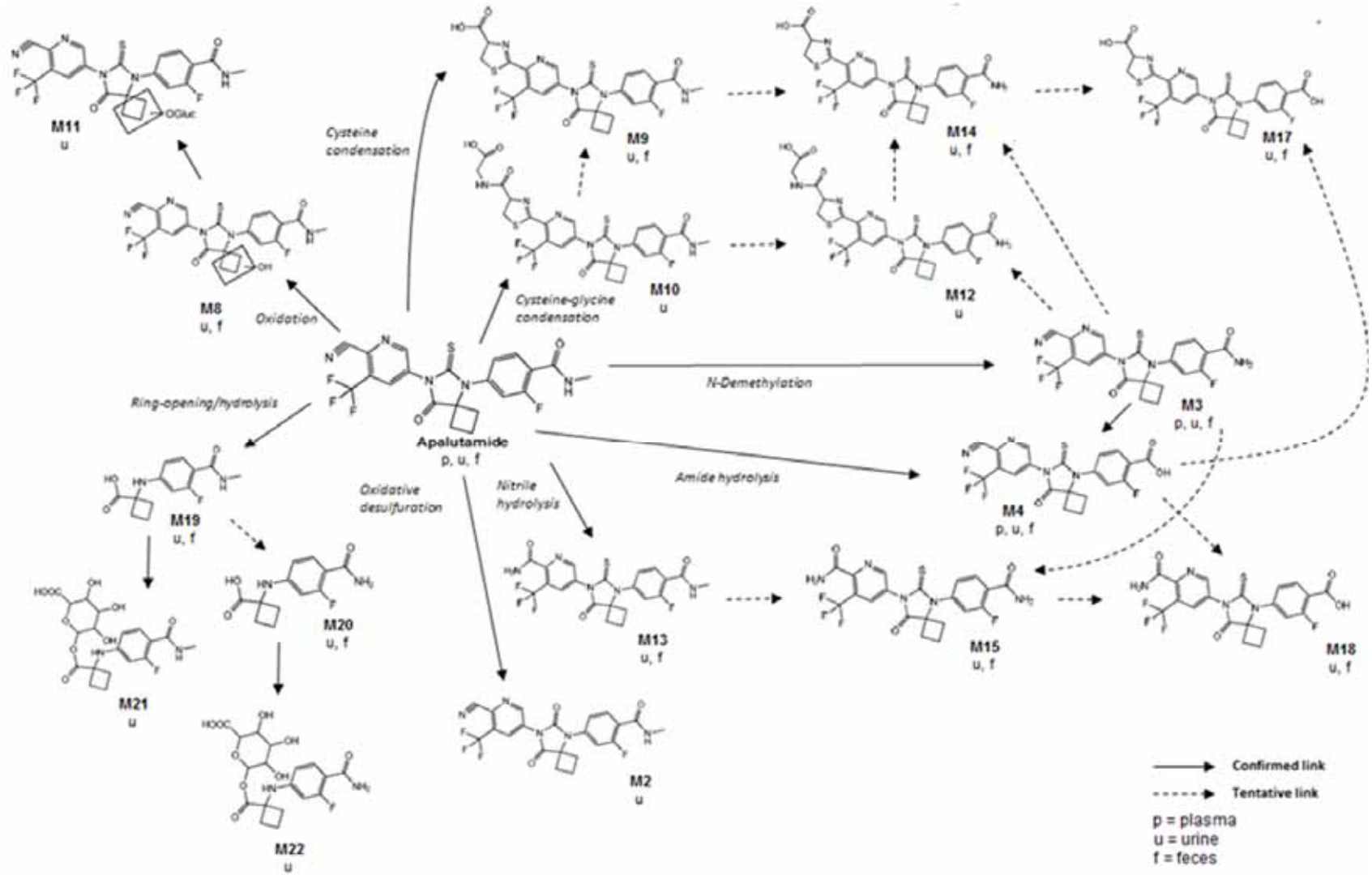
ラット及びヒトにおいて，M3 は *N*-脱メチル化によりアパルタミドから直接生成するが，イヌではアパルタミドの酸化生成物である M6（*N*-(ヒドロキシメチル)ベンズアミド）が M3 の前駆体であった。

ラットで同定された代謝物は少なかったが，すべてイヌ及びヒトでも検出されており，それらは M2/M5，M3/M4 及び M9/M17 のように親／誘導体の関係にあった。



Note: Species-specific pathways and metabolites are labeled as rat or dog.

図 2.6.4-1 ラット及びイヌにおけるアパルタミドの *in vivo* 推定代謝経路



Compounds with alternate identifiers: Apalutamide (JNJ-56021927; ARN-509); M2 (JNJ-56142047, ARN-273); M3 (JNJ-56142060, ARN-308); M4 (JNJ-56142021, ARN-66)

図 2.6.4-2 ヒトにおけるアパルタミドの *in vivo* 推定代謝経路

2.6.4.5.3 *In vitro* 代謝（代謝酵素含む）

【評価資料 4.2.2.4.5，4.2.2.4.6，参考資料 4.2.2.4.1】

ニコチンアミドアデニンジヌクレオチドリン酸（還元型：NADPH）のみの存在下，NADPH 及びグルタチオンの存在下，又は NADPH 及び 3'-ホスホアデノシン-5'-ホスホ硫酸の存在下で，ラット，イヌ及びヒトの肝 S9 画分とともに最長 80 分間インキュベーションしたとき，アパルタミドは非常に遅い代謝速度（長い $t_{1/2}$）を示した。NADPH のみの存在下，又は NADPH 及びウリジン二リン酸グルクロン酸の存在下，ラット，イヌ及びヒトの肝ミクロソームとともに最長 6 時間インキュベーションしたときも，アパルタミドの代謝速度は遅いことが示された【2.6.5.10A】（4.2.2.4.1 参照）。

アパルタミドの代謝速度が非常に遅いため，ヒト肝細胞を用いた *in vitro* リレー法により，M3 及び M4 の生成に関与する CYP 分子種及びエステラーゼを検討した。4 回のリレーで合計 16 時間インキュベーションした。本試験は，カルボキシエステラーゼ阻害薬である BNPP，又は各種 CYP 阻害薬［すなわち，ゲムフィブロジル（CYP2C8 阻害），イトラコナゾール及びトロレアンドマイシン（CYP3A4 阻害）並びにアミノベンゾトリアゾール（複数の CYP 分子種阻害）］の添加／非添加の条件下で実施した。その結果，M3 は主に CYP2C8 を介して生成し，それより若干程度は小さいが CYP3A4 を介しても生成した。M4 の生成は BNPP によって完全に阻害された【2.6.5.10B】（4.2.2.4.5 参照）。

引き続き実施した M3 の肝細胞リレー試験において，BNPP は M3 から M4 への代謝を完全に阻害したため，M4 の生成にはエステラーゼ（おそらくカルボキシエステラーゼ）のみが関与することが示された【2.6.5.10C】（4.2.2.4.6 参照）。

2.6.4.5.4 酵素誘導及び阻害

2.6.4.5.4.1 酵素誘導

【評価資料 4.2.2.4.7，4.2.2.4.8，4.2.3.2.3，4.2.3.2.10】

ヒト培養肝細胞を用いて CYP1A2，CYP2B6 及び CYP3A4 に対するアパルタミド及び M3 の誘導能を評価した。凍結保存された肝細胞（2 例のドナー由来）及び新規調製した単離肝細胞（1 例のドナー由来）を，アパルタミド又は M3（3，10 及び 30 μmol/L）とともに CYP 活性測定前 2 日間培養した。その結果，アパルタミド及び M3 はともに CYP2B6 及び CYP3A4 の活性を濃度依存的に増加させた。30 μmol/L における CYP2B6 及び CYP3A4 に対する最大誘導倍率（陽性対照に対する比率）は，アパルタミドでそれぞれ 117 及び 137%，M3 でそれぞれ 88 及び 72%であった。一方，アパルタミド及び M3 は CYP1A2 を誘導しなかった（溶媒対照に対する誘導倍率は 30 μmol/L において 1.2 以下）【2.6.5.12A】（4.2.2.4.7 参照）。

また，凍結保存されたヒト肝細胞（3 例のドナー由来）を 3～50 μmol/L のアパルタミド又は M3 とともに 2 日間培養した。CYP1A2 の mRNA は，50 μmol/L 以下のアパルタミドにより増加せず，M3 が細胞毒性を示さない最高濃度（2 ドナーで 30 μmol/L，1 ドナーで 50 μmol/L）まで増加しなかった【2.6.5.12B】（4.2.2.4.8 参照）。

一方，アパルタミドの 13 週間反復経口投与毒性試験において，雄ラット（25，50 又は 100 mg/kg/日投与）【2.6.5.12C】（4.2.3.2.3 参照）及び雄イヌ（2.5，5 又は 10 mg/kg/日投与）

【2.6.5.12D】（4.2.3.2.10 参照）から投与終了時及び回復期間終了時に採取した肝臓のミクロソームを用いて，CYP1A，CYP2B，CYP2C（ラットのみ），CYP3A 及び CYP4A の酵素活性を測定し，溶媒対照群と比較した。生物学的に重要と考えられる変化として，ラットの全群における CYP1A 活性の約 2 倍の増加及びイヌの 10 mg/kg/日群における CYP2B 活性の 2.3 倍の増加が認められた。

2.6.4.5.4.2 酵素阻害

【評価資料 4.2.2.4.9，4.2.2.4.10，4.2.2.4.11】

主要な CYP 分子種（CYP1A2，CYP2A6，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1 及び CYP3A4）に対するアパルタミド及び M3 の酵素阻害作用を，ヒト肝ミクロソームを用いて *in vitro* で評価した。

0.075～75 μmol/L の濃度範囲で検討したとき，アパルタミドは CYP2B6 及び CYP2C8 の中程度の阻害薬（IC_{50} はそれぞれ 36 及び 18 μmol/L）であり，CYP2C9，CYP2C19 及び CYP3A4（基質：テストステロン）の弱い阻害薬（IC_{50} は 54～67 μmol/L）であった。基質をミダゾラム又はニフェジピンとした場合の CYP3A4 に対するアパルタミド（75 μmol/L）の阻害はごくわずかであった（阻害率：15～21%）。また，75 μmol/L における CYP1A2，CYP2A6，CYP2D6 及び CYP2E1 の阻害率は 25%未満であった【2.6.5.12E】（4.2.2.4.9 参照）。

0.1～25 μmol/L のアパルタミド及び M3 を用いた試験において，アパルタミドは CYP2C8 を阻害し，その IC_{50} は 13.9 μmol/L であった【2.6.5.12F】（4.2.2.4.10 参照）。

0.075～75 μmol/L の濃度範囲で検討したとき，M3 は CYP2B6，CYP2C8 及び CYP2C9 の中程度の阻害薬（IC_{50} は 38～49 μmol/L）であり，CYP3A4（基質：テストステロン）の弱い阻害薬（IC_{50} は 69 μmol/L）であった。基質をミダゾラム又はニフェジピンとした場合の CYP3A4 に対する M3（75 μmol/L）の阻害はごくわずかであった（阻害率：12～21%）。また，75 μmol/L における CYP2C19 の阻害率は 50%，CYP1A2，CYP2A6，CYP2D6 及び CYP2E1 の阻害率は 16%未満であった【2.6.5.12G】（4.2.2.4.11 参照）。

2.6.4.6 排泄

2.6.4.6.1 排泄経路及び排泄率

2.6.4.6.1.1 ラット

【評価資料 4.2.2.4.2】

無処置及び胆管カニュレーションした雄のラット（各 3 例）に ^{14}C-アパルタミドを 50 mg/kg の用量で単回経口投与したときの放射能の排泄を評価した【2.6.5.13A 及び 2.6.5.14】（4.2.2.4.2 参照）。無処置ラットでは，投与 168 時間後までの放射能の累積排泄率は尿で投与量の 34.2%，糞で投与量の 58.2%であり，いずれも投与 48 時間後までにはほとんどの放射能が回収された。呼気中に回収された放射能は無視できる程度であった（投与 24 時間後までに投与量の 0.03%）。胆管カニュレーションしたラットでは，投与 72 時間後までの採取期間中に，投与量の 50.0%の放射能が胆汁中に排泄され，尿及び糞中にはそれぞれ 29.7 及び 11.2%が排泄された。

2.6.4.6.1.2 イヌ

【評価資料 4.2.2.4.3】

雄ビーグル犬（4 例）に ^{14}C-アパルタミドを 10 mg/kg の用量で単回経口投与したとき，放射能は投与 360 時間後までに尿及び糞中に同程度排泄された（それぞれ，投与量の 41.8 及び 40.2%）【2.6.5.13B】（4.2.2.4.3 参照）。不完全な回収率（投与量の 83.0%）は，薬物由来物質の放射能の排泄が遅いことに起因している可能性が考えられる。尿及び糞中の放射能濃度の $t_{1/2}$ はそれぞれ 74 及び 78 時間であり，イヌにおける ^{14}C-アパルタミド及びその代謝物の遅い排泄と一致した。

2.6.4.6.1.3 ヒト

【評価資料 4.2.2.4.4，参考資料 5.3.1.1.1】

ヒト男性被験者（6 例）に 240 mg の非標識アパルタミド（30 mg カプセル剤を 8 錠）及び 37 kBq（1000 nCi）の ^{14}C-アパルタミド（400 μg）を含有するカプセル剤 1 錠を単回経口投与し，尿，糞及び呼気中に排泄された放射能を加速器質量分析法によって測定した（5.3.1.1.1 参照）。試料は Day 1～10 並びに Day 15，22，29，43，57 及び 71 に 24 時間間隔で採取した。放射能の累積排泄率を Day 71 まで補間し，無限大時間まで外挿した（0 時間から無限大時間までの累積排泄率：$Ae_{0-\infty}$）。$Ae_{0-\infty}$は，尿及び糞並びに呼気中でそれぞれ投与量の 64.6 及び 24.3%並びに 0.06%未満であった。放射能の総回収率（尿及び糞中の $Ae_{0-\infty}$の合計）は 88.9%であった。

本試験の試料を用いた代謝物同定試験において，未変化体及び同定された代謝物の放射能の累積排泄率（投与量に対する%）は，投与 1680 時間後までの尿及び糞中でそれぞれ 64.2 及び 24.1%であった【2.6.5.13C】（4.2.2.4.4 参照）。

2.6.4.7　薬物動態学的薬物相互作用の可能性（トランスポーター）

【評価資料 4.2.2.6.1，4.2.2.6.2，4.2.2.6.3，4.2.2.6.4】

Human colon adenocarcinoma-2（Caco-2）細胞単層膜を用い，アパルタミド（5 μmol/L）の膜透過性を検討した結果，頂側膜側から側底膜側（A→B）方向でのアパルタミドの見掛けの透過係数（P_{app}：42×10^{-6} cm/s）は高く，側底膜側から頂側膜側（B→A）方向（P_{app}：31×10^{-6} cm/s）の透過よりも大きかった【2.6.5.15A】（4.2.2.6.1 参照）。アパルタミドの efflux ratio（B→A/A→B）が 0.7 であったことから，アパルタミドは用いた試験系において能動的な排出を受けないことが示された。

次に，トランスポーターを発現させた細胞系を用いてアパルタミド及び M3 がトランスポーターの基質又は阻害薬となる可能性を検討した。

アパルタミド及び M3（それぞれ 1 及び 5 μmol/L）の P_{app} は，BCRP を発現させた Madin-Darby canine kidney strain II（MDCKII）細胞で高く，親細胞でも同程度であった【2.6.5.15B】（4.2.2.6.2 参照）。アパルタミド及び M3 の efflux ratio は 0.7～0.9 であり，BCRP 阻害薬である KO143 の影響を受けなかった。したがって，アパルタミド及び M3 は BCRP の基質ではないことが示された。一方，アパルタミド及び M3 は，BCRP を介したトポテカン輸送の弱い阻害薬であった（30 μmol/L での最大阻害率はそれぞれ 45 及び 33%）。

Lilly Laboratories cell-porcine kidney（LLC-PK）1 細胞を用いて，アパルタミド及び M3 が MDR1（P-gp）の基質となるかについて評価した【2.6.5.15B】（4.2.2.6.2 参照）。1 及び 5 μmol/L の濃度における efflux ratio は，それぞれアパルタミドで 1.58 及び 1.32，M3 で 1.97 及び 1.56 であった。一方，陽性対照であるジゴキシンの efflux ratio は 7.5 であった。MDR1 阻害薬である GF120918（1 μmol/L）を添加すると，アパルタミド及び M3（それぞれ 1 及び 5 μmol/L）の efflux ratio はいずれも 0.6 となったことから，アパルタミド及び M3 は MDR1 の基質であることが示された。アパルタミド及び M3 はジゴキシンの輸送を 10 μmol/L でわずかに阻害し，30 μmol/L で最大の阻害率（アパルタミド：56%，M3：42%）を示したことから，MDR1 の弱い阻害薬であると考えられる。

アパルタミド及び M3（それぞれ 1 及び 5 μmol/L）の OATP1B1 又は OATP1B3 を発現させた human embryonic kidney293（HEK293）細胞での取り込みは，OATP 阻害薬であるリファンピシンの添加によらず，トランスポーターを発現させた細胞及び親細胞の両方において同程度であった【2.6.5.15C】（4.2.2.6.3 参照）。したがって，アパルタミド及び M3 は OATP1B1 及び OATP1B3 の基質ではないことが示された。^{3}H-エストラジオール-17β-グルクロン酸抱合体の OATP1B1 及び OATP1B3 を介した取り込みは，検討した最高濃度である 30 μmol/L において，アパルタミド（それぞれ阻害率 33 及び 52%）及び M3（それぞれ阻害率 42 及び 38%）によって阻害された。

OCT2［チャイニーズハムスター卵巣（CHO）細胞］並びに OAT3，MATE-1 及び MATE-2K（MDCKII 細胞）による基質輸送に対するアパルタミドの IC_{50} は，それぞれ 27.2 並びに 12.0，13.8 及び 37.9 μmol/L であった【2.6.5.15D】（4.2.2.6.4 参照）。また，OCT2，OAT3 及び MATE-1 による基質輸送に対する M3 の IC_{50} は，それぞれ 4.8，7.6 及び 17.6 μmol/L であり，検討した最高濃度である 50 μmol/L において，M3 は MATE-2K による基質輸送に対してわずかな阻害（32%）を示した。アパルタミド及び M3 は，OAT1（CHO 細胞）による基質取り込みを阻害しなかった。

2.6.4.8 考察及び結論

2.6.4.8.1 吸収

CD-1 マウス，Sprague-Dawley ラット及びビーグル犬を用い，アパルタミド並びにその代謝物である M3 及び M4 の非臨床薬物動態を単回及び反復投与試験において検討した。単回経口投与したとき，アパルタミドは速やかに吸収され，広い用量範囲において用量におおむね比例した薬物動態を示した。t_{max} は用量に依存せず，おおむね投与 2～12 時間後に認められた。

マウス，ラット及びイヌにおいて，アパルタミドは中程度の分布容積（2.1～2.8 L/kg）を有する低クリアランス（0.5～3.9 mL/min/kg）分子であった。脂質ベースのエマルジョンとして経口投与したときのバイオアベイラビリティは，マウスで高く（10 mg/kg の用量で 93%），イヌではほぼ 100%であった（10 mg/kg）。なお，ラットのバイオアベイラビリティは評価していない。

代謝物の血漿中濃度はおおむね投与 24 時間後までに最高となり，活性代謝物 M3 の曝露量は不活性代謝物 M4 の約 2 倍であった。単回投与後のアパルタミドの $t_{1/2}$ は用量依存性がなく，イヌ（69 時間）では，マウス（20 時間）及びラット（7 時間）よりも長かった。

連日反復経口投与したとき，ラットでは 2 週間後に，イヌでは 28 日以内に定常状態に達することが示された。マウスでは，投与 10 又は 14 日後の血漿中濃度は単回投与後の濃度よりも低く，最終相での消失がより速かったため，自己誘導が示唆された。

2.6.4.8.2 分布

全身オートラジオグラフィーの結果から，ラットに ^{14}C-アパルタミドを単回経口投与したとき，放射能は広範な組織に分布し，ほとんどの組織において投与 4～12 時間後に最高濃度を示した。放射能は，腹部及び褐色脂肪，肝臓，腎皮質及び髄質，副腎皮質，膵臓並びにハーダー腺において最も高い濃度を示し，水晶体，脳及び骨表面において最も濃度が低かった。微量の放射能が投与 504 時間後の皮膚に残存したが，有色皮膚内の放射能濃度は白色皮膚と同程度であり，ブドウ膜中に放射能の残存が認められなかったことから，薬物由来物質のメラニンへの結合はほとんどないと考えられる。

2.6.4.8.3 代謝及び排泄

アパルタミドは，ラット，イヌ及びヒトにおいて広範に代謝され，未変化体は，尿中では投与量のわずか 1～3%，糞中では 2～8%しか認められなかった。ラット，イヌ及びヒトにおける代謝経路は定性的に類似していた。

アパルタミドは，酸化，酸化的脱硫，ニトリル加水分解，グリシル-システイン縮合，システイン縮合，アミド加水分解，並びに開環及びそれに続く加水分解によって代謝された。M3（アミド）は主に CYP2C8，及びそれよりも若干程度は小さいが CYP3A4 により生成し，M3 から M4（カルボン酸）への代謝はカルボキシエステラーゼによって触媒される可能性が高かった。*In vitro* での代謝酵素に関する試験結果の臨床的意義は，【2.4】及び【2.7.2】で考察する。

M4 は，尿（ラット，イヌ及びヒト），糞（ラット及びイヌ）並びに胆汁（ラット）中に排泄された放射能の大部分を占める代謝物であった。したがって，検討したすべての動物種及びヒト

においてアミドへの *N*-メチルベンズアミド基の *N*-脱メチル化（M3）及びそれに続くカルボン酸へのアミド加水分解（M4），並びに *N*-メチルベンズアミド基の M4 への直接的アミド加水分解が，アパルタミドの主要な代謝経路であると考えられる。M19（アパルタミドの開環及び加水分解生成物）及びその *N*-脱メチル化による M20（アミド）の生成は，イヌ及びヒトにおける比較的重要な代謝経路であり，尿中で M20 は M4 以外のすべての代謝物の中で最も多かった。

イヌ及びヒト血漿中で確認された代謝物は M3 及び M4 のみであった。AUC に基づくと，血漿中曝露量は，アパルタミドと M3 で同程度又は M3 の方がわずかに高かったが，アパルタミドは M4 より 12～16 倍高かった。ヒトの主要代謝物である M3 に関して，定常状態の曝露量（C_{max} 及び AUC）を動物及びヒト被験者で比較すると，ヒトにおける M3 の曝露量を上回る曝露量が，イヌにおいて認められた。ヒト曝露量に対する動物の曝露量比の更に詳細な情報は【2.6.6.9】に示す。

2.6.4.8.4　薬物動態学的薬物相互作用の可能性

アパルタミド及び M3 は *in vitro* において CYP2B6 及び CYP3A4 を誘導したが，陽性対照に対する誘導倍率は M3 の方が低かった。アパルタミドの誘導作用は CYP3A4 で最も高く，10 及び 30 μmol/L における誘導倍率は陽性対照と同程度か又は若干高かった。

アパルタミドは CYP2B6 及び CYP2C8 の中程度の阻害薬であり，CYP2C9，CYP2C19 及び CYP3A4（基質：テストステロン）の弱い阻害薬であった。M3 は CYP2B6，CYP2C8，CYP2C9 及び CYP3A4（基質：テストステロン）の中程度又は弱い阻害薬であった。

ヒト薬物トランスポーターを発現させた細胞株を用いた検討において，アパルタミド及び M3 は MDR1（P-gp）の基質であったが，BCRP，OATP1B1 及び OATP1B3 の基質ではなかった。また，アパルタミド及び M3 は，MDR1（P-gp），BCRP，OATP1B1 及び OATP1B3 の弱い阻害薬であり（30 μmol/L までの最大阻害率は，最大で 56%），アパルタミド及び M3 は，OCT2，OAT3 及び MATE-1 の中程度の阻害薬であった（IC_{50} 範囲は，アパルタミド：12.0～27.2 μmol/L，M3：4.8～17.6 μmol/L）。MATE-2K に対する IC_{50} はアパルタミド及び M3 で，それぞれ 37.9 μmol/L 及び 50 μmol/L 超であった。一方，アパルタミド及び M3 は OAT1 を阻害しなかった。

In vitro における CYP 誘導／阻害及びトランスポーターに関する試験結果の臨床的意義については，【2.4】及び【2.7.2】において考察している。

2.6.4.9　図表

図表は本文中に記載した。

2.6.5 薬物動態試験概要表

2.6.5.1 薬物動態試験：一覧表

Test Article: JNJ-56021927

Type of Study	Test System	Route (Formulation)	GLP	Test Facility	Study No.	Location in CTD
Absorption						
Pharmacokinetics, single dose	CD-1 Mouse	IV (solution) and Oral (suspension)	No	Aragon San Diego, CA	DR-[redacted]-003-DSD	4.2.2.2.1
Pharmacokinetics, single dose	CD-1 Mouse	Oral (solution)	No	Aragon San Diego, CA	DR-[redacted]-004-DSD	4.2.2.2.2
Pharmacokinetics, single dose	CD-1 Mouse	Oral (solution*)	No	Aragon San Diego, CA	DR-[redacted]-010-DSD	4.2.2.2.3
Pharmacokinetics, single dose (Toxicity study)	CD-1 Mouse	Oral (solution*)	No	Janssen R&D Beerse, Belgium	TOX10822 (single-dose phase)	4.2.3.4.2.1
Pharmacokinetics, repeat-dose (Toxicity study)	CD-1 Mouse	Oral (solution*)	No	Janssen R&D Beerse, Belgium	TOX10822 (repeat-dose phase)	4.2.3.4.2.1
Pharmacokinetics, repeat-dose (Tolerability study)	CD-1 Mouse	Oral (solution*)	No	Janssen R&D Beerse, Belgium	TOX10940	4.2.3.4.2.2
Pharmacokinetics, repeat-dose (Toxicity study)	CByB6F1 Mouse	Oral (solution*)	No	[redacted]	TOX11241	4.2.3.4.2.3
Pharmacokinetics, single dose (Formulation study)[a]	Sprague-Dawley Rat	IV (solution) or Oral (7 formulations*)[a]	No	Aragon San Diego, CA	DR-[redacted]-001-DSD	4.2.2.2.4
Pharmacokinetics, single dose (Bridging study)[b]	Sprague-Dawley Rat	Oral (solution)	No	Janssen R&D Beerse, Belgium	TOX11320	4.2.3.1.1
Pharmacokinetics, single dose (Tolerability study)	Sprague-Dawley Rat	Oral (solution*)	No	Janssen R&D Beerse, Belgium	TOX10760 (single-dose phase)	4.2.3.2.5
Pharmacokinetics, single dose of M3	Sprague-Dawley Rat	IV (suspension) or Oral (solution*)	No	Aragon San Diego, CA	DR-[redacted]-014-DSD	4.2.2.2.5
Pharmacokinetics, single dose of apalutamide or M3	Sprague-Dawley Rat	Oral (solution*)	No	Aragon San Diego, CA	DR-[redacted]-003-DSD	4.2.2.2.6

Additional Information: A number of early development formulations were tested. Specific components of each formulation are indicated in the individual study tabulated summaries. When tested, the lipid-based emulsion (the original clinical formulation) is indicated with an asterisk (*).

* vehicle = the lipid-based emulsion: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15 %w/w/w/w)

[a] Summary of 3 in-life studies: Experiments PK001-09, PK003-09, and PK004-09. Results of PK001-09, Group 8 (IV solution) and PK-009 Formulation B* are reported.

[b] 2 API forms – The ethanolate salt (JNJ-56021927-ZAH) and the free base (JNJ-56021927-AAA)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; API = active pharmaceutical ingredient; Capmul MCM EP = mono-diglyceride of medium chain fatty acids; IV = intravenous; M3 = the *N*-desmethyl metabolite of apalutamide; PEG = polyethylene glycol; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate).

Continued

2.6.5.1 薬物動態試験：一覧表（続き）

Test Article: JNJ-56021927

Type of Study	Test System	Route (Formulation)	GLP	Test Facility	Study No.	Location in CTD
Pharmacokinetics, repeat-dose (Toxicity study)	Sprague-Dawley Rat	Oral (solution*)	No	[redacted]	VUM00002	4.2.3.2.1
Pharmacokinetics, repeat-dose (Toxicity study)	Sprague-Dawley Rat	Oral (solution*)	Yes	[redacted]	VUM00004	4.2.3.2.2
Pharmacokinetics, repeat-dose (Toxicity study)	Sprague-Dawley Rat	Oral (solution*)	Yes	[redacted]	TX-509-1001	4.2.3.2.3
Pharmacokinetics, repeat-dose (Toxicity study)	Sprague-Dawley Rat	Oral (solution*)	Yes	[redacted]	TOX10838	4.2.3.2.4
Pharmacokinetics, repeat-dose (Tolerability study)	Sprague-Dawley Rat	Oral (solution*)	No	Janssen R&D Beerse, Belgium	TOX10760 (repeat-dose phase)	4.2.3.2.5
Pharmacokinetics, repeat-dose (Toxicity study)	Sprague-Dawley Rat	Oral (solution*)	Yes	[redacted]	TOX10897	4.2.3.2.6
Pharmacokinetics, single dose (Formulation study)	Beagle Dog	IV (suspension) or 3 oral formulations (2 capsule - including Formulation B*, and one solution)	No	[redacted]	DR-[redacted]-002-DSD	4.2.2.2.7
Pharmacokinetics, single dose (Formulation study)	Beagle Dog	Oral (5 amorphous formulations, a reference suspension, and softgel – Formulation 7*)	No	Aragon San Diego, CA	DR-[redacted]-011-DSD	4.2.2.2.8
Pharmacokinetics, single dose (Formulation study)	Beagle Dog	Oral (5 tablet formulations and a softgel – Formulation F*)	No	Janssen R&D Beerse, Belgium	FK10587	4.2.2.2.9
Pharmacokinetics, single dose (Formulation study)	Beagle Dog	Oral (Three 60-mg concept formulations: softgel*, nanosuspension, and a microsuspension)	No	Janssen R&D Beerse, Belgium	FK10634	4.2.2.2.10
Pharmacokinetics, single dose (Formulation study)	Beagle Dog	Oral (60-mg softgel capsule formulations – 2 different API forms*)	No	Janssen R&D Beerse, Belgium	FK10786	4.2.2.2.11
Pharmacokinetics, single dose (Toxicity study)	Beagle Dog	Oral (2 formulations: oral solution* and capsule*)	No	Janssen R&D Beerse, Belgium	TOX10780 (single-dose phase)	4.2.3.2.12
Pharmacokinetics, repeat-dose (Toxicity study)	Beagle Dog	Oral (capsule*)	No	[redacted]	VUM00003	4.2.3.2.7
Pharmacokinetics, repeat-dose (Toxicity study)	Beagle Dog	Oral (capsule*)	Yes	[redacted]	VUM00005	4.2.3.2.8

Additional Information: A number of early development formulations were tested. Specific components of each formulation are indicated in the individual study tabulated summaries. When tested, the lipid-based emulsion (the original clinical formulation) is indicated with an asterisk (*).

* vehicle = the lipid-based emulsion: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15 %w/w/w/w)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; API = active pharmaceutical ingredient; Capmul MCM EP = mono-diglyceride of medium chain fatty acids; [redacted] = [redacted]; IV = intravenous; PEG = polyethylene glycol; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate).

Continued

2.6.5.1 薬物動態試験：一覧表（続き）

Test Article: JNJ-56021927

Type of Study	Test System	Route (Formulation)	GLP	Test Facility	Study No.	Location in CTD
Toxicokinetics of M3	Dog Plasma[a]	*In vitro*	No	[redacted]	DR-[redacted]-008-DSD	4.2.3.2.9
Pharmacokinetics, repeat-dose (TK and Toxicity study)	Beagle Dog	Oral (capsule*)	Yes	[redacted]	TX-509-1002	4.2.3.2.10
Pharmacokinetics, repeat-dose (Toxicity study)	Beagle Dog	Oral (capsule*)	Yes	[redacted]	TOX10839	4.2.3.2.11
Pharmacokinetics, repeat-dose (Toxicity study)	Beagle Dog	Oral (capsule*)	No	Janssen R&D Beerse, Belgium	TOX10780 (repeat-dose phase)	4.2.3.2.12
Pharmacokinetics, repeat-dose (Toxicity study)	Beagle Dog	Oral (capsule*)	Yes	[redacted]	TOX10895	4.2.3.2.13
Distribution						
Whole-body Quantitative Autoradiography	Sprague-Dawley Rat and Lister Hooded Rat	Oral (solution*)	Yes	[redacted]	FQA0001 (Phase A, B)	4.2.2.4.2
Brain and plasma concentrations	CD-1 Mouse	Oral (solution*)	No	Aragon San Diego, CA	DR-[redacted]-010-DSD	4.2.2.2.3
Brain and plasma concentrations	Sprague-Dawley Rat	Oral (solution*)	No	Aragon San Diego, CA	DR-[redacted]-014-DSD	4.2.2.2.5
Brain and plasma concentrations	Beagle Dog	Oral (capsule*)	No	Janssen R&D Beerse, Belgium	TOX10780 (repeat-dose phase)	4.2.3.2.12
Brain, plasma and CSF concentrations	Beagle Dog	Oral (capsule*)	Yes	[redacted]	TX-509-1002	4.2.3.2.10
PPB	Mouse, rat, dog, and human plasma	*In vitro* (DMSO/solution)	No	[redacted]	301010034	4.2.2.3.1
PPB	Mouse, rat, dog, and human plasma	*In vitro* (DMSO/solution)	No	[redacted]	301093931	4.2.2.3.2

Additional Information: A number of early development formulations were tested. Specific components of each formulation are indicated in the individual study tabulated summaries. When tested, the lipid-based emulsion (the original clinical formulation) is indicated with an asterisk (*).

* vehicle = the lipid-based emulsion: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15 %w/w/w/w)

[a] Plasma samples from Study VUM00005.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; API = active pharmaceutical ingredient; [redacted] = [redacted]; Capmul MCM EP = mono-diglyceride of medium chain fatty acids; [redacted] = [redacted]; CSF = cerebral spinal fluid; DMSO = dimethyl sulfoxide; [redacted] = [redacted]; M3 = the *N*-desmethyl metabolite of apalutamide; PEG = polyethylene glycol; PPB = plasma protein binding; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate).

