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略号一覧表

略号又は用語	定義（英語）	定義（日本語）
C3	complement component 3	補体 C3
C5a	complement component 5a	補体 C5a
C5b-9	terminal complement complex	終末補体複合体
PNH	paroxysmal nocturnal hemoglobinuria	発作性夜間ヘモグロビン尿症

2.6.1　緒言

1　薬理学的特性及び化学構造

ダニコパンは、補体介在性疾患に対する選択的補体 D 因子阻害剤である。

補体 D 因子は、補体第二経路の律速段階である補体 B 因子の開裂を触媒するセリンプロテアーゼである。補体 D 因子は血漿中に少量存在する補体タンパク質の 1 つであり、補体第二経路の活性化及び増幅に不可欠で、補体 C3（C3）フラグメントによるオプソニン化、アナフィラトキシン補体 C3a 及び補体 C5a（C5a）の放出、並びに終末補体複合体（C5b-9）の活性化をもたらす。補体 D 因子は急性期タンパク質ではない。補体 D 因子の阻害は補体第二経路を介した補体活性の増幅を阻害するが、補体古典経路及び補体レクチン経路は直接阻害しない。

発作性夜間ヘモグロビン尿症（PNH）は、終末補体経路の制御不能な活性化により生じる、生命を脅かす後天性の溶血性疾患であり、指定難病である。慢性的で制御不能な補体 C5 の開裂及び C5a の放出、並びに C5b-9 の形成により、赤血球が溶血する。

ダニコパンは補体 D 因子活性部位に可逆的かつ高親和性で結合し、その酵素活性を強力に阻害した。ダニコパンは、ex vivo で PNH 赤血球への C3 フラグメントの沈着を阻害した。さらに、ダニコパンは補体古典経路及び補体レクチン経路に特異的な成分を阻害せず、終末補体経路の成分も阻害しないが、補体古典経路及び補体レクチン経路を介して開始される補体第二経路を介した補体活性の増幅を阻害した。

ダニコパンの構造式を Figure 1 に示す。

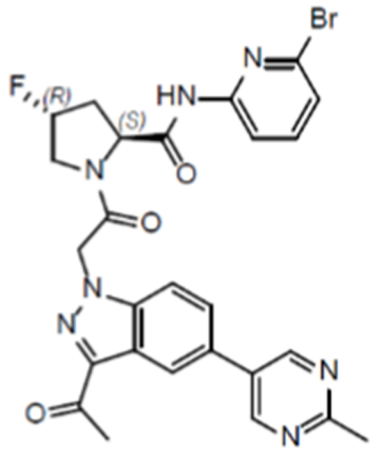


Figure 1:　　**ダニコパンの構造式**

2　効能・効果及び用法・用量

[効能・効果]

発作性夜間ヘモグロビン尿症

[用法・用量]

通常、成人には、補体（C5）阻害剤との併用において、ダニコパンとして 1 回 150 mg を 1 日 3 回食後に経口投与する。なお、効果不十分な場合には、1 回 200 mg まで増量することができる。

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略号一覧表

略語又は用語	定義（英語）	定義（日本語）
aHUS	atypical hemolytic uremic syndrome	非典型溶血性尿毒症症候群
AP	(complement) alternative pathway	補体第二経路
AUC_{0-24}	area under the plasma concentration-time curve from time 0 to 24 hours	投与 0 時間から 24 時間後までの血漿中濃度-時間曲線下面積
BSEP	bile salt export pump	胆汁酸塩排出ポンプ
C3	component complement 3	補体 C3
C3b	C3b fragment of C3 complement protein	補体 C3b
C3bB	C3bB complement component complex; comprising factor B [FB] in complex with C3b, an activation product of complement component 3 [C3]	補体 C3（C3）の活性化産物である C3b と補体 B 因子（FB）の複合体
C5	complement component 5	補体 C5
CC_{50}	50% cytotoxic concentration	50%細胞毒性濃度
C_{max}	maximum observed concentration	最高血漿中濃度
CNS	central nervous system	中枢神経系
CP	(complement) classical pathway	補体古典経路
ECG	electrocardiography	心電図
ELISA	enzyme-linked immunosorbent assay	エンザイムイムノアッセイ（酵素免疫定量法）
EVH	extravascular hemolysis	血管外溶血
GLP	Good Laboratory Practice	医薬品の安全性に関する非臨床試験の実施の基準
FXR	Farnesyl X nuclear receptor	ファルネシル X 核内受容体
hERG	Human ether-à-go-go-related gene	ヒト ether-à-go-go 関連遺伝子
IC_{50} (or IC_{90})	50% (or 90%) inhibitory concentration	50%（又は 90%）阻害濃度
k_a	association rate constant	結合速度定数
k_d	dissociation rate constant	解離速度定数
K_D	equilibrium dissociation constant	平衡解離定数
K_i	inhibitory constant	阻害定数
MDR3	multidrug resistance protein 3	-
MRP4	multidrug resistance protein 4	-
MT_1	Melatonin receptor subtype 1	メラトニン受容体サブタイプ 1
MT4	A human T-cell line	-
NCTP	sodium taurocholate co-transporting polypeptide	タウロコール酸ナトリウム共輸送ポリペプチド
PD	pharmacodynamic	薬力学的作用
PIGA	phosphatidylinositol N-acetylglucosaminyltransferase subunit A	-
PK	pharmacokinetic	薬物動態

略語又は用語	定義（英語）	定義（日本語）
PNH	paroxysmal nocturnal hemoglobinuria	発作性夜間ヘモグロビン尿症
QRS	group of electrocardiogram waves comprising the Q, R, and S waves	-
QT	time interval between Q wave and T wave in the heart's electrical cycle	-
QTc	corrected QT interval	補正 QT 間隔
SBA	serum bactericidal activity	血清殺菌活性
SPR	surface plasmon resonance	表面プラズモン共鳴
UGT1A1	uridine diphosphate glucuronosyltransferase 1A1	ウリジン二リン酸グルクロノシルトランスフェラーゼ 1A1
UGT2B7	uridine diphosphate glucuronosyltransferase 2B7	ウリジン二リン酸グルクロノシルトランスフェラーゼ 2B7

2.6.2　薬理試験の概要文

1　まとめ

ダニコパン（ALXN2040 又は ACH-0144471）は、発作性夜間ヘモグロビン尿症（PNH）などの補体介在性疾患の治療のためにアレクシオンファーマ合同会社が開発している経口低分子補体 D 因子阻害薬である。

ダニコパンは活性部位で補体 D 因子に可逆的に結合し、その親和性はナノモル以下であることが X 線結晶構造解析により確認された（ACH-15-147 試験；ACH-15-082 試験）。ダニコパンは合成基質（ACH-15-083 試験）及びその天然基質 C3bB（補体 C3［C3］の活性化産物である C3b と、補体 B 因子の複合体）に対するアッセイにより補体 D 因子セリンプロテアーゼ活性を阻害することが示された（ACH-15-084 試験；ACH-15-085 試験）。

血清中において、ダニコパンは補体古典経路及び補体レクチン経路ではなく、補体第二経路（AP）を選択的に阻害する（ACH-15-086 試験）。ダニコパンは、溶血試験及び Wieslab 酵素免疫定量法（ELISA）での AP 活性を強力かつ選択的に阻害する（ACH-15-086 試験；ACH-15-088 試験；ACH-15-089 試験）。さらに、ダニコパンは PNH 患者から採取した赤血球の溶血を阻害する（ACH-17-074 試験；Yuan, 2017）。ダニコパンは、異種（ウサギ）PNH 様赤血球及び PNH 患者由来赤血球を含む感受性細胞上の補体 AP 介在性 C3 フラグメント沈着を効果的に阻害する（ACH-15-091 試験；；Yuan, 2017）。

ダニコパンは、ヒト血清並びにサル、イヌ及びウサギの血清中の AP 活性を阻害する（ウサギでは阻害の程度は低い）が、ラット及びマウスの AP 活性は阻害せず、種特異性を示す（ACH-15-087 試験；ACH-15-088 試験；ACH-15-089 試験）。ダニコパンを経口投与したサル血清の ex vivo 分析で、AP 活性のほぼ完全な濃度依存的抑制が認められた（ACH-15-094 試験）。ビーグル犬にダニコパンを経口投与して得られた血清の ex vivo 分析により、この動物種における薬理活性が確認され（ACH-15-093 試験）、5 日間の GLP 非適用忍容性及び毒性試験（ACH-15-119 試験）、並びに 14 日間、13 週間及び 39 週間の GLP 適用反復投与毒性試験の 4 試験において、迅速かつ用量依存的な AP 阻害が確認された（ACH-15-073 試験；ACH-15-160 試験；ACH-16-057 試験）。胚・胎児発生に関する試験で得られた妊娠ウサギの母動物検体でも AP 阻害が認められた（ACH-15-163 試験）。

In vitro オフターゲット活性実験の結果から、ダニコパンの細胞毒性は限定的で（ACH-15-095 試験）、ミトコンドリア毒性のリスクは低く（ACH-15-096 試験）、骨髄前駆細胞増殖に対する作用は最小である（ACH-15-110 試験；ACH-15-133 試験）ことが示された。

ダニコパンは、オフターゲットの非特異的セリンプロテアーゼのパネルに対して、10 µmol/L 濃度で 50%以上の阻害と定義した有意な阻害を示さなかった（ACH-15-063 試験）。受容体、イオンチャネル及びトランスポーターの広範なパネルに対して、ダニコパンは検討した 55 のオフターゲット受容体及びイオンチャネルのうち 52 へのリガンド結合を阻害せず（ACH-15-043 試験）、ヒト A_1 及び A_3 アデノシン受容体サブタイプ並びに、より高い活性で、ヒトメラトニン受容体サブタイプ 1（MT_1）受容体に対してのみ阻害作用を示した（ACH-15-061 試験）。また、ダニコパンはヒト MT_1 機能（ACH-15-061 試験）及びイヌ MT_1 へのリガンド結合（ACH-17-132 試験）の強力なアンタゴニストとして作用するが、組織を用いた機能アッセイではラット MT_1 の弱い阻害剤であった（ACH-16-048 試験）。しかし、イヌ MT_1 に対する強力な作用にもかかわらず、イヌ又はラットを用いた毒性試験では中枢神経系（CNS）関連の徴候は認められていない（モジュール 2.6.6）。

さらに 2 つの安全性パネルを用いて評価したところ、ダニコパンは 12 種類の心臓イオンチャ

ネルのうち $hCa_v1.2$ カルシウムチャネルのみを阻害し（ACH-15-069 試験）、44 種類の薬物乱用に関連する受容体に対して有意な作用を示さなかった（ACH-20-021 試験）。

一連の肝胆道系に対する影響を検討したところ、ダニコパンはヒト、イヌ及びラットの肝細胞からの胆汁酸塩排出ポンプ（BSEP）胆汁酸塩排出トランスポーターをある程度阻害した（ACH-16-097 試験）。また、ダニコパンは、多剤耐性トランスポーターである MDR3 及び MRP4（ACH-16-085 試験；ACH-16-082 試験）、ウリジン二リン酸グルクロノシルトランスフェラーゼ 1A1（UGT1A1）及びウリジン二リン酸グルクロノシルトランスフェラーゼ 2B7（UGT2B7）（ACH-15-106 試験）、並びにファルネシル X 核内受容体（FXR）（ACH-16-093 試験）を阻害した。サンドイッチ培養肝細胞を用いた in vitro 試験では、複数の機序を介してダニコパンが胆汁酸恒常性を阻害する可能性が示されたが、胆汁酸排出は阻害されなかった（ACH-16-021 試験）。

被験微生物として大腸菌（*E coli*）及び髄膜炎菌（*N meningitidis*）を用いた in vitro 試験において、ダニコパン投与の潜在的な感染リスクを評価した。大腸菌を用いた試験において、ダニコパンは血清殺菌活性（SBA）（ACH-15-143 試験）並びにヒト単球及び顆粒球によるオプソニン化食食作用（ACH-15-143 試験；ACH-16-066 試験）を阻害しなかった。髄膜炎菌の複数の分離株を用いたところ、対照薬の C3 阻害剤及び補体 C5（C5）阻害剤と比較して、ダニコパンの全血及び血清による殺作用の阻害は低かった（ACH-19-015 試験）。これらの所見は、特にワクチン接種した個人において、ダニコパンは髄膜炎菌を含む臨床的に意味のある病原体に対して感染リスクが低いことを示唆する。

ダニコパンの安全性薬理を包括的評価した。ラット及びマウスでは薬理作用を示さないことから、ビーグル犬を用いて in vivo 安全性薬理試験を実施した。安全性薬理試験において、ダニコパンの 10 µmol/L でヒト ether-à-go-go 関連遺伝子（hERG）イオンチャネルがわずかに 29.9%阻害されたが（ACH-15-109 試験）、ALXN2040-PNH-301 試験においてヒトに 200 mg を 1 日 3 回投与したときの最高血漿中濃度（C_{max}）と比較して、hERG に対する安全域が約 117 倍高いことから、ダニコパンは QT 延長のリスクは低いと考えられた。さらに、イヌに最高 500 mg/kg を単回投与しても、血行動態又は心電図（ECG）の評価項目に影響は認められなかった（ACH-15-072 試験）。イヌでは、1 日総投与量が 1000 mg/kg/日まで、呼吸機能又は CNS 機能に対する影響は認められなかった（ACH-15-073 試験）。

非臨床薬理試験の要約を Table 1 に示す。これらの試験結果は、血管外溶血（EVH）を発現している PNH 患者に対する治療薬としてのダニコパンの使用を支持すると考えられる。

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
Primary Pharmacodynamics			
ACH-15-082	Binding by danicopan to human FD protein as assessed by SPR (in vitro)	Danicopan	● High-affinity and reversible binding to recombinant human FD: ○ K_D = 0.00054 µmol/L (0.540 nmol/L) k_a = 2.93E6 M^{-1} s^{-1} k_d = 0.00157 s^{-1}
ACH-15-083	Inhibition by danicopan of catalytic activity of human FD protein (in vitro)	Danicopan	● Inhibition of proteolytic activity of purified human FD against a nonspecific thioester substrate (Z-Lys-SBzl): ○ IC_{50} = 0.035 µmol/L
ACH-15-084	Inhibition by danicopan of catalytic activity of human FD protein (in vitro)	Danicopan	● Inhibition of proteolytic activity of purified human FD against its natural substrate (C3bB): ○ IC_{50} = 0.018 µmol/L
ACH-15-085	Inhibition by danicopan of catalytic activity of human FD protein (in vitro)	Danicopan	● Inhibition of proteolytic activity of purified human FD against its natural substrate (C3bB) (kinetic parameters determined using competitive inhibition model): ○ K_i = 0.0057 µmol/L (= 5.7 nmol/L)
ACH-15-147	Co-crystal structure of danicopan in complex with human FD protein	Danicopan	● Compound binding to FD enzyme at active site

Table 1: 非臨床薬理試験一覧

Study Number	**Species/Design**	**Test Article**	**Noteworthy Findings**
ACH-15-086	Inhibition by danicopan of human serum AP, CP, and LP activity (in vitro)	Danicopan	● Inhibition of human serum AP activity in hemolysis and Wieslab assays (dilute serum format): ○ AP hemolysis assay (8% serum): IC_{50} = 0.027 µmol/L IC_{90} = 0.092 µmol/L ○ AP Wieslab assay (5.6% serum): IC_{50} = 0.034 µmol/L IC_{90} = 0.11 µmol/L ● No inhibition of human serum CP or LP activity

