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略号及び用語の定義一覧

略号・用語	省略していない表現又は用語の定義
API	tucatinib freebase[redacted]([redacted])：有効成分
ARRY-380	tucatinib：ツカチニブ
AUC	area under the plasma concentration-time curve：血漿中濃度-時間曲線下面積
AUC_4	area under the curve from time 0 to 4 hours：0 時間から投与後 4 時間までの血漿中濃度-時間曲線下面積
AUC_{inf}	area under the plasma concentration-time curve from time zero to infinity：0 時間から無限大時間までの血漿中濃度-時間曲線下面積
AUC_{last}	area under the plasma concentration-time curve to the last measureable concentration：0 時間から最終定量可能時間までの血漿中濃度-時間曲線下面積
BA	bioavailability：バイオアベイラビリティ
BID	twice daily：1 日 2 回
CI	confidence interval：信頼区間
C_{max}	maximum observed plasma concentration：最高血漿中濃度
CSF	cerebrospinal fluid：脳脊髄液
CV	coefficient of variation：変動係数（幾何平均値に対しては幾何変動係数）
CYP	cytochrome p450：シトクロム P450
DDI	drug-drug interaction：薬物相互作用
DLT	dose-limiting toxicity：用量制限毒性
DP	drug product：製剤
HER2	human epidermal growth factor receptor-2：ヒト上皮増殖因子受容体 2 型
HPLC	high-performance liquid chromatography：高速液体クロマトグラフィー
IV	intravenous：静脈内
ISR	incurred sample reproducibility：実試料再現性
k_{el}	elimination rate constant：消失速度定数
LC	liquid chromatography：液体クロマトグラフィー
LC-MS/MS	liquid chromatography with tandem mass spectrometry：液体クロマトグラフィー・タンデム質量分析
LLOQ	lower limit of quantification：定量下限
mBC	metastatic breast cancer：転移性乳癌
MATE	multidrug and toxin extrusion：有機カチオン/H^+交換輸送体
MR_{AUC}	metabolite-to-parent total exposure ratio：未変化体に対する代謝物の総曝露量比
MS/MS	tandem mass spectrometry：タンデム質量分析
MTD	maximum tolerated dose：最大耐量
ONT-380	tucatinib：ツカチニブ
PIC	powder-in-capsule：原薬を充填したカプセル製剤
PK	pharmacokinetic(s)：薬物動態
QC	quality control：品質管理
QD	once daily：1 日 1 回
RSD	relative standard deviation：相対標準偏差
SD	standard deviation：標準偏差
SDD	spray-dried dispersion：噴霧乾燥分散体
$t_{½}$	terminal half-life：終末相の消失半減期
T-DM1	trastuzumab emtansin：トラスツズマブ エムタンシン
T_{lag}	lag time：ラグタイム
T_{max}	time to maximum concentration：最高血漿中濃度到達時間

略号・用語	省略していない表現又は用語の定義
ULOQ	upper limit of quantification：定量上限

1. 背景及び概観

本 M2.7.1 ではツカチニブの生物薬剤学及び関連する分析法について以下を要約する。

- 臨床試験で用いた各種製剤及び製剤開発（1.2 項）
- 臨床試験で採取した生体試料中のツカチニブ及びその代謝物（ONT-993）の濃度を測定した分析法（1.3 項）
- ツカチニブ即放性経口製剤を投与したときの以下の生物薬剤学（2 項）
    - 開発製剤の食事の影響（ARRAY-380-101 試験）
    - ツカチニブ製剤の相対的 BA（ARRAY-380-102 試験）
    - ツカチニブ錠の相対的 BA、食事の影響及び制酸薬との DDI（ARRAY-380-103 試験）

本 M2.7.1 及び M2.7.2 では、未変化体の名称としてツカチニブ（旧称：ARRY-380、ONT-380）あるいは本薬を使用する。ツカチニブの主要な循環血中代謝物の名称は、各臨床試験や分析法では ONT-993（旧称：AR00440993）を使用する。ツカチニブの臨床開発で用いた臨床試験及び各製剤の概要を Table 1 に示す。臨床試験では市販用製剤と同じ処方成分で製造した錠剤を用いた。臨床試験で用いた錠剤及び市販用製剤の名称としてツカチニブ錠あるいは本剤を使用する。

Table 1. Overview of formulations evaluated in the tucatinib clinical development program

Study Number	Formulation		Study Rationale
ARRAY-380-101	PIC	Early development	First-in-Human evaluation of tucatinib in subjects with a variety of cancers.
ARRAY-380-102	PIC Micronized PIC Micronized aqueous suspension Micronized solution in 20% Captisol® and apple juice	Early development	Evaluation of a potential change in the oral dosage form from the current PIC formulation to improve the between- and within-subject variability in plasma tucatinib exposure.
ARRAY-380-103	PIC Tablet[a]	Early development/ Commercial formulation	Evaluation of the tucatinib tablet formulation with the current PIC formulation to assess the relative bioavailability between the 2 formulations, assessment of the potential positive effect of food as a means of decreasing inter-subject variability, and assessment of the effect of omeprazole on tucatinib pharmacokinetics.
ONT-380-004	Tablet[a]	Commercial formulation	Dose-finding study to determine the MTD of tucatinib and the recommended phase 2 dose of tucatinib to be given in combination with the approved dose of T-DM1. Combining tucatinib with T-DM1 explored dual inhibition of HER2 by potentially improving the efficacy of T-DM1 by overcoming/delaying the development of resistance.
ONT-380-005	Tablet[a]	Commercial formulation	Dose-finding study to determine the MTD dose of tucatinib and the recommended phase 2 dose of tucatinib to be given in combination with the approved dose of capecitabine, trastuzumab alone, or with both trastuzumab and capecitabine. Combining tucatinib with both trastuzumab and capecitabine, explored the efficacy of dual inhibition of HER2 plus a cytotoxic agent.
ONT-380-008	[^{14}C]-tucatinib oral dose solution Radiolabeled [^{14}C]-tucatinib in ethanol solution combined with non-radiolabeled tucatinib powder	Exploratory formulation	To determine the absorption, metabolism, and excretion of [^{14}C]-tucatinib and the likelihood of effects of renal or hepatic impairment on tucatinib disposition and the potential for DDI with tucatinib.
ONT-380-009	Tablet[a]	Commercial formulation	To provide information for development of dosing recommendations for tucatinib in subjects with hepatic impairment. To compare the PK and safety profiles after tucatinib administration between mild, moderate, and severe hepatic impairment subjects versus matched healthy volunteers.

Study Number	Formulation		Study Rationale
ONT-380-011	Tablet[a]	Commercial formulation	To assess tucatinib for potential effects on cardiac repolarization through electrocardiographic evaluation in a TQT study by evaluating the QT effects of tucatinib at steady-state therapeutic plasma exposure.
ONT-380-012	Tablet[a]	Commercial formulation	To evaluate the magnitude of potential clinical drug interactions by tucatinib. This study was undertaken due to in vitro inhibition of the metabolizing enzymes cytochrome p450 (CYP)3A4, CYP2C8, and CYP2C9, and of the transporter P-gp by tucatinib in the nonclinical tucatinib development program.
ONT-380-206 (HER2CLIMB)	Tablet[a]	Commercial formulation	To determine if tucatinib provides additional benefit to the combination of trastuzumab plus capecitabine in subjects with incurable, progressing, locally advanced unresectable or metastatic HER2+ breast cancer.
MK-7119-001 (C4251013)	Tablet[a]	Commercial formulation	To evaluate the efficacy and safety of tucatinib in combination with trastuzumab and capecitabine in Japanese, South Korean, and Taiwanese participants.
SGNTUC-015	Tablet[a]	Commercial formulation	To investigate the PK, safety and tolerability of tucatinib in healthy male and female Caucasian and Japanese subjects. Results from this study will support future studies of tucatinib in Japan and assist in identifying appropriate, well-tolerated doses that can be administered in subsequent studies in healthy subjects and subjects with solid malignancies (including breast cancer) that are associated with HER2 overexpression or mutation.
SGNTUC-020	Tablet[a]	Commercial formulation	A drug-drug interaction study to evaluate the effects of tucatinib on the pharmacokinetics of a MATE substrate (metformin) in healthy male and female subjects.

DDI=drug-drug interactions; MATE=multidrug and toxin extrusion; P-gp=P-glycoprotein; PIC=powder-in-capsule; T-DM1=trastuzumab emtansine; TQT=thorough QT/corrected QT interval.

a Tablets contain [redacted]% tucatinib in [redacted].

Source: CSRs (ARRAY-380-101, -102, -103; ONT-380-004, -005, -008, -009, -011, -012, -206; MK-7119-001; SGNTUC-015, -020).

1.1. 要旨

ツカチニブの生物薬剤学に関する全体的な結論は以下のとおりである。

- 市販用製剤のツカチニブ 50 mg 錠及び 150 mg 錠に含まれる処方成分は共通しており、素錠の含有量は比例している。本剤の in vitro 溶出プロファイルは類似し、各含量及びロットで一貫した in vitro 溶出性を示した。このため、市販用製剤のツカチニブ 50 mg 錠と 150 mg 錠は生物学的に同等である。各臨床試験で用いた錠剤と市販用製剤の処方成分は同一である。

- ツカチニブ投与時の血漿中濃度測定のために用いた生物学的分析法は、高い感度、選択性、真度及び再現性を示した。各種の試料調製及び長期保存条件で、分析対象物質の安定性が示された。ツカチニブ及び ONT-993 の定量に用いた複数の分析法についてクロスバリデーションを実施した結果、分析法による違いは認められなかった。

- 粉末ツカチニブのカプセル（PIC）製剤投与時の本薬の経口 BA は、水性懸濁液より低く、PIC 製剤投与時の本薬の T_{max} は、水性懸濁液と同程度であった。微粉化ツカチニブ PIC 製剤投与時の本薬の経口 BA は、非微粉化の PIC 製剤投与時と大きな違いはなく、微粉化 PIC 製剤投与時の本薬の T_{max} は、非微粉化の PIC 製剤投与時と同程度であった。

- ツカチニブ PIC 製剤を絶食下及び食後に投与したときの PK を評価した結果、全体集団で食事の影響は認められなかった。ツカチニブ錠を食後投与したときの本薬の AUC は、絶食下と比べて 1.5 倍以下の増加であり、T_{max} はやや遅くなったものの変動幅は概ね重なり、C_{max} への影響は軽微であった。ツカチニブの曝露量に対する食事の影響は大きくなかったことから、ONT-380-206 試験及び MK-7119-001 試験において、ツカチニブの用法は食事に関係なく BID 経口投与に設定した。これらの臨床試験の結果に基づき、ツカチニブ錠は食事の有無にかかわらず服用可能である。

- ツカチニブ錠を制酸薬オメプラゾールと併用投与したときの本薬の BA に及ぼす影響は軽微であり、T_{max} に変化は認められなかった。ツカチニブ錠は制酸薬と併用可能である。

1.2. 製剤開発

初期の臨床試験ではツカチニブの PIC 製剤、水性懸濁液及び溶液を使用した（Table 2）。その後、非臨床の PK で認められた変動を低減し、胃内 pH の変化による吸収への潜在的影響を最小限に抑える目的で錠剤を開発した（M3.2.P.2.2）。市販用製剤のツカチニブ錠を用いた臨床試験の要約を Table 2（ARRAY-380-103 試験）及び Table 3 に示す。

Table 2. Overview of tucatinib early development clinical studies and exploratory formulations

Study Number	Study Description	Final Presentation	Dosage Form Strength	Ingredient (Tucatinib)	DP Lot Number
ARRAY-380-101	A phase 1, open-label, multiple dose study to assess the safety, tolerability, and pharmacokinetics of tucatinib given on a daily oral regimen in subjects with advanced cancer	Powder filled into hard gelatin capsules	25 mg	Tucatinib API	DP-ARR07-147
				Tucatinib API	DP-ARR08-139-A
			100 mg	Tucatinib API	DP-ARR07-148
				Tucatinib API	DP-ARR09-134-A
				Tucatinib API	DP-ARR09-132-A
				Tucatinib API	DP-ARR10-104-A
				Tucatinib API	DP-ARR10-124-A
				Tucatinib API	DP-ARR11-111-A
ARRAY-380-102	An exploratory, open-label, single dose, four-period study evaluating the pharmacokinetics, relative bioavailability, and safety of four oral tucatinib formulations in healthy subjects	Powder filled into hard gelatin capsules	100 mg	Tucatinib API	DP-ARR09-134-A
		Micronized powder filled into hard gelatin capsules	100 mg	Micronized bulk powder	L-ARR07-165-A[b]
		Micronized powder in SyrSpend® SF Dry and water	300 mg/60 mL	Micronized bulk powder	L-ARR07-165-A[b]
		Micronized powder in 20% Captisol® and apple juice	300 mg/60 mL	Micronized bulk powder	L-ARR07-165-A[b]
ARRAY-380-103	An open-label, single-dose four-period study evaluating the relative bioavailability, potential food effect, and omeprazole drug interaction of oral tucatinib capsules and tablets in healthy subjects	Powder filled into hard gelatin capsules	100 mg	Tucatinib API	DP-ARR10-124-A
		Tablet[a]	150 mg	Tucatinib API	DP-ARR11-112-A

Study Number	Study Description	Final Presentation	Dosage Form Strength	Ingredient (Tucatinib)	DP Lot Number
ONT-380-008	Phase 1, open-label study of the absorption, metabolism, and excretion of [^{14}C]-tucatinib following a single oral dose in healthy male and female subjects	[^{14}C]-tucatinib oral dose solution Radiolabeled [^{14}C]-tucatinib in ethanol solution combined with non-radiolabeled tucatinib powder	5 mg/mL (2.5 µCi/mL)	Non-radiolabeled powder Radiolabeled in ethanol solution	15JAN2018-8372735BLK

API =tucatinib freebase [redacted] ([redacted])
a Tablets contain [redacted]% tucatinib in [redacted].
b This is the drug substance lot number for the micronized API.
Source: CSRs (ARRAY-380-101, -102, -103, ONT-380-008).

Table 3. Overview of tucatinib clinical studies using the planned commercial formulation

Study Number	Study Description	Final Presentation	Dosage Form Strength	DP Lot Number
ONT-380-004	A phase 1b, open-label study to assess the safety and tolerability of tucatinib combined with ado-trastuzumab emtansine (trastuzumab emtansine; T-DM1) in patients with HER2+ breast cancer	Tablet[a]	50 mg	DP-ARR14-111-A DP-ARR14-111-B KH16/0046
		Tablet[a]	150 mg	DP-ARR13-111-A DP-ARR13-111-B DP-ARR13-111-C DP-ARR13-111-D DP-ARR14-105-B
ONT-380-005	A phase 1b, open-label study to assess the safety and tolerability of tucatinib combined with capecitabine and trastuzumab, alone and in combination in HER2+ metastatic breast cancer	Tablet[a]	50 mg	DP-ARR14-111-A DP-ARR14-111-B KH16/0046
		Tablet[a]	150 mg	DP-ARR14-105-A DP-ARR14-105-B DP-ARR13-111-A DP-ARR13-111-B DP-ARR13-111-D
ONT-380-009	An open-label, nonrandomized, single-dose, parallel-group, safety, tolerability, and pharmacokinetic study of tucatinib administered at 300 mg in fasted, hepatically-impaired male and female subjects and fasted matched control healthy subjects	Tablet[a]	150 mg	KH17/0064
ONT-380-011	A phase 1, randomized, partially double blind, placebo and positive controlled study to evaluate the effect of tucatinib on cardiac repolarization in healthy subjects	Tablet[a]	150 mg	N140208-0012L001.A1
ONT-380-012	A phase 1, open-label, fixed-sequence, 5-part, drug-drug interaction study of tucatinib to evaluate the effects of CYP3A4 and CYP2C8 inhibition and induction on the pharmacokinetics of tucatinib and to evaluate the effects of tucatinib on the pharmacokinetics of substrates of CYP3A4, CYP2C8, CYP2C9, and P-glycoprotein in healthy subjects	Tablet[a]	150 mg	KH17/0064

Study Number	Study Description	Final Presentation	Dosage Form Strength	DP Lot Number
ONT-380-206 (HER2CLIMB)	Phase 2 randomized, double-blinded, controlled study of tucatinib vs. placebo in combination with capecitabine and trastuzumab in patients with pretreated unresectable locally advanced or metastatic HER2+ breast carcinoma	Tablet[a]	50 mg	DP-ARR14-111-A KH16/0046 KH18/0207
		Tablet[a]	150 mg	145649.02 DP-ARR14-105-A KH16/0045 KH17/0064 KH17/0065 KH17/0071 KH17/0072 KH18/0208
MK-7119-001 (C4251013)	Phase 2 open-label, single arm study of tucatinib in combination with trastuzumab and capecitabine in participants with previously treated locally advanced unresectable or metastatic HER2+ breast carcinoma	Tablet[a]	50 mg	T042226[b] U022730[b] W016973[b] W036467[b] X008363[b] Y007362[b]
			150 mg	T042233[b] U022169[b] W016939[b] W039295[b] X018392[b] X019163[b]
SGNTUC-015	Phase 1, open-label, safety, tolerability and pharmacokinetic study of tucatinib (ONT-380) in healthy Japanese and Caucasian subjects	Tablet[a]	50 mg	KH16/0046
		Tablet[a]	150 mg	KH17/0065
SGNTUC-020	Phase 1, single center, open-label, fixed sequence drug-drug interaction study in healthy subjects evaluating the effects of tucatinib on the PK of a substrate probe of the MATE1/2-K transporters	Tablet[a]	150 mg	KH17/0065

MATE=multidrug and toxin extrusion; T-DM1=trastuzumab emtansine
a Tablets contain [redacted]% tucatinib in [redacted].
b Merck Lot identifications
Source: CSRs (ONT-380-004, -005, -009, -011, -012, -206; MK-7119-001; SGNTUC-015, -020).

1.2.1. 市販用製剤

市販用製剤は即放性のフィルムコーティング錠であり、含量規格は 2 種類である（50 mg 及び 150 mg）。市販用製剤のツカチニブ 50 mg 錠及び 150 mg 錠に含まれる処方成分は共通しており、素錠の含有量は比例している（M2.3.P.1）。各臨床試験で用いた錠剤と市販用製剤の組成は同一である。

1.2.2. ツカチニブ錠の溶出性

市販用製剤のツカチニブ 50 mg 錠及び 150 mg 錠の溶出挙動は速やかであり、2 つの含量規格の溶出挙動は同等であった（M2.3.P.2.2）。

1.3. 分析法の概要

ツカチニブの臨床開発では、関連する規制ガイドラインに従い、特異的かつ高感度にバリデートされた LC-MS/MS 分析法を用いてツカチニブ、ONT-993、併用薬及び代謝物を定量した（Table 4）。安定同位体標識内部標準物質を血漿又は尿と混合し、抽出した。分取した抽出液を LC-MS/MS システムに注入し、HPLC カラムを用いてツカチニブ、ONT-993、その他の分析対象物及び内部標準物質を分離した後、定量対象化合物ごとのプロダクトイオンスキャンを伴う MS/MS で分析した。内部標準物質のピーク面積に対する化合物（ツカチニブ、ONT-993 など）のプロダクトイオンのピーク面積の比で作成した 8 点から成る検量線を用いて定量した。

Table 4. Summary of validated analytical methods for tucatinib and other compounds used in the clinical development program

Analyte[a]	Validation Laboratory	Method Number	Validation Report Number/ Summary Table in Appendices	Bioanalytical Report	Clinical Study Number
Tucatinib ONT-993	[redacted]	STM1916[b]	1916[d] 1916 Amendment 2 1916 Amendment 3 Appendix 2, Appendix 3	1827 CP10-001 BA-12-001	ARRAY-380-101 ARRAY-380-102 ARRAY-380-103
Tucatinib ONT-993	[redacted]	ONT3HPP[b] (Plasma)	8373099[d] Appendix 4, Appendix 5	8373100 8373071 8373072 8373070	ONT-380-008 ONT-380-009 ONT-380-011 ONT-380-012
Tucatinib ONT-993	[redacted]	ONT3HUP (Urine)	8385605[d] Appendix 6, Appendix 7	8373100	ONT-380-008
Tucatinib ONT-993	[redacted]	TM17-396[b]	AV14-OTN380-01 Addendum 1[d] AV14-ONT380-01 Addendum 3[d,e] Appendix 8, Appendix 9, Appendix 10	AD16-950 AD19-849	ONT-380-206 SGNTUC-020
Tucatinib ONT-993	[redacted]	TM17-433[b]	AV17-ONT380-01[d] AV17-ONT380-01 Addendum 1[d,e] Appendix 11, Appendix 12	AD19-931	SGNTUC-015
Tucatinib ONT-993 Capecitabine 5'-DFCR 5'-DFUR	[redacted]	TM14-281[b]	AV14-OTN380-01[d], AV14-ONT380-01 Addendum 2[d,e] Appendix 8, Appendix 9, Appendix 10	AD14-747 AD14-734 Amendment 1 AD16-950	ONT-380-004 ONT-380-005 ONT-380-206
Tucatinib ONT-993	[redacted]	TM17-433	AV17-ONT380-01 Addendum 2 Appendix 13	AD20-1125	380-IST-004 (plasma samples)

Analyte[a]	Validation Laboratory	Method Number	Validation Report Number/ Summary Table in Appendices	Bioanalytical Report	Clinical Study Number
Tucatinib ONT-993	[redacted]	TM18-495	AV18-ONT380-03 AV18-ONT380-03 Addendum 1 Appendix 14, Appendix 15	AD20-1125	380-IST-004 (CSF samples)
Tucatinib ONT-993	[redacted]	TM20-593	AV20-123 Amendment 1 (MK-7119-001 CSR, Appendix 16.2.5.10) AV17-ONT380-01[d] Appendix 11	AD23-1554	MK-7119-001
5-FU FBAL	[redacted]	TM14-286	AV14-5FU-01[d], AV14-5FU-01 Addendum 2[d,e] Appendix 16, Appendix 17	AD14-734 AD14-734 Amendment 1	ONT-380-005
Moxifloxacin	[redacted]	MXNHPC[c]	2100-788, 8225508 Appendix 18, Appendix 19, Appendix 20	8373072	ONT-380-011
DM1[f]	[redacted]	TM14-277	AV14-DM1-01[d] AV14-DM1-01 Addendum 1[d,e] Appendix 21, Appendix 22	AD14-826[f]	ONT-380-004
Metformin	[redacted]	TM18-500 (Plasma)	AV18-Metformin-01 Addendum 1[e,g] Appendix 23, Appendix 24	AD19-849	SGNTUC-020
Metformin	[redacted]	TM18-503 (Urine)	AV18-Metformin-02 Addendum 1[e,g] Appendix 23, Appendix 25	AD19-849	SGNTUC-020
Gemfibrozil	[redacted]	GEMFHPC[c]	8395822 Appendix 28, Appendix 31	8373070	ONT-380-012
Iohexol	[redacted]	[redacted]-US-SML-0430	PRALABIN-186747-B Appendix 26, Appendix 27	SGB637UF-186372-A	SGNTUC-020
Rifampin	[redacted]	RIFHPC[c]	8251-203 Appendix 28, Appendix 29	8373070	ONT-380-012

Analyte[a]	Validation Laboratory	Method Number	Validation Report Number/ Summary Table in Appendices	Bioanalytical Report	Clinical Study Number
Digoxin	[redacted]	DGNHPC[c]	8274044 Appendix 28, Appendix 30	8373070	ONT-380-012
Repaglinide	[redacted]	REPGHPP	8395746[d] Appendix 28, Appendix 33	8373070	ONT-380-012
Itraconazole Hydroxy-Itraconazole	[redacted]	ITRHPC[c]	8294126 Appendix 28, Appendix 32	8373070	ONT-380-012
Midazolam 1'-hydroxymidazolam 4-hydroxymidazolam	[redacted]	MHHHPC[c]	8280288 Appendix 28, Appendix 34	8373070	ONT-380-012
Tolbutamide Hydroxy tolbutamide 4-Carboxy tolbutamide	[redacted]	TLBUHPC[c]	8352993 Appendix 28, Appendix 35	8373070	ONT-380-012

5-DFCR=5'-deoxy-5-fluorocytidine; 5'-DFUR=5'-deoxy-5-fluorouridine; 5-FU=5-fluorouracil; FBAL=fluoro-β-alanine.
a All assays were performed in human plasma, unless specified in Section 4.1.
b Cross-validation report available.
c This is a proprietary method, thus it is not included in this New Drug Application submission.
d Method included within validation report.
e Additional stability data presented in the addendum reports.
f Validation was successful. Sample analysis failed ISR, and data was not used.
g Validation report is proprietary. Please see Right of Reference Letter for AV18-Metformin-01 AV18-Metformin-02.

1.3.1. 分析法性能及びバリデーションの基準

すべての分析法の検量線標準試料データ及び QC 試料データの精度及び真度について、事前に規定した許容基準を用いた（4.1 項）。各濃度 2 試料で 8 濃度以上から成る検量線とした。検量線には、真度が理論値の±15.0%（LLOQ では±20.0%）の範囲内である正確な標準試料データのみを用いた。各バッチの標準試料の 3/4 以上がこの真度の基準を満たす場合、そのバッチを許容可能とした（LLOQ 及び ULOQ を含む）。

各バッチの試験試料と共に QC 試料を分析した。各 QC 試料濃度における RSD が 15.0%以下（LLOQ では 20.0%以下）で、真度が理論値の±15.0%（LLOQ では±20.0%）の範囲内を許容可能な基準とした。

全 QC 試料の 2/3 以上かつ各 QC 濃度で半数以上がこの真度の基準を満たす場合、各バッチを許容可能とした。

ISR は、再測定した試料の 2/3 以上で、測定された濃度が最初の測定値と再測定値の平均の±20%以内であった場合に許容可能とした。特に明記しない限り、ISR は PK 解析に使用したすべてのデータセットで許容基準内であった。

生物学的分析法の要約、分析法の性能の要約並びに分析法の変更及びクロスバリデーション結果の要約を 4.1 項に示す。

1.3.2. 分析法のクロスバリデーション

ツカチニブ及び ONT-993 の定量には複数の分析法（Table 5）を用いたため、クロスバリデーションを実施した（AV18-ONT380-04）。クロスバリデーションでは 5 つの分析法を評価した。このうち 3 つの分析法は [redacted].でバリデートし、1 つは [redacted]、1 つは [redacted]でバリデートした。[redacted].ですべての方法のクロスバリデーションを実施した。クロスバリデーションの要約を 4.1.1 項に示す。

分析法の同等性は、1）既知の濃度の QC 試料の評価、2）35 の実試料の分析で実証した。

QC 試料については、許容基準を平均精度が 15.0%以下かつ平均真度が理論値の±15.0%以内と設定した。クロスバリデーションを実施したすべての方法が、事前に規定した QC 許容基準を満たした。さらに方法間の平均真度を比較することにより、QC データを評価した。5 way クロスバリデーションは複雑なため、1 つの方法（TM17-396）を基本とし、他の 4 つの方法と比較した。各方法の TM17-396 と比較した QC 試料の平均真度の差（%バイアスで示す）は、ツカチニブで 12.5%以内、ONT-993 で 15.2%以内であった（Table 5）。

実試料については、最初の試験結果（TM17-396 法）を他の 4 つの方法の結果とそれぞれ比較した。ISR 許容基準を適用し、クロスバリデーションの合否を判定した。実試料を使用したクロスバリデーションは、事前に規定した許容基準を満たした（Table 6）。

クロスバリデーションの結果、5 つの異なる分析法は互いに類似した試料濃度結果を示し、これらの分析法を比較することにより、ツカチニブ及び ONT-993 の一貫した測定が可能であることを示した。

Table 5. Absolute difference of QC mean bias % between TM17-396 and cross validation methods

	Tucatinib			ONT-993		
Method	3.00 ng/mL	50.0 ng/mL	800 ng/mL	3.00 ng/mL	50.0 ng/mL	800 ng/mL
TM14-281	4.4	1.0	7.2	3.0	4.6	4.2
TM17-433	0.4	1.6	9.6	5.3	12.0	2.6
1916	0.3	9.0	4.0	10.6	15.2	0.5
ONT3HPP	3.6	5.0	12.5	11.7	9.4	10.4

Table 6. Summary of ISR results for assay bridging

Method	Analyte	Total No. ISR Samples	Passing ISR Samples	Failing ISR Samples	Overall % Passing
TM14-281	ONT-380	35	28	7	80.0%
	ONT-993	35	26	9	74.3%
TM17-433	ONT-380	35	32	3	91.4%
	ONT-993	35	28	7	80.0%
[redacted] 1916	ONT-380	35	34	1	97.1%
	ONT-993	35	35	0	100.0%
[redacted] ONT3HPP	ONT-380	35	30	5	85.7%
	ONT-993	35	35	0	100.0%

2. 個々の試験結果の要約

ツカチニブの生物薬剤学（相対的 BA、食事及び制酸薬の影響）に関する PK を評価した臨床試験の詳細を Table 7 に示す。

Table 7. Summary of biopharmaceutical studies

Study Number	Study Objective	Number of Subjects	Treatment Design Treatment Duration, Dose
ARRAY-380-101	Dose-Escalation Assessment of safety and tolerability of tucatinib in subjects with advanced solid malignancies Determination of the MTD as defined by dose-limiting toxicity Expansion Phase Further characterize the safety profile of tucatinib in subjects with HER2-positive metastatic breast cancer Further characterize the plasma PK of tucatinib and a metabolite (ONT-993) in subjects with HER2-positive metastatic breast cancer Assess expression/amplification and phosphorylation status changes in HER2 in postdose tumor samples relative to predose tumor samples	Dose-Escalation 33 subjects total Expansion Phase 17 subjects total	Open-label, standard single-arm design dose-escalation Dose-escalation Single dose on Cycle 1 Day 1 If no dose-limiting toxicity (DLT), then BID dosing began Cycle 1 Day 3 Dose-expansion 28-day cycles at declared MTD of 600 mg BID Subjects were allowed to continue treatment in 28-day cycles
ARRAY-380-102	Characterize the plasma PK of tucatinib and a metabolite, ONT-993, after separate single-dose administrations of 4 oral tucatinib formulations. Evaluate and compare the relative bioavailability and the variability in exposure-related PK parameters among the 4 tucatinib formulations	14 fasted healthy adults	Exploratory, open-label PK study 4 single oral doses of 300 mg tucatinib with 7-day washout administered as 4 different oral formulations: Tucatinib powder-in-capsule (PIC) formulation Tucatinib micronized PIC Tucatinib aqueous suspension Tucatinib 20% Captisol and apple juice solution

Study Number	Study Objective	Number of Subjects	Treatment Design Treatment Duration, Dose
ARRAY-380-103	Evaluate the oral bioavailability of tucatinib tablets relative to tucatinib capsules, both in the fasted state Evaluate the oral bioavailability of tucatinib tablets after consumption of a high-fat meal relative to tucatinib tablets in the fasted state Evaluate the oral bioavailability of tucatinib tablets after daily oral administration of omeprazole for 5 days relative to tucatinib tablets, all in the fasting state	12 healthy adults planned (9 completed)	Exploratory, open-label PK 4 treatments of 300 mg tucatinib with 7-day washout administered in fasted or fed, and with or without following omeprazole Tucatinib capsules/fasted Tucatinib tablets/fasted Tucatinib tablets/fed Tucatinib tablets following omeprazole for 5 days/fasted

MTD=maximum tolerated dose; PK=pharmacokinetics.

2.1. 進行がん患者を対象としたツカチニブの第 1 相試験：ARRAY-380-101 試験

本治験は、ツカチニブの First-in-human 試験であり、進行 HER2 陽性固形がん、特に HER2 陽性 mBC を有する患者を対象に、ツカチニブの MTD を決定し、安全性、忍容性、予備的な有効性、PK 及びバイオマーカーを評価する非盲検試験である。本治験では、HER2 陽性 mBC 患者 43 例を含む計 50 例を用量漸増期及び拡大期に組み入れた。本治験で評価した食事の影響評価の概要を以下に示す。PIC 製剤 25～800 mg の用量で BID 投与したときの本薬の曝露量は、用量に概ね比例して増加した。用量漸増時及び反復投与時の PK 評価の概要は M2.7.2 に示す。

2.1.1. 試験デザイン及び PK 評価

本治験の用量漸増期に計 33 例を組み入れ、標準的な 3 + 3 用量漸増デザインを用いた。本治験で評価した用量コホートを評価順に Table 8 に示す。ツカチニブの PIC 製剤を単回投与及び反復投与したときの PK を評価した。Cycle 1 Day 1 に絶食下で PIC 製剤を投与し、その後 Day 3 では食後投与し、PIC 製剤投与時の食事の影響を評価した。ツカチニブ及びツカチニブの主要な代謝物（ONT-993）の PK 評価用の血液試料は Table 9 に示すように採取した。

Table 8.　Dose-escalation cohorts

Dose Cohort	Cycle 1 Day 1 Single Dose (mg)	BID Dosing	
		Dose (mg)	Total Daily Dose (mg)
1	25	25	50
2	50	50	100
3	100	100	200
4	200	200	400
5	300	300	600
6	500	500	1000
7	650	650	1300
8	800	800	1600
9	600	600	1200

Table 9.　Pharmacokinetic sampling time points for the dose-escalation phase

		Post dose (hours)								
Cycle/Day	Predose	0.5	1	1.5	2	3	4	8	12	24
Cycle 1 Day 1	X	X	X	X	X	X	X	X	X	X
Cycle 1 Day 3	X		X		X		X			
Cycle 1 Day 8	X									
Cycle 1 Day 15	X	X	X	X	X	X	X	X	X	
Cycle 2 Day 1	X									
Cycle 3 Day 1	X									
Cycle 4 Day 1	X									
Study Termination Visit	X									

Note: On Cycle 1 Day 1, subjects were to fast for 2 hours before and 1 hour after study drug administration. On Cycle 1 Day 3, study drug was to have been taken with food if possible. Otherwise, study drug was to be administered irrespective of food.
Windows for PK blood sampling were 0.5 hr (±5 minutes), 1 hr (±5 minutes), 1.5 hr (±10 minutes), 2 hr (±10 minutes), 3 hr (±15 minutes), 4 hr (±20 minutes), 8 hr (±45 minutes), 12 hr (±1 hr), and 24 hr (±2 hr).

2.1.2. 食事の影響

ツカチニブ（ARRY-380）の曝露量（AUC_4）に対する食事の影響の推定値（絶食下投与時の AUC_4 に対する食後投与時の AUC_4）は、用量コホート間で変動が大きかったものの、全体集団で 0.861、90% CI は 0.610～1.22（p = 0.4662）であり、すべてのコホートに有意な食事の影響は認められなかった。ONT-993 の結果は、未変化体の解析結果と類似しており、全コホートでの絶食時に対する食後の比の推定値は 0.890、90% CI は 0.650～1.22（p = 0.5329）であった。ツカチニブ及び ONT-993 の曝露量に対して、食後投与による大きな影響は認められなかった。

2.2. 健康成人を対象としたツカチニブ開発製剤の相対的 BA 試験：ARRAY-380-102 試験

本治験は、健康成人に絶食条件下で 4 種類のツカチニブ経口製剤（PIC 製剤：非微粉化、微粉化 PIC 製剤、水性懸濁液及び 20% Captisol リンゴジュース液剤）としてツカチニブ 300 mg を単回投与したときの PK、相対的 BA 及び安全性を評価した探索的非盲検試験である。適格基準を満たした 14 例を本治験に組み入れた。

2.2.1. 試験デザイン及び PK 評価

本治験では、4 つの投与期間（各治験薬製剤につき 1 期間）を設け、各投与期間にツカチニブ各製剤をすべての治験参加者に投与した。各投与期間は 7 日間の休薬期間を設け、4 週間の試験期間中、各参加者に 4 回のツカチニブ単回経口投与を実施した。投与計画を Table 10 に示す。各投与期間中に投与直前から投与後 24 時間までの血液試料を採取し、ツカチニブ及び ONT-993 の血漿中濃度を測定した。

Table 10. Treatments administered in ARRAY-380-102

Treatment Period	Study Day	No. of Subjects	Treatment	Dose	Dosing Regimen	Route of Administration
1	Day 1	14	Tucatinib PIC formulation (control)	300 mg	Single dose	Oral
2	Day 8	14	Tucatinib micronized PIC formulation[a]	300 mg	Single dose	Oral
3	Day 15	14	Tucatinib aqueous suspension[a]	300 mg	Single dose	Oral
4	Day 22	14	Tucatinib 20% Captisol® and apple juice solution[a]	300 mg	Single dose	Oral

a Compounding was performed at the clinical research unit's on-site pharmacy.

2.2.2. PK の結果

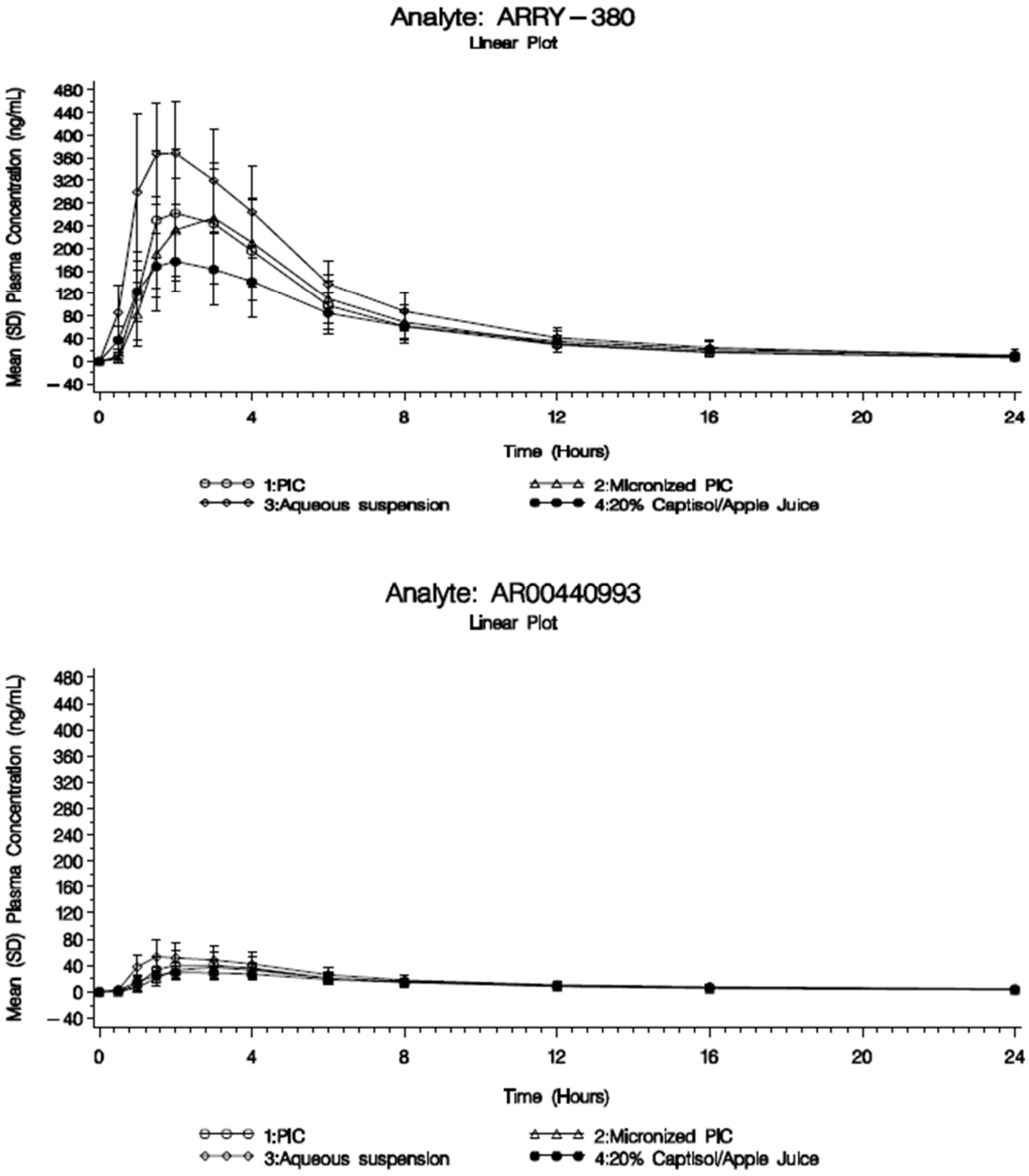
各投与製剤を投与したときのツカチニブ及び代謝物（ONT-993）の血漿中濃度の時間推移図を Figure 1 に示す。ツカチニブ及び ONT-993 の PK パラメータの要約を Table 11、ツカチニブの AUC 及び C_{max} の統計解析結果を Table 12 に示す。

いずれの製剤でも、ツカチニブの血漿中濃度は投与後 2～3 時間（中央値）で最高値に達した。C_{max} 到達後の血漿中濃度は二相性の低下を示した。概して、平均血漿中濃度は投与後 24 時間までに C_{max} の 20%未満に低下した。4 種類の製剤の中で、水性懸濁液では曝露量（AUC）が大きかった。PIC 製剤の曝露量は、微粉化 PIC 製剤及び 20% Captisol リンゴジュース液剤投与後の曝露量と大きな違いは認められなかった。微粉化 PIC 製剤投与時の本薬の T_{max} は、PIC 製剤投与

時と同程度であった。20% Captisol リンゴジュース液剤投与後のツカチニブの C_{max} は、各製剤の中で低い傾向を示した。曝露量の PK パラメータ（AUC_{inf} 及び C_{max}）の個体内変動は、PIC 製剤よりも他の 3 製剤で統計的に小さく（$p < 0.05$）、PIC 製剤以外での個体内変動は同程度であった（$p > 0.05$）。

ツカチニブに対する ONT-993 の総曝露量の比は、4 製剤すべてで 25%未満であった。ツカチニブ及び ONT-993 の $t_{½}$（算術平均値）は 4 製剤間で同程度である傾向を示し、ツカチニブで 5～7 時間、ONT-993 で 8～9 時間であった。

Figure 1　Mean (SD) plasma concentrations (ng/mL) over time (hours) of tucatinib (Upper Panel) and metabolite (Lower Panel) on linear scale by treatment formulation (pharmacokinetic population)



Abbreviations: PIC, powder-in-capsule.
Source: ARRAY-380-102 CSR, Section 15.2, Figure 15.2.1.1.

Table 11. Summary of plasma pharmacokinetic parameters of tucatinib and metabolite

	ARRY-380 Treatment			
Analyte Parameter	Micronized PIC (N =14)	Aqueous Suspension (N = 14)	20% Captisol/ Apple Juice (N = 14)	PIC (N = 14)
ARRY-380				
AUC_{inf} (h•ng/mL)[a]	1514.78 (34.2)	2212.16 (28.7)	1243.77 (35.3)[d]	1318.51 (41.2)
AUC_{last} (h•ng/mL)[a]	1454.20 (33.8)	2133.39 (28.2)	1243.47 (37.2)	1277.51 (41.2)
C_{max} (ng/mL)[a]	260.60 (31.5)	386.88 (27.3)	175.99 (32.4)	252.86 (40.3)
T_{max} (h)[b]	3.00	2.00	2.00	2.00
	(1.50, 4.02)	(1.00, 3.00)	(1.50, 3.00)	(1.50, 3.00)
$t_{1/2}$ (h)[c]	5.77 (28.3)	5.63 (24.6)	6.72 (31.4)	5.08 (26.7)
AR00440993				
AUC_{inf} (h•ng/mL)[a]	332.04 (24.2)[e]	443.17 (40.2)[e]	314.14 (29.9)[f]	318.49 (46.2)[e]
AUC_{last} (h•ng/mL)[a]	276.99 (29.0)	374.66 (40.9)	267.13 (31.2)	279.79 (46.2)
MR_{AUC}[c]	0.20 (30.6)	0.18 (30.5)	0.23 (40.4)	0.23 (38.6)
C_{max} (ng/mL)[a]	37.21 (27.1)	51.66 (44.7)	29.74 (33.6)	40.0 (47.5)
T_{max} (h)[b]	3.00	1.78	2.50	2.00
	(2.00, 4.02)	(1.00, 4.00)	(1.50, 4.07)	(1.50, 4.02)
$t_{1/2}$ (h)[c]	8.58 (26.0)	9.25 (22.3)	8.08 (21.2)[f]	7.70 (34.4)[e]

Abbreviations: PIC, powder-in-capsule.
a Geometric mean (coefficient of variation)
b Median (minimum, maximum)
c Mean (coefficient of variation)
d N = 12
e N = 13
f N = 9
Source: ARRAY-380-102 CSR, Section 15.2, Table 15.2.1.2

Table 12. Statistical analysis of plasma pharmacokinetic parameters of tucatinib

	Treatment [a]	N	Geometric Mean	Ratio of Geometric Means (test/reference)	90% CI of Ratio	Intersubject Variability	Intrasubject Variability	*P* Value[b]
AUC_{last} (ng•h/mL)	2	14	1454.20	1.14	0.90-1.44	0.10	0.32	0.0086
	3	14	2133.39	1.67	1.32-2.11	0.08	0.28	0.0016
	4	14	1243.47	0.97	0.77-1.23	0.12	0.35	0.0236
	1	14	1277.51			0.80	0.04	
	2/3	14		0.68	0.54-0.86	0.19	0.25	0.6004
	2/4	14		1.17	0.93-1.48	0.28	0.21	0.7005
	3/4	14		1.72	1.36-2.17	0.24	0.23	0.3660
AUC_{inf} (ng•h/mL)	2	14	1514.78	1.15	0.91-1.45	0.00	0.35	0.0096
	3	14	2212.16	1.68	1.33-2.12	0.08	0.29	0.0019
	4	12	1255.85	0.95	0.75-1.21	0.11	0.33	·
	1	14	1318.51			0.42	0.63	
	2/3	14		0.68	0.54-0.86	0.21	0.24	0.6098
	2/4	14		1.21	0.95-1.54	0.27	0.20	0.9854
	3/4	14		1.76	1.38-2.25	0.22	0.22	0.4774
C_{max}, (ng/mL)	2	14	260.60	1.03	0.82-1.29	0.33	0.02	0.0259
	3	14	386.88	1.53	1.22-1.92	0.08	0.28	0.0054
	4	14	175.99	0.70	0.55-0.87	0.00	0.34	0.0340
	1	14	252.86			0.35	0.57	
	2/3	14		0.67	0.54-0.85	0.20	0.23	0.6043
	2/4	14		1.48	1.18-1.86	0.29	0.16	0.8808
	3/4	14		2.20	1.75-2.76	0.22	0.22	0.5179

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; · denotes missing; PIC, powder-in-capsule.
a 1 = PIC; 2 = Micronized PIC; 3 = Aqueous suspension; 4 = 20% Captisol/apple juice.
b Pitman-Morgan test.
Notes: An ANOVA was performed on the natural logarithms of the parameters with treatment formulation as fixed effect and subject as random effect to allow both intersubject and intrasubject variance to be estimated. Point estimates and 90% CIs for differences on the log scale were exponentiated to obtain estimates for ratios of geometric means on the original scale.
Source: ARRAY-380-102 CSR, Section 15.2, Table 15.2.1.3.