Continued

2.6.5.1 薬物動態試験：一覧表（続き）

Test Article: JNJ-56021927

Type of Study	Test System	Route (Formulation)	GLP	Test Facility	Study No.	Location in CTD
PPB	Mouse, rat, rabbit, dog, and human plasma	*In vitro* (Ethanol/solution)	No	Janssen R&D Beerse, Belgium	FK10658	4.2.2.3.3
PPB	SHO Mouse plasma	*In vitro* (DMSO/solution)	No	Aragon San Diego, CA	DR-[redacted]-014-DSD	4.2.2.3.4
Metabolism						
Metabolite profiles; metabolic turnover	Rat, dog, human LM; rat and dog plasma	Oral (solution), Oral (capsule)[a]/*In vitro* (DMSO and acetonitrile solution)	No	Aragon San Diego, CA	DR-[redacted]-007-DSD	4.2.2.4.1
Metabolite profiles	Intact and bile-cannulated male rats	Oral (solution*)	Yes	[redacted], UK	FQA0001	4.2.2.4.2
Metabolite profiles	Beagle dog	Oral (capsule*)	No	Janssen R&D Beerse, Belgium	FK10704	4.2.2.4.3
Metabolite profiles	Human urine, feces, plasma	Oral (capsule)[b]/*In vitro*	No	Janssen R&D Beerse, Belgium	FK10840	4.2.2.4.4
CYP and esterase involvement in metabolite formation	Human hepatocytes	*In vitro* (methanol)	No	Janssen R&D Beerse, Belgium	FK10596	4.2.2.4.5
CYP and esterase involvement in metabolite formation (M3 to M4)	Human hepatocytes	*In vitro* (methanol)	No	Janssen R&D Beerse, Belgium	FK10924	4.2.2.4.6
Enzyme Induction and Inhibition						
Induction of CYP1A2	Human hepatocytes	*In vitro* (DMSO)	No	[redacted]	301091248	4.2.2.4.7
Induction of CYP1A2	Human hepatocytes	*In vitro* (DMSO)	No	Janssen R&D Beerse, Belgium	FK10955	4.2.2.4.8

Additional Information: A number of early development formulations were tested. Specific components of each formulation are indicated in the individual study tabulated summaries. When tested, the lipid-based emulsion (the original clinical formulation) is indicated with an asterisk (*).

* vehicle = the lipid-based emulsion: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15 %w/w/w/w)

[a] Rat and dog plasma samples from studies VUM00002 and VUM00003, respectively.

[b] Collected in Study ARN-509-006, part B

Acconon MC8-2 = caprylocaproyl macrogolglycerides; [redacted] = [redacted]; Capmul MCM EP = mono-diglyceride of medium chain fatty acids; CYP = cytochrome P450; DMSO = dimethyl sulfoxide; [redacted] = [redacted]; LM = liver microsomes; M3 = the *N*-desmethyl metabolite of apalutamide; M4 = the metabolite formed by amide hydrolysis of apalutamide or M3; PEG = polyethylene glycol; PPB = plasma protein binding; SHO = SCID Hairless Outbred; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate).

Continued

2.6.5.1 薬物動態試験：一覧表（続き）

Test Article: JNJ-56021927

Type of Study	Test System	Route (Formulation)	GLP	Test Facility	Study No.	Location in CTD
Apalutamide inhibition of CYP enzymes	Human LM	*In vitro* (2:1 methanol:DMSO solution)	No	Janssen R&D Beerse, Belgium	FK10651	4.2.2.4.9
Apalutamide and M3 inhibition of CYP enzymes	Human LM	*In vitro* (DMSO/solution)	No	[redacted], UK	CYP361_R6	4.2.2.4.10
M3 inhibition of CYP enzymes	Human LM	*In vitro* (2:1 methanol:DMSO solution)	No	Janssen R&D Beerse, Belgium	FK10660	4.2.2.4.11
Excretion						
Absorption, distribution, metabolism, and excretion	Sprague-Dawley Rat	Oral (solution*)	Yes	[redacted], UK	FQA0001 (Phase C, D)	4.2.2.4.2
Metabolism and excretion	Beagle Dog	Oral (solution*)	No	Janssen R&D Beerse, Belgium	FK10704	4.2.2.4.3
Absorption, metabolism and excretion	Human	*In vivo*[a] (capsule*)	No	Janssen R&D Beerse, Belgium	FK10840	4.2.2.4.4
Drug Transporter Studies						
Bidirectional membrane permeability	Caco-2 cells	*In vitro* (assay buffer[b]/solution)	No	[redacted]	[redacted]ARAGP1R1	4.2.2.6.1
ABCB1 and ABCG2 efflux transporters	LLC-PK1, LLC-PK1 MDR1 transfected, and MDCK-II cells	*In vitro* (DMSO solution)	No	Janssen R&D Beerse, Belgium	FK10661	4.2.2.6.2

Additional Information: A number of early development formulations were tested. Specific components of each formulation are indicated in the individual study tabulated summaries. When tested, the lipid-based emulsion (the original clinical formulation) is indicated with an asterisk (*).

* vehicle = the lipid-based emulsion: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15 %w/w/w/w)

[a] Samples collected in Clinical Study ARN-509-006, part B

[b] Assay buffer: Hanks Balanced Salt Solution containing 10mM HEPES and 15 mM glucose at a pH of 7.4

ABC = ATP-binding cassette; Acconon MC8-2 = caprylocaproyl macrogolglycerides; Capmul MCM EP = mono-diglyceride of medium chain fatty acids; CYP = cytochrome P450; DMSO = dimethyl sulfoxide; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; [redacted] = [redacted]; LLC-PK = Lilly Laboratories Cell - Porcine Kidney; LM = liver microsomes; M3 = the *N*-desmethyl metabolite of apalutamide; MDCK = Madin-Darby canine kidney; MDR = multidrug-resistant; PEG = polyethylene glycol; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate).

Continued

2.6.5.1 薬物動態試験：一覧表（続き）

Test Article: JNJ-56021927

Type of Study	Test System	Route (Formulation)	GLP	Test Facility	Study No.	Location in CTD
Drug Transporter Studies						
OATP1B1- and OATP1B3-mediated transport	Parental and stably transfected HEK293 cells	*In vitro* (DMSO/solution)	No	Janssen R&D Beerse, Belgium	FK10693	4.2.2.6.3
Inhibition potential on OCT2, OAT1, OAT3, MATE-1, and MATE-2K transporters	Parental and stably transfected OCT2, OAT1, OAT3, MATE-1, and MATE-2K cells	*In vitro* (DMSO/solution)	No	Janssen R&D Beerse, Belgium	FK11028	4.2.2.6.4

DMSO = dimethyl sulfoxide; HEK = human embryonic kidney; MATE = multi-antimicrobial extrusion protein; OAT = organic anion transporter; OATP = organic anion-transporting polypeptide; OCT = organic cation transporter

2.6.5.2 分析方法及びバリデーション試験

Information on analytical methods and validation reports can be found in Mod 2.6.4.

2.6.5.3 A 薬物動態試験：単回投与後の吸収（静脈内）

Test Article: JNJ-56021927

Location in CTD	4.2.2.2.1, 4.2.2.2.4, 4.2.2.2.7		
Study No.	DR-[redacted]-003-DSD	DR-[redacted]-001-DSD	DR-[redacted]-002-DSD
Species	**CD-1 Mouse**	**Sprague-Dawley Rat**	**Beagle Dog**
Feeding Condition	Fasted	Fasted	Fasted
Sample	Plasma	Plasma	Plasma
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Analyte	JNJ-56021927	JNJ-56021927	JNJ-56021927
LLOQ	0.005 µg/mL	0.0122 µg/mL	0.0122 µg/mL
Vehicle/Formulation	Solution[a]	Solution[a]	Solution[a]
Route	IV bolus	IV bolus	IV bolus
Gender/Number of Animals	M: 12	M: 4[b]	M: 3
Dose (mg/kg)	3	3	3
Mean (SD) Pharmacokinetic Parameters[c]			
C_{max} (µg/mL)	-[d]	-[d]	-[d]
t_{max} (h)	-[d]	-[d]	-[d]
$AUC_{0-\infty}$ (µg·h/mL)	37.7	13 (1.2)	105 (19)
$t_{½}$ (h)	19.5	7.3 (1.3)	68.8 (25.6)
V_z (L/kg)	-	2.43 (0.31)	2.77 (0.58)
V_{ss} (L/kg)	2.10	2.21 (0.09)	2.76 (0.51)
Clearance (CL_p; mL/min/kg)	1.33	3.88 (0.35)	0.486 (0.092)

Additional Information: Data for DR-[redacted]-001-DSD are from Experiment PK001-09, Group 8 and data for DR-[redacted]-002-DSD are from the intravenous arm of experiment AGN-002.

[a] Vehicle: DMSO, PEG 400, PVP k30, Tween-80, Ethanol, HP-β-CD (30% w/v) (5:18:1:1:5:70) – lipid IV formulation.

[b] Data reported for a mean of 3 rats. One animal died after the 2 hour time point.

[c] For the calculation of AUC values after single dosing, 0 h concentrations were added and equated with 0.

[d] C_0 for intravenous; not calculated.

- = not calculated/not applicable; AUC= area under the plasma concentration-time curve; $AUC_{0-\infty}$ = AUC from time 0 to infinity; C_0 = plasma concentration at time 0; CL_p = total plasma clearance; C_{max} = maximum observed plasma concentration; DMSO = dimethyl sulfoxide; HP-β-CD = hydroxypropyl-β-cyclodextrin; IV = intravenous; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; PVP = polyvinylpyrrolidone; $t_{½}$ = terminal half-life; t_{max} = time correspondent to maximum plasma concentration; Tween = polyoxyethylene sorbitan monooleate; V_{ss} = volume of distribution at steady-state; V_z = volume of distribution based on the terminal phase

2.6.5.3 B 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Location in CTD	4.2.2.2.1, 4.2.2.2.2			
Study No.	DR-[redacted]-003-DSD		DR-[redacted]-004-DSD	
Species	**CD-1 Mouse**		**CD-1 Mouse**	
Feeding Condition	Fasted		Fasted	
Route	Oral gavage		Oral gavage	
Vehicle/Formulation	Suspension[a]		Solution[b]	
Sample	Plasma		Plasma	
Analyte	JNJ-56021927		JNJ-56021927	
Assay	LC-MS/MS		LC-MS/MS	
LLOQ	0.005 µg/mL		0.196 µg/mL	
Gender/Number of Animals	M: 9	M: 9	M: 9	M: 9
Dose (mg/kg)	10	10	30	100
Mean Pharmacokinetic Parameters[c]				
C_{max} (µg/mL)	3.51	10.9	20.6	56.7
t_{max} (h)	1.0	8.0	2.0	8.0
$AUC_{0-\infty}$ (µg·h/mL)	118	212	595	1730
$t_{½}$ (h)	17.8	8.7	17.8	15.0
$Bioavailability_{AUC0-\infty}$ (%)	93.3	-	-	-

[a] Vehicle: PEG-400, Tween-80, PVP-k30, VitE-TPGS, and 20 mM Citrate Buffer (pH 4) in 0.5% HPMC in water; 18:1:1:15:65
[b] Vehicle: PEG-400, Tween-80, PVP-k30, VitE-TPGS, and 20 mM Citrate Buffer (pH 4) in CMC in water (0.5% w/v)
[c] N = 3 samples/time point.

- = not calculated/not applicable; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 to infinity; C_{max} = maximum observed plasma concentration; CMC = carboxymethyl cellulose; HPMC = hydroxypropyl methylcellulose; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; N = number; PEG = polyethylene glycol; PVP = polyvinylpyrrolidone; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; Tween = polyoxyethylenesorbitan monooleate; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3 C 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	DR-[redacted]-010-DSD					
Location in CTD	4.2.2.2.3					
Species	**CD-1 Mouse**					
Feeding Condition	Fasted					
Vehicle/Formulation	Acconon MC8-2 EP/NP, Capmul MCM EP, PEG-400, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral gavage					
Sample	Plasma					
Analyte	JNJ-56021927			M3 (JNJ-56142060)[a]		
Assay	LC-MS/MS			LC-MS/MS		
LLOQ	0.0976 µg/mL			0.0976 µg/mL		
Gender/Number of Animals	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9
Dose (mg/kg)	10	30	100	10	30	100
Mean Pharmacokinetic Parameters						
C_{max} (µg/mL)	3.26	10.0	28.7	0.88	2.88	10.0
t_{max} (h)	4.0	4.0	8.0	24.0	16.0	24.0
$t_{½}$ (h)	34.8	29.2	13.2	-	-	-
AUC_{0-24h} (µg·h/mL)	57.6	188	483	12.6	40.3	149
$AUC_{0-\infty}$ (µg·h/mL)	152	416	720	-	-	-
M/P $Ratio_{Cmax}$	-	-	-	0.270	0.288	0.348
M/P $Ratio_{AUC0-24h}$	-	-	-	0.219	0.214	0.308

Additional Information: DR-[redacted]-010-DSD is a compilation of 4 experiments, 2 of which dosed apalutamide – one in mice (PK096-10) and one in rats (PK031-10). Results listed are from the former which assessed pharmacokinetics and brain exposure.

[a] The *N*-desmethyl metabolite of apalutamide.

- = not calculated; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0-\infty}$ = AUC from time 0 to infinity; AUC_{0-24h} = AUC from time 0 to 24 hours postdose; Capmul MCM = mono-diglyceride of medium chain fatty acids; C_{max} = maximum observed plasma concentration; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M/P = metabolite-to-parent; PEG = polyethylene glycol; $t_{½}$ = terminal half-life; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3 D 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Location in CTD	4.2.3.4.2.1											
Study No.	TOX10822 (Single-dose phase)											
Species	**CD-1 Mouse**											
Feeding Condition	Fed											
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15 %w/w/w/w)/solution											
Route	Oral gavage											
Sample	Plasma											
Analyte	JNJ-56021927						M3 (JNJ-56142060)[a]					
Assay	LC-MS/MS						LC-MS/MS					
LLOQ	0.010 µg/mL						0.010 µg/mL					
Gender/Number of Animals	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F: 6
Dose (mg/kg)	20	20	100	100	500	500	20	20	100	100	500	500
Mean Pharmacokinetic Parameters[b]												
C_{max} (µg/mL)	4.69	6.25	24.1	28.1	71	84.7	0.896	0.866	6.68	6.66	17.8	7.29
t_{max} (h)	7	7	7	3	7	24	24	24	24	24	24	48
AUC_{0-48h} (µg·h/mL)	156	168	649	662	1750	2470	26.8	25.7	180	174	474	244
$AUC_{0-\infty}$ (µg·h/mL)	191	214	661	704	1760	2480	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] N ≤3/dose/sex/timepoint due to mortality. C_{max} and AUC values reported as ng/mL and ng·h/mL, respectively in study report.

[c] Not calculated; AUC_{ext} > 25%

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0-\infty}$ = AUC from time 0 to infinity; AUC_{0-48h} = AUC from time 0 to 48 hours postdose; AUC_{ext} = AUC extrapolated from time 0 to infinity; Capmul MCM = mono-diglyceride of medium chain fatty acids; C_{max} = maximum observed plasma concentration; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3 E 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927[a]

Study No.	DR-[redacted]-001-DSD		
Location in CTD	4.2.2.2.4		
Species	**Sprague-Dawley Rat**		
Feeding Condition	Fed		
Route	Oral gavage		
Sample	Plasma		
Analyte	JNJ-56021927		
Assay	LC-MS/MS		
LLOQ	0.0488 µg/mL		
Vehicle/Formulation	Formulation A[b]/suspension	Formulation B[c]/suspension	Formulation C[d]/suspension
Gender /Number of Animals	M:4	M:4	M:4
Dose (mg/kg)	1000	1000	1000
Mean (SD) Pharmacokinetic Parameters			
C_{max} (µg/mL)	18.9 (3.6)	13.2 (2.5)	37.8 (5.2)
t_{max} (h)	8.0 (4.0)	7.0 (2.0)	10.0 (2.3)
$AUC_{0\text{-}72h}$ (µg·h/mL)	456 (117)	291 (49)	1100 (56)
$AUC_{0\text{-}\infty}$ (µg·h/mL)	458 (117)	295 (49)	1110 (56)
k_{el} (h^{-1})	0.081 (0.007)	0.063 (0.010)	0.078 (0.006)
$t_{1/2}$ (h)	8.6 (0.8)	11.2 (1.7)	8.9 (0.7)

Additional Information: DR-[redacted]-001-DSD is a compilation of 3 early in-life studies. The results listed are from Groups 1 – 3 of experiment PK004-09. Formulation B is the lipid-based emulsion.

[a] Two lots of the test apalutamide were used (3.0 grams of Lot #[redacted] and 1.5 grams of Lot #[redacted]). The 2 lots were thoroughly blended as dry powders before manufacture of the dose formulations.

[b] Vehicle: Acconon MC8-2, EP/NF 20% (v/v); PEG-400 30% (v/v); Tween-80 1% (v/v); and Capmul MCM, EP 49% (a lipid formulation)

[c] Vehicle: Acconon MC8-2, EP/NF 10% (v/v); PEG-400 30% (v/v); VitE-TPGS NF 15% (w/v); and Capmul MCM, EP 45% (a lipid formulation)

[d] Vehicle: PEG-400 40% (v/v); VitE-TPGS 18% (w/v); Tween-80 2% (v/v); Poloxamer 407 2% (w/v) and 20 mM citrate buffer (pH 3) 38% (an aqueous formulation)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}72h}$ = AUC from time 0 to 72 hours postdose; $AUC_{0\text{-}\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; kel = elimination rate constant; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; Poloxamer = a nonionic surfactant, a copolymer consisting of polypropylene glycol and polyethylene glycol; SD = standard deviation; $t_{½}$ = terminal half-life; tmax = time correspondent to maximum plasma concentration; Tween = polyoxyethylenesorbitan monooleate; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3　F　薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX11320							
Location in CTD	4.2.3.1.1							
Species	**Sprague-Dawley Rat**							
Feeding Condition	Fed							
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15 %w/w/w/w)/ solution							
Route	Oral gavage							
Sample	Plasma							
Analyte	JNJ-56021927				M3 (JNJ-56142060)[a]			
Assay	LC-MS/MS				LC-MS/MS			
LLOQ	0.025 µg/mL				0.025 µg/mL			
API form	JNJ-56021927-AAA[b]		JNJ-56021927-ZAH[c]		JNJ-56021927-AAA[b]		JNJ-56021927-ZAH[c]	
Gender/Number of Animals	M:3	M:3	M:3	M:3	M:3	M:3	M:3	M:3
Dose (mg/kg)	5	50	5	50	5	50	5	50
Mean (SD) Pharmacokinetic Parameters								
C_{max} (µg/mL)	1.21 (0.274)	11.6 (0.416)	1.22 (0.288)	11.5 (0.608)	-[d]	0.389 (0.243)	0.284 (0.293)	0.286 (0.0265)
t_{max} (h)	13 (9.2)	8.0 (0.0)	8.0 (0.0)	8.0 (0.0)	-[d]	12 (11)	6.0 (2.8)	13 (9.2)
$AUC_{0-\infty}$ (µg·h/mL)	29.8 (10.1)	239 (36.2)	22.5 (5.20)	238 (12.3)	-[d]	-[d]	-[d]	-[d]

Additional Information: A bridging toxicokinetic study to compare exposures of apalutamide (JNJ-56021927) and the *N*-desmethyl metabolite of apalutamide(JNJ-56142060) after single oral dosing of the 2 different APIs: the free-base form of apalutamide (JNJ-56021927-AAA) and the ethanolate salt (JNJ-56021927-ZAH) to support selection of dose levels for future studies using the free-base form.

[a] The *N*-desmethyl metabolite of apalutamide.
[b] Free base form.
[c] Ethanolate form.
[d] Insufficient plasma concentration data.

- = not calculated; API = active pharmaceutical ingredient; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3 G 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10760 (Single-dose phase)							
Location in CTD	4.2.3.2.5							
Species	**Sprague-Dawley Rat**							
Feeding Condition	Fed							
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS (10:45:30:15 %w/w/w/w)/solution							
Route	Oral gavage							
Sample	Plasma							
Analyte	JNJ-56021927				M3 (JNJ-56142060)[a]			
Assay	LC-MS/MS				LC-MS/MS			
LLOQ	0.025 µg/mL				0.025 µg/mL			
Gender /Number of Animals	F:3	F:3	F:3	F:3	F:3	F:3	F:3	F:3
Dose (mg/kg)	50	100	150	250	50	100	150	250
Mean (SD) Pharmacokinetic Parameters[b]								
C_{max} (µg/mL)	15.6 (0.586)	30.8 (2.61)	37.5 (4.22)	29.1 (0.379)	0.158 (0.0475)	0.487 (0.0675)	0.723 (0.208)	0.326 (0.0267)
t_{max} (h)	9.3 (2.3)	11 (2.3)	12 (0.0)	9.3 (2.3)	12 (0.0)	20 (6.9)	26 (4.0)	24 (0.0)
t_{last} (h)	72 (0.0)	72 (0.0)	72 (0.0)	96 (0.0)	48 (0.0)	64 (14)	72 (0.0)	80 (14)
AUC_{0-24h} (µg·h/mL)	267 (17.9)	548 (79.6)	724 (78.0)	521 (35.1)	2.73 (0.765)	8.44 (1.51)	9.98 (3.17)	5.10 (0.602)
AUC_{last} (µg·h/mL)	446 (24.2)	963 (267)	1320 (205)	908 (28.3)	4.65 (1.12)	17.0 (6.3)	23.9 (7.41)	10.4 (1.25)
$AUC_{0-\infty}$ (µg·h/mL)	465 (32.1)	1030 (314)	1380 (232)	918 (27.0)	5.69 (1.71)	18.0 (7.09)	24.4 (7.65)	10.5 (1.20)

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] C_{max} and AUC values reported as ng/mL and ng·h/mL, respectively in study report.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; AUC_{0-24h} = AUC from time 0 to 24 hours postdose; $AUC_{0-\infty}$ = AUC from time 0 to infinity; AUC_{last} = AUC from time 0 to the last quantifiable concentration; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; t_{last} = time correspondent to last quantifiable plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3 H 薬物動態試験：単回投与後の吸収（経口又は静脈内）

Test Article: M3(JNJ-56142060)[a]

Study No.	DR-[redacted]-014-DSD			
Location in CTD	4.2.2.2.5			
Species	**Sprague-Dawley Rat**			
Feeding Condition	Fasted			
Sample	Plasma			
Assay	LC-MS/MS			
Analyte	M3 (JNJ-56142060)[a]		M4 (JNJ-56142021)[b]	
LLOQ	0.0012 µg/mL		0.0012 µg/mL	
Vehicle/Formulation	Suspension[c]	Solution[d]	Suspension[c]	Solution[d]
Route	Intravenous	Oral gavage	Intravenous	Oral gavage
Gender/Number of Animals	M: 4	M: 4	M: 4	M: 4
Dose (mg/kg)	3	10	3	10
Mean (SD) Pharmacokinetic Parameters				
C_{max} (µg/mL)	-	0.53 (0.13)	0.352 (0.014)	0.204 (0.054)
t_{max} (h)	-	7.00 (3.83)	0.92 (0.17)	7.00 (3.83)
$AUC_{0\text{-}24h}$ (µg·h/mL)	3.07 (0.16)	7.21 (0.90)	1.74 (0.21)	3.04 (0.35)
$AUC_{0\text{-}\infty}$ (µg·h/mL)	3.09 (0.16)	8.02 (0.46)	1.78 (0.21)	4.18 (0.61)
$t_{1/2}$ (h)	4.91 (0.22)	6.15 (1.80)	3.80 (1.52)	11.0 (5.51)
V_{ss} (L/kg)	2.63 (0.43)	-	-	-
Clearance (CL_p; mL/min/kg)	16.2 (0.8)	-	-	-
$Bioavailability_{AUC0\text{-}\infty}$ (%)	-	77.9	-	-
$M4/M3\ Ratio_{AUC0\text{-}24h}$	-	-	0.57 (0.07)	0.42 (0.04)

[a] The *N*-desmethyl metabolite of apalutamide.
[b] The metabolite formed by amide hydrolysis of apalutamide or M3
[c] Vehicle: DMSO, ethanol:[PEG-400:Tween-80:PVP (9:0.5:0.5)]:30% HP-β-CD (5:5:20:70)
[d] Vehicle: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15 %w/w/w/w)

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}\infty}$ = AUC from time 0 to infinity; $AUC_{0\text{-}24h}$ = AUC from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; CLp = total plasma clearance; DMSO = dimethyl sulfoxide; HP-β-CD = hydroxypropyl-β-cyclodextrin; IV = intravenous; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M3 = The *N*-desmethyl metabolite of apalutamide; M4 = the metabolite formed by amide hydrolysis of apalutamide or M3; PEG = polyethylene glycol; PVP = polyvinylpyrrolidone; SD = standard deviation; $t_{½}$ = terminal half-life; t_{max} = time correspondent to maximum plasma concentration; Tween = polyoxyethylenesorbitan monooleate; V_{ss} = volume of distribution at steady-state; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3 I 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	DR-[redacted]-003-DSD								
Location in CTD	4.2.2.2.6								
Species	**Sprague-Dawley Rat**								
Feeding Condition	Fed								
Sample	Plasma								
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 NF, VitE-TPGS (10:45:30:15 %w/w/w/w)/ solution								
Route	Oral gavage								
Analyte	JNJ-56021927			M3 (JNJ-56142060)[a]			M4 (JNJ-56142021)[b]		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS		
LLOQ	0.05 µg/mL			0.05 µg/mL			0.05 µg/mL		
Gender /Number of Animals	M:4	M:4	M:4	M:4	M:4	M:4	M:4	M:4	M:4
Dose (mg/kg)	500	1000	2000	500	1000	2000	500	1000	2000
Mean (SD) Pharmacokinetic Parameters									
C_{max} (µg/mL)	18.6 (2.32)	13.8 (3.43)	13.4 (1.87)	1.41 (0.330)	0.874 (0.204)	0.700 (0.020)	2.33 (0.574)	1.49 (0.254)	1.68 (0.170)
t_{max} (h)	11 (2.0)	12 (0.0)	12 (0.0)	21 (6.0)	24 (0.0)	18 (6.9)	24 (0.0)	14 (6.9)	14 (6.9)
$AUC_{0-\infty}$ (µg·h/mL)	516 (18.7)	521 (273)	410 (18.8)	42.8 (5.07)	34.3 (18.8)	23.6 (2.25)	73.0 (8.61)	64.5 (40.6)	56.8 (7.66)
Ratio of M3/$P_{AUC0-\infty}$ (%)	-	-	-	8.28 (0.82)	6.60 (0.24)	5.76 (0.46)	-	-	-
Ratio of M3/P_{Cmax} (%)	-	-	-	7.59 (1.80)	6.10 (0.72)	5.32 (0.73)	-	-	-
Ratio of M4/$P_{AUC0-\infty}$ (%)	-	-	-	-	-	-	14.1 (1.6)	12.0 (1.8)	13.9 (1.8)
Ratio of M4/P_{Cmax} (%)	-	-	-	-	-	-	12.5 (2.8)	10.0 (1.0)	12.6 (1.0)

Additional Information: This study is the compilation of 2 reports (PK052-13 and PK053-13) in which apalutamide and its M3 metabolite were dosed, respectively. The pharmacokinetics of apalutamide, M3 and M4 were calculated in PK052-13, and the pharmacokinetics of M3 and M4 were determined in PK053-13.

[a] The *N*-desmethyl metabolite of apalutamide.
[b] The metabolite formed by amide hydrolysis of apalutamide or M3

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M3 = the *N*-desmethyl metabolite of apalutamide; M3/P = M3 to parent; M4 = the metabolite formed by amide hydrolysis of apalutamide or M3; M4/P = M4 to parent; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; SD = standard deviation; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

Test Article: M3 (JNJ-56142060)[a]

Study No.	DR-[redacted]-003-DSD					
Location in CTD	4.2.2.2.6					
Species	**Sprague-Dawley Rat**					
Feeding Condition	Fed					
Sample	Plasma					
Vehicle	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 NF, VitE-TPGS (10:45:30:15 %w/w/w/w)/ solution					
Route	Oral gavage					
Analyte	M3 (JNJ-56142060)[a]			M4 (JNJ-56142021)[b]		
Assay	LC-MS/MS			LC-MS/MS		
LLOQ	0.05 µg/mL			0.05 µg/mL		
Gender /Number of Animals	M:4	M:4	M:4	M:4	M:4	M:4
Dose (mg/kg)	500	1000	2000	500	1000	2000
Mean (SD) Pharmacokinetic Parameters						
C_{max} (µg/mL)	7.49 (1.84)	5.34 (0.389)	5.03 (0.898)	3.13 (0.785)	2.43 (0.201)	2.43 (0.530)
t_{max} (h)	3.3 (3.2)	11.0 (2.0)	10 (2.3)	6.0 (2.3)	9.0 (2.0)	12.0 (8.0)
$AUC_{0-\infty}$ (µg·h/mL)	122 (31.1)	102 (17.5)	146 (84.4)	71.9 (27.0)	65.9 (26.1)	95.3 (76.4)
Ratio of $M4/M3_{AUC0-\infty}$ (%)	-	-	-	57.6 (9.31)	54.1 (5.58)	60.7 (16.2)
Ratio of $M4/M3_{Cmax}$ (%)	-	-	-	42.1 (5.01)	46.6 (7.05)	49.4 (12.7)

Additional Information: This study is the compilation of 2 reports (PK052-13 and PK053-13) in which apalutamide and its M3 metabolite were dosed, respectively. The pharmacokinetics of apalutamide, M3 and M4 were calculated in PK052-13, and the pharmacokinetics of M3 and M4 were determined in PK053-13.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] The metabolite formed by amide hydrolysis of apalutamide or M3

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M3 = the *N*-desmethyl metabolite of apalutamide; M4 = the metabolite formed by amide hydrolysis of apalutamide or M3; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3 J 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.		DR-[redacted]-002-DSD	
Location in CTD		4.2.2.2.7	
Species		**Beagle Dog**	
Feeding Condition		Fasted	
Sample		Plasma	
Assay		LC-MS/MS	
Analyte		JNJ-56021927	
LLOQ		0.0122 µg/mL	
Vehicle/Formulation	Formulation A[a]/Capsule	Formulation B[b]/Capsule	Formulation C[c]/Solution
Route	Oral	Oral	Oral gavage
Gender /Number of Animals	M: 3	M: 3	M: 3
Dose (mg/kg)	10	10	10
Mean (SD) Pharmacokinetic Parameters			
C_{max} (µg/mL)	4.9 (0.3)	5.0 (0.8)	5.4 (1.1)
t_{max} (h)	11.7 (11.0)	3.3 (1.2)	1.3 (0.6)
$AUC_{0\text{-}72h}$ (µg·h/mL)	232 (30)	209 (53)	198 (22)
$AUC_{0\text{-}\infty}$ (µg·h/mL)	590 (256)	505 (177)	368 (16)
$t_{1/2}$ (h)	88.23 (35.8)	90.7 (33.1)	63.0 (7.2)
Bioavailability (%)	-	149 (63)[d]	-

Additional Information: DR-[redacted]-002-DSD is a compilation of 2 early in-life experiments (AGN-001 and AGN-002) to determine the bioavailability of various formulations of apalutamide. In AGN-001, dogs were dosed with 2 lipid-based capsule formulations (Formulations A and B), an aqueous solution (Formulation C) and an IV formulation. In AGN-002, dogs were dosed with an oral capsule (Formulation D) and the IV formulation. (The IV data, reported in Mod2.6.5.3A, were used to derive the bioavailability.)
Data from the AGN-001 IV dosing results are not reported due to incomplete washout which resulted in high predose JNJ-56021927 plasma concentrations. Results for Formulation D were skewed due to emesis in 1 animal and are therefore not reported.

[a] Vehicle: Acconon MC8-2/Capmul MCM/PEG-400/Tween-80 (20:50:29:1) – lipid oral formulation (AGN-001).
[b] Vehicle: Acconon MC8-2/Capmul MCM/PEG-400/VitE-TPGS (10:45:30:15) – lipid oral formulation (AGN-001) used in the clinical softgel.
[c] Vehicle: PEG-400/VitE-TPGS/Tween-80/Citrate buffer/Poloxamer 407 (40:18:2:38:2) – aqueous oral formulation (AGN-001).
[d] Calculated using the IV data for the same 3 dogs in AGN-002 (reported in 2.6.5.3A).

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}72h}$ = AUC from time 0 to 72 hours postdose; $AUC_{0\text{-}\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; HP-β-CD = hydroxypropyl-β-cyclodextrin; IV = intravenous; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; Poloxamer = a nonionic surfactant, a copolymer consisting of polypropylene glycol and polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; Tween = polyoxyethylenesorbitan monooleate; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3 K 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	DR-[redacted]-011-DSD						
Location in CTD	4.2.2.2.8						
Species	**Beagle Dog**						
Feeding Condition	Fasted						
Route	Oral capsule						
Sample	Plasma						
Analyte	JNJ-56021927						
Assay	LC-MS/MS						
LLOQ	0.01 µg/mL						
Vehicle/Formulation[a]	Formulation 1 Amorphous	Formulation 2 Amorphous	Formulation 3 Amorphous	Formulation 4 Amorphous	Formulation 5 Amorphous	Formulation 6 Suspension	Formulation 7 Solution
Gender /Number of Animals	M:3	M:3	M:3	M:3	M:3	M:3	M:3
Dose (mg)	57	57	57	57	57	60	60
Mean (SD) Pharmacokinetic Parameters							
C_{max} (µg/mL)	2.7 (0.4)	1.8 (1.3)	3.2 (1.1)	2.5 (1.5)	2.4 (0.7)	1.4 (0.1)	3.5 (0.8)
t_{max} (h)	3.0 (1.0)	10 (12)	2.3 (0.6)	2.3 (0.6)	9.7 (12)	1.2 (0.8)	1.7 (0.6)
$AUC_{0-\infty}$ (µg·h/mL)	138 (39.5)	95.4 (38.5)	205 (152)	128 (24.9)	117 (19.1)	53.5 (3.10)	175 (146)
Relative Bioavailability (%)[b]	79.0	54.6	117.5	73.6	66.7	30.6	-

Additional Information: In an effort to develop a solid oral formulation, the pharmacokinetics of 5 new oral amorphous dosage formulations were compared with an aqueous reference suspension of the crystalline drug product (Formulation 6) and the current clinical trial formulation (Formulation 7)

[a] Formulation 1 - 25% Form A HME with HPMCAS-LF
Formulation 2 - 25% Form A HME with PVP-VA64
Formulation 3 - 25% Form B HME with HPMCAS-LF
Formulation 4 - 20% Form B spray dried with HPMCAS-LF
Formulation 5 - 20% Form B spray dried with PVP-VA64
Formulation 6 - 5% w/w Form B microfluidized suspension – aqueous reference suspension
Formulation 7- Acconon MC8-2/Capmul MCM/PEG-400/VitE-TPGS (10:45:30:15) – lipid oral formulation; current clinical trial formulation

[b] Bioavailability (%F) relative to Formulation 7 (reference formulation) as a function of $AUC_{0-\infty}$.