Table 1: 非臨床薬理試験一覧

Study Number	**Species/Design**	**Test Article**	**Noteworthy Findings**
ACH-15-087	Inhibition by danicopan of animal serum AP activity (in vitro)	Danicopan	● Inhibition of AP activity in serum from cynomolgus monkeys, dogs, and rabbits ○ Cynomolgus monkey, AP hemolysis assay (8% serum): IC_{50} = 0.0091 µmol/L IC_{90} = 0.026 µmol/L ○ Dog, AP hemolysis assay (8% serum): IC_{50} = 0.025 µmol/L IC_{90} = 0.073 µmol/L ○ Rabbit, AP hemolysis assay (10% serum): IC_{50} = 0.13 µmol/L IC_{90} = 0.453 µmol/L ● No inhibition of AP activity in serum from rats or mice ○ Rat, AP hemolysis assay (22% serum): IC_{50} > 10 µmol/L ○ Mouse, AP hemolysis assay (25% serum): IC_{50} > 10 µmol/L

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-088	Inhibition by danicopan of human and animal serum AP activity in "undiluted serum" format (in vitro)	Danicopan	● Inhibition of AP activity in human serum in "undiluted serum" format: o Human, AP hemolysis ("undiluted serum" format, final 8.3% serum in assay reactions) IC_{50} = 0.22 µmol/L IC_{90} = 0.84 µmol/L o Human AP Wieslab ("undiluted serum" format, final 5.5% serum in assay reactions) IC_{50} = 0.27 µmol/L IC_{90} = 0.86 µmol/L ● Inhibition of AP activity in cynomolgus monkey and beagle dog serum in "undiluted serum" format: o Cynomolgus monkey, AP hemolysis assay ("undiluted serum" format, final 8.3% serum in assay reactions): IC_{50} = 0.078 µmol/L IC_{90} = 0.27 µmol/L o Cynomolgus monkey, AP Wieslab assay ("undiluted serum" format, final 5.5% serum in assay reactions): IC_{50} = 0.066 µmol/L IC_{90} = 0.27 µmol/L o Beagle dog, AP hemolysis assay ("undiluted serum" format, final 8.3% serum in assay reactions): IC_{50} = 0.43 µmol/L IC_{90} => 1.0 µmol/L

ACH-15-089	Inhibition by danicopan of AP activity in human, cynomolgus monkey, beagle dog serum from individual donors (in vitro)	Danicopan	● Inhibition of AP activity in human serum from healthy individual donors: ○ Human, AP hemolysis (8% serum) IC_{50} = 0.0020 – 0.048 µmol/L IC_{90} = 0.0094 – 0.12 µmol/L (range, 20 individuals) ○ Human, AP Wieslab (“undiluted serum” format, final 5.6% serum in assay reactions) IC_{50} = 0.043 – 0.80 µmol/L IC_{90} = 0.093 – 1.9 µmol/L (range, 20 individuals) ● Inhibition of AP activity in cynomolgus monkey and beagle dog serum from individual animals. ○ Cynomolgus monkey, AP hemolysis (8% serum) IC_{50} = 0.0059 –0.033 µmol/L IC_{90} = 0.024 – 0.089 µmol/L (range, 10 animals) ○ Cynomolgus monkey, AP Wieslab (“undiluted serum” format, final 5.6% serum in assay reactions) IC_{50} = 0.022 – 0.31 µmol/L IC_{90} = 0.12 – 0.76 µmol/L (range, 10 animals) ○ Beagle dog, AP hemolysis (8% serum) IC_{50} = 0.044 – 0.31 µmol/L IC_{90} = 0.13 – 0.76 µmol/L (range, 10 animals)

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-090	Inhibition by danicopan of AP-dependent hemolysis using artificial PNH cells (in vitro)	Danicopan	● Inhibition of AP-dependent hemolysis in a PNH model comprising artificial PNH erythrocytes (normal erythrocytes treated with 2-amino-ethylisothiouronium bromide [AET]) and acidified human serum ○ Hemolysis assay (20% serum) IC_{50} = 0.021 µmol/L
ACH-15-091	Inhibition by danicopan of C3 fragment deposition on rabbit erythrocytes and on artificial PNH cells (in vitro)	Danicopan	● Inhibition of C3 fragment deposition on rabbit erythrocytes (20% C5-depleted serum) ○ IC_{50} = 0.096 µmol/L ● Inhibition of C3 fragment deposition on artificial PNH cells (erythrocytes treated with 2-amino-ethylisothiouronium bromide [AET]) (32% acidified C5-depleted serum) ○ IC_{50} = 0.027 µmol/L

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
（Yuan, 2017）	Inhibition by danicopan of AP-mediated hemolysis and C3-fragment deposition using erythrocytes from patients with PNH (ex vivo), and, Inhibition by danicopan of AP-mediated killing in a modified Ham test using serum from patients with aHUS (ex vivo)	Danicopan	● Inhibition of AP-mediated hemolysis of erythrocytes collected from 3 patients with PNH (20% acidified serum) ○ Patient 1 (type III clone size, 67%) IC_{50} = 0.027 µmol/L IC_{90} = 0.11 µmol/L ○ Patient 2 (type III clone size, 98%) IC_{50} = 0.0040 µmol/L IC_{90} = 0.015 µmol/L ○ Patient 3 (type III clone size, 13%) IC_{50} = 0.011 µmol/L IC_{90} = 0.029 µmol/L ● Inhibition of C3-fragment deposition on erythrocytes from patients with PNH (60% acidified C5-depleted serum) ○ IC_{50} = 0.031 µmol/L IC_{90} = 0.089 µmol/L (1 PNH patient) ● Inhibition of AP-mediated killing of PIGA-null reagent cells using serum collected from 5 patients with aHUS (20% serum) ○ Substantial reduction in cell killing at ≥ 0.01 µmol/L danicopan (combined analysis, serum from 4 patients with aHUS)

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-17-074	Inhibition by danicopan of AP activity in 2 standard functional assays (in vitro) and, Inhibition by danicopan of AP-mediated hemolysis erythrocytes from patients with PNH under "physiologic" conditions (ex vivo) (Comparison of test article vs eculizumab)	Danicopan	● Inhibition of AP activity in human serum in "undiluted serum" format: o AP hemolysis ("undiluted serum" format, final 8.3% serum in assay reactions) IC_{50} = 0.20 µmol/L IC_{90} = 0.85 µmol/L (eculizumab, incomplete inhibition) o AP Wieslab ("undiluted serum" format, final 5.6% serum in assay reactions) IC_{50} = 0.24 µmol/L (eculizumab, 0.16 µmol/L) IC_{90} = 1.1 µmol/L (eculizumab, 0.35 µmol/L) ● Inhibition of AP-mediated hemolysis of erythrocytes collected from 2 patients with PNH ("physiologic" conditions, 72% serum) o IC_{50} = 0.037 µmol/L IC_{90} = 0.18 µmol/L (combined analysis, erythrocytes from 2 patients with PNH) (eculizumab, 50% inhibition at 1.3 µmol/L)
ACH-15-093	Combined analyses of the PK-PD relationship following danicopan administration to beagle dogs in 2 PK studies and one tolerability/toxicity study	Danicopan	● Analysis: PK-PD relationship was evaluated using combined results from the following studies: o Single dose PK studies: ACH-15-036 and ACH-15-039 o Multiple dose-tolerability/toxicity study: PK results reported in ACH-15-056, PD results reported separately in ACH-15-119 ● Findings: concentration-dependent inhibition of ex vivo AP activity: o AP hemolysis ("undiluted serum" format, final 8.3% serum in assay reactions) IC_{50} = 198 ng/mL (0.341 µmol/L) (IC_{90} not determined)

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-094	Combined analysis of the PK-PD relationship following danicopan administration to cynomolgus monkeys in single-dose and multiple-dose PK-PD studies	Danicopan	● Analysis: PK-PD relationship was evaluated using combined results from the following studies: o Single-dose and multiple-dose PK-PD study: ACH-15-040 (Module 2.6.4) o Multiple-dose PK-PD study: ACH-15-107 (Module 2.6.4) ● Findings: concentration-dependent inhibition of ex vivo AP activity: o AP hemolysis (“undiluted serum” format, final 8.3% serum in assay reactions) IC_{50} = 46 ng/mL (0.079 µmol/L) (IC_{90} not determined) o AP Wieslab (“undiluted serum” format, final 5.6% serum in assay reactions) IC_{50} = 31 ng/mL (0.053 µmol/L) (IC_{90} not determined) o IC_{90}s not determined, but ≥ 80% (hemolysis assay) or ≥ 90% (Wieslab assay) inhibition was noted at danicopan ≥ 150 ng/mL (0.258 µmol/L) ● Effects on additional complement components: o Reversible reduction in concentration of FB activation product Bb (a direct product of FD activity) o No perturbations in CP activity or FD concentration

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-119	PD analysis of samples from non-GLP tolerability and toxicity study of danicopan in beagle dogs	Danicopan	● PD analysis using samples obtained from ACH-15-056 ● Dose-dependent and reversible AP inhibition in ex vivo serum assays following twice-daily oral administration for 5 days: o Nearly complete AP inhibition through 6 h following administration at 375 mg/kg bid o No perturbations in CP activity or in concentrations of complement C3, C4, or FD o Results from this study contributed to the integrated PK-PD analysis reported elsewhere (ACH-15-093)
ACH-15-073	Exploratory non-GLP complement analysis in GLP 14-day toxicity and toxicokinetic study of danicopan in beagle dogs	Danicopan	● PD results in this toxicity study are presented in ACH-15-073, Appendix 18 ● Dose-dependent and reversible AP inhibition in ex vivo serum assays following twice-daily oral administration to beagle dogs o Nearly complete AP inhibition through at least 4-6 h following administration at all doses o 71% or greater AP inhibition sustained through 12 h following administration at 1000 mg/kg/day (NOAEL) ● Concentration-dependent inhibition established by PK-PD analysis o AP hemolysis (“undiluted serum” format, final 8.3% serum in assay reactions) IC_{50} = 510 ng/mL (0.88 µmol/L) (IC_{90} not determined) ● Gradual and reversible elevations of plasma C3 concentrations by approximately 86% at the highest dose

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-160	Exploratory non-GLP complement analysis in GLP 13-week toxicity and toxicokinetic study of danicopan in beagle dogs	Danicopan	● PD results in this toxicity study are presented in ACH-15-160, Appendix 14 ● Dose-dependent and reversible AP inhibition in ex vivo serum assays following twice-daily oral administration to beagle dogs o Nearly complete AP inhibition through at least 3-4 h following administration at all doses o Nearly complete AP inhibition sustained through 3-4 h following administration at 100 mg/kg/day (NOAEL) ● Concentration-dependent inhibition established by PK-PD analysis o AP hemolysis (“undiluted serum” format, final 8.3% serum in assay reactions) IC_{50} = 555 ng/mL (0.956 µmol/L) (IC_{90} not determined) ● Gradual elevations of plasma C3 concentrations by approximately 100% at higher doses

Table 1: 非臨床薬理試験一覧

Study Number	**Species/Design**	**Test Article**	**Noteworthy Findings**
ACH-16-057	Exploratory non-GLP complement analysis in GLP 39-week toxicity and toxicokinetic study of danicopan in beagle dogs	Danicopan	● PD results in this toxicity study are presented in ACH-16-057, Appendix 17 ● Dose-dependent and reversible AP inhibition in ex vivo serum assays following twice-daily oral administration to beagle dogs o Nearly complete AP inhibition through at least 3-4 h following administration at all doses o Nearly complete AP inhibition sustained through 3-4 h following administration at 75 mg/kg/day (NOAEL) ● Concentration-dependent inhibition established by PK-PD analysis o AP hemolysis (“undiluted serum” format, final 8.3% serum in assay reactions) IC_{50} = 385 ng/mL (0.663 µmol/L) (IC_{90} not determined) ● Gradual and reversible elevations of plasma C3 concentrations by approximately 50% at higher doses

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-163	Exploratory non-GLP complement analysis in GLP embryo/fetal development (EFD) study of danicopan in New Zealand White rabbits (administration from gestational day [GD] 7 through GD20)	Danicopan	● PD results in this toxicity study are presented in ACH-15-163, Appendix I ● Dose-dependent and reversible AP inhibition in ex vivo assays of serum from gravid female New Zealand White rabbits following danicopan administration: o Nearly complete AP inhibition through at least 4-8 h following administration at all doses. o Nearly complete inhibition through 4-8 h following administration at 500 mg/kg/day (NOAEL) ● Concentration-dependent inhibition established by PK-PD analysis o AP hemolysis (“undiluted serum” format, final 28% serum in assay reactions) IC_{50} = 1285 ng/mL (2.21 µmol/L) (IC_{90} not determined)
Secondary Pharmacodynamics			
ACH-15-095	Evaluation of cellular toxicity of danicopan in human cell lines and primary cells (in vitro)	Danicopan	● Limited cytotoxicity in 4 human cell lines and 2 primary human cell types o Cell lines: HepG2: CC_{50} = 40.2 µmol/L Huh7: CC_{50} = 38.9 µmol/L MT4: CC_{50} > 50 µmol/L Hep2: CC_{50} = 42.4 µmol/L o Primary cells: Hepatocytes: CC_{50} > 50 µmol/L PBMCs: CC_{50} > 50 µmol/L

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-096	Evaluation of mitochondrial toxicity potential of danicopan in human cell lines cultured under conditions of dependence on mitochondrial respiration (in vitro)	Danicopan	● No mitochondrial toxicity in 2 human cell lines ○ MT4 cells Glucose media: CC_{50} = 66.6 µmol/L Galactose media: CC_{50} = 58.3 µmol/L Ratio: 1.1 ○ HepG2 cells Glucose media: CC_{50} > 100 µmol/L Galactose media: CC_{50} > 100 µmol/L Ratio: NA
ACH-15-110	Effect of danicopan on human myeloid, erythroid, and megakaryocyte progenitors (in vitro)	Danicopan	● Minimal inhibition of colony formation from clonogenic progenitor cells by 50 µmol/L danicopan: ○ Erythroid and myeloid: no effect Megakaryocyte: reduced proliferation (at 50 µmol/L danicopan)
ACH-15-133	Effect of danicopan on human megakaryocyte progenitors (in vitro)	Danicopan	● Limited inhibition of colony formation from clonogenic progenitor cells: ○ Megakaryocyte: IC_{50} = 49.5 µmol/L
ACH-15-063	Effect of danicopan on human serine protease panel (in vitro)	Danicopan	● Limited inhibition of a panel of 10 serine proteases ○ All targets: < 50% inhibition at 10 µmol/L danicopan

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-043	Effect of danicopan on a panel of 55 targets comprising receptors, ion channels, and transporters (in vitro)	Danicopan	● Limited off-target inhibition of panel of receptors, ion channels, and transporters: ○ Ligand-binding assays: Human A_1 adenosine receptor: 89% inhibition Human A_3 adenosine receptor: 77.2% inhibition Human MT_1 melatonin receptor: 99.2% inhibition Other targets (n = 52): < 50% (percentage inhibition at 10 µmol/L danicopan)
ACH-15-061	Effect of danicopan on human A_1, A_3, and MT_1 receptors (in vitro)	Danicopan	● Inhibition of selected molecular targets: ○ Ligand-binding assays: Human A_1: IC_{50} = 2.7 µmol/L; K_i = 1.7 µmol/L Human A_3: IC_{50} = 2.7 µmol/L; K_i = 1.6 µmol/L Human MT_1: IC_{50} = 0.0082 µmol/L; K_i = 0.0065 µmol/L ○ Functional assays (antagonist effect): Human A_3: IC_{50} = 0.68 µmol/L; K_B = 6.1 µmol/L Human MT_1: IC_{50} = 0.038 µmol/L; K_B = 0.0035 µmol/L
ACH-17-132	Effect of danicopan on dog MT_1 receptor (in vitro)	Danicopan	● Inhibition of ligand binding to dog MT_1 receptor: ○ Ligand-binding assay: Dog MT_1: IC_{50} = 0.0057 µmol/L; K_i = 0.0046 µmol/L
ACH-16-048	Effect of danicopan on rat MT_1 receptor in tissue based functional assay (in vitro)	Danicopan	● Inhibition of rat MT_1 receptor in tissue-based functional assay ○ Rat caudal artery ring contraction: Rat MT_1: IC_{50} = 7.80 µmol/L