2.3. 健康成人を対象としたツカチニブ錠及び PIC 製剤の相対的 BA、食事の影響及びオメプラゾールとの薬物相互作用試験：ARRAY-380-103 試験

本治験は、健康成人に絶食下でツカチニブ 300 mg 用量の錠剤と PIC 製剤の BA を比較する非盲検試験であり、ツカチニブ錠を絶食下及び食後に投与したときの食事の影響も評価した。さらに、ツカチニブ錠を投与したときのツカチニブの曝露量にプロトンポンプ阻害薬であるオメプラゾールが及ぼす影響を評価し、ツカチニブの吸収に対する胃内 pH 上昇の影響を評価した。本治験に男性 12 例を組み入れ、全例を対象に PK 解析を実施した。

2.3.1. 試験デザイン及び PK 評価

本治験は 4 つの投与期間を設け、各投与期間にすべての治験参加者に同じ投与条件でツカチニブを投与することとした。食後投与期（第 3 期）を除き、各投与期でほぼ同じ時刻（8 時間の絶食下）にツカチニブを投与した。各投与期間の間に 7 日間の休薬期間を設け、4 週間の試験期間中、各参加者に間隔を空けて 4 回のツカチニブ単回経口投与を実施した（Table 13）。オメプラゾールがツカチニブに及ぼす影響は、オメプラゾール 40 mg を絶食下で 1 日 1 回 5 日間投与し、5 日目にツカチニブ錠 300 mg とオメプラゾールを単回併用投与し、ツカチニブとオメプラゾール併用時と、絶食下でツカチニブ単剤投与時の PK を比較した。

各投与期間中に投与直前から投与後 24 時間までの血液試料を採取し、ツカチニブ及び ONT-993 の血漿中濃度を測定した。

Table 13.　Treatments administered in ARRAY-380-103

Treatment Period	Study Day	No. of Planned Subjects	Treatment	Dose	Fasted or Fed State	Route of Administration
1	Day 1	12	Tucatinib capsules	300 mg (3 × 100 mg)	Fasted	Oral (single dose)
2	Day 8	12	Tucatinib tablets	300 mg (2 × 150 mg)	Fasted	Oral (single dose)
3	Day 15	12[a]	Tucatinib tablets	300 mg (2 × 150 mg)	Fed	Oral (single dose)
4	Day 22	12[b]	Tucatinib tablets following omeprazole for 5 days	Tucatinib 300 mg (2 × 150 mg) Omeprazole 40 mg (2 × 20 mg)	Fasted	Oral (single dose)

a　Eleven subjects were treated in Period 3.
b　Nine subjects were treated in Period 4.

2.3.2. PK の結果

ツカチニブ錠及び PIC 製剤の相対的 BA、ツカチニブ錠の食事の影響及びオメプラゾール併用の影響を評価したときの血漿中ツカチニブ濃度の時間推移図をそれぞれ Figure 2、Figure 3 及び Figure 4 に示す。ツカチニブ及び ONT-993 の PK パラメータの要約を Table 14、ツカチニブの AUC 及び C_{max} の統計解析結果をそれぞれ Table 15、Table 16 及び Table 17、T_{max} の統計解析結果を Table 18 に示す。

- 錠剤と PIC 製剤の相対的 BA

各ツカチニブ製剤 300 mg（150 mg 錠を 2 錠又は PIC 製剤 100 mg を 3 カプセル）を単回投与したときの血漿中ツカチニブ濃度-時間推移は各製剤間で類似していた。ツカチニブの PK パラメータを統計解析した結果、AUC_{last} 及び AUC_{inf} の幾何平均値は PIC 製剤に比べて錠剤では約 10%大きかったが、C_{max} は両製剤間で同程度であった（Table 15）。

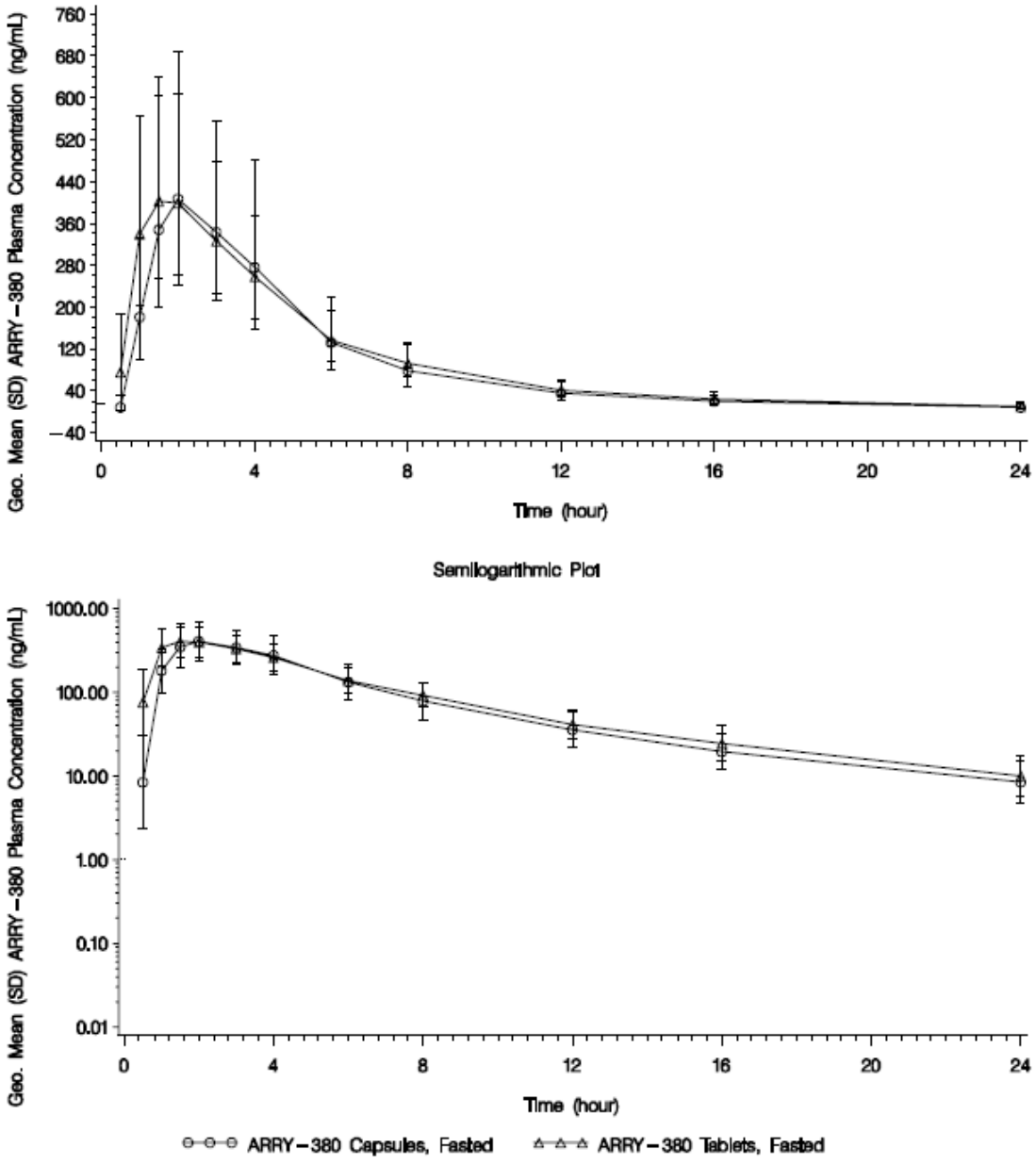
- ツカチニブ錠の食事の影響

ツカチニブ錠を食後に投与したときのツカチニブ及び ONT-993 の血漿中濃度の吸収速度は、絶食下よりも延長し、絶食下及び食後の T_{max} の中央値はそれぞれ 1.5 時間及び 4 時間であった（Table 14）。食後投与時のツカチニブの AUC_{last} は絶食下投与時に比べて約 48%大きく、C_{max} は約 8%高かった（Table 16）。

- オメプラゾール併用の影響

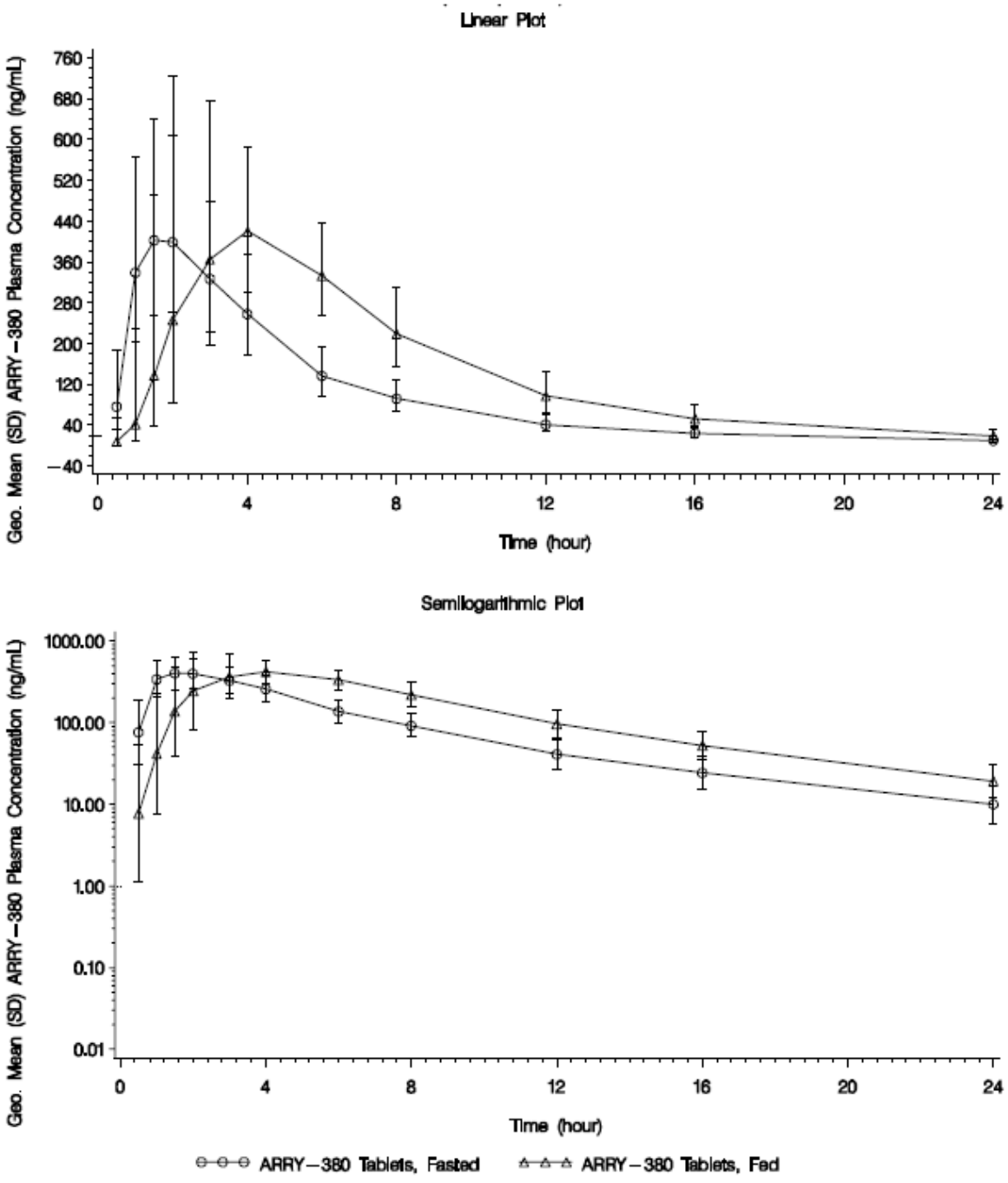
ツカチニブ錠をオメプラゾール併用及び非併用で投与したときの血漿中濃度-時間推移は、ツカチニブ及び ONT-993 のいずれにおいてもオメプラゾール併用及び非併用で類似していた。ツカチニブ錠をオメプラゾールと併用して投与したときでは、曝露量（AUC_{last} 及び AUC_{inf}）及び C_{max} はそれぞれ約 12%～13%低下したが、ツカチニブの T_{max}（中央値）への影響は認められなかった（Table 17 及び Table 18）。胃内 pH の上昇がツカチニブの吸収に影響する可能性は低いことが示された。

Figure 2　Geometric mean (geometric SD) plasma concentrations of tucatinib versus time (tablet versus capsule) linear and semilogarithmic scales (pharmacokinetic population)



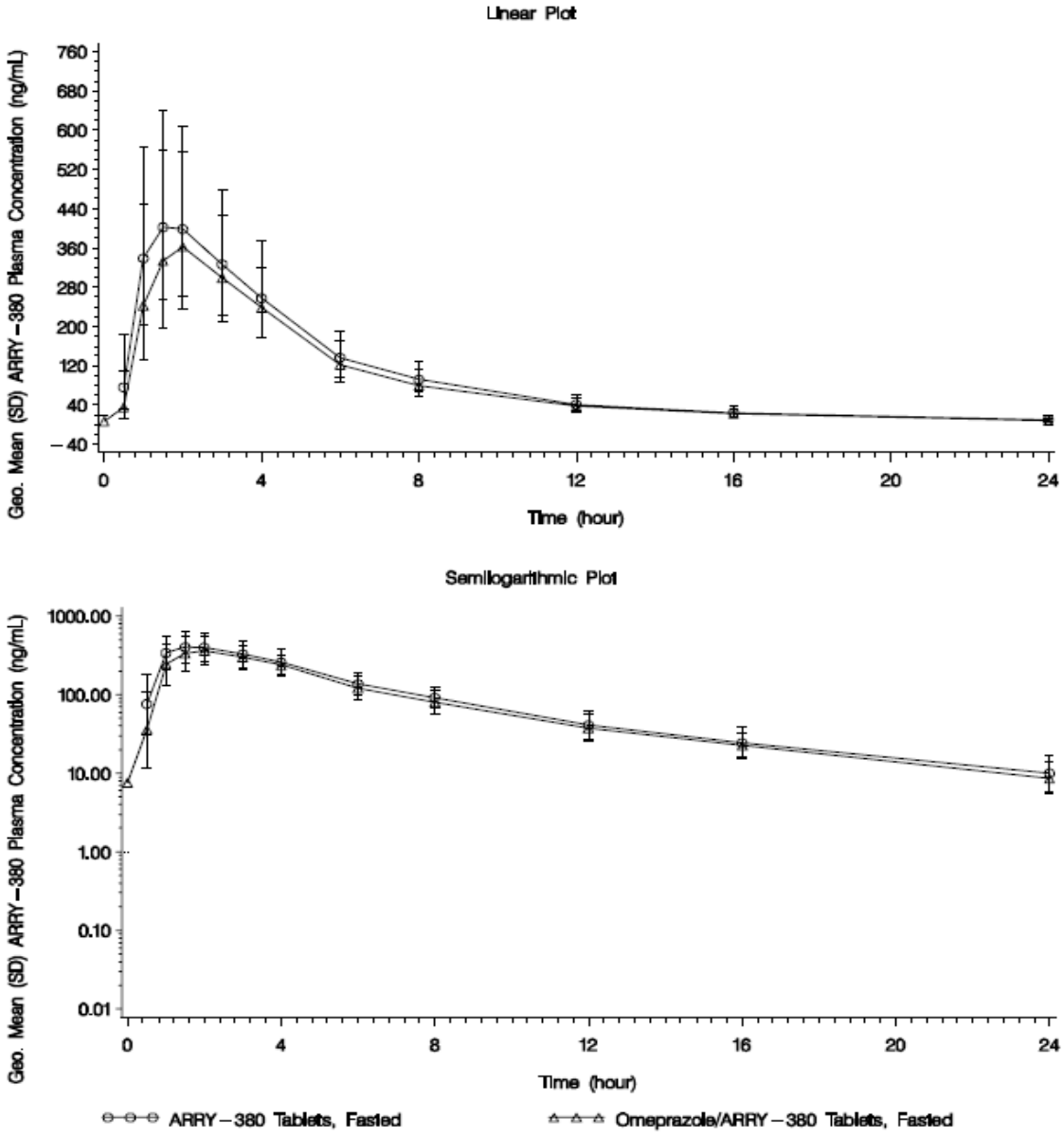
Upper: Linear plot, Bottom: semilogarithmic plot
Source: ARRAY-380-103 CSR, Figure 2

Figure 3　Geometric mean (geometric SD) plasma concentrations of tucatinib versus time (fed versus fasted) linear and semilogarithmic scales (pharmacokinetic population)



Source: ARRAY-380-103 CSR, Figure 3

Figure 4　Geometric mean (geometric SD) plasma concentrations of tucatinib versus time (tablet [fasted]/omeprazole versus tablet [fasted]) linear and semilogarithmic scales (pharmacokinetic population)



Source: ARRAY-380-103 CSR, Figure 4

Table 14. Summary of plasma pharmacokinetic parameters of tucatinib and ONT-993

Analyte Parameter	Treatment Tucatinib Capsules (Fasted) (Period 1) (N = 12)	Tucatinib Tablets (Fasted) (Period 2) (N = 12)	Tucatinib Tablets (Fed) (Period 3) (N = 11)	Omeprazole/Tucatinib Tablets (Fasted) (Period 4) (N = 9)
Tucatinib				
AUC_{inf} (hr*ng/mL)[a]	2200 (49.4)	2410 (36.2)	3660 (32.9)	2120 (33.1)
AUC_{last} (hr*ng/mL)[a]	2130 (49.2)	2330 (36.2)	3510 (32.9)	2040 (33.4)
MR_{AUC}[b]	0.187 (32.9)	0.161 (33.3)	0.118 (33.4)	0.172 (31.2)
C_{max} (ng/mL)[a]	428 (55.4)	429 (47.8)	479 (40.3)	379 (42.8)
T_{max} (hr)[c]	2.00 (1.00, 4.00)	1.51 (1.50, 2.00)	4.00 (2.00, 6.02)	2.00 (1.50, 3.00)
k_{el} (1/hr)[b]	0.130 (15.0)	0.132 (15.7)	0.140 (12.0)	0.133 (17.5)
T_{lag} (hr)[c]	0 (0, 0)	0 (0, 0)	0 (0, 0.517)	0 (0, 0)
$t_{½}$ (hr)[c]	5.30 (4.07, 7.00)	5.58 (4.17, 6.84)	5.07 (4.07, 6.03)	5.46 (4.34, 6.58)
ONT-993				
AUC_{inf} (hr*ng/mL)[a]	417 (77.9)[d]	383 (46.3)[e]	469 (32.5)[d]	436 (30.5)[f]
AUC_{last} (hr*ng/mL)[a]	376 (73.7)	354 (48.2)	390 (34.6)	336 (37.9)
C_{max} (ng/mL)[a]	54.2 (75.8)	46.8 (59.7)	42.4 (46.8)	44.1 (55.8)
T_{max} (hr)[c]	2.50 (1.50, 4.00)	1.51 (1.00, 3.00)	4.00 (1.00, 6.02)	2.00 (1.00, 4.00)
k_{el} (1/hr)[b]	0.0989 (24.6)[d]	0.0973 (24.4)[e]	0.0857 (20.4)	0.0841 (18.1)[g]
T_{lag} (hr)[c]	0.500 (0, 0.550)	0 (0, 0.500)	0.500 (0, 1.03)	0 (0, 0.500)
$t_{½}$ (hr)[c]	7.26 (4.87, 10.7)[d]	6.79 (4.90, 10.6)[e]	7.85 (6.44, 13.7)	8.29 (6.44, 11.0)[g]

a Geometric mean (coefficient of variation)
b Median (coefficient of variation)
c Median (minimum, maximum)
d N = 10
e N = 11
f N = 7
g N = 8

Note: Several subjects did not meet the requirements in order to retain k_{el} and its associated parameters ($t_{1/2}$ and AUC_{inf}). For ONT-993 plasma concentrations, Subject 1008 (capsule, fasted), Subject 1010 (tablet, fasted), and Subject 1004 (omeprazole/tablet, fasted) did not meet the predefined criteria. The AUC_{inf} for ONT-993 was set to missing for Subjects 1004 (tablet, fed) and 1006 (omeprazole/tablet, fasted) due to AUC extrapolated being >20%. One subject was not dosed during Period 3 (Subject 1002).
Source: ARRAY-380-103 CSR, Table 7, Table 15.2.1.2.

Table 15. Statistical analysis of plasma pharmacokinetic parameters of tucatinib (relative bioavailability)

	Treatment[a]	N	Geometric Mean	Ratio (%) of Geometric Means (Test/Reference)	90% CI of Ratio	Intersubject Variability	Intrasubject Variability	P value[b]
AUC_{last} (hr∗ng/mL)	B	12	2325.49	109.35	90.19–132.58	11.65	34.02	0.2766
	A	12	2126.69			0.00	49.16	
AUC_{inf} (hr∗ng/mL)	B	12	2410.82	109.68	90.51–132.91	36.24	0.00	0.2709
	A	12	2198.09			19.95	44.27	
C_{max} (ng/mL)	B	12	429.26	100.40	81.12–124.25	18.93	43.17	0.5965
	A	12	427.57			0.00	55.43	

CI=confidence interval; N=number of subjects
Note: A repeated measures analysis of variance was performed on the natural logarithms of the parameters with treatment as a fixed, repeated effect and subject as a random effect to allow both inter- and intrasubject variance to be estimated. Point estimates and 90% CIs for differences on the log scale were exponentiated to obtain estimates for ratios of geometric means on the original scale.
a A=tucatinib capsules 300 mg, fasted; B=tucatinib tablets 300 mg, fasted
b Pitman-Morgan test
Source: ARRAY-380-103 CSR, Table 8

Table 16. Statistical analysis of plasma pharmacokinetic parameters of tucatinib (food effect)

	Treatment[a]	N	Geometric Mean	Ratio (%) of Geometric Means (Fed/Fasted)	90% CI of Ratio	P value[b]
AUC_{last} (hr∗ng/mL)	C	11	3513.58	147.77	124.88–174.84	0.0018
	B	11	2377.82			
AUC_{inf} (hr∗ng/mL)	C	11	3661.64	148.51	125.90–175.17	0.0015
	B	11	2465.60			
C_{max} (ng/mL)	C	11	478.93	107.73	85.36–135.96	0.5749
	B	11	444.57			

CI=confidence interval; N=number of subjects
Note: A repeated measures analysis of variance was performed on the natural logarithms of the parameters with treatment as a fixed, repeated effect and subject as a random effect. Point estimates and 90% CIs for differences on the log scale were exponentiated to obtain estimates for ratios of geometric means on the original scale.
a B=tucatinib tablets 300 mg, fasted; C=tucatinib tablets 300 mg, fed
b For the treatment effect from linear mixed model
Source: ARRAY-380-103 CSR, Table 9

Table 17. Statistical analysis of plasma pharmacokinetic parameters of tucatinib (drug interaction)

	Treatment[a]	N	Geometric Mean	Ratio (%) of Geometric Means (Test/Reference)	90% CI of Ratio	P value[b]
AUC_{last} (hr∗ng/mL)	D	9	2044.11	87.89	73.79–104.70	0.2074
	B	9	2325.65			
AUC_{inf} (hr∗ng/mL)	D	9	2118.88	87.76	74.00–104.09	0.1927
	B	9	2414.28			
C_{max} (ng/mL)	D	9	378.62	86.96	66.80–113.21	0.3536
	B	9	435.38			

CI=confidence interval; N=number of subjects
Note: A repeated measures analysis of variance was performed on the natural logarithms of the parameters with treatment as a fixed, repeated effect and subject as a random effect. Point estimates and 90% CIs for differences on the log scale were exponentiated to obtain estimates for ratios of geometric means on the original scale.
a B=tucatinib tablets 300 mg, fasted; D= omeprazole 40 mg/tucatinib tablets 300 mg, fasted
b For the treatment effect from linear mixed model
Source: ARRAY-380-103 CSR, Table 10

Table 18. Statistical analysis for T_{max} difference of tucatinib (drug interaction)

	Treatment[a]	N	Median	Treatment Comparison	Difference[b]	90% CI of the Difference[b]
T_{max} (hr)	B	12	1.5	B-A	–0.5	–1.0, –0.3
	C	11	4.0	C-B	2.3	1.7, 3.0
	D	9	2.0	D-B	0.0	–0.0, 0.5
	A	12	2.0			

CI=confidence interval; hr=hour(s); N=number of subjects
a A=tucatinib capsules 300 mg, fasted; B=tucatinib tablets 300 mg, fasted; C=tucatinib tablets 300 mg, fed; D=omeprazole 40 mg/tucatinib tablets 300 mg, fasted
b From the Hodges-Lehmann method
Source: ARRAY-380-103 CSR, Table 11, Table 15.2.1.6

3. 全試験を通しての結果の比較と解析

初期のツカチニブの臨床試験では PIC 製剤、水性懸濁液及び溶液を使用し、その後、非臨床の PK で認められた変動を低減し、胃内 pH の変化による吸収への潜在的影響を最小限に抑える目的で錠剤を開発し、以後の臨床試験では錠剤を使用した。食事による変動が最も小さかった錠剤を市販用製剤として選択した。

全試験を通してツカチニブの各製剤の相対的 BA、食事の影響、制酸薬の影響を以下に示す。

3.1. 各製剤の相対的 BA

ツカチニブ臨床開発における最初の ARRAY-380-101 試験では、PIC 製剤を用いて、25～800 mg の用量における PK を評価した。血漿中ツカチニブ曝露量の個体間及び個体内変動を改善し、pH の変化の影響を最小化する製剤開発のために、ARRAY-380-102 試験では 4 種類の製剤（PIC 製剤、微粉化 PIC 製剤、水性懸濁液及び 20% Captisol リンゴジュース液剤）を用いて相対的 BA を評価し、PIC 製剤以外の製剤では AUC 及び C_{max} の変動が小さくなる可能性が示唆された。非臨床の PK で認められた変動を低減し、胃内 pH の変化による吸収への潜在的影響を最小限に抑える目的で錠剤を開発し、以後の臨床試験では錠剤を使用した。ARRAY-380-103 試験は、錠剤と PIC 製剤の比較試験であり、絶食下で両製剤を投与し、製剤間の相対的 BA を評価し、錠剤投与時の AUC は、PIC 製剤よりも高い傾向であった。ツカチニブ錠を単回及び反復投与した ARRAY-380-103 試験及び ONT-380-004 試験における 300 mg 経口投与後の曝露量は、ARRAY-380-101 試験における MTD（PIC 製剤として 600 mg を BID 経口投与）の曝露量と同程度であった（M2.7.2.4.1）。

3.2. 食事の影響

ARRAY-380-101 試験では、ツカチニブ PIC 製剤を絶食下及び食後に投与したときの PK を評価した結果、用量コホート間で変動が大きかったものの、全体集団で食事の影響は認められなかった。ARRAY-380-103 試験では、ツカチニブ錠を絶食下及び食後に投与したときの PK を評価した結果、食後投与時のツカチニブの AUC の増加はみられたものの 1.5 倍以下であり、T_{max} の変動は他の絶食下での試験結果（M2.7.2）も踏まえ概ね重なっており、C_{max} は 10%以下の上昇で大きな影響は認められなかった。ツカチニブの曝露量に対する食事の影響は大きくなかったことから、ONT-380-206 試験及び MK-7119-001 試験において、ツカチニブの用法は食事に関係なく BID 経口投与に設定した。ARRAY-380-101 試験及び ARRAY-380-103 試験の結果よりツカチニブの PK に及ぼす食事の影響は大きくなかったこと、ONT-380-206 試験及び MK-7119-001 試験の結果より食事に関係なくツカチニブ投与時の有効性と安全性が確認されたこと（M2.7.3.4）から、ツカチニブを食事に関係なく経口投与することは妥当と考える。

3.3. 制酸薬の影響

ARRAY-380-103 試験において、ツカチニブ錠とプロトンポンプ阻害薬オメプラゾールを併用投与したときの PK を評価した結果、本薬の BA に及ぼす影響は軽微であり、T_{max} に変化は認められなかったことから、胃内 pH の上昇がツカチニブの吸収に影響する可能性は低いことが示された。このため、ツカチニブ錠は制酸薬と併用可能と考える。

4. 付録

4.1. Summary Tables of Bioanalytical Assay and Performance

4.1.1. Cross Validation AV18-ONT380-04

Appendix 1. Summary of Method Modifications and Cross-Validation Results - AV18-ONT380-04

Bioanalytical method validation report name and hyperlink	HPLC-MS/MS Cross Validation for Bridging Multiple Validated Assays AV18-ONT380-04		
Changes in method	Cross validation of TM17-396 to TM14-281, TM17-433, 1916, and ONT3HPP for quantification of Tucatinib and ONT-993		
New validated assay range if any	NA		
Validation parameters	Cross-validation performance		Source location
Standard calibration curve performance during accuracy and precision runs	Cumulative accuracy (%bias) in standard calibrators from LLOQ to ULOQ	Tucatinib TM17-396: -3.9 to 8.0% ONT-993 TM17-396: -3.1 to 2.0%	AV18-ONT380-04 Table 3-2 Table 3-3
	Cumulative precision (%CV) from LLOQ to ULOQ	Tucatinib TM17-396: ≤ 9.9% ONT-993 TM17-396: ≤ 7.9%	AV18-ONT380-04 Table 3-2 Table 3-3
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 8 QCs	Tucatinib TM17-396: -8.0 to 5.5% ONT-993 TM17-396: -5.4 to 3.1%	AV18-ONT380-04 Table 3-13 Table 3-14
	Inter-batch %CV	Tucatinib TM17-396: ≤ 8.7% ONT-993 TM17-396: ≤ 6.3%	AV18-ONT380-04 Table 3-13 Table 3-14
	Percent TE	Tucatinib TM17-396: ≤ 16.7% ONT-993 TM17-396: ≤ 11.7%	Sum of largest error from %bias and %CV

Standard calibration curve performance during cross validation sample analysis	Cumulative accuracy (%bias) in standard calibrators from LLOQ to ULOQ	Tucatinib TM17-396: -3.9 to 8.0% TM14-281: -3.9 to 4.4% TM17-433: -9.8 to 10.2 1916: -5.0 to 9.0% ONT3HPP: -7.5 to 5.6% ONT-993 TM17-396: -3.1 to 2.0% TM14-281: -4.8 to 4.0% TM17-433: -2.9 to 3.8% 1916: -5.2 to 7.0% ONT3HPP: -3.1 to 6.5%	AV18-ONT380-04 Table 3-2 Table 3-4 Table 3-6 Table 3-8 Table 3-10 Table 3-3 Table 3-5 Table 3-7 Table 3-9 Table 3-11
	Cumulative precision (%CV) from LLOQ to ULOQ	Tucatinib TM17-396: ≤ 9.9% TM14-281: ≤ 9.9% TM17-433: ≤ 12.5% 1916: ≤ 10.2% ONT3HPP: ≤ 10.1% ONT-993 TM17-396: ≤ 7.9% TM14-281: ≤ 11.7% TM17-433: ≤ 11.7% 1916: ≤ 12.1% ONT3HPP: ≤ 8.1	AV18-ONT380-04 Table 3-2 Table 3-4 Table 3-6 Table 3-8 Table 3-10 Table 3-3 Table 3-5 Table 3-7 Table 3-9 Table 3-11
Performance of QCs during cross validation sample analysis	Cumulative accuracy[b] (%bias)	Tucatinib TM17-396: -8.0 to 5.5% TM14-281: -1.8 to 25.3%[b] TM17-433: 0.0 to 9.4% 1916: -9.3 to 2.0% ONT3HPP: 2.0 to 11.8% ONT-993 TM17-396: -5.4 to 3.1% TM14-281: -3.4 to 11.7% TM17-433: -3.4 to 1.0% 1916: -7.0 to 5.2% ONT3HPP: 0.4 to 4.7%	AV18-ONT380-04 Table 3-13 Table 3-15 Table 3-17 Table 3-19 Table 3-21 Table 3-14 Table 3-16 Table 3-18 Table 3-20 Table 3-22

	Inter-batch %CV	Tucatinib TM17-396: ≤ 8.7% TM14-281: ≤ 29.8%[b] TM17-433: ≤ 22.2%[b] 1916: ≤22.5%[b] ONT3HPP: ≤ 10.8% ONT-993 TM17-396: ≤ 6.3% TM14-281: ≤ 33.1%[b] TM17-433: ≤ 15.8%[b] 1916: ≤ 13.8 ONT3HPP: ≤ 7.4%	 Table 3-13 Table 3-15 Table 3-17 Table 3-19 Table 3-21 Table 3-14 Table 3-16 Table 3-18 Table 3-20 Table 3-22
Cross-validation	Analysis of cross validation QCs (n=6)[a] at 3.00, 50.0, and 800 ng/mL of Tucatinib and ONT-993. QCs qualified using TM17-396. Cross validation with QCs passed acceptance criteria for all methods: TM14-281, TM17-433, 1916, and ONT3HPP.		AV18-ONT380-04 Table 3-23 to 3-30
Performance of Cross Validation QCs[a]	Cumulative accuracy (%bias)	Tucatinib TM14-281: -0.8 to 5.7 TM17-433: 0.8 to 1.7% 1916: -4.0 to 8.2% ONT3HPP: -2.3 to 4.5% ONT-993 TM14-281: -0.8 to 3.7% TM17-433: 0.0 to 6.6% 1916: -3.1 to 11.3% ONT3HPP: -14.8 to -11%	AV18-ONT380-04 Table 3-23 Table 3-25 Table 3-27 Table 3-29 Table 3-24 Table 3-26 Table 3-28 Table 3-30
	Inter-batch %CV	Tucatinib TM14-281: ≤ 8.1% TM17-433: ≤ 11.9% 1916: ≤ 11.0% ONT3HPP: ≤ 3.9% ONT-993 TM14-281: ≤ 9.4% TM17-433: ≤ 13.1% 1916: ≤ 14.9% ONT3HPP: ≤ 6.1%	AV18-ONT380-04 Table 3-23 Table 3-25 Table 3-27 Table 3-29 Table 3-24 Table 3-26 Table 3-28 Table 3-30

<table>
<tr><td>Cross-validation</td><td colspan="5">Analysis of 35 incurred samples passed ISR acceptance criteria, compared to original analysis using TM17-396.

<table><tr><th>Method</th><th>Analyte</th><th>Passing ISR Samples</th><th>Failing ISR Samples</th><th>Overall % Passing</th></tr><tr><td rowspan="2">TM14-281</td><td>ONT-380</td><td>28</td><td>7</td><td>80.0 %</td></tr><tr><td>ONT-993</td><td>26</td><td>9</td><td>74.3 %</td></tr><tr><td rowspan="2">TM17-433</td><td>ONT-380</td><td>32</td><td>3</td><td>91.4 %</td></tr><tr><td>ONT-993</td><td>28</td><td>7</td><td>80.0 %</td></tr><tr><td rowspan="2">1916</td><td>ONT-380</td><td>34</td><td>1</td><td>97.1 %</td></tr><tr><td>ONT-993</td><td>35</td><td>0</td><td>100.0 %</td></tr><tr><td rowspan="2">ONT3HPP</td><td>ONT-380</td><td>30</td><td>5</td><td>85.7 %</td></tr><tr><td>ONT-993</td><td>35</td><td>0</td><td>100.0 %</td></tr></table></td><td>Table 3-31</td></tr>
<tr><td>List other parameters</td><td colspan="5">NA</td><td>NA</td></tr>
</table>

CV=coefficient of variation; HPLC/MS/MS=high-performance liquid chromatography tandem mass spectrometry; ISR=incurred sample reproducibility; LLOQ=lower limit of quality; NA=not applicable; TE=total error; ULOQ=upper limit of quality.

a QCs qualified using TM17-396 in accuracy and precision batch 1 (AV18-ONT380-04, Table 3-14).

b Values outside of acceptance criteria (±15% theoretical) included in summary statistics. All batches pass QC acceptance criteria for sample analysis (AV18-ONT380-04, Page 36).

4.1.2. Validation 1916

Appendix 2. Bioanalytical Method Life Cycle Information - 1916 - Tucatinib and ONT-993

	1916	ARRAY-380-101	ARRAY-380-102	ARRAY-380-103
Analyte	Tucatinib ONT-993	Tucatinib ONT-993	Tucatinib ONT-993	Tucatinib ONT-993
Validation type	Full	In study	In study	In study
eCTD reference number	NA	NA	NA	NA
Method ID	STM1916	STM1916	STM1916	STM1916
Duration of time method is in use	10Apr2008-21Oct2011	28Jun2008-17Apr2012	05Feb2010-10Mar2010	27Oct2011-17Nov2011
Bioanalytical site	[redacted]			
Matrix	K_2EDTA Plasma (Human)			
Platform	HPLC/MS/MS method			
Format	Following liquid-liquid extraction, extracts were analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) in selective reaction monitoring (SRM) mode under optimized conditions for detection of tucatinib, ONT-993 and ONT-572 (internal standard) positive ions formed by TurboIonSpray ionization.			

	1916	ARRAY-380-101	ARRAY-380-102	ARRAY-380-103
Stock reference Lot number Expiration date	Tucatinib AR00432380-29 08Apr2009 ONT-993 AR00440993-02 01Nov2008 ONT-572 AR00442572-02 03Mar2009	Tucatinib L-ARR07-165 07Nov2012 ONT-993 AR00440993-02 13Feb2013 ONT-572 AR00442572-02 09Feb2012	Tucatinib AR00432380-29 31Mar2010 ONT-993 AR00440993-02 15Jan2010 ONT-572 AR00442572-02 02Mar2010	Tucatinib L-ARR07-165 07Nov2012 ONT-993 AR00440993-02 07Feb2012 ONT-572 AR00442572-02 09Feb2012
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL
Matrix study population	Normal	Diseased, breast cancer patients	Normal	Normal
Link to reports and applicable amendments	Validation reports: 1916 1916 Amendment 2 1916 Amendment 3	BioA report: 1827	BioA report: CP10-001	BioA report: BA-12-001
Synopsis of amendment history	Long-term stock solution stability and matrix stability updated			

Appendix 3. Summary Method Performance - 1916

Bioanalytical method validation report name, amendments, and hyperlinks	1916 1916 Amendment 2 1916 Amendment 3		
Method description	Liquid-liquid extraction and HPLC-MS/MS were used to determine the concentration of tucatinib and ONT-993 from human K_2EDTA plasma. An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API3000). A X-bridge C18, 3.5 mm HPLC column (2.1 × 50 mm) was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. ONT-572 served as the internal standard for tucatinib and ONT-993. The peak area of the product ion of each analyte was measured against the peak area of the product ion of the respective internal standard.		
Materials used for standard calibration curve and concentration	Tucatinib, ONT-993: 1.00, 2.50, 10.0, 50.0, 100, 500, 800, 1000 ng/mL		
Validated assay range	Tucatinib, ONT-993: 1.00 to 1000 ng/mL		
Material used for quality controls (QCs) and concentration	Tucatinib, ONT-993: 3.00, 300, 850 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Tucatinib, ONT-993: quadratic regression with 1/x weighting		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	1916 Section 2.4.4.1
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Tucatinib: -6.4% to 9% ONT-993: -4.6% to 7%	1916 Table 6
	Cumulative precision (%CV) from LLOQ to ULOQ	Tucatinib: ≤5.9% ONT-993: ≤9.2%	1916 Table 4
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 3 QCs	Tucatinib: -0.4% to 9% ONT-993: -1.6% to 8%	1916 Table 8 Table 10

	Inter-batch %CV QCs	Tucatinib: 1.3% to 2.8% ONT-993: 0.90% to 5.3%	1916 Table 8 Table 10
	Total Error (TE)	Tucatinib: 11.8% ONT-993: 13.3%	Sum of largest bias and CV reported
Selectivity & matrix effect	Selectivity 6 lots tested There were no detectable peaks observed at the retention times of tucatinib or ONT-993 in either the 0 ng/mL samples or the control blanks. There were no detectable peaks at the retention time of ONT-572 internal standard in the control blanks.		1916 Page 19, 20
Interference & specificity	Concomitant Drug Interference Screen A neat solution containing 100 ng/mL acetaminophen, 100 ng/mL acetylsalicylic acid, and 100 ng/mL of caffeine was injected using the same chromatographic method used for tucatinib and ONT-993. The peak observed at the retention time of tucatinib was less ≤20% of the average LLOQ. There were no detectable peaks observed at the retention time of ONT-993 or the internal standard.		1916 Page 20
Hemolysis effect	NA		NA
Lipemic effect	NA		NA
Dilution linearity & hook effect	NA		NA
Bench-top/process stability	Tucatinib and ONT-993 are stable when stored in matrix for up to 26 hours at ambient temperature. Tucatinib: %Bias was 1 to 2%; %CV ≤2.7%. ONT-993: %Bias was 1 to 2%; %CV ≤6.4%.		1916 Table 23 Table 24
Freeze-Thaw stability	Tucatinib and ONT-993 are stable after 7 freeze/thaw cycles. Tucatinib: %Bias was -2.6 to -0.7%; %CV ≤6.4%. ONT-993: %Bias was 6 to 15%; %CV ≤12%.		1916 Table 27 Table 28
Long-term storage	-70°C 1093 days Tucatinib: %bias ≤ 6%; %CV ≤ 4.5% ONT-993: %bias ≤ 5%; %CV ≤ 5.4% -20°C 448 days Tucatinib: %bias ≤ 8.0%; %CV ≤ 4.2% ONT-993: %bias ≤ 9.0%; %CV ≤ 4.0%		1916 Amendment 3 Table 6 Table 7 Amendment 2 Table 10 Table 11

Parallelism	NA	NA
Carry over	During method validation, carryover was found to be persistent in blank plasma samples injected following the ULOQ standard; autosampler washes were optimized to reduce carryover. To circumvent carryover from standards and QC samples to the study samples, the sample analysis queue was assigned as follows: standards in ascending order of concentration, two carryover blank samples, one specificity/selectivity plasma blank sample, QC samples in ascending order of concentration, one carryover plasma blank sample, one specificity/selectivity plasma blank sample, and then study samples in increasing order of sample collection time to minimize large changes in sample concentration. Following a series of study samples, the sequence (plasma blank sample, QC samples in ascending order of concentration, followed by one carryover blank sample, and one specificity/selectivity plasma blank sample) was repeated. The final calibration curve was analyzed in ascending order of concentration.	NA
Method performance in study ARRAY-380-101 A Phase I, Open-label, Multiple Dose Study to Assess the Safety, Tolerability and Pharmacokinetics of ARRY-380 Given on a Daily Oral Regimen in Subjects with Advanced Cancer Report 1827		
Assay passing rate	Tucatinib: 50 out of 65 batches were accepted. ONT-993: 44 out of 65 batches were accepted.	1827 Table 1 Table 2
Standard curve performance	Cumulative %Bias Tucatinib: -1.2% to 3% ONT-993: -1.5% to 2% Cumulative %CV Tucatinib: ≤21% ONT-993: ≤30%	1827 Table 14 Table 16
QC performance	Cumulative %Bias Tucatinib: -2.8% to 3% ONT-993: -2.5% to 2% Cumulative %CV Tucatinib: ≤9.6% ONT-993: ≤15%	1827 Table 17 Table 18

Method reproducibility	Samples reanalyzed: 112 of 1176 (Tucatinib) 90 of 1176 (ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 97.3% (Tucatinib) 81.1% (ONT-993) ISR passes acceptance criteria.	1827 Table 11 Table 12
Study sample analysis/ stability	The sample with the longest storage time before final assay for tucatinib was extracted following a maximum storage period of 708 days. Long-term matrix stability was established for at least 1,093 days.	NA
Standard calibration curve performance during accuracy and precision runs	See above	
Method performance in study ARRAY-380-102 Please use bioanalytical reports for this portion of the table Source cited on right hand side An Exploratory, Open-Label, Single-Dose, Four-Period Study Evaluating the Pharmacokinetics, Relative Bioavailability and Safety of Four Oral ARRY-380 Formulations in Healthy Subjects Report CP10-001		
Assay passing rate	Tucatinib: 26 out of 27 batches were accepted. ONT-993: 22 out of 27 batches were accepted.	CP10-001 Table 1
Standard curve performance	Cumulative %Bias Tucatinib: -2.1% to 3% ONT-993:-2.5% to 2% Cumulative %CV Tucatinib: ≤19% ONT-993: ≤14%	CP10-001 Table 9 Table 11
QC performance	Cumulative %Bias Tucatinib: -0.9% to 2% ONT-993: -2.3% to 4% Cumulative %CV Tucatinib: ≤7.6% ONT-993: ≤12%	CP10-001 Table 12 Table 13

Method reproducibility	Samples reanalyzed: 111 out of 672 (Tucatinib and ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 87.4% (Tucatinib) 78.4% (ONT-993) ISR passes acceptance criteria.	CP10-001 Table 6 Table 7
Study sample analysis/ stability	The samples with the longest storage time before final assay were extracted for bioanalysis following a maximum storage period of 17 days. Long-term matrix stability was established for at least 1,093 days.	NA
Standard calibration curve performance during accuracy and precision runs	See above	
Method performance in study ARRAY-380-103 An Open-Label, Single-Dose, Four-Period Study Evaluating the Relative Bioavailability, Potential Food Effect and Omeprazole Drug Interaction of Oral ARRY-380 Capsules and Tablets in Healthy Subjects Report BA-12-001		
Assay passing rate	Tucatinib: 14 out of 14 batches were accepted. ONT-993: 14 out of 14 batches were accepted.	BA-12-001 Table 1
Standard curve performance	Cumulative %Bias Tucatinib: -5.2% to 7% ONT-993: -6.2% to 3% Cumulative %CV Tucatinib: ≤16% ONT-993: ≤22%	BA-12-001 Table 8 Table 10
QC performance	Cumulative %Bias Tucatinib: -1.6% to 4% ONT-993: -2.5% to 11% Cumulative %CV Tucatinib: ≤16% ONT-993: ≤17%	BA-12-001 Table 11 Table 12

Method reproducibility	Samples reanalyzed: 91 out of 552 (Tucatinib) 92 out of 552 (ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 90.1% (Tucatinib) 75.0% (ONT-993) ISR passes acceptance criteria.	BA-12-001 Table 5 Table 6
Study sample analysis/ stability	The samples with the longest storage time before final assay were extracted for incurred sample reproducibility analysis following a maximum storage period of 28 days. Long-term matrix stability was established for at least 1,093 days.	NA
Standard calibration curve performance during accuracy and precision runs	See above	

4.1.3. Validation: 8373099

Appendix 4. Bioanalytical Method Life Cycle Information - 8373099

	8373099	ONT-380-008	ONT-380-009	ONT-380-011	ONT-380-012
Analyte	Tucatinib ONT-993	Tucatinib ONT-993	Tucatinib ONT-993	Tucatinib ONT-993	Tucatinib ONT-993
Validation type	Full	In study	In study	In study	In study
eCTD reference number	NA	NA	NA	NA	NA
Method ID	ONT3HPP	ONT3HPP	ONT3HPP	ONT3HPP	ONT3HPP
Duration of time method is in use	16Jan2018-24Jan2018	19Mar2018-23Mar2018	19Dec2018-29May2019	15Feb2019-27Mar2019	26Oct2018-22Jan2019
Bioanalytical site	[redacted]				
Matrix	K_2EDTA Plasma				
Platform	HPLC/MS/MS				
Format	Acetonitrile precipitation and HPLC/MS/MS were used to determine the concentration of tucatinib and ONT-993 from human K_2EDTA plasma. An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API4500). A Luna C18(2) 100 Å HPLC column (2.0 x 50 mm) from Phenomenex was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. ONT-380-d_6 and ONT-993-d_4 served as the internal standard for ONT-380 and ONT-993 respectively. The peak area of the product ion of each analyte was measured against the peak area of the product ion of the respective internal standard.				