- = not applicable; Acconon MC8-2 = caprylocaproyl macrgologlycerides; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; HME = hot melt extrusion; HPMCAS-LF = hydroxypropyl methylcellulose acetate succinate LF grade; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; PVP = polyvinylpyrollindone (Copovidone); SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.3　L　薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.			FK10587			
Location in CTD			4.2.2.2.9			
Species			**Beagle Dog**			
Feeding Condition			Fasted			
Route			Oral gavage			
Sample			Plasma			
Analyte			JNJ-56021927			
Assay			LC-MS/MS			
LLOQ			0.025 µg/mL			
Vehicle/Formulation	Formulation A[a]/ tablet	Formulation B[b]/ tablet	Formulation C[c]/ tablet	Formulation D[d]/ tablet	Formulation E[e]/ tablet	Formulation F[f]/ reference softgel
Gender/Number of Animals	M:4	M:4	M:4	M:4	M:4	M:4
Dose (mg)	60	60	60	60	60	60
Mean (SD) Pharmacokinetic Parameters[g]						
C_{max} (µg/mL)	3.9 (0.86)	2.25 (1.13)	4.21 (0.16)	2.86 (1.40)	2.63 (0.15)	4.11 (0.66)
t_{max} (h)	0.88 (0.25)	4.3 (5.2)	1.8 (0.50)	7.1 (11)	1.8 (0.50)	1.3 (0.50)
t_{last} (h)	170 (0.0)	170 (0.0)	140 (45)	170 (0.0)	170 (0.0)	140 (48)
$AUC_{0\text{-}24h}$ (µg·h/mL)	43.7 (13.8)	33.4 (15.0)	57.4 (3.7)	38.7 (13.1)	38.7 (4.03)	62.1 (6.8)
AUC_{last} (µg·h/mL)	183 (52.9)	132 (82.6)	190 (105)	149 (71.5)	159 (50.8)	157 (41.3)
$AUC_{0\text{-}\infty}$ (µg·h/mL)	183 (85.1)[h]	164 (144)[i]	105 (52.7)[h]	94.6 (2.83)[h]	151 (21.4)[i]	167 (43.4)
Relative Bioavailability (AUC_{last} [%])[j]	117	84	121	95	101	-

Additional Information: Comparison of the relative bioavailability of 5 early development tablet formulations (either SDP or HME formulations) against the clinical softgel formulation (reference). The test article was dosed as a single 60-mg tablet (Formulations A-E) or two 30-mg softgel capsules (Formulation F).

Footnotes and abbreviations on following page.

Test Article: JNJ-56021927

Study No.	FK10587
Location in CTD	4.2.2.2.9

Footnotes and abbreviations:

[a] SDP, HPMC AS-LF ratio 1:3
[b] SDP, PVP-VA 64 ratio 1:3
[c] SDP, Eudragit L 100-55 ratio 1:2
[d] HME, Eudragit L 100-55 ratio 1:2
[e] HME, HPMC AS-LF ratio 1:3
[f] softgel reference 30-mg capsule; 2/dose (apalutamide, Lot number: [redacted])
[g] Cmax and AUC values reported as ng/mL and ng·h/mL, respectively in study report.
[h] N=2; AUC_{ext} >25% for other animals
[i] N=3; AUC_{ext} >25% for the other animal
[j] Bioavailability (%F) relative to Formulation F (reference softgel; current clinical trial material).

- = not applicable; AUC = area under the plasma concentration-time curve; AUC_{0-24h} = AUC from time 0 to 24 hours postdose; $AUC_{0-\infty}$ = AUC from time 0 to infinity; AUC_{ext} = AUC extrapolated from the time corresponding to the last quantifiable concentration to infinity, expressed as a percentage of AUC from time 0 to infinity; AUC_{last} = AUC from time 0 to the time corresponding to the last quantifiable concentration; C_{max} = maximum observed plasma concentration; Eudragit = a polymethacrylate-based copolymer; HME = heat melt extrusion; HPMC = hydroxypropyl methylcellulose; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; N = number; PVP = polyvinylpyrrolidone; SD = standard deviation; SDP = spray-dried powder; t_{max} = time correspondent to maximum plasma concentration; t_{last} = time correspondent to last quantifiable plasma concentration

2.6.5.3　M　薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.		FK10634	
Location in CTD		4.2.2.2.10	
Species		**Beagle Dog**	
Feeding Condition		Fasted	
Sample		Plasma	
Analyte		JNJ-56021927	
Assay		LC-MS/MS	
LLOQ		0.025 µg/mL	
Vehicle/Formulation	Formulation A[a]/softgel	Formulation B[b]/ nanosuspension	Formulation C[c]/microsuspension
Route	Oral gavage	Oral gavage	Oral gavage
Gender /Number of Animals	M: 4	M: 4	M: 4
Dose (mg)	60	52.3	51.8
Mean (SD) Pharmacokinetic Parameters[d]			
C_{max} (µg/mL)	2.78 (0.403)	1.21 (0.496)	0.469 (0.0971)
t_{max} (h)	1.8 (0.50)	7.3 (11)	0.75 (0.29)
t_{last} (h)	170 (0.0)	140 (48)	150 (12)
AUC_{0-24h} (µg·h/mL)	35.2(5.92)	17.7 (8.96)	8.63 (1.89)
AUC_{0-168h} (µg·h/mL)	144 (43.2)	74.3 (56.9)	27.3 (8.88)
AUC_{last} (µg·h/mL)	144 (43.2)	73.6(57.8)	26.8 (9.15)
$AUC_{0-\infty}$ (µg·h/mL)	-[e]	59.6[f] (55.8)	30.8 (13.5)
F_{rel} (C_{max} [%])[g]	-	50	19
F_{rel} (AUC_{last} [%])[g]	-	59	22

Additional Information: Three 60-mg concept formulations of apalutamide were dosed. Upon final analysis, actual doses for Formulations B and C were lower (~52 mg).

[a] 30-mg softgel; JNJ-56021927 (Lot number: [redacted]); 2 capsules dosed.
[b] 6 mg/mL; Ref. JNJ-56021927-n001-00179
[c] 6 mg/mL Ref. JNJ-56021927-n001-00182
[d] C_{max} and AUC values reported as ng/mL and ng·h/mL, respectively in study report. SD reported, if available.
[e] N=1; insufficient data (For most, AUC_{ext} >25%)
[f] N=3 (For one, AUCext >25%)
[g] Values were dose normalized prior to Frel calculations.

- = not calculated/not applicable; AUC = area under the plasma concentration-time curve; AUC_{0-24h} = AUC from time 0 to 24 hours postdose; AUC_{0-168h} = AUC from time 0 to 168 hours postdose; $AUC_{0-\infty}$ = AUC from time 0 to infinity; AUC_{ext} = AUC extrapolated from time 0 to infinity; AUC_{last} = AUC from time 0 to the time corresponding to the last quantifiable concentration; C_{max} = maximum observed plasma concentration; Frel = relative bioavailability; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; N = number; Ref. = API reference number; SD = standard deviation.

2.6.5.3 N 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.		FK10786	
Location in CTD		4.2.2.2.11	
Species		**Beagle Dog**	
Feeding Condition		Fasted	
Sample		Plasma	
Analyte		JNJ-56021927	
Assay		LC-MS/MS	
LLOQ		0.025 µg/mL	
Vehicle/Formulation	Formulation 1[a]/softgel capsule		Formulation 2[b]/softgel capsule
Route	Oral capsule		Oral capsule
Gender/Number of Animals	M:4		M:4
Dose (mg)	60		60
Mean (SD) Pharmacokinetic Parameters[c]			
C_{max} (µg/mL)	0.904 (0.279)		0.897 (0.393)
t_{max} (h)	1 – 3.25		2 – 24[d]
$t_{1/2}$ (h)	41 (17)		59 (31)
AUC_{last} (µg·h/mL)[e]	43.8 (22.0)		52.6 (21.6)
$AUC_{0-\infty}$ (µg·h/mL)	47.8 (23.9)		64.3 (28.5)
MRT (h)	61 (24)		89 (48)

Additional Information: Two 60-mg concept formulations of apalutamide (Form B) were dosed as softgel capsules.

[a] JNJ-56021927-n001-00521 (API – Form B, Labrasol, triethyl citrate, ethyl lactate [6:9:61:24; w/w/w/w])

[b] JNJ-56021927-n001-00522 (API – Form B, triethyl citrate, lactic acid USP/FCC (88.8%) [6:90.5:3.5; w/w/w])

[c] Mean values, except for t_{max} for which the range is reported. C_{max} and AUC values reported as ng/mL and ng·h/mL, respectively in study report.

[d] t_{max} ranged from 2 - 3.25h except for one dog where t_{max} was 24h.

[e] For all animals, JNJ-56021927 plasma levels were detectable up to the last scheduled time point (168h postdose).

API = active pharmaceutical ingredient; AUC = area under the plasma concentration-time curve; $AUC_{0-\infty}$ = AUC from time 0 to infinity; AUC_{last} = AUC from time 0 to the time corresponding to the last quantifiable concentration; C_{max} = maximum observed plasma concentration; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; MRT = mean residence time; SD = standard deviation; $t_{½}$ = terminal half-life; t_{max} = time correspondent to maximum plasma concentration.

2.6.5.3 O 薬物動態試験：単回投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10780 (Single-dose phase)					
Location in CTD	4.2.3.2.12					
Species	**Beagle Dog**					
Feeding Condition	Fasted					
Vehicle/Formulation	Acconon MC8-2 EP/NP, Capmul MCM EP, PEG-400, VitE-TPGS NF (10:45:30:15)/solution					
Sample	Plasma					
Analyte	JNJ-56021927					
Assay	LC-MS/MS					
LLOQ	0.025 µg/mL					
Route		Oral gavage			Oral capsule	
Gender/Number of Animals	F:3	F:3	F:3	F:3	F:3	F:3
Dose (mg/kg)	5	10	20	5	10	20
Mean (SD) Pharmacokinetic Parameters[a]						
C_{max} (µg/mL)	1.99 (0.764)	3.94 (1.56)	5.23 (2.67)	1.93 (0.302)	2.39 (1.42)	8.02 (0.907)
t_{max} (h)	3.3 (1.2)	1.7 (0.58)	3.3 (3.2)	2.7 (1.2)	2.0 (0.0)	4.0 (0.0)
t_{last} (h)	72 (0.0)	72 (0.0)	72 (0.0)	72 (0.0)	72 (0.0)	72 (0.0)
AUC_{0-24h} (µg·h/mL)	28.9 (6.47)	65.2 (33.1)	88.5 (41.2)	32.5 (5.8)	37.3 (27.4)	136 (10.9)
AUC_{last} (µg·h/mL)	59.5(11.3)	162 (89.8)	202 (102)	73.8 (12.2)	80.6 (54.8)	325 (21.9)
$AUC_{0-\infty}$ (µg·h/mL)	84.2 (12.4)[b]	-[c]	-[c]	-[c]	-[c]	-[c]

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] C_{max} and AUC values reported as ng/mL and ng·h/mL, respectively in study report.

[b] N=2. AUC_{ext} >25% for one animal, therefore not included.

[c] AUC_{ext} >25% for all values, therefore not calculated.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; AUC_{0-24h} = AUC from time 0 to 24 hours postdose; AUC_{last} = AUC from time 0 to the time corresponding to the last quantifiable concentration; $AUC_{0-\infty}$ = AUC from time 0 to infinity; AUC_{ext} = AUC extrapolated from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; PEG = polyethylene glycol; SD = standard deviation; ; t_{last} = time correspondent to last quantifiable plasma concentration; t_{max} = time correspondent to maximum plasma concentration ; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.3 O 薬物動態試験：単回投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TOX10780 (Single-dose phase)					
Location in CTD	4.2.3.2.12					
Species	**Beagle Dog**					
Feeding Condition	Fasted					
Vehicle/Formulation	Acconon MC8-2 EP/NP, Capmul MCM EP, PEG-400, VitE-TPGS NF (10:45:30:15)/solution					
Sample	Plasma					
Analyte	M3 (JNJ-56142060)[a]					
Assay	LC-MS/MS					
LLOQ	0.025 µg/mL					
Route		Oral Gavage			Oral capsule	
Gender/Number of Animals	F:3	F:3	F:3	F:3	F:3	F:3
Dose (mg/kg)	5	10	20	5	10	20
Mean (SD) Pharmacokinetic Parameters[b]						
C_{max} (µg/mL)	0.825 (0.129)	0.830 (0.171)	2.190 (0.730)	0.631 (0.186)	0.914 (0.872)	2.320 (0.538)
t_{max} (h)	72 (0.0)	72 (0.0)	64 (14)	72 (0.0)	72 (0.0)	72 (0.0)
t_{last} (h)	72 (0.0)	72 (0.0)	72 (0.0)	72 (0.0)	72 (0.0)	72 (0.0)
AUC_{0-24h} (µg·h/mL)	6.70 (1.79)	5.89 (0.728)	19.1(5.50)	4.84 (2.06)	7.77 (8.21)	18.5 (6.65)
AUC_{last} (µg·h/mL)	42.9 (8.39)	39.4 (7.27)	113(34.4)	30.4 (11.3)	46.7 (47.4)	111 (33.2)
$AUC_{0-\infty}$ (µg·h/mL)	-	-[c]	-[c]	-[c]	-[c]	-[c]

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] C_{max} and AUC values reported as ng/mL and ng·h/mL, respectively in study report.

[c] AUC_{ext} >25% for all values, therefore not calculated.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; AUC_{0-24h} = AUC from time 0 to 24 hours postdose; $AUC_{0-\infty}$ = AUC from time 0 to infinity; AUC_{ext} = AUC extrapolated from time 0 to infinity; AUC_{last} = AUC from time 0 to the last quantifiable concentration; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; N = number; PEG = polyethylene glycol; t_{last} = time correspondent to last quantifiable plasma concentration; t_{max} = time correspondent to maximum plasma concentration ; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 A 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10822 (Repeat-Dose Phase)											
Location in CTD	4.2.3.4.2.1											
Species	**CD-1 Mouse**											
Feeding condition	Fed											
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15)/solution											
Route	Oral gavage											
Sample	Plasma											
Analyte	JNJ-56021927						M3 (JNJ-56142060)[a]					
Assay	LC-MS/MS						LC-MS/MS					
LLOQ	0.01 µg/mL						0.01 µg/mL					
Gender/Number of Animals[b]	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6
Dose (mg/kg/day)	20		50		100		20		50		100	
Duration of dosing (days)[c]	1		1		1		1		1		1	
Pharmacokinetic Parameters[d,e]												
C_{max} (µg/mL)	5.04	5.85	13.5	11.8	23.1	27.5	0.674	0.658	2.09	1.56	7.39	3.91
t_{max} (h)	7	7	7	7	7	3	24	24	24	24	24	24
$AUC_{0\text{-}24h}$ (µg·h/mL)	102	109	256	244	491	503	7.97	7.74	23.6	19.6	72.6	42.9

Additional information: The ethanolate salt (JNJ-56021927-ZAH) was administered. During the single-dose phase of the study, MTD: 100 mg/kg.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] Separated into 2 groups of 3, each group sampled at 2 time points.

[c] First day of dosing designated as Day 0; duration shown as actual calendar days (ie. Day 0 = 1 day duration and Day 9 = 10 days duration).

[d] Mean pharmacokinetic data for N≤3 concentrationsper dose/day/sex/time point due to mortality.

[e] C_{max} and $AUC_{0\text{-}24h}$ reported as ng/mL and ng·h/mL, respectively in study report. For the calculation of AUC values after repeated dosing, 0 hour concentrations were added and equated with the 24 hour concentrations.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; MTD = maximum tolerated dose; N = number; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.4 A 薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TOX10822 (Repeat-Dose Phase)											
Location in CTD	4.2.3.4.2.1											
Species	**CD-1 Mouse**											
Feeding condition	Fed											
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15)/solution											
Route	Oral gavage											
Sample	Plasma											
Analyte	JNJ-56021927						M3 (JNJ-56142060)[a]					
Assay	LC-MS/MS						LC-MS/MS					
LLOQ	0.01 µg/mL						0.01 µg/mL					
Gender/Number of Animals[b]	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6
Dose (mg/kg/day)	20		50		100		20		50		100	
Duration of dosing (days)[c]	10		10		10		10		10		10	
Mean Pharmacokinetic Parameters[d,e]												
C_{max} (µg/mL)	6.75	11.1	11.6	15.1	13.9	-[f]	1.62	1.24	4.06	3.78	7.58	-[f]
t_{max} (h)	7	7	3	7	3	-[f]	7	7	3	7	7	-[f]
$AUC_{0\text{-}24h}$ (µg·h/mL)	113	170	136	228	140	-[f]	35.7	28.8	84.9	71.6	127	-[f]

Additional information: The ethanolate salt (JNJ-56021927-ZAH) was administered. During the single-dose phase of the study, MTD: 100 mg/kg.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] Separated into 2 groups of 3, each group sampled at 2 time points.

[c] First day of dosing designated as Day 0; duration shown as actual calendar days (ie. Day 0 = 1 day duration and Day 9 = 10 days duration).

[d] Mean pharmacokinetic data for N≤ 3 concentrations/per dose/day/sex/time point due to mortality.

[e] Cmax and $AUC_{0\text{-}24h}$ reported as ng/mL and ng·h/mL, respectively in study report. For the calculation of AUC values after repeated dosing, 0 hour concentrations were added and equated with the 24-hour concentrations.

[f] Not calculated; insufficient data due to mortality (4/6) in the TK animals

- = not calculated/not applicable/insufficient data; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; MTD = maximum tolerated dose; N = number; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; TK = toxicokinetic; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 B 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10940 (Phase 1 and Phase 3)											
Location in CTD	4.2.3.4.2.2											
Species	**CD-1 Mouse**											
Feeding condition	Fed											
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15)/solution											
Route	Oral gavage											
Sample	Plasma											
Analyte	JNJ-56021927											
Assay	LC-MS/MS											
LLOQ	0.025 µg/mL											
Gender/Number of Animals	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6
Dose (mg/kg/day)	100[a]		175[b]		250[b]		100[a]		175[b]		250[b]	
Duration of dosing (days) [c]	1		1		1		10		10		10	
Pharmacokinetic Parameters[d]												
C_{max} (µg/mL)	-[e]	-[e]	52.5	48.5	42.8	42.6	17.6	25.9	30.0	32.2	31.1	35.2
t_{max} (h)	-[e]	-[e]	7.0	3.0	3.0	7.0	3.0-7.0	3.0	3.0	3.0	3.0	3.0
AUC_{0-24h} (µg・h/mL)	-[e]	-[e]	861	790	727	657	175	262	195	248	210	283

Additional information: The ethanolate salt (JNJ-56021927-ZAH) was administered. During Phase 1 of the study, male and female CD-1 mice were administered 100 mg/kg/day by oral gavage. The purpose of Phase 1 was to optimize the oral gavage procedure. Blood samples were drawn after 10 days of dosing for TK analysis. Phase 2 further optimized the gavage procedure, but no blood samples were drawn for TK analysis. Phase 3 was the main TK experiment. Male and female CD-1 mice (new animals) were administered 175 or 250 mg/kg/day for 10 days and blood was drawn for TK analysis on the first and last day of dosing (Day 0 and Day 9, respectively).

[a] Phase 1 dosing data; data from 6 mice/sex/dose.

[b] Phase 3 dosing data; 6 mice/sex/dose dosed, but blood drawn from 3/sex/dose per timepoint. Two mortalities for males in the 250 mg/kg/day dose group: 1 on Day 0 and 1 euthanized on Day 3.

[c] The first day of dosing is referred to as “Day 0” in the report and the tenth day is referred to as “Day 9”.

[d] Cmax and AUC_{0-24h} reported as ng/mL and ng·h/mL, respectively in study report.

[e] No TK sampling was done on the first day of dosing (Day 0) during Phase 1.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC_{0-24h} = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; TK = toxicokinetic; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.4 B 薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TOX10940 (Phase 1 and Phase 3)											
Location in CTD	4.2.3.4.2.2											
Species	**CD-1 Mouse**											
Feeding condition	Fed											
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15)/solution											
Route	Oral gavage											
Sample	Plasma											
Analyte	M3 (JNJ-56142060)[a]											
Assay	LC-MS/MS											
LLOQ	0.025 µg/mL											
Gender/Number of Animals	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6
Dose (mg/kg/day)	100[b]		175[c]		250[c]		100[b]		175[c]		250[c]	
Duration of dosing (days) [d]	1		1		1		10		10		10	
Pharmacokinetic Parameters[e]												
C_{max} (µg/mL)	-[f]	-[f]	18.4	14.1	14.0	9.46	12.3	12.0	17.6	18.3	21.4	20.6
t_{max} (h)	-[f]	-[f]	24	24	24	24	7.0	7.0	7.0	7.0	3.0	7.0
$AUC_{0\text{-}24h}$ (µg・h/mL)	-[f]	-[f]	207	153	156	130	186	179	244	235	246	255

Additional information: The ethanolate salt (JNJ-56021927-ZAH) was administered. During Phase 1 of the study, male and female CD-1 mice were administered 100 mg/kg/day by oral gavage. The purpose of Phase 1 was to optimize the oral gavage procedure. Blood samples were drawn after 10 days of dosing for TK analysis. Phase 2 further optimized the gavage procedure, but no blood samples were drawn for TK analysis. Phase 3 was the main TK experiment. Male and female CD-1 mice (new animals) were administered 175 or 250 mg/kg/day for 10 days and blood was drawn for TK analysis on the first and last day of dosing (Day 0 and Day 9, respectively).

[a] The *N*-desmethyl metabolite of apalutamide.
[b] Phase 1 dosing data; data from 6 mice/sex/dose.
[c] Phase 3 dosing data; 6 mice/sex/dose dosed, but blood drawn from 3/sex/dose per timepoint. Two mortalities for males in the 250 mg/kg/day dose group: 1 on Day 0 and 1 euthanized on Day 3.
[d] In the report, the first day of dosing is referred to as “Day 0” and the tenth day is referred to as “Day 9”.
[e] C_{max} and $AUC_{0\text{-}24h}$ reported as ng/mL and ng·h/mL, respectively in study report.
[f] No TK sampling was done on the first day of dosing (Day 0) during Phase 1.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; TK = toxicokinetic; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 C 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX11241															
Location in CTD	4.2.3.4.2.3															
Species	**CByB6F1 Hybrid Mouse**															
Feeding condition	Fed															
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS (10:45:30:15)/solution															
Route	Oral gavage															
Sample	Plasma															
Analyte	JNJ-56021927								M3 (JNJ-56142060)[a]							
Assay	LC-MS/MS								LC-MS/MS							
LLOQ	0.025 µg/mL								0.025 µg/mL							
Gender/Number of Animals[b]	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6	M:6	F:6
Dose (mg/kg/day)	75				150				75				150			
Duration of dosing (days)	1		14		1		14		1		14		1		14	
Mean Pharmacokinetic Parameters[c]																
C_{max} (µg/mL)	18.3	23.9	16.1	18.6	36.2	41.1	-[d]	-[d]	2.78	3.21	10.0	6.85	7.56	8.05	-[d]	-[d]
t_{max} (h)	7	3	3	3	3	7	-[d]	-[d]	24	24	7	7	24	24	-[d]	-[d]
$AUC_{0\text{-}24}$ (µg·h/mL)	342	428	160	213	759	761	-[d]	-[d]	34.5	37.9	155	110	86.5	83.6	-[d]	-[d]

Additional information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] 13 animals dosed, 6/sex/dose group, plus 1 spare; 3 sampled/time point. The extra 75 mg/kg/day animals were not used for blood collection. The extra 150 mg/kg/day animals were euthanized on Day 3 when the dose group was terminated.

[c] C_{max} and $AUC_{0\text{-}24h}$ reported as ng/mL and ng·h/mL, respectively in study report. For the calculation of AUC values after repeated dosing, 0 hour concentrations were added and equated with the 24 hour concentrations.

[d] No data available. All 12 mice were sacrificed on Day 3 due to adverse clinical signs.

- = not calculated/not applicable/insufficient data; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 D 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	VUM00002					
Location in CTD	4.2.3.2.1					
Species	**Sprague-Dawley Rat**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral gavage					
Sample	Plasma					
Analyte	JNJ-56021927					
Assay	LC-MS/MS					
LLOQ	0.781 µg/mL					
Gender/Number of Animals	M: 4	M: 4	M: 4	M: 4	M: 4	M: 4
Dose (mg/kg/day)	150		300[a]		600[b]	
Duration of dosing (days)	1	14	1	14	1	14
Mean Pharmacokinetic Parameters[c]						
C_{max} (µg/mL)	28.9	31.4	55	39.3	26.0	33.5
t_{max} (h)	12	8	24	12	12	12
AUC_{0-t} (µg·h/mL)[d]	977	550	2420	743	913	650
$t_{1/2}$ (h)	11.7	10.3	24.6	15.7	11.8	12.8

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered. Rats were dosed once daily except not on Day 2 so that an accurate 48h plasma drug sample could be obtained.

[a] Two animals died within 5 days of study start therefore N=2 for Day 14.

[b] One animal died within 5 days of study start therefore N=3 for Day 14.

[c] Dosing groups were subdivided for toxicokinetic blood draws. N = 2/time point on Day 1 and 1 or 2/time point on Day 14.

[d] AUC calculated as $AUC_{0-\infty}$ (time 0 to infinity) on Day 1 and AUC_{0-24h} (time 0 to 24 hours) on Day 14.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; AUC_{0-t} = AUC from time 0 to time t; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; N = number; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 E 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	VUM00004					
Location in CTD	4.2.3.2.2					
Species	**Sprague-Dawley Rat**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral gavage					
Sample	Plasma					
Analyte	JNJ-56021927					
Assay	LC-MS/MS					
LLOQ	0.1 µg/mL					
Gender/Number of Animals[a]	M: 9	M: 9	M: 9[b]	M: 9[b]	M: 9[c]	M: 9[c]
Dose (mg/kg/day)	50		150		250	
Duration of dosing (days)	1	28	1	28	1	28
Mean Pharmacokinetic Parameters						
C_{max} (µg/mL)	8.37	11.9	23.3	31.5	41.7	-
t_{max} (h)	12.0	8.00	12.0	12.0	24.0	-
$AUC_{0\text{-}24h}$ (µg·h/mL)	138	201	430	572	688	-
$AUC_{0\text{-}\infty}$ (µg·h/mL)	-	265	-	723	-	-
$t_{1/2}$ (h)	-	8.98	-	7.01	-	-
Accumulation Index	-	1.19	-	1.10	-	-

Additional Information: NOAEL: 50 mg/kg/day. The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] Dosing groups were subdivided for toxicokinetic blood draws. N=3/subgroup/time point on Day 1 and Day 28.

[b] 3 animals died: 1 was found dead on Day 3, 1 was euthanized moribund on Day 4, and one was found dead on Day 13.

[c] All 9 animals were found dead or were euthanized moribund on Day 3.

- = not calculated/not applicable/insufficient data; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24h}$ = AUC from time 0 to 24 hours postdose; $AUC_{0\text{-}\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; N = number; NOAEL = No observed adverse effect level; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4　F　薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TX-509-1001								
Location in CTD	4.2.3.2.3								
Species	**Sprague-Dawley Rat**								
Feeding Condition	Fed								
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution								
Route	Oral gavage								
Sample	Plasma								
Analyte	JNJ-56021927								
Assay	LC-MS/MS								
LLOQ	0.025 µg/mL								
Gender/Number of Animals	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9
Dose (mg/kg/day)[a]	25	50	100	25[b]	50	100	25[b]	50[c]	100[d]
Duration of Dosing (days)	1	1	1	28	28	28	91	91	91
Mean (SE) Pharmacokinetic Parameters[e]									
C_{max} (µg/mL)	4.12 (0.455)	7.49 (0.241)	15.5 (0.233)	4.76 (0.312)	9.79 (0.199)	20.2 (0.784)	7.48 (2.51)	11.9 (0.650)	30.1 (5.83)
t_{max} (h)	8.00	12.0	12.0	8.00	8.00	8.00	12.0	8.00	8.00
$AUC_{0\text{-}24h}$ (µg·h/mL)	67.9 (2.11)	128 (4.44)	272 (2.96)	84.5 (1.36)	168 (3.58)	341 (3.80)	136 (23.7)	240 (7.78)	521 (40.1)
Acc Ratio $AUC_{0\text{-}24h\ (Day\ 91/Day1)}$	-	-	-	-	-	-	2.00	1.88	1.92

Additional Information: NOAEL = 100 mg/kg/day. The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] Spiked mid-dose with 2% degradants (1.0 mg/kg 不純物F＊ and 1.0 mg/kg 不純物G＊) but resultant plasma exposure for these not assessed.

[b] One animal euthanized moribund on Day 21, therefore no data for Day 28 or 91. Death considered incidental; not test-article related.

[c] One animal euthanized moribund on Day 66, therefore no data for Day 91. Death considered incidental; not test-article related. Another had clotted 24 hour sample on Day 1.

[d] One animal found dead on Day 76, therefore no data for Day 91. Death considered incidental; not test-article related.

[e] Mean concentrations were determined from 3 animals/group/time point. Insufficient terminal phase data, therefore values for $t_{1/2}$ and $AUC_{0\text{-}\infty}$ are not reported. SE reported, if available.

- = not applicable; Acc Ratio = accumulation ratio; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24h}$ = AUC from time 0 to 24 hours postdose; $AUC_{0\text{-}\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; NOAEL = No observed adverse effect level; PEG = polyethylene glycol; SE = standard error; $t_{½}$ = terminal half-life; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

*新薬承認情報提供時に置き換え

2.6.5.4　F　薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TX-509-1001								
Location in CTD	4.2.3.2.3								
Species	**Sprague-Dawley Rat**								
Feeding Condition	Fed								
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution								
Route	Oral gavage								
Sample	Plasma								
Analyte	M3 (JNJ-56142060)[a]								
Assay	LC-MS/MS								
LLOQ	0.010 µg/mL								
Gender/Number of Animals	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9
Dose (mg/kg/day)[b]	25	50	100	25[c]	50	100	25[c]	50[d]	100[e]
Duration of Dosing (days)	1	1	1	28	28	28	91	91	91
Mean (SE) Pharmacokinetic Parameters[f]									
C_{max} (µg/mL)	0.152 (0.0162)	0.252 (0.0299)	0.554 (0.0235)	0.191 (0.010)	0.324 (0.00888)	0.740 (0.0727)	0.200 (0.009)	0.413 (0.0156)	1.49 (0.237)
t_{max} (h)	12.0	12.0	12.0	12.0	8.00	8.00	12.0	12.0	8.00
$AUC_{0\text{-}24h}$ (µg·h/mL)	2.37 (0.183)	4.07 (0.268)	9.68 (0.318)	3.28 (0.141)	5.76 (0.284)	13.7 (0.685)	3.86 (0.152)	8.57 (0.405)	28.5 (2.30)
M/P Ratios	0.035	0.032	0.036	0.039	0.034	0.040	0.028	0.036	0.055
Acc Ratio $AUC_{0\text{-}24h\ (Day\ 91/Day1)}$	-	-	-	-	-	-	1.63	2.11	2.94

Additional Information: NOAEL = 100 mg/kg/day. The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] Spiked mid-dose with 2% degradants (1.0 mg/kg 不純物F* and 1.0 mg/kg 不純物G*) but resultant plasma exposure for these not assessed.

[c] One animal euthanized moribund on Day 21, therefore no data for Day 28 or 91. Death considered incidental; not test-article related.

[d] One animal euthanized moribund on Day 66, therefore no data for Day 91. Death considered incidental; not test-article related. Another had clotted 24 hour sample on Day 1.

[e] One animal found dead on Day 76, therefore no data for Day 91. Death considered incidental; not test-article related.

[f] Mean concentrations were determined from 3 animals/group/time point. Insufficient terminal phase data, therefore values for $t_{1/2}$ and $AUC_{0\text{-}\infty}$ are not reported. SE reported, if available.

- = not applicable; Acc Ratio = accumulation ratio; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24h}$ = AUC from time 0 to 24 hours postdose; $AUC_{0\text{-}\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M/P Ratio = metabolite-to-parent ratio; NOAEL = No observed adverse effect level; PEG = polyethylene glycol; SE = standard error; $t_{½}$ = terminal half-life; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

*新薬承認情報提供時に置き換え

2.6.5.4　F　薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TX-509-1001								
Location in CTD	4.2.3.2.3								
Species	**Sprague-Dawley Rat**								
Feeding Condition	Fed								
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution								
Route	Oral gavage								
Sample	Plasma								
Analyte	M4 (JNJ-56142021)[a]								
Assay	LC-MS/MS								
LLOQ	0.010 µg/mL								
Gender/Number of Animals	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9	M: 9
Dose (mg/kg/day)[b]	25	50	100	25[c]	50	100	25[c]	50[d]	100[e]
Duration of Dosing (days)	1	1	1	28	28	28	91	91	91
Mean (SE) Pharmacokinetic Parameters[f]									
C_{max} (µg/mL)	0.315 (0.00851)	0.629 (0.156)	1.79 (0.432)	0.460 (0.055)	0.901 (0.0489)	1.83 (0.127)	0.482 (0.0356)	0.962 (0.0969)	2.48 (0.100)
t_{max} (h)	8.00	6.00	24.0	8.00	8.00	6.00	24.0	12.0	8.00
$AUC_{0\text{-}24h}$ (µg·h/mL)	6.37 (0.237)	11.7 (0.295)	28.4(3.01)	8.75 (0.624)	17.7 (0.683)	34.2(1.82)	10.5 (0.731)	21.6(1.52)	52.4(2.13)
M/P Ratios	0.094	0.091	0.104	0.104	0.105	0.100	0.077	0.090	0.101
Acc Ratio $AUC_{0\text{-}24h(Day\ 91/Day1)}$	-	-	-	-	-	-	1.65	1.85	1.85

Additional Information: NOAEL = 100 mg/kg/day. The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] M4 = the metabolite formed by amide hydrolysis of apalutamide or M3

[b] Spiked mid-dose with 2% degradants (1.0 mg/kg 不純物F* and 1.0 mg/kg 不純物G*) but resultant plasma exposure for these not assessed.

[c] One animal euthanized moribund on Day 21, therefore no data for Day 28 or 91. Death considered incidental; not test-article related.

[d] One animal euthanized moribund on Day 66, therefore no data for Day 91. Death considered incidental; not test-article related. Another had clotted 24 hour sample on Day 1.

[e] One animal found dead on Day 76, therefore no data for Day 91. Death considered incidental; not test-article related.

[f] Mean concentrations were determined from 3 animals/group/time point. Insufficient terminal phase data, therefore values for $t_{1/2}$ and $AUC_{0\text{-}\infty}$ are not reported. SE reported, if available.

- = not applicable; Acc Ratio = accumulation ratio; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24h}$ = AUC from time 0 to 24 hours postdose; $AUC_{0\text{-}\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M/P Ratio = metabolite-to-parent ratio; NOAEL = No observed adverse effect level; PEG = polyethylene glycol; $t_{½}$ = terminal half-life; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

*新薬承認情報提供時に置き換え

2.6.5.4 G 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10838					
Location in CTD	4.2.3.2.4					
Species	**Sprague-Dawley Rat**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral gavage					
Sample	Plasma					
Analyte	JNJ-56021927			M3 (JNJ-56142060)[a]		
Assay	LC-MS/MS			LC-MS/MS		
LLOQ	0.025 µg/mL			0.025 µg/mL		
Gender/Number of Animals	M:8[b]	M:8	M:8[b]	M:8[b]	M:8	M:8[b]
Dose (mg/kg/day)	25	75	150	25	75	150
Duration of Dosing (days)	182	182	182	182	182	182
Mean (SE) Pharmacokinetic Parameters[c]						
C_{max} (µg/mL)	7.20 (0.673)	20.4 (1.56)	27.7 (4.66)	0.228 (0.00649)	1.10 (0.0413)	1.40 (0.127)
t_{max} (h)	4.00	4.00	4.00	12.0	12.0	12.0
$AUC_{0\text{-}24h}$ (µg·h/mL)	135 (7.43)	389 (20.8)	508 (53.7)	4.58 (0.361)	20.6 (0.572)	27.7 (1.58)
M/P Ratio ($AUC_{0\text{-}24h}$)	-	-	-	0.0338	0.0531	0.0545

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] Three animals died prior to toxicokinetic evaluation on Day 182 due to dosing errors. Two animals were not replaced: 1 from the 25 mg/kg/day dosing group which died on Day 153 and 1 animal in the 150 mg/kg/day which died on Day 17. Therefore, for these two groups, parameters are based on N=7. One animal in the 150 mg/kg/day group which died on Day 4 was replaced.