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-20-025	Analysis of MT_1 sequences from human, dog, rabbit, and rat	NA	● Sequence alignment and phylogenetic analysis: o High identity among human, dog, and rabbit MT_1 Greater distance between human and rat MT_1
ACH-15-069	Effect of danicopan on cardiac ion channels expressed in mammalian cells (in vitro)	Danicopan	● Limited off-target inhibition of cardiac ion channel activity: o Functional assays (patch clamp): Human calcium channel $hCa_V1.2$: 59.2% inhibition Other targets (n = 11): < 50% inhibition (percentage inhibition at 5 µmol/L danicopan)
ACH-20-021	Effect of danicopan on receptors, ion channels, and transporters associated with abuse liability (in vitro)	Danicopan	● No inhibition of ligand binding to 44 abuse liability receptors o Ligand-binding assays: All targets (n = 44): < 50% inhibition (percentage inhibition at 10 µmol/L danicopan)
ACH-16-097	Effect of danicopan on BSEP bile salt efflux transporter in primary human, dog, and rat hepatocytes (in vitro)	Danicopan	● Inhibition of BSEP bile salt efflux transporter activity: o Transporter assay in hepatocytes: Human BSEP: IC_{50} = 33.8 µmol/L Dog BSEP: IC_{50} = 18.9 µmol/L Rat BSEP: IC_{50} = 3.4 µmol/L
ACH-16-085	Effect of danicopan on MDR3 activity in primary human hepatocytes (in vitro)	Danicopan	● Inhibition of MDR3 transporter activity in human hepatocytes o Transporter assay in hepatocytes IC_{50} = 10.9 µmol/L

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-16-082	Effect of danicopan on human MRP3, MRP4, and NTCP transporter activity (in vitro)	Danicopan	● Inhibition of MRP3, MRP4, and NTCP transporter activity o Transporter assay in Sf9 membrane vesicles: MRP3: IC_{50} = 66.6 µmol/L MRP4: IC_{50} = 3.46 µmol/L o Transporter assay in MDCK cells: NTCP: < 50% inhibition at 30 µmol/L danicopan
ACH-15-106	Effect of danicopan on catalytic activity of uridine 5'-diphosphoglucuronosyl transferase (UGT) enzymes in human liver microsomes (in vitro)	Danicopan	● Inhibition of UGT metabolic enzymes o Enzymatic assay in human liver microsomes: UGT1A1: IC_{50} = 0.81 µmol/L UGT2B7: IC_{50} = 0.82 µmol/L
ACH-16-093	Effect of danicopan on activity of Farnesoid X Receptor (FXR) (in vitro)	Danicopan	● Modulation of FXR activity o Reporter cell assay: agonist activity: none antagonist activity: 54% inhibition at 33.3 µmol/L danicopan
ACH-16-021	Effect of danicopan on bile acid hepatobiliary disposition using hepatocytes in a sandwich-culture system (Sandwich-Cultured Transporter Certified™ Human Hepatocytes, SCHH) (in vitro)	Danicopan	● No effect on bile acid biliary efflux in SCHH system ● Disruption of bile acid homeostasis in SCHH system o Reduced endogenous bile acid accumulation and content o Reduced responses of FXR target genes at 3 µmol/L and 30 µmol/L danicopan ● Multiple potential mechanisms including FXR antagonism and cytotoxicity

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-16-065	Evaluation of pharmacological profile (AP inhibition, cytotoxicity, mitochondrial toxicity) of danicopan metabolites ACH-0144240, ACH-0144709, ACH-0145069, and ACH-0145071	Danicopan metabolites: ACH-0144240 and ACH-0144709 (amide hydrolysis) ACH-0145069 (carbonyl reduction) ACH-0145071 (amide hydrolysis and carbonyl reduction)	● No significant inhibition of AP activity by danicopan metabolites ○ AP hemolysis (8.3% serum) ACH-0144709: IC_{50} > 1 µmol/L; IC_{90} > 1 µmol/L ACH-0145069: IC_{50} = 9.27 µmol/L; IC_{90} = 37.8 µmol/L ACH-0145071: IC_{50} > 1 µmol/L; IC_{90} > 1 µmol/L ● Limited cytotoxicity of danicopan metabolites in 2 human cell lines ○ Huh7 ACH-0145069: CC_{50} = 88.00 µmol/L ACH-0145071: CC_{50} > 200 µmol/L ○ MT4 ACH-0145069: CC_{50} > 100 µmol/L ACH-0145071: CC_{50} > 100 µmol/L ● No mitochondrial toxicity of danicopan metabolites in one human cell line ○ HepG2 All 4 metabolites Glucose media: CC_{50} > 100 µmol/L Galactose media: CC_{50} > 100 µmol/L Ratio: NA
RTR-0065	Evaluation of pharmacological activity (AP inhibition) of danicopan metabolite ACH-0145072	Danicopan metabolite: ACH-0145072 (amide hydrolysis and carbonyl reduction)	● No significant inhibition of AP activity by danicopan metabolite ○ AP hemolysis (8.3% serum) IC_{50} > 1 µmol/L IC_{90} > 1 µmol/L

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-143	Effect of danicopan on human serum bactericidal activity (SBA) against *E. coli* (in vitro)	Danicopan	● Inhibition of serum bactericidal activity (SBA) by pooled normal human serum: No inhibition up to 31.6 µmol/L danicopan
ACH-16-066	Effect of danicopan on opsonophagocytosis of *E. coli* using human monocytes and granulocytes (in vitro)	Danicopan	● Inhibition of opsonophagocytosis by human donor monocytes and granulocytes: No inhibition up to 10 µmol/L danicopan
ACH-19-015	Effect of danicopan on human serum bactericidal activity (SBA) against encapsulated *N meningitidis* isolates (in vitro)	Danicopan	● Inhibition of SBA by pre-vaccination and post-vaccination serum from 1 donor: ○ Pre-vaccination: partial inhibition (2 of 3 isolates) Post-vaccination: no inhibition (0 of 5 isolates) (complete inhibition by comparator complement inhibitors compstatin and eculizumab)
Safety Pharmacology			
ACH-15-026	Effect of danicopan on hERG ion channels	Danicopan	● 6.9% inhibition of hERG channel at 3 µmol/L; precipitation observed at 30 µmol/L
ACH-15-066	Effect of danicopan on hERG ion channels	Danicopan	● 11.8% inhibition of hERG channel at the highest concentration (5 µmol/L) tested
ACH-15-109	Effect of danicopan on hERG ion channels	Danicopan	● 29.9% hERG channel inhibition at the highest concentration without precipitation, 10 µmol/L
ACH-15-072	Cardiovascular telemetry study in conscious beagle dogs	Danicopan	● No effect on qualitative or quantitative ECG parameters of PR interval, QRS duration, QT and QTc interval or on heart rate, blood pressure or body temperature measured at up to 24 h postdose

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-15-073	14-day toxicity study in beagle dogs	Danicopan	● No effect on respiratory function as assessed by measurements of tidal volume, respiratory rate, and minute volume. ● No overt effect on neurobehavioral parameters.
Pharmacodynamic Drug Interactions			
ACH-15-100	Evaluation of combinatory effect of danicopan with other complement inhibitors including compstatin and an anti-C5 antibody (in vitro)	Danicopan	● Overall: moderate to strong synergistic interactions, with no strong antagonistic interactions, as determined by 2 analytical methods ● Danicopan and compstatin (AP hemolysis, 8.3% serum) ○ Three-dimensional surface graphing method: strongly synergistic (slightly antagonistic); (Prichard and Shipman, 1990) ○ Median-effect plot method: additive (moderately synergistic to slightly antagonistic); (Chou and Talalay, 1983) ● Danicopan and anti-C5 antibody (AP Wieslab, 5.6% serum) ○ Three-dimensional surface graphing method: moderately synergistic (Prichard and Shipman, 1990) ○ Median-effect plot method: additive (synergistic to additive); (Chou and Talalay, 1983)

Table 1: 非臨床薬理試験一覧

Study Number	Species/Design	Test Article	Noteworthy Findings
ACH-17-073	Evaluation of combinatory effect of danicopan with compstatin and eculizumab against complement-mediated hemolysis of erythrocytes from patients with PNH (in vivo)	Danicopan	● Overall: moderate to strong synergistic interactions, with no antagonistic interactions, as determined by one or 2 analytical methods ● Danicopan and compstatin (hemolysis of PNH erythrocytes, 20% serum) ○ Three-dimensional surface graphing method: strongly synergistic (Prichard and Shipman, 1990) ○ Median-effect plot method: moderately synergistic (Chou and Talalay, 1983) ● Danicopan and eculizumab (hemolysis of PNH erythrocytes, 20% serum) ○ Three-dimensional surface graphing method: strongly synergistic (Prichard and Shipman, 1990)

Abbreviations: aHUS = atypical hemolytic-uremic syndrome; AP = (complement) alternative pathway; BSEP = bile salt export pump; C3 = complement component 3; C5 = complement component 5; CC_{50} = 50% cytotoxic concentration; CP = (complement) classical pathway; EVH = extravascular hemolysis; FD = factor D; hERG = human ether-à-go-go-related gene; IC_{50} = 50% inhibitory concentration; IC_{90} = 90% inhibitory concentration; K_a = association rate constant; K_d = dissociation rate constant; K_D = equilibrium dissociation constant; K_i = inhibitory constant; GLP = Good Laboratory Practice; LP = (complement) Lectin pathway; NA = not available or not applicable; NOAEL = no-observed-adverse-effect-level; PD = pharmacodynamic; PIGA = phosphatidylinositol N-acetylglucosaminyltransferase subunit A; PK = pharmacokinetic; PNH = paroxysmal nocturnal hemoglobinuria; QRS = group of electrocardiogram waves comprising the Q, R, and S waves; QT = time interval between Q wave and T wave in the heart's electrical cycle; QTc = corrected QT interval; SCHH = Sandwich Cultured Transporter Certified™ Human Hepatocytes; SPR = surface plasmon resonance

2　効力を裏付ける試験

ダニコパンの主要な薬理学的特徴は、ヒト補体 D 因子に対する高い親和性と可逆的結合、ヒト補体 D 因子酵素のセリンプロテアーゼ活性の強力な阻害、及び複数のアッセイ系を用いて確立されたヒト血清中の補体 AP 活性の選択的阻害である。

2.1　In vitro 薬理試験

補体 D 因子酵素に対するダニコパンの試験を以下及び Table 2 に示す。

表面プラズモン共鳴（SPR）実験により、ダニコパンはナノモル以下の親和性でヒト補体 D 因子に可逆的に結合することが確認された（ACH-15-082 試験）。

ダニコパンは、天然基質 C3bB（補体 B 因子と C3b との複合体で、C3 の活性化産物）又は非特異的最小チオエステル基質を用いた酵素アッセイにおいて、精製ヒト補体 D 因子のセリンプロテアーゼ活性を阻害することが示された（Table 2；ACH-15-083 試験；ACH-15-084 試験；ACH-15-085 試験）。最小基質に対する阻害により、ダニコパンは、その基質蛋白質との相互作用よりむしろ補体 D 因子酵素に対する直接作用により補体 D 因子を阻害することが示された。

触媒部位補体 D 因子阻害剤としてのダニコパンの作用は X 線結晶構造解析を用いて確認した（ACH-15-147 試験）。

Table 2:　　ヒト補体 D 因子プロテアーゼに対するダニコパンの結合と阻害

Danicopan Assessment vs FD Binding/Activity	**Result (µmol/L)[a]**	**Study Number**
FD binding, SPR assay	K_D = 0.00054 (n = 1)	ACH-15-082
FD activity, minimal substrate	IC_{50} = 0.035 ± 0.001 (n = 3)	ACH-15-083
FD activity, C3bB substrate	IC_{50} = 0.018 ± 0.007 (n = 5) K_i = 0.0057 ± 0.003 (n = 3)	ACH-15-084 ACH-15-085
FD + danicopan co-crystal structure	Compound binding at active site	ACH-15-147

[a] Where applicable: In units of µmol/L; mean ± SD (n = number of replicates)
Abbreviations: FD = factor D; IC_{50} = 50% inhibitory concentration; K_D = equilibrium dissociation constant; K_i = inhibitory constant; SPR = surface plasmon resonance

血清 AP 活性に対するダニコパンの試験結果を以下及び Table 3 に示す。

ダニコパンは、広く使用されている 2 種類の機能アッセイ、すなわち異種細胞（ウサギ赤血球）の溶血並びに AP 活性化後の終末補体複合体の形成及び沈着を測定する機能的 ELISA（Wieslab アッセイ）を用いて評価した場合、正常ヒト血清中の補体 AP 活性を阻害する（ACH-15-086 試験）。ダニコパンは AP 活性に選択的であり、機能アッセイにおいて補体古典経路又は補体レクチン経路活性の直接的な阻害は認められないが、補体古典経路及び補体レクチン経路を介して開始される AP を介した補体活性の増幅を阻害すると考えられる（ACH-15-086 試験）。

AP 溶血試験及び Wieslab アッセイでは、希釈及び測定の前に無希釈血清にダニコパンを添加する無希釈血清法も実施した（ACH-15-088 試験）。IC_{50} 及び IC_{90} は、ダニコパンの初期血清中濃度に対する相対値であり、したがって、これらの無希釈血清アッセイは、非臨床試験及び臨床試験で薬力学的作用（PD）及び薬物動態（PK）-PD 解析に使用する ex vivo アッセイと in vitro の相関性を示す。さらに、正常ビーグル犬、正常カニクイザル及び健康なヒトドナーの 20 検体から得られた血清パネルを用いて、標準方法及び無希釈血清法の両方において阻害活性が示された（ACH-15-089 試験）。

さらに、ヒト血清中の PNH 赤血球の AP 介在性溶血を評価するアッセイにおいて、ダニコパンを評価した。次に挙げる複数の試験において、ダニコパンが溶血を抑制することが示された：健康な赤血球を 20%血清に添加した人工的な PNH 様赤血球を用いた試験（ACH-15-090 試験）; PNH 患者から採取した赤血球を 20%血清に添加した ex vivo 試験（Yuan, 2017）; PNH 患者から採取した赤血球を 72%血清に添加した生理的状態を反映した ex vivo 試験（ACH-17-074 試験）。さらに、ダニコパンはウサギ赤血球（ACH-15-091 試験）、PNH 様赤血球（ACH-15-091 試験）、及び PNH 患者から採取し ex vivo で血清処理した赤血球（Yuan, 2017）上の AP 介在性 C3 フラグメント沈着を効果的に抑制した。