	8373099	ONT-380-008	ONT-380-009	ONT-380-011	ONT-380-012
Stock reference (storage temperature) **Lot number** **Expiration date**	Tucatinib L-ARR14-123 (Corden BN BO1406P006) 28Feb2018 ONT-993 ES6156-23-PI 30Nov2018 ONT-380-d_6 AR00442571-03 30Nov2018 ONT-993-d_4 ES6139-19 30Nov2018	Tucatinib L-ARR14-123 20Jun2018 ONT-993 ES6156-23-P1 30Nov2018 ONT-380-d_6 AR00442572-03 30Nov2018 ONT-993-d_4 ES6139-19 30Nov2018	Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 ES6156-23-PI 30Nov2018 ONT-993 AR00440993-03 21Nov2020 ONT-380-d_6 AR00442572-03 30Nov2018 ONT-993-d_4 ES6139-19 30Nov2018	Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 ES6156-23-PI 30Nov2018 ONT-993 AR00440993-03 21Nov2020 ONT-380-d_6 AR00442572-03 30Nov2018 ONT-993-d_4 ES6139-19 30Nov2018	Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 ES6156-23-PI 30Nov2018 ONT-380-d_6 AR00442572-03-R 30Nov2018 ONT-993-d_4 ES6139-19 30Nov2018
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL
Matrix study population	Normal	Normal	Normal and hepatically-impaired male and female subjects	Normal	Normal
Link to reports and applicable amendments	Validation report: 8373099	Bioanalytical report: 8373100	Bioanalytical report: 8373071	Bioanalytical report: 8373072	Bioanalytical report: 8373070
Synopsis of amendment history	NA	NA	NA	NA	NA

Appendix 5. Summary Method Performance – 8373099

Bioanalytical method validation report name, amendments, and hyperlinks	Validation report: 8373099 Method: ONT3HPP		
Method description	Acetonitrile precipitation and HPLC/MS/MS were used to determine the concentration of tucatinib and ONT-993 from human K_2EDTA plasma. An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API4500). A Luna C18(2) 100 Å HPLC column (2.0 × 50 mm) from Phenomenex was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. ONT-380-d_6 and ONT-993-d_4 served as the internal standard for ONT-380 and ONT-993 respectively. The peak area of the product ion of each analyte was measured against the peak area of the product ion of the respective internal standard.		
Materials used for standard calibration curve and concentration	Tucatinib and ONT-993: 1.00, 2.00, 10.0, 25.0, 100, 400, 800, 1000 ng/mL		
Validated assay range	Tucatinib and ONT-993: 1.00 to 1000 ng/mL		
Material used for quality controls (QCs) and concentration	Tucatinib and ONT-993: 3.00, 30.0, 400, 750 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Tucatinib and ONT-993: Weighted $1/x^2$ linear regression		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	8373099 Table 6.2 Table 6.3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Tucatinib: -2.0% to 1.0% ONT-993 -3.3% to 1.5%	8373099 Table 6.2 Table 6.3
	Cumulative precision (%CV) from LLOQ to ULOQ	Tucatinib: ≤5.7% ONT-993: ≤8.0%	8373099 Table 6.2 Table 6.3

Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	Tucatinib: -0.7% to 6.3% ONT-993: -4.0% to 2.0%	8373099 Table 6.6 Table 6.7
	Inter-batch %CV QCs	Tucatinib: ≤7.5% ONT-993: ≤7.0%	8373099 Table 6.6 Table 6.7
	Total Error (TE)	Tucatinib: 13.8% ONT-993: 11.0%	Sum of largest bias and CV reported
Selectivity & matrix effect	Selectivity 6 lots tested No significant interference (≤20.0% of the mean (acceptable) LLOQ calibration standard response and ≤5.0% of ISTD response in the control zero sample) in the chromatographic regions of the analytes and ISTD. Matrix Effect 6 lots tested Tucatinib: %bias -3.8%; %CV ≤7.2% ONT-993: %bias -6.3%; %CV ≤6.8% Matrix Factor 6 lots tested Internal standard normalized matrix factor Tucatinib: LQC 0.922, %CV 8.4%; HQC 1.01, %CV 3.5% ONT-993: LQC 0.921, %CV 3.0%; HQC 0.959, %CV 1.6%		8373099 Table 6.12 Table 6.13 Table 6.14 Table 6.15

Interference & specificity	Run Blanks and Interference of Analyte on other Analytes or Internal Standard No significant interference (≤20.0% of the mean (acceptable) LLOQ calibration standard response and ≤5.0% of ISTD response in the control zero sample) in the chromatographic regions of the ISTD and other non-spiked analytes. With the exception when carryover was observed, which did not impact the integrity of the data. Interference and Specificity in the presence of coadministered compounds in study ONT-380-012 was evaluated during sample analysis (report 8373070) The blank samples spiked with Itraconazole, Rifampin, Gemfibrozil, Repaglinide, Midazolam, 1′-Hydroxymidazolam, Tolbutamide, 4-Hydroxy Tolbutamide, and Digoxin did not contain any significant peaks at the retention time of tucatinib and ONT-993 or ISTD. This confirms that the presence of Itraconazole, Rifampin, Gemfibrozil, Repaglinide, Midazolam, 1′-Hydroxymidazolam, Tolbutamide, 4-Hydroxy Tolbutamide, and Digoxin does not affect the selectivity of Tucatinib and ONT-993.	8373099 Section 4.7 Section 5.2 8373070 Section 4.5
	Accuracy and precision of tucatinib and ONT-993 QCs in the presence of co administered compounds and metabolites were within acceptance criteria. The data confirms that the presence of Itraconazole, Rifampin, Gemfibrozil, Repaglinide, Midazolam, 1′-Hydroxymidazolam, Tolbutamide, 4-Hydroxy Tolbutamide, and Digoxin does not affect the quantification of ONT-380 and ONT-993.	8373070 Table 8.61-8.80
Hemolysis effect	The data were within the acceptance criteria. The number of lots tested are the following: 1 (Tucatinib), 1 (ONT-993) Tucatinib: %bias within 7.7%; %CV ≤6.9% ONT-993: %bias within 4.3%; %CV ≤5.8%	8373099 Table 6.18 Table 6.19
Lipemic effect	The data were within the acceptance criteria. The number of lots tested are the following: 1 (Tucatinib), 1 (ONT-993) Tucatinib: %bias within 1.7%; %CV ≤9.8% ONT-993: %bias within 1.3%; %CV ≤5.1%	8373099 Table 6.20 Table 6.21
Dilution linearity & hook effect	NA	NA

Bench-top/process stability	Stability was demonstrated for bench-top samples at ambient temperature after 27 hours. Tucatinib: %bias within 4.7%; %CV ≤9.5% ONT-993: %bias within 4.0%; %CV ≤7.8%	8373099 Table 6.30 Table 6.31
Freeze-Thaw stability	The stability of tucatinib and ONT-993 was confirmed for up to 4 freeze-thaw cycles. -10 to -30°C Tucatinib: %bias within 9.7%; %CV ≤5.1% ONT-993: %bias within 4.7%; %CV ≤10.2% -60 to -80°C Tucatinib: %bias within 4.7%; %CV ≤7.0% ONT-993: %bias within 3.7%; %CV ≤7.2%	8373099 Table 6.32 Table 6.33
Long-term storage	Long-term stability for tucatinib (1119 days at -70°C) and ONT-993 (1124 days at -70°C) was established elsewhere.	AV14-ONT380-01 Addendum 2 Table 4-26 AV14-ONT380-01 Addendum 1 Table 3-40
Parallelism	NA	NA
Carry over	In analytical runs 2, 4, 6, 7 and 8, carryover >20.0% of the ONT-380 peak area of the mean utilized LLOQ was observed. In analytical run 8, carryover >20.0% of the ONT-993 peak area of the mean utilized LLOQ was observed.	8373099 Section 5.2
Method performance in study ONT-380-008 Determination of ONT-380 and ONT-993 in Human Plasma and Urine by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-008 Report 8373100		
Assay passing rate	Tucatinib: 4 out of 5 batches were accepted. ONT-993: 4 out of 4 batches were accepted.	8373100 Table 10.1
Standard curve performance	<u>Cumulative %Bias</u> Tucatinib: -2.7% to 5.0% ONT-993: -5.5% to 6.5% <u>Cumulative %CV</u> Tucatinib: ≤7.6% ONT-993: ≤7.2%	8373100 Table 10.6 Table 10.7

QC performance	Cumulative %Bias Tucatinib: 0.7% to 7.5% ONT-993: -2.0% to 3.9% Cumulative %CV Tucatinib: ≤9.1% ONT-993: ≤10.7%	8373100 Table 10.14 Table 10.15
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 24 of 136 (18%, Tucatinib) 24 of 136 (18%, ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 88% (Tucatinib) 96% (ONT-993)	8373100 Table 10.20 Table 10.21
Study sample analysis/ stability	All plasma samples were analyzed within the validated stability range. Long-term stability for tucatinib (1119 days at -70°C) and ONT-993 (1124 days at -70°C) was established in AV14-ONT380-01 Addendum 1 and Addendum 2.	AV14-ONT380-01 Addendum 2 Table 4-26 AV14-ONT380-01 Addendum 1 Table 3-40
Standard calibration curve performance during accuracy and precision runs	See above.	
Method performance in study ONT-380-009 Determination of ONT-380 and ONT-993 in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-009 Report 8373071		
Assay passing rate	Tucatinib: 12 out of 16 batches were accepted. ONT-993: 12 out of 15 batches were accepted.	8373071 Table 8.1
Standard curve performance	Cumulative %Bias Tucatinib: -1.5% to 1.0% ONT-993: -2.0% to 1.5% Cumulative %CV Tucatinib: -≤7.5% ONT-993: ≤9.7%	8373071 Table 8.3 Table 8.4

QC performance	Cumulative %Bias Tucatinib: 2.3% to 4.0% ONT-993: -0.3% to 24.3% (with Grubbs outlier) ONT-993: -0.3% to 0.7% (minus Grubbs outlier) Cumulative %CV Tucatinib: -≤5.9% ONT-993: ≤93.6% (with Grubbs outlier) ONT-993: ≤5.4% (with Grubbs outlier)	8373071 Table 8.7 Table 8.8
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 50 of 481 (10.4%, Tucatinib) 50 of 481 (10.4%, ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100% (Tucatinib) 98.0% (ONT-993)	8373071 Table 8.11 Table 8.12
Study sample analysis/ stability	All plasma samples were analyzed within the validated stability range. The longest possible duration of sample storage was calculated to be 283 days [Day 1 signed approval of the protocol (20Aug2018) to the end of analysis (29May2019)]. Long-term stability for tucatinib (1119 days at -70°C) and ONT-993 (1124 days at -70°C) was established in AV14-ONT380-01 Addendum 1 and Addendum 2.	8373071 Page 8
Standard calibration curve performance during accuracy and precision runs	See above.	
Method performance in study ONT-380-011 Determination of ONT-380, ONT-993, and Moxifloxacin in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-011 Report 8373072		
Assay passing rate	Tucatinib: 14 out of 18 batches were accepted. ONT-993: 14 out of 18 batches were accepted.	8373072 Table 9.1
Standard curve performance	Cumulative %Bias Tucatinib: -1.0% to 1.0% ONT-993: -1.0% to 1.0% Cumulative %CV Tucatinib: ≤5.2% ONT-993: ≤4.6%	8373072 Table 9.4 Table 9.5

QC performance	Cumulative %Bias Tucatinib: 2.3% to 3.7% ONT-993: -2.3% to -0.4% Cumulative %CV Tucatinib: ≤4.1% ONT-993: ≤4.7%	8373072 Table 9.10 Table 9.11
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 70 of 663 (10.6%, Tucatinib) 70 of 663 (10.6%, ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100% (Tucatinib) 98.6% (ONT-993)	8373072 Table 9.16 Table 9.17
Study sample analysis/ stability	All plasma samples were analyzed within the validated stability range. The longest possible duration of sample storage was calculated to be 171 days. Long-term stability for tucatinib (1119 days at -70°C) and ONT-993 (1124 days at -70°C) was established in AV14-ONT380-01 Addendum 1 and Addendum 2.	8373072 Table 1
Standard calibration curve performance during accuracy and precision runs	See above.	
Method performance in study ONT-380-012 Determination of Digoxin, Itraconazole, Rifampin, Tolbutamide, Hydroxy Tolbutamide, Gemfibrozil, Midazolam, 1'-Hydroxymidazolam, ONT-380, ONT-993, and Repaglinide in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-012 Report 8373070		
Assay passing rate	Tucatinib: 68 out of 69 batches were accepted. ONT-993: 68 out of 69 batches were accepted.	8373070 Table 8.1
Standard curve performance	Cumulative %Bias Tucatinib: -4.0% to 2.0% ONT-993: -1.6% to 1.6% Cumulative %CV Tucatinib: ≤6.0% ONT-993: ≤7.5%	8373070 Table 8.13 Table 8.14

QC performance	Cumulative %Bias Tucatinib: -2.3% to 2.7% ONT-993: 0.3% to 2.3% Cumulative %CV Tucatinib: ≤6.0% ONT-993: ≤6.2%	8373070 Table 8.35 Table 8.36
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): Tucatinib: 227 of 3359 (7%) ONT-993: 227 of 3358 (7%) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 94.3% (Tucatinib) 98.7% (ONT-993)	8373070 Table 8.51 Table 8.52
Study sample analysis/ stability	All plasma samples were analyzed within the validated stability range. The longest possible duration of sample storage was calculated to be 155 days [Day 1 signed approval of the protocol (20Aug2018) to the end of analysis (22Jan2019)]. Long-term stability for tucatinib (1119 days at -70°C) and ONT-993 (1124 days at -70°C) was established in AV14-ONT380-01 Addendum 1 and Addendum 2.	8373070 Table 1
Standard calibration curve performance during accuracy and precision runs	See above.	

4.1.4. Validation: 8385605

Appendix 6. Bioanalytical Method Life Cycle Information - 8385605

	8385605	**ONT-380-008**
Analyte	Tucatinib ONT-993	Tucatinib ONT-993
Validation type	Full	In Study
eCTD reference number	NA	NA
Method ID	ONT3HUP	ONT3HUP
Duration of time method is in use	03May2018-present	23May2018-30May2018
Bioanalytical site	[redacted]	
Matrix	Urine (treated 10:1 with 5% Tween 80)	
Platform	HPLC/MS/MS method	
Format	A validated LC-MS/MS method was developed at [redacted] to quantify Tucatinib and ONT-993 from urine treated with Tween. Urine samples treated 10:1 with 5% Tween 80 were extracted using acetonitrile, diluted with water and subsequently injected onto a Luna C18(2) 50 × 2 mm 5 µm particle sizle (Phenomenex)column and analyzed using a Sceix 5500 mass spectrometer. ONT-380-d6 and ONT-993-d4 served as internal standards for tucatinib and ONT-993 respectively. The peak area of the product ions of the compounds are measured against the peak area of the product ions of the respective internal standards.	

	8385605	**ONT-380-008**
Stock reference **Lot number** **Expiration date**	Tucatinib: L-ARR14-123 20Jun2018 Tucatinib: 6451-P54/17-001 14Nov2019 ONT-993: ES6156-23-P1 30Nov2018 ONT-380-d_6 AR00442572-03 30Nov2018 ONT-993-d_4 ES6139-19 30Nov2018	Tucatinib: L-ARR14-123 20Jun2018 ONT-993: ES6156-23-P1 30Nov2018 ONT-380-d_6 AR00442571-03 30Nov2018 ONT-993-d_4 ES6139-19 30Nov2018
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	Tucatinib, ONT-993: 0.300 to 300 ng/mL	Tucatinib, ONT-993: 0.300 to 300 ng/mL
Matrix study population	Normal	Normal
Link to reports and applicable amendments	Validation report: 8385605	Bioanalytical report: 8373100
Synopsis of amendment history	NA	NA

Appendix 7. Summary Method Performance - 8385605

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: 8385605		
Method description	A validated LC-MS/MS method was developed at [redacted] to quantify Tucatinib and ONT-993 from urine treated with Tween. Urine samples treated 10:1 with 5% Tween 80 were extracted using acetonitrile, diluted with water and subsequently injected onto a Luna C18(2) 50x2 mm 5 µm particle sizle (Phenomenex)column and analyzed using a Sceix 5500 mass spectrometer. ONT-380-d6 and ONT-993-d4 served as internal standards for Tucatinib and ONT-993 respectively. The peak area of the product ions of the compounds are measured against the peak area of the product ions of the respective internal standards.		
Materials used for standard calibration curve and concentration	Tucatinib, ONT-993: 0.300, 0.600, 2.50, 5.00, 25.0, 100, 250, 300 ng/mL		
Validated assay range	Tucatinib, ONT-993: 0.300 to 300 ng/mL		
Material used for quality controls (QCs) and concentration	Tucatinib, ONT-993: 0.900, 12.5, 150, 225 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model and weighting	Tucatinib, ONT-993: Linear regression; weighted $1/x^2$		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	8385605 Table 7.2 Table 7.3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Tucatinib: -1.8 to 1.6% ONT-993: -2.8 to 2.0%	8385605 Table 7.2 Table 7.3
	Cumulative precision (%CV) from LLOQ to ULOQ	Tucatinib: ≤6.5% ONT-993: ≤7.3%	8385605 Table 7.2 Table 7.3
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	Tucatinib: -8.7 to -3.3% ONT-993: -5.8 to -2.0%	8385605 Table 7.6 Table 7.7

	Inter-batch %CV	Tucatinib: ≤5.3% ONT-993: ≤12.3%	8385605 Table 7.6 Table 7.7
	Total Error (TE)	Tucatinib: ≤14.0% ONT-993: ≤18.1%	Sum of largest bias and CV reported
Selectivity & matrix effect	Matrix selectivity 6 lots were tested. Passes acceptance criteria_ Tucatinib: %Bias -10.3%; %CV 2.9% ONT-993: %Bias 6.7%; %CV 4.5% Matrix Factor 6 lots tested Internal standard normalized matrix factor Tucatinib: LQC 1.02, %CV 3.5%; HQC 0.998, %CV 1.5% ONT-993: LQC 1.03, %CV 5.7%; HQC 1.02, %CV 1.9%		8385605 Table 7.10 Table 7.11 Table 7.12 Table 7.13
Interference & specificity	Passes acceptance criteria		8385605 Section 4.7
Hemolysis effect	NA		NA
Lipemic effect	NA		NA
Dilution linearity & hook effect	NA		NA
Bench-top/process stability	The stability of tucatinib and ONT-993 was confirmed for up to 24 hours on the benchtop at ambient conditions. Tucatinib: %Bias: ≤8.9% %CV: ≤4.0% ONT-993: %Bias: ≤9.9% %CV: ≤9.2% Processed Sample Stability Tucatinib 2 to 8°C for 167 hours: %Bias: ≤6.7% %CV: ≤4.7% ONT-993 2 to 8°C for 167 hours: %Bias: ≤8.4% %CV: ≤8.3%		8385605 Table 7.22 Table 7.23 Table 7.32 Table 7.33

Freeze-Thaw stability	The stability of tucatinib and ONT-993 was confirmed for up to 5 freeze-thaw cycles. Tucatinib: %Bias: -≤6.9% %CV: ≤4.9% ONT-993: %Bias: -≤11.2% %CV: ≤6.4%	8385605 Table 7.24 Table 7.25
Long-term storage	Tucatinib and ONT-993 were stable in samples stored for 160 days at -10 to -30°C/-60 to -80°C and in processed extracts stored for 167 hours at 2 to 8°C. Tucatinib: -10 to -30°C for 160 days: %Bias: ≤4.7% %CV: ≤3.7% -60 to -80°C for 160 days: %Bias: ≤2.7% (excluding Grubbs outlier) ≤9.0% (including Grubbs outlier) %CV: ≤4.0% (excluding Grubbs outlier) ≤17.1% (including Grubbs outlier) ONT-993: -10 to -30°C for 160 days: %Bias: ≤1.8% %CV: ≤5.9% -60 to -80°C for 160 days: %Bias: ≤5.2% (excluding Grubbs outlier) ≤12.9% (including Grubbs outlier) %CV: ≤6.2% (excluding Grubbs outlier) ≤17.4% (including Grubbs outlier)	8385605 Table 7.30 Table 7.31
Parallelism	NA	NA
Carry over	Carryover >20.0% of the peak area of the mean utilized LLOQ was monitored and observed in select runs. The carryover observed in runs 4 and 7 was not significant and did not affect the integrity of the data generated. Passes acceptance criteria.	8385605 Section 5.1

Determination of ONT-380 and ONT-993 in Human Plasma and Urine by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-008 Report 8373100		
Assay passing rate	Tucatinib: 4 out of 4 batches were accepted. ONT-993: 4 out of 4 batches were accepted.	8373100 Table 10.1
Standard curve performance	Cumulative %Bias Tucatinib: -4.8% to 6.0% ONT-993: -4.4 to 5.6% Cumulative %CV Tucatinib: -≤8.9% ONT-993: ≤8.6%	8373100 Table 10.8 Table 10.9
QC performance	Cumulative %Bias Tucatinib: -3.6% to 1.0% ONT-993: -2.4% to 0.7% Cumulative %CV Tucatinib: -≤6.5% ONT-993: ≤9.5	8373100 Table 10.16 Table 10.17
Method reproducibility	Samples reanalyzed (% total analyzed): 24 of 113 samples (21%, Tucatinib) 24 of 113 samples (21%, ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 96% (Tucatinib) 100% (ONT-993) ISR passes acceptance criteria.	8373100 Table 10.22 Table 10.23
Study sample analysis/stability	All treated urine samples were analyzed within 12 days of being treated with Tween 80, which is within the established frozen matrix stability duration of 32 days for treated urine following storage at -60°C to -80°C. All untreated urine samples were treated with Tween 80 within 122 days of collection following storage at -60 to -80°C; however, frozen matrix stability has not yet been determined in untreated urine, and testing is ongoing.	8373100 Section 6.3
Standard calibration curve performance during accuracy and precision runs	See above	

4.1.5. Validation AV14-OTN380-01

Appendix 8. Bioanalytical Method Life Cycle Information - AV14-OTN380-01

	AV14-OTN380-01	AV14-ONT380-01 Addendum 1	ONT-380-004	ONT-380-005	ONT-380-206	SGNTUC-020
Analyte	Tucatinib ONT-993 Capecitabine 5'-DFCR 5'-DFUR	Tucatinib ONT-993	Tucatinib ONT-993	Tucatinib ONT-993 Capecitabine 5'-DFCR 5'-DFUR	Tucatinib ONT-993	Tucatinib ONT-993
Validation type	Full	Partial	In study	In study	In study	In study
eCTD reference number	NA	NA	NA	NA	NA	NA
Method ID	TM14-281	TM17-396	TM14-281	TM14-281	TM14-281 TM17-396	TM17-396
Duration of time method is in use	06Feb2014-present	17Feb2017-present	06Feb2014- 07Dec2016	06Feb2014- 01Mar2017	22Aug2016- 13Sep2019	06Mar2019- 18Mar2019
Bioanalytical site	[redacted]					
Matrix	K_2EDTA Plasma +/- THU	K_2EDTA Plasma +/- THU	K_2EDTA Plasma +/- THU	K_2EDTA Plasma +/- THU	K_2EDTA Plasma +/- THU	K_2EDTA Plasma +/- THU Human Urine
Platform	HPLC/MS/MS method					

	AV14-OTN380-01	AV14-ONT380-01 Addendum 1	ONT-380-004	ONT-380-005	ONT-380-206	SGNTUC-020
Format	A validated HPLC/MS/MS method was developed at [redacted], Inc. to determine concentrations of tucatinib, ONT-993, capecitabine, 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR) in human K_2EDTA plasma containing 1.2 µM tetrahydrouridine (THU). Methanol precipitation is followed by separation on a Discovery HS-C18, 3 µm HPLC column (2.1 × 50 mm), and quantification on a Sciex API5500 or API6500. The peak area of the product ions of the compounds are measured against the peak area of the product ions of the respective internal standards.					
Stock reference **Lot number** **Expiration date**	Tucatinib AR00432380-25 NA ONT-993 AR00440993-03 NA OTN-572 AR00442572-01 NA Capecitabine 0431797 23Aug2014 D11-Capecitabine A1212 NA	Tucatinib AR00432380-25 NA ARM-0221 20Jun2017 NA 6451-P54/17-001 14Nov2019 ONT-572 AR00442572-01 NA AR00442572-03 04Oct2018 ONT-993 AR00440993-03 NA	Tucatinib AR00432380-25 NA ONT-993 AR00440993-03 NA OTN-572 AR00442572-01 NA Capecitabine 0431797 23Aug2016 10-XJZ-158-1 05Feb2019	Tucatinib AR00432380-25 NA ONT-993 AR00440993-03 NA OTN-572 AR00442572-01 NA Capecitabine 0431797 23Aug2016 10-XJZ-158-1 05Feb2019	Tucatinib AR00432380-25 NA Tucatinib ARM-0221 20Jun2018 Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 AR00440993-03 30Nov2018 ONT-993 ES6156-23-P1 30Nov2018	Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 AR00440993-03 21Nov2020 ONT-572 AR00442572-03 30Nov2018 d4-ONT-993 ES6139-19 30Nov2018

	AV14-OTN380-01	AV14-ONT380-01 Addendum 1	ONT-380-004	ONT-380-005	ONT-380-206	SGNTUC-020
Stock reference **Lot number** **Expiration date** **(continued)**	5'-DFCR L2111 NA $^{13}C,^{15}N_2$-5'-DFCR 22-ABY-48-1 27Apr2016 5'-DFUR 0439624-4 10Jul2014 $^{13}C,^{15}N_2$-5'-DFUR 8-SHG-1-1 29Apr2016	ONT-993 ES6156-23-P1 30Nov2018 Capecitabine 10-XJZ-158-1 05Feb2019 5'-DFCR 1-BKG-58-1 12Jun2020 5'-DFUR 3-MAR-46-3 19Oct2021 5-FU 11-SCC-140-1 07Sep2021 FBAL K2618 26Nov2023	D_{11}-Capecitabine A1212 NA D_{11}-Capecitabine X145P3 05Jun2017 5'-DFCR L2111 NA D1414 NA 1-BKG-58-1 12Jun2020 $^{13}C,^{15}N_2$-5'-DFCR 22-ABY-48-1 27Apr2016 5'-DFUR 0439624 10Jan2016	D_{11}-Capecitabine A1212 NA D_{11}-Capecitabine X145P3 05Jun2017 5'-DFCR L2111 NA 5'-DFCR D1414 NA 5'-DFCR 1-BKG-58-1 12Jun2020 $^{13}C,^{15}N_2$-5'-DFCR 22-ABY-48-1 27Apr2016 5'-DFUR 0439624 10Jan2016	ONT-993 AR00440993-03 21Nov2020 d4-ONT-993 ES6139-19 30Nov2018 Capecitabine 0431797 23Aug2016 Capecitabine 10-XJZ-158-1 05Feb2019 5'-DFCR D1414 NA 5'-DFCR 1-BKG-58-1 12Jun2020 5'-DFUR 0439624 10Jan2016	

	AV14-OTN380-01	AV14-ONT380-01 Addendum 1	ONT-380-004	ONT-380-005	ONT-380-206	SGNTUC-020
Stock reference **Lot number** **Expiration date** **(continued)**		DM1 24442 28Nov2020 d_4-ONT-993 E6139-19 30Nov2018	 3-MAR-46-3 19Oct2021 $^{13}C,^{15}N_2$-5'-DFUR 8-SHG-1-1 29Apr2016	5'-DFUR 3-MAR-46-3 19Oct2021 $^{13}C,^{15}N_2$-5'-DFUR 8-SHG-1-1 29Apr2016 FBAL MKBK1527V NA E2016 18Oct2017 $^{13}C_3$-FBAL (Int. Std.) 4-SDJ-123-3 16May2017	5'-DFUR 3-MAR-46-3 19Oct2021 Capecitabine-d11 X145P3 05Jun2017 5'-DFCR $^{13}C,^{15}N_2$ 22-ABY-48-1 27Apr2016 5'-DFUR $^{13}C,^{15}N_2$ 8-SHG-1-1 29Apr2016	
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	Tucatinib, ONT-993: 1.00 to 1000 ng/mL Capecitabine, 5'-DFCR, 5'-DFUR: 5.00 to 5000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL Capecitabine, 5'-DFCR, 5'-DFUR: 5.00 to 5000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL	Tucatinib, ONT-993: 1.00 to 1000 ng/mL
Matrix study population	Normal	Normal	Diseased, breast cancer patients	Diseased, breast cancer patients	Diseased, breast cancer patients	Normal

	AV14-OTN380-01	AV14-ONT380-01 Addendum 1	ONT-380-004	ONT-380-005	ONT-380-206	SGNTUC-020
Link to reports and applicable amendments	Validation report: AV14-OTN380-01, AV14-OTN380-01 Addendum 2	Validation report: AV14-OTN380-01 Addendum 1, AV14-OTN380-01 Addendum 3	Bioanalytical reports: AD14-747	Bioanalytical reports: AD14-734, AD14-734 Amendment 1	Bioanalytical reports: AD16-950	Bioanalytical report: AD19-849
Synopsis of amendment history	Addendum 2 includes stock and working solution stability, plasma stability, and whole blood stability.	Addendum 1 includes the following modifications to method: removal of capecitabine, 5'-DFCR and 5'-DFUR, addition of d_4-ONT993 as internal standard for ONT-993. Additional tests: (1) whole blood stability of tucatinib and ONT993 on ice. (2) interference and stability of tucatinib and ONT-993 in the presence of DM1 with no THU present.	NA	Report Amendment 1 re-issues the report in its entirety to denote 131 of 134 samples identified by the sponsor that did not have THU added to the collected samples. The capecitabine and metabolite results are therefore considered unreliable because of pre-analytical technical issues.	NA	NA

	AV14-OTN380-01	AV14-ONT380-01 Addendum 1	ONT-380-004	ONT-380-005	ONT-380-206	SGNTUC-020
Synopsis of amendment history (continued)		(3) interference and stability of tucatinib and ONT-993 in the presence of capecitabine and metabolites (5'-DFCR, 5'-DFUR, 5-FU, and FBAL) with no THU. Addendum 3 is an extension of interference and stability testing of tucatinib and ONT-993: (1) in the presence of DM1 with no THU present. (2) in the presence of capecitabine and metabolites (5'-DFCR, 5'-DFUR, 5-FU, and FBAL) with no THU present.				

Appendix 9. Summary Method Performance - AV14-OTN380-01

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: AV14-OTN380-01, AV14-OTN380-01 Addendum 2		
Method description	Methanol precipitation and HPLC-MS/MS were used to determine the concentration of tucatinib, ONT-993, capecitabine, 5'-DFUR, and 5'-DFCR from human K_2EDTA plasma containing 1.2 µM tetrahydrouridine (THU). An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API5500 or API6500). A Discovery HS-C18, 3 µm HPLC column (2.1 × 50 mm) from Supelco was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. OTN-572 served as the internal standard for tucatinib and ONT-993. D_{11}-Capecitabine served as the internal standard for capecitabine. $^{13}C,^{15}N_2$-5'-DFCR served as the internal standard for 5'-DFCR. $^{13}C,^{15}N_2$-5'-DFUR served as the internal standard for 5'-DFUR. The peak area of the product ion of each analyte were measured against the peak area of the product ion of the respective internal standard.		
Materials used for standard calibration curve and concentration	Tucatinib, ONT-993: 1.00, 2.00, 5.00, 10.0, 100, 500, 900, 1000 ng/mL Capecitabine, 5'-DFCR, 5'-DFUR: 5.00, 10.0, 25.0, 50.0, 500, 2500, 4500, 5000 ng/mL		
Validated assay range	Tucatinib, ONT-993: 1.00 to 1000 ng/mL Capecitabine, 5'-DFCR, 5'-DFUR: 5.00 to 5000 ng/mL		
Material used for quality controls (QCs) and concentration	Tucatinib, ONT-993: 3.00, 50.0 and 800 ng/mL Capecitabine, 5'-DFCR, 5'-DFUR: 15.0, 250 and 4000 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Tucatinib, 5'-DFCR and 5'-DFUR: Linear regression; weighted ($1/x^2$) linear regression analysis ONT-993, capecitabine: quadratic regression analysis; weighted ($1/x^2$)		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	AV14-ONT380-01 Table 3-1 Table 3-2 Table 3-3 Table 3-4 Table 3-5

	Cumulative accuracy (%bias) from LLOQ to ULOQ	Tucatinib: -1.9% to 3.6% ONT-993: -1.5% to 1.0% Capecitabine: -3.8% to 2.8% 5'-DFCR: -1.0% to 1.0% 5'-DFUR: -2.0% to 2.0%	AV14-ONT380-01 Table 3-1 Table 3-2 Table 3-3 Table 3-4 Table 3-5
	Cumulative precision (%CV) from LLOQ to ULOQ	Tucatinib: ≤5.8% ONT-993: ≤6.8% Capecitabine: ≤6.1% 5'-DFCR: ≤6.1% 5'-DFUR: ≤7.7%	AV14-ONT380-01 Table 3-1 Table 3-2 Table 3-3 Table 3-4 Table 3-5
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	Tucatinib: -4.4% to 7.0% ONT-993: -5.2% to 5.7% Capecitabine: -3.3% to 3.4% 5'-DFCR: -2.8% to 3.8% 5'-DFUR: -2.0% to 8.0%	AV14-ONT380-01 Table 3-6 Table 3-7 Table 3-8 Table 3-9 Table 3-10
	Inter-batch %CV QCs	Tucatinib: ≤8.7% ONT-993: ≤11.6% Capecitabine: ≤8.2% 5'-DFCR: ≤9.1% 5'-DFUR: ≤12.7%	AV14-ONT380-01 Table 3-6 Table 3-7 Table 3-8 Table 3-9 Table 3-10
	Total Error (TE)	Tucatinib: 15.7% ONT-993: 17.3% Capecitabine: 11.6% 5'-DFCR: 12.9% 5'-DFUR: 20.7%	Sum of largest bias and CV reported for analytes.

Selectivity & matrix effect	Blank interference 6 lots were tested, no significant interfering peaks were found that eluted at the retention times of all analytes. Matrix factor 6 lots of unpooled matrix tested The precision of the IS normalized matrix factor was acceptable for all analytes IS-Normalized Matrix Factor %CV Tucatinib: 3.2% ONT-993: 13.0% Capecitabine: 3.2% 5'-DFCR: 2.2% 5'-DFUR: 9.3%	AV14-ONT380-01 Table 3-46 Table 3-47 Table 3-48 Table 3-49 Table 3-50 Table 3-31 Table 3-32 Table 3-33 Table 3-34 Table 3-35
Interference & specificity	Multi-analyte selectivity Individual QCs of tucatinib, ONT-993, capecitabine, 5'-DFCR, and 5'-DFUR were quantified against the calibration curve containing all 5. All passed the QC criteria. Coadmin selectivity Tucatinib, ONT-993, capecitabine, 5'-DFCR and 5'-DFUR will not be affected by the presence of DM1 (50 ng/mL), which is released by the coadiminstered ADC, T-DM1. Metabolite selectivity All analytes passed and will not be affected by 5-fluorouracil (250 ng/mL) and α-fluoro-β-alanine (10000 ng/mL).	AV14-ONT380-01 Table 3-51 Table 3-52 Table 3-53 Table 3-54 Table 3-55 Table 3-61 Table 3-62 Table 3-63 Table 3-64 Table 3-65 Table 3-56 Table 3-57 Table 3-58 Table 3-59 Table 3-60
Hemolysis effect	Analysis of tucatinib, ONT-993, capecitabine, 5'-DFCR, and 5'-DFUR has been validated in up to 1% hemolyzed human K_2EDTA plasma. One lot was tested. All analytes passed in 10% hemolyzed human K_2EDTA plasma, except ONT-993 which did not meet acceptance criteria. Tucatinib: %Bias <11.7%; %CV <6.6% Capecitabine: %Bias <9.5%; %CV <6.4% 5'-DFCR: %Bias <8.0%; %CV <2.5% 5'-DFUR: %Bias <10.0%; %CV <9.3% 1% hemolyzed human K_2EDTA plasma ONT-993: %Bias <12.0%; %CV <10.1%	AV14-ONT380-01 Table 3-16 Table 3-17 Table 3-18 Table 3-19 Table 3-20

Lipemic effect	All samples passed. One lot was tested. Tucatinib: %Bias within 5.2%; %CV within 7.8% ONT-993: %Bias within 14.4%; %CV within 7.3% Capecitabine: %Bias within 8.0%; %CV was within 6.0% 5'-DFCR: %Bias was within 6.0%; %CV within 4.5% 5'-DFUR: %Bias was within 4.7%; %CV within 5.6%	AV14-ONT380-01 Table 3-21 Table 3-22 Table 3-23 Table 3-24 Table 3-25
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	Bench-top plasma stability demonstrated for 6 hours at ambient conditions. Tucatinib: %Bias within 11.5%; %CV within 5.9% ONT-993: %Bias within 7.0%; %CV within 5.5% Capecitabine: %Bias within 6.4%; %CV was within 9.5% 5'-DFCR: %Bias was within 7.6%; %CV was within 5.4% 5'-DFUR: %Bias was within 9.3%; %CV was within 9.3% Bench-top plasma stability demonstrated for 6 hours on ice Tucatinib: %Bias within 12.5%; %CV within 4.9% ONT-993: %Bias within 10.7%; %CV within 4.4% Capecitabine: %Bias within 12.4%; %CV was within 6.6% 5'-DFCR: %Bias was within 7.6%; %CV was within 12.2% 5'-DFUR: %Bias was within 10.7%; %CV was within 5.6%	AV14-ONT380-01 Table 3-71 Table 3-72 Table 3-73 Table 3-74 Table 3-75 AV14-ONT380-01 Addendum 2 Table 4-21 Table 4-22 Table 4-23 Table 4-24 Table 4-25
Freeze-Thaw stability	3 freeze-thaw cycles across analytes tucatinib, ONT-993, capecitabine, 5'-DFCR and 5'-DFUR. Tucatinib: %Bias within 10.5%, %CV within 8.7% ONT-993: %Bias within 15.0%; %CV within 9.8% Capecitabine: %Bias within 5.3%; %CV within 10.8% 5'-DFCR: %Bias within 6.4%; %CV within 7.3% 5'-DFUR: %Bias within 11.3%; %CV within 10.1%	AV14-ONT380-01 Table 3-76 Table 3-77 Table 3-78 Table 3-79 Table 3-80

Long-term storage	Tucatinib, capecitabine, 5'-DFCR and 5'-DFUR samples stored at -70°C are stable for up to 1119 days and for 95 days at -20°C. For ONT-993, QCs are stable for up to 602 days at -70ºC and DQCs are stable up to 1119 days at -70 ºC. Stability at -20ºC for ONT-993 has been established within AV14-ONT-380-01 Addendum 1 (Appendix 10). Long-term storage testing is ongoing. <u>-70°C</u> Tucatinib: %Bias within 3.5%; %CV was within 3.9% ONT-993: %Bias within 9.0%; %CV within 5.4% (DQC only) Capecitabine: %Bias within 5.5%; %CV within 4.5% 5'-DFCR: %Bias within 12.7%; %CV within 3.0% 5'-DFUR: %Bias within 4.7%; %CV within 11.2% <u>-20°C</u> Tucatinib: %Bias within 5.0%; %CV was within 5.1% Capecitabine: %Bias within 9.3%; %CV within 10.0% 5'-DFCR: %Bias within 14.7%; %CV within 5.6% 5'-DFUR: %Bias within 5.3%; %CV within 7.8%	AV14-ONT380-01 Addendum 2 Table 4-26 Table 4-27 Table 4-28 Table 4-29 Table 4-30 Table 4-31 Table 4-32 Table 4-33 Table 4-34 Table 4-35
Parallelism	NA	NA
Carry over	Analyte carryover met acceptance criteria for all batches for capecitabine, 5'-DFCR and 5'-DFUR. Analyte carryover failed for 10 out of 15 analytical batches for tucatinib. Analyte carryover failed for 15 out of 20 analytical batches for ONT-993. These results indicate that bioanalytical sample batches should be monitored for carryover.	AV14-ONT380-01 Table 3-66 Table 3-67 Table 3-68 Table 3-69 Table 3-70
Method performance in study ONT-380-004 Bioanalytical Sample Analysis for the Determination of ONT-380 and ONT-993 in Human Plasma by HPLC/MS/MS Report AD14-747		
Assay passing rate	Tucatinib: A total of 24 batches were collected, 23 batches were used. ONT-993: A total of 22 batches were collected, 20 were used.	AD14-747 Table 3-3 Table 3-4
Standard curve performance	<u>Cumulative %Bias</u> Tucatinib: -2.0% to 1.4% ONT-993: -1.5% to 1.1% <u>Cumulative %CV</u> Tucatinib: ≤9.7% ONT-993: ≤8.2%	AD14-747 Table 3-9 Table 3-10

QC performance	Cumulative %Bias Tucatinib: 0.0% to 1.8% ONT-993: -9.0% to -0.7% Cumulative %CV Tucatinib: ≤13.5% ONT-993: ≤13.5%	AD14-747 Table 3-11 Table 3-12
Method reproducibility	Samples reanalyzed (% total analyzed): Tucatinib: 121 (18%) ONT-993: 80 (11.9%) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: Tucatinib: 91.7% ONT-993: 67.5% ISR passed acceptance criteria.	AD14-747 Table 3-13 Table 3-14 Table 3-15
Study sample analysis/stability	All plasma samples were analyzed within the validated stability range. The longest period from collection to analysis was 620 and 641 days for tucatinib and ONT-993 respectively. All samples were stored at -70ºC. Validated stability at -70ºC: Tucatinib: 1119 days ONT-993:1124 days	AD14-747 Table 3-2
Standard calibration curve performance during accuracy and precision runs	See above	
Method performance in study ONT-380-005 Amendment 1 to Bioanalytical Sample Analysis for the Determination of ONT-380, ONT-993, Capecitabine, 5'-DFUR, 5'-DFCR, 5-FU, and FBAL in Human Plasma by HPLC/MS/MS Report AD14-734 Amendment 1		
Assay passing rate	Across two bioanalytical methods, a total of 36 batches were collected. Tucatinib: 16 of 18 batches were used. ONT-993: 14 of 16 batches were used. Capecitabine: 15 of 19 batches were used. 5'-DFUR: 16 of 17 batches were used. 5'-DFCR: 15 of 16 batches were used.	AD14-734 Amdendment 1 Table 3-3 Table 3-4 Table 3-5 Table 3-6 Table 3-7

Standard curve performance	Cumulative %Bias Tucatinib: -2.6% to 1.7% ONT-993: -3.9% to 1.5% Capecitabine: -1.4% to 1.9% 5'-DFUR: -2.3% to 3.8% 5'-DFCR: -0.8% to 1.3% Cumulative %CV Tucatinib: ≤9.6% ONT-993: ≤7.4% Capecitabine: ≤6.1% 5'-DFUR: ≤8.45% 5'-DFCR: ≤7.6%	AD14-734 Amdendment 1 Table 3-24 Table 3-25 Table 3-26 Table 3-27 Table 3-28
QC performance	Cumulative %Bias Tucatinib: 0.5% to 3.3% ONT-993: -5.9% to -0.6% Capecitabine: -2.5% to 3.0% 5'-DFUR: -1.8% to 4.1% 5'-DFCR: -0.3% to 1.5% Cumulative %CV Tucatinib: ≤12.9% ONT-993: ≤10.2% Capecitabine: ≤9.7% 5'-DFUR: ≤6.8% 5'-DFCR: ≤8.0%	AD14-734 Amdendment 1 Table 3-31 Table 3-32 Table 3-33 Table 3-34 Table 3-35
Method reproducibility	Samples reanalyzed (% total analyzed): Tucatinib: 33 (12.3%) ONT-993: 35 (13.0%) Capecitabine: 34 (12.6%) 5'-DFUR: 34 (12.6%) 5'-DFCR: 34 (12.6%) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: Tucatinib: 90.6% ONT-993: 68.6% Capecitabine:85.3% 5'-DFUR: 82.4% 5'-DFCR: 82.4% ISR passed acceptance criteria.	AD14-734 Amdendment 1 Table 3-38

Study sample analysis/stability	All plasma samples were analyzed within the validated stability range. The longest period from collection to analysis was 671 for tucatinib, ONT-993, capecitabine, 5'-DFUR, and 5'-DFCR, and 680 days for 5-FU and FBAL. All samples were stored at -70°C. Validated Stability at -70°C: Tucatinib:1119 days ONT-993:1124 days Capecitabine: 1119 days 5'-DFUR: 1119 days 5'-DFCR: 1119 days	AD14-734 Amendment 1 Table 3-2
Standard calibration curve performance during accuracy and precision runs	See above.	