[c] On Day 182, the animals were subdivided equally within a dose group and toxicokinetic blood samples at each timepoint were drawn from alternate subgroups (ie, for most dose groups, N = 4/group/time point). SE reported, if available.

- = not applicable/not calculated; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M/P Ratio = metabolite-to-parent ratio; N = number; PEG = polyethylene glycol; SE = standard error; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4　H　薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10760 (Repeat-Dose Phase)											
Location in CTD	4.2.3.2.5											
Species	**Sprague-Dawley Rat**											
Feeding Condition	Fed[a]											
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution											
Route	Oral gavage											
Sample	Plasma											
Analyte	JNJ-56021927						M3 (JNJ-56142060)[b]					
Assay	LC-MS/MS						LC-MS/MS					
LLOQ	0.025 µg/mL						0.025 µg/mL					
Gender/Number of Animals	F: 10	F: 10	F: 10	F: 10	F: 10	F: 10	F: 10	F: 10	F: 10	F: 10	F: 10	F: 10
Dose (mg/kg/day)[c]	25	50	100	25	50	100	25	50	100	25	50	100
Duration of Dosing (days)	56[d]	56[d]	56[d]	69[e]	69[e]	69[e]	56[d]	56[d]	56[d]	69[e]	69[e]	69[e]
Mean Pharmacokinetic Parameters												
C_{max} (µg/mL)	10.4	24.1	38.5	11.3	23.7	33.9	0.093	0.328	0.737	0.111	0.349	0.662
t_{max} (h)	4.0	8.0	4.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
$AUC_{0\text{-}24h}$ (µg・h/mL)	186	411	710	195	416	659	1.89	5.67	14.8	2.18	6.25	13.5

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered. Toxicokinetics done by microsampling.

[a] Except prior to 24-hour blood draw at which time animals were fasted.
[b] The *N*-desmethyl metabolite of apalutamide.
[c] 25, 50, and 100 mg/kg/day correspond to Low, Medium, and High dose, respectively.
[d] Corresponds to 2 weeks of repeat dosing following the single-dose phase.
[e] Corresponds to 4 weeks of repeat dosing following the single-dose phase.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to the time corresponding to the last quantifiable concentration; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 I 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10897							
Location in CTD	4.2.3.2.6							
Species	**Sprague-Dawley Rat**							
Feeding Condition	Fed							
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15 %w/w/w/w)/solution							
Route	Oral gavage							
Sample	Plasma							
Analyte	JNJ-56021927				M3 (JNJ-56142060)[a]			
Assay	LC-MS/MS				LC-MS/MS			
LLOQ	0.025 µg/mL				0.025 µg/mL			
Gender/Number of Animals	F: 6	F: 6	F: 6[b]	F: 6	F: 6	F: 6	F: 6[b]	F: 6
Dose (mg/kg/day)	25	50	100	150	25	50	100	150
Duration of Dosing (days)	1	1	1	1	1	1	1	1
Mean (SE) Pharmacokinetic Parameters[c]								
C_{max} (µg/mL)	5.17 (0.128)	9.63 (0.436)	17.4 (1.31)	23.8 (1.31)	0.0558 (0.00751)	0.109 (0.0160)	0.283 (0.0502)	0.683 (0.0977)
t_{max} (h)	8.00	8.00	12.0	8.00	12.0	12.0	12.0	24.0
$AUC_{0\text{-}24h}$ (µg·h/mL)	90.2 (2.63)	173 (8.31)	321 (13.3)	477 (22.0)	0.769 (0.113)	1.92 (0.184)	4.55 (0.502)	8.61 (0.886)
M/P Ratio ($AUC_{0\text{-}24h}$)	-	-	-	-	0.00852	0.0111	0.0141	0.0180

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide.

[b] On Day 1, 2 animals in the 100 mg/kg/day dose group were euthanized moribund due to accidental injuries and were replaced.

[c] SE reported, if available.

- = not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M/P Ratio = metabolite-to-parent ratio; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; SE = standard error; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.4 I 薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TOX10897							
Location in CTD	4.2.3.2.6							
Species	**Sprague-Dawley Rat**							
Feeding Condition	Fed							
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15%w/w/w/w)/solution							
Route	Oral gavage							
Sample	Plasma							
Analyte	JNJ-56021927				M3 (JNJ-56142060)[a]			
Assay	LC-MS/MS				LC-MS/MS			
LLOQ	0.025 µg/mL				0.025 µg/mL			
Gender/Number of Animals	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]
Dose (mg/kg/day)	25	50	100	150	25	50	100	150
Duration of Dosing (days)	28	28	28	28	28	28	28	28
Mean (SE) Pharmacokinetic Parameters[d]								
C_{max} (µg/mL)	9.14 (0.237)	18.9 (0.200)	28.3 (2.64)	31.9 (3.89)	0.114 (0.0159)	0.325 (0.0540)	0.879 (0.122)	1.40 (0.230)
t_{max} (h)	4.00	12.00	4.00	4.00	12.0	12.0	12.0	8.00
$AUC_{0\text{-}24h}$ (µg·h/mL)	169 (7.69)	346 (26.1)	521 (37.2)	599(46.8)	2.09 (0.245)	5.80 (0.890)	14.0 (1.80)	22.6 (3.35)
Accumulation Ratio $AUC_{0\text{-}24h}$ (Day 28/Day 1)	1.87	2.00	1.62	1.25	2.72	3.02	3.08	2.63
M/P Ratio ($AUC_{0\text{-}24h}$)	-	-	-	-	0.0124	0.0168	0.0269	0.0378

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide

[b] Three animals were found dead prior to scheduled terminal euthanasia: 1 in the 50 mg/kg/day dose group on Day 14 and 2 in the 150 mg/kg/day group on Days 19 and 92, respectively. The deaths were incidental or due to dosing errors; they were not considered to be JNJ-56021927-related.

[c] N=5 for the 50 mg/kg/day and N=4 for the 150 mg/kg/day group due to premature deaths listed above.

[d] SE reported, if available.

- = not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M/P Ratio = metabolite-to-parent ratio; N = number; PEG = polyethylene glycol; SE = standard error; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.4 I 薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TOX10897							
Location in CTD	4.2.3.2.6							
Species	**Sprague-Dawley Rat**							
Feeding Condition	Fed							
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15%w/w/w/w)/solution							
Route	Oral gavage							
Sample	Plasma							
Analyte	JNJ-56021927				M3 (JNJ-56142060)[a]			
Assay	LC-MS/MS				LC-MS/MS			
LLOQ	0.025 µg/mL				0.025 µg/mL			
Gender/Number of Animals	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]
Dose (mg/kg/day)	25	50	100	150	25	50	100	150
Duration of Dosing (days)	92	92	92	92	92	92	92	92
Mean (SE) Pharmacokinetic Parameters[d]								
C_{max} (µg/mL)	11.7 (0.945)	26.3 (2.55)	38.3 (2.10)	38.8 (3.45)	0.189 (0.0335)	0.532 (0.0395)	1.57 (0.234)	1.16 (0.125)
t_{max} (h)	4.00	4.00	4.00	4.00	12.0	12.0	12.0	8.00
$AUC_{0\text{-}24h}$ (µg·h/mL)	230 (13.3)	462 (46.3)	693 (56.7)	732 (63.7)	3.44 (0.505)	10.5 (1.63)	22.9 (2.42)	23.5 (3.96)
Accumulation Ratio $AUC_{0\text{-}24h\ (Day\ 92/Day\ 1)}$	2.55	2.67	2.15	1.53	4.48	5.47	5.04	2.73
M/P Ratio ($AUC_{0\text{-}24h}$)	-	-	-	-	0.0149	0.0227	0.0331	0.0321

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide

[b] Three animals were found dead prior to scheduled terminal euthanasia: 1 in the 50 mg/kg/day dose group on Day 14 and 2 in the 150 mg/kg/day group on Days 19 and 92, respectively. The deaths were incidental or due to dosing errors; they were not considered to be JNJ-56021927-related.

[c] N=5 for the 50mg/kg/day and N=4 for the 150 mg/kg/day group due to premature deaths listed above.

[d] SE reported, if available.

- = not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M/P Ratio = metabolite-to-parent ratio; N = number; PEG = polyethylene glycol; SE = standard error; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.4 I 薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TOX10897							
Location in CTD	4.2.3.2.6							
Species	**Sprague-Dawley Rat**							
Feeding Condition	Fed							
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15%w/w/w/w)/solution							
Route	Oral gavage							
Sample	Plasma							
Analyte	JNJ-56021927				M3 (JNJ-56142060)[a]			
Assay	LC-MS/MS				LC-MS/MS			
LLOQ	0.025 µg/mL				0.025 µg/mL			
Gender/Number of Animals	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]	F: 6	F: 6[b,c]
Dose (mg/kg/day)	25	50	100	150	25	50	100	150
Duration of Dosing (days)	182	182	182	182	182	182	182	182
Mean (SE) Pharmacokinetic Parameters[d]								
C_{max} (µg/mL)	13.4 (1.19)	32.3 (0.950)	43.3 (5.69)	42.3 (0.950)	0.250 (0.0616)	0.702 (0.0920)	1.79 (0.186)	1.57 (0.695)
t_{max} (h)	4.00	4.00	4.00	4.00	12.0	12.0	12.0	12.0
$AUC_{0\text{-}24h}$ (µg·h/mL)	282 (20.1)	566 (59.4)	834 (55.4)	785 (27.3)	4.88 (0.913)	14.5 (2.47)	31.3 (2.32)	29.0 (8.80)
Accumulation Ratio $AUC_{0\text{-}24h\ (Day\ 182/Day\ 1)}$	3.13	3.27	2.59	1.64	6.35	7.55	6.89	3.37
M/P Ratio ($AUC_{0\text{-}24h}$)	-	-	-	-	0.0173	0.0256	0.0375	0.0369

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide

[b] Three animals were found dead prior to scheduled terminal euthanasia: 1 in the 50 mg/kg/day dose group on Day 14 and 2 in the 150 mg/kg/day group on Days 19 and 92, respectively. The deaths were incidental or due to dosing errors; they were not considered to be JNJ-56021927-related.

[c] N=5 for the 50 mg/kg/day and N=4 for the 150 mg/kg/day group due to premature deaths listed above.

[d] SE reported, if available.

- = not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M/P Ratio = metabolite-to-parent ratio; PEG = polyethylene glycol; SE = standard error; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS= water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 J 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.			VUM00003			
Location in CTD			4.2.3.2.7			
Species			**Beagle Dog**			
Feeding Condition			Fed			
Vehicle/Formulation			Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15%w/w/w/w)/solution			
Route			Oral capsule			
Sample			Plasma			
Analyte			JNJ-56021927			
Assay			LC-MS/MS			
LLOQ			0.781 µg/mL			
Gender/Number of Animals	M: 2	M: 2	M: 2	M: 2	M: 2	M: 2
Dose (mg/kg/day)	10	20	40	10	20	40
Duration of Dosing (days)	1	1	1	21	21	21
Mean Pharmacokinetic Parameters						
C_{max} (µg/mL)	3.19	7.98	16.2	17.4	47.5	-[a]
t_{max} (h)	4.0	2.0	3.0	18.0	12.5	-[a]
$AUC_{0\text{-}24h}$ (µg·h/mL)	-	-	-	338	1020	-[a]
$AUC_{0\text{-}\infty}$ (µg·h/mL)	326	611	1510	-	-	-[a]
$t_{½}$ (h)	69.4	54.3	78.3	-[b]	-[b]	-[a]
CL/F (mL/min/kg)	0.89	0.55	0.49	-	-	-[a]

Additional Information: Animals were dosed Day 1 and Days 3-21. No dose was administered on Day 2 to allow collection of 48 hour postdose sample. Test article was administered in 2 capsules/dose (~6 mL/ capsule). The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] Not determined; both high dose animals were euthanized before Day 15.
[b] Not calculable because of increasing concentrations 24 hours postdose.

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24h}$ = AUC from time 0 to 24 hours postdose; $AUC_{0\text{-}\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; CL/F = apparent total systemic clearance after extravascular administration; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 K 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	VUM00005					
Location in CTD	4.2.3.2.8					
Species	**Beagle Dog**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral capsule					
Sample	Plasma					
Analyte	JNJ-56021927					
Assay	LC-MS/MS					
LLOQ	0.100 µg/mL					
Gender/Number of Animals	M: 5	M: 8[a]	M: 8[a]	M: 5	M: 8[a]	M: 8[a,f]
Dose (mg/kg/day)	5	10	25	5	10	25
Duration of Dosing (days)	1	1	1	28	28	28
Mean (SD) Pharmacokinetic Parameters[b]						
C_{max} (µg/mL)	1.26 (0.143)	2.68 (0.411)	6.77 (0.805)	5.97 (1.29)	13.2 (1.98)	-
t_{max} (h)	2.40 (1.14)	2.75 (2.19)	5.38 (4.10)	3.50 (4.80)	7.13 (7.74)	-
AUC_{0-24h} (µg·h/mL)	21.0 (3.34)	47.4 (8.63)	127 (22.5)	116 (20.2)	290 (56.6)	-
$AUC_{0-\infty}$ (µg·h/mL)	53.5 (10.5)	148 (52.0)	480 (210)[c]	388[d]	1570[e]	-
$t_{1/2}$ (h)	34.4 (15.0)	41.7 (16.2)	49.3 (20.1)[c]	57.4[d]	86.2[e]	-
Accumulation Ratio $C_{max\ (Day\ 28/Day\ 1)}$	-	-	-	4.88 (1.62)	5.01 (0.906)	-
Accumulation Ratio $AUC_{0-24h\ (Day\ 28/Day\ 1)}$	-	-	-	5.78 (1.99)	6.30 (1.69)	-

Additional Information: No Day 28 PK data for high dose group due to adverse clinical signs (intermittent tremors, convulsion, and decreased activity)[f]. Dogs were dosed 2 capsules (each with a fill volume ~6 mL). The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] Includes 3 recovery animals.

[b] AUC_{0-24h} = AUC_{last} since quantifiable concentrations were observed throughout the 24-hour sampling schedule. SD reported, if available.

[c] N=7

[d] N=1

[e] N=2

[f] Three animals euthanized for humane reasons on Days 7, 8, and 9, respectively. Per protocol, remaining animals placed on dosing holiday on Day 9.

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; AUC_{0-24h} = AUC from time 0 to 24 hours postdose; $AUC_{0-\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; N = number; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 L 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	DR-[redacted]-008-DSD			
Location in CTD	4.2.3.2.9			
Species	**Beagle Dog**			
Feeding Condition	Fed			
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution			
Route	Oral capsule			
Sample	Plasma[a]			
Analyte	JNJ-56021927[b]		M3 (JNJ-56142060)[c]	
Assay	LC-MS/MS		LC-MS/MS	
LLOQ	0.100 µg/mL		0.100 µg/mL	
Gender/Number of Animals	M: 5	M: 8	M: 5	M: 8
Dose (mg/kg/day)	5	10	5	10
Duration of Dosing (days)	28	28	28	28
Mean (SD) Pharmacokinetic Parameters[d]				
C_{max} (µg/mL)	5.97 (1.29)[e]	13.2 (1.98)[e]	4.35 (0.7)	7.69 (1.04)
t_{max} (h)	3.5[e]	7.1[e]	5.1	3.6
$AUC_{0\text{-}24h}$ (µg·h/mL)[d]	116 (20.2)[e]	290 (56.6)[e]	96.5 (16.9)	172 (26.0)
M/P Ratio (C_{max})	-	-	0.776	0.599
M/P Ratio ($AUC_{0\text{-}24h}$)	-	-	0.859	0.620

Additional Information: The purpose of this study was to determine the steady-state plasma concentrations of JNJ-56142060 (M3)[c] using Day 28 plasma obtained from thawed plasma from surviving animals in the definitive dog toxicology study (VUM00005) and to determine M/P exposure ratios previously not calculated. For this reason, data from both studies are presented. The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] Day 28 plasma from Study VUM00005

[b] Pharmacokinetic parameters from Study VUM00005.

[c] The *N*-desmethyl metabolite of apalutamide. Pharmacokinetic parameters calculated using plasma from Day 28 of Study VUM00005.

[d] $AUC_{0\text{-}24h} = AUC_{last}$ since quantifiable concentrations were observed throughout the 24-hour sampling schedule. SD reported, if available.

[e] Data for 5 and 10 mg/kg/day from VUM00005 report included for comparison.

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma concentration-time curve; $AUC_{0\text{-}24h}$ = AUC from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M/P Ratio = metabolite-to-parent ratio; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4　M　薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TX-509-1002								
Location in CTD	4.2.3.2.10								
Species	**Beagle Dog**								
Feeding Condition	Fed								
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution								
Route	Oral capsule								
Sample	Plasma								
Analyte	JNJ-56021927								
Assay	LC-MS/MS								
LLOQ	0.025 µg/mL								
Gender/Number of Animals	M: 5	M: 8[c]	M: 8[c]	M: 5	M: 8[c]	M: 8[c]	M: 5	M: 8[c]	M: 8[c]
Dose (mg/kg/day)	2.5	5	10	2.5	5	10	2.5	5	10
Duration of Dosing (days)	1	1	1	28	28	28	91	91	91
Mean (SD) Pharmacokinetic Parameters									
C_{max} (µg/mL)	0.558 (0.294)[a]	1.56 (0.274)[d]	2.85 (0.469)	2.85 (0.397)	7.60 (1.17)	15.2 (2.00)	3.78 (0.907)	10.3 (4.92)	19.6 (3.14)
t_{max} (h)	5 (4)[a]	7 (4)[d]	7 (8)	4 (3)	11 (9)	10 (10)	4 (3)	5 (8)	5 (8)
$AUC_{0\text{-}24h}$ (µg·h/mL)	10.6 (5.84)[a]	31.9 (7.27)[d]	56.2 (10.1)	64.6 (9.14)	168 (24.9)	343 (44.3)	84.2 (21.9)	202 (57.4)	419 (58.8)
Acc Ratio $C_{max\ (Day\ 91/Day\ 1)}$	-	-	-	-	-	-	6.77	6.60	6.88
Acc Ratio $AUC_{0\text{-}24h\ (Day\ 91/Day\ 1)}$	-	-	-	-	-	-	7.94	6.33	7.46
Steady-State $t_{½}$ (h)[b]	-	-	-	-	-	-	-	-	78.6 (54.7)

Additional Information: Each dog was dosed at least 2 capsules (each with a fill volume of ~6 mL). The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] Report states that N=4 due to presumed missed dose for one dog on Day 1, but reported values include all animals (ie, N=5).

[b] Based on samples collected from recovery animals (N = 3) Day 91 postdose, Day 98, Day 105, and Day 112.

[c] Main 5 + Recovery 3

[d] N = 7

- = not calculated/not applicable; Acc Ratio = accumulation ratio; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; N = number; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.4 M 薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TX-509-1002								
Location in CTD	4.2.3.2.10								
Species	**Beagle Dog**								
Feeding Condition	Fed								
Sample	Plasma								
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution								
Route	Oral capsule								
Analyte	M3 (JNJ-56142060)[a]								
Assay	LC-MS/MS								
LLOQ	0.01 µg/mL Plasma								
Gender/Number of Animals	M: 5	M: 8[c]	M: 8[c]	M: 5	M: 8[c]	M: 8[c]	M: 5	M: 8[c]	M: 8[c]
Dose (mg/kg/day)	2.5	5	10	2.5	5	10	2.5	5	10
Duration of Dosing (days)	1	1	1	28	28	28	91	91	91
Mean (SD) Pharmacokinetic Parameters									
C_{max} (µg/mL)	0.127 (0.0787)[b]	0.459 (0.212)[d]	0.588 (0.251)	2.63 (0.488)	4.78 (0.846)	7.78 (0.990)	2.55 (0.270)	4.63 (0.881)	8.93 (1.64)
t_{max} (h)	24 (0)[b]	24 (0)[d]	21 (6)	21 (7)	6 (5)	12 (11)	1 (1)	3 (4)	9 (10)
$AUC_{0\text{-}24h}$ (µg·h/mL)	1.51 (0.956)[b]	5.77 (2.56)[d]	8.01 (3.81)	57.3 (11.1)	108 (18.8)	175 (25.1)	57.4 (6.23)	100 (18.3)	196 (32.0)
Acc Ratio $C_{max\ (Day\ 91/Day\ 1)}$	-	-	-	-	-	-	20.1	10.1	15.2
Acc Ratio $AUC_{0\text{-}24h\ (Day\ 91/Day\ 1)}$	-	-	-	-	-	-	38.0	17.3	24.5
M/P Ratio $AUC_{0\text{-}24h}$	24.9 (24.9)	18.4 (7.72)	14.9 (8.49)	91.5 (26.6)	65.7 (16.5)	51.9 (10.9)	71.6 (17.5)	52.3 (14.3)	47.8 (12.0)
Steady-State $t_{½}$ (h)[e]	-	-	-	-	-	-	-	-	188 (171)

Additional Information: Each dog was dosed at least 2 capsules (each with a fill volume of ~6 mL). The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] The *N*-desmethyl metabolite of apalutamide

[b] N=4 due to presumed missed dose for one dog on Day 1, but reported values include all animals (ie, N=5).

[c] Main 5 + Recovery 3

[d] N = 7

[e] Based on samples collected from recovery animals (N = 3) Day 91 postdose, Day 98, Day 105, and Day 112.

- = not calculated/not applicable; Acc Ratio = accumulation ratio; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M/P Ratio = metabolite-to-parent ratio; N = number; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.4 M 薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TX-509-1002								
Location in CTD	4.2.3.2.10								
Species	**Beagle Dog**								
Feeding Condition	Fed								
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution								
Route	Oral capsule								
Sample	Plasma								
Analyte	M4 (JNJ-56142021)[a]								
Assay	LC-MS/MS								
LLOQ	0.01 µg/mL								
Gender/Number of Animals	M: 5	M: 8[c]	M: 8[c]	M: 5	M: 8[c]	M: 8[c]	M: 5	M: 8[c]	M: 8[c]
Dose (mg/kg/day)	2.5	5	10	2.5	5	10	2.5	5	10
Duration of Dosing (days)	1	1	1	28	28	28	91	91	91
Mean (SD) Pharmacokinetic Parameters									
C_{max} (µg/mL)	0.0223 (0.0135)[b]	0.0818 (0.0289)[d]	0.134 (0.0354)	0.451 (0.129)	0.873 (0.140)	2.15 (0.378)	0.469 (0.103)	1.22 (0.349)	2.85 (0.812)
t_{max} (h)	21 (6)[b]	22 (6)[d]	18 (6)	4 (5)	2 (4)	2 (3)	3 (5)	1 (1)	2 (2)
$AUC_{0\text{-}24h}$ (µg·h/mL)	0.310 (0.192)[b]	1.18 (0.375)[d]	2.32 (0.732)	6.81 (1.46)	15.1 (3.40)	35.3 (7.25)	7.44 (0.906)	17.9 (5.63)	45.6 (12.6)
Acc Ratio $C_{max\ (Day\ 91/Day\ 1)}$	-	-	-	-	-	-	21.0	14.9	21.3
Acc Ratio $AUC_{0\text{-}24h\ (Day\ 91/Day\ 1)}$	-	-	-	-	-	-	24.0	15.2	19.7
M/P Ratio $AUC_{0\text{-}24h}$	2.39 (1.54)	3.81 (1.15)	4.34 (1.93)	10.5 (1.69)	9.03 (1.95)	10.6 (3.13)	9.15 (1.79)	9.14 (3.03)	11.1 (3.52)
Steady-State $t_{½}$ (h)[e]	-	-	-	-	-	-	-	-	123 (69.7)

Additional Information: Each dog was dosed at least 2 capsules (each with a fill volume of ~6 mL). The ethanolate salt (JNJ-56021927-ZAH) was administered.

[a] M4 = the metabolite formed by amide hydrolysis of apalutamide or M3

[b] N=4 due to presumed missed dose for one dog on Day 1, but reported values include all animals (ie, N=5).

[c] Main 5 + Recovery 3

[d] N = 7

[e] Based on samples collected from recovery animals (N = 3) Day 91 postdose, Day 98, Day 105, and Day 112.

- = not calculated/not applicable; Acc Ratio = accumulation ratio; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M/P Ratio = metabolite-to-parent ratio; N = number; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma concentration; $t_{½}$ = terminal half-life; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 N 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10839					
Location in CTD	4.2.3.2.11					
Species	**Beagle Dog**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral capsule					
Sample	Plasma					
Analyte	JNJ-56021927					
Assay	LC-MS/MS					
LLOQ	0.025 µg/mL					
Gender/Number of Animals	M: 4	M: 4	M: 4	M: 4	M: 4	M: 4
Dose (mg/kg/day)	2.5	5	10	2.5	5	10
Duration of Dosing (days)	182	182	182	273	273	273
Mean (SD) Pharmacokinetic Parameters						
C_{max} (µg/mL)	3.10 (0.659)	7.43 (1.29)	16.2 (2.82)	3.82 (0.643)	8.60 (1.28)	18.7 (3.89)
t_{max} (h)	6.50 (6.40)	10.5 (9.98)	9.00 (10.4)	13.5 (12.2)	12.5 (9.00)	10.0 (10.5)
$AUC_{0\text{-}24h}$ (µg·h/mL)	69.4 (15.5)	167 (31.8)	362 (78.1)	86.6 (15.2)	197 (29.9)	427 (95.1)
Accumulation Ratio $AUC_{0\text{-}24h\ (Day\ 273/Day\ 182)}$	-	-	-	1.26 (0.0908)	1.19 (0.0959)	1.18 (0.0276)
Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered. NOAEL could not be determined.						

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; NOAEL = no observed adverse effect level; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.4 N 薬物動態試験：反復投与後の吸収（経口）（続き）

Test Article: JNJ-56021927

Study No.	TOX10839					
Location in CTD	4.2.3.2.11					
Species	**Beagle Dog**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral capsule					
Sample	Plasma					
Analyte	M3 (JNJ-56142060)[a]					
Assay	LC-MS/MS					
LLOQ	0.025 µg/mL					
Gender/Number of Animals	M: 4	M: 4	M: 4	M: 4	M: 4	M: 4
Dose (mg/kg/day)	2.5	5	10	2.5	5	10
Duration of Dosing (days)	182	182	182	273	273	273
Mean (SD) Pharmacokinetic Parameters						
C_{max} (µg/mL)	2.89 (0.575)	4.78 (0.993)	7.71 (0.895)	2.99 (0.532)	5.04 (1.13)	8.32 (0.978)
t_{max} (h)	4.50 (5.26)	7.00 (11.5)	6.00 (2.31)	18.5 (11.0)	15.0 (11.5)	13.5 (12.2)
$AUC_{0\text{-}24h}$ (µg·h/mL)	66.4 (14.2)	109 (23.1)	177 (19.9)	69.7 (12.6)	116 (25.5)	189 (24.0)
Accumulation Ratio $AUC_{0\text{-}24h\ (Day\ 273/Day\ 182)}$	-	-	-	1.06 (0.0354)	1.06 (0.0861)	1.07 (0.0229)
M/P Ratio ($AUC_{0\text{-}24h}$)	0.962 (0.0888)	0.671 (0.180)	0.505 (0.101)	0.811 (0.0975)	0.593 (0.133)	0.458 (0.0971)

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered. NOAEL could not be determined.

[a] The *N*-desmethyl metabolite of apalutamide

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; M/P Ratio = metabolite-to-parent ratio; NOAEL = no observed adverse effect level; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4　O　薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10780 (Repeat-Dose Phase)					
Location in CTD	4.2.3.2.12					
Species	**Beagle Dog**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral capsule					
Sample	Plasma					
Analyte	JNJ-56021927			M3 (JNJ-56142060)[a]		
Assay	LC-MS/MS			LC-MS/MS		
LLOQ	0.025 µg/mL			0.025 µg/mL		
Gender/Number of Animals	F: 3	F: 3	F: 3	F: 3	F: 3	F:3
Dose (mg/kg/day)	5	10	20	5	10	20
Mean (SD) Pharmacokinetic Parameters[b]						
Following 2 weeks of dosing[c]						
C_{max} (µg/mL)	4.87 (0.838)	12.1 (2.12)	28.8 (5.65)	3.20 (1.24)	7.72 (0.161)	13.2 (3.5)
t_{max} (h)	3.3 (1.2)	1.3 (0.58)	1.3 (0.58)	18 (9.8)	11 (12)	1.0 (0.0)
AUC_{last} (µg·h/mL)	102 (18.3)	268 (43)	620 (98.2)	74.6 (29.9)	176 (2.69)	294 (66.5)
Following 3 weeks of dosing[c]						
C_{max} (µg/mL)	5.16 (0.70)	14.2 (1.06)	37.1 (9.97)	3.72 (1.31)	9.44 (0.681)	16.5 (1.27)
t_{max} (h)	3.3 (3.2)	3.0 (3.5)	13 (16)	4.7 (4.0)	3.0 (3.6)	16 (12)
AUC_{last} (µg·h/mL)	111 (11.7)	308 (26.3)	806 (245)	86.6 (29.5)	210 (11)	371 (29.9)

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered. MTD: 10 mg/kg/day

[a] The *N*-desmethyl metabolite of apalutamide

[b] C_{max} and AUC_{last} reported as ng/mL and ng·h/mL, respectively in study report.

[c] Study Day 63 and Day 70 correspond to 2 and 3 weeks of repeated dosing, respectively.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC_{last} = area under the plasma concentration-time curve from time 0 to the time corresponding to the last quantifiable concentration; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; MTD = maximum tolerated dose; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.4 P 薬物動態試験：反復投与後の吸収（経口）

Test Article: JNJ-56021927

Study No.	TOX10895					
Location in CTD	4.2.3.2.13					
Species	**Beagle Dog**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral capsule					
Sample	Plasma					
Analyte	JNJ-56021927			M3 (JNJ-56142060)[a]		
Assay	LC-MS/MS			LC-MS/MS		
LLOQ	0.025 µg/mL			0.025 µg/mL		
Gender/Number of Animals	F: 4	F: 4	F: 6[b]	F: 4	F: 4	F: 4
Dose (mg/kg/day)	2.5	5	10	2.5	5	10
Duration of Dosing (days)	90	90	90	90	90	90
Mean (SD) Pharmacokinetic Parameters						
C_{max} (µg/mL)	3.49 (0.824)	8.33 (1.28)	16.7 (3.32)	2.77 (0.387)	4.80 (0.243)	9.05 (1.32)
t_{max} (h)	18 (12)	4.50 (4.04)	10.5 (10.7)	24 (0)	6.75 (11.5)	12.3 (12.8)
$AUC_{0\text{-}24h}$ (µg·h/mL)	79.8 (18.8)	192 (28.4)	385 (84.8)	63.2 (8.61)	112 (6.38)	206 (29.4)
M/P Ratio ($AUC_{0\text{-}24h}$)	-	-	-	0.832 (0.232)	0.592 (0.118)	0.562 (0.153)

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered. NOAEL: 10 mg/kg/day.