Table 3: ヒト血清 AP 活性に対するダニコパンの阻害試験

Danicopan Assessment vs AP Activity	**Result (µmol/L)[a]**	**Study Number**
In vitro		
Hemolysis assay (values relative to 8% serum)	IC_{50} = 0.027 ± 0.013 IC_{90} = 0.092 ± 0.026 (n = 16)	ACH-15-086
Wieslab assay (values relative to 5.6% serum)	IC_{50} = 0.034 ± 0.000 IC_{90} = 0.11 ± 0.00 (n = 2)	ACH-15-086
Hemolysis assay, “undiluted serum format” (values relative to 100% serum) [b]	IC_{50} = 0.22 ± 0.06 IC_{90} = 0.84 ± 0.35 (n = 9)	ACH-15-088
Wieslab assay, “undiluted serum format” (values relative to 100% serum) [b]	IC_{50} = 0.27 ± 0.12 IC_{90} = 0.86 ± 0.43 (n = 21)	ACH-15-088
Hemolysis assay, serum from individual donors (values relative to 8% serum)	IC_{50} = 0.0020 - 0.048 IC_{90} = 0.0094 - 0.12 (range, 20 individuals)	ACH-15-089
Wieslab assay, serum from healthy individual donors, “undiluted serum format” (values relative to 100% serum) [b]	IC_{50} = 0.043 - 0.80 IC_{90} = 0.093 - 1.9 (range, 20 individuals)	ACH-15-089
C3 fragment deposition on rabbit erythrocytes, serum depleted of C5 to prevent hemolysis (values relative to 20% serum)	IC_{50} = 0.096 ± 0.021 (n = 2)	ACH-15-091
PNH erythrocytes, ex vivo		

Hemolysis of “PNH-like” erythrocytes produced by chemical treatment of healthy erythrocytes (values relative to 20% serum)	IC_{50} = 0.021 ± 0.0064 (n = 2)	ACH-15-090
Hemolysis of patient PNH erythrocytes (values relative to 20% serum)	IC_{50} = 0.0040 - 0.027 IC_{90} = 0.015 - 0.11 (range, 3 patients with PNH)	(Yuan, 2017)
Hemolysis of patient PNH erythrocytes, “physiologic” conditions (values relative to 72% serum)	IC_{50} = 0.037 ± 0.018 IC_{90} = 0.18 ± 0.08 (combined analysis, 2 patients with PNH)	ACH-17-074
C3 fragment deposition on “PNH-like” erythrocytes, serum depleted of C5 to prevent hemolysis (value relative to 32% serum)	IC_{50} = 0.027 ± 0.0047 (n = 2)	ACH-15-091
C3 fragment deposition on patient PNH erythrocytes (values relative to 60% serum)	IC_{50} = 0.031 IC_{90} = 0.089 (n = 1) (1 PNH patient)	(Yuan, 2017)

[a] Where applicable: in units of µmol/L; mean ± SD (n = number of replicates)
[b] In “undiluted serum” format, compound was added to undiluted serum prior to dilution and assay. IC_{50} and IC_{90} values are expressed relative to concentrations in undiluted serum, providing in vitro correlates of the ex vivo assays used in nonclinical and clinical studies.
Abbreviations: C3 = complement component 3, C5 = complement component 5; IC_{50} = 50% inhibitory concentration; IC_{90} = 90% inhibitory concentration; PNH = paroxysmal nocturnal hemoglobinuria

Table 3 に含まれていない追加試験において、非典型溶血性尿毒症症候群（aHUS）患者から採取された血清下で PIGA 欠損細胞株の死滅をダニコパンが阻害することが示された（Yuan, 2017）。この細胞は、膜結合性補体制御因子 CD55 及び CD59 が欠損しており aHUS 血清に感受性であることから、これらの試験から得られた知見は AP 阻害剤としてのダニコパンの薬理活性を示すものと考えられる。

2.1.1 適切な動物種の選択

ダニコパンのヒト以外の動物種における AP 阻害活性は、Wieslab アッセイ（カニクイザル）及び溶血アッセイ（評価したすべての動物種）を用いて in vitro で評価された。ダニコパンは血清中の AP 活性をカニクイザル及びビーグル犬では阻害し、ウサギでは程度は低いものの AP 活性を阻害したが、ラット及びマウスの血清中では阻害しなかった（ACH-15-087 試験；ACH-15-088 試験；ACH-15-089 試験）。これらの知見から、ダニコパンはビーグル犬及びウサギにおいて薬理活性を示すことが確認され、2.2 項で言及する安全性薬理試験及び毒性試験に使用する動物種として選択した。

2.2 In vivo 薬理試験

複数の非臨床試験で使用した動物種にダニコパンを投与し、Wieslab アッセイ（カニクイザル）

及び溶血アッセイ（評価したすべての動物種）による ex vivo 血清アッセイを実施した結果、全身性 AP 活性の迅速、可逆的かつ用量依存的な阻害が確認された。カニクイザルを用いた複数の PK-PD 試験において、血漿中ダニコパン濃度が 150 ng/mL 以上のとき、ダニコパンは AP 活性を 80%以上（溶血アッセイ）又は 90%以上（Wieslab アッセイ）阻害した（ACH-15-094 試験）。ビーグル犬を用いた複数の PK-PD 試験においても同様の薬理活性が観察され（ACH-15-093 試験）、GLP 非適用の 5 日間忍容性及び毒性試験の検体（ACH-15-119 試験）並びに GLP 適用の 14 日間、13 週間及び 39 週間反復投与毒性試験の検体（ACH-15-073 試験 ; ACH-15-160 試験 ; ACH-16-057 試験）でも用量依存的な AP 阻害作用が認められた。AP 阻害作用は、GLP 適用のウサギを用いた胚・胎児発生に関する試験の母体の検体おいても認められた（ACH-15-163 試験）。

3　副次的薬理試験

ダニコパンの副次的薬理試験では、ヒト細胞株、初代培養細胞及び骨髄前駆細胞を用いた試験において顕著な細胞毒性及びミトコンドリア毒性が認められなかったこと、並びにダニコパンの安全性及び選択性を明らかにするためにデザインされた一連の広範かつ集中的な試験で様々な分子標的に対するオフターゲット作用が限定的であったことが示された。

3.1　In vitro オフターゲット活性

複数のヒト細胞を用いた in vitro 試験において、ダニコパンの細胞毒性の欠如を確認した。細胞毒性作用を直接評価したところ、4 種類のヒト細胞株（HepG2、Huh7、MT4 及び Hep2）に対する CC_{50} は 38.9 µmol/L 以上であり、ヒト初代肝細胞及び末梢血単核細胞（PBMC）に対する CC_{50} は 50 µmol/L 超であった（ACH-15-095 試験）。ダニコパンの細胞毒性は限定的であった。ダニコパンは、2 種類のヒト細胞株（HepG2 及び MT4）を用いて、解糖系に依存せずミトコンドリア機能に完全に依存する培養条件下（ガラクトース培地）で細胞毒性を評価したところ、ミトコンドリア毒性のリスクは低いことが示された（ACH-15-096 試験）。また、ダニコパンは 50 µmol/L の濃度でヒト赤血球又は骨髄細胞のコロニー数又はサイズに影響を及ぼさず、巨核球前駆細胞の増殖をわずかに阻害し、IC_{50} は 49.5 µmol/L であった（ACH-15-110 試験及び ACH-15-133 試験）。

ダニコパンは、構造的に関連のある 10 種類のセリンプロテアーゼパネルに対して評価したところ、10 µmol/L の濃度で 50%以上の阻害と定義した有意な阻害を示さず、補体 D 因子阻害に対する選択性が示された（ACH-15-063 試験）。

広範なプロファイリングの一環として、受容体、イオンチャネル及びトランスポーターのパネルを用いたリガンド結合アッセイにおいて、ダニコパンのオフターゲット作用を 10 µmol/L で評価した。55 種類の標的のうち 52 種類ではリガンド結合の有意な阻害は認められなかったが、ヒト A_1 及び A_3 アデノシン受容体サブタイプ（それぞれ、89%及び 77.2%阻害）並びにヒト MT_1 メラトニン受容体（99.2%阻害）では 50%以上の阻害が認められた（ACH-15-043 試験）。アデノシン受容体を用いたフォローアップ評価において、ヒト A_1 及び A_3 に対する K_i 値はそれぞれ 1.7 µmol/L 及び 1.6 µmol/L であり、機能アッセイにおいてヒト A_3 に対するアンタゴニスト活性が認められた（K_B = 6.1 µmol/L）（ACH-15-061 試験）。ヒト MT_1 のフォローアップ評価では、リガンド結合（K_i = 0.0065 µmol/L）及びアンタゴニスト活性（K_B = 0.0035 µmol/L）のいずれに対しても強力な阻害作用が確認された（ACH-15-061 試験）。非臨床試験動物種の MT_1 に対する阻害作用も評価した。ダニコパンはイヌ MT_1 リガンド結合に対して同様に強力であったが（K_i = 0.0046 µmol/L）（ACH-17-132 試験）、組織ベースの機能アッセイにおいてラット MT_1 に対する効力ははるかに弱

かった（7.80 μmol/L）（ACH-16-048 試験）。これらの相対的効力は、ヒト MT_1 配列がラット MT_1 よりもイヌ MT_1 に類似していることを示した系統発生解析と一致する（ACH-20-025 試験）。なお、イヌ及びラットを用いた毒性試験で CNS 関連の変化は認められなかったことから、観察された強力な MT_1 阻害は毒性学的に重要ではないことが示唆される。

ダニコパンの、心機能に関連するイオンチャネルのパネルに対する影響についても評価した。12 種類のチャネルのうち 11 種類に対して有意な阻害は認められず（5 μmol/L で 50%未満の阻害と定義）、ヒト L 型カルシウムチャネルである $hCa_v1.2$ に対して限定的な阻害（5 μmol/L で 59.2%）がみられた（ACH-15-069 試験）。

ダニコパンは、薬物乱用に関連する 44 種類の受容体パネルに対して 10 μmol/L で試験したとき、リガンド結合の有意な阻害を示さなかった（ACH-20-021 試験）。

一連の肝胆道系に対する影響を検討したところ、ダニコパンは、ヒト及びイヌの肝細胞において BSEP を阻害し、ラットの肝細胞においてより強力な BSEP 活性阻害を示した（IC_{50} 値はそれぞれ 33.8、18.9 及び 3.4 μmol/L）（ACH-16-097 試験）。また、ダニコパンは、機能アッセイにおいて、ヒト MDR3 及び MRP4 トランスポーター（IC_{50} は、それぞれ、10.9 及び 3.46 μmol/L）（ACH-16-085 試験；ACH-16-082 試験）、UGT1A1 及び UGT2B7（IC_{50} は、それぞれ、0.81 及び 0.82 μmol/L）（ACH-15-106 試験）、並びに FXR（33.3 μmol/L で 54%阻害）（ACH-16-093 試験）を阻害した。ダニコパンは、機能アッセイにおいて、ヒト MRP3（IC_{50} = 66.6 μmol/L）及びタウロコール酸ナトリウム共輸送ポリペプチド（NCTP）トランスポーターに対して有意な阻害を示さなかった（30 μmol/L で 50%未満の阻害）（ACH-16-082 試験）。サンドイッチ培養の Transporter Certified™ ヒト肝細胞（SCHH）を用いて内因性胆汁酸動態の修飾作用及び FXR 活性化の阻害作用に対するダニコパンの影響を評価した in vitro 試験においては、ダニコパンは複数の機序により胆汁酸の恒常性を阻害する可能性が示されたが、評価した濃度では胆汁酸排泄の阻害は認められなかった（ACH-16-021 試験）。

3.2 代謝物の薬理作用

非臨床試験（モジュール 2.6.4、4 項）及び臨床試験（モジュール 2.7.2、3 項）で同定された次の 5 種類のダニコパンの代謝物における主要な薬理作用、細胞毒性及びミトコンドリア毒性を評価した：ACH-0144240 及び ACH-0144709（M173 及び M426、それぞれアミド加水分解物）；ACH-0145069（M582, カルボニル還元生成物、S 体）並びに ACH-0145071 及び ACH-0145072（M428、アミド加水分解とカルボニル還元の生成物、それぞれ S 体と R 体）。

4 種類の代謝物（ACH-0144709、ACH-0145069、ACH-0145071 及び ACH-0145072）について、in vitro 溶血アッセイにより血清 AP 活性阻害を検討した。ACH-0144240 は小さな断片であり、適切な濃度で阻害作用を示すとは考えられない。これらの代謝物は、血清 AP 活性に対し阻害作用をほとんど又は全く示さず（IC_{50}＞1 μmol/L）（ACH-16-065 試験；RTR-0065 試験）、これはダニコパン（IC_{50}=0.027 μmol/L）とは対照的であった（ACH-15-086 試験）。

2 種類のヒト細胞株（Huh7 及び MT4）を用いて 2 種類の代謝物（ACH-0145069 及び ACH-0145071）の細胞毒性を検討したところ、ダニコパンと同様に細胞毒性はほとんど又は全く認められなかった（CC_{50} 値 88 μmol/L 以上）。代謝物のうち 4 種類（ACH-0144240、ACH-0144709、ACH-0145069 及び ACH-0145071）については、1 種類の細胞株（HepG2）を用いてミトコンドリア毒性を検討した結果、グルコースを含まない培地で増殖した細胞に対する作用が異なっていなかったことから、低リスクであることが示された（ガラクトース培地; CC_{50}＞100 μmol/L）。同時に、こ

れら 4 種類の代謝物は標準条件下（グルコース培地、CC_{50}＞100 µmol/L）においても細胞毒性を示さなかった（ACH-16-065 試験）。

これらの結果を総合すると、これらのダニコパン代謝物は AP 阻害剤として薬理活性を示さず、ダニコパンと同様に細胞毒性及びミトコンドリア毒性のリスクが低いことが確認された。ダニコパンの代謝物プロファイルの要約を Table 4 に示す。

Table 4: ダニコパン代謝物の主要な薬理作用、細胞毒性及びミトコンドリア毒性

Compound		Primary	Cellular Toxicity		Mitochondrial Toxicity		
ID	Reaction (Metabolite)	AP	Huh7	MT4	HepG2		Ratio
		IC_{50} (µmol/L)[a]	CC_{50} (µmol/L)	CC_{50} (µmol/L)	Glucose CC_{50} (µmol/L)	Galactose CC_{50} (µmol/L)	
ACH-0144240	AH (M173)	ND	ND	ND	> 100 [b]	> 100 [b]	NA
ACH-0144709	AH (M426)	> 1 [b]	ND	ND	> 100 [b]	> 100 [b]	NA
ACH-0145069	CR (M582)	9.27 [c]	88 [b]	> 100 [b]	> 100 [b]	> 100 [b]	NA
ACH-0145071	AH+CR (M428)	> 1 [c]	> 200 [b]	> 100 [b]	> 100 [b]	> 100 [b]	NA
ACH-0145072	AH+CR (M428)	> 1 [c]	ND	ND	ND	ND	ND

[a] AP inhibition assessed by hemolysis of rabbit erythrocytes in 8.3% serum. Compounds were tested at maximum concentration of 1 µM, or 200 µM for ACH-0145069, in n = 1 to 3 independent experiments.
[b] n = 3
[c] n = 1
Abbreviations: AH = amide hydrolysis; CC_{50} = 50% cytotoxic concentration; CR = carbonyl reduction; IC_{50} = 50% inhibitory concentration; NA = not available or not applicable; ND = not determined
Source: ACH-16-065 and RTR-0065

3.3 In vitro 感染リスク評価

ダニコパンの防御的補体依存性殺菌活性及びオプソニン化貪食作用を補体保存血液検体を用いた複数のアッセイで評価した。

実験菌として大腸菌を用いたところ、ダニコパンは SBA（ACH-15-143 試験）及びヒト単球と顆粒球によるオプソニン化貪食作用（ACH-16-066 試験）を阻害しなかった。

臨床的に重要な病原体である髄膜炎菌の莢膜を有する菌株を用いた試験において、ダニコパンは補体阻害剤であるコンプスタチン及びエクリズマブよりも SBA に対する阻害作用が弱かった（ACH-19-015 試験）。特筆すべきこととして、髄膜炎菌に対するワクチン接種後に得られた健康ドナーの血清において、ダニコパンは髄膜炎菌 5 株に対していずれも SBA を阻害しなかったのに対し、コンプスタチン及びエクリズマブはいずれも全 5 株に対して SBA を阻害した。