Note: Any variance values reported as %RSD in the validation or sample analysis reports are presented as %CV in this table.

Appendix 10. Summary of Method Modifications and Cross-Validation Results - AV14 ONT380-01

Bioanalytical method validation report name and hyperlink	Validation AV14-ONT380-01 Addendum 1, AV14-ONT380-01 Addendum 3 Method TM17-396		
Changes in method	Validated changes to the original method, TM14-281, include: removal of capecitabine, 5'-DFCR and 5'-DFUR. addition of d_4-ONT993 as the internal standard for ONT-993		
New validated assay range if any	None		
Validation parameters	Cross-validation performance		Source location
Standard calibration curve performance during accuracy and precision runs[a]	Cumulative accuracy (%Bias) in standard calibrators from LLOQ to ULOQ	Tucatinib: -1.0 to 3.0% ONT-993: -1.6 to 1.5%	AV14-ONT380-01 Addendum 1 Table 3-1 Table 3-2
	Cumulative precision (%CV) from LLOQ to ULOQ	Tucatinib: ≤7.0% ONT-993: ≤9.2%	AV14-ONT380-01 Addendum 1 Table 3-1 Table 3-2
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%Bias) in 5 QCs	Tucatinib: 0.3 to 3.8% ONT-993: -0.8 to 1.7%	AV14-ONT380-01 Addendum 1 Table 3-9 Table 3-10
	Inter-batch %CV	Tucatinib: ≤7.4% ONT-993: ≤7.2%	AV14-ONT380-01 Addendum 1 Table 3-9 Table 3-10
	Percent TE	Tucatinib: 11.2% ONT-993: 8.9%	Sum of largest bias and CV reported for analytes
Cross-validation	NA		NA

List other parameters	<u>Absence of capecitabine, 5'-DFUR, and 5'-DFCR</u> %Bias Tucatinib: -4.1% to 3.0% ONT-993: -2.6% to 3.0% <u>DM-1 in the absence of THU</u> %Bias Tucatinib: -9.3% to 3.0% ONT-993: -11.4% to -4.7% <u>With capecitabine and associated metabolites in the absence of THU</u> %Bias Tucatinib: 5.0% to 0.4% ONT-993: -11.5% to -7.4% <u>With and without THU:</u> This analysis showed that a standard curve prepared with THU may be used to quantify samples collected without THU. Tucatinib: %Bias was within 4.7%; %CV within 5.4% ONT-993: %Bias was within 3.7%; %CV within 6.2% <u>Whole blood stability of tucatinib and ONT-993 on ice for 2 hours</u> Tucatinib: within 9.3% of control ONT-993: within 11.3% of control <u>Blank interference</u> One lot was tested. No substantial interfering peaks were found that eluted at the retention times of tucatinib and ONT-572, or ONT-993 and d_4-ONT-993 in any of the blank plasma samples analyzed during the validation batches. No substantial interfering peaks were found that eluted at the retention times of tucatinib and ONT-572, or ONT-993 and d_4-ONT-993 when coadministered with capecitabine and metabolites in the blank plasma selectivity samples analyzed. <u>Coadmin with DM1</u> No substantial interfering peaks were found that eluted at the retention times of tucatinib and ONT-572, or ONT-993 and d_4-ONT-993 when coadministered with DM1 in the blank plasma selectivity samples analyzed.	AV14-ONT380-01 Addendum 1 Table 3-3 Table 3-4 Table 3-5 Table 3-6 Table 3-7 Table 3-8 Table 3-17 Table 3-18 Table 3-44 Table 3-45 Table 3-19 Table 3-20 Table 3-23 Table 3-24 Table 3-21 Table 3-22

List other parameters (continued)	Benchtop stability	AV14-ONT380-01 Addendum 1
	Bench-top plasma stability demonstrated for 6 hours on ice	
	With DM1	
	Tucatinib: %Bias ≤11.0%: %CV ≤8.6%	Table 3-25
	ONT-993: %Bias ≤8.4%: %CV ≤8.1%	Table 3-26
	With capecitabine and Metabolites	
	Tucatinib: %Bias ≤15.0%: %CV ≤ 8.6%	Table 3-27
	ONT-993: %Bias ≤13.0%: %CV ≤9.0%	Table 3-28
	Bench-top plasma stability demonstrated for 6 hours at ambient conditions	
	With capecitabine and Metabolites	
	Tucatinib: %Bias ≤11.9%: %CV ≤6.9%	Table 3-29
	ONT-993: %Bias ≤13.3%: %CV ≤6.0%	Table 3-30
	Freeze-thaw stability	
	4 cycles at -70°C	
	With DM1	Table 3-31
	Tucatinib: %Bias ≤11.7%: %CV ≤9.0%	Table 3-32
	ONT-993: %Bias ≤11.4%: %CV ≤4.9%	
	With capecitabine and Metabolites	
	Tucatinib: %Bias ≤10.8%: %CV ≤7.2%	Table 3-33
	ONT-993: %Bias ≤13.1%: %CV ≤8.0%	Table 3-34
	8 cycles at -70°C without THU	
	Tucatinib: %Bias ≤5.2%: %CV w≤5.8%	AV14-OTN380-01 Addendum 3 (Ad3)
	ONT-993: %Bias ≤11.3%: %CV ≤12.8%	Table 3-12
	Long-term stability	Table 3-13
	-70°C with THU, capecitabine, and metabolites	
	ONT-993: 1124 days %Bias ≤12.0%: %CV ≤9.4%	Table 3-40
	-20°C with THU (with capecitabine and metabolites ONT-993 only)	
	Tucatinib: 330 days: %Bias ≤14.2%: %CV ≤3.7%	Table 3-37
	ONT-993: 365 days %Bias ≤9.4%: %CV ≤6.2%	Table 3-43

List other parameters (continued)	Long-term stability cont. -70°C without THU Tucatinib: 132 days: %Bias ≤7.3%: %CV ≤5.1% ONT-993: 132 days %Bias ≤4.8%: %CV ≤5.9% -20°C without THU Tucatinib: 132 days: %Bias ≤6.0%: %CV ≤6.0% ONT-993: 132 days %Bias w≤6.5%: %CV ≤4.5% -70°C in the presence of capecitabine and metabolites without THU Tucatinib: 125 days: %Bias ≤7.0%: %CV ≤5.0% ONT-993: 125 days %Bias ≤12.3%: %CV ≤5.9% -20°C in the presence of capecitabine and metabolites without THU Tucatinib: 16 days: %Bias ≤6.9%: %CV ≤6.2% ONT-993: 16 days %Bias ≤4.7%: %CV ≤5.9% -70°C in the presence of DM1 without THU Tucatinib: 126 days: %Bias ≤3.5%: %CV ≤6.3% ONT-993: 126 days %Bias ≤12.0%: %CV ≤6.6%	AV14-ONT380-01 Addendum 3 Ad3:Table 3-23 Ad3:Table 3-24 Ad3:Table 3-23 Ad3:Table 3-24 Ad3:Table 3-18 Ad3:Table 3-19 Ad3:Table 3-20 Ad3:Table 3-21 Ad3:Table 3-16 Ad3:Table 3-17
a Data presented for standard calibration curve performance was for all runs, not just during accuracy and precision runs		
Method performance in study ONT-380-206 Bioanalytical Sample Analysis for the Determination of ONT-380 and ONT-993 in Human Plasma by HPLC/MS/MS Report AD16-950		
Assay passing rate	Tucatinib: 89 batches total, 70 batches used. ONT-993: 90 batches total, 71 batches used	AD16-950 Table 3-4 Table 3-5
Standard curve performance	Cumulative %Bias Tucatinib: -2.4% to 3.0% ONT-993: -1.4% to 1.0% Cumulative %CV Tucatinib: -≤8.3% ONT-993: ≤7.0%	AD16-950 Table 3-10 Table 3-11

QC performance	Cumulative %Bias Tucatinib: -3.4% to 1.4% ONT-993: -6.8% to 1.7% Cumulative %CV Tucatinib: ≤9.4% ONT-993: ≤11.6%	AD16-950 Table 3-12 Table 3-13
Method reproducibility	Samples reanalyzed (% total analyzed): 198 (7.1%, Tucatinib) 198 (7.1%, ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 94.9% (Tucatinib) 79.5% (ONT-993) ISR passes acceptance criteria.	AD16-950 Table 3-14 Table 3-15 Table 3-16
Study sample analysis/stability	Study samples for tucatinib and ONT-993 were analyzed within 880 days from collection. Validated stability in the presence of capecitabine, metabolites, and THU additive: Tucatinib: 1119 days ONT-993:1124 days (Additional stability summarized in AD16-950 Table 3-1 and 3-2)	AD16-950 Table 3-1 Table 3-2
Standard calibration curve performance during accuracy and precision runs	See Above	
Method performance in study SGNTUC-020 Bioanalytical Sample Analysis for the Determination of Tucatinib and ONT-993 from Human Plasma and Metformin from Human Plasma and Urine Report AD19-849		
Assay passing rate[a]	Tucatinib: 4 batches total, 3 batches used ONT-993: 4 batches total, 3 batches used	AD19-849 Table 3-2 Table 3-3

Standard curve performance	Cumulative %Bias Tucatinib: -3.4% to 3.0% ONT-993: -3.2% to 4.5% Cumulative %CV Tucatinib: -≤7.5% ONT-993: ≤8.4%	AD19-849 Table 3-11 Table 3-12
QC performance	Cumulative %Bias Tucatinib: 0.0% to 7.0% ONT-993: -2.5% to 9.7% Cumulative %CV Tucatinib: -≤7.7% ONT-993: ≤8.5%	AD19-849 Table 3-15 Table 3-16
Method reproducibility	Samples reanalyzed (% total analyzed): 22 (16%, Tucatinib) 22 (16%, ONT-993) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 95.5% (Tucatinib) 90.9% (ONT-993) ISR passes acceptance criteria.	AD19-849 Table 3-19 Table 3-20 Table 3-21
Study sample analysis/stability	Study samples for ONT-380 and ONT-993 were analyzed within 13 days from collection. Long-term stability for tucatinib and ONT-993 in human K_2EDTA plasma in the presence of iohexhol and metformin has been validated for up to 111 days; see AV17-ONT380-01 Addendum 1.	AD19-849 Table 3-1 AV17-ONT380-01 Addendum 1, Table 3 23, and Table 3 24
Standard calibration curve performance during accuracy and precision runs	See above	

a Data presented for standard calibration curve performance was for all runs, not just during accuracy and precision runs.

4.1.6. Validation AV17-ONT380-01

Appendix 11. Bioanalytical Method Life Cycle Information - AV17-ONT380-01

	AV17-ONT380-01	SGNTUC-015	MK-7119-001
Analyte	Tucatinib ONT-993 Palbociclib	Tucatinib ONT-993	Tucatinib ONT-993
Validation type	Full	In Study	In Study
eCTD reference number	NA	NA	NA
Method ID	TM17-433	TM17-433	TM20-593 (identical to TM17-433)
Duration of time method is in use	08Nov2017-present	08Nov2018-21Aug2019	13Oct2021-08Aug2023
Bioanalytical site	[redacted]		
Matrix	K_2EDTA Human Plasma		
Platform	HPLC/MS/MS method		
Format	Methanol precipitation and HPLC/MS/MS were used to determine the concentration of Tucatinib, ONT-993, and Palbociclib in human K_2EDTA plasma. An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API 6500 or API 5500). A C_{18} HPLC column was used to separate Tucatinib, ONT-993, and palbociclib and the internal standards (IS), ONT-572, D_4-ONT-993 and d_8-palbociclib from interfering compounds that may be present in the sample extract. The peak areas of the product ions of the compounds (Tucatinib, ONT-993, and palbociclib) was measured against the peak areas of the product ions of the internal standards.		

	AV17-ONT380-01	SGNTUC-015	MK-7119-001
Stock reference Lot number Expiration date	Tucatinib ARM-0221 12Jul2017 Tucatinib 6451-P54/17-001 14Nov2019 ONT-572 AR00442572-03 30Nov2018 and 21Nov2020 ONT-993 ES6156-23-P1 30Nov2018 ONT-993 AR00440993-03 21Nov2020 D_4-ONT-993 ES6139-19 20Dec2017 and 30Nov2018 Palbociclib 1-ZPK-125-1 06Mar2020 D_8-palbociclib SA-ALS-15-120-P1 08Dec2018 Metformin HCl Q6020 31Dec2019 Iohexol BCBV6710 31Jul2020	Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 AR00440993-03 21Nov2020 ONT-993 ES6156-23-P1 30Nov2018 ONT-572 AR00442572-03 30Nov2018 and 21Nov2020 D_4ONT-993 ES6139-19 20Dec2017 and 30Nov2018	Tucatinib 19-0004 14Nov2021 and 13Sep2023 ONT-993 PC07328-5-FP-S 11May2023

	AV17-ONT380-01	SGNTUC-015	MK-7119-001
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	1.00 to 1000 ng/mL	1.00 to 1000 ng/mL	1.00 to 1000 ng/mL
Matrix study population	Normal	Normal	Normal
Link to reports and applicable amendments	Validation: AV17-ONT380-01, AV17-ONT380-01 Addendum 1	Bioanalytical Report: AD19-931	NA
Synopsis of amendment history	Addendum 1 includes selectivity, stability and long-term storage experiments in presence of co-administered compounds metformin and iohexol at 2.00 µg/mL and 300 µg/mL, respectively. Additionally, it was demonstrated that palbociclib can be removed from the method and does not impact the quantification of Tucatinib or ONT-993.	NA	NA

Appendix 12. Bioanalytical Method Performance - AV17-ONT380-01

Bioanalytical method validation report name, amendments, and hyperlinks	Validation reports: AV17-ONT380-01, AV17-ONT380-01 Addendum 1		
Method description	Methanol precipitation and HPLC/MS/MS were used to determine the concentration of Tucatinib, ONT-993, and Palbociclib in human K_2EDTA plasma. An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API 6500 or API 5500). A C_{18} HPLC column was used to separate Tucatinib, ONT-993, and palbociclib and the internal standards (IS), ONT-572, D_4-ONT-993 and d_8-palbociclib from interfering compounds that may be present in the sample extract. The peak areas of the product ions of the compounds (Tucatinib, ONT-993, and palbociclib) was measured against the peak areas of the product ions of the internal standards.		
Materials used for standard calibration curve and concentration	Tucatinib, ONT-993, and Palbociclib: 1.00, 2.00, 5.00, 10.0, 100, 500, 900, and 1000 ng/mL		
Validated assay range	1.00 -1000 ng/mL		
Material used for quality controls (QCs) and concentration	3.00, 50.0, and 800 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model and weighting	Linear $1/x^2$ regression		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	AV17-ONT380-01 Table 3-1 Table 3-2 Table 3-3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Tucatinib: -2.2% to 3.0% ONT-993: -1.5% to 1.8% Palbociclib: -0.8% to 1.0%	AV17-ONT380-01 Table 3-1 Table 3-2 Table 3-3
	Cumulative precision (%CV) from LLOQ to ULOQ	Tucatinib: ≤7.0% ONT-993: ≤8.0% Palbociclib: ≤7.0%	AV17-ONT380-01 Table 3-1 Table 3-2 Table 3-3

Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	Tucatinib: -11.8% to -1.2% ONT-993: -10.4% to 0.0% Palbociclib: -9.5% to -2.5%	AV17-ONT380-01 Table 3-5 Table 3-7 Table 3-9
	Inter-batch %CV	Tucatinib: ≤13.9% ONT-993: ≤10.2% Palbociclib: ≤9.4%	AV17-ONT380-01 Table 3-5 Table 3-7 Table 3-9
	Total Error (TE)	Tucatinib: 25.7% ONT-993: 20.6% Palbociclib: 18.9%	Sum of largest bias and CV reported.
Selectivity & matrix effect	Blank interference 6 lots were tested, no significant interfering peaks were found that eluted at the retention times of all analytes in any of the plasma samples analyzed. Matrix selectivity and Matrix effect 6 lots were tested, no significant interfering peaks were found that eluted at the retention times of all analyte in the blank plasma selectivity samples Matrix factor Six lots tested The precision of the matrix factor was acceptable for all 3 analytes. IS normalized matrix factor Tucatinib: LQC %CV 11.3% HQC %CV 5.2% ONT-993 LQC %CV 8.0% HQC %CV 4.0% Palbociclib LQC %CV 10.4% HQC %CV 3.2%		AV17-ONT380-01 Table 3-28 Table 3-29 Table 3-30 Table 3-31 Table 3-32 Table 3-33 Table 3-22 Table 3-23 Table 3-24

Interference & specificity	Multi-analyte selectivity Individual QCs of Tucatinib, ONT-993, Palbociclib were quantified against the calibration curve containing all 3. All passed the QC criteria. Tucatinib did not pass in batch 6 but did in batch 13. Assay selectivity LLOQ, ULOQ and QC samples containing tucatinib and ONT-993 with and without palbociclib quantified against the standard curve containing Tucatinib and ONT-993, with and without palbociclib. For both analyses the samples passed QC criteria. Addendum 1 Blank interference in the presence of metformin and iohexol: No significant interfering peaks were found that eluted at the retention times of tucatinib and ONT-572 or ONT-993 and D4-ONT-993 in any of the plasma samples in the presence of metformin and iohexol. Specificity in the presence of metformin and iohexol LLOQ, ULOQ and QCs of tucatinib and ONT-993 also containing iohexol and metformin passed QC criteria.	AV17-ONT380-01 Table 3-34 Table 3-35 Table 3-36 Table 3-37 Table 3-38 Table 3-39 Table 3-40 Table 3-41 Table 3-42 Table 3-43 Table 3-44 Table 3-45 AV17-ONT380-01 Addendum 1 Table 3-14 Table 3-15 AV17-ONT380-01 Addendum 1 Table 3-7 Table 3-8
Hemolysis effect	One lot tested:10% lysed whole blood Tucatinib LQC %Bias 5.0%, %CV 6.3% HQC %Bias 4.9%, %CV 1.6% ONT-993 LQC %Bias 2.0%, %CV 3.7% HQC %Bias 7.8%, %CV 8.1% Palbociclib LQC %Bias -1.3%, %CV 5.4% HQC %Bias 5.1%, %CV 1.1%	AV17-ONT380-01 Table 3-16 Table 3-17 Table 3-18

Lipemic effect	Two lots tested: natural high lipid content ≥300 mg/dL Tucatinib LQC %Bias 5.7%, %CV 7.1% HQC %Bias -5.1%, %CV 4.5% One lot tested. ONT-993 LQC %Bias 2.3%, %CV 2.8% HQC %Bias 0.9%, %CV 1.8% One lot tested. Palbociclib LQC %Bias 3.7%, %CV 4.0% HQC %Bias -0.8%, %CV 1.0%	AV17-ONT380-01 Table 3-19 Table 3-20 Table 3-21
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	Bench-top matrix stability 6 hours at room temperature Tucatinib: %Bias -9.3% to -5.4%; %CV ≤8.1% ONT-993: %Bias -7.9% to -2.0%; %CV ≤5.5% Palbociclib: %Bias -10.3% to -4.4%; %CV ≤6.7% Bench-top matrix stability 6 hours on ice Tucatinib: %Bias 3.7% to 8.8%; %CV ≤12.0% ONT-993: %Bias -6.8% to 2.0%; %CV ≤14.1% Palbociclib: %Bias -8.8% to 6.0%; %CV ≤15.0% Reinjection stability at 10°C, 33.1 hours in the autosampler Tucatinib: %Bias -1.3% to 5.0%; %CV ≤8.9% ONT-993: %Bias -5.3% to 2.7%; %CV ≤5.3% Palbociclib: %Bias -3.9% to -0.7%; %CV ≤6.9% Tucatinib: Whole blood stability 2 hours room temperature: %Bias -2.4% wet ice: %bias -9.4% ONT-993: Whole blood stability 2 hours room temperature: %Bias -5.5% wet ice: %bias -10.3% Palbociclib: Whole blood stability 2 hours room temperature: %Bias -5.5% wet ice: %bias -5.8%	AV17-ONT380-01 Table 3-49 Table 3-50 Table 3-51 Table 3-52 Table 3-53 Table 3-54 Table 3-61 Table 3-62 Table 3-63 Table 3-64 Table 3-65 Table 3-66

	Addendum 1 Bench-top stability 6 hours at room temperature in the presence of metformin and isohexol: Tucatinib: %Bias -9.6% to 1.0%; %CV ≤6.3% ONT-993: %Bias -8.6% to -0.6%; %CV ≤4.8%	AV17-ONT380-01 Addendum 1 Table 3-16 Table 3-17
Freeze-Thaw stability	4 cycles Tucatinib: %Bias -9.2% to 3.0%; %CV ≤7.0% ONT-993: %Bias -8.8% to -2.4%; %CV ≤9.8% Palbociclib: %Bias -7.0% to -4.8%; %CV ≤4.1% Addendum 1 4 cycles in the presence of metformin and isohexol: Tucatinib: %Bias -9.8% to -0.8%; %CV ≤5.9% ONT-993: %Bias -9.4% to 0.3%; %CV ≤7.6%	AV17-ONT380-01 Table 3-55 Table 3-56 Table 3-57 AV17-ONT380-01 Addendum 1 Table 3-18 Table 3-19
Long-term storage	363 days at -70°C Tucatinib: %Bias -4.6% to 13.0%; %CV ≤12.8% ONT-993: %Bias -6.3% to 1.4%; %CV ≤12.2% Palbociclib: %Bias -2.0% to 2.0%; %CV ≤5.9% 31 days at -20°C Tucatinib: %Bias -10.3% to 0.4%; %CV ≤10.6% ONT-993: %Bias -14.4% to -7.4; %CV ≤10.0% Palbociclib: %Bias -11.8% to -2.8%; %CV ≤11.6% 111 days at -70°C in presence of metformin and iohexol Tucatinib: %Bias -7.8% to 5.8%; %CV ≤13.7% ONT-993: %Bias 3.0% to 16.4%; %CV ≤10.9%	AV17-ONT380-01 Table 3-58 Table 3-59 Table 3-60 AV17-380-01 Addendum 1 Table 3-20 Table 3-21 Table 3-22 AV17-380-01 Addendum 1 Table 3-23 Table 3-24
Parallelism	NA	NA
Carry over	Carryover was considered acceptable.	AV17-ONT380-01 Table 3-46 Table 3-47 Table 3-48
Method performance in study SGNTUC-015 Bioanalytical Sample Analysis for the Determination of ONT-380 (Tucatinib) and ONT-993 in Human Plasma by HPLC/MS/MS Report AD19-931		

Assay passing rate	Tucatinib: 35 batches were collected, 31 were accepted. ONT-993: 35 batches were collected, 30 were accepted.	AD19-931 Table 3-2 Table 3-3
Standard curve performance	Tucatinib: %Bias: -3.4% to 4.0%; %CV ≤6.8% ONT-993: %Bias: -2.1% to 2.0%; %CV ≤8.1%	AD19-931 Table 3-8 Table 3-9
QC performance	Tucatinib: %Bias: -4.5% to 4.8%; %CV ≤9.1% ONT-993: %Bias: -1.6% to 4.8%; %CV ≤9.3%	AD19-931 Table 3-10 Table 3-11
Method reproducibility	The criteria for reproducibility were met for Tucatinib with 93% within specification. One sample not calculable. The criteria for reproducibility were met for ONT-993 with 90.5% within specification. One sample not calculable.	AD19-931 Table 3-14 Table 3-15
Study sample analysis/ stability	Samples were stored at nominal -70 °C. Study samples for Tucatinib were analyzed within 68 days from collection and samples for ONT-993 were analyzed within 45 days from collection.	AD19-931 Table 3-1
Standard calibration curve performance during accuracy and precision runs	See above	

Appendix 13. Summary Method Performance - AV17-ONT380-01 Addendum 2

Bioanalytical method validation report name, amendments, and hyperlinks	AV17-ONT380-01 Addendum 2		
Method description	Methanol precipitation and HPLC/MS/MS were used to determine the concentration of tucatinib and ONT-993, with or without palbociclib, in human K_2EDTA plasma. An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API 6500 or API 5500). A C_{18} HPLC column was used to separate tucatinib, ONT-993, and palbociclib and the internal standards (IS), ONT-572, D_4-ONT-993 and D_8-palbociclib from interfering compounds that may be present in the sample extract. The peak areas of the product ions of the compounds (tucatinib, ONT-993, and palbociclib) was measured against the peak areas of the product ions of the internal standards.		
Materials used for standard calibration curve and concentration	Tucatinib, ONT-993, and Palbociclib: 1.00, 2.00, 5.00, 10.0, 100, 500, 900, and 1000 ng/mL		
Validated assay range	1.00 -1000 ng/mL		
Material used for quality controls (QCs) and concentration	3.00, 50.0, and 800 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model and weighting	Linear $1/x^2$ regression		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs[a]	Number of standard calibrators from LLOQ to ULOQ	8	AV17-ONT380-01 Addendum 2 Table 3-1 Table 3-2 Table 3-3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	TM17-433 Tucatinib: -1.8% to 3.5% ONT-993: -2.1% to 1.0% Palbociclib: -1.0% to 1.0% TM20-593 Tucatinib: -3.4% to 4.0% ONT-993: -4.0% to 2.3%	AV17-ONT380-01 Addendum 2 Table 3-1 Table 3-2 Table 3-3 Table 4-1 Table 4-2

	Cumulative precision (%CV) from LLOQ to ULOQ	TM17-433 Tucatinib: ≤6.9% ONT-993: ≤9.8% Palbociclib: ≤6.1% TM20-593 Tucatinib: ≤6.4% ONT-993: ≤9.2%	AV17-ONT380-01 Addendum 2 Table 3-1 Table 3-2 Table 3-3 Table 4-1 Table 4-2
Performance of QCs during accuracy and precision runs[a]	Cumulative accuracy (%bias) in 5 QCs	TM17-433 Tucatinib: -1.6% to 6.4% ONT-993: 0.4% to 6.8% Palbociclib: 2.5% to 5.3% TM20-593 (5QCs) Tucatinib: 1.1% to 18.0% ONT-993: 0.3% to 19.0%	AV17-ONT380-01 Addendum 2 Table 3-4 Table 3-5 Table 3-6 Table 4-3 Table 4-4
	Inter-batch %CV	TM17-433 Tucatinib: ≤6.2% ONT-993: ≤13.8% Palbociclib: ≤12.8% TM20-593 (5QCs) Tucatinib: ≤6.9% ONT-993: ≤13.7%	AV17-ONT380-01 Addendum 2 Table 3-4 Table 3-5 Table 3-6 Table 4-3 Table 4-4
	Total Error (TE)	TM17-433 Tucatinib: 12.6% ONT-993: 20.6% Palbociclib: 18.1% TM20-593 (5QCs) Tucatinib: 24.9% ONT-993: 32.7%	Sum of largest bias and CV reported.

Selectivity & matrix effect	Blank interference No significant interfering peaks were found that eluted at the retention times of all analytes in any of the blank plasma samples analyzed.	AV17-ONT380-01 Addendum 2 Table 3-10 Table 3-11 Table 3-12 Table 4-7 Table 4-8
Dilution linearity & hook effect	NA	NA
Bench-top/process stability N=3 aliquots	Solution Stability (N=6 replicates) Bench-top stability at 6.3 hours of IS working solution at 2.00 ng/mL ONT-572: %Difference vs. Control -5.5%; %CV ≤3.0% D_4-ONT-993: %Difference vs. Control -2.6%; %CV ≤4.2% Confirmed in TM20-593 Accuracy and Precision QC batch 40 TM17-433 Bench-top matrix stability 6.5 hours at room temperature Tucatinib: %Bias -7.4% to 2.0%; %CV ≤7.5% ONT-993: %Bias -9.8% to 2.7%; %CV ≤7.8% Palbociclib: %Bias -9.0% to -1.6%; %CV ≤4.8% TM20-593 Processed Stability Extract stability at 10°C for 116 hours for tucatinib and ONT-993 was established.	AV17-ONT380-01 Addendum 2 Table 2-4 Table 2-5 Table 3-13 Table 3-14 Table 3-15 Table 4-1 Table 4-2 Table 4-3 Table 4-4
Freeze-Thaw stability N=3 aliquots	TM17-433 4 cycles at -70°C Tucatinib: %Bias -3.3% to 0.2%; %CV ≤7.4% ONT-993: %Bias -9.7% to -4.1%; %CV ≤6.6% Palbociclib: %Bias -3.2% to -0.1%; %CV ≤7.1%	AV17-ONT380-01 Addendum 2 Table 3-16 Table 3-17 Table 3-18

Long-term storage N=3 aliquots	TM17-433 522 days at -70°C (529 days ONT-993 DQC) Tucatinib: %Bias -3.6% to 9.6%; %CV ≤13.8% ONT-993: %Bias 4.8% to 6.6%; %CV ≤7.0% Palbociclib: %Bias 10.0% to 13.3%; %CV ≤6.9% (Palbociclib DQC failure, passes at 204 days -70°C) 456 days at -20°C Tucatinib: %Bias -4.3% to 4.4%; %CV ≤7.7% ONT-993: %Bias -11.0% to -0.8%; %CV ≤6.8% Palbociclib: %Bias -10.3% to -5.0%; %CV ≤7.1% TM20-593 1019 days at -70°C Tucatinib: %Bias -0.3% to 1.3%; %CV ≤4.0% ONT-993: %Bias 6.4% to 8.2%; %CV ≤7.6%	AV17-ONT380-01 Addendum 2 Table 3-20 Table 3-22 Table 3-23 Table 3-25 Table 3-24 Table 3-26 Table 3-27 Table 3-28 Table 4-9 Table 4-10
Parallelism	NA	NA
Carry over	Previously evaluated/acceptable in AV17-ONT380-01	
a Data presented for standard calibration curve and quality control performance was for all runs; accuracy and precision not included AV17-ONT380-01 Addendum 2, except for TM20-593 quality controls in batch 40		
Method performance for plasma samples in study 380-IST-004 (TBCRC 049/UAB 1794) Bioanalytical Sample Analysis for the Determination of Tucatinib (ONT-380) and ONT-993 in Human Plasma and Cerebrospinal Fluid by HPLC/MS/MS Report AD20-1125		
Assay passing rate	Tucatinib: A total of 10 plasma batches were collected, 8 batches were used. ONT-993: A total of 10 plasma batches were collected, 8 batches were used.	AD20-1125 Table 3-3 Table 3-4
Standard curve performance	<u>Cumulative %Bias for calibrators used to quantitate plasma samples</u> Tucatinib: -5.5 to 4.0% ONT-993: -2.9 to 2.0% <u>Cumulative %CV for calibrators used to quantitate plasma samples</u> Tucatinib: ≤ 7.9% ONT-993: ≤ 8.8%	AD20-1125 Table 3-13 Table 3-14

QC performance	Cumulative %Bias for QC samples in plasma batches Tucatinib:　-1.0 to 6.4% ONT-993: -4.7 to 2.4% Cumulative %CV for QC samples in plasma batches Tucatinib: ≤ 11.3% ONT-993: ≤ 10.3%	AD20-1125 Table 3-17 Table 3-18
Method reproducibility	Plasma samples reanalyzed (% total analyzed) Tucatinib: 21 (22.6%) ONT-993: 21 (22.6%) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: Tucatinib: 100% ONT-993: 85.7% ISR passed acceptance criteria.	AD20-1125 Table 3-21
Study sample analysis/ stability	Plasma samples were analyzed within 358 days of collection. No collection data was available for 8 ONT-380 plasma samples and 4 ONT-993 plasma samples; however, these samples were analyzed 314 days from receipt. All samples were stored at -70°C. Validated stability for tucatinib and ONT-993 at -70°C: 1019 days in human plasma (no DQC evaluated) 375 days in human plasma, DQC level	AD20-1125 Table 3-2

4.1.7. Validation AV18-380-03

Appendix 14. Bioanalytical Method Life Cycle Information - AV18-380-03

	AV18-380-03	380-IST-004 (TBCRC 049/UAB 1794)
Analyte	Tucatinib ONT-993 Abemaciclib	Tucatinib ONT-993
Validation type	Full	In-Study
eCTD reference number	NA	NA
Method ID	TM18-495	TM18-495
Duration of time method is in use	27Nov2018 – present	06Aug2019 – 23Jun2021
Bioanalytical site	[redacted]	
Matrix	Human K_2EDTA plasma and CSF	Human CSF [a]
Platform	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)	
Format	A validated HPLC-MS/MS method was developed at [redacted], Inc. to determine concentrations of tucatinib, ONT-993, and abemaciclib in human K_2EDTA plasma or CSF (diluted 1:1 with plasma). The method can be used to quantitate tucatinib, ONT-993, and abemaciclib, or tucatinib and ONT-993 alone. Methanol precipitation is followed by separation on a Discovery HS-C18, 3 µm HPLC column (2.1 x 50 mm), and quantification on a Sciex API-5500 or API-6500. The peak area of the product ions of the compounds are measured against the peak area of the product ions of the respective internal standards.	
Stock reference, lot number, expiration date	Tucatinib 6451-P54/17-001 14Nov2019 19-004 13Sep2023 ARM-0221.03 15Jun2020 ONT-993 ES6156-23-P1 30Nov2018 AR00440993-03 21Nov2020 PC07328-5-FP-S 23May2022 Abemaciclib S7158 batch 03 14Aug2021 S7158 batch 02 16Apr2022	Tucatinib 6451-P54/17-001 14Nov2019 ARM-0221.03 15Jun2020 ONT-993 AR00440993-03 21Nov2020 Abemaciclib S7158 batch 02 16Apr2022 ONT-572 AR00442572-03 21Nov2020 AR00442572-03 30Nov2018 D_4-ONT-993 ES6139-19 30Nov2018

	ONT-572 AR00442572-03 30Nov2018 D_4-ONT-993 ES6139-19 30Nov2018 D_8-Abemaciclib MS-ALS-17-015-P1 02May2020 Capecitabine 10-XJZ-158-1 05Feb2019 5'-DFCR 1-BKG-58-1 12Jun2020 5'DFUR 3-MAR-46-3 19Oct2021 FBAL K2618 26Nov2023 5-FU 11-SCC-140-1 07Sep2021	ES6139-19 20Dec2017 D_8-Abemaciclib MS-ALS-17-015-P1 02May2020 D_8-Palbociclib [b] SA-ALS-15-120-P1 08 Dec 2018
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	0.100 – 100 ng/mL (plasma) 0.200 – 200 ng/mL (CSF) [c]	0.200 – 200 ng/mL (CSF) [c]
Matrix study population	Normal	Diseased, breast cancer patients
Link to reports and applicable amendments	Validation report: AV18-ONT380-03 AV18-ONT380-03 Addendum 1	Bioanalytical report: AD20-1125
Synopsis of amendment history	Addendum 1 contains long-term stability testing in plasma and in 1:1 CSF:plasma of tucatinib, ONT-993, and abemaciclib, as well as tucatinib and ONT-993 in the presence of capecitabine and metabolites.	NA

a See Appendix 13 for information related to plasma samples in this study.
b Internal standard present in solutions but not used for analysis.
c Calibration curve is 0.100-100 ng/mL in plasma, but samples are diluted 1:1 CSF/plasma.

Appendix 15. Summary Method Performance - AV18-380-03

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: AV18-ONT380-03, AV18-380-03 Addendum 1		
Method description	Methanol precipitation and HPLC-MS/MS were used to determine the concentration of tucatinib, ONT-993, and abemaciclib in human K_2EDTA plasma or CSF. An aliquot of the extract was injected onto an HPLC-MS/MS triple quadrupole mass spectrometer (Sciex API-5500 or API-6500). A Discovery HS-C18, 3 µm HPLC column (2.1 x 50 mm) from Supelco was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. ONT-572 served as the internal standard for tucatinib. D_4-ONT-993 served as the internal standard for ONT-993. D_8-Abemaciclib served as the internal standard for abemaciclib. The combination of tucatinib and ONT-993 was also validated in the presence of capecitabine and metabolites, 5'-deoxyfluorocytidine (5'-DFCR), 5'-deoxyfluorouridine (5'-DFUR), alpha-fluorobeta-alanine (FBAL) and 5-fluorouracil (5-FU). The peak area of the product ion of each analyte was measured against the peak area of the product ion of its respective internal standard.		
Materials used for standard calibration curve and concentration	Tucatinib, ONT-993, abemaciclib: 0.100, 0.200, 0.800, 3.00, 20.0, 50.0, 90.0, 100 ng/mL		
Validated assay range	Tucatinib, ONT-993, abemaciclib in plasma: 0.100 – 100 ng/mL Tucatinib, ONT-993, abemaciclib in CSF: 0.200 – 200 ng/mL [a]		
Material used for quality controls (QCs) and concentration	Tucatinib, ONT-993, abemaciclib: 0.300, 5.00, 80.0 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model and weighting	Tucatinib, ONT-993, abemaciclib: Linear regression; weighted $1/x^2$		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	AV18-ONT380-03 Table 3-1 Table 3-2 Table 3-3
	Cumulative accuracy (%bias) from LLOQ to ULOQ [b]	Tucatinib: -1.4 to 1.6% ONT-993: -1.2 to 1.0% Abemaciclib: -0.9 to 1.4%	AV18-ONT380-03 Table 3-1 Table 3-2 Table 3-3
	Cumulative precision (%CV) from LLOQ to ULOQ [b]	Tucatinib: ≤5.6% ONT-993: ≤8.4% Abemaciclib: ≤7.0%	AV18-ONT380-03 Table 3-1 Table 3-2 Table 3-3
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	Tucatinib, ONT-993, and abemaciclib Plasma:	AV18-ONT380-03

		Tucatinib: -5.1 to 1.3% ONT-993: -1.7 to 2.3% Abemaciclib: -8.1 to -0.6% CSF (1:1 with plasma): Tucatinib: -4.3 to 3.7% ONT-993: -4.8 to 2.0% Abemaciclib: -5.2 to -2.0% Tucatinib and ONT-993 in the presence of capecitabine and metabolites CSF (1:1 with plasma): Tucatinib: -0.5 to 9.0% ONT-993: 2.3 to 9.8%	Table 3-4 Table 3-5 Table 3-6 Table 3-16 Table 3-17 Table 3-18 Table 3-22 Table 3-23
	Inter-batch %CV	Tucatinib, ONT-993, and abemaciclib Plasma: Tucatinib: ≤12.5% ONT-993: ≤11.2% Abemaciclib: ≤12.4% CSF (1:1 with plasma): [c] Tucatinib: ≤11.0% ONT-993: ≤20.0% Abemaciclib: ≤15.6% Tucatinib and ONT-993 in the presence of capecitabine and metabolites CSF (1:1 with plasma): [c] Tucatinib: ≤6.3% ONT-993: ≤10.3%	AV18-ONT380-03 Table 3-4 Table 3-5 Table 3-6 Table 3-16 Table 3-17 Table 3-18 Table 3-22 Table 3-23
	Total Error (TE)	Tucatinib, ONT-993, and abemaciclib Plasma: Tucatinib: 17.6% ONT-993: 13.5% Abemaciclib: 20.5% CSF (1:1 with plasma): Tucatinib: 15.3% ONT-993: 24.8% Abemaciclib: 20.8%	Sum of largest bias and CV reported for analytes

		Tucatinib and ONT-993 in the presence of capecitabine and metabolites CSF (1:1 with plasma): Tucatinib: 15.3% ONT-993: 20.1%	

Table continued

Selectivity & matrix effect	Blank interference in plasma 6 lots were tested; no significant interfering peaks were found that eluted at the retention times of all analytes. Blank interference in CSF 6 lots were mixed 1:1 with pooled plasma and tested; no significant interfering peaks were found that eluted at the retention times of all analytes. Matrix factor in plasma 6 lots of unpooled plasma tested The precision of the IS normalized matrix factor was acceptable for all analytes IS-Normalized Matrix Factor %CV: Tucatinib: 2.8% ONT-993: 3.4% Abemaciclib: 2.0% Matrix factor in CSF 6 lots of unpooled CSF were mixed 1:1 with pooled plasma and tested The precision of the IS normalized matrix factor was acceptable for all analytes IS-Normalized Matrix Factor %CV: Tucatinib: 4.1% ONT-993: 4.2% Abemaciclib: 3.0%	AV18-ONT380-03 Table 3-51 Table 3-52 Table 3-53 Table 3-54 Table 3-55 Table 3-56 Table 3-30 Table 3-31 Table 3-32 Table 3-33 Table 3-34 Table 3-35
Interference & specificity	Multi-analyte selectivity in plasma Individual QCs of tucatinib, ONT-993, and abemaciclib were quantified against the calibration curve containing all 3 analytes. All passed the acceptance criteria. Tucatinib: %Bias within 2.6%; %CV within 7.3% ONT-993: %Bias within 9.0%; %CV within 11.6% Abemaciclib: %Bias within 3.2%; %CV within 4.2% Multi-analyte selectivity in CSF (diluted 1:1 in plasma) Individual QCs of tucatinib, ONT-993, and abemaciclib were quantified against the calibration curve containing all 3 analytes. Tucatinib: %Bias within 6.4%; %CV within 2.2% ONT-993: %Bias within 7.2%; %CV within 7.0% Abemaciclib: %Bias within 4.7%; %CV within 5.0% Selectivity in CSF (diluted 1:1 in plasma) QCs containing tucatinib and ONT-993 were quantified against the calibration curve containing only these analytes. Tucatinib: %Bias within 9.8%; %CV within 4.8% ONT-993: %Bias within 6.3%; %CV within 6.7% QCs containing tucatinib and ONT-993 were quantified against the calibration curve containing tucatinib, ONT-993, and abemaciclib. Tucatinib: %Bias within 3.4%; %CV within 4.8% ONT-993: %Bias within 5.5%; %CV within 5.9%	AV18-ONT380-03 Table 3-66 Table 3-67 Table 3-68 Table 3-69 Table 3-70 Table 3-71 Table 3-57 Table 3-58 Table 3-59 Table 3-60