[a] The *N*-desmethyl metabolite of apalutamide

[b] Main 4 + Recovery 2

- = not calculated/not applicable; Acconon MC8-2 = caprylocaproyl macrogolglycerides; $AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve time 0 to 24 hours postdose; C_{max} = maximum observed plasma concentration; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M/P Ratio = metabolite-to-parent ratio; NOAEL = no observed adverse effectt level; PEG = polyethylene glycol; SD = standard deviation; t_{max} = time correspondent to maximum plasma concentration; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.5 薬物動態試験：組織分布

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase A)
Location in CTD	4.2.2.4.2
Species	**Sprague-Dawley Rat (albino)**
Gender (M/F)/Number of Animals	M:7
Feeding Condition	Fed
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]
Route	Oral gavage
Dose (mg/kg)	50
Radionuclide	[^{14}C]JNJ-56021927
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)
Analytical methods	Quantitative whole-body autoradiography (tissue distribution) and liquid scintillation counting (LSC) for plasma

	Tissue:Plasma Concentration Ratios						**Pharmacokinetic Parameters**				
	Sampling Time[b]						C_{max}	t_{max}	AUC_{last}	T:P	$t_{½}$
	1h	4h	8h	12h	24h	72h	(µg eq/g)	(h)	(µg eq h/g)	$Ratio_{AUClast}$	(h)
Albino animals (Sprague-Dawley)											
Tissue type Tissues/Organs											
Circulatory: Plasma (by LSC)	1.0	1.0	1.0	1.0	1.0	1.00	11.8	12	422	1.0	25.5
Aorta	3.7	1.7	1.6	1.2	1.1	BQL	14.7	12	297	0.70	19.2
Whole blood (cardiac)	1.8	1.5	1.2	0.97	0.77	BQL	11.4	12	223	0.53	15.1
Vena cava	1.2	1.4	0.85	1.3	1.2	BQL	15.8	12	263	0.62	19.0
Nervous: Brain	1.4	0.87	0.90	0.96	0.65	BQL	11.3	12	187	0.44	11.6
Pineal gland	2.7	2.6	2.3	2.0	1.5	BQL	23.3	12	433	1.0	13.9
Spinal cord	1.0	1.0	1.2	1.4	0.83	BQL	16.5	12	250	0.59	9.70
Meninges	0.39	1.7	0.68	1.5	0.38	BQL	17.4	12	232	0.55	4.90
Ocular: Uveal tract/retina	1.9	1.1	0.99	0.68	0.67	BQL	9.12	8	174	0.41	23.5
Eye (lens)	0.11	0.35	0.23	0.33	0.51	20	4.38	24	486	1.2	369
Choroid plexus	1.3	0.96	1.8	1.5	0.78	BQL	17.9	12	281	0.67	8.40
Visceral organs: Kidney cortex	6.1	5.8	6.5	5.6	4.3	9.0	66.4	12	2070	4.9	9.80
Kidney medulla	4.2	3.1	4.9	2.5	2.2	2.4	45.2	8	1100	2.6	8.50

Additional information: Plasma concentrations used in T:P calculations (µg eq./g)

Time	1h	4h	8h	12h	24h	72h
Concentration	2.67	7.66	9.24	11.8	8.52	0.113

[a] Vehicle: Acconon MC8-2 EP/NF: Capmul MCM EP: PEG-400 USP: VitE-TPGS (10:45:30:15, w/w)

[b] Samples were also taken at 168h postdose, but only 4 tissues had quantifiable concentrations – eye (lens), liver, Hardarian gland, and skin (2.98, 0.921, 1.06, and 2.35 µg eq/g, respectively. T:P ratios were not calculable because plasma concentrations at 168h were BQL.)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC_{last} = area under the plasma or tissue time-concentration curve from time 0 to the last quantifiable concentration; BQL = below quantification limit (<0.209 µg eq/g); C_{max} = maximal plasma or tissue concentration; M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma or tissue concentration; T:P = tissue-to-plasma; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.5 薬物動態試験：組織分布（続き）

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase A)
Location in CTD	4.2.2.4.2
Species	**Sprague-Dawley Rat (albino)**
Gender (M/F)/Number of Animals	M:7
Feeding Condition	Fed
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]
Route	Oral gavage
Dose (mg/kg)	50
Radionuclide	[^{14}C]JNJ-56021927
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)
Analytical methods	Quantitative whole-body autoradiography (tissue distribution) and liquid scintillation counting (LSC) for plasma

	Tissue:Plasma Concentration Ratios						**Pharmacokinetic Parameters**				
	Sampling Time[b]						C_{max}	t_{max}	AUC_{last}	T:P	$t_{½}$
	1h	4h	8h	12h	24h	72h	(µg eq/g)	(h)	(µg eq h/g)	$Ratio_{AUClast}$	(h)
Tissue type Tissues/Organs											
Liver	15	9.3	10	9.1	6.5	35.0	107	12	3550	8.4	45.6
Lungs	2.8	1.8	1.6	1.6	0.99	BQL	19.2	12	328	0.78	10.2
Heart (myocardium)	3.9	4.0	3.6	2.8	2.2	BQL	33.3	8	639	1.5	15.2
Spleen	1.8	2.7	2.9	2.2	1.5	BQL	26.7	8	473	1.1	11.2
Glandular/ Adrenal cortex	5.5	6.5	5.9	4.6	3.7	BQL	54.2	8	1040	2.5	16.0
Secretory: Adrenal medulla	3.5	3.4	2.7	2.3	1.5	BQL	27.0	12	501	1.2	11.3
Bulbourethral gland	1.4	1.9	1.7	1.1	1.0	BQL	16.0	8	280	0.66	18.5
Exorbital lacrimal gland	4.8	3.2	3.1	3.6	2.4	3.5	42.1	12	1190	2.8	8.70
Harderian gland	9.7	8.0	4.9	3.8	3.8	25	61.0	4	2010	4.8	15.4
Intra-orbital lacrimal gland	4.6	4.4	3.1	3.3	2.8	5.2	39.1	12	1300	3.1	9.60
Mandibular lymph node	2.8	2.0	1.9	1.2	1.1	BQL	17.3	8	303	0.72	17.6
Mesenteric lymph node	2.6	2.2	1.7	1.5	1.2	BQL	17.8	12	342	0.81	15.5
Nasal mucosa	1.1	1.3	1.3	1.1	0.64	BQL	13.2	12	229	0.54	9.40
Pancreas	6.3	5.7	4.4	3.9	3.0	2.8	45.6	12	1480	3.5	8.10

Additional information: Plasma concentrations used in T:P calculations (µg eq./g)

Time	1h	4h	8h	12h	24h	72h
Concentration	2.67	7.66	9.24	11.8	8.52	0.113

[a] Vehicle: Acconon MC8-2 EP/NF: Capmul MCM EP: PEG-400 USP: VitE-TPGS (10:45:30:15, w/w)

[b] Samples were also taken at 168h postdose, but only 4 tissues had quantifiable concentrations – eye (lens), liver, Hardarian gland, and skin (2.98, 0.921, 1.06, and 2.35 µg eq/g, respectively. T: P ratios were not calculable because plasma concentrations at 168h were below quantification limit.)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC_{last} = area under the plasma or tissue time-concentration curve from time 0 to the last quantifiable concentration; BQL = below quantification limit (<0.209 µg eq/g); C_{max} = maximal plasma or tissue concentration; M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma or tissue concentration; T:P = tissue-to-plasma; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase A)
Location in CTD	4.2.2.4.2
Species	**Sprague-Dawley Rat (albino)**
Gender (M/F)/Number of Animals	M:7
Feeding Condition	Fed
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]
Route	Oral gavage
Dose (mg/kg)	50
Radionuclide	[^{14}C]JNJ-56021927
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)
Analytical methods	Quantitative whole-body autoradiography (tissue distribution) and liquid scintillation counting (LSC) for plasma

	Tissue:Plasma Concentration Ratios						**Pharmacokinetic Parameters**				
	Sampling Time[b]						C_{max}	t_{max}	AUC_{last}	T:P	$t_{½}$
	1h	4h	8h	12h	24h	72h	(µg eq/g)	(h)	(µg eq h/g)	$Ratio_{AUClast}$	(h)
Tissue type Tissues/Organs											
Pituitary	3.4	3.8	2.7	2.3	1.7	BQL	28.8	4	522	1.2	13.0
Preputial gland	3.4	4.4	3.1	3.1	3.4	BQL	36.2	12	715	1.7	38.1
Salivary gland	4.7	4.0	2.8	2.2	2.1	2.7	30.9	4	977	2.3	9.00
Thymus	2.7	2.1	1.5	1.5	1.0	BQL	17.3	12	317	0.75	12.3
Thyroid	4.9	2.8	2.2	2.5	2.2	13	29.6	12	1010	2.4	13.5
Reproductive: Testis	1.3	1.5	1.4	1.2	0.86	1.7	14.0	12	434	1.0	9.50
Prostate	1.2	2.3	1.8	1.8	1.2	BQL	21.0	12	361	0.86	10.9
Seminal vesicles	1.2	1.6	0.82	1.1	0.69	BQL	12.4	12	213	0.50	11.1
Epididymis	1.7	3.2	2.1	2.6	1.6	BQL	31.0	12	499	1.2	9.70
Musculo- Bone marrow	2.2	2.0	2.1	1.3	1.1	BQL	19.7	8	322	0.76	15.2
skeletal: Bone surface	1.3	0.97	0.84	0.49	0.11	BQL	7.79	8	116	0.27	5.10
Fat (abdominal)	3.9	4.9	13	16	9.9	9.4	190	12	4700	11	7.90
Fat (brown)	4.4	4.4	4.5	7.5	4.3	BQL	88.2	12	1230	2.9	9.30
Muscle (skeletal)	1.7	2.3	2.8	2.6	1.6	BQL	30.3	12	497	1.2	10.0
Skin	1.6	1.9	2.3	2.3	1.4	42	27.6	12	1180	2.8	64.8

Additional information: Plasma concentrations used in T:P calculations (µg eq./g)

Time	1h	4h	8h	12h	24h	72h
Concentration	2.67	7.66	9.24	11.8	8.52	0.113

[a] Vehicle: Acconon MC8-2 EP/NF: Capmul MCM EP: PEG-400 USP: VitE-TPGS (10:45:30:15, w/w)

[b] Samples were also taken at 168h postdose, but only 4 tissues had quantifiable concentrations – eye (lens), liver, Hardarian gland, and skin (2.98, 0.921, 1.06, and 2.35 µg eq/g, respectively. T:P ratios were not calculable because plasma concentrations at 168h were below quantification limit.)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC_{last} = area under the plasma or tissue time-concentration curve from time 0 to the last quantifiable concentration; BQL = below quantification limit (<0.209 µg eq/g); C_{max} = maximal plasma or tissue concentration; M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma or tissue concentration; T:P = tissue-to-plasma; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.5 薬物動態試験：組織分布（続き）

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase A)
Location in CTD	4.2.2.4.2
Species	**Sprague-Dawley Rat (albino)**
Gender (M/F)/Number of Animals	M:7
Feeding Condition	Fed
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]
Route	Oral gavage
Dose (mg/kg)	50
Radionuclide	[^{14}C]JNJ-56021927
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)
Analytical methods	Quantitative whole-body autoradiography (tissue distribution) and liquid scintillation counting (LSC) for plasma

	Tissue:Plasma Concentration Ratios						**Pharmacokinetic Parameters**				
	Sampling Time[b]						C_{max}	t_{max}	AUC_{last}	T:P	$t_{½}$
	1h	4h	8h	12h	24h	72h	(µg eq/g)	(h)	(µg eq·h/g)	$Ratio_{AUClast}$	(h)
Tissue type Tissues/Organs											
Tongue	2.9	3.6	2.7	2.3	1.9	BQL	27.2	4	520	1.0	15.6
Oesophagus wall	3.5	1.4	1.3	0.97	0.93	17	12.0	8	478	1.1	23.0
Excretory: Stomach wall	5.2	4.2	5.3	3.4	2.6	2.2	49.3	8	1340	3.2	7.90
Small intestine wall	16	6.3	3.3	3.8	1.4	5.0	48.5	4	1120	2.7	9.80
Caecum wall	2.9	4.0	2.2	2.3	1.5	5.0	30.6	4	819	1.9	10.7
Large intestine wall	4.1	4.3	1.8	2.4	1.1	4.6	32.8	4	713	1.7	10.7
Rectum wall	2.5	4.0	1.5	2.2	2.4	BQL	30.3	4	509	1.2	33.0
Urinary bladder wall	1.7	7.5	2.6	2.5	1.4	2.1	57.7	4	903	2.1	8.60

Additional information: Plasma concentrations used in T:P calculations (µg eq./g)

Time	1h	4h	8h	12h	24h	72h
Concentration	2.67	7.66	9.24	11.8	8.52	0.113

[a] Vehicle: Acconon MC8-2 EP/NF: Capmul MCM EP: PEG-400 USP: VitE-TPGS (10:45:30:15, w/w)

[b] Samples were also taken at 168h postdose, but only 4 tissues had quantifiable concentrations – eye (lens), liver, Hardarian gland, and skin (2.98, 0.921, 1.06, and 2.35 µg eq/g, respectively. T:P ratios were not calculable because plasma concentrations at 168h were below quantification limit.)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC_{last} = area under the plasma or tissue time-concentration curve from time 0 to the last quantifiable concentration; BQL = below quantification limit (<0.209 µg eq/g); C_{max} = maximal plasma or tissue concentration; M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; t_{max} = time correspondent to maximum plasma or tissue concentration; T:P = tissue-to-plasma; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.5 薬物動態試験：組織分布（続き）

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase B)			
Location in CTD	4.2.2.4.2			
Species	**Lister Hooded Rat (pigmented)**			
Gender (M/F)/Number of Animals	M:3			
Feeding Condition	Fed			
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]			
Route	Oral gavage			
Dose (mg/kg)	50			
Radionuclide	[^{14}C]JNJ-56021927			
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)			
Analytical methods	Quantitative whole-body autoradiography (tissue distribution) and liquid scintillation counting (LSC) for plasma			
	Radioactivity Concentrations (µg eq/g tissue)[b]			
	Sampling Time			**Tissue: Plasma Ratio$_{24h}$**
	24h	168h	504h	
Pigmented Animals (Lister Hooded)				
Tissue type Tissues/Organs				
Circulatory: Plasma (by LSC)	6.74	BQL	BQL	1.0
Aorta	11.2	BQL	BQL	1.7
Whole blood	8.21	BQL	BQL	1.2
Vena cava	9.67	BQL	BQL	1.4
Nervous: Brain	4.67	BQL	BQL	0.69
Pineal gland	11.8	BQL	BQL	1.8
Spinal cord	5.87	BQL	BQL	0.87
Meninges	8.01	BQL	BQL	1.2
Ocular: Uveal tract/retina	9.28	BQL	BQL	1.4
Eye (lens)	7.44	3.71	BQL	1.1
Choroid plexus	6.05	BQL	BQL	0.90
Visceral organs: Kidney cortex	30.7	BQL	BQL	4.6
Kidney medulla	20.6	BQL	BQL	3.1
Liver	53.2	0.745	BQL	7.9

Additional information: Plasma concentrations (by LSC) used in T:P calculations (µg eq./g)

Time	24h	168h	504h
Concentration	6.74	BQL	BQL

[a] Vehicle: Acconon MC8-2 EP/NF: Capmul MCM EP: PEG-400 USP: VitE-TPGS (10:45:30:15, w/w)

[b] Maximal tissue [^{14}C]JNJ-56021927 concentrations (C_{max}) were recorded in all tissues 24 hours postdose.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; BQL = below quantification limit (<0.209 µg eq/g); M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; Tissue: Plasma Ratio$_{24h}$ = tissue-to-plasma concentration calculated using 24h values; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase B)			
Location in CTD	4.2.2.4.2			
Species	**Lister Hooded Rat (pigmented)**			
Gender (M/F)/Number of Animals	M:3			
Feeding Condition	Fed			
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]			
Route	Oral gavage			
Dose (mg/kg)	50			
Radionuclide	[^{14}C]JNJ-56021927			
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)			
Analytical methods	Quantitative whole-body autoradiography (tissue distribution) and liquid scintillation counting (LSC) for plasma			
	Radioactivity Concentrations (µg eq/g tissue)			
	Sampling Time			**Tissue: Plasma Ratio$_{24h}$**
	24h[b]	168h	504h	
Tissue type Tissues/Organs				
Lungs	10.6	BQL	BQL	1.6
Heart (myocardium)	16.4	BQL	BQL	2.4
Spleen	10.2	BQL	BQL	1.5
Glandular/ Adrenal cortex	28.4	BQL	BQL	4.2
Secretory: Adrenal medulla	16.8	BQL	BQL	2.5
Bulbourethral gland	7.57	BQL	BQL	1.1
Exorbital lacrimal gland	17.9	BQL	BQL	2.7
Harderian gland	29.6	1.28	BQL	4.4
Intra-orbital lacrimal gland	19.9	BQL	BQL	3.0
Mandibular lymph node	9.25	BQL	BQL	1.4
Mesenteric lymph node	10.7	BQL	BQL	1.6
Nasal mucosa	5.99	BQL	BQL	0.89
Pancreas	22.2	BQL	BQL	3.3
Pituitary	11.8	BQL	BQL	1.8
Preputial gland	21.5	BQL	BQL	3.2
Salivary gland	15.4	BQL	BQL	2.3

Additional information: Plasma concentrations (by LSC) used in T:P calculations (µg eq./g)

Time	24h	168h	504h
Concentration	6.74	BQL	BQL

[a] Vehicle: Acconon MC8-2 EP/NF: Capmul MCM EP: PEG-400 USP: VitE-TPGS (10:45:30:15, w/w)

[b] Maximal tissue [^{14}C]JNJ-56021927 concentrations (C_{max}) were recorded in all tissues 24 hours postdose.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; BQL = below quantification limit (<0.209 µg eq/g); M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; Tissue: Plasma Ratio$_{24h}$ = tissue-to-plasma concentration calculated using 24h values; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase B)
Location in CTD	4.2.2.4.2
Species	**Lister Hooded Rat (pigmented)**
Gender (M/F)/Number of Animals	M:3
Feeding Condition	Fed
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]
Route	Oral gavage
Dose (mg/kg)	50
Radionuclide	[^{14}C]JNJ-56021927
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)
Methods	Quantitative whole-body autoradiography (tissue distribution) and liquid scintillation counting (LSC) for plasma

	Radioactivity Concentrations (µg eq/g tissue)			
	Sampling Time			**Tissue: Plasma Ratio$_{24h}$**
	24h[b]	168h	504h	
Tissue type Tissues/Organs				
Thymus	8.71	BQL	BQL	1.3
Thyroid	15.4	BQL	BQL	2.3
Reproductive: Testis	7.27	BQL	BQL	1.1
Prostate	7.72	BQL	BQL	1.1
Seminal vesicles	7.35	BQL	BQL	1.1
Epididymis	12.2	BQL	BQL	1.8
Musculo- Bone marrow	14.6	BQL	BQL	2.2
skeletal: Bone surface[c]	4.94	BQL	BQL	0.73
Fat (abdominal)[c]	84.9	BQL	BQL	13
Fat (brown)	32.2	BQL	BQL	4.8
Muscle (skeletal)	12.0	BQL	BQL	1.8
Skin (nonpigmented)	11.6	0.322	0.453	1.7
Skin (pigmented)	17.7	0.927	2.46	2.6

Additional information: Plasma concentrations (by LSC) used in T:P calculations (µg eq./g)

Time	24h	168h	504h
Concentration	6.74	BQL	BQL

[a] Vehicle: Acconon MC8-2 EP/NF: Capmul MCM EP: PEG-400 USP: VitE-TPGS (10:45:30:15, w/w)

[b] Maximal tissue [^{14}C]JNJ-56021927 concentrations (C_{max}) were recorded in all tissues 24 hours postdose.

[c] Tissue corrected for quenching

Acconon MC8-2 = caprylocaproyl macrogolglycerides; BQL = below quantification limit (<0.209 µg eq/g); M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; Tissue:Plasma Ratio$_{24h}$ = tissue-to-plasma concentration calculated using 24h values; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.5 薬物動態試験：組織分布（続き）

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase B)
Location in CTD	4.2.2.4.2
Species	**Lister Hooded Rat (pigmented)**
Gender (M/F)/Number of Animals	M:3
Feeding Condition	Fed
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]
Route	Oral gavage
Dose (mg/kg)	50
Radionuclide	[^{14}C]JNJ-56021927
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)
Methods	Quantitative whole-body autoradiography (tissue distribution) and liquid scintillation counting (LSC) for plasma

Radioactivity Concentrations (µg eq/g tissue)

	Sampling Time			**Tissue: Plasma Ratio$_{24h}$**
	24h[b]	168h	504h	
Tissue type Tissues/Organs				
Tongue	15.0	BQL	BQL	2.2
Oesophagus wall	12.8	BQL	BQL	1.9
Excretory: Stomach wall	19.4	BQL	BQL	2.9
Small intestine wall	27.3	BQL	BQL	4.1
Caecum wall	22.8	BQL	BQL	3.4
Large intestine wall	12.5	0.386	BQL	1.9
Rectum wall	16.3	BQL	BQL	2.4
Urinary bladder wall	NS	0.511	BQL	NC

Additional information: Plasma concentrations (by LSC) used in T:P calculations (µg eq./g)

Time	24h	168h	504h
Concentration	6.74	BQL	BQL

[a] Vehicle: Acconon MC8-2 EP/NF: Capmul MCM EP: PEG-400 USP: VitE-TPGS (10:45:30:15, w/w)

[b] Maximal tissue [^{14}C]JNJ-56021927 concentrations (C_{max}) were recorded in all tissues 24 hours postdose.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; BQL = below quantification limit (<0.209 µg eq/g); M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; NC = not calculable; NS = no sample. PEG = polyethylene glycol; Tissue:Plasma Ratio$_{24h}$ = tissue-to-plasma concentration calculated using 24h values; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.6 A 薬物動態試験：血漿たん白結合及び血球移行

Test Article: JNJ-56021927

Study Number	301010034	301093931
Location in CTD	4.2.2.3.1	4.2.2.3.2
Type of Study	**Degree of Plasma Protein Binding of Apalutamide**	**Degree of Plasma Protein Binding of M3, the *N*-Desmethyl Metabolite of apalutamide**
Study System	*In vitro*	*In vitro*
Test System	For both studies: A rapid equilibrium dialysis (RED) system was used. The test article (apalutamide or the *N*-desmethyl metabolite of apalutamide; Study No. 301010034 or 301093931, respectively) was added to plasma of various species to achieve a final concentration of 10 µg/mL and a 300 µL of the spiked plasma was placed in the donor chamber. A 500 µL aliquot of isotonic buffer (0.1M KH_2PO_4, 0.6% NaCl, pH 7.4) was added to the receiver chamber. The sealed plates were incubated for 4 hours at 37°C. An additional aliquot of spiked plasma was retained separately at 37°C to serve as a standard to evaluate relative recovery. Following incubation and centrifugation of the chamber contents, the supernatant from each chamber was assayed for test article using LC-MS/MS. Triplicate samples were tested for each species' plasma. Warfarin (10µM) was used as the positive control.	
Vehicle/Formulation	DMSO/solution	DMSO/solution
Sample	Plasma[a]	Plasma[a]
Analyte	JNJ-56021927	M3 (JNJ-56142060)[b]
Assay	LC-MS/MS	LC-MS/MS
Tabulated Results		
	Percent Bound (range)	
	JNJ-56021927 (10 µg/mL)	JNJ-56142060 (10 µg/mL)[b]
Gender/Species		
Male Mouse, CD-1	87.9 (86.5 – 89.4)	86.5 (85.6 – 88.1)
Male Rat Sprague-Dawley	86.8 (80.7 – 89.9)	88.8 (87.5 – 89.7)
Male Dog Beagle	89.4 (86.8 – 91.2)	90.8 (90.4 – 91.2)
Male Human	95.6 (95.4 – 95.9)	95.5 (95.4 – 95.7)

Additional Information: For both studies, the RED test system methodology, the source of plasma samples, and the assay technique was identical.

[a] Frozen pooled tripotassium EDTA plasma.
[b] The *N*-desmethyl metabolite of apalutamide.

DMSO = dimethyl sulfoxide; EDTA = ethylenediaminetetraacetic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; KH_2PO_4 = potassium phosphate; NaCl = sodium chloride; RED = rapid equilibrium dialysis

2.6.5.6 B 薬物動態試験：血漿たん白結合及び血球移行

Test Article: JNJ-56021927

Study No.:	FK10658
Location in CTD:	4.2.2.3.3
Study System:	*In vitro* plasma protein binding and blood distribution
Test Systems:	Stability of JNJ-56021927: Stability of ^{14}C-labeled JNJ-56021927 was evaluated by spiking human plasma at 10 µg/mL (39 kBq/mL) and incubating the samples at 22°C and 37°C for 0h, 0.5h, 1h, and 2h and then assaying for the test article with radio-HPLC. Blood distribution: ^{14}C-labeled JNJ-56021927 or M3 (JNJ-56142060) was added to blood of various species to achieve concentrations ranging from 0.1-30 µg/mL (23.1×10^3 – 1.02×10^6 dpm/mL). These were incubated at 37°C for 15 minutes. At the end of the incubation, the blood was centrifuged to harvest plasma (22°C at 1700 g for 10 minutes). Plasma and plasma ultrafiltrate samples were assayed for radioactive content with liquid scintillation counting. Plasma protein binding: JNJ-56021927 or M3 (JNJ-56142060) was added to pooled plasma of various species or specific plasma proteins (HSA and AGP) to achieve concentrations ranging from 0.1-30 µg/mL and incubated at 37°C for 15 minutes. PPB was determined by first ultrafiltrating aliquots of spiked plasma with a centrifugal filter unit (at both 22°C and (for an additional 10µg/mLsample) at 37°C) and then analyzing filtrate or protein solutions with LC-MS/MS.
Vehicle/Formulation:	Ethanol/solution
Sample:	Pooled blood, plasma, ultrafiltrate, and protein solutions[a]
Assay:	LC-MS/MS and radio-HPLC or liquid scintillation counting (for ^{14}C-labeled assays)

	JNJ-56021927 (10 µg/mL)[b]					M3 (JNJ-56142060, 10 µg/mL)[b,c]				
Species	**Mouse**	**Rat**	**Rabbit**	**Dog**	**Human**	**Mouse**	**Rat**	**Rabbit**	**Dog**	**Human**
Gender	M+F	M+F	F	M+F	M	M+F	M+F	F	M+F	M
Mean blood values										
Plasma % Bound[d]	91.8	92.8	88.6	93.7	95.8	90.6	91.5	86.8	91.8	94.9
Plasma % Free	8.21	7.16	11.4	6.30	4.18	9.44	8.55	13.2	8.24	5.11
Blood-to-Plasma Ratio	0.83	0.86	0.96	0.85	0.79	0.89	0.92	0.97	0.86	0.78
Distribution to Plasma Water (%)	5.55	4.97	6.65	3.71	2.29	5.96	5.52	7.59	4.80	3.64
Plasma Proteins (%)	62.0	64.5	51.9	55.1	68.5	57.2	59.1	50.0	53.5	67.5
Blood Cells (%)	32.4	30.5	41.4	41.2	28.5	36.9	35.4	42.4	41.7	28.8
Mean binding to human blood proteins[d]										
α_1-acid glycoprotein (AGP; 0.07%, w/v)	-	-	-	-	22.2	-	-	-	-	18.0
Albumin (HSA; 4.3%, w/v)	-	-	-	-	93.6	-	-	-	-	93.2
AGP (0.07%)/HSA (4.3%) mixture	-	-	-	-	94.2	-	-	-	-	93.7

Additional Information: Although PPB experiments were run at both 22°C and 37°C, only the 37°C results were considered relevant and are presented. Plasma protein binding of either JNJ-56021927 or its M3 metabolite, JNJ-56142060, was concentration (0.1-30 µg/mL) and species independent. Nearly all human plasma protein binding is to albumin.

[a] The sample is a function of the parameter measured.

[b] Determination of test article distribution in blood was made with ^{14}C-labeled compounds, whereas the protein binding experiments were carried out with unlabeled compounds.

[c] The *N*-desmethyl metabolite of apalutamide.

[d] Calculated by subtracting the corresponding mean percent free value from 100.

- = not assayed/not applicable; AGP = α_1-acid glycoprotein; F = female; HPLC = high-performance liquid chromatography; HSA = human serum albumin; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; PPB = plasma protein binding;

2.6.5.6 C 薬物動態試験：たん白結合及び血球移行

Test Article: JNJ-56021927

Study No.	DR-[redacted]-014-DSD
Location in CTD	4.2.2.3.4
Type of Study	***In vitro* Plasma Protein Binding in SHO Mouse Plasma**
Method:	Equilibrium dialysis was used to determine the amount of unbound apalutamide after 6 and 24 hours. Apalutamide (JNJ-56021927) was added to plasma to achieve a final 10 µM concentration. A 250-µL aliquot of the spiked plasma was added to the donor chamber and a 250-µL aliquot of phosphate buffer solution with 0.1% DMSO was added to the receiver chamber. The sealed chambers were incubated for either 6 or 24 hours at 37°C while rotated in an orbital shaker at 200 rpm. Following incubation and centrifugation of the chamber contents, the supernatant from each chamber was assayed for test article with LC-MS/MS and the amount of unbound apalutamide was determined by the ratio of the test article in the receiver chamber (unbound) to that in the donor chamber (bound and unbound).
Gender/Species:	Male SHO Mouse
Vehicle/Formulation:	10 mM DMSO/solution
Sample:	Plasma
Assay:	LC-MS/MS
Analyte:	JNJ-56021927

Tabulated Results:

Incubation time	Unbound JNJ-56021927 (mean [SD])	Mass Balance (%, mean [SD])
6 hours	0.090 (0.006)	77.6 (6.3)
24 hours	0.138 (0.009)	60.5 (3.9)

Additional Information: The instability of JNJ-56021927 in mouse plasma at 24 hours resulted in poor (low) mass balance results and in the higher amount of unbound JNJ-56021927.

DMSO = dimethylsulfoxide; LC-MS/MS = liquid chromatography-tandem mass spectrometry; SD = standard deviation; SHO = SCID hairless outbred (immunodeficient)

2.6.5.7　薬物動態試験：妊娠又は授乳動物における試験

Test Article: JNJ-56021927

Not Applicable

2.6.5.8 A 薬物動態試験：その他の分布試験

Test Article: JNJ-56021927

Study No.	DR-[redacted]-010-DSD							
Location in CTD	4.2.2.2.3							
Species	**CD-1 Mouse**							
Feeding Condition	Fasted							
Vehicle/Formulation	Acconon MC8-2 EP/NP, Capmul MCM EP, PEG-400, VitE-TPGS NF (10:45:30:15)/solution							
Route	Oral gavage							
Sample/LLOQ	Plasma/0.0976 µg/mL, Brain/0.0244 µg/mL							
Analyte	JNJ-56021927							
Assay	LC-MS/MS							
Gender/Number of Animals	M: 3		M: 3		M: 6			
Dose (mg/kg)	10		30		100			
Mean (SD) Brain and Plasma Exposure								
Time Postdose (h)	16	24	16	24	4	8	16	24
Brain Concentration (µg/g)	1.27 (0.21)	0.57 (0.37)	3.37 (0.63)	1.74 (0.29)	19.9 (11.0)	9.61 (4.74)	10.4 (4.29)	5.55 (2.44)
Plasma Concentration (µg/mL)	2.89 (0.20)	1.77 (0.72)	9.31 (2.00)	4.83 (1.00)	19.4 (1.54)	22.7 (4.20)	18.7 (9.05)	12.4 (2.04)
Brain/Plasma Concentration (%)	43.9 (4.56)	31.5 (8.46)	36.8 (6.29)	37.6 (11.1)	106 (64.5)	41.4 (18.2)	59.5 (22.3)	43.9 (18.3)
Analyte	M3 (JNJ-56142060)[a]							
Assay	LC-MS/MS							
Gender/Number of Animals	M: 3		M: 3		M: 6			
Dose (mg/kg)	10		30		100			
Mean (SD) Brain and Plasma Exposure								
Time Postdose (h)	16	24	16	24	4	8	16	24
Brain Concentration (µg/g)	0.44 (0.13)	0.40 (0.17)	1.20 (0.14)	1.24 (0.54)	1.59 (0.98)	1.94 (0.76)	4.38 (1.43)	4.96 (1.11)
Plasma Concentration (µg/mL)	0.87 (0.16)	0.80 (0.42)	2.88 (0.80)	2.63 (0.67)	2.17 (0.30)	4.71 (3.36)	8.93 (1.02)	9.96 (2.23)
Brain/Plasma Concentration (%)	50.3 (0.16)	50.6 (11.7)	44.0 (13.1)	47.0 (13.3)	78.3 (59.4)	50.0 (18.8)	48.9 (14.7)	50.6 (10.7)

Additional Information: DR-[redacted]-010-DSD is a compilation of 4 experiments, 2 of which dosed apalutamide – one in mice (PK096-10) and one in rats (PK031-10). Results listed are from the former.

[a] The *N*-desmethyl metabolite of apalutamide.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

Continued

2.6.5.8 A 薬物動態試験：その他の分布試験（続き）

Test Article: JNJ-56021927

Study No.	DR-[redacted]-010-DSD
Location in CTD	4.2.2.2.3
Species	**Sprague-Dawley Rat**
Feeding Condition	Fasted
Vehicle/Formulation	PEG-400, Tween 80, PVP, and 0.5%CMC (9:0.5:0.5:90)/solution
Route	Oral gavage
Sample/LLOQ	Plasma/0.00122 µg/mL, Brain/0.00122 µg/mL
Analyte	JNJ-56021927
Assay	LC-MS/MS
Gender/Number of Animals	M: 5
Dose (mg/kg)	10
Mean (SD) Brain and Plasma Exposure	
Time Postdose (h)	24
Brain Concentration (µg/g)	0.48 (0.18)
Plasma Concentration (µg/mL)	0.63 (0.21)
Brain/Plasma Concentration (%)	75.7 (9.08)

Additional Information: DR-[redacted]-010-DSD is a compilation of 4 experiments, 2 of which dosed apalutamide – one in mice (PK096-10) and one in rats (PK031-10). Results listed are from the latter.

CMC = carboxymethyl cellulose; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; PVP = polyvinylpyrrolidone; Tween = polyoxyethylenesorbitan monooleate

2.6.5.8 B 薬物動態試験：その他の分布試験

Test Article: M3 (JNJ-56142060)[a]

Study No.	DR-[redacted]-014-DSD
Location in CTD	4.2.2.2.5
Species	**Sprague-Dawley Rat**
Feeding Condition	Fasted
Sample/LLOQ	Plasma/0.0012 µg/mL, Brain/0.0019 µg/mL
Assay	LC-MS/MS
Analyte	M3 (JNJ-56142060)[a]
Formulation	Solution[b]
Route	Oral gavage
Gender/Number of Animals	M: 4
Dose (mg/kg)	10
Mean (SD) Brain Exposure	
Time Postdose (h)	24
Brain Concentration$_{24h}$ (µg/g)	0.046 (0.030)
Plasma Concentration$_{24h}$ (µg/mL)	0.083 (0.037)
Brain: Plasma Concentration$_{24h}$ (%)	52.5 (11.6)

[a] The *N*-desmethyl metabolite of apalutamide.
[b] Vehicle: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS (10:45:30:15)

Acconon MC8-2 = caprylocaproyl macrogolglycerides; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; PEG = polyethylene glycol; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.8 C 薬物動態試験：その他の分布試験

Test Article: JNJ-56021927

Study No.	TOX10780 (Repeat-Dose Phase)					
Location in CTD	4.2.3.2.12					
Species	**Beagle Dog**					
Feeding Condition	Fed					
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400 USP, VitE-TPGS NF (10:45:30:15)/solution					
Route	Oral capsule					
Sample	Plasma and Brain					
Analyte	JNJ-56021927			M3 (JNJ-56142060)[a]		
Assay	LC-MS/MS			LC-MS/MS		
LLOQ	0.025 µg/mL			0.025 µg/mL		
Gender/Number of Animals	F: 3	F: 3	F: 3	F: 3	F: 3	F: 3
Dose (mg/kg/day)	5	10	20	5	10	20
Brain and Plasma Concentrations following 3 weeks of dosing [b,c]						
Brain Concentration (µg/g)	4.487	13.133	35.600	4.010	10.570	24.133
Plasma Concentration (µg/mL)	4.333	12.000	34.500	3.630	8.670	17.067
Brain/Plasma ratio	1.0	1.1	1.0	1.1	1.2	1.4

Additional Information: The ethanolate salt (JNJ-56021927-ZAH) was administered. MTD: 10 mg/kg/day

[a] The *N*-desmethyl metabolite of apalutamide

[b] Study Day 70 corresponds to 3 weeks of repeated dosing.

[c] Brain and plasma concentrations reported as ng/g and ng/mL, respectively in study report.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; Capmul MCM = mono-diglyceride of medium chain fatty acids; F = female; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; MTD = maximum tolerated dose; PEG = polyethylene glycol; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.8 D 薬物動態試験：その他の分布試験

Test Article: JNJ-56021927

Study No.	TX-509-1002								
Location in CTD	4.2.3.2.10								
Species	**Beagle Dog**								
Feeding Condition	Fed								
Vehicle/Formulation	Acconon MC8-2 EP/NF, Capmul MCM EP, PEG-400, VitE-TPGS NF (10:45:30:15)/solution								
Route	Oral capsule								
Sample	Plasma, CSF and Brain								
Analyte	JNJ-56021927			M3 (JNJ-56142060)[a]			M4 (JNJ-56142021)[b]		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS		
LLOQ	Plasma: 0.025 µg/mL, CSF: 0.005 µg/mL, Brain: 0.005 µg/mL			Plasma: 0.01 µg/mL, CSF: 0.005 µg/mL, Brain: 0.005 µg/mL			Plasma: 0.01 µg/mL, CSF: 0.005 µg/mL, Brain: 0.005 µg/mL		
Gender/Number of Animals	M: 5	M: 5	M: 5	M: 5	M: 5	M: 5	M: 5	M: 5	M: 5
Dose (mg/kg/day)	2.5	5	10	2.5	5	10	2.5	5	10
Mean (SD) Brain and CSF concentrations at Steady State[c,d]									
Brain Concentration (µg/g)	2.46 (0.85)	4.73 (1.42)	13.5 (1.41)	2.29 (0.16)	3.32 (0.45)	7.98 (0.90)	0.15 (0.038)	0.27 (0.038)	0.92 (0.104)
CSF Concentration (µg/mL)	0.18 (0.05)	0.37 (0.18)[e]	0.84 (0.14)	0.12 (0.02)	0.17 (0.02)[e]	0.37 (0.08)	0.007 (0.001)	0.013 (0.004)[e]	0.042 (0.007)
CSF/Brain Concentration (%)	7.59 (0.69)	7.87 (0.91)[e]	6.20 (0.88)	5.11 (0.52)	5.24 (0.88)[e]	4.60 (0.80)	4.74 (0.37)	4.89 (0.96)[e]	4.60 (0.76)
Mean (SD) Plasma and CSF concentrations at Steady State[c]									
Plasma Concentration (µg/mL)	3.43 (0.92)	10.3 (6.72)	17.9 (2.41)	2.38 (0.294)	4.03 (0.916)	8.29 (0.957)	0.257 (0.075)	0.663 (0.340)	1.72 (0.790)
CSF Concentration (µg/mL)	0.18 (0.05)	0.37 (0.18)[e]	0.84 (0.14)	0.12 (0.02)	0.17 (0.02)[e]	0.37 (0.08)	0.007 (0.001)	0.013 (0.004)[e]	0.042 (0.007)
CSF/Plasma Concentration (%)	5.38 (0.70)	3.85 (0.71)[e]	4.69 (0.63)	4.95 (0.589)	4.57 (1.00)[e]	4.41 (0.586)	2.88 (0.604)	2.14 (1.13)[e]	2.94 (1.39)

Additional Information: Each dog was dosed at least 2 capsules (each with a fill volume of ~6 mL).