同様に、公表されている 2 試験では、ワクチン接種した健康なドナーから採取した全血による莢膜形成髄膜炎菌分離株に対するオプソニン化貪食作用（Konar, 2017）、並びにエクリズマブ投与中の患者から採取した莢膜が形成されていない血清群分類不能の髄膜炎菌株に対するワクチン接種前及びワクチン接種後のドナー血清の SBA に対するダニコパンの阻害作用はエクリズマブよりも低いことが報告されている（Granoff, 2019）。

これらの結果を総合すると、十分な抗体価で達成される頑健な抗菌活性は AP 活性とは無関係であることが示され、補体 D 因子阻害は C3 阻害及び C5 阻害よりも髄膜炎菌感染症のリスクが相対的に低いという結論が裏付けられる。

4　安全性薬理試験

心血管系、呼吸系及び CNS の機能に対するダニコパンの効果を、広範囲にわたる in vitro 及び in vivo 安全性薬理試験で評価した。その結果、心血管系、呼吸系及び CNS の機能に影響を及ぼすことなく、ダニコパンを治療用量でヒトに安全に投与できることが示された。ラット及びマウスでは薬理作用を示さないことから、ビーグル犬を用いて in vivo 安全性薬理試験を実施した。

ダニコパンの 10 µmol/L でヒト hERG イオンチャネルがわずかに 29.9%阻害されたが（ACH-15-109 試験）、ALXN2040-PNH-301 試験[PMX-0038]においてヒトに 200 mg を 1 日 3 回投与したときの C_{max} と比較して、hERG に対する安全域が約 117 倍高いことから、ダニコパンは QT 延長のリスクは低いと考えられた。さらに、イヌに最高 500 mg/kg を単回投与しても、血行動態又は ECG の評価項目に影響は認められなかった（ACH-15-072 試験）。

1 日総投与量が 1000 mg/kg/日まで、呼吸機能又は CNS 機能に対する影響は認められなかった（ACH-15-073 試験）。

4.1　In vitro 安全性薬理試験

In vitro hERG アッセイにより心血管系に対するダニコパンの作用を評価した。

2 種類の GLP 非適用（ACH-15-026 試験；ACH-15-066 試験）及び 1 種類の GLP 適用（ACH-15-109 試験）の in vitro 試験を実施し、hERG イオンチャネル電流に対するダニコパンの影響を評価した。生理的温度付近（33℃～35℃）で hERG チャネル電流に及ぼすダニコパンの影響を検討するため、hERG チャネルを発現する HEK293 細胞を用いて GLP に適用した in vitro 試験を実施した（ACH-15-109 試験）。その結果、ダニコパンは最大溶解濃度である 10 µmol/L で hERG チャネル電流を 29.9%阻害した。この結果は 2 つの in vitro 試験（GLP 非適用）の結果と同等であった。したがって、IC_{50} は 10 µmol/L 超と推定された。in vitro hERG アッセイ（GLP 適用）の結果を Table 5 に示す。

Table 5:　GLP 適用 in vitro hERG アッセイ（ACH-15-109 試験）の結果

Concentration (µmol/L)	Mean Reduction	SD	SEM	N
0	0.8%	1.5%	0.9%	3
1	4.9%	1.4%	0.8%	3
3	14.7%	1.5%	0.9%	3
5	21.3%	2.1%	1.2%	3
10	29.9%	4.7%	2.7%	3

Abbreviations: hERG = human ether-à-go-go-related gene
Source: ACH-15-109

4.2　In vivo 安全性薬理試験

4.2.1　中枢神経系に及ぼす影響

ビーグル犬を用いたダニコパンの 14 日間反復投与毒性試験（GLP 適用、ACH-15-073 試験）において CNS 機能を評価した。神経行動学的評価は、行動変化、姿勢反応及び脊髄と脳神経の機能

等を評価した。反復投与毒性を評価する一環として、血漿検体を用いてトキシコキネティクスパラメータを測定した。

Week 1（Day 4）及び Week 2（Day 10）において、いずれの用量（100、500 又は 1000 mg/kg/日のダニコパンをそれぞれ 50、250 又は 500 mg/kg の用量で 1 日 2 回投与）でも神経行動学的パラメータへの影響は認められなかった。本試験における神経行動学的機能に対する無影響量は 1000 mg/kg/日であり、その際のダニコパンの平均 C_{max} 及び AUC_{0-24} は、それぞれ 23,100 ng/mL 及び 274,500 ng•h/mL であった。

4.2.2 呼吸系に及ぼす影響

ビーグル犬を用いたダニコパンの 14 日間反復投与試験（GLP 適用、ACH-15-073 試験）において呼吸系の機能を評価した。評価項目は 1 回換気量、呼吸数、及び算出された分時換気量であった。反復投与毒性を評価する一環として、血漿検体を用いてトキシコキネティクスパラメータを測定した。

Week 1（Day 3）及び Week 2（Day 12）において、いずれの用量（100、500 又は 1000 mg/kg/日のダニコパンをそれぞれ 50、250 又は 500 mg/kg の用量で 1 日 2 回投与）でも呼吸系の機能への影響は認められなかった。本試験における呼吸系の機能に対する無影響量は 1000 mg/kg/日であり、その際のダニコパンの平均 C_{max} 及び AUC_{0-24} は、それぞれ 23,100 ng/mL 及び 274,500 ng•h/mL であった。

4.2.3 心血管系に及ぼす影響

GLP 適用試験において、テレメトリーを装着した雄ビーグル犬に 50、150 又は 500 mg/kg を単回強制経口投与し、24 時間までの心血管系に対するダニコパンの影響を評価した（ACH-15-072 試験）。

死亡例はみられなかった。ダニコパン投与による一般状態の異常及び体温の変化は認められなかった。ダニコパンに起因する異常な ECG 波形はみられなかった。ダニコパンの投与は ECG パラメータ（PR、QT、QTc、QRS）又は血行動態パラメータ（収縮期及び拡張期血圧、平均動脈圧、脈圧及び心拍数）に影響を及ぼさなかった。

イヌを用いた 14 日間反復投与毒性試験（GLP 適用、ACH-15-073 試験）では、1000 mg/kg/日（500 mg/kg、1 日 2 回）までの用量で、ダニコパンに関連した ECG 波形/パラメータの変化は認められなかった。しかし、GLP 非適用のイヌを用いた忍容性試験で設定した用量（750 mg/kg/日）の 5 日間投与期において、375 mg/kg の各投与（午前及び午後）後、Day 4 に有害ではない心拍数増加が認められた（ACH-15-056 試験）。この増加は投与後 30 分に認められ、投与後約 5 時間まで持続した。ACH-15-056 試験で認められた心拍数増加は、投与開始前に認められた心拍数の範囲内であった。この有害ではない心拍数増加は、イヌを用いた心血管系安全性薬理試験（ACH-15-072 試験）及びイヌを用いた 14 日間反復投与毒性試験（ACH-15-073 試験）のいずれにおいても、同等以上の用量（用量制限 1000 mg/kg/日）で心拍数増加が認められなかったことから、偶発的な所見である可能性が示唆された。

5 薬力学的薬物相互作用

相乗的又は拮抗的相互作用の可能性を明らかにするため、2 試験を実施した。最初の試験では、AP 依存性ウサギ細胞溶血アッセイ及び AP Wieslab アッセイを用いて、補体阻害剤 FUT-175、コ

ンプスタチン及び抗 C5 抗体とダニコパンを併用した際の作用を評価した（ACH-15-100 試験）。2 つ目の試験では、PNH 患者由来の赤血球に対する AP 依存性溶血を用いて、ダニコパンをコンプスタチン及びエクリズマブとを併用した際の作用を評価した（ACH-17-073 試験）。いずれの試験でも、相乗的及び拮抗的相互作用を、公表されている分析指標（Prichard and Shipman, 1990；Chou and Talalay, 1983）を用いて評価した。

全体として、ダニコパンと他の補体阻害剤との間で中等度から強力な相乗的相互作用が認められ、強力な拮抗的相互作用は示唆されなかった。PNH 患者の赤血球を用いた際、ダニコパンとエクリズマブの相互作用は強い相乗作用を示し、拮抗作用は認められなかった。これらの結果は、C5 阻害剤であるエクリズマブ及びラブリズマブを含む他の補体阻害剤と併用したときのダニコパンの臨床的有用性を裏付けるものである。

6　考察及び結論

ダニコパンの主要な薬力学的作用、副次的薬力学的作用、安全性薬理及び薬力学的薬物相互作用を明らかにするために、ヒト、サル、ウサギ及びイヌを含む複数の動物種を用いて、in vitro 及び in vivo におけるダニコパンの標的に対する親和性、効力及び選択性を評価する非臨床薬理試験を実施した。主な所見の要約を以下に示す。

効力を裏付ける試験

In vitro:

- ダニコパンは補体 D 因子活性部位に可逆的かつ高親和性に結合し（Kd = 0.00054 µmol/L）、天然基質 C3bB（C3 の活性化産物である C3b と、補体 B 因子の複合体）に対して補体 D 因子セリンプロテアーゼ活性を阻害した（IC_{50} = 0.018 µmol/L、Ki = 0.0057 µmol/L）。
- ダニコパンはヒト血清中の AP 活性を阻害することが、溶血アッセイ（IC_{50} = 0.027 µmol/L）及び機能的 ELISA（Wieslab アッセイ）（IC_{50} = 0.034 µmol/L）により示された。また、ダニコパンは「無希釈血清」を用いた場合でも阻害作用を示し（AP 溶血アッセイ及び Wieslab アッセイでそれぞれ IC_{50} = 0.22 µmol/L 及び 0.27 µmol/L）、非臨床試験及び臨床試験で用いた ex vivo アッセイと in vitro での相関性が示された。
- ダニコパンは AP 活性に選択的であり、対応する機能アッセイにおいて補体古典経路又は補体レクチン経路活性を直接阻害しないが、補体古典経路及び補体レクチン経路を介して開始される AP を介した補体活性の増幅を阻害すると考えられた。
- ダニコパンは、健康なヒトドナー20 例から採取した血清パネルにおいて効力を保持しており、あらゆる人種で有用である可能性が示された（AP 溶血アッセイ、IC_{50} = 0.002～0.048 µmol/L、Wieslab アッセイ「無希釈血清」、IC_{50} = 0.043～0.80 µmol/L）。さらに、ヒト PNH 患者由来の検体を用いたアッセイにおいて、ダニコパンは AP 活性化後の溶血から赤血球を保護し、AP 調節不全の aHUS 患者血清において細胞溶解活性を抑制した。

- ダニコパンは、終末補体反応の阻害に加えて、C3 フラグメントによる標的細胞のオプソニン化を用量依存的に阻害した（ウサギ赤血球で IC_{50} = 0.096 µmol/L、PNH 患者赤血球で IC_{50} = 0.031 µmol/L）。したがって、ダニコパンは、エクリズマブ又はラブリズマブの投与を受けている一部の PNH 患者で認められる EVH おける補体依存性プロセスを阻害する。

In vivo:

- カニクイザル、イヌ及び程度は低いがウサギを含む非臨床試験に使用した動物種の血清では、ダニコパンは in vitro で活性を示したが、ラット及びマウスでは活性を示さなかった。ビーグル犬を用いた GLP 適用の反復投与毒性試験を含む複数の非臨床試験で使用した動物種及びウサギを用いた胚・胎児発生に関する試験の母動物から得られた検体において、経口投与後の ex vivo アッセイで薬理活性が認められた。

副次的薬理試験

- in vitro オフターゲット活性実験から、ダニコパンの細胞毒性は限定的で、ミトコンドリア毒性のリスクは低く、骨髄前駆細胞増殖に対する作用は最小であることが示された。
- ダニコパンは非特異的セリンプロテアーゼのパネルに対して不活性であったことから、ダニコパンの補体 D 因子触媒部位に対する選択性が確認され、非特異的プロテアーゼ阻害によるオフターゲット作用のリスクが低いことが示唆された。
- 受容体、イオンチャネル及びトランスポーターの広範なパネルに対して、ダニコパンはヒト A_1 及び A_3 受容体に対して阻害作用を示し（A_1 及び A_3、それぞれ、K_i = 1.7 µmol/L 及び 1.6 µmol/L、リガンド結合アッセイ；A_3、K_i = 6.1 µmol/L、機能アッセイ）、また、ヒト及びイヌの MT_1 受容体に高親和性のリガンドであり、ヒト MT_1 に対して強力なアンタゴニスト活性を示した（K_B = 0.0035 µmol/L）。ラット及びイヌを用いた毒性試験で示されたように、MT_1 受容体拮抗作用は CNS に影響を及ぼさない。
- 2 つの安全性パネルを用いて評価したところ、ダニコパンは 12 種類の心臓イオンチャネルのうち $hCa_v1.2$ カルシウムチャネルのみを阻害し（59.2%、5 µmol/L）、44 種類の薬物乱用に関連する受容体に対して有意な作用を示さなかった。
- ダニコパンはヒト、イヌ及びラットの BSEP を阻害した（IC_{50} は、それぞれ、33.8、18.9 及び 3.4 µmol/L）。また、MRP3 トランスポーター（IC_{50} = 66.6 µmol/L）、UGT1A1（IC_{50} = 0.81 µmol/L）及び UGT2B7（IC_{50} = 0.82 µmol/L）並びに FXR（33 µmol/L で 54%阻害）など、他の肝胆道系トランスポーターを阻害した。
- ダニコパンの 4 種類の代謝物の AP 阻害を溶血アッセイにより検討したところ、血清 AP に対する阻害活性はほとんど又は全くなかった。ダニコパンの代謝物は、細胞毒性（2 種類の代謝物で試験）及びミトコンドリア毒性（4 種類の代謝物で試験）の可能性もほとんど示さなかった。アミド加水分解及び/又はカルボニル還元の生成物であ

るこれらの化合物は、in vitro 及び in vivo の非臨床試験で使用した動物種及びヒトにおける最も顕著な代謝物であった。

- 大腸菌（*E coli*）及び髄膜炎菌（*N meningitidis*）を用いた in vitro 試験において、ダニコパンは細菌感染を引き起こすリスクが低いことが示唆された。髄膜炎菌の複数の分離株を用いたところ、対照薬の C3 阻害剤及び C5 阻害剤と比較して、ダニコパンの全血及び血清による殺作用の阻害は低く、特にワクチン接種後の感染リスクが低いことが示唆された。

安全性薬理試験

- in vitro ヒト hERG アッセイにおける QT 延長の評価（安全域は約 117 倍）、並びに心臓イオンチャネルの阻害が限定的であること、テレメトリーを装着したビーグル犬を用いた GLP 適用の心血管系安全性薬理試験において、収縮期血圧、拡張期血圧、脈圧、ECG 波形及び ECG パラメータ（PR、QRS、QT、及び QTc）に影響がなく、ダニコパンは潜在的な心血管リスクを示さなかった。
- ビーグル犬を用いた安全性薬理評価の結果、最高用量（C_{max} = 23,100 ng/mL = 40 μmol/L）でも呼吸系及び CNS に対する有害作用は認められなかった。

薬力学的薬物相互作用試験

- in vitro において、ダニコパンと他の補体阻害剤との間で拮抗的相互作用は認められなかったことから、補体関連疾患に対してダニコパンを他の補体阻害剤（エクリズマブ及びラブリズマブを含む）と併用することの妥当性が裏付けられた。

以上から、非臨床薬理試験の結果は、ダニコパンによる AP 阻害が臨床的に重大な EVH を発現している PNH 患者の治療に実行可能な戦略であるという仮説を支持すると考えられる。