	QCs containing tucatinib, ONT-993, and abemaciclib were quantified against the calibration curve containing tucatinib and ONT-993. Tucatinib: %Bias within 8.0%; %CV within 5.9% ONT-993: %Bias within 5.4%; %CV within 11.4% QCs containing tucatinib, ONT-993, and abemaciclib were quantified against the calibration curve containing all 3 analytes. Tucatinib: %Bias within 3.4%; %CV within 7.2% ONT-993: %Bias within 8.0%; %CV within 10.7% Abemaciclib: %Bias within 4.3%; %CV within 6.9%	 Table 3-61 Table 3-62 Table 3-63 Table 3-64 Table 3-65
Hemolysis effect	Analysis of tucatinib, ONT-993, and abemaciclib has been validated in up to 10% hemolyzed human K_2EDTA plasma. One lot was tested. All analytes passed acceptance criteria. Tucatinib: %Bias within 4.8%; %CV within 6.0% ONT-993: %Bias within 8.6%; %CV within 14.1% Abemaciclib: %Bias within 3.8%; %CV within 8.9%	AV18-ONT380-03 Table 3-24 Table 3-25 Table 3-26
Lipemic effect	Human plasma with naturally high lipid content did not affect the quantitation of tucatinib, ONT-993, and abemaciclib in the method. One lot was tested Tucatinib: %Bias within 0.8%; %CV within 9.1% ONT-993: %Bias within 1.9%; %CV within 6.3% Abemaciclib: %Bias within 1.0%; %CV within 6.8%	AV18-ONT380-03 Table 3-27 Table 3-28 Table 3-29
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	<u>In plasma on ice</u> Benchtop stability in plasma was demonstrated for 6.5 hours on ice. Tucatinib: %Bias within 10.4%; %CV within 5.9% ONT-993: %Bias within 6.8%; %CV within 7.3% Abemaciclib: %Bias within 9.8%; %CV within 5.2% <u>In plasma at ambient temperature</u> Benchtop stability in plasma was demonstrated for 6.5 hours at ambient temperature for ONT-993 and abemaciclib, and 5.8 hours for tucatinib. Tucatinib: %Bias within 6.7%; %CV within 8.8% ONT-993: %Bias within 12.0%; %CV within 5.4% Abemaciclib: %Bias within 13.6%; %CV within 6.9% <u>In CSF (1:1 with plasma) on ice</u> Benchtop stability in CSF (1:1 with plasma) was demonstrated for 6.6 hours on ice. Tucatinib: %Bias within 6.3%; %CV within 8.6% ONT-993: %Bias within 6.5%; %CV within 6.8% Abemaciclib: %Bias within 5.8%; %CV within 7.6% <u>In CSF (1:1 with plasma) at ambient temperature</u> Benchtop stability in CSF (1:1 with plasma) was demonstrated for 6.6 hours at ambient temperature.	AV18-ONT380-03 Table 3-83 Table 3-85 Table 3-87 Table 3-81 Table 3-82 Table 3-84 Table 3-86 Table 3-89 Table 3-91 Table 3-93

	Tucatinib: %Bias within 7.3%; %CV within 5.6% ONT-993: %Bias within 6.0%; %CV within 2.5% Abemaciclib: %Bias within 7.9%; %CV within 4.6% <u>In CSF (1:1 with plasma) on ice in the presence of capecitabine and metabolites</u> Benchtop stability in CSF (1:1 with plasma) in the presence of capecitabine and metabolites was demonstrated for 6.5 hours on ice. Tucatinib: %Bias within 3.9%; %CV within 2.6% ONT-993: %Bias within 6.3%; %CV within 7.1% <u>In CSF (1:1 with plasma) at ambient temperature in the presence of capecitabine and metabolites</u> Benchtop stability in CSF (1:1 with plasma) in the presence of capecitabine and metabolites was demonstrated for 6.5 hours at ambient temperature. Tucatinib: %Bias within 2.0%; %CV within 7.2% ONT-993: %Bias within 6.0%; %CV within 4.3% <u>In CSF on ice (prior to being diluted 1:1 with plasma)</u> Benchtop stability in CSF was demonstrated for 30 minutes on ice prior to 1:1 dilution with plasma, mimicking sample collection conditions. Tucatinib: %Bias within 5.0%; %CV within 3.2% ONT-993: %Bias within 4.4%; %CV within 4.7% Abemaciclib: %Bias within 6.0%; %CV within 3.2% <u>In CSF on ice (prior to being diluted 1:1 with plasma) in the presence of capecitabine and metabolites</u> Benchtop stability in CSF in the presence of capecitabine and metabolites was demonstrated for 30 minutes on ice prior to 1:1 dilution with plasma, mimicking sample collection conditions. Tucatinib: %Bias within 8.0%; %CV within 4.0% ONT-993: %Bias within 12.0%; %CV within 6.5% <u>Reinjection stability in plasma</u> Extracts containing tucatinib, ONT-993, and abemaciclib reinjected 80.1 hours after first injection, stored in autosampler at 10°C. Tucatinib: %Bias within 6.3%; %CV within 5.3% ONT-993: %Bias within 10.0%; %CV within 11.3% Extracts containing tucatinib, ONT-993, and abemaciclib reinjected 20.6 hours after first injection, stored in autosampler at 10°C Abemaciclib: %Bias within 3.9%; %CV within 4.7% <u>Reinjection stability in CSF (1:1 with plasma)</u> Extracts reinjected 48.7 hours after first injection, stored in autosampler at 10°C. Tucatinib: %Bias within 2.7%; %CV within 3.3% ONT-993: %Bias within 8.3%; %CV within 2.4% Abemaciclib: %Bias within 4.0%; %CV within 2.8%	Table 3-88 Table 3-90 Table 3-92 Table 3-95 Table 3-97 Table 3-94 Table 3-96 Table 3-76 Table 3-77 Table 3-78 Table 3-79 Table 3-80 Table 3-120 Table 3-121 Table 3-123 Table 3-124 Table 3-125 Table 3-126

	Reinjection stability in CSF (1:1 with plasma) in the presence of capecitabine and metabolites Extracts reinjected 105 hours after first injection, stored in autosampler at 10°C. Tucatinib: %Bias within 4.5%; %CV within 2.0% ONT-993: %Bias within 4.0%; %CV within 4.7%	 Table 3-127 Table 3-128
Freeze-Thaw stability	In plasma Stability of tucatinib, ONT-993, and abemaciclib in plasma was demonstrated for up to 4 freeze-thaw cycles. Tucatinib: %Bias within 8.2%; %CV within 10.2% ONT-993: %Bias within 5.2%; %CV within 8.7% Abemaciclib: %Bias within 8.3%; %CV within 4.2% In CSF (1:1 with plasma) Stability of tucatinib, ONT-993, and abemaciclib in CSF (1:1 with plasma) was demonstrated for up to 4 freeze-thaw cycles. Tucatinib: %Bias within 11.3%; %CV within 4.8% ONT-993: %Bias within 7.3%; %CV within 4.1% Abemaciclib: %Bias within 7.8%; %CV within 4.7% In CSF (1:1 with plasma) in the presence of capecitabine and metabolites Stability of tucatinib and ONT-993 in CSF (1:1 with plasma) in the presence of capecitabine and metabolites was demonstrated for up to 4 freeze-thaw cycles. Tucatinib: %Bias within 3.4%; %CV within 3.0% ONT-993: %Bias within 6.3%; %CV within 2.8%	AV18-ONT380-03 Table 3-98 Table 3-99 Table 3-100 Table 3-101 Table 3-102 Table 3-103 Table 3-104 Table 3-105
Long-term storage	In plasma at -70°C Tucatinib, ONT-993, and abemaciclib plasma samples stored at -70°C are stable for up to 375 days. Tucatinib: %Bias within 3.6%; %CV within 3.3% ONT-993: %Bias within 3.0%; %CV within 5.9% Abemaciclib: %Bias within 7.6%; %CV within 7.2% In CSF (1:1 with plasma) at -70°C Tucatinib, ONT-993, and abemaciclib CSF (1:1 with plasma) samples stored at -70°C are stable for up to 474 days. Tucatinib: %Bias within 5.0%; %CV within 5.9% ONT-993: %Bias within 14.6%; %CV within 8.4% Abemaciclib: %Bias within 6.1%; %CV within 8.7% Tucatinib and ONT-993 CSF (1:1 with plasma) samples stored at -70°C are stable for up to 375 days. Tucatinib: %Bias within 3.6%; %CV within 4.9% ONT-993: %Bias within 3.1%; %CV within 3.9% In CSF (1:1 with plasma) in the presence of capecitabine and metabolites at -70°C	AV18-ONT380-03 Addendum 1 Table 3-13 Table 3-14 Table 3-15 Table 3-18 Table 3-19 Table 3-20 Table 3-16 Table 3-17

	Tucatinib and ONT-993 CSF (1:1 with plasma) samples in the presence of capecitabine and metabolites stored at -70°C are stable for up to 927 days. Tucatinib: %Bias within 5.4%; %CV within 14.1% ONT-993: %Bias within 9.0%; %CV within 14.2% <u>In CSF (1:1 with plasma) at -20°C</u> Tucatinib and abemaciclib CSF (1:1 with plasma) samples stored at 70°C are stable for up to 72 days. ONT-993 was also present in the samples but did not meet acceptance criteria. Tucatinib: %Bias within 10.3%; %CV within 2.7% Abemaciclib: %Bias within 11.3%; %CV within 4.6% Tucatinib and ONT-993 CSF (1:1 with plasma) samples stored at 70°C are stable for up to 68 days. Tucatinib: %Bias within 3.0%; %CV within 4.0% ONT-993: %Bias within 2.8%; %CV within 4.3%	Table 3-21 Table 3-22 AV18-ONT380-03 Table 3-109 Table 3-111 Table 3-114 Table 3-115
Parallelism	NA	NA
Carry over	Analyte carryover failed for tucatinib, ONT-993, and abemaciclib in human plasma, indicating that bioanalytical sample batches should be monitored for carryover.	AV18-ONT380-03 Table 3-72 Table 3-73 Table 3-74
Method performance for CSF samples in study 380-IST-004 (TBCRC 049/UAB 1794) [d] Bioanalytical Sample Analysis for the Determination of Tucatinib (ONT-380) and ONT-993 in Human Plasma and Cerebrospinal Fluid by HPLC/MS/MS Report AD20-1125		
Assay passing rate	Tucatinib: A total of 9 CSF batches were collected, 7 batches were used. ONT-993: A total of 9 CSF batches were collected, 7 batches were used.	AD20-1125 Table 3-3 Table 3-4
Standard curve performance	<u>Cumulative %Bias for calibrators used to quantitate CSF samples</u> Tucatinib: -3.3 to 2.3% ONT-993: -3.2 to 2.5% <u>Cumulative %CV for calibrators used to quantitate CSF samples</u> Tucatinib: ≤ 6.1% ONT-993: ≤ 9.9%	AD20-1125 Table 3-15 Table 3-16
QC performance	<u>Cumulative %Bias for QC samples in CSF batches</u> Tucatinib: 1.1 to 4.0% ONT-993: -0.5 to 6.6% <u>Cumulative %CV for QC samples in CSF batches</u> Tucatinib: ≤ 9.6% ONT-993: ≤ 10.7%	AD20-1125 Table 3-19 Table 3-20
Method reproducibility	CSF samples reanalyzed (% total analyzed) Tucatinib: 20 (23.8%) ONT-993: 20 (23.8%) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: Tucatinib: 89.5%	AD20-1125 Table 3-21

	ONT-993: 75.0% ISR passed acceptance criteria.	
Study sample analysis/ stability	All CSF samples were analyzed within the validated stability range. The longest period from collection to analysis was 467 days. All samples were stored at -70°C. Validated stability for tucatinib and ONT-993 at -70°C: 927 days in 1:1 human plasma:CSF with capecitabine and metabolites present, validated for ONT-380 and ONT-993	AD20-1125 Table 3-2
Standard calibration curve performance during accuracy and precision runs	See above	

a Calibration curve is 0.100-100 ng/mL in plasma, but samples are diluted 1:1 CSF/plasma
b Data presented for standard calibration curve performance was for all runs in AV18-380-03, not just during accuracy and precision runs.
c Data presented for %CV in 1:1 CSF:plasma is for 6 replicates in a single batch (intra-batch rather that inter-batch).
d See Appendix 13 for method performance of plasma samples in this study.

4.1.8. Validation AV14-5FU-01

Appendix 16. Bioanalytical Method Life Cycle Information - AV14-5FU-01

	AV14-5FU-01	ONT-380-005
Analyte	5-Fluorouracil (5-FU) α-Fluoro-β-Alanine (FBAL)	5-Fluorouracil (5-FU) α-Fluoro-β-Alanine (FBAL)
Validation type	Full	In study
eCTD reference number	NA	NA
Method ID	TM14-286	TM14-286
Duration of time method is in use	14May2015-Present	14May2015- 28 Dec2016
Bioanalytical site	[redacted]	
Matrix	K_2EDTA Plasma	
Platform	HPLC/MS/MS method	
Format	Acetonitrile precipitation and HPLC/MS/MS were used to determine the concentration of 5-Fluorouracil (5-FU) and α-Fluoro-β-Alanine (FBAL) from human K_2EDTA plasma containing 1.2 µM tetrahydrouridine (THU). An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API6500). A Luna NH2, 100 Å HPLC column (2.0 x 50 mm) from Supelco was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. Compounds $^{13}C,^{15}N_2$-5-FU and $^{13}C_3$-FBAL served as the internal standard for 5-FU and FBAL respectively. The peak area of the product ion of each analyte were measured against the peak area of the product ion of the respective internal standard.	

	AV14-5FU-01	ONT-380-005
Stock reference Lot number Expiration date	5-FU SLBC6849V Oct2015 5-FU MKBS2555V 30Jun2016 5-FU 11-SCC-140-1 07Sep2021 $^{13}C,^{15}N_2$-5-FU G2613 NA $^{13}C,^{15}N_2$-5-FU L1318 13Dec2023 FBAL MKBK1527V NA FBAL E2016 18Oct2017 FBAL K2618 26Nov2023 $^{13}C_3$-FBAL 4-SDJ-123-3 16May2017 $^{13}C_3$-FBAL C2217 19Nov2019	5-FU SLBC6849V 31Oct2015 5-FU MKBS2555V 30Jun2016 5-FU 11-SCC-140-1 07Sep2021 FBAL MKBK1527V NA FBAL E2016 18Oct2017

	AV14-5FU-01	ONT-380-005
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	5-FU: 1.00 ng/mL to 1000 ng/mL FBAL: 10.0 ng/mL to 10,000 ng/mL	5-FU: 1.00 ng/mL to 1000 ng/mL FBAL: 10.0 ng/mL to 10,000 ng/mL
Matrix study population	Normal	Patients with HER2+ metastatic breast cancer
Link to reports and applicable amendments	AV14-5FU-01 AV14-5FU-01 Addendum 1 AV14-5FU-01 Addendum 2	AD14-734 Amendment 1
Synopsis of amendment history	Addendum 1 Continuation of long-term stability Selectivity in presence of co-administered compounds and metabolites Additional plasma stability Addendum 2 Continuation of long-term stability	Report Amendment 1 re-issues the report in its entirety to denote 131 of 134 samples identified by the sponsor that did not have THU added to the collected samples. The capecitabine and metabolite results are therefore considered unreliable because of pre-analytical technical issues.

Appendix 17. Summary Method Performance - AV14-5FU-01

Bioanalytical method validation report name, amendments, and hyperlinks	Validation report: AV14-5FU-01, AV14-5FU-01 Addendum 1, AV14-5FU-01 Addendum 2		
Method description	Acetonitrile precipitation and HPLC/MS/MS were used to determine the concentration of 5-Fluorouracil (5-FU) and α-Fluoro-β-Alanine (FBAL) from human K_2EDTA plasma containing 1.2 µM tetrahydrouridine (THU). An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API6500). A Luna NH2, 100 Å HPLC column (2.0 x 50 mm) from Supelco was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. Compounds $^{13}C,^{15}N_2$ 5-FU and $^{13}C_3$-FBAL served as the internal standard for 5-FU and FBAL respectively. The peak area of the product ion of each analyte were measured against the peak area of the product ion of the respective internal standard.		
Materials used for standard calibration curve and concentration	5-FU: 1.00, 2.00, 5.00, 10.0, 100, 500, 900, 1000 ng/mL FBAL: 10.0, 20.0, 50.0, 100, 1000, 5000, 9000, 10000 ng/mL		
Validated assay range	5-FU: 1.00 to 1000 ng/mL FBAL: 10.0 to 10000 ng/mL		
Material used for quality controls (QCs) and concentration	5-FU: 3.00, 50.0, 800 ng/mL FBAL: 30.0, 500, 8000 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model and weighting	5-FU and FBAL: linear regression; weighted $1/x^2$		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	AV14-5FU-01 Table 3-1
	Cumulative accuracy (%bias) from LLOQ to ULOQ	5-FU: -2.8 to 2% FBAL: -2.0% to 2.0%	AV14-5FU-01 Table 3-1 Table 3-2
	Cumulative precision (%CV) from LLOQ to ULOQ	5-FU: ≤6.4% FBAL: ≤8.1%	AV14-5FU-01 Table 3-1 Table 3-2
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	5-FU: -1.0% to 4.0% FBAL: 0.0% to 7.0%	AV14-5FU-01 Table 3-3 Table 3-4

	Inter-batch %CV	5-FU: ≤9.4% FBAL: ≤8.4%	AV14-5FU-01 Table 3-3 Table 3-4
	Total Error (TE)	5-FU: 13.4% FBAL: 15.4%	Sum of largest bias and CV reported.
Selectivity & matrix effect	Blank interference 6 lots were tested. No significant interfering peaks were found that eluted at the retention times of FBAL or $^{13}C_3$-FBAL in any of the blank plasma samples analyzed during the validation batches. No significant interfering peaks were found that eluted at the retention times of 5-FU or $^{13}C,^{15}N_2$-5-FU in the blank plasma selectivity samples analyzed. Matrix factor 6 lots were tested. The precision of the IS-normalized MF was acceptable for 5-FU (%CV 11.5%) and FBAL (%CV 4.2%).		AV14-5FU-01 Table 3-17 Table 3-18 Table 3-19 Table 3-20 Table 3-13 Table 3-14

Interference & specificity	Multi-analyte selectivity Individual QCs quantified using calibration curves containing both analytes 5-FU: %bias ≤4.0%; %CV was ≤4.1% FBAL: %bias ≤5.2%; %CV ≤3.2% Coadministration and Metabolite selectivity QC samples in the presence of Tucatinib, ONT-993, Capecitabine, 5'-DFCR, and 5'-DFUR Samples were considered to be acceptable for FBAL, 5-FU did not pass the criteria. Two lots were tested for 5-FU. 5-FU: %bias ≤209.7% (LQC), MQC and HQC ≤ 7.8%; %CV was ≤3.1% FBAL: %bias ≤8.0%; %CV ≤3.3% Because these reference standards will not be used in the dosing process, the low level of contamination in the stock solutions causing failure at the LQC level for 5-FU will not affect the accurate quantitation of 5-FU from human K_2EDTA plasma containing the coadministration and metabolite compounds listed above for Tucatinib, ONT-993, Capecitabine, 5'-DFCR, 5'-DFUR. Additional specificity in the presence of Tucatinib, ONT-993, Capecitabine, 5'-DFCR, 5'-DFUR was performed in Addendum 1	AV14-5FU-01 Table 3-21 Table 3-22 AV14-5FU-01 Table 3-23 Table 3-24 AV14-5FU-01 Addendum 1 Table 5-5 Table 5-6 Table 5-7 Table 5-8 Table 5-9 Table 5-10
Hemolysis effect	The samples were considered acceptable in 10% hemolyzed human K_2EDTA plasma. One lot tested. 5-FU: %bias ≤1.3%; %CV ≤3.5% FBAL: %bias ≤1.3%; %CV ≤4.0%	AV14-5FU-01 Table 3-7 Table 3-8
Lipemic effect	The samples were considered acceptable. One lot tested. 5-FU: %bias ≤1.4%; %CV ≤4.3% FBAL: %bias ≤1.6%; %CV ≤5.1%	AV14-5FU-01 Table 3-9 Table 3-10
Dilution linearity & hook effect	NA	NA

Bench-top/process stability	The stability of 5-FU and FBAL was confirmed for up to 7 hours on the benchtop at ambient conditions. One lot tested. 5-FU: %bias ≤5.3%; %CV ≤2.2% FBAL: %bias ≤5.8%; %CV ≤4.6% Stability of 5-FU and FBAL in the presence of Tucatinib was confirmed for up to 6 hours on wet ice 5-FU: %bias ≤4.7%; %CV ≤6.2% FBAL: %bias ≤3.0%; %CV ≤7.4%	AV14-5FU-01 Table 3-27 Table 3-28 AV14-5FU-01 Addendum 1 Table 5-11 Table 5-12
Freeze-Thaw stability	The stability of 5-FU and FBAL was confirmed for up to 4 freeze-thaw cycles. One lot tested. 5-FU: %bias ≤5.5%; %CV ≤2.3% FBAL: %bias ≤5.7%; %CV ≤6.0% The stability of 5-FU and FBAL was confirmed for up to 4 freeze-thaw cycles in the presence of Tucatinib. 5-FU: %bias ≤4.0%; %CV ≤4.6% FBAL: %bias ≤4.5%; %CV ≤5.5%	AV14-5FU-01 Table 3-29 Table 3-30 AV14-5FU-01 Addendum 1 Table 5-13 Table 5-14
Long-term storage	5-FU and FBAL undiluted samples stored at nominal -70°C are stable for up to 1037 days (1036 days for diluted samples) prior to extraction and analysis. 5-FU: %bias ≤-12.4%; %CV ≤3.1% FBAL: %bias ≤4.6%; %CV ≤1.9% Long-term storage at -70°C in the presence of Tucatinib 139 days 5-FU: %bias ≤12.0%; %CV ≤3.8% FBAL: %bias ≤4.7%; %CV ≤2.3%	AV14-5FU-01 Addendum 1 Table 4-3 Table 4-4 AV14-5FU-01 Addendum 2 Table 3-7 Table 3-9
Parallelism	NA	NA
Carry over	Analyte carryover was acceptable per SOP for all batches for 5-FU. Carryover failed for FBAL in all batches, indicating carry over should be evaluated during sample analysis.	AV14-5FU-01 Table 3-25 Table 3-26
Method performance in study ONT-380-005 Amendment 1 to Bioanalytical Sample Analysis for the Determination of ONT-380, ONT-993, Capecitabine, 5'-DFUR, 5'-DFCR, 5-FU, and FBAL in Human Plasma by HPLC/MS/MS Report AD14-734 Amendment 1 *Information presented here is only for 5-FU and FBAL*		

Assay passing rate[a]	5-FU: A total of 14 batches were collected, 10 batches were used. FBAL: A total of 15 batches were collected, 12 batches were used.	AD14-734 Amendment 1 Table 3-8 Table 3-9
Standard curve performance	Cumulative %Bias 5-FU: -4.8% to 6.7% FBAL: -11.6% to -6.1% Cumulative %CV 5-FU: ≤7.0% FBAL: ≤31.0%	AD14-734 Amendment 1 Table 3-29 Table 3-30
QC performance	Cumulative %Bias 5-FU: -6.9% to 1.3% FBAL: -3.4% to 1.2% Cumulative %CV 5-FU: ≤8.5% FBAL: ≤10.7%	AD14-734 Amendment 1 Table 3-36 Table 3-37
Method reproducibility	Samples reanalyzed (% total analyzed): 36 (13.4%, 5-FU) 44 (16.4%, FBAL) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 75.0% (5-FU) 100% (FBAL) ISR passes acceptance criteria.	AD14-734 Amendment 1 Table 3-38
Study sample analysis/ stability	All plasma samples were analyzed within 680 days of collection. Long-term stability has been validated out to 1037 days at -70°C for 5-FU and FBAL.	AD14-734 Amendment 1 Table 3-2
Standard calibration curve performance during accuracy and precision runs	See above	

a　A total of 36 total batches were collected. The 36 batches includes those collected for analytes in AV14-OTN380-01 (Tucatinib, ONT-993, Capecitabine, 5'-DFCR, 5'-DFUR, 5-FU, and FBAL).

4.1.9. Validation 2100-788 and 8225508

Appendix 18. Bioanalytical Method Life Cycle Information - 2100-788

	2100-788	**8225508**	**ONT-380-011**
Analyte	Moxifloxacin	Moxifloxacin	Moxifloxacin
Validation type	Full	Partial	In Study
eCTD reference number	NA	NA	NA
Method ID	MOXHPC	MXNHPC	MXNHPC
Duration of time method is used	12Jan2006-10Feb2010	06Apr2010-Present	15Feb2019-26Apr2019
Bioanalytical site	[redacted]		
Matrix	K_3EDTA Plasma	K_3EDTA and K_2EDTA Plasma	K_2EDTA Plasma
Format	LC-MS/MS method proprietary to [redacted]		
Platform	Protein precipitation and LC/MS/MS were used to determine the concentration of moxifloxacin from human K_3EDTA plasma. An aliquot of the extract was injected onto an LC/MS/MS triple quadrupole mass spectrometer. A LC column was used to separate analyte and internal standard from interfering endogenous compounds that may be present in the sample extract. Gatifloxacin served as the internal standard for moxifloxacin. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard. All	Protein precipitation and LC/MS/MS were used to determine the concentration of moxifloxacin from human K_2EDTA plasma. An aliquot of the extract was injected onto an LC/MS/MS triple quadrupole mass spectrometer. A LC column was used to separate analyte and internal standard from interfering endogenous compounds that may be present in the sample extract. Moxifloxacin-d_4 served as the internal standard for moxifloxacin. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard. All methodologies are proprietary to [redacted]	

	methodologies are proprietary to [redacted]		
Stock reference/ Lot number/ Expiration date	Moxifloxacin 2397707 27Feb2004 Moxifloxacin 2590803 31May2008 Gatifloxacin (IS) 2399104 31Oct2007	Moxifloxacin HCL F0H454 Jan2014 Moxifloxacin HCL monohydrate 3-JHY-159-1 29Jul2013 Moxifloxacin-d4 HCL 1-STR-68-5 23Mar2013 and 26Sept2015	Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 ES6156-23-P1 30Nov2018 ONT-993 AR00440993-03 21Nov2020 Moxifloxacin R03640 Current lot per USP
Stock reference/Lot number/Expiration date (continued)		Moxifloxacin-d4 HCL 021 30Jun2011[a] Moxifloxacin HCl SVI-ALS-16-098 29Apr2019 Moxifloxacin-d4 HCL 6-JUZ-142-2 09Dec2021	ONT-380-d6 AR00442572-03 30Nov2018 ONT-993-d4 ES6139-19 30Nov2018 Moxifloxacin-d4 BDG 9151.8 06Nov2023
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	25.0 to 5000 ng/mL	25.0 to 5000 ng/mL	25.0 to 5000 ng/mL
Matrix study population	Normal	Normal	Normal
Link to reports and applicable amendments	Validation report: 2100-788, 2100-788 Addendum 1	Validation report: 8225508, 8225508 Addendum 1, 8225508 Addendum 2, 8225508 Addendum 3, 8225508 Addendum 4	Bioavailability report: 8373072

Synopsis of amendment history	2100-788 Addendum 1 Method transfer to [redacted]- [redacted] Selectivity Matrix Factor in K_2EDTA Matrix effects Matrix Stability in K_2EDTA Long-Term Frozen Matrix Stability	8225508 Addendum 1 Accuracy and Precision 8225508 Addendum 2: Method Transfer in K_2EDTA plasma. Hemolysis Carryover 8225508 Addendum 3: Selectivity Matrix Factor ISTD Normalized Matrix Factor Processed-Sample Stability Sample Collection Stability Freeze/Thaw Matrix Stability Room Temperature Matrix Stability Long-Term Frozen Matrix Stability Hemolysis Assessment Hyperlipidemia 8225508 Addendum 4 Method transfer and assessment for K_2EDTA plasma Calibration performance	

a Retest date stated as June 2011 and assigned per [redacted] SOP as 30-Jun-2011.

Appendix 19. Summary Method Performance - 2100-788

Bioanalytical method validation report name, amendments, and hyperlinks	Validation report: 2100-788, 2100-788 Addendum 1 Method: MOXHPC		
Method description	Protein precipitation and LC/MS/MS were used to determine the concentration of moxifloxacin from human K_3EDTA plasma. An aliquot of the extract was injected onto an LC/MS/MS triple quadrupole mass spectrometer. A LC column was used to separate analyte and internal standard from interfering endogenous compounds that may be present in the sample extract. Gatifloxacin served as the internal standard for moxifloxacin. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard. All methodologies are proprietary to [redacted].		
Materials used for standard calibration curve and concentration	Moxifloxacin 25.0, 50.0, 100, 200, 1000, 2000, 4000, 5000 ng/mL		
Validated assay range	25.0 to 5000 ng/mL		
Material used for quality controls (QCs) and concentration	Moxifloxacin: 75.0, 750, 3500 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Linear regression $1/x^2$		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	2100-788 Table 4
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-4.0% to 4.0%	2100-788 Table 4
	Cumulative precision (%CV[a]) from LLOQ to ULOQ	≤8.6%	2100-788 Table 4
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5QCs	-0.6% to 1.6%	2100-788 Table 5

	Inter-batch %CV QCs	≤6.7%	2100-788 Table 5
	Total Error (TE)	8.3%	Sum of largest bias and RSD reported.
Selectivity & matrix effect	Selectivity 6 lots tested %Bias -1.9%; %CV 2.9% Matrix Factor 6 lots tested The matrix effect did not significantly impact assay performance. Moxifloxacin: %matrix effect: -1.7%, %CV 11.1% Gatifloxacin: %matrix effect: -6.5%, %CV 10.3% Selectivity in K_2EDTA Similar to above, the moxifloxacin and ISTD regions were free from significant interference. The method demonstrated acceptable selectivity. %Bias 5.6%; %CV 6.1% Matrix Effect Similar to above the matrix effect did not significantly impact assay performance. Moxifloxacin: %matrix effect ≤11.4% Gatifloxacin: %matrix effect ≤14.8% Aliquots of blank human plasma from six different individuals were tested for endogenous interferences. In all cases, the moxifloxacin and ISTD regions were free from significant interference.		2100-788 Table 1 Table 2 2100-788 Addendum 1 Table 6.6 Table 6.7 2100-788 Addendum 1 Section 4.3
Interference & specificity	NA		NA
Hemolysis effect	NA		NA
Lipemic effect	NA		NA
Dilution linearity & hook effect	NA		NA

Bench-top/process stability	Room temperature matrix stability Room temperature storage stability of moxifloxacin in human plasma was confirmed for at least 24 hours. %Bias 0% to 2.6% and %CV ≤2.8% Processed sample stability: The stability of moxifloxacin in the extracts was found to be acceptable. Stability was confirmed for 71 hours at 2ºC to 8°C. %Bias 1.2% to 4.3%	2100-788 Table 11 Table 13
Freeze-Thaw stability	Stability of moxifloxacin in human plasma was confirmed for 7 freeze/thaw cycles at -10°C to -30°C in Addendum 1. %Bias was 0.3% and %CV ≤4.2%	2100-788 Addendum 1 Table 6.11
Long-term storage	Moxifloxacin in human plasma is stable for 1001 days when stored in a freezer set to maintain -60ºC to -80ºC. %Bias -2.4% to 1.9%, %CV ≤3.5% Moxifloxacin in human plasma is stable for up to 394 days when stored in a freezer set to maintain -10ºC to -30ºC. %Bias 0.7% to 8.4% and %CV ≤8.2%	2100-788 Table 12 2100-788 Addendum 1 Table 6.13
Parallelism	NA	NA
Carry over	There was no significant carryover in the chromatographic regions of interest for Moxifloxacin.	2100-788

Appendix 20. Summary Method Performance - 8225508

Bioanalytical method validation report name, amendments, and hyperlinks	Validation report: 8225508, 8225508 Addendum 1, 8225508 Addendum 2, 8225508 Addendum 3, and 8225508 Addendum 4[a] Method: MXNHPC		
Method description	Protein precipitation and LC/MS/MS were used to determine the concentration of moxifloxacin from human K_2EDTA plasma. An aliquot of the extract was injected onto an LC/MS/MS triple quadrupole mass spectrometer. A LC column was used to separate analyte and internal standard from interfering endogenous compounds that may be present in the sample extract. Moxifloxacin-d_4 served as the internal standard for moxifloxacin. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard. All methodologies are proprietary to [redacted]		
Materials used for standard calibration curve and concentration	Moxifloxacin 25.0, 50.0, 100, 200, 1000, 2000, 4000, 5000 ng/mL		
Validated assay range	25.0 to 5000 ng/mL		
Material used for quality controls (QCs) and concentration	75.0, 750, 3500 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Weighted ($1/x^2$) linear least-squares regression		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	8225508 Addendum 1 Table 7.5
	Cumulative accuracy (%bias[b]) from LLOQ to ULOQ	-5.2% to 3.0%	8225508 Addendum 1 Table 7.5
	Cumulative precision (%CV[c]) from LLOQ to ULOQ	≤5.0%	8225508 Addendum 1 Table 7.5

Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias[b]) in 5 QCs	2.3% to 7.6%	8225508 Addendum 1 Table 7.6
	Inter-batch %CV[c] QCs	≤5.5%	8225508 Addendum 1 Table 7.6
	Total Error (TE)	13.1%	Sum of highest bias and CV
Selectivity & matrix effect	Selectivity 6 lots tested There were no interference peaks detected at the ISTD retention time (>5% of the internal standard peak response in the control zero sample) for the six individual blank matrix samples. There were no interference peaks (>20% of the LLOQ calibration standard response) detected in the six individual blank matrix samples at the retention time of moxifloxacin. Matrix Factor 6 lots tested Internal standard normalized matrix factor LQC mean 0.978, %CV 3.5% HQC mean 1.01, %CV 0.9% The precision of the matrix factor evaluation met the acceptance criteria as stated and the matrix factor was not considered to impact assay performance.		8225508 Addendum 3 Figures 9.1 through 9.4 Table 7.5 Table 7.6
Interference & specificity	The blank samples spiked with tucatinib and ONT-993 did not contain any significant peaks at the retention time of Moxifloxacin or internal standard. This confirms that the presence of tucatinib and ONT-993 does not affect the selectivity of Moxifloxacin.		8373072 Section 4.5
Hemolysis effect	The presence of 2% hemolyzed red blood cells in the human plasma did not affect the quantitation of moxifloxacin in this method. %Bias ≤-2.5%, %CV ≤1.6%		8225508 Addendum 2 Table 7.9
Lipemic effect	The human plasma with naturally high lipid content did not affect the quantification of moxifloxacin in this assay. One lot was tested. %Bias within 4.5%; %CV was within 3.2%		8225508 Addendum 3 Table 7.13
Dilution linearity & hook effect	NA		NA

Bench-top/process stability	Processed sample stability: The stability of moxifloxacin in the extracts was found to be acceptable. Stability was confirmed for 173 hours at 2ºC to 8°C. %Bias within 3.7%; %CV within 2.0% Room temperature matrix stability Room temperature storage stability of moxifloxacin in human plasma was confirmed for 26 hours. %Bias within 4.8%; %CV within 3.3%	8225508 Addendum 3 Table 7.7 Table 7.10
Freeze-Thaw stability	Stability of moxifloxacin in human plasma was confirmed for 7 freeze/thaw cycles at -60ºC to -80°C. %Bias within 3.1%; %CV within 4.3%	8225508 Addendum 3 Table 7.9
Long-term storage	Moxifloxacin in human K_2EDTA plasma is stable for 419 days when stored in a freezer set to maintain -60ºC to -80ºC. %Bias within 1.1%; %CV within 2.1%	8225508 Addendum 3 Table 7.11
Parallelism	NA	NA
Carryover	There was no significant carryover in the chromatographic regions of interest for moxifloxacin and moxifloxacin-d4.	8225508 Addendum 4 Figure 7.6

Method performance in study ONT-380-011
Determination of ONT-380, ONT-993, and Moxifloxacin in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-011
Report 8373072

Assay passing rate	A total of 15 batches of moxifloxacin were run, 13 were accepted.	8373072 Table 9.1
Standard curve performance	Moxifloxacin: %bias -7.6% to 5.0% Moxifloxacin: %CV ≤4.6%	8373072 Table 9.6
QC performance	Moxifloxacin: %bias 2.7% to 7.6% Moxifloxacin: %CV ≤6.1%	8373072 Table 9.12
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 72 of 674 (10.7%) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100%	8373072 Section 4.4 Table 9.18

Study sample analysis/ stability	All plasma samples were analyzed within the validated stability range. Moxifloxacin in human K_2EDTA plasma is stable for 419 days when stored in a freezer set to maintain -60ºC to -80ºC.	8225508 Addendum 3 Table 7.11
Standard calibration curve performance during accuracy and precision runs	See above.	

a 8225508 Addenda 2, 3, and 4 pertain to testing K_2EDTA plasma in support of sample analysis for ONT-380-011.
b Reported as %RE in 8225508 Addendum 1.
c CV is used interchangeably with RSD. Validation and sample analysis reports report RSD.

4.1.10. Validation AV14-DM1-01

Appendix 21. Bioanalytical Method Life Cycle Information (Study data not used due to ISR failures) - AV14-DM1-01

	AV14-DM1-01[a]	ONT-380-004
Analyte	DM1	DM1
Validation type	Full	In study
eCTD reference number	NA	NA
Method ID	TM14-277	TM14-277
Duration of time method is in use	11Feb2014-Jan2019	07Apr2014-28Dec2016
Bioanalytical site	[redacted]	[redacted]
Matrix	K_2EDTA plasma	
Platform	HPLC/MS/MS method	
Format	Tris(2-carboxyelthyl)phosphine (TCEP) reduction, acetonitrile precipitation, N-ethyl maleimide (NEM) derivatization and HPLC MS/MS were used to determine the concentration of DM1 from human K_2EDTA plasma. An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API5000 or API5500). A Discovery HS-C18, 3 µm HPLC column (2.1 x 50 mm) from Supelco column was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. Ansamitocin P-3 served as the internal standard for DM1. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard.	

	AV14-DM1-01[a]	**ONT-380-004**
Stock reference (storage temperature) **Lot number** **Expiration date**	DM1 FD334531399 NA Ansamitocin-P3 10361 11Dec2015 Addendum 1 DM1 FD334531399 30Nov2015 1-LWJ-28-1 28May2019 24442 28Nov2020 Ansamitocin P-3 10361 11Dec2015 Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 ES6156-23-P1 30Nov2018	DM1 FD334531399 30Nov2015 1-LWJ-28-1 28May2019 Ansamitocin P-3 10361 11Dec2015
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	DM1: 0.500 to 100 ng/mL	DM1: 0.500 to 100 ng/mL
Matrix study population	Normal	Diseased, breast cancer patients
Link to reports and applicable amendments	Validation reports: AV14-DM1-01[a] AV14-DM1-01 Addendum 1	BioA report: AD14-826[a]
Synopsis of amendment history	Addendum 1 Additional matrix stability and specificity in the presence of Tucatinib and ONT-993 Cross validation between plasma and serum. FAILED	NA

a Validation was successful, but Sample analysis failed ISR, and data was not used for PK evaluation.

Appendix 22. Summary Method Performance - AV14-DM1-01

Bioanalytical method validation report name, amendments, and hyperlinks	AV14-DM1-01 *Please note: Validation was successful, but sample analysis failed ISR, and data was not used for PK evaluation.*		
Method description	Tris(2-carboxyelthyl)phosphine (TCEP) reduction, acetonitrile precipitation, N-ethyl maleimide (NEM) derivatization and HPLC MS/MS were used to determine the concentration of DM1 from human K_2EDTA plasma. An aliquot of the extract was injected onto an HPLC/MS/MS triple quadrupole mass spectrometer (Sciex API5000 or API5500). A Discovery HS-C18, 3 µm HPLC column (2.1 × 50 mm) from Supelco column was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. Ansamitocin P-3 served as the internal standard for DM1. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard.		
Materials used for standard calibration curve and concentration	DM1: 0.500, 1.00, 3.00, 12.0, 25.0, 50.0, 90.0, 100 ng/mL		
Validated assay range	DM1: 0.500 to 100 ng/mL		
Material used for quality controls (QCs) and concentration	DM1: 1.50, 10.0, and 80.0 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	DM1: linear regression, weighted ($1/x^2$)		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	AV14-DM1-01 Table 3-1
	Cumulative accuracy (%bias) from LLOQ to ULOQ	DM1: -5.4% to 7.0%	AV14-DM1-01 Table 3-1

	Cumulative precision (%CV) from LLOQ to ULOQ	DM1: ≤11.4%	AV14-DM1-01 Table 3-1
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 3 QCs	DM1: -1.7% to 10.0%	AV14-DM1-01 Table 3-2
	Inter-batch %CV QCs	Tucatinib: ≤27.8% (includes outlier)	AV14-DM1-01 Table 3-2
	Total Error (TE)	37.8%	Sum of largest bias and CV reported
Selectivity & matrix effect	<u>Selectivity</u> No significant interfering peaks were found that eluted at the retention times of DM1 or ansamitocin P-3 in the blank plasma selectivity samples analyzed. Six lots tested. Matrix Factor Six lots tested. Internal standard normalized matrix factor was within acceptance criteria, %CV ≤3.5 Selectivity in the presence of Tucatinib and ONT-993 No substantial interfering peaks were found that eluted at the retention times of DM1 or ansamitocin P-3.		AV14-DM1-01 Table 3-9 Table 3-6 AV14-DM1-01 Addendum 1 Table 4-7
Interference & specificity	<u>Concomitant Drug Interference Screen</u> For DM1 in the presence of tucatinib, the coadmin selectivity at each nominal concentration level was acceptable %Bias ≤5.3%; %CV ≤6.0% DM1 in the presence of Tucatinib and ONT-993 %Bias ≤10.7% (Outlier excluded); %CV ≤17.6%		AV14-DM1-01 Table 3-10 AV14-DM1-01 Addendum 1 Table 4-5
Hemolysis effect	These results indicate that if sample hemolysis does not exceed 1%, accurate quantitation of DM1 will not be affected. QC samples did not pass acceptance criteria at 10% hemolysis. DM1 in 1% hemolyzed human K_2EDTA plasma %Bias ≤6.0%; %CV ≤3.8%		AV14-DM1-01 Table 3-4

Lipemic effect	The mean lipemic human K_2EDTA plasma was within the nominal value and thus was acceptable. %Bias within 2.4%; %CV within 11.3%	AV14-DM1-01 Table 3-5
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	The stability of DM1 in human K_2EDTA plasma samples was confirmed for up to 6 hours on the benchtop at ambient conditions. %Bias within 5.3%; %CV within 8.5% Bench-top stability in the presence of Tucatinib and ONT-993 4.2 hours on the benchtop at ambient conditions %Bias within 14.0%; %CV within 5.7% Bench-top stability in the presence of Tucatinib and ONT-993 6.3 hours on wet ice %Bias within 9.5%; %CV within 8.3%	AV14-DM1-01 Table 3-12 AV14-DM1-01 Addendum 1 Table 4-9 Table 4-10
Freeze-Thaw stability	The stability of DM1 in human K_2EDTA plasma was therefore confirmed for up to 6 freeze-thaw cycles. %Bias within 14.0%; %CV within 10.3% Freeze-thaw stability in the presence of Tucatinib and ONT-993 4 cycles at -70°C %Bias within 15.0%; %CV within 6.7%	AV14-DM1-01 Addendum 1 Table 4-1 Table 4-11
Long-term storage	DM1 samples stored at -70 ºC are stable for up to 1267 days %Bias within 9.0%; %CV within 5.3%	AV14-DM1-01 Addendum 1 Table 4-3
Parallelism	NA	NA
Carry over	Carryover was within acceptable limits.	Table 3-11
Method performance in study ONT-380-004 Bioanalytical Sample Analysis for the Determination of DM1 in Human Plasma and Serum by HPLC-MS/MS Report AD14-826		
Assay passing rate	17 out of 18 batches were accepted. 217 serum samples were analyzed prior to the matrix cross validation failure, results are flagged. ISR failure for the study prevented use of data in PK analyses etc.	
Standard curve performance	DM1: %Bias -2.5 to 3.0; %CV ≤7.0%	AD14-826 Table 3-6

QC performance	DM1: %Bias -6.5 to 2.0; %CV ≤9.3%	AD14-826 Table 3-7
Method reproducibility	ISR failed acceptance criteria. Data was not used for this analyte. DM1 Samples reanalyzed: 6 of 62 samples (15.3% of total number of samples) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 9.7% passed	AD14-826 Table 3-8
Study sample analysis/ stability	Samples were tested within 898 days of collection. Long-term storage of plasma samples was 1267 days at -70°C.	AD14-826 Table 3-2
Standard calibration curve performance during accuracy and precision runs	See above.	

4.1.11. Validation AV18-Metformin-01 and AV18-Metformin-02

Appendix 23. Bioanalytical Method Life Cycle Information - AV18-Metformin-01 and AV18-Metformin-02-01

	AV18-Metformin-01	AV18-Metformin-02	SGNTUC-020
Analyte	Metformin	Metformin	Metformin
Validation type	Full	Full	In study
eCTD reference number	NA	NA	NA
Method ID	TM18-500	TM18-503	TM18-500 TM18-503
Duration of time method is in use	06Dec2018-present	06Dec2018-Present	06Mar2019-18Mar2019
Bioanalytical site	[redacted]		
Matrix	K_2EDTA Plasma	Urine	K_2EDTA Plasma Urine
Platform	HPLC/MS/MS method		
Format	Acetonitrile precipitation and LC/MS/MS were used to determine the concentration of metformin from human K_2EDTA plasma and urine. An aliquot of the extract was injected onto an LC/MS/MS triple quadrupole mass spectrometer (Sciex API-5500 or 6500). A Discovery HS-C18, 2.1×50mm, 3 µm HPLC column from Supelco was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. Compound d6-metformin served as the internal standard for metformin. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard.		