[a] The *N*-desmethyl metabolite of apalutamide.
[b] The metabolite formed by amide hydrolysis of apalutamide or M3.
[c] Based on samples collected from main study animals Day 91 postdose.
[d] Results are recorded in Toxicokinetic Report, Appendix 1 as Study DR-[redacted]-013-DSD.
[e] N=4 due to contaminated blood sample.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; Capmul MCM = mono-diglyceride of medium chain fatty acids; CSF = cerebral-spinal fluid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; LLOQ = lower limit of quantification; M = male; N = number; PEG = polyethylene glycol; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.9　A　薬物動態試験：*In vivo* における代謝

Test Article: JNJ-56021927

Study No.			DR-[redacted]-007-DSD	
Location in CTD:			4.2.2.4.1	
Species:			**Sprague-Dawley Rat**	**Beagle Dog**
Gender /Number of Animals:			M:4	M:2
Feeding Condition:			Fed	Fed
Dose (mg/kg/day):			150	10
Vehicle/Formulation:			Solution[a]	Solution[a]
Route:			Oral gavage	Oral capsule
Assay:			LC-MS/MS	LC-MS/MS
Sample:			Plasma[b]	Plasma[c]
Metabolite		Retention Time (min)	% Peak Area[d]	
M1, di-amide	ARN-925[e]	18.6	>0.5	Detectable, but <0.5
M2, hydantoin	JNJ-56142047 (ARN-273)	24.8	>10	>0.5
M3, *N*-desmthyl	JNJ-56142060 (ARN-308)	26.8	>0.5	>25
M4, amide hydrolysis	JNJ-56142021 (ARN-066)	30.6	>5	>10

[a] Vehicle: Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, VitE-TPGS NF (10:45:30:15%w/w/w/w)
[b] Rat plasma obtained from the Day 14, 8-12 hour postdose sample on Day 14 of the nonGLP toxicity study in male rats administered 150 mg/kg/day apalutamide (Mod4.2.3.2.1)
[c] Dog plasma obtained from the Day 21, 8-12 hour postdose sample of the nonGLP toxicity study in male dogs administered 10 mg/kg/day apalutamide (Mod4.2.3.2.7)
[d] Calculated as a percentage of the total normalized signal for all metabolites plus apalutamide.
[e] No JNJ number assigned.

Acconon MC8-2 = caprylocaproyl macrogolglycerides; Capmul MCM = mono-diglyceride of medium chain fatty acids; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M = male; M2 = oxidative desulfuration metabolite of apalutamide, JNJ 56142047; M3 = The *N*-desmethyl metabolite of apalutamide, JNJ-56142060; M4 = the metabolite formed by amide hydrolysis of apalutamide or M3, JNJ-56142021; PEG = polyethylene glycol; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.9 B 薬物動態試験：*In vivo* における代謝

Test Article: JNJ-56021927

Study No.	FQA0001 Urinary, Fecal, Biliary, and Plasma Metabolites after Single-Dose administration of ^{14}C-labeled apalutamide
Location in CTD	4.2.2.4.2
Species	**Sprague-Dawley Rat (albino)**
Gender (M/F)/Number of Animals	M:3 (Phase C), M:5 (Phase D), M:6 (Phase E)
Feeding Condition	Fed
Vehicle/Formulation	solution[a]/vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)
Route	Oral gavage
Dose (mg/kg)	50
Radionuclide	[^{14}C]JNJ-56021927
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)
Analytical method	HPLC

Component		Retention Time (min)	Phase C – Excretion/Balance Uncannulated (% of radioactive components)			Phase D – Biliary Excretion Bile-duct Cannulated (% of radioactive components)				Phase E Blood sample[b] (% radioactive components)	
			Urine		Feces	Bile		Urine	Feces	Plasma	
			0 - 24h	24 - 48h	0 - 48h	0 - 24h	24 - 48h	0 - 48h	0 - 48h	12h	24h
M1[c] M9[d]	(RM4) (RM4+m/z582)	11	2[e]	3[e]	4	7[f]	12[f]	3[e]	1	1	12
M2[g]	(RM7)	17.5	2	2	4	1	BQL	BQL	5	8	BQL
M3[h]	(RM8)	19.5	BQL	BQL	1	BQL	BQL	2	3	3	5
M4[i]	(RM11)	24	81	77	49	48	39	79	8	12	15
M5[j]	(RM9)	21	8	9	15	1	BQL	BQL	2	1	BQL
M17[k]	(RM5)	13	2	8	3	6	6	7	BQL	BQL	2
UD[l]	(RM10)	22	BQL	BQL	14	22	20	4	72	65	53

Additional information: This is the only report in which the metabolites are referred to by their "RM" number. In later reports, the metabolite number (eg, "M4") and/or a compound number is referenced therefore, for consistency, both are listed. Eleven components were measured and reported in study report. Only the metabolites that have been identified are tabulated and so totals are <100%.

Footnotes and abbreviations on following page.

2.6.5.9 B 薬物動態試験：*In vivo* における代謝（続き）

Test Article: JNJ-56021927

Study No.	FQA0001
	Urinary, Fecal, Biliary, and Plasma Metabolites after Single-Dose administration of ^{14}C-labeled apalutamide

Footnotes and abbreviations

[a] Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, VitE TPGS NF (10:45:30:15%w/w)
[b] Terminal blood sample collected from 3 rats/time interval postdose prior to preparation for quantitative whole body autoradiography imaging procedures.
[c] ARN-925
[d] Co-elutes with M1 (ARN-925); cysteine condensate of apalutamide.
[e] Ratio of ARN-925 (M1) to cysteine condensate of apalutamide , calculated from peak area to be 22:1 in urine.
[f] Ratio of ARN-925 (M1) to cysteine condensate of apalutamide , calculated from peak area to be 3:1 in bile.
[g] JNJ-56142047, a.k.a. ARN-273
[h] JNJ-56142060, a.k.a. ARN-308
[i] JNJ-56142021, a.k.a. ARN-066
[j] JNJ-56142099, a.k.a. ARN1908
[k] Identified in study report as RM5 – the cysteine condensate of M4 (JNJ-56142021)
[l] unchanged drug, JNJ-56141927 (apalutamide) a.k.a. ARN-509; identified in study report as RM10

Acconon MC8-2 = caprylocaproyl macrogolglycerides; a.k.a. = also known as; BQL = below quantification limit; HPLC = high-performance liquid chromatography; M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; UD = unchanged drug, apalutamide; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.9　C　薬物動態試験：*In vivo* における代謝

Test Article: JNJ-56021927

Location in CTD	4.2.2.4.3		5.3.1.1.1	
	Plasma Metabolites after Single-Dose Administration of ^{14}C-labeled apalutamide			
Study No.	FK10704		ARN-509-006	
Species	Beagle Dog		Human	
Assay	LSC or LC-MS/MS		LC-MS/MS or HPLC followed by AMS	
Vehicle/Formulation	Solution[b]/Capsule		Solution[b]/Capsule	
Route	Oral		Oral	
Gender/Number of Animals	M: 4		M: 6	
Dose	10 mg/kg		240 mg	
Specific Activity	2.37 µCi/mg (0.0877 MBq/mg)		1000 nCi (37 kBq)	
	$AUC_{0\text{-}528h}$ (µg (eq.)·h/mL)[a]	Percentage of TR	$AUC_{0\text{-}t}$ (µg·h/mL)[a]	Percentage of TR
Component				
Total radioactivity	632.3	-	413	-
UD	260.2	41	175	42
M3	322.2	51	169	41
M4	22.6	4	11.1	2.7

[a] AUCs are reported as arithmetic means are reported for the dog (Study FK10704) and as geometric means for the human data (Clinical Study ARN-509-006).
[b] Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, VitE TPGS NF (10:45:30:15%w/w)

- = not calculated; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AUC = area under the plasma time-concentration curve; $AUC_{0\text{-}528h}$ = AUC from time 0 to 528 hours postdose; $AUC_{0\text{-}672h}$ = AUC from time 0 to 672 hours postdose; AMS = accelerated mass spectrometry; Capmul MCM = mono-diglyceride of medium chain fatty acids; HPLC = high-performance liquid chromatography; PEG = polyethylene glycol; TR = total radioactivity; UD = unchanged drug; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.9 D 薬物動態試験：*In vivo* における代謝

Test Article: JNJ-56021927

Location in CTD	4.2.2.4.2, 4.2.2.4.3, 4.2.2.4.4 Urinary and Fecal Metabolites after Single-Dose administration of ^{14}C-labeled apalutamide					
Study No.	FQA0001 (Phase C)		FK10704		FK10840	
Species	**Sprague-Dawley Rat**		**Beagle Dog**		**Human**[a]	
Assay	HPLC		HPLC		HPLC followed by AMS	
Vehicle/Formulation	Solution[b]/Gavage		Solution[b]/Capsule		Solution[b]/Capsule	
Route	Oral		Oral		Oral	
Gender/Number of Animals	M: 3		M: 4		M: 6	
Dose	50 mg/kg		10 mg/kg		240 mg	
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)		2.37 µCi/mg (0.0877 MBq/mg)		1000 nCi (37 kBq)	
	Mass Balance of ^{14}C-labeled apalutamide and its Metabolites (% of administered dose)					
	Urine (0-48h)	Feces (0-48h)	Urine (0-360h)	Feces (0-360h)	Urine (0-1680 h)	Feces (0-1680h)
Component						
UD[c]	BQL	7.56	2.66	4.73	1.20	1.54
Metabolites						
M1	-[d]	-[d]	-	-	-	-
M2[e]	0.66	2.16	0.19	0.14	0.23	-
M3[f]	BQL	0.54	3.58	2.56	2.73	1.97
M4[g]	26.31	26.46	15.81	18.58	31.12	2.38
M5[h]	2.75	8.10	0.16	0.06	-	-
M6	-	-	0.34	0.71	-	-
M7	-	-	0.29	0.22	-	-
M8	-	-	0.02	-	0.90	0.39
M9	**0.77**	**2.16**	**0.29**[i]	**2.42**[i]	0.50	3.49[j]
M10	-	-			trace	-
M11	-	-	-	-	1.11	-

Additional Information: For some metabolites, the result is reported as the sum of 2 or more metabolites because their peaks almost co-elute and cannot be distinguished. These are listed in **bold**.

[a] Human samples from Clinical Study Mod5.3.1.1.1/ARN-509-006.
[b] Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, VitE-TPGS NF (10:45:30:15 %w/w/w/w)
[c] apalutamide; JNJ-56021927 or ARN-509
[d] Amount added to M9 because peaks almost co-elute and cannot be distinguished, see note in Additional Information.
[e] JNJ-56142047 or ARN-273
[f] JNJ-56142060 or ARN-308
[g] JNJ-56142021 or ARN-066
[h] JNJ-56142099 or ARN1908
[i] M9 + M10, see note in Additional Information.
[j] M9 + M16, see note in Additional Information.

Continued, abbreviations on next page

2.6.5.9 D 薬物動態試験：*In vivo* における代謝（続き）

Test Article: JNJ-56021927

Location in CTD	4.2.2.4.2, 4.2.2.4.3, 4.2.2.4.4 Urinary and Fecal Metabolites after Single-Dose administration of ^{14}C-labeled apalutamide					
Study No.	FQA0001 (Phase C)		FK10704		FK10840	
Species	**Sprague-Dawley Rat**		**Beagle Dog**		**Human**[a]	
Assay	HPLC		HPLC		HPLC followed by AMS	
Vehicle/Formulation	Solution[b]/Gavage		Solution[b]/Capsule		Solution[b]/Capsule	
Route	Oral		Oral		Oral	
Gender/Number of Animals	M: 3		M: 4		M: 6	
Dose	50 mg/kg		10 mg/kg		240 mg	
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)		2.37 µCi/mg (0.0877 MBq/mg)		1000 nCi (37 kBq)	
	Mass Balance of ^{14}C-labeled apalutamide and its Metabolites (% of administered dose)					
	Urine (0-48h)	Feces (0-48h)	Urine (0-360h)	Feces (0-360h)	Urine (0-1680 h)	Feces (0-1680h)
Metabolites						
M12	-	-	-	2.08	**4.73**[d]	-
M13+M14	-	-	**0.59**[c]	**1.03**[c]		**4.67**[d]
M15	-	-	0.93	0.13	1.54	trace
M16	-	-	**0.41**[e]	**1.98**[e]	-	-[f]
M17	-	-			trace	trace
M18	-	-	0.04	0.22	0.27	0.90
M19	-	-	2.83	0.71	5.31	0.41
M20	-	-	6.06	0.61	6.85	0.29
M21	-	-	1.83	-	2.44	-
M22	-	-	1.65	-		
Sum of observed metabolites	31.79	48.60	38.3	37.2	64.2	24.1

Additional Information: For some metabolites the result is reported as the sum of 2 or more metabolites because their peaks almost co-elute and cannot be distinguished. These are listed in **bold**.

[a] Human samples from Clinical Study Mod5.3.1.1.1/ARN-509-006.
[b] Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, VitE-TPGS NF (10:45:30:15 %w/w/w/w)
[c] M13 + M14, see note in Additional Information.
[d] M12 + M13 + M14, see note in Additional Information.
[e] M16 + M17, see note in Additional Information.
[f] Amount added to M9 because peaks almost co-elute and cannot be distinguished, see note in Additional Information.

- = not detected; Acconon MC8-2 = caprylocaproyl macrogolglycerides; AMS = accelerated mass spectrometry; BQL = below quantification limit (<0.5%); HPLC = high-performance liquid chromatography; M = male; Capmul MCM = mono-diglyceride of medium chain fatty acids; PEG = polyethylene glycol; UD = unchanged drug, apalutamide; VitE-TPGS = water-soluble Vitamin E (D-α-Tocopherol polyethylene glycol succinate conjugate)

2.6.5.10　A　薬物動態試験：*In vitro* における代謝

Test Article: JNJ-56021927

Species	**Rat, Dog, Human**
Study No.	DR-[redacted]-007-DSD
Location in CTD:	4.2.2.4.1
Study Type	Quantitative Metabolic Stability in Liver Microsomes (rat, dog) or S9 Fractions (rat, dog, human)
Test System	Cyropreserved liver microsomes (0.5 mg/mL protein) in 3 incubation buffers[a] or boiled liver microsomes in the NADPH only buffer
Vehicle/Formulation	0.1% DMSO plus 0.5% acetonitrile/solution
Dose	10 µM JNJ-56021927
Sample	120-minute liver microsome incubates
Assay	LC-MS/MS

Apalutamide Half-life (SD) in Liver Microsomes (minutes)

Incubation Condition	Sprague-Dawley Rat (male)	Beagle Dog (male)
NADPH only	627 (26)	>780
NADPH + UDPGA	599 (51)	745 (35)
NADPH + GSH	587 (37)	699 (68)
Boiled + NADPH	>780	>780

[a] All buffers contained: 100 mM KH_2PO_4 buffer (pH 7.4), 3 mM $MgCl_2$, 1mM EDTA, 5 mM glucose-6-phosphate, 1 U/mL G6PD, 25 µg/mg protein alamethicin, 1mM NADP. In addition, Liver microsomes contained either only NADPH; NADPH plus 5 mM UDPGA and 1.3 mM D-saccharic acid 1,4 lactone; or 10 mM L-glutathione.

Continued, abbreviations on next page

2.6.5.10　A　薬物動態試験：*In vitro* における代謝（続き）

Test Article: JNJ-56021927

Species	**Rat, Dog, Human**
Study No.	DR-[redacted]-007-DSD
Location in CTD:	4.2.2.4.1
Test System	S9 fractions (1.0 mg/mL protein) in 3 incubation buffers[a] or boiled S9 fractions in the NADPH only buffer
Vehicle/Formulation	0.1% DMSO plus 0.5% acetonitrile/solution
Dose	10 µM JNJ-56021927
Sample	80-minute S9 fraction incubates
Assay	LC-MS/MS

Apalutamide Half-life in S9 Fractions (minutes)

Incubation Condition	Sprague-Dawley Rat (male)	Beagle Dog (male)	Human (male/female)
NADPH only	>520	>520	466
NADPH + GSH	>520	>520	420
NADPH + PAPS	>520	>520	>520
Boiled + NADPH	>520	>520	>520

[a] All buffers contained: 100 mM KH_2PO_4 buffer (pH 7.4), 3 mM $MgCl_2$, 1mM EDTA, 5 mM glucose-6-phosphate, 1 U/mL G6PD, 25 µg/mg protein alamethicin, 1 mM NADP. In addition, S9 fractions contained either only NADPH; NADPH plus 0.8 mM PAPS; or NADPH plus 10 mM GSH

DMSO = dimethyl sulfoxide; G6PD = glucose-6-phosphate dehydrogenase; GSH = reduced L-glutathione; KH_2PO_4 = potassium phosphate; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NADP = β-nicotinamide adenine dinucleotide phosphate; NADPH = β-nicotinamide adenine dinucleotide phosphate sodium salt hydrate; PAPS = adenosine 3'phosphate 5'phosphosulfate; S9 = liver homogenate containing cytosol and microsomes

Continued

2.6.5.10　A　薬物動態試験：*In vitro* における代謝（続き）

Test Article: JNJ-56021927

Species			**Rat, Dog, Human**		
Study No.			DR-[redacted]-007-DSD		
Location in CTD:			4.2.2.4.1		
Study Type			Qualitative Metabolic Profiling in Liver Microsomes (rat, dog, and human)		
Test System:			Cyropreserved liver microsomes (1.0 mg/mL protein) in incubation buffer[a]		
Vehicle/Formulation			0.5% DMSO/solution		
Dose			30 µM JNJ-56021927		
Sample			6-hour liver microsome incubates		
Assay			LC-MS/MS		
			% Peak Area[b]		
Metabolite		Retention Time (min)	Sprague-Dawley Rat (male)	Beagle Dog (male)	Human (male)
M1, di-amide	ARN-925[c]	18.6	detectible, but <0.5%	detectible, but <0.5%	detectible, but <0.5%
M2, hydantoin	JNJ-56142047 (ARN-273)	24.8	detectible, but <0.5%	detectible, but <0.5%	detectible, but <0.5%
M3, *N*-desmthyl	JNJ-56142060 (ARN-308)	26.8	detectible, but <0.5%	>0.5%	detectible, but <0.5%
M4, amide hydrolysis	JNJ-56142021 (ARN-066)	30.6	>10%	>0.5%	>0.5%

Additional Information: Liver microsomes (final protein concentration, 1.0 mg/mL) were incubated with 30 µM apalutamide at 37°C for 0, 2, 4, and 6 hours in triplicate. At the end of the 6-hour incubation, the solutions were centrifuged, the protein was removed, and the supernatant was assayed for the presence of UD and various metabolites.

[a] Incubation buffer: 100 mM KH_2PO_4 buffer (pH 7.4), 3 mM $MgCl_2$, 1mM EDTA, 5 mM glucose-5-phosphate, 1 U/mL G5PD, 25 µg/mg protein alamethicin, 1mM NADP with and without 5 mM UDPGA

[b] Calculated as a percentage of the total normalized signal for all metabolites plus apalutamide.

[c] No JNJ number assigned.

DMSO = dimethyl sulfoxide; EDTA = ethylenediaminetetraacetic acid; G6PD = glucose-6-phosphate dehydrogenase; GSH = reduced L-glutathione; KH_2PO_4 = potassium phosphate; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M2 = oxidative desulfuration metabolite of apalutamide, JNJ 56142047; M3 = the *N*-desmethyl metabolite of apalutamide, JNJ-56142060; M4 = the metabolite formed by amide hydrolysis of apalutamide or M3, JNJ-56142021; NADP = β-nicotinamide adenine dinucleotide phosphate; NADPH = β-nicotinamide adenine dinucleotide phosphate sodium salt hydrate; PAPS = adenosine 3'phosphate 5'phosphosulfate; SD = standard deviation; UD = unchanged drug; UDPGA = uridine 5′-diphosphoglucuronic acid triammonium salt

2.6.5.10　B　薬物動態試験：*In vitro* における代謝

Test Article: JNJ-56021927

Species	**Human**
Study No.	FK10596
Location in CTD	4.2.2.4.5
Study System	Human cryopreserved hepatocytes (20-donor pool, male/female)
Dose	1 and 5 µM JNJ-56021927[a]
Assay	LC-MS/MS
Analytes	M3 (JNJ-56142060) and M4 (JNJ-56142021)

Purpose and Methods: The purpose of this study was to measure the formation of the *N*-desmethyl and ester hydrolysis metabolites of apalutamide (M3 and M4, respectively). The relay method was used because apalutamide is a low clearance molecule and successive incubations with human hepatocytes enabled quantitation of metabolite formation. Thawed hepatocyte suspensions (0.5x106 cells/mL) were spiked with apalutamide in the presence or absence of CYP and esterase inhibitors and incubated at 37°C for 4 hours. After 4 hours (Relay 1) a 100 µL aliquot of the supernatant was removed for analysis of metabolite formation, new hepatocytes were introduced, and incubation continued for an additional 4 hours (Relay 2). The process was repeated 2 more times and at the end of Relay 4 (16 hours) a final analysis was performed. Data presented are for Relay 4, following 16 hours of incubation.

		Effect of CYP Inhibitors on Apalutamide Metabolism		
Inhibitor (Concentration)	Compound Inhibited		% Inhibition of Metabolite Formation after Relay 4 (16-hour incubation)	
		N[b]	M3 (JNJ-56142060)	M4 (JNJ-56142021)
Vehicle - CH_3OH (0.1%)	-	6	0	0
Itaconazole (1 µM)	CYP3A4	2	17	11
Gemfibrozil (30 µM)[c]	CYP2C8	3	55	4.8
Troleandomycin (100 µM)	CYP3A4	1	23	0
ABT (1000 µM)	Pan CYP	1	98	18
BNPP (25 µM)	Carboxyl esterase	2	42	98
BNPP+ABT (25 µM, 1000 µM, respectively)	Carboxyl esterase + Pan CYP	4	100	100

Additional Information: Both enzymatic activity and cytotoxicity were compared with the enzymatic activity and cytotoxicity of a satellite experiment in which hepatocytes were incubated under the same conditions for 4 hours with the inhibitors alone. Cytotoxicity was increased only in the presence of (BNPP + ABT). None was seen with any of the other inhibitors or with ABT or BNPP alone.

[a] Data are the mean of the results of the 2 concnetrations (1 and 5 µM JNJ-56021927)

[b] Number of wells sampled.

[c] Additionally, 10.5 µM gemfibrozil glucuronide added during Relay 1.

ABT = aminobenzotriazole; BNPP = bis nitrophenol phosphate; CH_3OH = methanol; CYP = cytochrome P450; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M2 = oxidative desulfuration metabolite of apalutamide, JNJ-56142047; M3 = the *N*-desmethyl metabolite of apalutamide, JNJ-56142060; M4 =the metabolite formed by amide hydrolysis of apalutamide or M3

2.6.5.10　C　薬物動態試験：*In vitro* における代謝

Test Article: M3 (JNJ-56142060)

Species	**Human**
Study No.	FK10924
Location in CTD	4.2.2.4.6
Study System:	Human cryopreserved hepatocytes (20-donor pool, male/female)
Assay:	LC-MS/MS
Analyte:	M4 (JNJ-56142021)

Purpose and Methods: The purpose of this study was to measure the clearance of the M3 metabolite by investigating the formation of M4 and the influence of esterases on that formation. The relay method was used because apalutamide is a low clearance molecule and successive incubations with human hepatocytes enabled quantitation of metabolite formation. Thawed hepatocyte suspensions ($0.5x10^6$ cells/mL) were spiked with M3 in the presence or absence of CYP and esterase inhibitors and incubated at 37°C for 4 hours. After 4 hours (Relay 1) a 100 µL aliquot of the supernatant was removed for analysis of metabolite formation, new hepatocytes were introduced, and incubation continued for an additional 4 hours (Relay 2). This process was repeated 2 more times and at the end of Relay 4 (16 hours).

		Effect of CYP Inhibitors on M3 (JNJ-56142060) Metabolism
Inhibitor (Concentration)	Compound Inhibited	% Inhibition of Metabolite Formation after 4 Relays (16 hours)
		M4 (JNJ-56142021)
Vehicle - CH_3OH (0.1%)	-	0
ABT (1000 µM)	Pan CYP	22
BNPP (25 µM)	Carboxyl esterase	100
BNPP+ABT (25 µM, 1000 µM, respectively)	Carboxyl esterase + Pan CYP	100

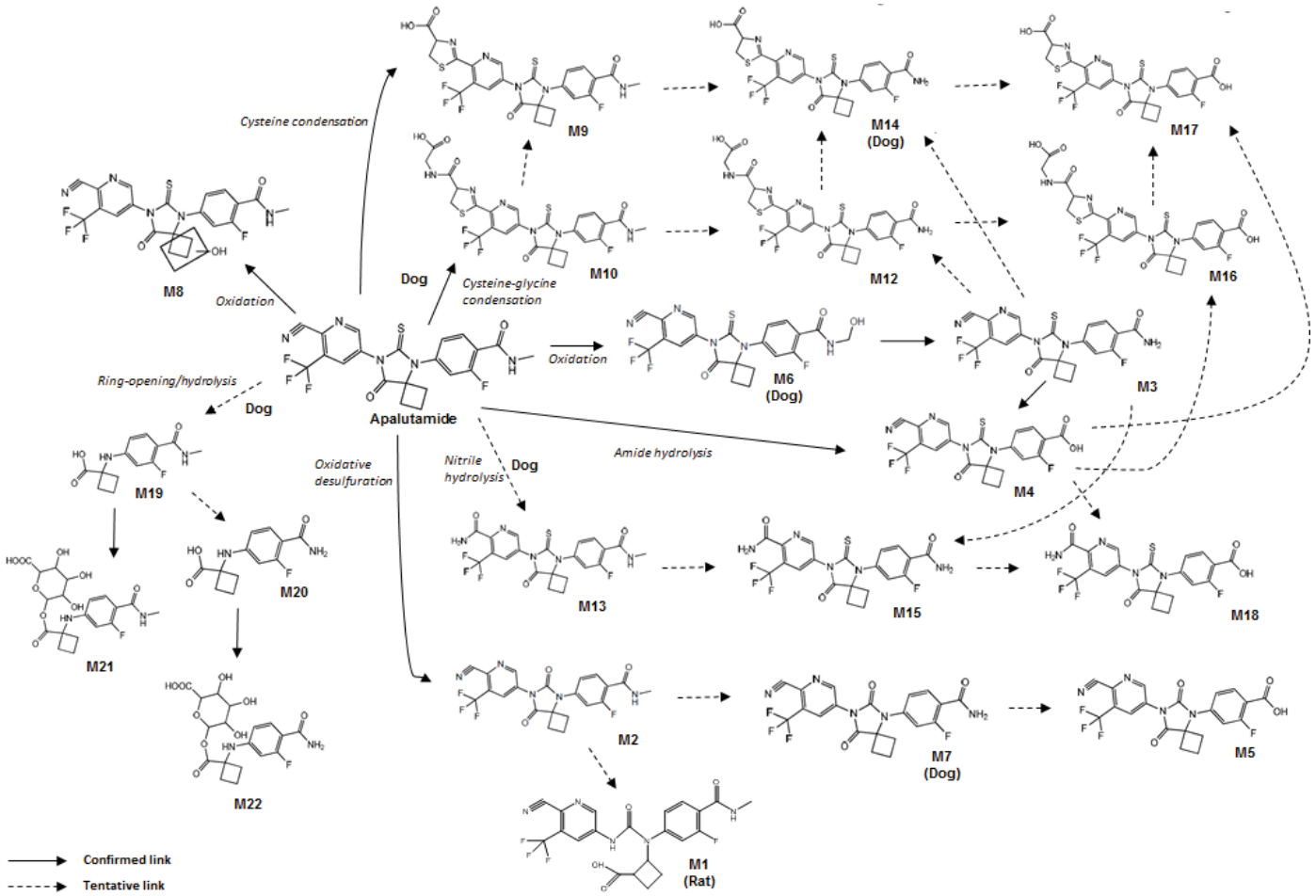
Additional Information: Both enzymatic activity and cytotoxicity were compared with the enzymatic activity and cytotoxicity of a satellite experiment in which hepatocytes were incubated under the same conditions for 4 hours with the inhibitors alone. Cytotoxicity was increased in the presence of ABT and (BNPP + ABT). None was seen with any of the other inhibitors or with BNPP alone.

ABT = aminobenzotriazole; BNPP = bis nitrophenol phosphate; CH_3OH = methanol; CYP = cytochrome P450; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M2 = oxidative desulfuration metabolite of apalutamide, JNJ-56142047; M3 = the *N*-desmethyl metabolite of apalutamide, JNJ-56142060; M4 = the metabolite formed by amide hydrolysis of M3, JNJ-56142021.

2.6.5.11　A　薬物動態試験：推定代謝経路（ラット，イヌ）

Test Article: JNJ-56021927

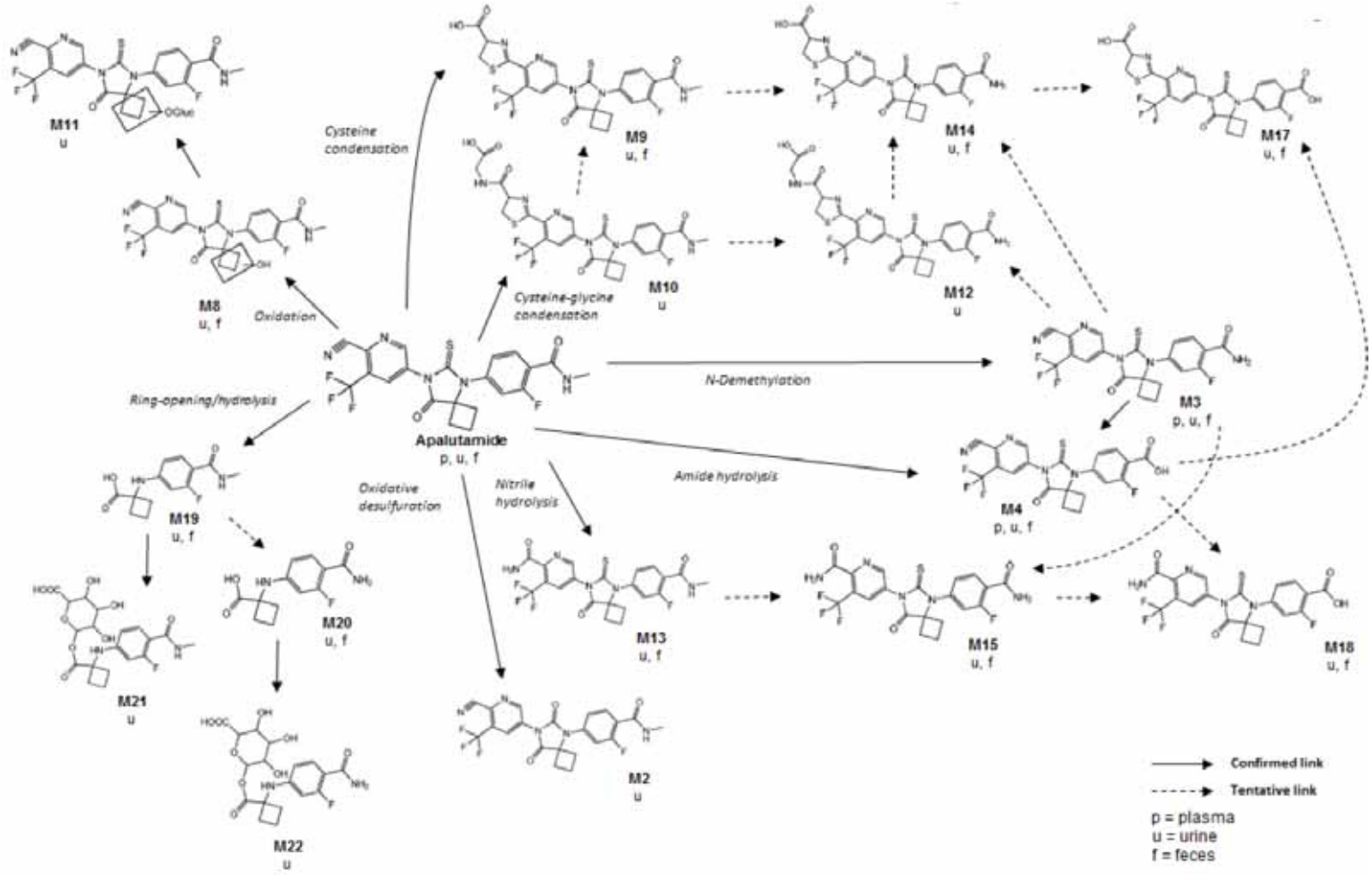
Study No. FQA0001 (Rat); FK10704 (Dog)



Note: Species-specific pathways and metabolites are labeled as rat or dog

2.6.5.11　B　薬物動態試験：推定代謝経路（ヒト）

Test Article: JNJ-56021927 **Study No.** FK10840



2.6.5.12　A　薬物動態試験：薬物代謝酵素の誘導／阻害：*In vitro* 試験

Test Article: JNJ-56021927

Study No.	301091248
Location in CTD	4.2.2.4.7
Type of Study:	Induction of CYPs in Cryopreserved Human Hepatocytes *in vitro*

Method: Cryopreserved human hepatocytes from 3 donors (not pooled) were treated with apalutamide, its *N*-desmethyl metabolite M3 (JNJ-56142060), and a positive control as separate preparations. Omeprazole, phenobarbital, and rifampicin were used as positive controls for induction of CYP1A2, CYP2B6, and CYP3A4, respectively. The cells were incubated for 2 days and the medium, containing the test article or positive control, was changed daily. After incubation, the supernatant was assayed for the appropriate CYP-mediate metabolite. Data are expressed as % induction relative to the positive control and the mean fold change of the treated samples relative to the vehicle control.