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2.6.3 薬理試験概要表

2.6.3.1 薬理試験：一覧表

Overview		**Test Article: Danicopan (ALXN2040; ACH-0144471)**			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location (Module 4)**
Primary Pharmacodynamics					
Binding by danicopan to human FD protein as assessed by SPR	Binding assay using factor D conjugated to planar metal and measuring adsorption of ligand binding	In Vitro	[redacted], United Kingdom	ACH-15-082	4.2.1.1-1
Inhibition by danicopan of catalytic activity of human FD protein	Biochemical assay using factor D and the synthetic substrate Z-Lys-SBzl	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-083	4.2.1.1-2
Inhibition by danicopan of catalytic activity of human FD protein	Biochemical assay using factor D and natural substrate C3b and factor B measuring cleavage product Bb by ELISA	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-084	4.2.1.1-3
Inhibition by danicopan of catalytic activity of human FD protein	Biochemical assay using factor D and natural substrate C3b and factor B measuring cleavage product C3b-fB by ELISA	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-085	4.2.1.1-4
Co-crystal structure of danicopan in complex with human FD protein	Crystallization, synchrotron x-ray diffraction and molecular replacement	In Vitro	[redacted], United Kingdom	ACH-15-147	4.2.1.1-5

Overview		**Test Article: Danicopan (ALXN2040; ACH-0144471)**			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location (Module 4)**
Inhibition by danicopan of human serum AP, CP, and LP activity	Hemolysis of red blood cells via serum complement activation, and Wieslab functional ELISA measurement of serum complement activation	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-086	4.2.1.1-6
Inhibition by danicopan of animal serum AP activity	Hemolysis of red blood cells via serum complement activation	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-087	4.2.1.1-7
Inhibition by danicopan of human and animal serum AP activity in “undiluted serum” format	Hemolysis of red blood cells via serum complement activation, and Wieslab functional ELISA measurement of serum complement activation	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-088	4.2.1.1-8
Inhibition by danicopan of AP activity in human and animal serum from individual donors	Hemolysis of red blood cells via serum complement activation, and Wieslab functional ELISA measurement of serum complement activation	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-089	4.2.1.1-9
Inhibition by danicopan of AP-dependent hemolysis using artificial PNH cells	Hemolysis of red blood cells via serum complement activation	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-090	4.2.1.1-10

Overview		**Test Article: Danicopan (ALXN2040; ACH-0144471)**			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location (Module 4)**
Inhibition by danicopan of C3 fragment deposition on rabbit erythrocytes and on artificial PNH cells	Antibody recognition of C3 fragments on cells assessed by flow cytometry	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-091	4.2.1.1-11
Inhibition by danicopan of AP-mediated hemolysis and C3-fragment deposition using erythrocytes from patients with PNH and Inhibition by danicopan of AP-mediated killing in a modified Ham test using serum from patients with aHUS	Factor D binding assay; factor D protease and other biochemical assays using various methods; cell lysis/killing by serum complement; antibody recognition of C3 fragments on cells by flow cytometry; cynomolgus monkeys	Ex Vivo	[redacted] Achillion Pharmaceuticals, Inc., New Haven, CT 06511	（Yuan, 2017）	4.3-12
Inhibition by danicopan of AP activity in two standard functional assays (in vitro) and Inhibition by danicopan of AP-mediated hemolysis of erythrocytes from patients with PNH under “physiologic” conditions (ex vivo) (Comparison of test article vs eculizumab)	Hemolysis of red blood cells via serum complement activation, and Wieslab functional ELISA measurement of serum complement activation	In Vitro/Ex Vivo	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-17-074	4.2.1.1-12
Combined analyses of the PK-PD relationship following danicopan administration to beagle dogs in two PK studies and one tolerability/toxicity study	Beagle Dogs	Ex Vivo	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-093	4.2.1.1-13

Overview		**Test Article: Danicopan (ALXN2040; ACH-0144471)**			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location (Module 4)**
Combined analysis of the PK-PD relationship following danicopan administration to cynomolgus monkeys in single-dose and multiple-dose PK-PD studies	Cynomolgus Monkeys	Ex Vivo	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-094	4.2.1.1-14
PD analysis of samples from non-GLP tolerability and toxicity study of danicopan in beagle dogs	Beagle Dogs	Ex Vivo	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-119	4.2.1.1-15
Exploratory non-GLP complement analysis in GLP 14-day toxicity and toxicokinetic study of danicopan in beagle dogs	Beagle Dog	Ex Vivo	[redacted], Canada	ACH-15-073 Appendix 18	4.2.1.1-16
Exploratory non-GLP complement analysis in GLP 13-week toxicity and toxicokinetic study of danicopan in beagle dogs	Beagle Dog	Ex Vivo	[redacted], Canada	ACH-15-160 Appendix 14	4.2.1.1-17
Exploratory non-GLP complement analysis in GLP 39-week toxicity and toxicokinetic study of danicopan in beagle dogs	Beagle Dog	Ex Vivo	[redacted], Canada	ACH-16-057 Appendix 17	4.2.1.1-18
Exploratory non-GLP complement analysis in GLP embryo/fetal development (EFD) study of danicopan in New Zealand White rabbits (administration from gestational day [GD] 7 through GD20)	New Zealand White rabbit	Ex Vivo	[redacted]	ACH-15-163 Appendix I	4.2.1.1-19
Secondary Pharmacodynamics					
Evaluation of cellular toxicity of danicopan in human cell lines and primary cells	Human-derived cell lines and primary human cells	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-095	4.2.1.2-1
Evaluation of mitochondrial toxicity potential of danicopan in human cell lines cultured under conditions of dependence on mitochondrial respiration	Human derived (hepatic and non-hepatic) cell lines	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-096	4.2.1.2-2

Overview		**Test Article: Danicopan (ALXN2040; ACH-0144471)**			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location (Module 4)**
Effect of danicopan on human myeloid, erythroid, and megakaryocyte progenitors	Progenitor assays with human erythroid, myeloid, multipotential and megakaryocytic cell lineages	In Vitro	[redacted]	ACH-15-110	4.2.1.2-3
Effect of danicopan on human megakaryocyte progenitors	Human megakaryocyte progenitor proliferation assay	In Vitro	[redacted]	ACH-15-133	4.2.1.2-4
Effect of danicopan on human serine protease panel	Biochemical enzyme activity assays	In Vitro	[redacted], France	ACH-15-063	4.2.1.2-5
Effect of danicopan on a panel of 55 targets comprising receptors, ion channels, and transporters	Binding assay measures of inhibition of radioactively labeled ligand binding specific for each target	In Vitro	[redacted], France	ACH-15-043	4.2.1.2-6
Effect of danicopan on human A_1, A_3, and MT_1 receptors	Binding and functional receptor assays	In Vitro	[redacted], France	ACH-15-061	4.2.1.2-7
Effect of danicopan on dog MT_1 receptor	In vitro ligand binding assay with a stable cell line expressing the dog MT_1 receptor	In Vitro	[redacted], France	ACH-17-132	4.2.1.2-8
Effect of danicopan on rat MT_1 receptor in tissue based functional assay	Inhibition of melatonin induced contraction in rat caudal artery ring	In Vitro	[redacted], Taiwan	ACH-16-048	4.2.1.2-9

Overview		**Test Article: Danicopan (ALXN2040; ACH-0144471)**			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location (Module 4)**
Analysis of MT_1 amino acid sequences from human, dog, rabbit, and rat	Human, dog, rabbit, and rat MT_1 amino acid sequences	In Silico	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-20-025	4.2.1.2-10
Effect of danicopan on cardiac ion channels expressed in mammalian cells	In vitro assay with cells expressing human recombinant proteins/cardiac ion channels	In Vitro	[redacted]	ACH-15-069	4.2.1.2-11
Effect of danicopan on receptors, ion channels, and transporters associated with possible abuse liability	In vitro binding assays with cells expressing human recombinant proteins and with rat cerebral cortex cells	In Vitro	[redacted] France	ACH-20-021	4.2.1.2-12
Effect of danicopan on BSEP in primary human, dog, and rat hepatocytes	In vitro assay with primary rat, dog, and human hepatocytes	In Vitro	[redacted]	ACH-16-097	4.2.1.2-13
Effect of danicopan on MDR3 activity in primary human hepatocytes	In vitro assay with pooled human hepatocytes	In Vitro	[redacted]	ACH-16-085	4.2.1.2-14
Effect of danicopan on human MRP3, MRP4, and NTCP transporter activity	In vitro assays with Sf9 membrane vesicles containing human MRP3 and MRP4, and MDCK-II cells expressing NTCP	In Vitro	[redacted]	ACH-16-082	4.2.1.2-15
Effect of danicopan on catalytic activity of P450 and uridine 5'-diphosphoglucuronosyl transferase (UGT) enzymes in human liver microsomes	In vitro assay with human liver microsomes	In Vitro	[redacted]	ACH-15-106	4.2.1.2-16

Overview		**Test Article: Danicopan (ALXN2040; ACH-0144471)**			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location (Module 4)**
Effect of danicopan and 4 of its metabolites on Farnesoid X Receptor (FXR) modulation	In vitro assay with reporter cells expressing human FXR receptor	In Vitro	[redacted]	ACH-16-093	4.2.1.2-17
Effect of danicopan on bile acid hepatobiliary disposition and inhibition of FXR activation using hepatocytes in a sandwich-culture system (Sandwich-Cultured Transporter Certified™ Human Hepatocytes)	In vitro assay with sandwich-cultured human primary hepatocytes	In Vitro	[redacted]	ACH-16-021	4.2.1.2-18
Evaluation of pharmacological profile (AP inhibition, cytotoxicity, potential mitochondrial toxicity) of danicopan metabolites ACH-0144240, ACH-0144709, ACH-0145069, and ACH-0145071	In vitro hemolysis of red blood cells via serum complement activation, and cytotoxicity and mitochondrial toxicity assays using human cell lines	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-16-065	4.2.1.2-19
Evaluation of pharmacological activity (AP inhibition) of danicopan metabolite ACH-0145072 as inhibitors of complement AP	In vitro hemolysis of red blood cells via serum complement activation	In Vitro	Alexion Pharmaceuticals, Inc., New Haven, CT 06511	RTR-0065	4.2.1.2-20
Effect of danicopan on human serum bactericidal activity (SBA) against *E coli*	Bacterial death assessed by flow cytometry	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-143	4.2.1.2-21
Effect of danicopan on opsonophagocytosis of *E coli* using human monocytes and granulocytes	In Vitro assay with primary white blood cells	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-16-066	4.2.1.2-22
Effect of danicopan on human serum bactericidal activity (SBA) against encapsulated *Neisseria meningitidis* isolates	In vitro assay with *Neisseria meningitidis* isolates serogroups B, C, W, and Y and donor serum	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-19-015	4.2.1.2-23

Overview		**Test Article: Danicopan (ALXN2040; ACH-0144471)**			
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location (Module 4)**
Safety Pharmacology					
Effect of danicopan on hERG ion channels[a]	In vitro cultured cell line (human embryonic kidney cells)	In Vitro	[redacted]	ACH-15-109	4.2.1.3-3
Effect of danicopan on hERG Potassium Channels in Human Embryonic Kidney Cells (HEK293)	In vitro cultured cell line	In Vitro	[redacted]	ACH-15-026	4.2.1.3-1
Effect of danicopan on hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (HEK293)	In vitro cultured cell line	In Vitro	[redacted]	ACH-15-066	4.2.1.3-2
Cardiovascular telemetry study in conscious beagle dogs[a]	Beagle Dog	In Vivo	[redacted], Canada	ACH-15-072	4.2.1.3-4
14-day toxicity study in beagle dogs [a]	Beagle Dog	In Vivo	[redacted], Canada	ACH-15-073	4.2.1.3-5
Pharmacodynamic Drug Interactions					
Evaluation of combinatory effect of danicopan with other complement inhibitors including compstatin and an anti-C5 antibody	Hemolysis of red blood cells via serum complement activation, and Wieslab functional ELISA measurement of serum complement activation	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-15-100	4.2.1.4-1
Evaluation of combinatory effect of danicopan with compstatin and eculizumab against complement-mediated hemolysis of erythrocytes from PNH patients	Hemolysis of red blood cells via serum complement activation	In Vitro	Achillion Pharmaceuticals, Inc., New Haven, CT 06511	ACH-17-073	4.2.1.4-2

[a] Indicates that the study includes a GLP compliance statement.

Abbreviations: aHUS = atypical hemolytic uremic syndrome; AP = alternative pathway; BSEP = bile salt export pump; C3 = complement component 3; CP = classical pathway; EFD = embryo/fetal development; ELISA = enzyme-linked immunoassay; FD = Factor D; GD = gestational day; GLP = Good Laboratory Practice; HEK = human embryonic Kidney; hERG = human ether-à-go-go-related gene; LP = lectin pathway; MT1 receptor = melatonin receptor type 1; PD = pharmacodynamic(s); PK = pharmacokinetic(s); PNH = paroxysmal nocturnal hemoglobinuria; SPR = Surface Plasmon Resonance; UGT = uridine 5'-diphosphoglucuronosyl transferase

2.6.3.2 効力を裏付ける試験

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Binding by danicopan to human FD protein as assessed by SPR	Recombinant human FD	In Vitro	Conc. series[a]	NA	High-affinity and reversible binding to recombinant human FD: • K_D = 0.00054 µmol/L k_a = 2.93E6 mol/L^{-1} s^{-1} k_d = 0.00157 s^{-1}	ACH-15-082
Inhibition by danicopan of catalytic activity of human FD protein	Purified human FD	In Vitro	Conc. series[a]	NA	Inhibition of proteolytic activity of purified human FD against a nonspecific thioester substrate (Z-Lys-SBzl): • IC_{50} = 0.035 µmol/L	ACH-15-083
Inhibition by danicopan of catalytic activity of human FD protein	Purified human FD	In Vitro	Conc. series[a]	NA	Inhibition of proteolytic activity of purified human FD against its natural substrate (C3bB): • IC_{50} = 0.018 µmol/L	ACH-15-084
Inhibition by danicopan of catalytic activity of human FD protein	Purified human FD	In Vitro	Conc. series[a]	NA	Inhibition of proteolytic activity of purified human FD against its natural substrate (C3bB) (kinetic parameters determined using competitive inhibition model): • K_i = 0.0057 µmol/L (= 5.7 nmol/L)	ACH-15-085
Co-crystal structure of danicopan in complex with human FD protein	Recombinant human FD	NA	NA	NA	Compound binding to FD enzyme at active site	ACH-15-147
Inhibition by danicopan on AP, CP, and LP activity in human serum	Normal human serum	In Vitro	Conc. series[a]	NA	Inhibition of human serum AP activity in hemolysis and Wieslab assays (dilute serum format): • AP hemolysis assay (8% serum): IC_{50} = 0.027 µmol/L IC_{90} = 0.092 µmol/L • AP Wieslab assay (5.6% serum): IC_{50} = 0.034 µmol/L IC_{90} = 0.11 µmol/L No inhibition of human serum CP or LP activity	ACH-15-086
Inhibition by danicopan of animal serum AP activity	Cynomolgus monkey,	In Vitro	Conc. series[a]	NA	Inhibition of AP activity in serum from cynomolgus monkeys, dogs, and rabbits	ACH-15-087