	AV18-Metformin-01	AV18-Metformin-02	SGNTUC-020
Stock reference **Lot number** **Expiration date**	Metformin Hydrochloride Q6020 31Dec2019 Metformin D_6 Hydrochloride CRC-0308-037 22Jan2020 Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 AR00440993-03 21Nov2020 Iohexol BCBV6710 31Jul2020	Metformin Hydrochloride Q6020 31Dec2019 Metformin D_6 Hydrochloride CRC-0308-037 22Jan2020 Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 AR00440993-03 21Nov2020 Iohexol BCBV6710 31Jul2020	Metformin Hydrochloride Q6020 31Dec2019 Metformin D_6 Hydrochloride CRC-0308-037 22Jan2020
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	Metformin 2.00 ng/mL to 2000 ng/mL	Metformin 10.0 ng/mL to 10000 ng/mL	Plasma 2.00 ng/mL to 2000 ng/mL Urine 10.0 ng/mL to 10000 ng/mL
Matrix study population	Normal	Normal	Normal
Link to reports and applicable amendments	Validation reports: Right of Reference Letter AV18-Metformin-01 Addendum 1	Validation reports: Right of Reference Letter AV18-Metformin-02 Addendum 1	BioA report: AD19-849

	AV18-Metformin-01	AV18-Metformin-02	SGNTUC-020
Synopsis of amendment history	Interference testing in the presence of Tucatinib, ONT-993 and iohexol Matrix stability	Interference testing in the presence of Tucatinib, ONT-993 and iohexol Matrix stability	

Appendix 24. Summary Method Performance - AV18-Metformin-01, Human Plasma

Bioanalytical method validation report name, amendments, and hyperlinks	HPLC-MS/MS Assay Validation for the Determination of Metformin from Human Plasma		
Method description	Acetonitrile precipitation and HPLC/MS/MS were used to determine the concentration of metformin from human K_2EDTA plasma. An aliquot of the extract was injected onto an LC/MS/MS triple quadrupole mass spectrometer (Sciex API-5500 or 6500). A Discovery HS-C18, 2.1x50mm, 3µm HPLC column from Supelco was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. Compound d6-metformin served as the internal standard for metformin. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard.		
Materials used for standard calibration curve and concentration	Metformin: 2.00, 4.00, 20.0, 50.0, 250, 1000, 1800, and 2000 ng/mL		
Validated assay range	2.00 to 2000 ng/mL		
Material used for quality controls (QCs) and concentration	Metformin: 2.00, 6.00, 100, 1600 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model and weighting	linear $1/x^2$ weighted regression		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	Table 1-1
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.0% to 1.5%	Table 3-1
	Cumulative precision (%CV) from LLOQ to ULOQ	≤6.8%	Table 3-1
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	1.5% to 7.0%	Table 1-1
	Inter-batch %CV	≤10.0%	Table 1-1
	Total Error (TE)	17%	Sum of largest bias and CV reported.

Selectivity & matrix effect	6 lots tested, no %bias calculated. Internal Standard Normalized Matrix Factor: LQC Analyte Matrix Factor 1.14 ± 3.1% HQC Analyte Matrix Factor 1.08 ± 1.5%	Table 3-10
Interference & specificity	6 lots tested for interference, no interference observed. Selectivity Blanks with Co-Administered Compounds No interfering peaks were found that eluted at the retention times of metformin or metformin-D_6 in the co-administered selectivity sample analyzed in batch 3 Intrabatch Accuracy and Precision Quality Control Evaluation Data for Metformin in the Presence of ONT-380, ONT-993, and Iohexol in Human Urine Metformin: %bias -1.1 % to 6.5%, %CV ≤5.2%	Table 3-14 Addendum 1 Table 3-6 Addendum 1 Table 3-3
Hemolysis effect	1 lot, 5% hemolyzed plasma. %bias from -3.8% to 6.7% and %CV within 3.5%. 10% hemolyzed plasma was also tested but did not meet acceptance criteria.	Table 3-7
Lipemic effect	1 lot, %bias from -12.4% to 0.0%, %CV within 6.1%.	Table 3-9
Dilution linearity & hook effect	NA	
Bench-top/process stability	6 hours ambient benchtop plasma stability: %bias 0.0% to 6.2%, %CV ≤2.3% 6 hours on ice benchtop plasma stability: %bias -0.2 % to 3.7%, %CV ≤ 3.1% Bench-top Stability with Co-Administered Compounds 6.4 hours ambient: %bias -1.9% to 12.8%, %CV ≤6.5%	Ambient: Table 3-16 On Ice: Table 3-17 Addendum 1 Table 3-7
Freeze-Thaw stability	4 freeze-thaw cycles. %bias 0.6% to 4.8%, %CV ≤13.4 Stability with Co-Administered Compounds 4 freeze thaw cycles: %bias -3.1 % to 7.5%, %CV ≤8.4%	Table 3-18 Addendum 1 Table 3-8

Long-term storage	126 days plasma stability at -20°C, %bias -3.8% to 3.0%, %CV ±5.7% 125 days plasma stability at -70°C, %bias -0.6% to 1.0%, %CV ±6.1% Long-Term Stability with Co-Administered Compounds 113 days plasma stability at -70°C %bias -10% to 2.0%, %CV ≤ 3.4	-20°C stability: Table 3-21 -70°C stability: Table 3-20 Addendum 1 Table 3-9
Parallelism	NA	NA
Carry over	The analyte peak area of the carryover double blank was > 20% of the passing LLOQ curve points in 2 out of the 3 batches where carryover was tested during validation.	Table 3-5

Method performance in study SGNTUC-020
Bioanalytical Sample Analysis for the Determination of Tucatinib and ONT-993 from Human Plasma and Metformin from Human Plasma and Urine
Report AD19-849

Assay passing rate	13 batches were extracted, 11 batches were used.	AD19-849 Table 3-4
Standard curve performance	Metformin: %bias -2.5 % to 2.2%, %CV ≤6.4%	AD19-849 Table 3-13
QC performance	Metformin: %bias -3.1 % to 9.4%, %CV ≤6.2%	AD19-849 Table 3-17
Method reproducibility	ISR was performed on 58 plasma samples (11% of total), with 57 passing acceptance criteria. Overall passing of 98.3%	AD19-849 Table 3-19
Study sample analysis/ stability	Metformin plasma samples were analyzed within 16 days from collection. Long-term stability of metformin in plasma in the presence of Tucatinib, ONT-993 and iohexol has been established to 113 days plasma stability at -70°C.	AD19-849 Table 3-1
Standard calibration curve performance during accuracy and precision runs	See above	

Appendix 25. Summary Method Performance - AV18-Metformin-02, Human Urine

Bioanalytical method validation report name, amendments, and hyperlinks	HPLC-MS/MS Assay Validation for the Determination of Metformin from Human Urine		
Method description	Acetonitrile precipitation and HPLC/MS/MS were used to determine the concentration of metformin from human urine. An aliquot of the extract was injected onto an LC/MS/MS triple quadrupole mass spectrometer (Sciex API-5500 or 6500). A Discovery HS-C18, 2.1 × 50mm, 3µm HPLC column from Supelco was used to separate analytes and internal standards from interfering compounds that may be present in the sample extract. Compound d6-metformin served as the internal standard for metformin. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard.		
Materials used for standard calibration curve and concentration	Metformin: 10.0, 20.0, 100, 300, 1000, 5000, 9000, and 10000 ng/mL		
Validated assay range	10.0 to 10000 ng/mL		
Material used for quality controls (QCs) and concentration	Metformin: 30.0, 500, 8000 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model and weighting	linear $1/x^2$ weighted regression		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	Table 1-1
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-2.0% to 2.3%	Table 3-1
	Cumulative precision (%CV) from LLOQ to ULOQ	≤3.5%	Table 3-1
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	-6.0% to 1.0%	Table 1-1
	Inter-batch %CV	≤6.1%	Table 1-1
	Total Error (TE)	12.1%	Sum of largest bias and CV reported.

Selectivity & matrix effect	6 lots tested, no %bias calculated. Internal Standard Normalized Matrix Factor: LQC Analyte Matrix Factor 1.06 ± 3.5% HQC Analyte Matrix Factor 1.06 ± 1.5%	Table 3-6
Interference & specificity	6 urine lots (from individual donors) tested for interference, no interference observed Selectivity Blanks with Co-Administered Compounds No interfering peaks were found that eluted at the retention times of metformin or metformin-D_6 in the co-administered selectivity sample Intrabatch Accuracy and Precision Quality Control Evaluation Data for Metformin in the Presence of ONT-380, ONT-993, and Iohexol in Human Urine Metformin: %bias 3.1% to 9.0%, %CV ≤4.2%	Table 3-11 Addendum 1 Table 3-6 Addendum 1 Table 3-3
Hemolysis effect	NA	NA
Lipemic effect	NA	NA
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	6.5 hours ambient benchtop urine stability: %bias -1.3% to 1.0%, %CV ± 4.7% 5 hours for DQC. %bias -3.0% and %CV 4.8%. 6.5 hours on ice benchtop urine stability: %bias -1.3 % to 0.1%, %CV ± 7.4% Bench-top Stability with Co-Administered Compounds 6.4 hours ambient: %bias 1.5% to 12.6%, %CV ≤2.0%	Ambient: Table 3-15 Ambient, DQC, 5h: Table 3-16 On Ice: Table 3-17 Addendum 1 Table 3-7
Freeze-Thaw stability	4 freeze-thaw cycles. %bias -1.9% to 3.7%, %CV ±3.4 Stability with Co-Administered Compounds 4 freeze thaw cycles at -70°C: %bias 0.9% to 9.2%, %CV ≤2.6%	Table 3-19 Addendum 1 Table 3-8

Long-term storage	79 days urine stability at -20°C, %bias 0.8% to 10.8%, %CV ±4.1% 124 days urine stability at -70°C, %bias 3.0% to 11.0%, %CV ±3.9% <u>Long-Term Stability with Co-Administered Compounds</u> 114 days plasma stability at -70°C %bias -7.8%% to 3.2%, %CV ≤ 3.1	-20°C stability: Table 3-22 -70°C stability: Table 3-21 Addendum 1 Table 3-9
Parallelism	NA	NA
Carry over	The analyte peak area of the carryover double blank was > 20% of the passing LLOQ curve points in 2 out of the 3 batches where carryover was tested during validation.	Table 3-12

Method performance in study SGNTUC-020 Bioanalytical Sample Analysis for the Determination of Tucatinib and ONT-993 from Human Plasma and Metformin from Human Plasma and Urine Report AD19-849		
Assay passing rate	7 batches were extracted, 7 batches were used	AD19-849 Table 3-4
Standard curve performance	Metformin: %bias -2.8 % to 3.3%, %CV ≤4.5%	AD19-849 Table 3-14
QC performance	Metformin: %bias -1.8 % to 6.6%, %CV ≤12.1%	AD19-849 Table 3-18
Method reproducibility	ISR was performed on 21 urine samples (12% of total), with 15 passing acceptance criteria, and two with non calculable results. Overall passing of 78.9%	AD19-849 Table 3-19
Study sample analysis/ stability	Metformin plasma samples were analyzed within 16 days from collection. Long-term stability of metformin in urine in the presence of Tucatinib, ONT-993 and iohexol has been established to 114 days plasma stability at -70°C.	AD19-849 Table 3-1
Standard calibration curve performance during accuracy and precision runs	See above	

4.1.12. PRALABIN-186747-B

Appendix 26. Bioanalytical Method Life Cycle Information - PRALABIN-186747-B

	PRALABIN-186747-B	SGNTUC-020
Analyte	Iohexol	Iohexol
Validation type	Full	In study
eCTD reference number	NA	NA
Method ID	[redacted]-US-SML-0430	[redacted]-US-SML-0430
Duration of time method is in use	19Feb2019-present	06Mar2019-12Mar2019
Bioanalytical site	[redacted]	
Matrix	K_2EDTA plasma samples (human)	
Platform	HPLC/MS/MS method	
Format	Protein precipitation and UPLC/MS/MS were used to determine the concentration of Iohexol from human K_2EDTA plasma. An aliquot of the extract was injected onto an UPLC/MS/MS triple quadrupole mass spectrometer (Sciex AP5500). A BEH HILIC column (2.1 × 50 mm, 1.7 µm particles) from Waters was used to separate analyte and internal standard from interfering compounds that may be present in the sample extract. Iohexol-d5 served as the internal standard for Iohexol. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard.	

	PRALABIN-186747-B	**SGNTUC-020**
Stock reference **Lot number** **Expiration date**	Iohexol H0J211 11Feb2024 Iohexol-d_5 (Int. Std.) 10-THT-155-3 13Jun2021 Metformin Hydrochloride R069H0 NA	Iohexol H0J211 11Feb2024 Iohexol-d_5 10-THT-155-3 13Jun2021 Metformin Hydrochloride R069H0 Expiry verified on USP website prior to each use Tucatinib L-ARR14-123 15Jun2020
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	5.00 to 1000 µg/mL	5.00 to 1000 µg/mL
Matrix study population	Normal	Normal
Link to reports and applicable amendments	PRALABIN-186747-B	SGNTUC-020
Synopsis of amendment history	NA	NA

Appendix 27. Summary Method Performance - PRALABIN-186747-B

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: PRALABIN-186747-B Method: [redacted]-US-SML-0430		
Method description	Protein precipitation and UPLC/MS/MS were used to determine the concentration of Iohexol from human K_2EDTA plasma. An aliquot of the extract was injected onto an UPLC/MS/MS triple quadrupole mass spectrometer (Sciex AP5500). A BEH HILIC column (2.1 × 50 mm, 1.7 µm particles) from Waters was used to separate analyte and internal standard from interfering compounds that may be present in the sample extract. Iohexol-d5 served as the internal standard for Iohexol. The peak area of the product ion of the analyte was measured against the peak area of the product ion of the internal standard.		
Materials used for standard calibration curve and concentration	Iohexol: 5.00, 10.0, 25.0, 125, 200, 500, 800, 1000 µg/mL		
Validated assay range	5.00 to 1000 µg/mL		
Material used for quality controls (QCs) and concentration	15.0, 150, and 800 µg/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Linear regression; $1/x^2$		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	PRALABIN-186747 Table 2
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.1% to 4.0%	PRALABIN-186747 Table 2
	Cumulative precision (%CV) from LLOQ to ULOQ	≤4.0%	PRALABIN-186747 Table 2

Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 3 QCs	-1.6% to 1.6%	PRALABIN-186747 Table 4
	Inter-batch %CV QCs	≤3.0%%	PRALABIN-186747 Table 4
	Total Error (TE)	4.6%	Sum of largest bias and CV reported
Selectivity & matrix effect	<u>Selectivity</u> The selectivity of the method was considered acceptable. Six lots were tested. <u>Matrix factor</u> The degree of matrix variability is acceptable. Six lots were tested %Bias: -0.9%; %CV: 5.0% <u>Matrix effect</u> The matrix effect was acceptable. Internal Standard Normalized Matrix Factor LQC 0.985, %CV 1.6% HQC 0.987 %CV 1.9%		PRALABIN-186747 Table 7 Table 8 Table 13
Interference & specificity	<u>Concomitant Drug Interference Screen</u> The method is sufficiently selective with respect to metformin hydrochloride. One lot was tested. %bias ≤3.1% Specificity in the presence of tucatinib and metformin hydrochloride was evaluated during sample analysis Iohexol QCs Containing Tucatinib Bias ≤ 2.7% Iohexol QCs Containing Tucatinib and Metformin Hydrochloride Bias ≤ 4.7%		PRALABIN-186747 Table 11 Table 12 SGB637UF-186372-A Table 6 Table 7 Table 8 Table 9
Hemolysis effect	Selectivity for hemolytic plasma using 2% (v/v) lysed human whole blood spiked in human K_2EDTA was found to be within the criteria. One lot was tested. %Bias: ≤2.0%		PRALABIN-186747 Table 9

Lipemic effect	Calculation was done in relation to a calibration curve in non-lipemic plasma. Selectivity for lipemic plasma was found to be within the criteria. One lot was tested. %Bias: ≤8.8%	PRALABIN-186747 Table 10
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	Iohexol is stable up to 22 hours in plasma at room temperature. %Bias: -5.7 to -1.7% %CV: ≤2.8% Iohexol extracts are stable up to 50 hours in the autosampler (10°C) %Bias: 0.5% to 1.5% %CV: ≤2.5%	PRALABIN-186747 Table 21 Table 20
Freeze-Thaw stability	Iohexol is stable up to 5 cycles in plasma at -20°C %Bias:≤-7.0% %CV: ≤2.7% -70°C. %Bias: ≤-5.5% %CV: ≤1.8%	PRALABIN-186747 Table 22
Long-term storage	Iohexol is stable up to 38 days in plasma at -20°C and at -70°C. -20°C %Bias:≤-4.3% %CV: ≤7.2 -70°C. %Bias: ≤7.8% %CV: ≤3.0%	PRALABIN-186747 Table 23
Parallelism	NA	NA
Carry over	All acceptance criteria were met and demonstrated the method was not affected by carry-over.	PRALABIN-186747 Table 16 Table 17
DETERMINATION OF IOHEXOL IN HUMAN K2-EDTA PLASMA SAMPLES BY LC-MS/MS (API 5500)Report SGB637UF-186372-A		
Assay passing rate	4 out of 4 batches were accepted. Sample analysis occurred in 3 out of 4 batches, and all three batches were accepted.	SGB637UF-186372-A Table 2

Standard curve performance	%Bias: -3.4% to 7.0% %CV: ≤3.1%	SGB637UF-186372-A Table 10
QC performance	%Bias: -2.0% to -0.7% %CV: ≤5.1%	SGB637UF-186372-A Table 12
Method reproducibility	Samples reanalyzed: 28 of 175 (16% of total) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100% ISR passes acceptance criteria	SGB637UF-186372-A Table 13
Study sample analysis/ stability	The maximum storage period between blood sampling collection and sample preparation was 12 days. Stability was demonstrated at a nominal -20°C for 38 days.	SGB637UF-186372-A Table 17
Standard calibration curve performance during accuracy and precision runs	See above.	

4.1.13. Validation 8395822, 8251-203, 8274044, 8395746, 8294126, 8280288, AND 8352-993

Appendix 28. Bioanalytical Method Life Cycle Information - Multiple Analytes and Validation Reports

	8251-203	8274044	8395822	8294126	8395746	8280288	8352993	ONT-380-012
Analyte	Rifampin	Digoxin	Gemfibrozil	Itraconazole Hydroxy-Itraconazole	Repaglinide	Midazolam 1'Hydroxymidazolam 4-Hydroxymidazolam	Tolbutamide Hydroxy Tolbutamide 4-Carboxy Tolbutamide	Digoxin, Gemfibrozil, Itraconazole, Midazolam, 1'-Hydroxymidazolam, Rifampin, Tolbutamide, Hydroxy Tolbuatmide, Repaglinide
Validation type	Full	Full	Full	Full	Full	Full	Full	In Study
eCTD reference number	NA	NA	NA	NA	NA	NA	NA	NA
Method ID	RIFHPC	DGNHPC	GEMFHPC	ITRHPC	REPGHPP	MHHHPC	TLBUHPC	All in row
Duration of time method is in use	07Aug2012-Present	25Apr2013-Present	13May2019-Present	10Feb2014-Present	15May2019-Present	07Jun2013-Present	07Mar2018-Present	26Oct2018-31Jan2019
Bioanalytical site	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

	8251-203	8274044	8395822	8294126	8395746	8280288	8352993	ONT-380-012
Matrix	K_2EDTA Plasma	K_2EDTA Plasma	K_2EDTA Plasma	K_2EDTA Plasma	K_2EDTA Plasma	K_2EDTA Plasma, Sodium Heparin plasma	K_2EDTA Plasma	K_2EDTA Plasma
Platform	HPLC/MS/MS method	HPLC/MS/MS method	HPLC/MS/MS method	HPLC/MS/MS method	HPLC/MS/MS method	HPLC/MS/MS method	HPLC/MS/MS method	
Format	Protein precipitation, proprietary to [redacted]	Liquid-liquid extraction, proprietary to [redacted]	Protein precipitation, proprietary to [redacted]	Solid-phase extraction, proprietary to [redacted]	Supported-liquid extraction.	Liquid-liquid extraction, proprietary to [redacted]	Protein precipitation, proprietary to [redacted]	Protein precipitation, proprietary to [redacted]
Stock reference Lot number Expiration date	Rifampin K0G365 current USP lot Rifabutin 030M4714V 04Aug2011 Rifampin SLBD2314V 31Aug2018 Rifabutin H0I151 current USP lot Rifampicin-d4 1182-093A3 31Mar2014	Digoxin P0I054 current USP lot Digoxin SLBF1625V May2015 Digoxin 2-KAA-135-1 05Nov2017 Digoxin O0B096 30Apr2012 Digoxin-d3 5-GBH-106-1 08Aug2014 Digoxin-d3 5-GBH-106-1 24Sep2017 Digoxin-d3	Gemfibrozil H1F285 current USP lot Gemfibrozil-d6 2-TMH-101-1 17Jan2022	Hydroxy-Itraconazole 3-NSR-67-1 24Mar2016 Hydroxy-Itraconazole 8-SXG-134 1 25Feb2017 Hydroxy-Itraconazole 24-GHZ-39-1 27Apr2017 Hydroxy-Itraconazole 1634-033A2 31May2017 Hydroxy-Itraconazole-d5 3-AXF-99-4 22Aug2015 Itraconazole	Repaglinide R038K0 NA Repaglinide-d5 5-WLI-14-1 31Jul2022	1'Hydroxymidazolam 065K3262 20Jun2007 1'Hydroxymidazolam 1400293 30Jun2012 1'Hydroxymidazolam 1385527 31Jan2014 1'Hydroxymidazolam FN062209-01 30Jun2014 1'Hydroxymidazolam FN05291302 30Jun2018 1'Hydroxymidazolam 636-063-1V Jul2015 1'Hydroxymidazolam-D_4 FN111308-01	4-Carboxy Tolbutamide 1032-067A1 11Jul2019 4-Carboxy Tolbutamide-d9 9-XJZ-183-1 24Mar2019 Hydroxy Tolbutamide 1-PSB-27-2 06Nov2020 Hydroxy Tolbutamide-d9 1-PSB-135-1 25Apr2021 Tolbutamide J1010A 09Oct2018 Tolbutamide-d9 21-SHG-17-1	Digoxin BCBR4893V 31Dec2019 Digoxin-d3 8-ZCA-105-1 05Jun2021 Gemfibrozil H1F285 current USP lot Gemfibrozil-d6 2-TMH-101-1 17Jan2022 Hydroxy Tolbutamide-d9 1-PSB-135-1 23Apr2023 1'Hydroxymidazolam FN09021601 31Dec2021 1'Hydroxymidazolam-d4 FN01091504

	8251-203	8274044	8395822	8294126	8395746	8280288	8352993	ONT-380-012
		HL-ALS-12-059-P7 06Mar2016 Digoxin-d3 8-ZCA-103-2 29Nov2017		G0K425 30Nov2015 Itraconazole 13-GHZ-186-1 15Mar2015 Itraconazole-d5 6-MMH-193-4 04Jun2015 Itraconazole-d5 6-MMH-193-4 06Dec2019		30Nov2013 1'Hydroxymidazolam-D_4 FE093012-06 30Nov2017 1'Hydroxymidazolam-D_4 FN01091504 03Mar2020 1'Hydroxymidazolam-D_4 FE113012-03 Apr2018 1'Hydroxymidazolam-D_4 FN01091504 31Mar2010 4-Hydroxymidazolam 281-082 15Apr2007 4-Hydroxymidazolam 1422422 31Dec2012 4-Hydroxymidazolam 282-259V 30Jun2016 4-Hydroxymidazolam 281-259V 28Feb2017	30Apr2017	31Mar2020 4-Hydroxy Tolbutamide (Hydroxy Tolbutamide) 1-PSB-27-2 06Nov2022 Itraconazole 13-GHZ-186-1 24Sep2019 Itraconazole-d5 6-MMH-193-4 06Dec2019 Midazolam FE01221602 30Apr2021 Midazolam-d4 FE01271501 29Feb2020 Repaglinide R038K0 current USP lot Repaglinide-d5 5-WLI-14-1 31Jul2022 Rifampin SLBD2314V 31Aug2018 Rifampicin-d4 1182-093A3

	8251-203	8274044	8395822	8294126	8395746	8280288	8352993	ONT-380-012
						4-Hydroxymidazolam 282-459V Jun2016 4-Hydroxymidazolam 51152 22Apr2011 4-Hydroxymidazolam BCBK812IV 28Feb2017 Midazolam 116K4051 09Mar2008 Midazolam 039K4060 31Aug2010 Midazolam FE100110-01 31Oct2015 Midazolam FE042209-01 30Apr2014 Midazolam FE11111402 30Nov2019 Midazolam 059K3350 NA		26Apr2020 Tolbutamide 0500043 10Apr2019 Tolbutamide-d9 21-SHG-17-1 23Nov2023 Tucatinib 6451-P54/17-001 14Nov2019 ONT-993 ES6156-23-P1 30Nov2018 ONT-380-d6 AR00442572-03-R 30Nov2018 ONT-993-d4 ES6139-19 30Nov2018

	8251-203	8274044	8395822	8294126	8395746	8280288	8352993	ONT-380-012
						Midazolam-D_4 FN092209-01 30Sep2014 Midazolam-D_4 FE112110-01 30Nov2015 Midazolam-D_4 FE113012-04 31Dec2017 Midazolam-D_4 FE05131301 May2018 Midazolam-D_4 FE0121501 29Feb2020 Midazolam-D_4 FE01271501 29Feb2020		
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	Rifampin: 50.0 to 35,000 ng/mL	Digoxin: 0.0500 to 10.0 ng/mL	Gemfibrozil: 50.0 to 25000 ng/mL	Itraconazole, hydroxy-itraconazole: 2.00 to 1000 ng/mL	Repaglinide: 0.100 to 100 ng/mL	Midazolam, 1'-Hydroxymidazolam, 4-Hydroxymidazolam: 0.100 to 100 ng/mL	Tolbutamide 0.100 to 100 µg/mL 4-Carboxy Tolbutamide, Hydroxy Tolbutamide: 0.0250 to 5.00 µg/mL	Calibration curve ranges identical between validation and sample testing, see left.
Matrix study population	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

	8251-203	8274044	8395822	8294126	8395746	8280288	8352993	ONT-380-012
Link to reports and applicable amendments	8251-203	8274044	8395822	8294126	8395746	8280288	8352993	8373070
Synopsis of amendment history	8251-203 Addendum 1 Transfer of method to [redacted] Addendum 3: Qualification of Use of ISTD 8251-203 Addendum 3: Selectivity Carryover Matrix factor Freeze/Thaw Stability Bench-Top Stability Processed sample stability Hemolysis Lipemia	8274044 Addendum 1: Lipemia Addendum 2: Selectivity Matrix Factor Carryover Accuracy, Precision Recovery 8274044 Addendum 5: Bench-Top Stability Matrix Frozen Stability	NA	8294126 Addendum 1: Hemolysis 8294126 Addendum 2: Matrix Frozen Stability Selectivity Matrix Factor Carryover Accuracy, Precision Recovery Processed Sample Stability 8294126 Addendum 3: Selectivity Matrix Factor Hemolysis	NA	8280288 Addendum 1: Matrix Frozen Stability 8280288 Addendum 2: Solution Stability 8280288 Addendum 3: Hyperlipidemia	NA	NA

Appendix 29. Summary Method Performance -8251-203 Rifampin

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: 8251-203, 8251-203 Addendum 1 and 8251-203 Addendum 3		
Method description	Following protein precipitation, an aliquot of the extract will be injected onto an HPLC/MS/MS triple quadrupole mass spectrometer. The peak area of the product ion of the analyte will be measured against the peak area of the product ion of the internal standard.		
Materials used for standard calibration curve and concentration	Rifampin: 50.0, 100, 500, 1000, 5000, 10000, 20000, 30000, 35000 ng/mL		
Validated assay range	Rifampin: 50.0 to 35000 ng/mL		
Material used for quality controls (QCs) and concentration	Rifampin: 150, 2000, 28000 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Linear regression, $1/x^2$ weighting		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	9	8251-203 Table 7.5
	Cumulative accuracy (%bias[a]) from LLOQ to ULOQ	-2.3% to 4.0%	8251-203 Table 7.5
	Cumulative precision (%CV[b]) from LLOQ to ULOQ	≤7.3%	8251-203 Table 7.5
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	-0.4% to 6.5%	8251-203 Table 7.6
	Inter-batch (%CV) QCs	≤6.7%	8251-203 Table 7.6
	Total Error (TE)	13.2%	Sum of largest bias and CV reported.

Selectivity & matrix effect	Selectivity 6 lots tested. Rifampin and rifabutin regions were free from significant interference (<20.0% of the response from the single LLOQ used and <5.0% of internal standard response in the control zero sample). %Bias -2.0%; %CV 3.1 Batch blank matrix samples, 1 reagent blank, 1 blank matrix sample spiked with ISTD only (control zero), and 1 blank matrix sample spiked with rifampin alone at 35000 ng/mL and without ISTD were analyzed. In all cases, the rifampin and rifabutin regions were free from significant interference (<20.0% of the response from the single LLOQ used and <5.0% of internal standard response in the control zero sample). Aliquots of these individuals were spiked at the LQC concentration of rifampin and with internal standard. The accuracy of each sample was within the range of ±15.0%bias. Selectivity with Rifampicin-d4 as Internal Standard There were no interference peaks (>20% of the LLOQ calibration standard response) detected in the six individual blank matrix samples or the validation run blank samples at the retention time of rifampin. There were no interference peaks detected at the ISTD retention time (>5% of the internal standard peak response in the control zero sample) for the six individual blank matrix samples or validation run blank samples. Qualification using Rifampicin-d4 To evaluate selectivity for the run, one blank matrix sample spiked with rifampin alone at the ULOQ without ISTD was analyzed. The sample demonstrated a lack of significant interference in the chromatographic region of interest for the ISTD (≤5.0% of ISTD response in the control zero sample). %Bias within 3.4%; %CV within 3.8%	8251-203 Table 7.2 8251-203 Addendum 3 Table 7.2 8251-203 Addendum 3 Table 7.18

Selectivity & matrix effect (continued)	Run Blanks and Interference of Analyte on other Analytes or Internal Standard The extracts of blank matrix demonstrated a lack of significant carryover in the chromatographic regions of interest for rifampin (<20.0% of the response from the single LLOQ used) and rifabutin (<5.0% of the internal standard response in the control zero sample). LLOQ for rifampin in human plasma was at least 5 times greater than any response observed for the blank matrix. In this study, the LLOQ results met these criteria, and it was therefore concluded that the method demonstrated acceptable sensitivity. %Bias 5.0%; %CV 6.7 Matrix Factor 6 lots tested Internal standard normalized matrix factor The precision of the 6 individual matrix factor values for the/each analyte and the/each internal standard, as well as the internal standard normalized matrix factor values was <15.0% RSD, thus the precision of the matrix factor evaluation met the acceptance criteria and the matrix. mean: 1.02; %RSD ≤ 4.3% The matrix effect did not significantly impact assay performance. Matrix Factor With Rifampin-d4 as the Internal Standard The precision of the internal standard normalized matrix factor values must be <15.0% RSD. The precision of the matrix factor evaluation met the acceptance criteria and the matrix factor was not considered to impact assay performance. LQC mean: 1.04; %CV 2.5% HQC mean: 0.994; %CV 1.7%	8251-203 Table 7.9 8251-203 Table 7.3 8251-203 Addendum 3 Table 7.3
Interference & specificity	Specificity in the presence of Tucatinib and ONT-993 was assessed during sample analysis. Rifampin in the presence of Tucatinib and ONT-993 %Bias ≤ 10.7%; n=1	8373070 Table 8.97
Hemolysis effect	One lot tested. 2% hemolyzed red blood cells LQC: %bias 2.0%; %CV 4.9% HQC: %bias 3.9%; %CV 2.6%	8251-203 Addendum 3 Table 7.15
Lipemic effect	One lot tested high lipid content (≥300 mg/dL triglyceride) LQC: %bias -0.7%; %CV 2.3% HQC: %bias 2.5%; %CV 0.9%	8251-203 Addendum 3 Table 7.16
Dilution linearity & hook effect	NA	NA

Bench-top/process stability	Processed Sample Stability: 2ºC to 8°C: 136 hours Stability Using Rifampicin-d4 as Internal Standard Standard Solution Stability Intermediate standard solutions at room temperature: 6 hours % difference between the compared standard solutions at room temperature was ≤5.0% Intermediate standard solutions at 2°C to 8°C: 7 days % difference between the compared standard solutions was standard solutions at 2°C to 8°C was ≤10.0%. Processed Sample Stability: 2ºC to 8°C: 98 hours %Bias within 10.7%; %CV within 3.3% Sample Collection Stability in Whole Blood Room temperature or wet ice/whole blood samples: 2 hours %Bias within 4.7% Room Temperature Matrix Stability Room temperature: 24 hours. %Bias within 4.3%, %CV within 3.7%	8251-203 Table 7.15 8251-203 Addendum 3 Table 7.9 Table 7.10 Table 7.11 Table 7.12 Table 7.14
Freeze-Thaw stability	3 freeze-thaw cycles at -10°C to -30°C and -60°C to -80°C -10 to -30°C %bias within 5.0%; %CV within 4.0% -60 to -80°C %bias within 4.6%; %CV within 5.1%	8251-203 Addendum 3 Table 7.13
Long-term storage	Rifampin in human plasma is stable for 1886 days at -60 to -80°C This was determined elsewhere ([redacted]2100-365).	
Parallelism	NA	NA
Carry over	There was no significant in the original method. No evidence of carryover (>20% of the LLOQ calibration standard response) to the blank matrix extracts injected immediately after the 2 highest calibration standards except in Runs 4, 10, and 14 where interference peaks were noted in some of the carryover samples. At most, carryover was calculated at approximately 30% of the LLOQ. The impact of the carryover in Runs 4, 10, and 14 was considered negligible and had no impact on the integrity of the data in these runs.	8251-203 Addendum 3 Section 4.2.3

Method performance in study ONT-380-012 Determination of Digoxin, Itraconazole, Rifampin, Tolbutamide, Hydroxy Tolbutamide, Gemfibrozil, Midazolam, 1'-Hydroxymidazolam, ONT-380, ONT-993, and Repaglinide in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-012 Report 8373070 *Please note Appendix 29 through Appendix 35 are part of the same Study and Bioavailability Report*		
Assay passing rate	2 batches collected and accepted.	8373070 Table 8.1
Standard curve performance	Cumulative %Bias -1.7% to 2.0%	8373070 Table 8.20
QC performance	Cumulative %Bias -3.3% to -1.0% Cumulative %RSD ≤3.2%	8373070 Table 8.42
Method reproducibility	NA	NA
Study sample analysis/ stability	The presence of Tucatinib and ONT-993 does not affect the quantification of Rifampin. The blank samples spiked Rifampin did not contain any significant peaks at the retention time of Tucatinib an ONT-993 or ISTD. The blank samples spiked with ONT-380 and ONT-993 did not contain any significant peaks at the retention time of Rifampin or ISTD. Rifampin in human plasma is stable for 1886 days at -60 to -80°C This was determined elsewhere ([redacted] 2100-365). See 8251-203 Addendum 1.	8373070 Table 8.71 Table 8.97
Standard calibration curve performance during accuracy and precision runs	See above	

a Bias is used interchangeably with RE. Validation reports report RE.
b CV is used interchangeably with RSD. Validation reports report RSD.

Appendix 30. Summary Method Performance -8274044 Digoxin

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: 8274044, 8274044 Addendum 1, Addendum 2, and Addendum 5		
Method description	Method details are proprietary to [redacted]. Following liquid-liquid extraction of an aliquot of K_2EDTA plasma, the extract is injected into an HPLC-API5500 triple quadrupole mass spectrometer. The peak area of the product ion of digoxin is measured against the peak area of the product ion of the internal standard, digoxin-d_3		
Materials used for standard calibration curve and concentration	Digoxin: 0.0500, 0.100, 0.250, 1.00, 2.50, 5.00, 8.00, 10.0 ng/mL		
Validated assay range	0.0500 to 10.0 ng/mL		
Material used for quality controls (QCs) and concentration	0.150, 3.00, 7.50 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Linear regression, $1/x^2$ weighting		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	8274044 Table 7.5
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.0% to 6.0%	8274044 Table 7.5
	Cumulative precision (%CV[a]) from LLOQ to ULOQ	≤4.8%	8274044 Table 7.5
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	0.1% to 7.4%	8274044 Table 7.6
	Inter-batch (%CV) QCs	≤7.1%	8274044 Table 7.6

	Total Error (TE)	14.5%	Sum of largest bias and CV reported.
Selectivity & matrix effect	Selectivity The data met acceptance criteria for lack of significant interference. 6 lots tested, %Bias 0.0% to 6.0% In all cases, the digoxin and digoxin-d3 regions were free from significant interference (≤20.0% of the response from the single LLOQ used and ≤5.0% of internal standard response in the control zero sample). Matrix Factor 6 lots tested Internal standard normalized matrix factor LQC mean 1.03, %CV 9.9% HQC mean 1.03, %CV 4.4%		8274044 Table 7.2 Table 7.3
Interference & specificity	Specificity in the presence of Tucatinib and ONT-993 was assessed during sample analysis. Digoxin in the presence of Tucatinib %Bias ≤ 2.7%[b]; %CV ≤4.8% Digoxin in the presence of ONT-993 %Bias ≤ 5.0%[b]; %CV ≤2.2%		8373070 Table 8.81 Table 8.82
Hemolysis effect	One lot tested:2% lysed whole blood %Bias 4.7%, %CV 1.8%		8274044 Table 7.16
Lipemic effect	One lot tested: One value at the HQC level was confirmed as a Grubbs Test outlier, the value is excluded from the statistical analysis. LQC %Bias 0.7%, %CV 2.9% HQC %Bias -5.5%, %CV 2.0%		8274044 Addendum 1 Table 7.6
Dilution linearity & hook effect	NA		NA
Bench-top/process stability	Bench-top stability 24 hours at room temperature established in [redacted] 2100-863 Processed sample stability 98 hours at 2°C to 8°C %Bias -2.0% to 3.1%; %CV ≤4.4%		8274044 Table 7.14
Freeze-Thaw stability	Freeze-Thaw stability established in [redacted] 2100-863 10 cycles -10°C to -30°C and -60°C to -80°C.		8274044 Report Summary

Long-term storage	-10°C to -30°C 1092 days LQC: %Bias -4.7%; %CV ≤4.6% HQC: %Bias -3.5%; %CV ≤1.5% -60°C to -80°C: 490 days LQC: %Bias -4.7%; %CV ≤6.9% HQC: %Bias -4.0%; %CV ≤2.8%	8274044 Addendum 5 Table 6.6
Parallelism	NA	NA
Carry over	There was no significant carryover. There were no interference peaks detected at the ISTD retention time (>5% of the ISTD peak response in the control zero sample) in the carryover blank samples.	8274044 Section 4.5 Addendum 2 Section 4.4
Method performance in study ONT-380-012 Determination of Digoxin, Itraconazole, Rifampin, Tolbutamide, Hydroxy Tolbutamide, Gemfibrozil, Midazolam, 1'-Hydroxymidazolam, ONT-380, ONT-993, and Repaglinide in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-012 Report 8373070 *Please note Appendix 29 through Appendix 35 are part of the same Study and Bioavailability Report*		
Assay passing rate	A total of 9 batches were collected and accepted.	8373070 Table 8.1
Standard curve performance	Cumulative %Bias: -1.9% to 2.4% Cumulative %CV: ≤5.0%	8373070 Table 8.15
QC performance	Cumulative %Bias: -0.3% to 1.3% Cumulative %CV: ≤9.9%	8373070 Table 8.37
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 57 of 503 (11%) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 96.5%	8373070 Table 8.53
Study sample analysis/ stability	All samples were analyzed within the validated stability range.	8373070
Standard calibration curve performance during accuracy and precision runs	See above	

a CV is used interchangeably with RSD. Validation and sample analysis reports report RSD.
b Error in report 8373070 for digoxin QC concentrations in the presence of tucatinib and ONT-993, digoxin concentration should be 0.15 ng/mL, and not 1.5 ng/mL as used in calculation of %Bias.

Appendix 31. Summary Method Performance -8395822 Gemfibrozil

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: 8395822 Method: GEMFHPC (Proprietary to [redacted])		
Method description	Method details are proprietary to [redacted] Following protein precipitation of a 50 µL aliquot of K_2EDTA plasma, the extract is injected into an HPLC/MS/MS triple quadrupole mass spectrometer. The peak area of the product ion of gemfibrozil is measured against the peak area of the product ion of the internal standard, gemfibrozil-d_6		
Materials used for standard calibration curve and concentration	Gemfibrozil 50.0, 100, 400, 1000, 4000, 12500, 22500, 25000 ng/mL		
Validated assay range	50.0 to 25000 ng/mL		
Material used for quality controls (QCs) and concentration	150, 1250, 10000, 20000 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Linear regression, $1/x^2$		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	8395822 Table 6.2 Table 6.3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.4% to 3.0%	8395822 Table 6.2
	Cumulative precision (%CV[a]) from LLOQ to ULOQ	≤5.2%	8395822 Table 6.2
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	-3.0% to 2.0%	8395822 Table 6.4
	Inter-batch (%CV) QCs	≤5.8%	8395822 Table 6.4

	Total Error (TE)	8.8%	Sum of largest bias and CV reported.
Selectivity & matrix effect	Matrix selectivity and Matrix effect The data met acceptance criteria for lack of significant interference. 6 lots tested, %Bias -5.4%, %CV 6.7% Matrix factor Six lots tested Internal standard normalized matrix factor LQC mean 0.932, %CV 4.5% HQC mean 1.03, %CV 2.7%		8395822 Table 6.7 Table 6.8
Interference & specificity	Specificity in the presence of Tucatinib and ONT-993 was assessed during sample analysis. Gemfibrozil in the presence of Tucatinib %Bias ≤ 1.3%; %CV ≤3.6% Gemfibrozil in the presence of ONT-993 %Bias ≤ 7.3%; %CV ≤2.0%		8373070 Table 8.83 Table 8.84
Hemolysis effect	One lot tested:2% lysed whole blood LQC %Bias 2.7%, %CV 1.9% HQC %Bias 1.0%, %CV 0.5%		8395822 Table 6.10
Lipemic effect	One lot tested: natural high lipid content ≥300 mg/dL LQC %Bias 0.0%, %CV 3.1% HQC %Bias 0.0%, %CV 1.5%		8395822 Table 6.11
Dilution linearity & hook effect	NA		NA
Bench-top/process stability	Bench-top matrix stability 24 hours at room temperature %Bias -4.7% to -1.0%; %CV ≤2.2% Processed sample stability 190 hours at 2°C to 8°C %Bias -3.0% to 0.7%; %CV ≤2.6%		8395822 Table 6.15 Table 6.18
Freeze-Thaw stability	5 cycles -10°C to -30°C: %Bias -1.0% to -0.7%; %CV ≤3.3% -60°C to -80°C: %Bias -1.3% to -1.0%; %CV ≤4.2%		8395822 Table 6.16
Long-term storage	167 days -10°C to -30°C: %Bias -3.5% to 3.3%; %CV ≤2.0% -60°C to -80°C: %Bias -2.0% to 6.0%; %CV ≤1.9%		8395822 Table 6.17

Parallelism	NA	NA
Carry over	There was no evidence of carryover within the chromatographic regions of the analyte and ISTD.	NA
Method performance in study ONT-380-012 Determination of Digoxin, Itraconazole, Rifampin, Tolbutamide, Hydroxy Tolbutamide, Gemfibrozil, Midazolam, 1'-Hydroxymidazolam, ONT-380, ONT-993, and Repaglinide in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-012 Report 8373070 *Please note Appendix 29 through Appendix 35 are part of the same Study and Bioavailability Report*		
Assay passing rate	A total of 4 batches were collected. At total of 0 batches were rejected.	8373070 Table 8.1
Standard curve performance	Cumulative %Bias: -6.0% to 5.5% Cumulative %CV: ≤4.4%	8373070 Table 8.16
QC performance	Cumulative %Bias: -6.5% to 1.3% Cumulative %CV: ≤3.1%	8373070 Table 8.38
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 24 of 83 (28.9%) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100%	8373070 Table 8.54
Study sample analysis/ stability	All samples were analyzed within the validated stability range.	8373070
Standard calibration curve performance during accuracy and precision runs	See above	

a CV is used interchangeably with RSD. Validation and sample analysis reports report RSD.