Tabulated Results

	% of Positive Control[a]						**Fold Induction[a]**					
	Apalutamide (JNJ-56021927)			M3 (JNJ-56142060)			Apalutamide (JNJ-56021927)			M3 (JNJ-56142060)		
Concentration (μM)	3	10	30	3	10	30	3	10	30	3	10	30
CYP1A2	3	10	5	<1	4	<1	1.1	1.5	1.2	1.0	1.2	1.0
	<1	<1	1	2	<1	<1	0.92	1.0	1.1	1.1	1.1	1.0
	<1	<1	1	2	<1	<1	0.91	1.0	1.1	1.1	1.1	1.0
CYP2B6	56	76	117	33	66	88	3.5	4.3	6.1	2.4	3.9	4.9
	59	72	82	25	17	30	4.1	4.7	5.2	2.3	1.9	2.5
	46	61	82	25	27	43	4.3	5.4	6.9	2.8	3.0	4.1
CYP3A4	77	123	137	21	65	65	19	30	33	6.0	16	16
	58	90	97	24	58	66	14	21	23	6.5	14	16
	50	85	91	15	57	72	10	16	17	3.6	11	14

Additional Information:

Substrate (Concentration)	Positive Control (Concentration)
CYP1A2 phenacetin (100 μM)	omeprazole (50 μM)
CYP2B6 bupropion (250 μM)	phenobarbital (1 mM)
CYP3A4 testosterone (200 μM)	rifampicin (10 μM)

[a] Data are expressed as individual values for each donor (N=3 donors/concentration/inhibitor).

CYP = cytochrome P450; M3 = the *N*-desmethyl metabolite of apalutamide.

2.6.5.12　B　薬物動態試験：薬物代謝酵素の誘導／阻害：*In vitro* 試験

Test Article: JNJ-56021927

Study No.	FK10955
Location in CTD	4.2.2.4.8
Type of Study:	Induction of CYP1A2 in Cryopreserved Human Hepatocytes *in vitro*

Method: Cryopreserved human hepatocytes from 3 donors (not pooled) were treated with 4 different apalutamide or M3 concentrations ranging from 3 to 50 µM.

Tabulated Results

	Mean % of Positive Control[a]								**Mean Fold Induction[a]**							
	Apalutamide (JNJ-56021927)				M3 (JNJ-56142060)				Apalutamide (JNJ-56021927)				M3 (JNJ-56142060)			
Concentration (µM)	3	10	30	50	3	10	30	50	3	10	30	50	3	10	30	50
CYP marker substrate																
CYP1A2 (50 µM)	0.0	0.0	0.0	0.0	-0.1	-0.1	0.0	0.0	1.0	1.0	1.0	1.0	0.7	0.6	1.0	1.0
	-0.1	0.0	0.0	0.1	-0.1	-0.1	0.0	0.2	0.8	0.9	1.0	1.4	0.6	0.7	1.1	1.7
	0.0	0.0	0.0	0.0	-0.1	-0.1	0.0	0.2[b]	0.9	1.1	0.9	1.2	0.7	0.7	1.1	1.8[b]

Additional Information: All substances were prepared in 0.1% DMSO.

[a] Individual data expressed as the mean of triplicate samples for each donor (N=3 samples/donor/concentration).
[b] Toxicity evident in samples from 2 donors.

CYP = cytochrome P450; M3 = the *N*-desmethyl metabolite of apalutamide.

2.6.5.12 C 薬物動態試験：薬物代謝酵素の誘導／阻害：*Ex vivo* 試験

Test Article: JNJ-56021927

Study No.	TX-509-1001
Location in CTD	4.2.3.2.3
Type of Study:	Evaluation of *Ex vivo* CYP Enzyme Induction in Male Sprague-Dawley Rats

Method: Total CYP content; CYP activity; and testosterone metabolism were evaluated in hepatic microsomes prepared from livers of male Sprague-Dawley rats orally administered apalutamide 0, 25, 50, or 100 mg/kg/day for 13 weeks followed by a 30-day recovery period. Tissue was obtained from the first 5 animals necropsied in the Main Study groups and all 5 animals in the recovery control and high dose group the animals in the in vivo portion of the study. Apalutamide-naïve Sprague-Dawley rat hepatic microsomes were incubated in parallel with classic inducers to serve as positive controls. Microsomal pellets were suspended in Na_2HPO_4 buffer. After a 2-min incubation, reactions were initiated by addition of a cofactor. Activity was determined by fluorescence HPLC for ethoxyresorufin and pentoxyresorufin, radio and UV-HPLC for [^{14}C]testosterone and Radio-HPLC for [^{14}C]lauric acid.[a]

Total CYP Content

Mean (SD) CYP Content (nmol P450/mg protein)

Apalutamide Dose (mg/kg/day)	0	25	50	100
N	10	5	5	10
CYP Content	0.902 (0.115)	0.838 (0.142)	0.816 (0.128)	0.793 (0.084)
Fold Induction	-	NA	NA	NA

CYP Activity

				Mean (SD) Rate of Product Formation (pmol/mg/min)				Mean Fold Induction[b]			
Apalutamide Dose (mg/kg/day)				0	25	50	100	0	25	50	100
N				10	5	5	10	10	5	5	10
CYP	Substrate	Enzyme	Product								
1A	ethoxyresorufin	ethoxyresorufin O-dealkylase	resorufin	52.29 (20.8)	116.7 (33.9)*	107.4 (32.6)*	111.6 (69.7)*	-	2.2	2.1	2.1
2B	pentoxyresorufin	pentoxyresorufin O-dealkylase	resorufin	6.387 (1.96)	7.738 (1.70)	7.764 (1.17)	5.988 (1.59)	-	1.2	1.2	NA
3A	[^{14}C]testosterone	[^{14}C]testosterone 6β-hydroxylase	6β-OH-testosterone	866 (178)	1129 (375)	1160 (395)	994 (302)	-	1.3	1.3	1.1
2B	[^{14}C]testosterone	[^{14}C]testosterone 2α- hydroxylase	2α-OH-testosterone	2110 (486)	1847 (329)	1614 (1168)	1612 (841)	-	NA	NA	NA
4A	[^{14}C]lauric acid	[^{14}C]lauric acid 12-hydroxylase	12-OH lauric acid	947 (181)	1389 (147)*	1159 (287)	1046 (347)	-	1.5	1.2	1.1

Total Testosterone Metabolism

	Mean (SD) Rate of Metabolite Formation (pmol/mg/min)				Mean Fold Induction[b]			
Apalutamide Dose (mg/kg/day)	0	25	50	100	0	25	50	100
N	10	5	5	10	10	5	5	10
[^{14}C]testosterone	7035 (1242)	7687 (1106)	8179 (3092)	7450 (1255)	-	1.1	1.2	1.1

* Student's t-test, p-value ≤0.05

[a] *Ex vivo* portion of study conducted at [redacted] in [redacted], UK. For complete assay conditions see Appendix 17, Table 1.

[b] Compared to apalutamide vehicle control.

- = not applicable/not evaluated; CYP = cytochrome P450; HPLC = high-performance liquid chromatography; N = number; NA = not applicable - no increase as group mean was below control mean; Na_2HPO_4 = sodium phosphate; radio-HPLC = HPLC coupled with radioactivity detection; SD standard deviation; UV = ultraviolet; UV-HPLC = HPLC coupled with UV light absorption

Continued

2.6.5.12　C　薬物動態試験：薬物代謝酵素の誘導／阻害：*Ex vivo* 試験（続き）

Test Article: JNJ-56021927

Study No.	TX-509-1001
Location in CTD	4.2.3.2.3
Type of Study:	Evaluation of *Ex vivo* CYP Enzyme Induction in Male Sprague-Dawley Rats

Apalutamide-Naïve Rats – Positive Control Data

Mean Rate of Metabolite Formation (pmol/mg/min)

CYP	Inducer	No Pretreatment	Pretreated	Fold Induction
1A	β-napthoflavone	86.01	1150[a]	13.4
2B	phenobarbital	9.570	106.7[a]	11.1
3A	dexamethasone	1069[b]	7069	6.6
2C	phenobarbital	0	2130[c]	NC
4A	clofibric acid	1393	2880	2.1

[a] Approximate value derived from extrapolated data.
[b] Value from one determination.
[c] Value from a single sample as insufficient sample to repeat analysis.

CYP = cytochrome P450; NC = fold induction cannot be calculated as no formation in control animals

2.6.5.12 D 薬物動態試験：薬物代謝酵素の誘導／阻害：*Ex vivo* 試験

Test Article: JNJ-56021927

Study No.	TX-509-1002
Location in CTD	4.2.3.2.10
Type of Study:	Evaluation of *Ex vivo* CYP Enzyme Induction in Male Beagle Dogs

Method: Total CYP content; CYP activity; and testosterone metabolism were evaluated in hepatic microsomes repared from male Beagle dogs orally administered apalutamide 0, 2.5, 5, or 10 mg/kg/day for 13 weeks followed by a 60-day recovery period. Tissue was obtained from all the animals in the in vivo portion of the study. Apalutamide naïve Beagle dog hepatic microsomes were incubated in parallel with classic inducers to serve as positive controls. Microsomal pellets were suspended in Na_2HPO_4 buffer. After a 2-min incubation, reactions were initiated by addition of a cofactor. Activity was determined by fluorescence HPLC for ethoxyresorufin and pentoxyresorufin, radio and UV-HPLC for [^{14}C]testosterone and Radio-HPLC for [^{14}C]lauric acid.[a]

Total CYP Content

Mean (SD) CYP Content (nmol P450/mg protein)				
Apalutamide Dose (mg/kg/day)	0	2.5	5.0	10
N	8	5	8	8
CYP Content	0.451 (0.049)	0.507 (0.184)	0.572 (0.108)*	0.686 (0.171)*
Fold Induction	-	1.1	1.3	1.5

CYP Activity

			Experimental Parameters	Mean (SD) Rate of Product Formation (pmol/mg/min)				Mean Fold Induction[b]			
			Apalutamide Dose (mg/kg/day)	0	2.5	5.0	10	0	2.5	5.0	10
			N	8	5	8	8	8	5	8	8
CYP	Substrate	Enzyme	Product								
1A	ethoxyresorufin	ethoxyresorufin o-deethylase	resorufin	70.20 (23.85)	57.74 (17.12)	61.41 (18.65)	54.45 (13.30)	-	NA	NA	NA
2B	pentoxyresorufin	pentoxyresorufin o-depentyllase	resorufin	16.22 (3.97)	22.86 (4.45)*	26.32 (10.11)*	37.30 (20.42)*	-	1.4	1.6	2.3
3A	[^{14}C]testosterone	[^{14}C]testosterone 6β-hydroxylase	6β-OH-testosterone	531 (114)	698 (378)	633 (292)	653 (186)	-	1.3	1.2	1.2
2B	[^{14}C]testosterone	[^{14}C]testosterone 16β-hydroxylase	16β-OH-testosterone	109 (87)	162 (91)	179 (86)	253 (159)*	-	1.5	1.6	2.3
4A	[^{14}C]lauric acid	[^{14}C]lauric acid 12-hydroxylase	12-OH lauric acid	359 (111)	360 (155)	396 (180)	486 (204)	-	1.0	1.1	1.4

Effect of Treatment on Total Testosterone Metabolism

	Experimental Parameters	Mean (SD) Rate of Metabolite Formation (pmol/mg/min)				Mean Fold Induction[b]			
	Apalutamide Dose (mg/kg/day)	0	2.5	5.0	10	0	2.5	5.0	10
	N	8	5	8	8	8	5	8	8
	[^{14}C]testosterone	2586 (379)	3107 (578)	3138 (497)	3660 (994)*	-	1.2	1.2	1.4

* Student's t-test, p-value ≤0.05

[a] *Ex vivo* portion of the study conducted at [redacted] in [redacted], UK. For complete assay conditions see Appendix 18, Table 1.

[b] Compared to apalutamide vehicle control.

- = not applicable/not evaluated; CYP = cytochrome P450; HPLC = high-performance liquid chromatography; N = number; NA = not applicable - no increase as group mean was below control mean; Na_2HPO_4 = sodium phosphate; OH = hydroxyl; radio-HPLC = HPLC coupled with radioactivity detection; SD standard deviation; UV = ultraviolet; UV-HPLC = HPLC coupled with UV light absorption

Continued

Test Article: JNJ-56021927

Study No.	TX-509-1002[a]
Location in CTD	4.2.3.2.10
Type of Study:	Evaluation of *Ex vivo* CYP Enzyme Induction in Male Beagle Dogs

Apalutamide-Naïve Dogs – Positive Control Data
Mean Rate of Metabolite Formation (pmol/mg/min)

CYP	Inducer	No Pretreatment	Pretreated	Fold Induction
1A	β-napthoflavone	129.5	2680[b]	20.7
2B	phenobarbital[c]	12.74	71.23	5.6
3A	rifampin[d]	403	681	1.7
3A	phenobarbital[d]	185	700	3.8
2B	phenobarbital[e]	0	288	NC
3A	rifampin[f]	1969	2583	1.3
3A/2B	phenobarbital[f]	1874	4878	2.6
4A	clofibric acid	246[g]	372	1.5

* Student's t-test, p-value ≤0.05

[a] Appendix 18 to Study TX-509-1002; identified as Study TX-509-1002, conducted at [redacted] in [redacted], UK. For complete assay conditions see Appendix 18, Table 1.

[b] Value derived from extrapolated data.

[c] Control animals for pentoxyresorufin O-depentylase CYP activity.

[d] Control animals for [^{14}C]testosterone 6 β -hydroxylase CYP activity.

[e] Control animals for [^{14}C]testosterone 16β-hydroxylase CYP activity.

[f] Control animals for testosterone metabolism.

[g] Value from 1 sample; approximate value derived from a pooled sample.

CYP = cytochrome P450; NC = fold induction cannot be calculated as no formation in control animals

2.6.5.12　E　薬物動態試験：薬物代謝酵素の誘導／阻害：*In vitro* 試験

Test Article: JNJ-56021927

Study No.	FK10651
Location in CTD	4.2.2.4.9
Type of Study:	*In vitro* CYP Inhibition in Human Liver Microsomes

Method: Cryopreserved human liver microsomes from a pooled sample of 50 male/female donors were used to assess the ability of apalutamide to inhibit major human CYP isoforms. IC_{50} concentrations for each isoform were determined at a probe concentration at or below the substrate K_m value when incubated at 8 apalutamide concentrations ranging from 0 to 75 µM. Serial dilutions of a known inhibitor of each CYP isoform was included as a positive control. Following a preincubation period, reactions were initiated by adding NADPH, G6P, and G6PD to yield final incubation concentrations of 1.3 mM, 3.3 mM, and 0.4 U/mL, respectively. The microsomes were incubated in the solutions for times ranging from 5 to 25 minutes, depending on the substance. Inhibition was assayed using LC-MS/MS. Inhibition was measured as a percent reduction of probe substrate in the presence and absence of apalutamide.

CYP Isoform	Probe Substrate	Incubation Time (min)	Substrate Concentration (µM)	Microsome Concentration (mg/mL)	Apalutamide IC_{50} (µM)	Maximum Inhibition (%)	Positive Control Inhibitor	Concentration Range (µM)	Control IC_{50} (µM)
1A2	phenacetin	15	80	0.20	-	<5	α-naphthoflavone	0.001 – 1	0.0070
2A6	coumarin	15	2	0.20	-	6	tranylcypromine	0.02 – 20	0.46
2B6	bupropion	15	25	0.20	36	60	ticlopidine	0.005 – 5	0.061
2C8	amodiaquine	15	2	0.10	18	83	quercetin	0.03 – 30	2.6
2C9	tolbutamide	12	100	0.15	>75	48	sulphaphenazole	0.005 – 5	0.27
2C9	diclofenac	12	5	0.15	56	54	sulphaphenazole	0.005 – 5	0.23
2C19	s-(+)-mephenytoin	25	30	0.20	67	56	N-3-benzylPB	0.002 – 2	0.12
2D6	dextromethorphan	12	3	0.15	>75	24	quinidine	0.002 – 2	0.048
2E1	chlorzoxazone	16	45	0.20	-	<5	4-methylpyrazole	0.02 – 20	0.25
3A4	testosterone	12	25	0.15	54	54	ketoconazole	0.002 – 2	0.016
3A4	midazolam	5	2	0.15	>75	15	ketoconazole	0.002 – 2	0.019
3A4	nifedipine	5	10	0.10	>75	21	ketoconazole	0.002 – 2	0.025

Additional Information: All substrates were prepared in solutions of DMSO, methanol, and/or water with final organic solvent concentration ≤0.05%. Positive control inhibitors were prepared in methanol, acetonitrile and/or DMSO with final organic solvent concentration ≤0.07%. All were stored at 4°C until use.

- = no inhibition observed up to 75 µM apalutamide; CYP = cytochrome P450; DMSO = dimethyl sulfoxide; G6P = glucose-6-phosphate; G6PD = glucose-6-phophate dehydrogenase; IC_{50} = concentration producing 50% inhibition; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M3 = the *N*-desmethyl metabolite of apalutamide; NADPH = β-nicotinamide adenine dinucleotide phosphate sodium salt hydrate; N-3-BenzylPB = N-3-Benzylphenobarbital

2.6.5.12　F　薬物動態試験：薬物代謝酵素の誘導／阻害：*In vitro* 試験

Test Article: JNJ-56021927

Study No.	CYP361_R6
Location in CTD	4.2.2.4.10
Type of Study:	*In vitro* CYP Reversible Inhibition and Time-Dependent Assays

Method: For CYP reversible inhibition, cryopreserved human liver microsomes were incubated with 6 concentrations of apalutamide or M3 (0.10, 0.25, 1.0, 2.5, 10, and 25 µM) in DMSO (final concentrations: 0.25 to 0.30%) in the presence of the probe substrate for 5 minutes at 37°C. At the end of incubation, a decrease in the formation of metabolites compared to vehicle control was used to calculate the IC_{50}. For time-dependent inhibition, cryopreserved human microsomes were pre-incubated for 30 minutes with 25 µM apalutamide or M3 in DMSO (final concentration: 0.075%) ± NADPH. At the end of that period, the probe was added and after 5 minutes the reaction was terminated and metabolite formation was measured. In both assays, the positive control inhibitor was screened alongside the apalutamide.

CYP Reversible Inhibition

CYP Isoform	Probe Substrate (concentration, µM)	Microsome Concentration (mg/mL)	Apalutamide IC_{50} [µM (SE)]	JNJ-56142060, M3 IC_{50} (µM)	Maximum % Inhibition (apalutamide)	Positive Control Inhibitor	Control IC_{50} Limits (µM)	Control IC_{50} [µM (SE)]
1A	ethoxyresorufin (0.5)	0.25	>25	>25	-	α-naphthoflavone	0.01 - 0.15	0.0674 (0.0158)
2C8	paclitaxel(7.5)	0.25	13.9 (2.28)	>25	NA	montelukast	0.1 - 1.0	0.584 (0.142)
2C9	tolbutamide (120)	1.0	>25	>25	24.3	sulfaphenazole	0.3 – 1.2	0.799 (0.123)
2C19	s-mephenytoin (25)	0.5	>25	>25	31.0	tranylcypromine	3 – 18	8.27 (1.47)
2D6	dextromethorphan (5.0)	0.5	>25	>25	25.4	quinidine	0.01 – 0.1	0.0435 (0.00291)
3A4	midazolam (2.5)	0.1	>25	>25	-	ketoconazole	0.01 – 0.16	0.0253 (0.00156)
3A4	testosterone (50)	0.5	>25	>25	45.0	ketoconazole	0.01 – 0.16	0.0881 (0.0151)

Time-Dependent CYP Inhibition

CYP Isoform	Probe Substrate (concentration, µM)	Microsome Concentration (mg/mL)	Apalutamide Inhibition[a] (% (SD) at 25 µM)	JNJ-56142060 , M3 Inhibition[a] (% (SD) at 25 µM)	Positive Control Inhibitor	Minimum Control Inhibition (% at 25 µM)	Mean (SD) % Inhibition[a]
1A	ethoxyresorufin (2.5)	0.25	5.43 (8.32)	-0.146 (0.363)	furafylline	50	64.1 (0.904)
					resveratrol	45	62.6 (1.09)
2C9	diclofenac (100)	0.50	2.70 (6.47)	-6.13 (0.889)	tienilic acid	70	72.4 (5.42)[b]
2C19	s-mephenytoin (250)	0.20	12.9 (1.19)	12.3 (2.25)	ticlopidine	50	58.1 (0.636)[b]
					phenelzine	20	30.7 (1.73)
2D6	dextromethorphan (25)	0.50	7.88 (6.80)	-5.05 (8.44)	paroxetine	60	78.0 (2.36)
3A4	midazolam (12.5)	0.1	-24.7 (4.24)	-1.98 (4.43)	mibefradil HCl	40	48.6 (6.67)[b]
					mifepristone	65	80.9 (2.13)[b]

Additional Information: For CYP reversible inhibition, the following scheme was used to classify the extent of inhibition:
potent IC_{50} <1 µM
moderate IC_{50} between 1 and 10 µM
no or weak IC_{50} >10 µM

[a] Mean, corrected for vehicle

[b] Reversible inhibition observed

- = no inhibition observed up to 25 µM apalutamide; CYP = cytochrome P450; DMSO = dimethyl sulfoxide; IC_{50} = concentration producing 50% inhibition; NA = not applicable; NADPH = β-nicotinamide adenine dinucleotide phosphate sodium salt hydrate; M3 = the *N*-desmethyl metabolite of apalutamide

2.6.5.12　G　薬物動態試験：薬物代謝酵素の誘導／阻害：*In vitro* 試験

Test Article: JNJ-56142060[a]

Study No.	FK10660
Location in CTD	4.2.2.4.11
Type of Study:	*In vitro* CYP Inhibition in Human Liver Microsomes

Method: Cryopreserved human hepatocytes from a pooled sample of 50 male/female donors were used to assess the ability of JNJ-56142060 (M3, the *N*-desmethyl metabolite of apalutamide) to inhibit major human CYP isoforms. IC_{50} concentrations for each isoform were determined at a probe concentration at or below the substrate K_m value when incubated at 8 M3 concentrations ranging from 0 to 75 µM. Serial dilutions of a known inhibitor of each CYP isoform was included as a positive control. Following a preincubation period, reactions were initiated by adding NADPH, G6P, and G6PD to yield final incubation concentrations of 1.3 mM, 3.3 mM, and 0.4 U/mL, respectively. The microsomes were incubated in the solutions for times ranging from 5 to 25 minutes, depending on the substance. Inhibition was assayed using LC-MS/MS. Inhibition was measured as a percent reduction of probe substrate in the presence and absence of JNJ-56142060.

CYP Isoform	Probe Substrate	Incubation Time (min)	Substrate Concentration (µM)	Microsome Concentration (mg/mL)	JNJ-56142060[a] IC_{50} (µM)	Maximum Inhibition (%)	Positive Control Inhibitor	Concentration Range (µM)	Control IC_{50} (µM)
1A2	phenacetin	15	80	0.20	-	10	α-naphthoflavone	0.001 – 1	0.0070
2A6	coumarin	15	2	0.20	-	6	tranylcypromine	0.02 – 20	0.46
2B6	bupropion	15	25	0.20	49	60	ticlopidine	0.005 – 5	0.061
2C8	amodiaquine	15	2	0.10	47	62	quercetin	0.03 – 30	2.6
2C9	tolbutamide	12	100	0.15	49	64	sulphaphenazole	0.005 – 5	0.27
2C9	diclofenac	12	5	0.15	38	73	sulphaphenazole	0.005 – 5	0.23
2C19	s-(+)-mephenytoin	25	30	0.20	>75	50	N-3-benzylPB	0.002 – 2	0.12
2D6	dextromethorphan	12	3	0.15	>75	15	quinidine	0.002 – 2	0.048
2E1	chlorzoxazone	16	45	0.20	-	<5	4-methylpyrazole	0.02 – 20	0.25
3A4	testosterone	12	25	0.15	69	52	ketoconazole	0.002 – 2	0.016
3A4	midazolam	5	2	0.15	>75	21	ketoconazole	0.002 – 2	0.019
3A4	nifedipine	5	10	0.10	>75	12	ketoconazole	0.002 – 2	0.025

Additional Information: All substrates were prepared in solutions of DMSO, methanol, and/or water with final organic solvent concentration ≤0.05%. Positive control inhibitors were prepared in methanol, acetonitrile and/or DMSO with final organic solvent concentration ≤0.07%. All were stored at 4°C until use.

[a] M3, the *N*-desmthyl metabolite of apalutamide

- = no inhibition observed up to 75 µM JNJ-56142060; CYP = cytochrome P450; DMSO = dimethyl sulfoxide; G6P = glucose-6-phosphate; G6PD = glucose-6-phophate dehydrogenase; K_m = Michaelis constant; LC-MS/MS = liquid chromatography-tandem mass spectrometry; M3 = the *N*-desmethyl metabolite of apalutamide; N-3-BenzylPB = N-3-Benzylphenobarbital

2.6.5.13　A　薬物動態試験：尿･糞･呼気中排泄（ラット）

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase C)				
Location in CTD	4.2.2.4.2				
Species	**Sprague-Dawley Rat**				
Gender (M/F)/ Number of Animals	M:3				
Feeding Condition	Fed				
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[a]				
Route	Oral gavage				
Dose (mg/kg)	50				
Radionuclide	[^{14}C]JNJ-56021927				
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)				
Analytical method	HPLC				
	Mean Amount excreted [% dose per stated interval, (SD)]				
Excretion Route:	Urine	Feces	Aqueous Cage Wash	Expired Air Traps	Other[b]
Time (h)					
Predose	BQL	BQL	-	-	
0 – 6	2.13 (0.72)	32.07 (2.22)[c]	1.58 (0.41)[c]	0.03 (0.01)[c]	
6 – 24	20.21 (1.96)				
24 – 48	10.66 (0.80)	21.92 (2.53)	0.63 (0.32)	BQL	
48 – 72	0.97 (0.38)	3.58 (0.85)	0.09 (0.04)	BQL	
72 – 96	0.16 (0.06)	0.37 (0.15)	0.06 (0.03)	-	
96 – 120	0.06 (0.03)	0.17 (0.01)	0.06 (0.05)	-	
120 – 144	0.03 (0.02)	0.07 (0.02)	0.07 (0.09)	-	
144 – 168	0.01 (0.01)	0.05 (0.01)	0.02 (0.01)	-	
Methanol Cage Wash					0.05 (0.02)
Carcass					0.70 (0.17)
Totals[b]	34.23 (2.74)	58.23 (3.43)	2.52 (0.77)		
Overall Excretion[b]					95.76 (1.25)

Additional Information: Most of the fecal and urinary radioactivity was excreted during 0 – 48 hours postdose (mean totals of 53.99% and 33.01% dose, respectively).

[a] Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, vitamin E TPGS NF (10:45:30:15%w/w/w/w)
[b] All values represent 0 – 168 h data.
[c] 0 – 24 h data.

= not assessed; BQL = below quantification limit; HPLC = high-performance liquid chromatography; M = male; SD = standard deviation

2.6.5.13　B　薬物動態試験：尿･糞･呼気中排泄（イヌ）

Test Article: JNJ-56021927

Study No.	FK10704		
Location in CTD	4.2.2.4.3		
Species	**Beagle Dog**		
Gender (M/F)/ Number of Animals	M: 3[a]		
Feeding Condition:	Fasted		
Vehicle/Formulation	Vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)/solution[b]		
Route	Oral capsule		
Dose (mg/kg)	10		
Radionuclide	[^{14}C]JNJ-56021927		
Specific Activity	2.37 µCi/mg (0.0877 MBq/mg)		
Analytical method	Liquid scintillation counting		
	Mean Amount excreted [% dose per stated interval, (SD)]		
Excretion Route:	Urine	Feces	Other[c]
Time (h)			
0 – 24	3.80 (0.69)	2.73 (3.30)	
24 – 48	3.85 (0.72)	5.80 (3.50)	
48 – 72	5.22 (1.60)	3.06 (1.67)	
72 – 96	4.20 (1.26)	4.42 (2.05)	
96 – 120	3.83 (0.94)	3.06 (1.47)	
120 – 144	3.75 (0.62)	5.55 (1.05)	
144 – 168	3.33 (0.83)	2.82 (1.56)	
168 – 192	3.02 (0.43)	2.18 (0.95)	
192 – 216	2.26 (0.38)	2.70 (0.71)	
216 – 240	2.11 (0.24)	2.18 (1.07)	
240 – 264	1.36 (0.51)	1.46 (0.63)	
264 – 288	1.72 (0.18)	1.92 (0.39)	
288 – 312	1.49 (0.42)	0.75 (0.36)	
312 – 336	1.06 (0.27)	0.98 (0.71)	
336 – 360	0.85 (0.34)	0.60 (0.27)	
Cage Washing			0.92 (0.53)
Totals[c]	**41.80 (6.02)**	**40.20 (3.05)**	
Overall Excretion[c]			**83.00 (5.34)**

[a] Three dogs were dosed with apalutamide, but since 2 vomited within 30 minutes of dosing, a fourth dog was dosed the following day, at the same time a dog was dosed with the vehicle. The total radioactivity in the emesis was determined so that the total administered dose could be modified and be reflected in the results.

[b] Vehicle = Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, vitamin E TPGS NF (10:45:30:15%w/w/w/w)

[c] 0 – 360 h interval

M = male; SD = standard deviation

2.6.5.13　C　薬物動態試験：尿･糞･呼気中排泄（ヒト）

Test Article: JNJ-56021927

Study No.	FK10840
Location in CTD	4.2.2.4.4
Species	**Human**
Gender (M/F)/ Number of Animals	M: 6
Feeding Condition:	Fasted
Vehicle/Formulation	8 Capsules (240 mg apalutamide) plus one capsule containing 0.400 mg [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)
Route	Oral
Radionuclide	[^{14}C]JNJ-56021927
Specific Activity	92.5 kBq/mg
Analytical method	AMS or LSC

Mass Balance of unchanged [^{14}C]JNJ-56021927 and Its Metabolites (% dose per stated interval)

Excretion Route	Urine			Feces			Urine + Feces		
Time (h)	0 – 240 h	240 – 1680 h	0 – 1680 h	0 – 240 h	240 – 1680 h	0 – 1680 h	0 – 240 h	240 – 1680 h	0 – 1680 h
Component									
M21 + M22	0.91	1.53	2.44	-	-	-	0.91	1.53	2.44
M20	2.17	4.68	6.85	0.11	0.17	0.29	2.28	4.85	7.14
M19	3.29	2.02	5.31	0.20	0.21	0.41	3.49	2.22	5.71
M18	0.14	0.13	0.27	0.33	0.57	0.90	0.47	0.70	1.17
M17	trace	trace	trace	trace	trace	trace	trace	trace	trace
M15	0.84	0.71	1.54	trace	trace	trace	0.8	0.7	1.54
M13 + M14	-	-	-	0.87	3.79	4.67	-	-	-
M12 + M13 + M14	2.35	2.38	4.73	-	-	-	3.22	6.17	9.40
M11	0.66	0.45	1.11	-	-	-	0.66	0.45	1.11
M9	0.29	0.21	0.50	-	-	-	-	-	-
M9 + M16	-	-	-	1.61	1.88	3.49	1.91	2.09	4.00
M10	trace	trace	trace	-	-	-	trace	trace	trace
M4	12.53	18.59	31.12	0.69	1.69	2.38	13.22	20.29	33.50
M8	0.61	0.29	0.90	0.20	0.19	0.39	0.80	0.48	1.29
M2	0.17	0.07	0.23	-	-	-	0.17	0.07	0.23
M3	0.98	1.75	2.73	0.62	1.35	1.97	1.60	3.10	4.70
UD	0.92	0.28	1.20	0.91	0.64	1.54	1.82	0.92	2.74
Sum of reported entities	25.9	33.0	58.8	5.5	10.5	16.0	31.4	43.4	74.8
Sum of observed metabolites	**28.1**	**36.1**	**64.2**	**7.9**	**16.2**	**24.1**	**36.0**	**52.3**	**88.3**

[a] vehicle = Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, vitamin E TPGS NF (10:45:30:15%w/w/w/w)

- = not detected; AMS = accelerator mass spectrometry; LSC = liquid scintillation counting; M = male; M# = metabolite; UD = unchanged drug

2.6.5.14　薬物動態試験：胆汁中排泄

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase D)				
Location in CTD	4.2.2.4.2				
Species	**Bile-duct Cannulated Sprague-Dawley Rat**				
Gender (M/F)/ Number of Animals	M:3[a]				
Feeding Condition	Fed				
Vehicle/Formulation	Solution[b]/vehicle plus [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide)				
Route	Oral gavage				
Dose (mg/kg)	50				
Radionuclide	[^{14}C]JNJ-56021927				
Specific Activity	103.94 µCi/mg (3.85 MBq/mg)				
Analytical method	LSC				
	Mean Amount excreted [% dose per stated interval, (SD)]				
Excretion Route:	Bile	Urine	Feces	Aqueous Cage Wash	Other[c]
Time (h)					
Predose	BQL	-	-	-	
0 – 1	<0.01 (0.01)				
1 – 2	0.22 (0.05)				
2 – 3	0.74 (0.17)				
3 – 4	0.93 (0.24)				
4 – 5	1.01 (0.30)				
5 – 6	1.03 (0.27)	2.72 (0.63)[d]			
6 – 24	31.48 (1.72)	18.07 (2.58)	6.47 (2.19)[e]	2.62 (1.08)[e]	
24 – 48	12.97 (3.14)	8.08 (1.51)	4.39 (1.75)	0.040 (0.09)	
48 – 72	1.59 (0.40)	0.86 (0.19)	0.34 (0.08)	0.17 (0.10)	
Subtotals	**49.98 (4.69)**	**29.73 (3.10)**	**11.20 (0.65)**	**3.19 (1.25)**	
Methanol Cage Wash					0.08 (0.05)
Residual retained in tissues					
Liver					0.17 (0.02)
GI contents					0.03 (0.00)
GI wall					0.01 (0.00)
Carcass					1.37 (0.15)
Total 72-hour Excretion					**95.75 (0.57)**

Additional Information: Only a small portion of radioactivity was retained in the carcass and tissues 72 hours postdose indicating that elimination was almost complete by this time. Radioactivity was rapidly excreted through bile (49.98% by 72 hours postdose; ~97% of biliary activity)

Footnotes and abbreviations on following page.

2.6.5.14　薬物動態試験：胆汁中排泄（続き）

Test Article: JNJ-56021927

Study No.	FQA0001 (Phase D)
Location in CTD	4.2.2.4.2

[a] 5 male rats were cannulated, but 2 represented “spare” animals that were dosed to ensure that data from the required number (N=3) was available for analysis.
[b] Acconon MC8-2 EP/NF, Capmul MCM EP, PEG 400 USP, vitamin E TPGS NF (10:45:30:15%w/w/w/w)
[c] 0 – 72 h data.
[d] 0 – 6 h data.
[e] 0 – 24 h data.