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
	beagle dog, rabbit, rat, and mouse serum				• Cynomolgus monkey, AP hemolysis assay (8% serum): IC_{50} = 0.0091 µmol/L IC_{90} = 0.026 µmol/L • Dog, AP hemolysis assay (8% serum): IC_{50} = 0.025 µmol/L IC_{90} = 0.073 µmol/L • Rabbit, AP hemolysis assay (10% serum): IC_{50} = 0.13 µmol/L IC_{90} = 0.453 µmol/L No inhibition of AP activity in serum from rats or mice • Rat, AP hemolysis assay (22% serum): IC_{50} > 10 µmol/L • Mouse, AP hemolysis assay (25% serum): IC_{50} > 10 µmol/L	
Inhibition by danicopan of human and animal serum AP activity in “undiluted serum” format	Normal human, cynomolgus monkey, and beagle dog serum	In Vitro	Conc. series[a]	NA	Inhibition of AP activity in human serum in “undiluted serum” format: • Human, AP hemolysis (“undiluted serum” format, final 8.3% serum) IC_{50} = 0.027 µmol/L IC_{90} = 0.092 µmol/L • Human AP Wieslab (“undiluted serum” format, final 5.5% serum) IC_{50} = 0.034 µmol/L IC_{90} = 0.11 µmol/L Inhibition of AP activity in cynomolgus monkey and beagle dog serum in “undiluted serum” format: • Cynomolgus monkey, AP hemolysis assay (“undiluted serum” format, final 8.3% serum): IC_{50} = 0.010 µmol/L IC_{90} = 0.027 µmol/L • Cynomolgus monkey, AP Wieslab assay (“undiluted serum” format, final 5.5% serum):	ACH-15-088

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
					IC_{50} = 0.0033 µmol/L IC_{90} = 0.0086 µmol/L • Beagle dog, AP hemolysis assay ("undiluted serum" format, final 8.3% serum): IC_{50} = 0.031 µmol/L IC_{90} = 0.090 µmol/L	
Inhibition by danicopan of AP activity in human and animal serum from individual donors	Human, cynomolgus monkey, and beagle dog complement-preserved serum	In Vitro	Conc. series[a]	NA	Inhibition of AP activity in human serum from individual donors: • Human, AP hemolysis (8% serum) IC_{50} = 0.0020 –0.048 µmol/L IC_{90} = 0.0094 – 0.12 µmol/L (range, 20 individuals) • Human, AP Wieslab ("undiluted serum" format, final 5.6% serum) IC_{50} = 0.043 – 0.80 µmol/L IC_{90} = 0.093 – 1.9 µmol/L (range, 20 individuals) Inhibition of AP activity in cynomolgus monkey and beagle dog serum from individual animals. • Cynomolgus monkey, AP hemolysis (8% serum) IC_{50} = 0.0059 –0.033 µmol/L IC_{90} = 0.024 – 0.089 µmol/L (range, 10 individuals) • Cynomolgus monkey, AP Wieslab ("undiluted serum" format, final 5.6% serum) IC_{50} = 0.022 – 0.31 µmol/L IC_{90} = 0.12 – 0.76 µmol/L (range, 10 individuals) • Beagle dog, AP hemolysis (8% serum) IC_{50} = 0.044 –0.31 µmol/L IC_{90} = 0.13 – 0.76 µmol/L (range, 10 individuals)	ACH-15-089

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Inhibition by danicopan of AP-dependent hemolysis using artificial PNH cells	Artificial PNH erythrocytes (normal erythrocytes treated with AET) and acidified human serum	In Vitro	Conc. Series[a]	NA	Inhibition of AP-dependent hemolysis in a PNH model comprising artificial PNH erythrocytes (normal erythrocytes treated with 2-amino-ethylisothiouronium bromide [AET]) and acidified human serum • Hemolysis assay (20% serum) IC_{50} = 0.021 µmol/L	ACH-15-090
Inhibition by danicopan of C3 fragment deposition on rabbit erythrocytes and on artificial PNH cells	Artificial PNH erythrocytes and rabbit erythrocytes	In Vitro	0.0003 - 10 µmol/L	NA	Inhibition of C3 fragment deposition on rabbit erythrocytes (20% C5-depleted serum) • IC_{50} = 0.096 µmol/L Inhibition of C3 fragment deposition on artificial PNH cells (erythrocytes treated with AET) (32% acidified C5-depleted serum) • IC_{50} = 0.027 µmol/L	ACH-15-091
Inhibition by danicopan of AP-mediated hemolysis and C3-fragment deposition using erythrocytes from patients with PNH and, Inhibition by danicopan of AP-mediated killing in a modified Ham test using serum from patients with aHUS	Human PNH erythrocytes and aHUS serum	Ex Vivo	0.0003 – 1 µmol/L (hemolysis of PNH cells) 0.0001 – 1 µmol/L (C3 fragment deposition on PNH cells) 0.001 – 10 µmol/L (killing of PIGA-null	NA	Inhibition of AP-mediated hemolysis of erythrocytes collected from 3 patients with PNH (20% acidified serum) • Patient 1 (type III clone size, 67%) IC_{50} = 0.027 µmol/L IC_{90} = 0.11 µmol/L • Patient 2 (type III clone size, 98%) IC_{50} = 0.0040 µmol/L IC_{90} = 0.015 µmol/L • Patient 3 (type III clone size, 13%) IC_{50} = 0.011 µmol/L IC_{90} = 0.029 µmol/L Inhibition of C3-fragment deposition on erythrocytes from patients with PNH (60% acidified C5-depleted serum) • IC_{50} = 0.031 µmol/L IC_{90} = 0.089 µmol/L (1 PNH patient)	(Yuan, 2017)

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
			cells in aHUS serum)		Inhibition of AP-mediated killing of PIGA-null reagent cells using serum collected from 5 patients with aHUS (20% serum) • Substantial reduction in cell killing at ≥0.01 µmol/L danicopan (combined analysis, serum from 4 aHUS patient)	
Inhibition by danicopan of AP activity in two standard functional assays (in vitro) and, Inhibition by danicopan of AP-mediated hemolysis erythrocytes from patients with PNH under "physiologic" conditions (ex vivo) (Comparison of test article vs eculizumab)	Rabbit and human PNH erythrocytes and normal human serum	In Vitro/Ex Vivo	Conc. series[a]	NA	Inhibition of AP activity in human serum in "undiluted serum" format: • AP hemolysis ("undiluted serum" format, final 8.3% serum) IC_{50} = 0.20 µmol/L IC_{90} = 0.85 µmol/L (eculizumab, incomplete inhibition) • AP Wieslab ("undiluted serum" format, final 5.6% serum) IC_{50} = 0.24 µmol/L (eculizumab, 0.16 µmol/L) IC_{90} = 1.1 µmol/L (eculizumab, 0.35 µmol/L) Inhibition of AP-mediated hemolysis of erythrocytes collected from 2 patients with PNH ("physiologic" conditions, 72% serum) • IC_{50} = 0.037 µmol/L IC_{90} = 0.18 µmol/L (combined analysis, erythrocytes from 2 patients with PNH) (eculizumab, 50% inhibition at 1.3 µmol/L)	ACH-17-074
Combined analyses of the PK-PD relationship following danicopan administration to beagle dogs in two PK studies and one tolerability/toxicity study	Dog/Beagle	IV (PEG 400:Saline [50:50, w:w]) Oral (PEG 400:Kolliphor HS15 [90:10,	10 mg/kg single dose (IV) 20 mg/kg (± 10 mg/kg ritonavir)	Male N=21 (3 animals/ group)	Analysis: PK-PD relationship was evaluated using combined results from the following studies: • Single dose PK studies: ACH-15-036 and ACH-15-039 • Multiple dose-tolerability/toxicity study: PK results reported in ACH-15-056, PD results reported separately in ACH-15-119	ACH-15-093

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
		w:w]; or 0.5% HPMC: 0.5% Tween 80; or PEG400: water [70:30]; or PEG 400; or powder in capsule)	single dose (oral) 150 mg single dose (oral capsule) 375 mg/kg bid, 5 days (oral)		Findings: concentration-dependent inhibition of ex vivo AP activity: • AP hemolysis (“undiluted serum” format, final 8.3% serum) IC_{50} = 198 ng/mL (0.341 µmol/L) (IC_{90} not determined)	
Combined analysis of the PK-PD relationship following danicopan administration to cynomolgus monkeys in single-dose and multiple-dose PK-PD studies	Monkey/ Cynomolgus	IV (PEG 400:Saline [50:50, w:w]) Oral (PEG 400:water [70:30, w:w]; or PEG 400:Kolliphor RH40:Propylene Glycol [84:10.5: 5.5])	10 mg/kg single dose (IV) 50 mg/kg single dose (oral) 200 mg/kg bid, 1 day (oral) 75 mg/kg q12h, 5 days (oral)	Male N=12 (3 animals/ group)	Analysis: PK-PD relationship was evaluated using combined results from the following studies: • Single-dose and multiple-dose PK-PD study: ACH-15-040 (module 2.6.4) • Multiple-dose PK-PD study: ACH-15-107 (module 2.6.4) Findings: concentration-dependent inhibition of ex vivo AP activity: • AP hemolysis (“undiluted serum” format, final 8.3% serum) IC_{50} = 46 ng/mL (0.079 µmol/L) (IC_{90} not determined) • AP Wieslab (“undiluted serum” format, final 5.6% serum) IC_{50} = 31 ng/mL (0.053 µmol/L) (IC_{90} not determined) • IC_{90}s not determined, but ≥80% (hemolysis assay) or ≥90% (Wieslab assay) inhibition was noted at danicopan ≥150 ng/mL (0.258 µmol/L) Effects on additional complement components:	ACH-15-094

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
					• Reversible reduction in concentration of FB activation product Bb (a direct product of FD activity) • No perturbations in CP activity or FD concentration	
PD analysis of samples from non-GLP tolerability and toxicity study of danicopan in beagle dogs	Dog/Beagle	Oral (PEG 400)	0, 375 mg/kg bid/5 days	Male N=6 (3 animals/ group)	Sample used for PD analysis were obtained from ACH-15-056 Dose-dependent and reversible AP inhibition in ex vivo serum assays following twice-daily oral administration for 5 days: • Nearly complete CAP inhibition through 6 h following administration at 375 mg/kg bid • No perturbations in CP activity or in concentrations of complement C3, C4, or FD • Results from this study contributed to the integrated PK-PD analysis reported elsewhere (ACH-15-093)	ACH-15-119

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Exploratory non-GLP complement analysis in GLP 14-day toxicity and toxicokinetic study of danicopan in beagle dogs	Dog/Beagle	Oral (PEG 400:Kolliphor RH40: Propylene Glycol [84:10.5:5.5;w:w:w])	0, 50, 250, 500 mg/kg bid/14 days	Male N=16 Female N=16 (were subjected to AP analysis)	PD results in this toxicity study are presented in ACH-15-073, Appendix 18. Dose-dependent and reversible AP inhibition in ex vivo serum assays following twice-daily oral administration to beagle dogs • Nearly complete AP inhibition through at least 4-6 h following administration at all doses • ≥71% AP inhibition sustained through 12 h following administration at 1000 mg/kg/day (NOAEL) Concentration-dependent inhibition established by PK-PD analysis • AP hemolysis (“undiluted serum” format, final 8.3% serum) IC_{50} = 510 ng/mL (0.88 µmol/L) (IC_{90} not determined) • Gradual and reversible elevations of plasma C3 concentrations by approximately 86% at the highest dose	ACH-15-073

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Exploratory non-GLP complement analysis in GLP 13-week toxicity and toxicokinetic study of danicopan in beagle dogs	Dog/Beagle	Oral (PEG 400:Kolliphor RH40:Propylene Glycol [84:10.5:5.5;w:w:w])	0, 50, 125, 250, 500 mg/kg bid/13 weeks	Male N=16 Female N=16 (were subjected to AP analysis)	PD results in this toxicity study are presented in ACH-15-160, Appendix 14 Dose-dependent and reversible AP inhibition in ex vivo serum assays following twice-daily oral administration to beagle dogs • Nearly complete AP inhibition through at least 3-4 h following administration at all doses • Nearly complete AP inhibition sustained through 3-4 h following administration at 100 mg/kg/day (NOAEL) Concentration-dependent inhibition established by PK-PD analysis • AP hemolysis (“undiluted serum” format, final 8.3% serum) IC_{50} = 555 ng/mL (0.956 µmol/L) (IC_{90} not determined) Gradual elevations of plasma C3 concentrations by approximately 100% at higher doses	ACH-15-160

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Exploratory non-GLP complement analysis in GLP 39-week toxicity and toxicokinetic study of danicopan in beagle dogs	Dog/Beagle	Oral (PEG 400:Kolliphor RH40:Propylene Glycol [84:10.5:5.5;w:w:w])	0, 17.5, 37.5, 75 mg/kg bid/39 weeks	Male N=20 Female N=15 (were subjected to AP analysis)	PD results in this toxicity study are presented in ACH-16-057, Appendix 17 Dose-dependent and reversible AP inhibition in ex vivo serum assays following twice-daily oral administration to beagle dogs • Nearly complete AP inhibition through at least 3-4 h following administration at all doses • Nearly complete AP inhibition sustained through 3-4 h following administration at 75 mg/kg/day (NOAEL) Concentration-dependent inhibition established by PK-PD analysis • AP hemolysis (“undiluted serum” format, final 8.3% serum) IC_{50} = 385 ng/mL (0.663 µmol/L) (IC_{90} not determined) Gradual and reversible elevations of plasma C3 concentrations by approximately 50% at higher doses	ACH-16-057

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Exploratory non-GLP complement analysis in GLP embryo/fetal development (EFD) study of danicopan in New Zealand White rabbits (administration from gestational day [GD] 7 through GD20)	Rabbit/New Zealand White	Oral (0.5% HPMC [w:w] with 0.1% Tween® 80 [w:v] in deionized water)	0, 200, 500, 1000 mg/kg/day	Female N=88 (22 animals/ group)	PD results in this toxicity study are presented in ACH-15-163, Appendix I Dose-dependent and reversible AP inhibition in ex vivo assays of serum from gravid female New Zealand White rabbits following danicopan administration: • Nearly complete AP inhibition through at least 4-8 h following administration at all doses • Nearly complete inhibition through 4-8 h following administration at 500 mg/kg/day (NOAEL) Concentration-dependent inhibition established by PK-PD analysis • AP hemolysis (“undiluted serum” format, final 28% serum) IC_{50} = 1285 ng/mL (2.21 µmol/L) (IC_{90} not determined)	ACH-15-163

[a] Test concentrations are not specified in study report but were sufficient for determination of resulting parameters of binding (K_D, k_a, k_d) and/or inhibition (IC_{50}, IC_{90}, K_i).
Abbreviations: AET = 2-amino-ethylisothiouronium bromide; aHUS = atypical hemolytic-uremic syndrome; AP = (complement) alternate pathway; bid = twice daily; C3 = complement component 3; C5 = complement component 5; Conc. = concentration; CP = (complement) classical pathway; EFD = embryo-fetal development; FB = (complement) factor B; FD = (complement) factor D; GD = gestation day; GLP = Good Laboratory Practice; IC_{50} (or IC_{90}) = 50% (or 90%) inhibitory concentration; IV = intravenous(ly); k_a = association rate constant; k_d = dissociation rate constant; K_D = equilibrium dissociation constant; K_i = inhibitory constant; LP = (complement) lectin pathway; NA = not applicable; NOAEL = no-observed-adverse-effect level; PD = pharmacodynamic(s); PK = pharmacokinetic(s); PIGA = phosphatidylinositol N-acetylglucosaminyltransferase subunit A; PNH = paroxysmal nocturnal hemoglobinuria; SPR = surface plasmon resonance.