Appendix 32. Summary Method Performance -8294126 Itraconazole, Hydroxy Itraconazole

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: 8294126, 8294126 Addendum 1, Addendum 2, and Addendum 3		
Method description	Method details are proprietary to [redacted] Following solid phase extraction of an aliquot of K_2EDTA plasma, the extract is injected into an HPLC/MS/MS triple quadrupole mass spectrometer. The peak areas of the product ion of itraconazole and hydroxy-itraconazole are measured against the peak areas of the product ions of the respective internal standards, itraconazole-d_5 and hydroxy-itraconazole-d_5.		
Materials used for standard calibration curve and concentration	Itraconazole and hydroxy-itraconazole 2.00, 5.00, 10.0, 50.0, 100, 200, 500, 900, 1000 ng/mL		
Validated assay range	2.00 to 1000 ng/mL		
Material used for quality controls (QCs) and concentration	2.00, 6.00, 150, 750, 5000 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Weighted (1/x) linear least-squares regression		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	9	8294126 Table 7.2 Table 7.3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Itraconazole: -5.2% to 6.0% Hydroxy-Itraconazole: -9.0% to 20.6%	8294126 Table 7.2 Table 7.3
	Cumulative precision (%CV[a]) from LLOQ to ULOQ	NA	
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	Itraconazole: -5.3% to 13.5% Hydroxy-Itraconazole: -6.7% to 14.5%	8294126 Table 7.2 Table 7.3
	Inter-batch (%CV) QCs	Itraconazole: ≤10.3% Hydroxy-Itraconazole : ≤8.5%	8294126 Table 7.2 Table 7.3

	Total Error (TE)	Itraconazole: 23.8% Hydroxy-Itraconazole : 23.0%	Sum of highest reported bias and CV
Selectivity & matrix effect	<u>Selectivity</u> 6 lots tested. There were no interference peaks (>20.0% of the LLOQ calibration standard response) detected in the six individual blank matrix samples or the validation run blank samples at the retention time of itraconazole and hydroxy-itraconazole. The mean accuracy of each of the 6 spiked individual blank matrix samples was within the acceptance criteria. Itraconazole: mean %bias within 5.2% Hydroxy-Itraconazole: mean %bias within 10% <u>Matrix Factor</u> 6 lots tested. The precision of the matrix factor evaluation met the acceptance criteria. Itraconazole: %CV within 3.1% Hydroxy-Itraconazole: %CV within 6.5%		8294126 Addendum 2 Section 5.2 Figures 10.1-10.6 Table 8.2 Table 8.3 Table 8.4 Table 8.5
Interference & specificity	Specificity for Itraconazole in the presence of Tucatinib and ONT-993 was assessed during sample analysis. The blank samples spiked with ONT-380 and ONT-993 did not contain any significant peaks at the retention time of Itraconazole or ISTD. Itraconazole in the presence of Tucatinib %Bias ≤ 4.2%; %CV ≤4.7% Itraconazole in the presence of ONT-993 %Bias ≤ 3.7%; %CV ≤10%		8373070 Table 8.85 Table 8.86
Hemolysis effect	One lot tested. Hemolyzed red blood cells. Itraconazole: LQC: %bias -7.0%; %CV 4.4% HQC: %bias 2.0%; %CV 2.8% Hydroxy-Itraconazole: LQC: %bias -7.2%; %CV 6.6% HQC: %bias 2.0%; %CV 3.1% Confirmed in Addendum 2 and Addendum 3		8294126 Addendum 1 Table 6.8 Table 6.9 8294126 Addendum 2 Table 8.18 Table 8.19 8294126 Addendum 3 Table 6.12 Table 6.13

Lipemic effect	One lot tested. Naturally high lipid content. Itraconazole: LQC: %bias -0.3%; %CV 6.9% HQC: %bias -2.9%; %CV 2.9% Hydroxy-Itraconazole: LQC: %bias 0.8%; %CV 4.7% HQC: %bias 1.1%; %CV 2.7%	8294126 Addendum 2 Table 8.20 Table 8.21
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	Stability confirmed for 121 hours at 2°C to 8°C. Itraconazole: %bias within -10.5%; %CV within 4.0% Hydroxy-Itraconazole: %bias within -4.8%; %CV within 6.8%	8294126 Addendum 2 Table 8.16 Table 8.17
Freeze-Thaw stability	4 freeze/thaw cycles at -10°C to -30°C Itraconazole: %bias within -6.1%; %CV within 6.7% Hydroxy-Itraconazole: %bias within -9.3%; %CV within 5.9% 6 freeze/Thaw Cycles - 60°C to -80°C Itraconazole: %bias within -5.9%; %CV within 9.0% Hydroxy-Itraconazole: %bias within -0.9%; %CV within 6.2%	8294126 Addendum 2 Table 7.8 Table 7.9
Long-term storage	168 days at -60°C to -80°C Itraconazole: %bias within -4.8%; %CV within 4.9% Hydroxy-Itraconazole: %bias within -8.7%; %CV within 10.3%	8294126 Addendum 2 Table 7.10 Table 7.11
Parallelism	NA	NA
Carry over	There was no evidence of carryover at the itraconazole and hydroxy-itraconazole retention time (>20.0% of the LLOQ calibration standard response) to the blank matrix extracts injected immediately after the two highest calibration standards. There was no evidence of carryover at the ISTD retention time (>5.0% of the internal standard peak response in the control zero sample) of the ISTD peak area for the matrix blanks.	8294126 Addendum 2 Section 5.4
Method performance in study ONT-380-012 Determination of Digoxin, Itraconazole, Rifampin, Tolbutamide, Hydroxy Tolbutamide, Gemfibrozil, Midazolam, 1'-Hydroxymidazolam, ONT-380, ONT-993, and Repaglinide in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-012 Report 8373070 *Please note Appendix 29 through Appendix 35 are part of the same Study and Bioavailability Report*		

Assay passing rate	4 batches collected, 3 accepted.	8373070 Table 8.1
Standard curve performance	Cumulative %Bias -1.5% to 3.3%	8373070 Table 8.17
QC performance	Cumulative %Bias 2.0% to 6.0% Cumulative %CV ≤12.6%	8373070 Table 8.39
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 24 (29.6%, Itraconazole) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100% (Itraconazole)	8373070 Table 8.55
Study sample analysis/ stability	Samples were analyzed within established stability for itraconazole. Itraconazole in human plasma 168 days at -60°C to -80°C Itraconazole: %bias within -5.7%; %CV within 4.9%	8373070 Table 8.61 Table 8.70 Section 4.5
Standard calibration curve performance during accuracy and precision runs	See above	

a CV is used interchangeably with RSD. Validation and sample analysis reports report RSD.

Appendix 33. Summary Method Performance -8395746 Repaglinide

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: 8395746		
Method description	Following supported-liquid extraction of a 50 µL aliquot of K_2EDTA plasma, the extract is injected into Zorbax SB-C18, 50 × 2.1 mm, 5 µm HPLC column and analyzed using a Sciex API 4500 mass spectrometer. The peak area of the product ion of repaglinide is measured against the peak area of the product ion of the internal standard, repaglinide-d_5.		
Materials used for standard calibration curve and concentration	Repaglinide: 0.100, 0.200, 1.00, 5.00, 30.0, 60.0, 90.0, 100 ng/mL		
Validated assay range	0.100 to 100 ng/mL		
Material used for quality controls (QCs) and concentration	0.300, 4.00, 50.0, 80.0 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Linear regression, $1/x^2$ weighting		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	8395746 Table 6.2
	Cumulative accuracy (%bias[a]) from LLOQ to ULOQ	-1.4% to 3.0%	8395746 Table 6.2
	Cumulative precision (%CV[b]) from LLOQ to ULOQ	≤3.6%	8395746 Table 6.2
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	-2.0% to 6.5%	8395746 Table 6.4
	Inter-batch (%CV) QCs	≤4.7%	8395746 Table 6.4
	Total Error (TE)	-11.2%	Sum of largest bias and CV

Selectivity & matrix effect	Matrix selectivity and Matrix effect 6 lots tested Bias -1.6%, CV 3.5% Matrix factor Six lots were tested Internal standard normalized matrix factor LQC mean 0.981, CV 2.3% HQC mean 1.00, CV 1.4%	8395746 Table 6.13 Table 6-14
Interference & specificity	The sample extracts lacked significant interference, thus met acceptable criteria for blanks spiked with the analyte individually at the ULOQ without ISTD, blank matrix with ISTD, and blank matrix and reagent blank. Interference co medications Tucatinib 3000 ng/mL: LQC bias 5.7%, RSD 3.4% ONT-993 300 ng/mL: LQC bias 5.0%, RSD 1.6% Midazolam 20.0 ng/mL: LQC bias 3.3%, RSD 3.6% 1-hydroxymidazolam 15.0 ng/mL: LQC bias 5.3%, RSD 3.0% Tolbutamide 50000 ng/mL: LQC bias 4.3%, RSD 3.3% 4-hydroxytolbutamide 600 ng/mL: LQC bias 5.7%, RSD 1.5%	8395746 Table 6-7 Table 6-8 Table 6-9 Table 6-10 Table 6-11 Table 6-12
Hemolysis effect	One lot tested, containing 2% lysed whole blood LQC Bias -1.7%; %CV: 2.8 HQC Bias -1.6%; %CV: 1.7	8395746 Table 6.16
Lipemic effect	One lot tested: matrix with a natural high lipid content ≥300 mg/dL triglyceride level LQC Bias -1.3%; %CV: 2.0 HQC Bias -1.8%; %CV: 1.6	8395746 Table 6.17
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	24 hours at room temperature LQC Bias -2.7%; %CV: 2.9 HQC Bias -1.3%; %CV: 1.5	8395746 Table 6.28
Freeze-Thaw stability	5 freeze-thaw cycles -10°C to -30°C: %Bias: ≤3.7; %CV: ≤2.1 -60°C to -80°C: %Bias: -≤3.1; %CV: ≤2.7	8395746 Table 6.29
Long-term storage	78 days -10°C to -30°C: %Bias: 2.6; %CV: ≤3.0 -60°C to -80°C: %Bias: 1.9; %CV: ≤3.0	8395746 Table 6.30
Parallelism	NA	NA

Carry over	There was no evidence of carryover within the chromatographic regions of the analyte and ISTD, with the exception of runs 5 and 10. In analytical runs 3, 5, and 10, carryover >20.0% of the peak area of the mean utilized LLOQ was observed. The potential peak area contribution from carryover was reviewed by the Responsible Scientist. The review confirmed that the carryover observed was not significant and did not affect the integrity of the data generated.	8395746 Section 4.9
Method performance in study ONT-380-012 Determination of Digoxin, Itraconazole, Rifampin, Tolbutamide, Hydroxy Tolbutamide, Gemfibrozil, Midazolam, 1'-Hydroxymidazolam, ONT-380, ONT-993, and Repaglinide in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-012 Report 8373070 *Please note Appendix 29 through Appendix 35 are part of the same Study and Bioavailability Report*		
Assay passing rate	7 batches collected and accepted.	8373070 Table 8.1
Standard curve performance	Cumulative %Bias -7.5% to 4.0% Cumulative %CV ≤3.2%	8373070 Table 8.23
QC performance	Cumulative %Bias -6.0% to 1.4% Cumulative %CV ≤2.6%	8373070 Table 8.45
Method reproducibility	ISR passed acceptance criteria. Samples reanalyzed (% total analyzed): 40 (11.0%, Repaglinide) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100% (Repaglinide)	8373070 Table 8.60
Study sample analysis/ stability	Samples were analyzed within established stability.	8373070 page 18
Standard calibration curve performance during accuracy and precision runs	See above	

a Bias is reported in table for uniformity, source report may use bias or accuracy.
b CV is used interchangeably with RSD. Validation and sample analysis reports report RSD.

Appendix 34. Summary Method Performance -8280288 - Midazolam, 1 Hydroxymidazolam, 4' Hydroxymidazolam

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: 8280288, 8280288 Addendum 1, Addendum 2, and Addendum 3		
Method description	Method details are proprietary to [redacted]. Following liquid-liquid extraction of an aliquot of K_2EDTA plasma, the extract is injected into an HPLC-API5500 triple quadrupole mass spectrometer. The peak area of the product ion of Midazolam, 1-Hydroxymidazolam, and 4' Hydroxymidazolam are measured against the peak areas of the product ions of the internal standards Midazolam-D4 and 1'-Hydroxymidazolam-D4.		
Materials used for standard calibration curve and concentration	Midazolam, 1'-Hydroxymidazolam, 4-Hydroxymidazolam: 0.100, 0.200, 0.500, 2.00, 10.0, 40.0, 80.0, 100 ng/mL		
Validated assay range	Midazolam, 1'-Hydroxymidazolam, 4-Hydroxymidazolam: 0.100 to 100 ng/mL		
Material used for quality controls (QCs) and concentration	Midazolam, 1'-Hydroxymidazolam, 4-Hydroxymidazolam: 0.100, 0.300, 5.00, 75.0, 200 ng/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Midazolam, 1'-Hydroxymidazolam, 4-Hydroxymidazolam: weighted ($1/x^2$) linear least squares regression		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	8280288 Addendum 2 Table 6.2 Table 6.7 Table 6.12
	Cumulative accuracy (%bias) from LLOQ to ULOQ	Midazolam: -5.0% to 2.5% 1'-Hydroxymidazolam: -4.8 % to 4.0% 4-Hydroxymidazolam: -2.8 % to 4.5%	8280288 Table 8.13 Table 8.14 Table 8.15
	Cumulative precision (%CV[a]) from LLOQ to ULOQ	Midazolam: ≤4.3% 1'-Hydroxymidazolam: ≤8.3% 4-Hydroxymidazolam: ≤4.8%	8280288 Table 8.13 Table 8.14 Table 8.15

Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	Midazolam: 1.5% to 7.0 % 1'-Hydroxymidazolam: 1.7% to 8.5% 4-Hydroxymidazolam: -4.7 % to 5.0%	8280288 Table 8.16 Table 8.17 Table 8.18
	Inter-batch (%CV) QCs	Midazolam: ≤5.9% 1'-Hydroxymidazolam: ≤12.4% 4-Hydroxymidazolam: ≤12.4%	8280288 Table 8.16 Table 8.17 Table 8.18
	Total Error (TE)	Midazolam: 12.9% 1'-Hydroxymidazolam: 20.9% 4-Hydroxymidazolam: 17.4%	Sum of the largest bias and CV
Selectivity & matrix effect	Run Blanks and Interference of Analyte on other Analytes or Internal Standard No significant interference (≤20.0% of the response from the single LLOQ used and ≤5.0% of internal standard response in the control zero sample) in the chromatographic regions of the ISTD and other non-spiked analytes. With the exception of 1'-hydroxymidazolam in Run ID 2 where a peak was observed in the first carryover blank which was >20.0 of the LLOQ. Selectivity 6 lots tested. The method demonstrated acceptable selectivity. No significant interference (≤20.0% of the response from the single LLOQ used and ≤5.0% of internal standard response in the control zero sample). Matrix Factor The precision of the matrix factor evaluation met the acceptance criteria and the matrix factor was not considered to impact assay performance. Midazolam: LQC %CV 2.2%; HQC %CV 2.1 1'-Hydroxymidazolam: LQC %CV 3.8%; HQC %CV 1.0 4-Hydroxymidazolam: LQC %CV 3.3%; HQC %CV 1.2		8280288 Table 8.4 Table 8.5 Table 8.6
Interference & specificity	Specificity/interference in the presence of Tucatinib, ONT-993, Repaglinide, Tolbutamide, 4-hydroxy tolbutamide, was assessed during sample analysis. QCs of midazolam and 1'-hydroxymidazolam all were within acceptance criteria of %bias and %CV during the interference testing.		8373070 Table 8.87 to 8.96

Hemolysis effect	One lot tested. 2% lysed whole blood in K_2EDTA Midazolam: %Bias 0.7%; %CV 3.2% 1'-Hydroxymidazolam: %Bias 7.7%; %CV 3.6% 4-Hydroxymidazolam: %Bias 1.0%; %CV 3.3%	8280288 Table 8.54 Table 8.55 Table 8.56
Lipemic effect	One lot tested. One lot of matrix with a natural high lipid content ≥300 mg/dL triglyceride level. Midazolam: LQC Mean %Bias within 6.7%; %CV within 8.5% HQC Mean %Bias within 6.1%; %CV within 4.4% 1'-Hydroxymidazolam: LQC Mean %Bias within 2.0%; %CV within 7.8% HQC Mean %Bias within 1.7%; %CV within 3.8% 4-Hydroxymidazolam: LQC Mean %Bias within 9.3%; %CV within 4.5% HQC Mean %Bias within 5.5%; %CV within 4.6%	8280288 Addendum 3 Table 6.11 Table 6.12 Table 6.13
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	Processed Sample Stability Stability was confirmed for 192 hours at 2ºC to 8°C. Midazolam: LQC %Bias: 2.3%; HQC %Bias -0.5% 1'-Hydroxymidazolam: LQC %Bias: 3.7%; HQC %Bias 0.8% 4-Hydroxymidazolam: LQC %Bias: -94.6%; HQC %Bias -95.6% Room temperature Midazolam: %Bias: 0.6% 1'-Hydroxymidazolam: 0.6% 4-Hydroxymidazolam: -1.6% Wet ice Midazolam: %Bias: 3.3% 1'-Hydroxymidazolam: 7.4% 4-Hydroxymidazolam: 2.3% Short-term Matrix Stability 24 hours at room temperature Midazolam: LQC %Bias: 8.0%; HQC %Bias 4.0% 1'-Hydroxymidazolam: LQC %Bias: 6.3%; HQC %Bias 2.8% 4-Hydroxymidazolam: LQC %Bias: -2.7%; HQC %Bias -2.0%	8280288 Table 8.39 Table 8.40 Table 8.41 8280288 Table 8.45 Table 8.46 Table 8.47

Freeze-Thaw stability	5 freeze/thaw cycles at -10°C to -30°C Midazolam: LQC %Bias: 9.3%; HQC %Bias 4.9% 1'-Hydroxymidazolam: LQC %Bias: 3.7%; HQC %Bias 4.3% 4-Hydroxymidazolam: LQC %Bias: 6.0%; HQC %Bias -0.4% 5 freeze/thaw cycles at -60°C to -80°C Midazolam: LQC %Bias: 11.3%; HQC %Bias 3.6% 1'-Hydroxymidazolam: LQC %Bias: 7.3%; HQC %Bias 3.7% 4-Hydroxymidazolam: - LQC %Bias: 2.7%; HQC %Bias -1.3%	8280288 Table 8.45 Table 8.46 Table 8.47
Long-term storage	Long-term stability 1077 days at -10°C to -30°C K_3EDTA Midazolam: %Bias within 5.5% 1'-Hydroxymidazolam: %Bias within 5.5% 4-Hydroxymidazolam: %Bias within 11.5% Long-term stability 2143 days at -60°C to -80°C K_3EDTA Midazolam: %Bias within 8.7% 1'-Hydroxymidazolam: %Bias within 7.3% 4-Hydroxymidazolam: %Bias within 5.5%	8280288 Table 8.48 Table 8.49 Table 8.50 Table 8.51 Table 8.52 Table 8.53
Parallelism	NA	NA
Carry over	A lack of carryover was observed except for 1'-hydroxymidazolam in Run ID 2 a peak was observed in the first carryover blank which was >20.0 of the LLOQ matrix demonstrated a lack of significant carryover in the chromatographic regions of interest for midazolam, 1'-hydroxymidazolam, and 4-hydroxymidazolam.	
Method performance in study ONT-380-012 Determination of Digoxin, Itraconazole, Rifampin, Tolbutamide, Hydroxy Tolbutamide, Gemfibrozil, Midazolam, 1'-Hydroxymidazolam, ONT-380, ONT-993, and Repaglinide in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-012 Report 8373070 *Please note Appendix 29 through Appendix 35 are part of the same Study and Bioavailability Report*		
Assay passing rate	Midazolam: 10 batches collected and accepted. 1´-Hydroxymidazolam: 10 batches collected and accepted. 4-Hydrozymidazolam: 1 batch collected, no batches accepted.	8373070 Table 8.1
Standard curve performance	Cumulative %Bias Midazolam: -1.4% to 2.0% 1´-Hydroxymidazolam: -4.2% to 3.0%	8373070 Table 8.18 Table 8.19

QC performance	Cumulative %Bias Midazolam: -0.3% to 2.6% 1′-Hydroxymidazolam: -0.7% to 3.0% Cumulative %CV Midazolam: ≤3.7% 1′-Hydroxymidazolam: ≤2.3%	8373070 Table 8.40 Table 8.41
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 44 (11.1%, Midazolam) 44 (11.1%, 1'-Hydroxy-Midazolam) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100% (Midazolam e) 100% (1'-Hydroxy-Midazolam)	8373070 Table 8.56 Table 8.57
Study sample analysis/ stability	Samples were analyzed within known stability for all analytes.	8373070
Standard calibration curve performance during accuracy and precision runs	See above	

a CV is used interchangeably with RSD. Validation and sample analysis reports report RSD.

Appendix 35. Summary Method Performance - 8352993 Tolbutamide, 4-Carboxy Tolbutamide, Hydroxy Tolbutamide

Bioanalytical method validation report name, amendments, and hyperlinks	Validation: 8352993		
Method description	Method details are proprietary to [redacted]. Following protein precipitation of a 50 µL aliquot of K_2EDTA plasma, the extract is injected into an HPLC/MS/MS triple quadrupole mass spectrometer. The peak area of the product ion of tolbutamide, hydroxy tolbutamide, and 4-carboxy tolbutamide are measured against the peak area of the product ion of the internal standards, tolbutamide-d_9, hydroxy tolbutamide-d_9, and 4-carboxy tolbutamide-d_9 respectively.		
Materials used for standard calibration curve and concentration	Tolbutamide: 0.100, 0.200, 1.00, 2.00, 10.0, 50.0, 90.0, 100 µg/mL 4-Carboxy Tolbutamide, Hydroxy Tolbutamide: 0.0250, 0.0500, 0.250, 0.500, 1.00, 2.50, 4.50, 5.00 µg/mL		
Validated assay range	Tolbutamide: 0.100 to 100 µg/mL 4-Carboxy Tolbutamide, Hydroxy Tolbutamide: 0.0250 to 5.00 µg/mL		
Material used for quality controls (QCs) and concentration	Tolbutamide: 0.300, 3.50, 40.0, 80.0 µg/mL 4-Carboxy Tolbutamide and Hydroxy Tolbutamide: 0.300, 3.50, 40.0, 80.0 µg/mL		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	NA		
Regression model; weighting	Linear regression, $1/x^2$		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	8352993 Table 7.2 Table 7.4 Table 7.6
	Cumulative accuracy (%bias[a]) from LLOQ to ULOQ	Tolbutamide: -2.0% to 2.0% 4-Carboxy Tolbutamide: -3.2% to 4.4% Hydroxy Tolbutamide: -2.2% to 4.8%	8352993 Table 7.2 Table 7.4 Table 7.6

	Cumulative precision (%CV[b]) from LLOQ to ULOQ	Tolbutamide: ≤8.4% 4-Carboxy Tolbutamide: ≤7.7% Hydroxy Tolbutamide: ≤7.6%	8352993 Table 7.2 Table 7.4 Table 7.6
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	Tolbutamide: -3.6% to -0.3% 4-Carboxy Tolbutamide: -3.0% to 2.9% Hydroxy Tolbutamide: -4.0% to 4.5%	8352993 Table 7.14 Table 7.16 Table 7.18
	Inter-batch (%CV) QCs	Tolbutamide: ≤8.0% 4-Carboxy Tolbutamide: ≤11.5% Hydroxy Tolbutamide: ≤11.0%	8352993 Table 7.14 Table 7.16 Table 7.18
	Total Error (TE)	Tolbutamide: 7.7% 4-Carboxy Tolbutamide: 14.4% Hydroxy Tolbutamide: 15.5%	Sum of largest bias and CV reported
Selectivity & matrix effect	<u>Selectivity</u> There were no interference peaks detected at the retention time of the ISTD (>5% of the ISTD peak response in the control zero sample) in the run blank samples. Six lots tested at LQC Tolbutamide: %Bias ≤6.0%, CV 6.5% 4-Carboxy Tolbutamide: %Bias ≤2.8%, CV 11.6% Hydroxy Tolbutamide: %Bias ≤2.0%, CV 10.4% Internal standard normalized matrix effect Tolbutamide: LQC 0.973, CV 2.5% HQC 1.01, CV 1.1% 4-Carboxy Tolbutamide: LQC 1.04, CV 4.3% HQC 1.01, CV 1.2% Hydroxy Tolbutamide: LQC 1.02, CV 2.9% HQC 0.997, CV 1.5%		8352993 Table 7.26 Table 7.27 Table 7.28 Table 7.29 Table 7.30 Table 7.31
Interference & specificity	The data met acceptance criteria for interference and there was no evidence of carryover within the chromatographic regions of the analytes and ISTD. Specificity/interference in the presence of Tucatinib, ONT-993, repaglinide, midazolam, 1'-hydroxymidazolame, was assessed during sample analysis. QCs of tolbutamide and 4-hydroxy tolbutamide were all within acceptance criteria of %bias and %CV during the interference testing.		NA 8373070 Table 8.98-8.107

Hemolysis effect	One lot of plasma containing 2% lysed whole blood Tolbutamide: LQC %Bias ≤1.3%, %CV 4.4% HQC %Bias - ≤1.4%, %CV 3.8% 4-Carboxy Tolbutamide: LQC %Bias ≤0.7%, %CV 4.8% HQC %Bias ≤1.0%, %CV 3.3% Hydroxy Tolbutamide: LQC %Bias ≤0.1%, %CV2.6% HQC %Bias ≤1.8%, %CV 4.8%	8352993 Table 7.35 Table 7.36 Table 7.37
Lipemic effect	One lot of matrix with a natural high lipid content ≥300 mg/dL triglyceride level Tolbutamide: LQC %Bias ≤0.3%, %CV 4.0% HQC %Bias ≤1.6%, %CV 3.7% 4-Carboxy Tolbutamide: LQC %Bias ≤0.4%, %CV 4.4% HQC %Bias ≤1.5%, %CV 2.9% Hydroxy Tolbutamide: LQC %Bias ≤0.9%, %CV 4.2% HQC %Bias ≤2.8%, %CV 3.7%	8352993 Table 7.38 Table 7.39 Table 7.40
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	<u>Matrix bench-top stability 25 hours room temperature</u> Tolbutamide, Hydroxy Tolbutamide, and 4-Carboxy Tolbutamide: 25 hours at room temperature. Tolbutamide: %Bias -2.2% to 101.0%; %CV ≤5.2% 4-Carboxy Tolbutamide: %Bias 0.8% to 1.0%; %CV ≤6.6% Hydroxy Tolbutamide: %Bias -0.9% to 0.3%; %CV ≤4.8% <u>Processed sample stability</u> Tolbutamide, 4-Carboxy Tolbutamide, and Hydroxy Tolbutamide: 215 hours at 2°C to 8°C Tolbutamide: %Bias -2.2% to -2.0%; %CV ≤3.3% 4-Carboxy Tolbutamide: %Accuracy -2.9% to 1.5%; %CV ≤2.8% Hydroxy Tolbutamide: %Accuracy -4.3% to 2.0%; %CV ≤3.9%	8352993 Table 7.59 Table 7.60 Table 7.61 Table 7.68 Table 7.69 Table 7.70

Freeze-Thaw stability	6 cycles -10°C to -30°C Tolbutamide: %Accuracy 98.1% to 98.7%; %CV ≤5.6% 4-Carboxy Tolbutamide: %Accuracy 99.8% to 100.8%; %CV ≤7.7% Hydroxy Tolbutamide: %Accuracy 99.1% to 100.5%; %CV ≤3.5% -60°C to -80°C Tolbutamide: %Accuracy 97.3% to 99.7%; %CV ≤4.0% 4-Carboxy Tolbutamide: %Accuracy 99.8% to 99.9%; %CV ≤5.9% Hydroxy Tolbutamide: %Accuracy 98.7% to 101.3%; %CV≤4.3%	8352993 Table 7.62 Table 7.63 Table 7.64
Long-term storage	Matrix frozen stability 275 days -10 to -30°C Tolbutamide: %Accuracy 98.8% to 102.0%; %CV ≤3.8% 4-Carboxy Tolbutamide: %Accuracy 101.5% to 102.0%; %CV ≤2.3 Hydroxy Tolbutamide: %Accuracy 98.4% to 100.8%; %CV ≤3.5% -60 to -80°C Tolbutamide: %Accuracy 95.9% to 102.0%; %CV ≤2.4% 4-Carboxy Tolbutamide: %Accuracy 99.3% to 101.3%; %CV ≤2.1% Hydroxy Tolbutamide: %Accuracy 97.0% to 97.2%; %CV ≤2.4%	8352993 Table 7.65 Table 7.66 Table 7.67
Parallelism	NA	NA
Carry over	There was no evidence of carryover within the chromatographic regions of the analytes and ISTD.	
Method performance in study ONT-380-012 Determination of Digoxin, Itraconazole, Rifampin, Tolbutamide, Hydroxy Tolbutamide, Gemfibrozil, Midazolam, 1'-Hydroxymidazolam, ONT-380, ONT-993, and Repaglinide in Human Plasma by HPLC with MS/MS Detection for Sponsor Reference Number ONT-380-012 Report 8373070 *Please note Appendix 29 through Appendix 35 are part of the same Study and Bioavailability Report*		
Assay passing rate	10 batches of Tolbutamide were collected and accepted. 10 batches of Hydroxy Tolbutamide were collected and accepted. 1 batch for 4-Carboxy Tolbutamide was collected, no batches were accepted.	8373070 Table 8.1

Standard curve performance	Cumulative %Bias Tolbutamide: -3.4% to 3.0% Hydroxy Tolbutamide: -1.2% to 3.0% Cumulative %CV Tolbutamide: ≤5.6% Hydroxy Tolbutamide: ≤5.3%	8373070 Table 8.21 Table 8.22
QC performance	Cumulative %Bias Tolbutamide: -2.6% to 2.0% Hydroxy Tolbutamide: -0.8% to 1.3% Cumulative %CV Tolbutamide: ≤4..4% Hydroxy Tolbutamide: ≤3.6%	8373070 Table 8.43 Table 8.44
Method reproducibility	ISR passes acceptance criteria. Samples reanalyzed (% total analyzed): 48 (10.4%, Tolbutamide) 48 (10.4%, Hydroxy Tolbutamide) Percentage of samples (repeat and original) within 20% of the mean of the 2 values: 100% (Tolbutamide) 100% (Hydroxy Tolbutamide)	8373070 Table 8.58 Table 8.59
Study sample analysis/ stability	Samples were analyzed within known stability for all analytes. Matrix frozen stability is 275 days at -10 to -30°C and at -60 to -80°C (See 8352993).	8373070
Standard calibration curve performance during accuracy and precision runs	See above	

a Percent relative error (RE) is used interchangeably with percent bias.
b CV is used interchangeably with RSD. Validation and sample analysis reports report RSD.

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略号及び用語の定義一覧

略号・用語	省略していない表現又は用語の定義
Ae_{0-24}	amount excreted in urine from time 0 to 24 hours：0 時間から投与後 24 時間までの尿中排泄量
AE	adverse event：有害事象
AESI	adverse event of special interest：特に注目すべき有害事象
aGFR	actual glomerular filtration rate：実際の糸球体濾過量
AR	accumulation ratio：累積係数
AUC	area under the plasma concentration-time curve：血漿中濃度-時間曲線下面積
AUC_6, AUC_{0-6}	area under the plasma concentration-time curve from time 0 to 6 hours：0 時間から投与後 6 時間までの血漿中濃度-時間曲線下面積
AUC_{12}, AUC_{0-12}	area under the plasma concentration-time curve from time 0 to 12 hours：0 時間から投与後 12 時間までの血漿中濃度-時間曲線下面積
AUC_{24}	area under the plasma concentration-time curve from time 0 to 24 hours：0 時間から投与後 24 時間までの血漿中濃度-時間曲線下面積
AUC_{inf}, $AUC_{0-\infty}$	area under the plasma concentration-time curve from time 0 extrapolated to infinity：0 時間から無限大時間までの血漿中濃度-時間曲線下面積
AUC_{last}, AUC_{0-t}, AUC_t	area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration：0 時間から最終定量可能時間までの血漿中濃度-時間曲線下面積
AUC_{tau}	area under the plasma concentration-time curve during dosing period：投与間隔の血漿中濃度-時間曲線下面積
BICR	blinded independent central review：盲検独立中央判定
BID	twice daily：1 日 2 回
BLOQ	below limit of quantification：定量下限未満
BOR	best overall response：最良総合効果
BSV	between subject's variability：個体間変動
C_0	concentration at dosing time：投与時の血漿中濃度
CI	confidence interval：信頼区間
CL/F	apparent total plasma clearance：見かけのクリアランス
CL_R	renal clearance：腎クリアランス
C_{max}	maximum concentration：最高血漿中濃度
CNS	central nervous system：中枢神経系
CNSM	central nervous system metastases：中枢神経系転移
CRC	colorectal cancer：大腸癌
CSF	cerebrospinal fluid：脳脊髄液
CSR	clinical study report：治験総括報告書
C_{trough}	trough concentration：トラフ濃度
Cum A_{eu}	cumulative amount excreted in urine：累積尿中排泄量
Cum F_{eu}	cumulative percentage excreted in urine：累積尿中排泄率
CV	coefficient of variation：変動係数（幾何平均値に対しては幾何変動係数）
CYP	cytochrome p：シトクロム
DDI	drug-drug interaction：薬物相互作用
ECG	electrocardiogram：心電図
ECOG	Eastern Cooperative Oncology Group：米国東海岸癌臨床試験グループ
eGFR	estimated glomerular filtration rate：推定糸球体濾過量

略号・用語	省略していない表現又は用語の定義
Fe_{0-24}	fraction of excreted unchanged drug in urine from time 0 to 24 hours：0 時間から投与後 24 時間までの未変化体尿中排泄量
Frel	relative bioavailability：相対的バイオアベイラビリティ
GFR	glomerular filtration rate：糸球体濾過量
HER2	human epidermal growth factor receptor-2：ヒト上皮増殖因子受容体 2 型
HER2+	human epidermal growth factor receptor-2 positive：ヒト上皮増殖因子受容体 2 型陽性
HV	Healthy volunteer：健康成人
IC_{50}	50% inhibitory concentration：50%阻害濃度
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use：医薬品規制調和国際会議
ICR	independent central review：独立中央判定
ka	absorption rate constant：吸収速度定数
Ki	inhibition constant：阻害定数
LLOQ	lower limit of quantification：定量下限
LS	least squares：最小二乗
MATE	multidrug and toxin extrusion protein：有機カチオン/H^+交換輸送体
mBC	metastatic breast cancer：転移性乳癌
MR_{AUC}	metabolite-to-parent total exposure ratio：未変化体に対する代謝物の総曝露量比
MR_{Cmax}	metabolic ratio based on C_{max}：C_{max} の代謝物比
MRT	mean residence time：平均滞留時間
MTD	maximum tolerated dose：最大耐量
NCA	noncompartmental analysis：ノンコンパートメント解析
OATP	organic anion transporting polypeptide：有機アニオントランスポーターポリペプチド
OCT	organic cation transporter：有機カチオントランスポーター
PBPK	physiologically based pharmacokinetics：生理学的薬物速度論
PD	pharmacodynamics：薬力学
PFS	progression-free survival：無増悪生存期間
P-gp	P-glycoprotein：P 糖タンパク質
PIC	powder-in-capsule：原薬充填カプセル剤
PK	pharmacokinetics：薬物動態
PT	preferred term：基本語
QD	once daily：1 日 1 回
Q/F	apparent intercompartmental clearance：見かけのコンパートメント間のクリアランス
ORR	objective response rate：客観的奏効率
QTc	corrected QT interval：補正 QT 間隔
QTcF	QT interval corrected for HR using Fridericia's method：Fridericia 法の補正 QT 間隔
RP2D	recommended Phase 2 dose：Phase 2 試験の推奨用量
RSE	relative standard error：相対標準誤差
SD	standard deviation：標準偏差
SE	standard error：標準誤差
SMQ	standardized MedDRA Queries：MedDRA 標準検索式
ss	steady state：定常状態
T-DM1	trastuzumab emtansine：トラスツズマブ エムタンシン
$t_{1/2}$	terminal half-life：終末相の消失半減期

略号・用語	省略していない表現又は用語の定義
TEAE	treatment-emergent adverse event：治験治療下での有害事象
Tlag	lag time：ラグタイム
Tlast	time of last measurable concentration：定量可能な最終測定時点
T_{max}, t_{max}	time to maximum concentration：最高血漿中濃度到達時間
UGT	UDP (Uridine 5'-diphospho)-glucuronosyltransferase：UDP-グルクロン酸転移酵素
Vc/F	apparent volume of distribution in the central compartment：見かけの中央コンパートの分布容積
Vd/F	apparent volume of distribution：見かけの分布容積
Vz/F	apparent volume of distribution at terminal phase：見かけの終末相の分布容積

1. 背景及び概観

本 M2.7.2 に示すツカチニブの臨床薬理は、ツカチニブの PK 及び PD を評価した臨床試験の結果に基づいている。これらの臨床試験の臨床薬理上の目的及び要約を Table 1 に示す。

Table 1. Summary of tucatinib clinical pharmacology assessments

Objective(s)	Description	Trial Number
Pharmacokinetics (PK)	Evaluate single and multiple dose PK characteristics of tucatinib	ARRAY-380-101 ARRAY-380-103 ONT-380-004 ONT-380-005 ONT-380-009 ONT-380-011 ONT-380-012 SGNTUC-015 SGNTUC-020 ONT-380-206 (HER2CLIMB) MK-7119-001 (C4251013)
Hepatic Impairment	Evaluate the PK of tucatinib in subjects with hepatic impairment	ONT-380-009
QT/QTc	Evaluate the effect of multiple doses of tucatinib on cardiac repolarization	ONT-380-011
Population PK Analysis	Evaluate the influence of intrinsic and extrinsic factors on tucatinib PK using a population PK model	ARRAY-380-103 ONT-380-012 SGNTUC-015 SGNTUC-020 SGNTUC-017 ONT-380-004 ONT-380-005
Exposure-response Analysis and PK Covariate Analysis	Assess exposure-response for efficacy in the pivotal mBC study Assess exposure-response for safety in the pivotal mBC study Evaluate the influence of intrinsic factors on PK in the pivotal mBC study	ONT-380-206 MK-7119-001
Food Effect	Evaluate the effect of food on tucatinib PK	ARRAY-380-103
Absorption, Metabolism and Elimination	Evaluate the disposition of radiolabeled tucatinib	ONT-380-008
Drug-drug Interactions	Assess the effect of gastric pH modulation by omeprazole on the PK of tucatinib	ARRAY-380-103
	Determine the extent to which tucatinib inhibits the metabolism of CYP3A4, CYP2C8, CYP2C9, and transport of P-gp substrates Determine the impact of inhibitors/inducers of CYP3A4 and CYP2C8 on tucatinib exposure	ONT-380-012
	Determine the extent to which tucatinib inhibits the renal elimination of the multidrug and toxin extrusion protein (MATE)1/2-K substrate metformin Evaluate the effect of tucatinib on renal function	SGNTUC-020

CYP3A4 = cyctochrome P450 3A4; CYP2C8 = cyctochrome P450 2C8; CYP2C9 = cyctochrome P450 2C9; MATE = multidrug and toxin extrusion; mBC = metastatic breast cancer; P-gp = P-glycoprotein; PK = pharmacokinetics; QTc = corrected QT interval

1.1. 非臨床試験の PK 評価概要

ツカチニブの PK、トキシコキネティクス、分布、代謝、排泄及び DDI について、in vitro 試験系並びにマウス、ラット及びカニクイザルを用いた in vivo 試験で評価した非臨床試験結果の要約を以下に示す。非臨床試験の PK 評価の詳細な方法及び結果は M2.6.4 に示す。

- ラットに 0.5 mg/kg を静脈内投与と、溶液及び懸濁液 10 mg/kg を経口投与した結果、絶対的 BA は 23.8%（溶液）及び 21.9%（懸濁液）であった。
- ツカチニブは高い膜透過性を示した。
- ツカチニブの血漿タンパク結合の in vitro 評価により、ツカチニブ濃度 1 µmol/L における平均ヒト血漿タンパク結合率は 97.1%であった。ツカチニブ濃度 0.2、2.0 及び 20 µmol/L における in vitro 血液/血漿分配比はそれぞれ 1.02、1.00 及び 0.98 であったことから、本薬は血球及び血漿に同程度の割合で存在することが示唆された。
- ツカチニブは主に CYP2C8（主要な代謝酵素）及び CYP3A（副次的な代謝酵素）によって代謝され、アルデヒドオキシダーゼでも代謝される。
- ラット、カニクイザル及びヒトでは、^{14}C 標識ツカチニブの単回経口投与後、放射能の大部分が主に代謝物として糞便中から回収され、代謝及び肝胆道系排泄が主要な消失経路であることが示唆された。未変化体の腎排泄はわずかであった。
- ツカチニブは P-gp 及び乳癌耐性タンパク質の基質であり、in vitro で様々なトランスポーターを阻害した。In vitro 試験並びに PBPK モデリング及びシミュレーションから、臨床用量における血漿中ツカチニブ濃度が CYP1A2、CYP2B6、CYP2C9、CYP2C19、CYP2D6、UGT1A1 及び OATP1B1/3 を阻害する影響は低いと予測された。

1.2. 臨床薬理試験の概要

ツカチニブの臨床薬理を評価した 12 試験から PK 及び PD に関する結果が得られている。これらの試験を治験参加者集団別に Table 2 に示す。

第 1 相試験の 7 試験では、健康成人及び肝機能障害を有する治験参加者にツカチニブを単回又は反復投与し、ツカチニブ投与時の PK 及び PD を評価した。進行固形がん又は mBC を有する患者を対象にした第 1 相試験及び第 2 相試験の 5 試験では、患者集団において、ツカチニブ単剤又は併用投与におけるツカチニブ投与時の PK 及び PD を評価した。PK の分析法及びそのバリデーションは M2.7.1 に示す。

Table 2. Tucatinib clinical trials presenting clinical pharmacology data

Study Number	Description	Treatment Regimen	Number of Subjects (N)
Studies in Subjects with Advanced Cancer/Metastatic Breast Cancer			
Monotherapy Study			
ARRAY-380-101	Phase 1, open-label, multiple dose study to assess the safety, tolerability, and pharmacokinetics of tucatinib given on a daily oral regimen in subjects with advanced cancer	Dose escalation: 25 to 800 mg PO BID, 28-day cycles, PIC Expansion (at MTD): 600 mg PO BID, 28-day cycles, PIC	Total subjects; N=50 Dose escalation phase; N=33 N=3 each for 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, and 650 mg BID N=4 for 500 mg and 800 mg BID N=7 for 600 mg BID Dose expansion phase; N=17 N=17 for 600 mg BID
Combination Therapy Studies			
ONT-380-005	Phase 1b, open-label study to assess the safety and tolerability of ONT-380 combined with capecitabine and trastuzumab, alone and in combination in HER2+ metastatic breast cancer	Combination 1: Tucatinib (tablet) 300 mg or 350 mg PO BID of every 21-day cycle and either capecitabine 1000 mg/m^2 PO BID for 14 days of every 21-day cycle; Combination 2: Trastuzumab 8 mg/kg IV loading dose Day 1 Cycle 1, followed by 6 mg/kg IV Day 1 of all subsequent cycles; or Combination 3: Capecitabine 1000 mg/m^2 PO BID for 14 days of every 21-day cycle + trastuzumab 8 mg/kg IV loading dose Day 1 Cycle 1, followed by 6 mg/kg IV Day 1 of all subsequent cycles	Total subjects; N=60 Cohort 1A (300 mg Tucatinib BID + Cape) N=7 Cohort 1B (350 mg Tucatinib BID + Cape) N=4 Cohort 2A (300 mg Tucatinib BID + Tras) N=10 Cohort 2B (350 mg Tucatinib BID + Tras) N=4 Cohort 2F (300 mg Tucatinib BID + Tras CNS Expansion) N=8 Cohort 3A (300 mg Tucatinib BID+Cap+Tras) N=22 Cohort 3E (300 mg Tucatinib BID+Cap+Tras CNS Expansion) N=5
ONT-380-206 (HER2CLIMB)	Phase 2, randomized, double-blind, controlled study of tucatinib vs. placebo in combination with capecitabine and trastuzumab in patients with pretreated unresectable locally advanced or metastatic HER2+ breast carcinoma	300 mg tucatinib (tablet) or placebo PO BID every 21-day cycle plus capecitabine 1000 mg/m^2 PO BID for 14 days of every 21-day cycle plus trastuzumab 8 mg/kg IV loading dose Day 1 Cycle 1, followed by 6 mg/kg IV Day 1 of all subsequent cycles	Total subjects: N=612 Tucatinib: N=410 Placebo: N=202 (2:1 randomization) PK Analysis Set Tucatinib: N=385

Study Number	Description	Treatment Regimen	Number of Subjects (N)
MK-7119-001 (C4251013)	Phase 2 open-label, single arm study of tucatinib in combination with trastuzumab and capecitabine in participants with previously treated locally advanced unresectable or metastatic HER2+ breast carcinoma	300 mg tucatinib (tablet) PO BID every 21-day cycle plus capecitabine 1000 mg/m^2 PO BID for 14 days of every 21-day cycle plus trastuzumab 8 mg/kg IV loading dose Day 1 Cycle 1, followed by 6 mg/kg IV Day 1 of all subsequent cycles	Total subjects: N=66 (53 Japanese including 4 patients for safety run-in)
ONT-380-004	Phase 1b, open-label study to assess the safety and tolerability of tucatinib combined with ado-trastuzumab emtansine (trastuzumab emtansine; T-DM1) in patients with HER2+ breast cancer	Dose escalation: tucatinib (tablet) 300 mg or 350 mg PO BID and T-DM1 3.6 mg/kg IV on Day 1 of each 21-day cycle Expansion (at MTD): tucatinib (tablet) 300 mg BID and T-DM1 3.6 mg/kg IV on Day 1 of each 21-day cycle	Total subjects: N=57 Initial cohort (300 mg): N=8 Dose escalation cohort (350 mg): N=7 Expansion cohort: N=23 Expansion cohort with active CNS disease: N=19
Studies in Subjects Without Cancer			
ARRAY-380-103	Open-label, single-dose, four-period study evaluating the relative bioavailability, potential food effect, and omeprazole drug interaction of oral tucatinib capsules and tablets in healthy subjects	Single 300 mg PO dose in each of the four treatment periods: Tucatinib capsules (PIC; fasted) Tucatinib tablets (fasted) Tucatinib fasted (fed) Tucatinib tablets (fasted) following omeprazole (40 mg) for 5 days	Total subjects: N=12 Tucatinib capsules fasted: N=12 Tucatinib tablets fasted: N=12 Tucatinib tablets fed: N=11 Omeprazole/tucatinib tablets fasted: N=9
ONT-380-008	Phase 1, open-label study of the absorption, metabolism, and excretion of [^{14}C]-tucatinib following a single oral dose in healthy male and female subjects	Single dose of 300 mg [^{14}C]-tucatinib administered as an oral solution	Total subjects: N=8
ONT-380-009	Open-label, nonrandomized, single-dose, parallel-group, safety, tolerability, and pharmacokinetic study of tucatinib administered at 300 mg in fasted, hepatically-impaired male and female subjects and fasted matched-control healthy subjects	Single dose of 300 mg of tucatinib tablet	Total subjects: N=37 Normal hepatic function: N=15 Mild hepatic impairment: N=8 Moderate hepatic impairment: N=8 Severe hepatic impairment: N=6