- = Not assessed; BQL = below quantification limit (<2x background radioactivity); GI = gastrointestinal tract; LSC = liquid scintillation counting; M = male; N = number; SD = standard deviation

2.6.5.15　A　薬物動態試験：薬物相互作用

Test Article: JNJ-56021927

Study No.	12ARAGP1R1	
Location in CTD:	4.2.2.6.1	
Type of Study:	Evaluation of transepithelial transport of JNJ-56021927 in Caco-2 Cells	
Method: Caco-2 cells were used to investigate the bidirectional transepithelial transport of JNJ-56021927. Cells were incubated in a humidified chamber in a Hanks balanced salt solution[a] at 37°C with 5% CO_2.The cells were exposed to 5 µM JNJ-56021927 for up to 120 minutes and transport was assessed in the A-to-B and B-to-A directions. Samples were drawn from the donor chamber at 0 and 120 minutes and from the receiver chambers at 60 and 120 minutes; each in quadruplicate. The amount of radiolabeled compound permeated into the receiver compartment was measured by LC-MS/MS.		
Dose	5 µM	
Findings:	**A→B**	**B→A**
Recovery, %	80	99
Recovery, % (including cell lysate)	88	102
Average P_{app} ($x10^{-6}$ cm/s)	42.3	30.7
Additional Information	Conclusion: HIGH permeability with no significant efflux.	

[a] Hanks balanced salt solution containing 10mM HEPES and 15 mM glucose (pH7.4). The receiver chamber also contained 1% bovine serum albumin.

A→B = apical to basolateral; B→A = basolateral to apical; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LC-MS/MS = liquid chromatography-tandem mass spectrometry; Papp = apparent permeability

2.6.5.15　B　薬物動態試験：薬物相互作用

Test Article: JNJ-56021927

Study No.	FK10661
Location in CTD:	4.2.2.6.2
Type of Study:	Evaluation of transport of JNJ-56021927 by ABCB1 and ABCG2 Efflux Proteins

Method: On Day 0 the cells were seeded on a 24-well plate (400 µL/well, MDCKII cells; 122,500 cells/mL, LLC-PK-1 cells; 700,000 cells/mL,). For the MDCKII cells, after 24 hours and on Day 3 the media in both compartments was replaced and on Day 4 the transport study was conducted. For the LLC-PK1 cells, after 24 hours and on Day 4 the media in both compartments was replaced and on Day 5 the transport study was conducted. For transportation studies, all cells were preincubated for 60 minutes with transport medium with/without inhibitor. At time = 0, substrate, test compound or inhibitor was introduced and at t = 60 minutes the compartments were sampled and radioactivity was determined using liquid scintillation counting. All transport experiments were performed in triplicate.

BCRP-Mediated Transport of JNJ-56021927, JNJ-56142060 (M3), and Topotecan (positive control)/Inhibition of Topotecan transport by JNJ-56021927 and JNJ-56142060 (M3)

		MDCKII-Parent Cells			**MDCKII-BCRP Transfected Cells**		
Experimental Condition		Permeability Coefficient (P_{app}, 10^{-6} cm/s)		Ratio (BA/AB)	Permeability Coefficient (P_{app}, 10^{-6} cm/s)		Ratio (BA/AB)
Compound	Inhibitor	A→B	B→A		A→B	B→A	
JNJ-56021927	**KO143**						
1 µM	-	34.09 (0.76)	22.25 (1.29)	0.65 (0.04)	34.84 (2.33)	24.61 (0.09)	0.71 (0.05)
	1 µM	36.75 (1.06)	21.41 (0.83)	0.58 (0.03)	36.98 (1.31)	24.36 (1.02)	0.66 (0.04)
5 µM	-	35.91 (1.48)	26.66 (1.15)	0.74 (0.04)	35.45 (0.44)	28.03 (1.46)	0.79 (0.04)
	1 µM	36.45 (1.66)	24.48 (1.47)	0.67 (0.05)	37.33 (2.37)	26.76 (0.63)	0.72 (0.05)
JNJ-56142060 (M3)	**KO143**						
1 µM	-	36.57 (0.93)	23.31 (1.13)	0.64 (0.03)	32.67 (0.55)	28.88 (1.94)	0.88 (0.06)
	1 µM	38.38 (1.93)	23.47 (0.91)	0.61 (0.04)	37.12 (1.90)	25.70 (1.47)	0.69 (0.05)
5 µM	-	38.05 (1.95)	25.36 (0.97)	0.67 (0.04)	35.18 (2.43)	27.89 (0.78)	0.79 (0.06)
	1 µM	41.32 (0.19)	24.89 (0.47)	0.60 (0.01)	37.87 (0.72)	26.89 (0.83)	0.71 (0.03)
Digoxin	**KO143**						
0.03 µM	-	2.40 (0.33)	1.93 (0.07)	0.81 (0.11)	2.26 (0.21)	1.89 (0.07)	0.84 (0.08)
	1 µM	2.53 (0.44)	1.89 (0.07)	0.75 (0.13)	2.66 (0.06)	1.87 (0.11)	0.70 (0.04)
Topotecan	**KO143**						
1 µM	-	3.44 (0.16)	2.90 (0.14)	0.84 (0.06)	1.31 (0.10)	8.94 (0.08)	6.80 (0.53)
	1 µM	3.67 (0.38)	2.67 (0.30)	0.84 (0.11)	3.22 (0.31)	2.31 (0.06)	0.72 (0.07)

Additional Information: Radioisotopes used: [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3), [^{3}H]digoxin, [^{3}H] topotecan

A→B = apical to basolateral; B→A = basolateral to apical; ABC = ATP-binding cassette; BCRP = breast cancer resistance protein; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LLC-PK = Lilly Laboratories Cell - Porcine Kidney; M3 = the *N*-desmethyl metabolite of apalutamide; MDCKII = Madin-Darby canine kidney strain II; Papp = apparent permeability; t = time

Continued

2.6.5.15　B　薬物動態試験：薬物相互作用（続き）

Test Article: JNJ-56021927

Study No.	FK10661
Location in CTD:	4.2.2.6.2
Type of Study:	Evaluation of transport of JNJ-56021927 by ABCB1 and ABCG2 Efflux Proteins

BCRP-Mediated Transport of JNJ-56021927, JNJ-56142060 (M3), and Topotecan (positive control)/Inhibition of Topotecan transport by JNJ-56021927 and JNJ-56142060 (M3)

Experimental Condition		MDCKII-Parent Cells			MDCKII-BCRP Transfected Cells		
		Permeability Coefficient (P_{app}, 10^{-6} cm/s)		Ratio (BA/AB)	Permeability Coefficient (P_{app}, 10^{-6} cm/s)		Ratio (BA/AB)
Compound	Inhibitor	A→B	B→A		A→B	B→A	
Topotecan							
1 µM	-	3.44 (0.16)	2.90 (0.14)	0.84 (0.06)	1.31 (0.10)	8.94 (0.08)	6.80 (0.53)
	JNJ-56021927						
	0.3 µM	2.62 (0.27)	2.17 (0.19)	0.83 (0.11)	1.17 (0.07)	7.13 (0.27)	6.11 (0.42)
	1 µM	2.73 (0.21)	2.16 (0.09)	0.79 (0.07)	0.94 (0.06)	6.83 (0.16)	7.24 (0.50)
	3 µM	2.24 (0.09)	2.33 (0.06)	1.04 (0.05)	1.32 (0.08)	6.67 (0.19)	5.04 (0.34)
	10 µM	2.47 (0.09)	2.16 (0.18)	0.88 (0.08)	1.41 (0.08)	6.70 (0.30)	4.76 (0.35)
	30 µM	2.17 (0.18)	1.75 (0.05)	0.81 (0.07)	1.50 (0.05)	5.64 (0.26)	3.77 (0.21)
	JNJ-56142060 (M3)						
	0.3 µM	2.28 (0.34)	1.94 (0.12)	0.85 (0.14)	1.27 (0.14)	5.78 (0.47)	4.57 (0.61)
	1 µM	1.99 (0.21)	1.96 (0.16)	0.98 (0.13)	1.27 (0.15)	6.18 (0.39)	4.87 (0.64)
	3 µM	2.27 (0.36)	2.07 (0.19)	0.91 (0.17)	1.13 (0.06)	6.71 (0.33)	5.92 (0.42)
	10 µM	2.33 (0.20)	1.80 (0.14)	0.81 (0.10)	1.06 (0.01)	5.59 (0.16)	5.26 (0.16)
	30 µM	2.30 (0.16)	1.76 (0.16)	0.80 (0.09)	1.11 (0.08)	5.07 (0.27)	4.56 (0.39)

Additional Information: Radioisotopes used: [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3), [^{3}H]digoxin, [^{3}H] topotecan

A→B = apical to basolateral; B→A = basolateral to apical; ABC = ATP-binding cassette; BCRP = breast cancer resistance protein; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; M3 = the *N*-desmethyl metabolite of apalutamide; MDCKII = Madin-Darby canine kidney strain II; Papp = apparent permeability

Continued

Test Article: JNJ-56021927

Study No.	FK10661
Location in CTD:	4.2.2.6.2
Type of Study:	Evaluation of transport of JNJ-56021927 by ABCB1 and ABCG2 Efflux Proteins

MDR1-Mediated Transport of JNJ-56021927, JNJ-56142060 (M3), and Digoxin (positive control)/Inhibition of Digoxin transport by JNJ-56021927 and JNJ-56142060 (M3)

Experimental Condition		LLC-PK1 Parent Cells			LLC-PK1 MDR1-Transfected Cells		
		Permeability Coefficient (P_{app}, 10^{-6} cm/s)		Ratio (BA/AB)	Permeability Coefficient (P_{app}, 10^{-6} cm/s)		Ratio (BA/AB)
Compound	Inhibitor	A→B	B→A		A→B	B→A	
JNJ-56021927	**GF120918**						
1 µM	-	38.91 (3.87)	21.41 (0.29)	0.55 (0.06)	21.20 (0.66)	33.47 (0.86)	1.58 (0.06)
	1µM	40.01 (1.06)	22.61 (0.45)	0.57 (0.02)	39.03 (1.40)	23.20 (0.70)	0.59 (0.03)
5 µM	-	36.53 (1.75)	22.40 (0.68)	0.61 (0.03)	24.00 (1.10)	31.57 (0.23)	1.32 (0.06)
	1µM	39.99 (1.21)	22.85 (0.89)	0.57 (0.03)	40.84 (2.56)	22.88 (2.10)	0.56 (0.06)
JNJ-56142060 (M3)	**GF120918**						
1 µM	-	39.21 (1.34)	25.35 (0.62)	0.65 (0.03)	20.18 (1.29)	39.69 (1.35)	1.97 (0.14)
	1µM	41.95 (0.92)	24.53 (0.92)	0.58 (0.03)	41.82 (1.28)	23.72 (1.10)	0.57 (0.03)
5 µM	-	36.37 (0.23)	21.39 (0.64)	0.59 (0.02)	20.49 (1.06)	31.92 (0.99)	1.56 (0.09)
	1µM	38.02 (1.75)	21.46 (0.97)	0.56 (0.04)	38.70 (0.90)	21.86 (0.94)	0.56 (0.03)

Additional Information: Radioisotopes used: [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3)

A→B = apical to basolateral; B→A = basolateral to apical; ABC = ATP-binding cassette; BCRP = breast cancer resistance protein; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LLC-PK = Lilly Laboratories Cell - Porcine Kidney; M3 = the *N*-desmethyl metabolite of apalutamide; MDR = multidrug-resistant; Papp = apparent permeability

Continued

Test Article: JNJ-56021927

Study No. Study No.	FK10661
Location in CTD:	4.2.2.6.2
Type of Study:	Evaluation of transport of JNJ-56021927 by ABCB1 and ABCG2 Efflux Proteins

MDR1-Mediated Transport of JNJ-56021927, JNJ-56142060 (M3), and Digoxin (positive control)/Inhibition of Digoxin transport by JNJ-56021927 and JNJ-56142060 (M3)

Experimental Condition		LLC-PK1 Parent Cells			LLC-PK1 MDR1-Transfected Cells		
		Permeability Coefficient (P_{app},10^{-6} cm/s)		Ratio (BA/AB)	Permeability Coefficient (P_{app},10^{-6} cm/s)		Ratio (BA/AB)
Compound	Inhibitor	A→B	B→A		A→B	B→A	
Digoxin	**GF120918**						
0.03 µM	-	1.74 (0.11)	4.51 (0.14)	2.59 (0.18)	1.25 (0.11)	8.91 (0.27)	7.15 (0.66)
	JNJ-56021927						
	0.3 µM	2.19 (0.13)	4.83 (0.02)	2.21 (0.13)	1.56 (0.07)	9.29 (0.58)	5.95 (0.45)
	1 µM	2.26 (0.12)	4.30 (0.38)	1.90 (0.19)	1.39 (0.10)	9.65 (0.92)	6.93 (0.84)
	3 µM	1.82 (0.08)	3.13 (0.06)	1.72 (0.08)	1.37 (0.13)	9.06 (0.69)	6.61 (0.81)
	10 µM	2.33 (0.22)	3.06 (0.50)	1.31 (0.25)	1.81 (0.05)	8.97 (0.47)	4.97 (0.30)
	30 µM	2.50 (0.43)	2.59 (0.24)	1.03 (0.20)	2.50 (0.33)	7.80 (0.43)	3.12 (0.45)
	JNJ-56142060 (M3)						
	0.3 µM	2.03 (0.04)	5.08 (0.27)	2.50 (0.14)	1.48 (0.14)	9.67 (0.46)	6.54 (0.69)
	1 µM	2.21 (0.23)	4.61 (0.15)	2.08 (0.23)	1.45 (0.08)	10.28 (0.15)	7.10 (0.40)
	3 µM	2.42 (0.38)	4.11 (0.23)	1.69 (0.28)	1.37 (0.08)	9.73 (0.24)	7.11 (0.43)
	10 µM	2.51 (0.22)	3.76 (0.47)	1.50 (0.23)	1.79 (0.11)	10.38 (0.29)	5.81 (0.40)
	30 µM	2.68 (0.10)	3.08 (0.26)	1.15 (0.10)	2.12 (0.11)	8.77 (0.21)	4.14 (0.24)

Additional Information: Radioisotopes used: [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3), [^{3}H]digoxin

A→B = apical to basolateral; B→A = basolateral to apical; ABC = ATP-binding cassette; BCRP = breast cancer resistance protein; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LLC-PK = Lilly Laboratories Cell - Porcine Kidney; M3 = the *N*-desmethyl metabolite of apalutamide; MDR = multidrug-resistant; Papp = apparent permeability

2.6.5.15　C　薬物動態試験：薬物相互作用

Test Article: JNJ-56021927 (M3)

Study No. . FK10693
Location in CTD: 4.2.2.6.3
Type of Study: OATP -Mediated Transport of ^{14}C-labeled apalutamide or M3/Inhibition Potential of apalutamide or M3 on OATP Transporters in HEK Cells Overexpressing OATP Transporters

Method: All cell lines (HEK293-parent and OATP1B1/B3 cell lines) were seeded into 24-well plates (1 mL/well, 400,000 cells/well) in HBSS supplemented with 10 mM Hepes at pH 7.4. At time 0 the incubation medium, containing test substance or inhibitor was added. Following incubation, radioactivity and protein were determined by LSC and protein assay kit, respectively.

HEK-OATP1B1 Cell Lines

Experimental Condition **1 µM Test Substance**	Incubation Time (min)	HEK-Parent Cells (pmol/mg protein/min)	HEK-OATP1B1 Cells (pmol/mg protein/min)	% Transport	% Inhibition
1 µM E2G + DMSO	0.5	2.52 (0.11)	118 (15.9)	100	-
1 µM E2G + 25 µM rifampicin	0.5	2.98 (0.36)	14.6 (0.08)	12.3	87.7
1 µM apalutamide	1	31.5 (5.41)	26.8 (3.72)	100	-
1 µM apalutamide + 25 µM rifampicin	1	31.9 (4.52)	27.1 (6.17)	101	NI
1 µM apalutamide	5	4.31 (0.29)	5.36 (0.38)	100	-
1 µM apalutamide + 25 µM rifampicin	5	4.55 (0.22)	5.36 (0.40)	100	NI
1 µM apalutamide	10	2.63 (0.32)	2.69 (0.24)	100	-
1 µM apalutamide + 25 µM rifampicin	10	2.77 (0.13)	2.38 (0.50)	88.5	11.5
1 µM JNJ-56142060 (M3)	1	43.0 (2.97)	52.8 (1.34)	100	-
1 µM JNJ-56142060 (M3) + 25 µM rifampicin	1	63.0 (2.31)	54.6 (0.85)	103	NI
1 µM JNJ-56142060 (M3)	5	13.7 (0.22)	10.8 (2.28)	100	-
1 µM JNJ-56142060 (M3) + 25 µM rifampicin	5	14.3 (0.27)	14.2 (0.53)	131	NI
1 µM JNJ-56142060 (M3)	10	6.68 (0.37)	7.03 (0.14)	100	-
1 µM JNJ-56142060 (M3) + 25 µM rifampicin	10	6.40 (1.04)	7.09 (0.20)	101	NI

Additional Information: Radioisotopes used: [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3)

DMSO = dimethyl sulfoxide; E2G = estradiol-17β-glucuronide; HBSS = Hank's Balanced Salt Solution; HEK = human embryonic kidney; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LSC = liquid scintillation counting; M3 = JNJ-56142060, the *N*-desmethyl metabolite of apalutamide; OATP = organic anion-transporting polypeptide; NI = no inhibition

Continued

2.6.5.15　C　薬物動態試験：薬物相互作用（続き）

Test Article: JNJ-56021927 (M3)

Study No. . FK10693

Location in CTD: 4.2.2.6.3

Type of Study: OATP -Mediated Transport of ^{14}C-labeled apalutamide or M3/Inhibition Potential of apalutamide or M3 on OATP Transporters in HEK Cells Overexpressing OATP Transporters

HEK-OATP1B1 Cell Lines

Experimental Condition **5 µM Test Substance**	Incubation Time (min)	HEK-Parent Cells (pmol/mg protein/min)	HEK-OATP1B1 Cells (pmol/mg protein/min)	% Transport	% Inhibition
1 µM E2G + DMSO	0.5	2.52 (0.11)	118 (15.9)	100	-
1 µM E2G + 25 µM rifampicin	0.5	2.98 (0.36)	14.6 (0.08)	12.3	87.7
5 µM apalutamide	1	172 (13.7)	158 (11.7)	100	-
5 µM apalutamide + 25 µM rifampicin	1	199 (8.28)	199 (11.2)	126	NI
5 µM apalutamide	5	20.1 (1.02)	25.7 (4.05)	100	-
5 µM apalutamide + 25 µM rifampicin	5	23.2 (2.38)	28.9 (1.79)	113	NI
5 µM apalutamide	10	12.4 (0.35)	13 (0.74)	100	-
5 µM apalutamide + 25 µM rifampicin	10	14 (1.41)	13.3 (0.26)	102	NI
5 µM JNJ-56142060 (M3)	1	298 (3.38)	273 (1.96)	100	-
5 µM JNJ-56142060 (M3) + 25 µM rifampicin	1	291 (60.2)	309 (18.6)	113	NI
5 µM JNJ-56142060 (M3)	5	64.2 (1.94)	71.4 (0.77)	100	-
5 µM JNJ-56142060 (M3) + 25 µM rifampicin	5	67.6 (0.6)	75.1 (4.63)	105	NI
5 µM JNJ-56142060 (M3)	10	34 (2.47)	34.1 (1.11)	100	-
5 µM JNJ-56142060 (M3) + 25 µM rifampicin	10	39 (2.57)	34.3 (1.15)	100	NI

Additional Information: Radioisotopes used: [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3)

DMSO = dimethyl sulfoxide; E2G = estradiol-17β-glucuronide; HBSS = Hank's Balanced Salt Solution; HEK = human embryonic kidney; M3 = JNJ-56142060, the *N*-desmethyl metabolite of apalutamide; OATP = organic anion-transporting polypeptide; NI = no inhibition

Continued

2.6.5.15　C　薬物動態試験：薬物相互作用（続き）

Test Article: JNJ-56021927 (M3)

Study No. FK10693
Location in CTD: 4.2.2.6.3
Type of Study: OATP -Mediated Transport of ^{14}C-labeled apalutamide or M3/Inhibition Potential of apalutamide or M3 on OATP Transporters in HEK Cells Overexpressing OATP Transporters

HEK-OATP1B3 Cell Lines

Experimental Condition **1 µM Test Substance**	Incubation Time (min)	HEK-Parent Cells (pmol/mg protein/min)	HEK-OATP1B3 Cells (pmol/mg protein/min)	% Transport	% Inhibition
1 µM E2G + DMSO	0.5	2.52 (0.11)	27.6 (0.69)	100	-
1 µM E2G + 25 µM rifampicin	0.5	2.98 (0.36)	6.89 (1.5)	25	75
1 µM apalutamide	1	31.5 (5.41)	29.7 (1.85)	100	-
1 µM apalutamide + 25 µM rifampicin	1	31.9 (4.52)	24.4 (7.11)	82.1	17.9
1 µM apalutamide	5	4.31 (0.29)	5.81 (0.18)	100	-
1 µM apalutamide + 25 µM rifampicin	5	4.55 (0.22)	5.35 (0.08)	92.1	7.9
1 µM apalutamide	10	2.63 (0.32)	3.14 (0.14)	100	-
1 µM apalutamide + 25 µM rifampicin	10	2.77 (0.13)	2.73 (0.22)	87.1	12.9
1 µM JNJ-56142060 (M3)	1	43 (2.97)	51.7 (1.78)	100	-
1 µM JNJ-56142060 (M3) + 25 µM rifampicin	1	63 (2.31)	42.9 (12)	83.1	16.9
1 µM JNJ-56142060 (M3)	5	13.7 (0.22)	14.2 (0.53)	100	-
1 µM JNJ-56142060 (M3) + 25 µM rifampicin	5	14.3 (0.27)	13.9 (1.11)	98.1	1.9
1 µM JNJ-56142060 (M3)	10	6.68 (0.37)	6.29 (0.22)	100	-
1 µM JNJ-56142060 (M3) + 25 µM rifampicin	10	6.4 (1.04)	6.31 (0.27)	100	NI

Additional Information: Radioisotopes used: [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3)

DMSO = dimethyl sulfoxide; E2G = estradiol-17β-glucuronide; HBSS = Hank's Balanced Salt Solution; HEK = human embryonic kidney; M3 = JNJ-56142060, the *N*-desmethyl metabolite of apalutamide; OATP = organic anion-transporting polypeptide; NI = no inhibition

Continued

2.6.5.15　C　薬物動態試験：薬物相互作用（続き）

Test Article: JNJ-56021927 (M3)

Study No.	FK10693
Location in CTD:	4.2.2.6.3

Type of Study: OATP -Mediated Transport of ^{14}C-labeled apalutamide or M3/Inhibition Potential of apalutamide or M3 on OATP Transporters in HEK Cells Overexpressing OATP Transporters

HEK-OATP1B3 Cell Lines

Experimental Condition **5 µM Test Substance**	Incubation Time (min)	HEK-Parent Cells (pmol/mg protein/min)	HEK-OATP1B3 Cells (pmol/mg protein/min)	% Transport	% Inhibition
1 µM E2G + DMSO	0.5	2.52 (0.11)	27.6 (0.69)	100	-
1 µM E2G + 25 µM rifampicin	0.5	2.98 (0.36)	6.89 (1.5)	25	75
5 µM apalutamide	1	172 (13.7)	144 (5.77)	100	-
5 µM apalutamide + 25 µM rifampicin	1	199 (8.28)	159 (9.09)	110	NI
5 µM apalutamide	5	20.1 (1.02)	31.7 (5.6)	100	-
5 µM apalutamide + 25 µM rifampicin	5	23.2 (2.38)	31.3 (2.09)	98.8	1.2
5 µM apalutamide	10	12.4 (0.35)	15.2 (1.7)	100	-
5 µM apalutamide + 25 µM rifampicin	10	14 (1.41)	14.7 (2.02)	97.2	2.8
5 µM JNJ-56142060 (M3)	1	298 (3.38)	270 (10.9)	100	-
5 µM JNJ-56142060 (M3) + 25 µM rifampicin	1	291 (60.2)	273 (3.83)	101	NI
5 µM JNJ-56142060 (M3)	5	64.2 (1.94)	69.2 (0.5)	100	-
5 µM JNJ-56142060 (M3) + 25 µM rifampicin	5	67.6 (0.6)	69.9 (2.28)	101	NI
5 µM JNJ-56142060 (M3)	10	34 (2.47)	31.5 (0.5)	100	-
5 µM JNJ-56142060 (M3) + 25 µM rifampicin	10	39 (2.57)	33.3 (0.36)	106	NI

Additional Information: Radioisotopes used:[^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3)

DMSO = dimethyl sulfoxide; E2G = estradiol-17β-glucuronide; HBSS = Hank's Balanced Salt Solution; HEK = human embryonic kidney; M3 = JNJ-56142060, the *N*-desmethyl metabolite of apalutamide; OATP = organic anion-transporting polypeptide; NI = no inhibition

Continued

Test Article: JNJ-56021927 (M3)

Study No. FK10693
Location in CTD: 4.2.2.6.3
Type of Study: OATP -Mediated Transport of ^{14}C-labeled apalutamide or M3/Inhibition Potential of apalutamide or M3 on OATP Transporters in HEK Cells Overexpressing OATP Transporters

Inhibition of E2G Transport Experimental Condition	Incubation Time (min)	HEK-Parent Cells (pmol/mg protein/min)	HEK-OATP1B1 Cells[a] (pmol/mg protein/min)	% Transport	% Inhibition	HEK-OATP1B3 Cells[b] (pmol/mg protein/min)	% Transport	% Inhibition
1 µM E2G + DMSO	0.5	3.69 (0.56)	111 (1.42)	100	-	32.3 (4.19)	100	-
1 µM E2G + 25 µM rifampicin	0.5	3.96 (0.39)	11.9 (0.36)	10.8	89.2	2.92 (0.76)	9.05	90.9
1 µM E2G + 0 µM apalutamide	0.5	3.69 (0.56)	111 (1.42)	100	-	32.3 (4.19)	100	-
1 µM E2G + 0.3 µM apalutamide	0.5	4.19 (0.05)	98.3 (3.80)	88.9	11.1	32.4 (0.97)	100	NI
1 µM E2G + 1 µM apalutamide	0.5	4.78 (0.94)	98.6 (3.98)	89.2	10.8	27.8 (0.99)	86.0	14.0
1 µM E2G + 3 µM apalutamide	0.5	4.77 (0.87)	101 (3.21)	91.7	8.30	22.2 (0.92)	68.9	31.1
1 µM E2G + 10 µM apalutamide	0.5	5.05 (0.35)	88.7 (3.84)	80.2	19.8	21.7 (0.57)	67.3	32.7
1 µM E2G + 30 µM apalutamide	0.5	5.82 (0.28)	73.8 (1.26)	66.7	33.3	15.5 (1.03)	48.0	52.0
1 µM E2G + 0 µM JNJ-56142060 (M3)	0.5	4.08 (0.29)	92.1 (3.72)	100	-	24.5 (3.01)	100	-
1 µM E2G + 0.3 µM JNJ-56142060 (M3)	0.5	4.90 (0.50)	92.1 (4.18)	100	NI	27.5 (1.25)	112	NI
1 µM E2G + 1 µM JNJ-56142060 (M3)	0.5	4.84 (0.85)	95.0 (5.01)	103	NI	27.2 (2.59)	111	NI
1 µM E2G + 3 µM JNJ-56142060 (M3)	0.5	5.19 (0.41)	89.9 (2.45)	97.6	2.40	26.9 (1.68)	110	NI
1 µM E2G + 10 µM JNJ-56142060 (M3)	0.5	5.59 (1.02)	70.0 (2.50)	76.0	24.0	21.9 (1.96)	89.3	10.7
1 µM E2G + 30 µM JNJ-56142060 (M3)	0.5	7.39 (0.83)	53.0 (0.41)	57.6	42.4	15.2 (6.07)	62.0	38.0

Additional Information: Radioisotopes used: [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3) and [^{3}H]estradiol-17β-glucuronide.

[a] Dynamic range = 31 for 1 µM E2G + DMSO

[b] Dynamic range = 10 for 1 µM E2G + DMSO

DMSO = dimethyl sulfoxide; E2G = estradiol-17β-glucuronide; HBSS = Hank's Balanced Salt Solution; HEK = human embryonic kidney; M3 = JNJ-56142060, the *N*-desmethyl metabolite of apalutamide; OATP = organic anion-transporting polypeptide; NI = no inhibition

2.6.5.15　D　薬物動態試験：薬物相互作用

Test Article: JNJ-56021927

Study No.			FK11028		
Location in CTD:			4.2.2.6.4		
Type of Study: Inhibition potential of apalutamide and M3 on OCT2, OAT1, OAT3, MATE-1 and MATE-2K in cell lines overexpressing these transporters					
Method: All cell lines were seeded into 24-well plates (1 mL/well, 625,000 cells/well). After a 24-hour incubation, the cells were washed and stock solutions of inhibitors of substrates were added, resulting apalutamide and M3 concentrations of 0.5 to 50 µM (the maximum nominal concentration of solubility) and 1% DMSO. Cells were incubated either 3 or 10 minutes for OAT or OCT and MATE transporters, respectively. Following incubation, radioactivity and protein were determined by LSC and protein assay kit, respectively					
	OAT1	**OCT2**	**OAT3**	**MATE-1**	**MATE-2K**
Substrate	Para-immuno-hippurate	Metformin	Estrone-3-Sulfate	Tetra-ethyl-ammonium bromide	Tetra-ethyl-ammonium bromide
Concentration (µM)	5	10	1	40	40
Incubation time (min)	3	10	3	10	10
Control inhibitor	Probenecid	Quinidine	Probenecid	Quinidine	Pyrimethamine
Inhibitor concentration (µM)	300	300	300	100	100
			Transport (pmol/mg·min)		
Parent	0.36 (0.02)	0.40 (0.02)	0.76 (0.13)	3.72 (0.32)	3.78 (0.11)
Transfected	80.9 (5.2)	39.5 (2.9)	7.1 (0.5)	76.7 (3.2)	90.8 (1.9)
Ratio	225	99.2	9.4	20.6	24.0
Parent + inhibitor	0.44 (0.01)	0.23 (0.02)	0.74 (0.05)	4.96 (0.28)	2.23 (0.19)
Transfected + inhibitor	3.63 (0.35)	0.46 (0.04)	0.92 (0.05)	13.8 (0.94)	2.89 (0.47)
Ratio	8.21	2.06	1.24	2.78	1.29
Concentration			Inhibition with JNJ-56021927 (% of inhibition)		
0 µM	0.0 (5.8)	0.0 (4.2)	0.0 (6.4)	0.0 (1.5)	0.0 (3.0)
0.50 µM	-1.9 (2.6)	4.9 (1.0)	7.5 (1.9)	0.7 (3.7)	-3.2 (3.6)
2.0 µM	-4.6 (1.0)	9.0 (1.1)	16.8 (4.0)	12.4 (5.1)	2.0 (1.4)
5.0 µM	-12.5 (5.6)	13.2 (5.5)	30.6 (0.8)	23.2 (4.1)	9.0 (2.8)
10 µM	-4.5 (1.2)	28.5 (0.3)	49.8 (3.1)	39.8 (1.6)	22.8 (1.7)
25 µM	-14.1 (1.7)	50.4 (0.0)	66.7 (1.0)	68.3 (0.4)	40.0 (1.6)
50 µM	-13.1 (0.2)	66.4 (0.3)	76.6 (1.2)	86.7 (0.8)	56.7 (1.3)
IC_{50} (µM)	> 50	27.2 (1.69)	12.0 (1.0)	13.8 (0.81)	37.9 (2.61)
Additional Information: Radioisotopes used were [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3), [^{3}H]esterone-3-sulfate, P-[glycyl-1-^{14}C]aminohippuric acid, [^{14}C]metformin, and [^{14}C]TEA. Cell lines were: OCT2 (SLC22A2), OAT1 (SLC22A6), OAT3 (SLC22A8), MATE1 (SLC47A1), and MATE-2K (SLC47A2).					
DMSO = dimethyl sulfoxide; IC_{50} = concentration producing 50% inhibition; LSC = liquid scintillation counting; OAT = organic anion transporter; OCT = organic cation transporter; M3 = JNJ-56142060, the *N*-desmethyl metabolite of apalutamide; MATE = multi-antimicrobial extrusion protein; TEA = tetraethylammonium					

Continued

2.6.5.15　D　薬物動態試験：薬物相互作用（続き）

Test Article: JNJ-56021927 (M3)

Study No.			FK11028		
Location in CTD:			4.2.2.6.4		
Type of Study: Inhibition potential of apalutamide and M3 on OCT2, OAT1, OAT3, MATE-1 and MATE-2K in cell lines overexpressing these transporters					
	OAT1	**OCT2**	**OAT3**	**MATE-1**	**MATE-2K**
Substrate	Para-immuno-hippurate	Metformin	Estrone-3-Sulfate	Tetra-ethyl-ammonium bromide	Tetra-ethyl-ammonium bromide
Concentration (μM)	5	10	1	40	40
Incubation time (min)	3	10	3	10	10
Control inhibitor	Probenecid	Quinidine	Probenecid	Quinidine	Pyrimethamine
Inhibitor concentration (μM)	300	300	300	100	100
Concentration			Inhibition with JNJ-56142060, M3 (% of inhibition)		
0 μM	0.0 (2.4)	0.0 (1.6)	0.0 (14.2)	0.0 (1.0)	0.0 (3.1)
0.50 μM	-1.9 (0.9)	10.0 (0.9)	14.9 (1.3)	0.2 (2.8)	-6.7 (3.5)
2.0 μM	-5.6 (7.3)	29.2 (2.9)	27.0 (1.5)	4.2 (4.1)	-8.0 (3.7)
5.0 μM	-5.0 (2.8)	50.9 (0.9)	42.4 (2.9)	11.9 (2.6)	-5.6 (3.8)
10 μM	-7.5 (4.0)	69.0 (0.2)	60.7 (1.4)	34.5 (2.4)	11.4 (3.0)
25 μM	-5.9 (3.2)	88.3 (0.4)	73.2 (3.3)	59.8 (1.6)	21.4 (2.9)
50 μM	-9.9 (0.3)	90.9 (0.3)	83.9 (0.8)	80.1 (0.7)	32.3 (1.2)
IC_{50} (μM)	**> 50**	**4.76 (0.18)**	**7.59 (1.25)**	**17.6 (0.83)**	**> 50**

Additional Information: Radioisotopes used were [^{14}C]JNJ-56021927 (^{14}C-labeled apalutamide), [^{14}C]JNJ-56142060 (^{14}C-labeled M3) [^{3}H]esterone-3-sulfate, P-[glycyl-1-^{14}C]aminohippuric acid, [^{14}C]metformin, and [^{14}C]TEA. Cell lines were: OCT2 (SLC22A2), OAT1 (SLC22A6), OAT3 (SLC22A8), MATE1 (SLC47A1), and MATE-2K (SLC47A2).

DMSO = dimethyl sulfoxide; IC_{50} = concentration producing 50% inhibition; LSC = liquid scintillation counting;OAT = organic anion transporter; OCT = organic cation transporter; M3 = JNJ-56142060, the *N*-desmethyl metabolite of apalutamide; MATE = multi-antimicrobial extrusion protein; TEA = tetraethylammonium

2.6.5.16　薬物動態試験：その他

Test Article: JNJ-56021927

Not Applicable