2.6.3.3 副次的薬理試験

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Evaluation of cellular toxicity of danicopan in human cell lines and primary cells	HepG2, Huh7, MT4, and Hep2 cell lines; primary human hepatocytes and PBMCs	In Vitro	0.316 – 100 µmol/L (Hep2G, Huh7, and Hep 2 cells) 20000 – 200000 cells/well (MT4 and primary PBMCs) 0.14 – 100 µmol/L (primary hepatocytes)	NA	Limited cytotoxicity in 4 human cell lines and two primary human cell types • Cell lines: HepG2: CC_{50} = 40.2 µmol/L Huh7: CC_{50} = 38.9 µmol/L MT4: CC_{50} > 50 µmol/L Hep2: CC_{50} = 42.4 µmol/L • Primary cells: Hepatocytes: CC_{50} > 50 µmol/L PBMCs: CC_{50} > 50 µmol/L	ACH-15-095
Evaluation of mitochondrial toxicity potential of danicopan in human cell lines cultured under conditions of dependence on mitochondrial respiration	MT4 and HepG2 cell lines	In Vitro	Conc. series[a]	NA	No mitochondrial toxicity in two human cell lines • MT4 cells Glucose media: CC_{50} = 66.6 µmol/L Galactose media: CC_{50} = 58.3 µmol/L Ratio: 1.1 • HepG2 cells Glucose media: CC_{50} > 100 µmol/L Galactose media: CC_{50} > 100 µmol/L Ratio: NA	ACH-15-096

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Effect of danicopan on human myeloid, erythroid, and megakaryocyte progenitors	Human erythroid, myeloid, multi-potential, and megakaryo-cytic lines	In Vitro	50 µmol/L	NA	Limited inhibition colony formation from clonogenic progenitor cells by 50 µmol/L danicopan: • Erythroid and myeloid: no effect Megakaryocyte: reduced proliferation (at 50 µmol/L danicopan)	ACH-15-110
Effect of danicopan on human megakaryocyte progenitors	Human megakaryo-ctye clonogenic progenitor cells	In Vitro	1 – 100 µmol/L	NA	Limited inhibition of colony formation from clonogenic progenitor cells: • Megakaryocyte: IC_{50} = 49.5 µmol/L	ACH-15-133
Effect of danicopan on human serine protease panel	Human serine proteases	In Vitro	10 µmol/L	NA	Limited inhibition of a panel of 10 serine proteases • All targets: <50% inhibition at 10 µmol/L danicopan	ACH-15-063
Effect of danicopan on a panel of 55 targets comprising receptors, ion channels, and transporters	Human receptors, ion channels, and transporters	In Vitro	10 µmol/L	NA	Limited off-target inhibition of panel of receptors, ion channels, and transporters: • Ligand-binding assays: Human A_1 adenosine receptor: 89% inhibition Human A_3 adenosine receptor: 77.2% inhibition Human MT_1 melatonin receptor: 99.2% inhibition Other targets (n=52): <50% (% inhibition at 10 µmol/L danicopan)	ACH-15-043

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Effect of danicopan on human A_1, A_3, and MT_1 receptors	Human molecular targets	In Vitro	0.001 – 30 µmol/L	NA	Inhibition of selected molecular targets: • Ligand-binding assays: Human A_1: IC_{50} = 2.7 µmol/L; K_i = 1.7 µmol/L Human A_3: IC_{50} = 2.7 µmol/L; K_i = 1.6 µmol/L Human MT_1: IC_{50} = 0.0082 µmol/L; K_i = 0.0065 µmol/L • Functional assays (antagonist effect): Human A_3: IC_{50} = 0.68 µmol/L; K_B = 6.1 µmol/L Human MT_1: IC_{50} = 0.038 µmol/L; K_B = 0.0035 µmol/L	ACH-15-061
Effect of danicopan on dog MT_1 receptor	CHO-S cells with dog MT_1 receptor plasmid	In Vitro	$1x10^{-7}$ – 10 µmol/L	NA	Inhibition of ligand binding to dog MT_1 receptor: • Ligand-binding assay: Dog MT_1: IC_{50} = 0.0057 µmol/L; K_i = 0.0046 µmol/L	ACH-17-132
Effect of danicopan on rat MT_1 receptor in tissue based functional assay	Rat MT_1	In Vitro	0.03 – 10 µmol/L	NA	Inhibition of rat MT_1 receptor in tissue-based functional assay • Rat caudal artery ring contraction: Rat MT_1: IC_{50} = 7.80 µmol/L	ACH-16-048
Analysis of MT_1 amino acid sequences from human, dog, rabbit, and rat	NA	NA	NA	NA	Sequence alignment and phylogenetic analysis: • High identity among human, dog, and rabbit MT_1 Greater distance between human and rat MT_1	ACH-20-025
Effect of danicopan on cardiac ion channels expressed in mammalian cells	Mammalian cells expressing cardiac ion channels	In Vitro	5 µmol/L	NA	Limited off-target inhibition of cardiac ion channel activity: • Functional assays (patch clamp): Human calcium channel $hC_{AV}1.2$: 59.2% inhibition Other targets (n=11): <50% inhibition (% inhibition at 5 µmol/L danicopan)	ACH-15-069

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Effect of danicopan on receptors, ion channels, and transporters associated with abuse liability	Human ligands	In Vitro	10 µmol/L	NA	No inhibition of ligand binding to 44 abuse liability receptors • Ligand-binding assays: All targets (n=44): <50% inhibition (% inhibition at 10 µmol/L danicopan)	ACH-20-021
Effect of danicopan on BSEP bile salt efflux transporter in primary human, dog, and rat hepatocytes	Primary rat, dog, and human hepatocytes	In Vitro	0.1 - 100 µmol/L	NA	Inhibition of BSEP bile salt efflux transporter activity: • Transporter assay in hepatocytes: Human BSEP: IC_{50} = 33.8 µmol/L Dog BSEP: IC_{50} = 18.9 µmol/L Rat BSEP: IC_{50} = 3.4 µmol/L	ACH-16-097
Effect of danicopan on MDR3 activity in primary human hepatocytes	Primary human hepatocytes	In Vitro	0.1 - 100 µmol/L	NA	Inhibition of MDR3 transporter activity in human hepatocytes • Transporter assay in hepatocytes IC_{50} = 10.9 µmol/L	ACH-16-085
Effect of danicopan on human MRP3, MRP4, and NTCP transporter activity	Sf9 membrane vesicles containing human MRP3 and MRP4 transporters	In Vitro	30 µmol/L (NTCP) 0.3 – 100 µmol/L (MRP3 and MRP4)	NA	Inhibition of MRP3, MRP4, and NTCP transporter activity • Transporter assay in Sf9 membrane vesicles: MRP3: IC_{50} = 66.6 µmol/L MRP4: IC_{50} = 3.46 µmol/L • Transporter assay in MDCK cells: NTCP: <50% inhibition at 30 µmol/L danicopan	ACH-16-082
Effect of danicopan on catalytic activity of UGT enzymes in human liver microsomes	Model substrates and human liver microsomes	In Vitro	0.03 - 30 µmol/L	NA	Inhibition of UGT metabolic enzymes • Enzymatic assay in human liver microsomes: UGT1A1: IC_{50} = 0.81 µmol/L UGT2B7: IC_{50} = 0.82 µmol/L	ACH-15-106

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Effect of danicopan on activity of FXR	Reporter cells expressing human FXR receptor	In Vitro	0.0457 - 100 µmol/L	NA	Modulation of FXR nuclear receptor activity • Reporter cell assay: agonist activity: none antagonist activity: 54% inhibition at 33.3 µmol/L danicopan (inhibition at 100 µmol/L danicopan to be interpreted with caution due to cytotoxicity)	ACH-16-093
Effect of danicopan on bile acid hepatobiliary disposition using hepatocytes in a sandwich-culture system (Sandwich-Cultured Transporter Certified™ Human Hepatocytes)	Sandwich-cultured human primary hepatocytes	In Vitro	0.3, 3, 30 µmol/L	NA	No effect on bile acid biliary efflux in SCHH system Disruption of bile acid homeostasis in SCHH system • Reduced endogenous bile acid accumulation and content • Reduced responses of FXR target genes at 3 µmol/L and 30 µmol/L danicopan Multiple potential mechanisms including FXR antagonism and cytotoxicity	ACH-16-021
Evaluation of pharmacological profile (AP inhibition, cytotoxicity, mitochondrial toxicity) of danicopan metabolites ACH-0144240, ACH-0144709, ACH-0145069, and ACH-0145071	Huh7, MT4, and HepG2 cell lines	In Vitro	Conc series (AP hemolysis)[a] 0.632 – 200 µmol/L (Huh7 cytotoxicity) 0.316 – 100 µmol/L (MT4 cytotoxicity, HepG2 mitochondrial toxicity)	NA	No significant inhibition of AP activity by danicopan metabolites • AP hemolysis (8.3% serum) ACH-0144709: IC_{50} > 1 µmol/L; IC_{90} > 1 µmol/L ACH-0145069: IC_{50} = 9.27 µmol/L; IC_{90} = 37.8 µmol/L ACH-0145071: IC_{50} > 1 µmol/L; IC_{90} > 1 µmol/L Limited cytotoxicity of danicopan metabolites in two human cell lines • Huh7 ACH-0145069: CC_{50} = 88.00 µmol/L ACH-0145071: CC_{50} > 200 µmol/L • MT4 ACH-0145069: CC_{50} > 100 µmol/L ACH-0145071: CC_{50} > 100 µmol/L No mitochondrial toxicity of danicopan metabolites in one human cell line	ACH-16-065

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
					• HepG2 All four metabolites Glucose media: CC_{50} >100 µmol/L Galactose media: CC_{50} >100 µmol/L Ratio: NA	
Evaluation of pharmacological activity (AP inhibition) of danicopan metabolite ACH-0145072	Red blood cells	In Vitro	0.0003 – 1 µmol/L	NA	No significant inhibition of AP activity by danicopan metabolite • AP hemolysis (8.3% serum) IC_{50} > 1 µmol/L IC_{90} > 1 µmol/L	RTR-0065
Effect of danicopan on human SBA against *E coli*	Normal human serum and *E coli*	In Vitro	0.1 – 31.6 µmol/L	NA	Inhibition of SBA by pooled normal human serum: • No inhibition up to 31.6 µmol/L danicopan	ACH-15-143
Effect of danicopan on opsonophagocytosis of *E coli* using human monocytes and granulocytes	*E coli* and human monocytes and granulocytes	In Vitro	0.001 – 10 µmol/L	NA	Inhibition of opsonophagocytosis by human donor monocytes and granulocytes: • No inhibition up to 10 µmol/L danicopan	ACH-16-066
Effect of danicopan on human SBA against encapsulated *Neisseria meningitidis* isolates	*N meningitidis* isolates serogroups B, C, W, and Y and donor serum	In Vitro	1 µmol/L	NA	Inhibition of SBA by pre-vaccination and post-vaccination serum from 1 donor: • Pre-vaccination: partial inhibition (2 of 3 isolates) Post-vaccination: no inhibition (0 of 5 isolates) (complete inhibition by comparator complement inhibitors compstatin and eculizumab)	ACH-19-015

[a] Test concentrations are not specified in study report but were sufficient for determination of resulting parameters of inhibition (IC_{50}, IC_{90}) or cytotoxicity (CC_{50}).
Abbreviations: AP = (complement) alternative pathway; BSEP = bile salt export pump; CC_{50} = 50% cytotoxic concentration; CHO = Chinese hamster ovary; Conc = concentration; FXR = Farnesyl X nuclear receptor; IC_{50} = 50% inhibitory concentration; K_i = inhibitory constant; MDCK = Madin-Darby canine kidney; MDR3 = multidrug resistance protein 3; MRP4 = multidrug resistance protein 4; MT_1 = melatonin receptor subtype 1; NA = not applicable; NTCP = sodium

taurocholate co-transporting polypeptide; PBMC = peripheral blood mononuclear cells; SBA = serum bactericidal activity; SCHH = sandwich cultured human hepatocytes; UGT = uridine diphosphate glucuronosyltransferase.

2.6.3.4 安全性薬理試験

Test Article: Danicopan							
Organ Systems Evaluated	**Species/ Strain**	**Method of Admin.**	**Concentrations (µmol/L) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**GLP Compliance**	**Study Number**
Cardiovascular (hERG channels)	Human Embryonic Kidney Cells	In Vitro	0, 1, 3, 5, and 10 µmol/L	NA	29.9% hERG channel inhibition at the highest concentration without precipitation, 10 µmol/L.	Yes	ACH-15-109
Cardiovascular (hERG channels)	Human Embryonic Kidney Cells	In Vitro	3 and 30 µmol/L	NA	Screening at 3 µmol/L gave 6.9% ± 1.4% inhibition, but precipitation was seen at 30 µmol/L.	No	ACH-15-026
Cardiovascular (hERG channels)	Human Embryonic Kidney Cells	In Vitro	5 µmol/L	NA	Screened up to highest concentration without precipitation 11.8% ± 1.0% inhibition at 5 µmol/L.	No	ACH-15-066
Cardiovascular	Dog/Beagle	Oral gavage	0, 50, 150, and 500 mg/kg (single dose)	Male N=16 (4 animals/ group)	No effect on qualitative or quantitative ECG parameters of PR interval, QRS duration, QT and QTc interval or on heart rate, blood pressure or body temperature measured at up to 24 h postdose.	Yes	ACH-15-072
Respiratory/ Neurobehavioral	Dog/Beagle	Oral gavage	0, 50, 250, 500 mg/kg bid	Male/ Female N=40 (Main study: 4 animals/ group/sex; Recovery: 2 animals/ group/sex, only 2 groups tested)	No effect on respiratory function (tidal volume, respiratory rate and minute volume). No overt effect on neurobehavioral parameters.	Yes	ACH-15-073

Abbreviations: bid = twice daily; ECG = electrocardiogram; GLP = Good Laboratory Practice; hERG = human ether-à-go-go-related gene; NA = not applicable.

2.6.3.5 薬力学的薬物相互作用試験

Test Article: Danicopan						
Type of Study	**Species/ Strain**	**Method of Admin.**	**Concentrations (µM) or Doses (mg/kg)**	**Sex and No. per Group**	**Noteworthy Findings**	**Study Number**
Evaluation of combinatory effect of danicopan with other complement inhibitors including compstatin and an anti-C5 antibody	Red blood cells	In Vitro	0.00234 – 0.15 µmol/L and 0.0234 – 1.5 µmol/L	NA	Overall: moderate to strong synergistic interactions, with no significant antagonistic interactions, as determined by two analytical methods Danicopan and compstatin (AP hemolysis, 8.3% serum) • Three-dimensional surface graphing method: strongly synergistic (slightly antagonistic) • Median-effect plot method: additive (moderately synergistic to slightly antagonistic) Danicopan and anti-C5 antibody (AP Wieslab, 5.6% serum) • Three-dimensional surface graphing method: moderately synergistic • Median-effect plot method: additive (synergistic to additive)	ACH-15-100
Evaluation of combinatory effect of danicopan with compstatin and eculizumab against complement-mediated hemolysis of erythrocytes from patients with PNH	PNH erythro-cytes	In Vitro	0.0234 – 1.5 µmol/L of eculizumab and 0.0938 – 6 µmol/L of compstatin	NA	Overall: moderate to strong synergistic interactions, with no antagonistic interactions, as determined by one or two analytical methods Danicopan and compstatin (hemolysis of PNH erythrocytes, 20% serum) • Three-dimensional surface graphing method: strongly synergistic • Median-effect plot method: moderately synergistic Danicopan and eculizumab (hemolysis of PNH erythrocytes, 20% serum) • Three-dimensional surface graphing method: strongly synergistic	ACH-17-073

Abbreviations: AP = (complement) alternative pathway; C5 = complement component 5; NA = not applicable; PNH = paroxysmal nocturnal hemoglobinuria.