Study Number	Description	Treatment Regimen	Number of Subjects (N)
ONT-380-011	Phase 1, randomized, partially double-blind, placebo and positive-controlled study to evaluate the effect of tucatinib on cardiac repolarization in healthy subjects	Treatment A: tucatinib (tablet) 300 mg for 4 days Treatment B: tucatinib matching placebo for 4 days Treatment C: Single dose of moxifloxacin 400 mg	Total subjects: N=53 Sequence ABC: N=9 Sequence CAB: N=8 Sequence BCA: N=9 Sequence CBA: N=9 Sequence ACB: N=9 Sequence BAC: N=9
ONT-380-012	Phase 1, open-label, fixed-sequence, 5-part, drug-drug interaction study of tucatinib to evaluate the effects of CYP3A4 and CYP2C8 inhibition and induction on the pharmacokinetics of tucatinib and to evaluate the effects of tucatinib on the pharmacokinetics of substrates of CYP3A4, CYP2C8, CYP2C9, and P-glycoprotein in healthy male and female subjects	Parts A-C: Single dose of 300 mg tucatinib (tablet) Parts D-E: Tucatinib (tablet) 300 mg BID for 10 or 14 days Part A: itraconazole 200 mg BID Part B: rifampicin 600 mg Part C: gemfibrozil 600 mg BID Part D: Single doses of repaglinide 0.5 mg, tolbutamide 500 mg, midazolam 2 mg Part E: digoxin 0.5 mg	Total subjects: N=116 Part A: N=28 Part B: N=28 Part C: N=28 Part D: N=17 Part E: N=13
SGNTUC-020	Phase 1, single center, open-label, fixed sequence drug-drug interaction study in healthy subjects evaluating the effects of tucatinib on the PK of a substrate probe of the MATE1/MATE2-K transporters	Tucatinib (tablet) 300 mg BID Metformin 850 mg for oral administration Iohexol, 1500 mg iodine (3235 mg iohexol) for IV administration	Total subjects: N=18
SGNTUC-015	Phase 1, open-label, safety, tolerability and pharmacokinetic study of tucatinib (ONT-380) in healthy Japanese and Caucasian subjects	Cohort 1: Tucatinib (tablet) 50 mg BID Days 1 to 13, and QD on Day 14 Cohort 2: Tucatinib (tablet) 150 mg BID Days 1 to 13, and QD on Day 14 Cohort 3: Tucatinib (tablet) 300 mg BID on Days 1 to 13, and QD on Day 14	Total subjects: N=36 Cohort 1: 50 mg: Caucasian, N=6; Japanese, N=6 Cohort 2: 150 mg: Caucasian, N=6; Japanese, N=6 Cohort 3: 300 mg: Caucasian, N=6; Japanese, N=6

BID = twice daily; CSR = clinical study report; HER2 = human epidermal growth factor receptor-2; IV = intravenous; MATE = multidrug and toxin extrusion; MTD = maximum tolerated dose; PIC = powder-in-capsule; PO = oral; QD = once daily

1.3. 要旨

ツカチニブの臨床薬理に関する全体的な結論は以下のとおりである。

- ツカチニブ PIC 製剤を 800 mg までの用量及びツカチニブ錠 50～300 mg の用量で BID 投与したとき、ツカチニブの C_{max} 及び AUC は用量に概ね比例して増加し、T_{max}（中央値）は約 2 時間であった。ツカチニブ錠 300 mg 投与時の $t_{½}$（算術平均値）は、約 4～9 時間であり、BID 反復投与後、5 日目までには概ね定常状態に達し、AR_{AUC} は約 2 であった。

- ツカチニブ錠 300 mg BID 経口投与後の曝露量は、PIC 製剤 600 mg（MTD）を BID 経口投与したときと同程度であった。

- ツカチニブの代謝に主に関与する酵素は CYP2C8 であり、CYP3A 及びアルデヒドオキシダーゼによっても代謝される。ツカチニブは主に肝胆道系を介して排泄される（放射性標識ツカチニブ投与量の約 86%が便中から回収された）。ツカチニブ消失における腎排泄の寄与は小さかった（放射性標識ツカチニブ投与量の約 4%が尿中で回収された）。

- ツカチニブ 300 mg BID の反復投与は QT 間隔を延長しなかった。

- 母集団 PK 解析の結果、体重及びアルブミンはツカチニブの PK に臨床的に意味のある影響を及ぼさなかった。年齢、クレアチニンクリアランス及び人種はツカチニブの PK の予測因子と特定されなかった。日本人及び白人の健康成人にツカチニブを投与したときの PK に人種差は認められなかった。

- ツカチニブの曝露量は正常肝機能群と比べ、軽度肝機能障害群では変化はなく、中等度肝機能障害群では AUC の増加は 15%以下であり、重度肝機能障害群の AUC_{last} 及び AUC_{inf} はそれぞれ 143.0%及び 161.0%の増加が認められた。重度肝機能障害を有する患者では、ツカチニブの開始用量を減量する必要があると考えられる。

- ツカチニブは主に代謝及び胆汁中排泄を介して消失するため、腎機能障害はツカチニブの PK にほとんど影響しないと考えられる。

- 曝露-反応解析の結果、ONT-380-206 試験において、ツカチニブの C_{trough} の上昇に伴って PFS が延長する傾向が示されたものの、すべての四分位範囲で信頼区間が重なり、すべての四分位範囲の治験参加者に治療効果が認められた。各四分位範囲における AESI の発現頻度は低かったものの、AESI の発現割合と Cycle 3 Day 1 のツカチニブの C_{trough} との関係に明らかな傾向は認められなかった。MK-7119-001 試験において全集団及び日本人集団ともに第 2～3 四分位では、他の四分位範囲よりも確定 ORR は高めの傾向であり、第 1 四分位及び第 1～2 四分位の確定 ORR は同様の傾向であった。全集団と日本人集団で各四分位の確定 ORR の傾向に明らかな違いは認められなかった。第 3～4 四分位では肝毒性発現率は高めの傾向であったものの管理可能であり、全集団と日本人集団で各四分位の肝毒性発現率の傾向に明らかな違いは認められなかった。

- ツカチニブは CYP3A4 の強い阻害薬であり、DDI 試験ではミダゾラム曝露量が 5.7 倍に増加した。強い CYP3A4 誘導薬及び中程度の CYP2C8 誘導薬との併用投与により、ツカチニブの曝露量は約 50%減少した。強い CYP2C8 阻害薬との併用投与により、ツカチニブの曝露

量は約 3.0 倍に増加した。ツカチニブと CYP3A4 の高感受性基質との併用は避けることが推奨される。ツカチニブと強い CYP3A4 誘導薬あるいは中程度の CYP2C8 誘導薬との併用は避けることが推奨される。ツカチニブと強い CYP2C8 阻害薬との併用は避け、併用が必要な場合にはツカチニブの用量を減量することが推奨される。強い CYP3A4 阻害薬の併用投与による明らかな DDI の影響は認められなかったため併用可能である。

- ツカチニブは P-gp の弱い阻害薬である。ジゴキシン又は治療域の狭い他の P-gp 基質との併用では慎重な投与が推奨される。

- ツカチニブは CYP2C8 及び MATE1/2-K の弱い阻害薬であり、CYP2C9 の阻害薬ではなかった。ツカチニブは、MATE1/MATE2-K を介したメトホルミンの輸送及び OCT2/MATE1 を介したクレアチニンの輸送を弱く阻害する。ツカチニブの臨床試験で認められた血清クレアチニン増加は、OCT2 及び MATE1 を介したクレアチニンの尿細管分泌の阻害によるものである。

- In vitro 及び PBPK 解析の結果、臨床用量のツカチニブを投与したとき、CYP1A2、CYP2B6、CYP2C9、CYP2C19、CYP2D6、UGT1A1 及び OATP1B1/3 による阻害の影響は低い。

- ツカチニブ及び ONT-993 はツカチニブ初回投与後から CSF 中に検出され、定常状態では、CSF と遊離型血漿中濃度は同程度であることが示された。ツカチニブの CSF への移行性により、脳転移患者でツカチニブの有効性が示されたことが示唆された。

本 M2.7.2 の臨床薬理及び生物製剤学の結果（M2.7.1）は、HER2 陽性 mBC 患者において、トラスツズマブ及びカペシタビンと併用したツカチニブの用法・用量として、ツカチニブ錠 300 mg BID を食事の有無を問わず連続経口投与とすることを支持する。

2. 個々の試験結果の要約

臨床試験で評価した PK 及び PD の概要について、各臨床試験の結果を 2.1 及び 2.2 に示し、全試験を通しての結果を 3 に示す。各臨床試験の有効性及び安全性の概要は M2.7.3、M2.7.4 及び M2.7.6 に示す。

2.1. 固形癌患者を対象とした臨床試験

2.1.1. 進行固形癌患者にツカチニブ単剤を投与した臨床試験

2.1.1.1. 進行固形癌患者を対象としたツカチニブの first-in-human 第 1 相試験：ARRAY-380-101 試験

本治験は、ツカチニブの first-in-human 試験であり、進行 HER2 陽性固形癌、特に HER2 陽性 mBC を有する患者を対象に、ツカチニブの最大耐量（MTD）を決定し、安全性、忍容性、予備的な有効性、PK 及びバイオマーカーを評価する非盲検試験である。本治験では、HER2 陽性 mBC 患者 43 例を含む計 50 例を用量漸増期及び拡大期（用量漸増期 33 例、拡大期 17 例）に組み入れ、全 50 例を PK 解析に含めた。本項では PIC 製剤 25～800 mg の用量で BID 投与したときの用量漸増時及び反復投与時の PK 評価の概要を示す。本治験の用量漸増期では、Cycle 1 Day 1 にツカチニブを単回投与した。DLT が認められなかった場合、Cycle 1 Day 3 に BID 反復投与を開始した。本治験で評価した食事の影響評価の概要は M2.7.1.2.1 に示す。

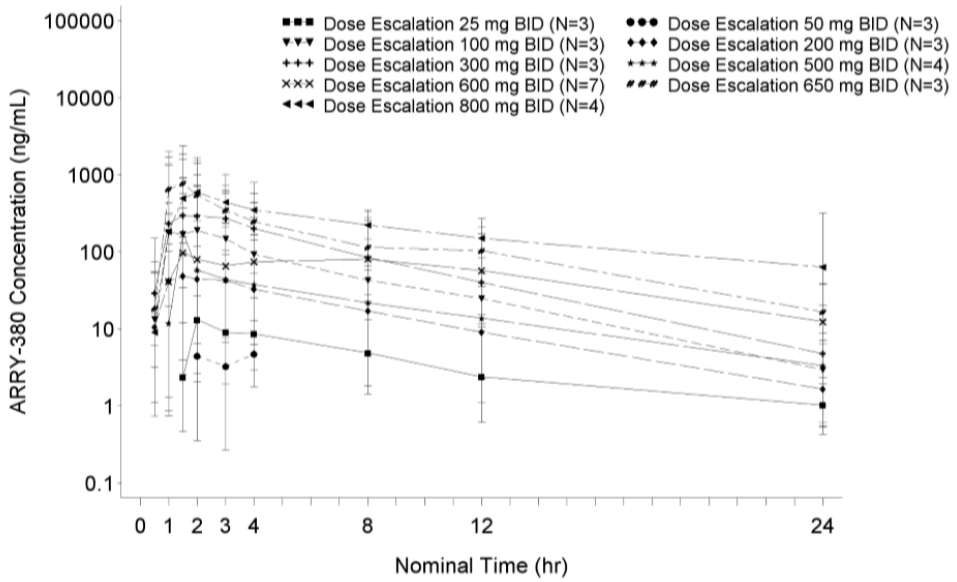
- ツカチニブ及び ONT-993 の血漿中濃度

ツカチニブ単回投与後（Cycle 1 Day 1）の血漿中ツカチニブ濃度-時間推移は、各用量間及び単回投与時と反復投与時で吸収及び消失の推移は類似しており、用量増量に伴って上昇する傾向を示した（Figure 1、Figure 2）。血漿中 ONT-993 濃度-時間推移は、ツカチニブの濃度推移の形状と概ね類似し、血漿中濃度の推移範囲はツカチニブの約 1/10 であった（Figure 3、Figure 4）。

- ツカチニブ及び ONT-993 の PK パラメータ

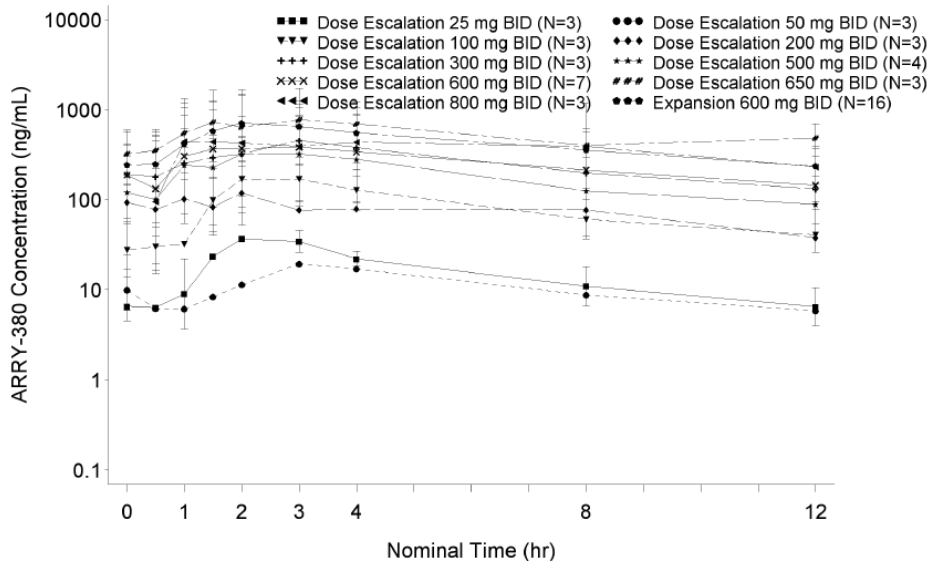
Cycle 1 Day 1及びCycle 1 Day 15におけるツカチニブのPKパラメータの要約をTable 3に示す。ONT-993の曝露量は、ツカチニブ曝露量の約11～12%（幾何平均MR_{AUC}）であった（ARRAY-380-101 CSR Section 11.3.2.2）。Cycle 1 Day 1及びCycle 1 Day 15のいずれにおいても、ツカチニブのT_{max}（中央値）は全体で約2時間、$t_{½}$（中央値）は全体で約5時間であり、ツカチニブの曝露量（AUC_{tau}）は反復投与により約2倍に増加した。PKの結果を総合すると、ツカチニブの曝露量は用量に応じて増加し、T_{max}及び$t_{½}$は用量に依存しない傾向が示された。
ツカチニブの AUC_{tau} 及び C_{max} と投与量との関係について検討した結果、関数の傾きは、Cycle 1 Day 1 及び Cycle 1 Day 15 のいずれにおいても約 1（範囲：0.868～0.99）と推定され、傾きの 90% CI はいずれも 1 を跨いだ（Figure 5、Figure 6、ARRAY-380-101 CSR Table 15.4.6）。曝露量のばらつきはやや大きかったものの、ツカチニブを単回及び反復投与したときの曝露量は用量に比例することが示された。

Figure 1　Geometric mean ± SD plasma tucatinib concentrations on Cycle 1 Day 1 (semi log scale)



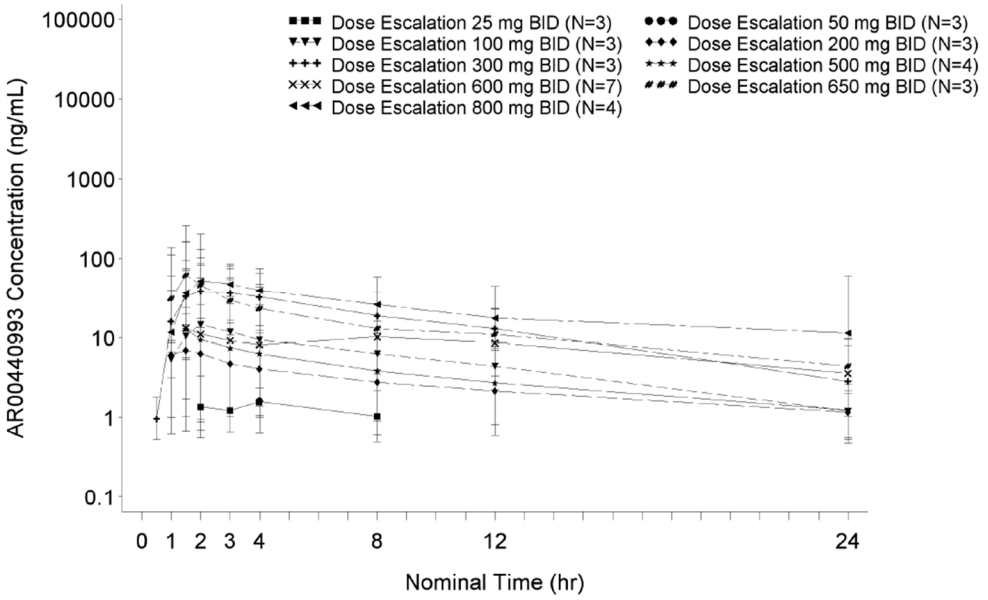
BID = twice daily; hr = hour(s); mg = milligram(s); mL = milliliter(s); N = number; ng = nanogram(s); SD = standard deviation; log = logarithmic
Source: ARRAY-380-101 CSR, Figure 1

Figure 2　Geometric mean ± SD plasma tucatinib concentrations on Cycle 1 Day 15 (semi log scale)



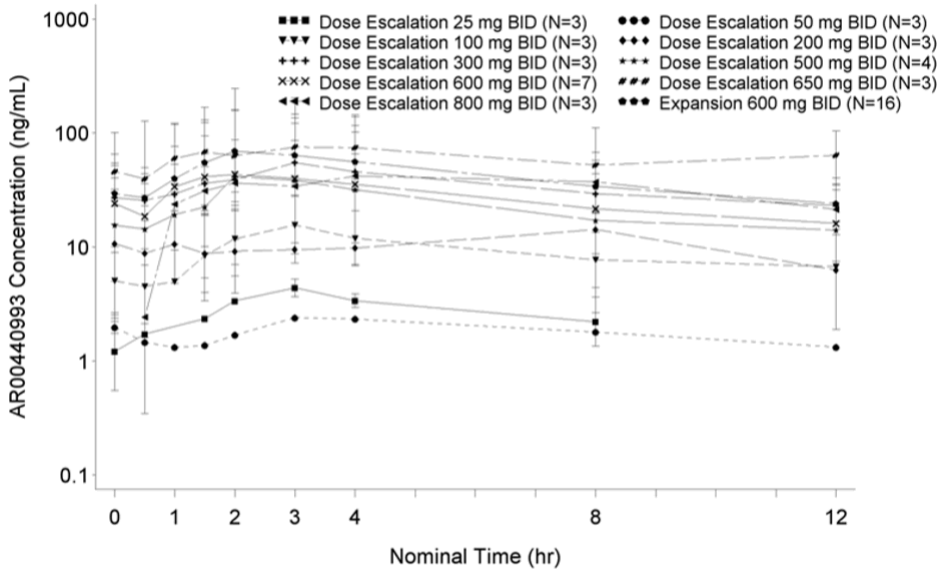
BID = twice daily; hr = hour(s); mg = milligram(s); mL = milliliter(s); N = number; ng = nanogram(s); SD = standard deviation; log = logarithmic
Source: ARRAY-380-101 CSR, Figure 2

Figure 3　Geometric mean ± SD plasma ONT-993 concentrations on Cycle 1 Day 1 (semi log scale)



Abbreviations: BID = twice daily; hr = hour(s); mg = milligram(s); mL = milliliter(s); N = number; ng = nanogram(s); SD = standard deviation; log = logarithmic
Source: ARRAY-380-101 CSR, Figure 15.4.2.1b

Figure 4　Geometric mean ± SD plasma ONT-993 concentrations on Cycle 1 Day 15 (semi log scale)



Abbreviations: BID = twice daily; hr = hour(s); mg = milligram(s); mL = milliliter(s); N = number; ng = nanogram(s); SD = standard deviation; log = logarithmic
Source: ARRAY-380-101 CSR, Figure 3

Table 3. Pharmacokinetic parameters for tucatinib on Cycle 1 Day 1 and Cycle 1 Day 15 (dose-escalation pharmacokinetic population)

Day	Parameter (Units)	Dose Escalation									Dose Escalation Total (N=33)
		25 mg BID (N=3)	50 mg BID (N=3)	100 mg BID (N=3)	200 mg BID (N=3)	300 mg BID (N=3)	500 mg BID (N=4)	600 mg BID (N=7)	650 mg BID (N=3)	800 mg BID (N=4)	
1	AUC_{last} (hr*ng/mL)[a]	93.3 (106)	26.9 (NC)	1020 (58.3)	370 (652)	1910 (136)	1470 (185)	1490 (288)	3100 (70.1)	5350 (46.2)	NR
	AUC_{tau} (hr*ng/mL)[a]	80.4 (76.5)	302 (NC)	901 (55.1)	347 (589)	1670 (122)	1280 (210)	1140 (278)	2650 (67.6)	3660 (23.3)	NR
	AUC_{tau}/D (hr*ng/mL/mg)[a]	3.22 (76.5)	6.05 (NC)	9.01 (55.1)	1.74 (589)	5.57 (122)	2.57 (210)	1.90 (278)	4.08 (67.6)	4.58 (23.3)	3.36 (157)
	C_{max} (ng/mL)[a]	19.1 (39.6)	15.4 (NC)	212 (49.4)	97.3 (1860)	366 (82.2)	285 (215)	255 (488)	776 (82.8)	876 (31.2)	NR
	C_{max}/D (ng/mL/mg)[a]	0.766 (39.6)	0.307 (NC)	2.12 (49.4)	0.487 (1860)	1.22 (82.2)	0.569 (215)	0.425 (488)	1.19 (82.8)	1.10 (31.2)	0.742 (225)
	T_{max} (hr)[b]	2.12 (0.983 - 2.93)	1.74 (1.48 - 2.00)	1.37 (1.00 - 3.00)	1.00 (1.00 - 3.00)	2.00 (1.50 - 3.00)	2.00 (2.00 - 2.00)	2.00 (1.05 - 8.00)	1.50 (1.00 - 1.50)	1.75 (1.50 - 24.3)	2.00 (0.983 - 24.3)
	$t_{½}$ (hr)[b]	5.38 (2.15 - 7.66)	2.02 (2.02 - 2.02)	3.84 (3.43 - 6.04)	4.74 (4.30 - 5.44)	3.86 (3.14 - 6.51)	5.50 (4.49 - 7.39)	5.98 (5.00 - 8.85)	6.34 (4.74 - 6.60)	6.06 (5.37 - 6.59)	5.38 (2.02 - 8.85)
15	AUC_{last} (hr*ng/mL)[a]	182 (28.4)	122 (NC)	954 (47.6)	453 (145)	3020 (35.4)	2210 (127)	2430 (220)	4530 (59.9)	4240 (91.8)	NR
	AUC_{tau} (hr*ng/mL)[a]	187 (29.3)	128 (NC)	1040 (44.2)	800 (NC)	3090 (37.5)	1940 (157)	2900 (188)	6680 (NC)	4480 (94.7)	NR
	AUC_{tau}/D (hr*ng/mL/mg)[a]	7.48 (29.3)	2.56 (NC)	10.4 (44.2)	4.00 (NC)	10.3 (37.5)	3.88 (157)	4.83 (188)	10.3 (NC)	6.61 (72.8)	6.04 (100)
	C_{max} (ng/mL)[a]	35.8 (35.4)	19.5 (NC)	205 (33.1)	136 (69.5)	485 (62.8)	507 (128)	506 (240)	967 (26.1)	655 (55.0)	NR
	C_{max}/D (ng/mL/mg)[a]	1.43 (35.4)	0.389 (NC)	2.05 (33.1)	0.679 (69.5)	1.62 (62.8)	1.01 (128)	0.844 (240)	1.49 (26.1)	0.966 (41.6)	1.06 (105)
	R_{AUC}[a]	2.33 (100)	0.775 (NC)	1.15 (50.9)	5.15 (NC)	1.85 (192)	1.43 (NC)	2.92 (76.3)	1.77 (NC)	1.20 (132)	1.95 (104)
	T_{max} (hr)[b]	3.00 (2.00 - 3.00)	3.03 (3.00 - 3.05)	2.98 (1.58 - 3.07)	1.75 (1.50 - 2.00)	2.98 (1.50 - 4.00)	2.08 (1.00 - 2.98)	1.73 (1.07 - 4.00)	3.10 (1.50 - 4.00)	2.08 (1.00 - 4.00)	2.08 (1.00 - 4.00)
	$t_{½}$ (hr)[b]	3.48 (3.19 - 5.40)	6.31 (2.97 - 9.66)	3.93 (3.25 - 4.61)	8.13 (5.96 - 10.3)	4.88 (4.12 - 5.38)	5.80 (5.07 - 9.60)	5.33 (3.15 - 8.57)	17.2 (4.95 - 29.4)	8.43 (5.53 - 12.1)	5.38 (2.97 - 29.4)

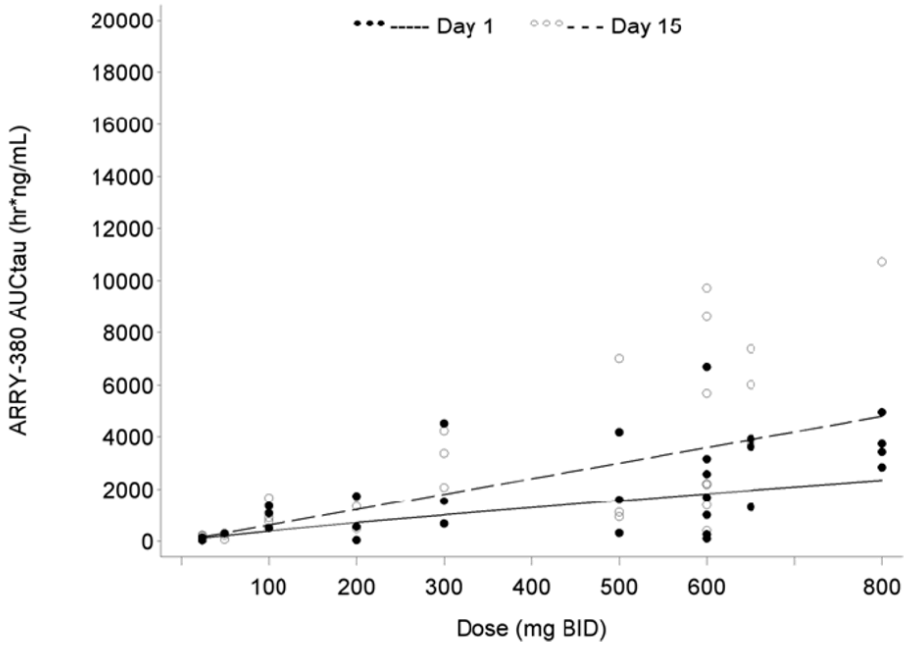
Abbreviations: AUC_{last} = area under the plasma concentration-time curve from time 0 to the last measurable concentration; AUC_{tau} = area under the plasma concentration-time curve for the dosing interval; BID = twice daily; C_{max} = maximum observed plasma concentration; CV = coefficient of variation; D = dose; hr = hour(s); Max = maximum; mg = milligram(s); Min = minimum; mL = milliliter(s); N = number; NC = not calculated; ng = nanogram(s); NR = not reported; R_{AUC} = accumulation ratio based on area under the plasma concentration-time curve values for the dosing interval; $t_{1/2}$ = apparent terminal half-life; T_{max} = time of maximum observed plasma concentration

[a] Geometric mean (%CV)

[b] Median (Min-Max)

Source: ARRAY-380-101 CSR, Table 15

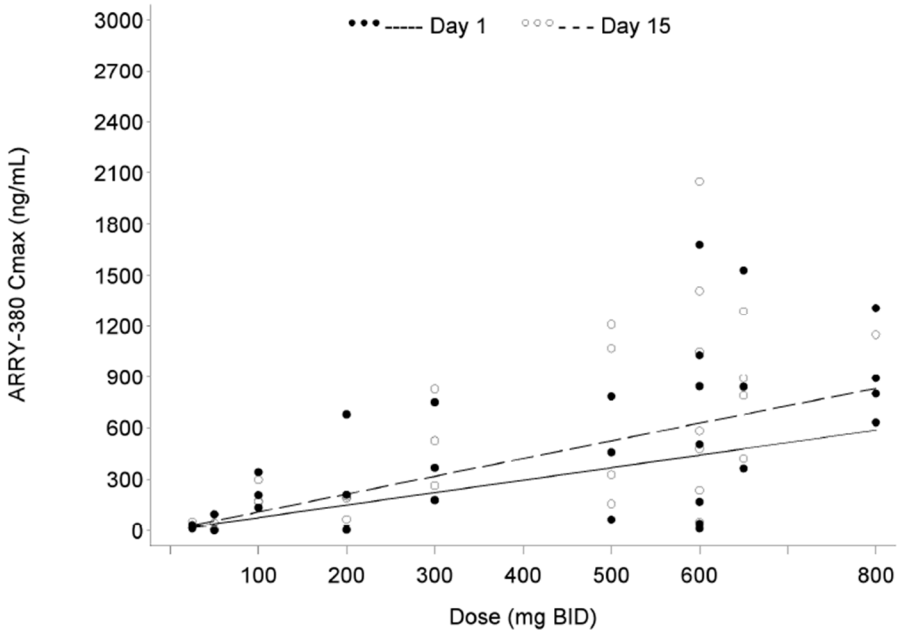
Figure 5　Evaluation of dose proportionality for tucatinib AUC_{tau}



Abbreviations: AUC_{tau} = area under the plasma concentration-time curve for the dosing interval; BID = twice daily; hr = hour(s); mg = milligram(s); mL = milliliter(s); ng = nanogram(s)

Source: ARRAY-380-101 CSR, Figure 4

Figure 6　Evaluation of dose proportionality for tucatinib C_{max}



Source: ARRAY-380-101 CSR, Figure 15.4.6b

2.1.2. HER2 陽性 mBC 患者にツカチニブを併用投与した臨床試験

2.1.2.1. HER2 陽性 mBC 患者にツカチニブとカペシタビン、トラスツズマブ及び両剤を併用投与した第 1b 相：ONT-380-005 試験

本治験は、HER2 陽性 mBC 患者にツカチニブ（Tuc）とトラスツズマブ（Tras）、カペシタビン（Cap）及び両剤（Tras + Cap）を併用したときのツカチニブの RP2D を特定し、ONT-380-206 試験の用量の設定根拠を示すための非盲検試験である。本治験で検討した併用は、ツカチニブとカペシタビン（併用 1A：Tuc 300 mg BID + Cap 及び併用 1B：Tuc 350 mg BID + Cap）、ツカチニブとトラスツズマブ（併用 2：Tuc 300 mg BID + Tras 及び Tuc 350 mg BID + Tras）及びツカチニブとカペシタビン及びトラスツズマブ（併用 3：Tuc 300 mg BID + Cap + Tras）である。

本治験では 3 + 3 デザインを用いた用量漸増法により、各併用時のツカチニブ（錠剤）の MTD 及び RP2D を決定した。併用 1 及び併用 2 について 3 つの用量コホートを計画し、安全性データに基づいてコホートを進めた。これら 2 つの併用の両方で忍容性が認められた場合、併用 3 を評価することとした。本治験に計 60 例を組み入れ、うち 24 例（併用 1 は 11 例、併用 3 は 13 例）を PK 解析に含めた。PK 評価は、用量漸増コホート又は拡大コホートにおいて併用 1 及び併用 3 の投与を受けた患者のみを対象に実施した。併用 3 は CNS 転移無しの患者をコホート 3A、転移有りの患者をコホート 3E として評価した。

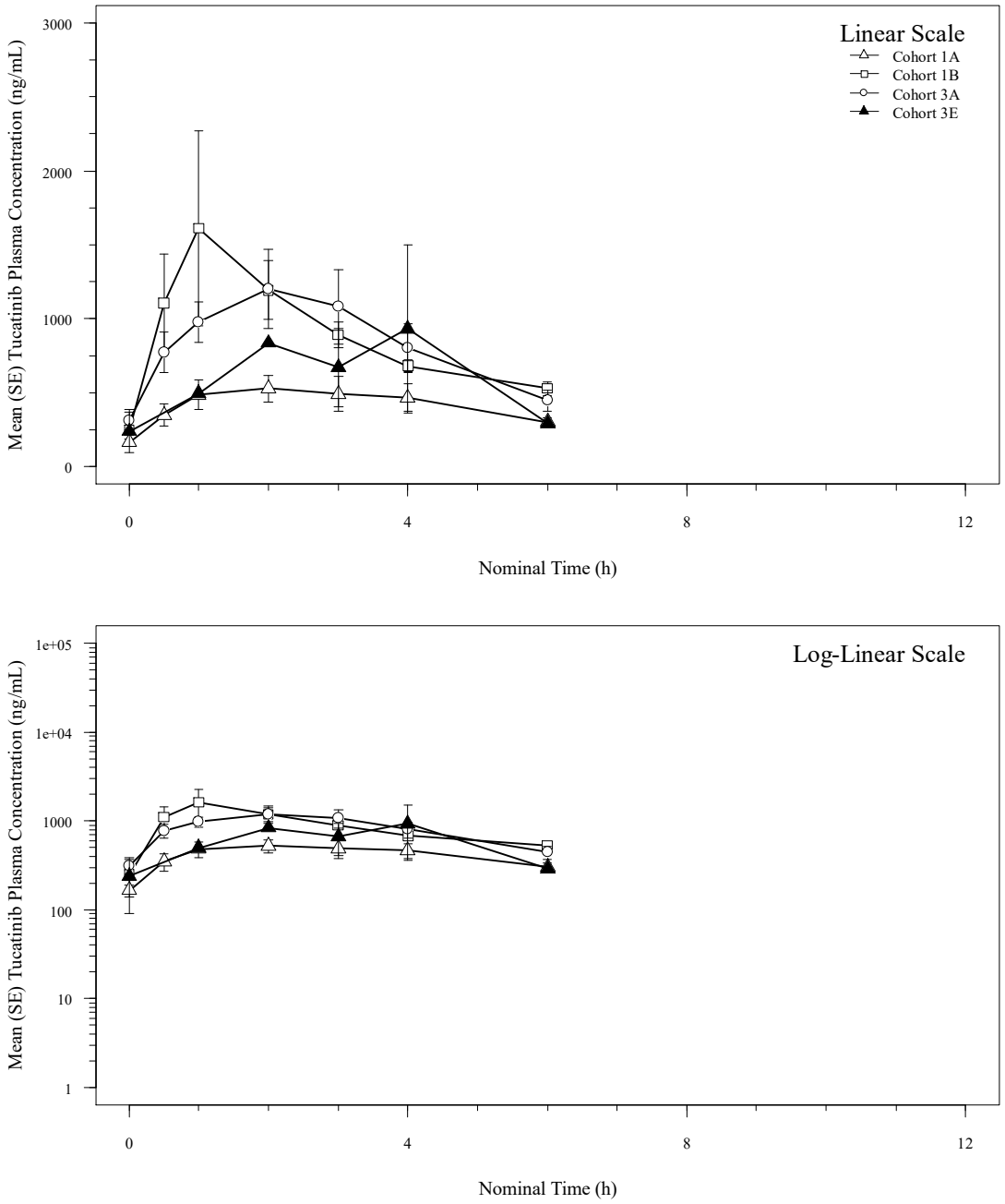
- ツカチニブ及び ONT-993 の血漿中濃度

ツカチニブをカペシタビン又はカペシタビン+トラスツズマブとの併用で反復投与したときのツカチニブ及び ONT-993 の血漿中濃度-時間推移をそれぞれ Figure 7 及び Figure 8 に示す（Cycle 1 Day 14）。血漿中ツカチニブ濃度-時間推移は、ツカチニブ投与後 6 時間まで評価し、ツカチニブ 300 mg 各コホートの推移は変動が認められたものの、吸収から消失への推移の形状は概ね同様であった。血漿中 ONT-993 濃度-時間推移は、ツカチニブの濃度推移の形状と概ね類似していた。

- ツカチニブ及び ONT-993 の PK パラメータ

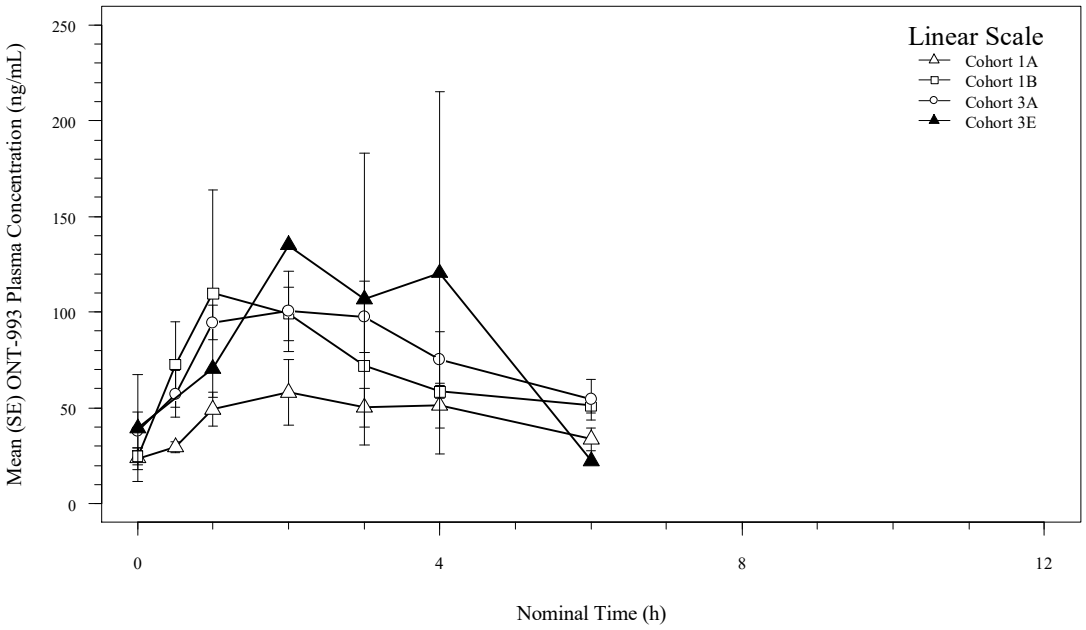
反復投与後のツカチニブの PK パラメータを Table 4 に要約する。ツカチニブの T_{max}（中央値）は 1～3 時間（Cycle 1 Day 14）であった。個々の患者の曝露量は変動があり、コホート 3A ではやや高値を示したものの、ツカチニブ単剤初回投与時の曝露量（2.1.2.4）及び AR（3.5.1）から想定される傾向であり、ツカチニブ 300 mg コホート間（併用 1A 及びコホート 3E）では大きな違いは認められなかった。コホート 3A の Cycle 1 Day 14 の C_{trough}（算術平均値）は 310.0 ng/mL であり、Cycle 1～5 のツカチニブの C_{trough}（Day 1 の投与前）も変動が認められたが、ツカチニブ 300 mg コホート間では比較的一貫していた。ONT-993 の PK パラメータを Table 5 に要約する。ツカチニブと同様に、曝露量は変動があったものの、ツカチニブ 300 mg コホート間（併用 1A 及びコホート 3E）では大きな違いは認められなかった。併用 3 におけるツカチニブの T_{max}（中央値）は 2～3 時間（Cycle 1 Day 14）、消失相における PK 用採血時点は限定的であったもののコホート 3A では $t_{½}$（幾何平均値）は約 3 時間、MR_{AUC} は約 0.1 であった。

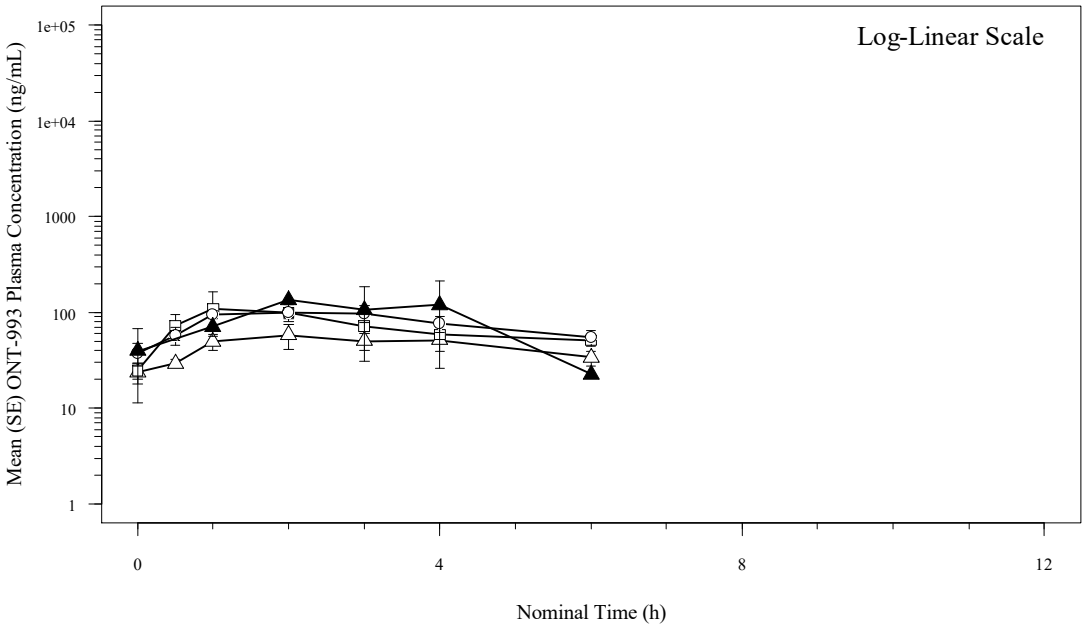
Figure 7　Mean ± SE plasma concentrations of tucatinib following multiple dose administrations of tucatinib in combination with capecitabine alone or capecitabine + trastuzumab – Cycle 1, Day 14 (linear and log-linear scales)



Cohort 1A: 300 mg Tucatinib BID + 1000 mg/m² Capecitabine BID in Subjects with HER2+ Metastatic Breast Cancer
Cohort 1B: 350 mg Tucatinib BID + 1000 mg/m² Capecitabine BID in Subjects with HER2+ Metastatic Breast Cancer
Cohort 3A: 300 mg Tucatinib BID + 1000 mg/m² Capecitabine BID + 8 mg/kg C1D1 and 6 mg/kg q21 IV Infusion of Trastuzumab in Subjects with HER2+ Metastatic Breast Cancer
Cohort 3E: 300 mg Tucatinib BID + 1000 mg/m² Capecitabine BID + 8 mg/kg C1D1 and 6 mg/kg q21 IV Infusion of Trastuzumab in Subjects with Central Nervous System Metastases
Source: ONT-380-005 PK Report; Figure 5-1

Figure 8　Mean ± SE plasma concentrations of ONT-993 following multiple dose administrations of tucatinib in combination with capecitabine alone or capecitabine + trastuzumab – Cycle 1, Day 14 (linear and log-linear scales)





Cohort 1A: 300 mg Tucatinib BID + 1000 mg/m² Capecitabine BID in Subjects with HER2+ Metastatic Breast Cancer
Cohort 1B: 350 mg Tucatinib BID + 1000 mg/m² Capecitabine BID in Subjects with HER2+ Metastatic Breast Cancer
Cohort 3A: 300 mg Tucatinib BID + 1000 mg/m² Capecitabine BID + 8 mg/kg C1D1 and 6 mg/kg q21 IV Infusion of Trastuzumab in Subjects with HER2+ Metastatic Breast Cancer
Cohort 3E: 300 mg Tucatinib BID + 1000 mg/m² Capecitabine BID + 8 mg/kg C1D1 and 6 mg/kg q21 IV Infusion of Trastuzumab in Subjects with Central Nervous System Metastases
Source: ONT-380-005 PK Report, Figure 5-5

Table 4. Geometric mean (Geo Mean CV%) PK parameters for plasma tucatinib following multiple dose administrations of tucatinib in combination with capecitabine alone or capecitabine + trastuzumab

Cohort	1A		1B	3A	3E
Subject Population	mBC		mBC	mBC	CNSM
Tucatinib	300 mg BID		350 mg BID	300 mg BID	300 mg BID
Capecitabine	1000 mg/m^2 BID		1000 mg/m^2 BID	1000 mg/m^2 BID	1000 mg/m^2 BID
Trastuzumab	-		-	8 mg/kg then 6 mg/kg q21	8 mg/kg then 6 mg/kg q21
Plasma PK Parameters[a]	Cycle 1, Day 14	Cycle 1, Day 21	Cycle 1, Day 14	Cycle 1, Day 14	Cycle 1, Day 14
N	6[c]	6[c]	3[d]	9[e]	2
AUC_{0-6} (ng·h/mL)	2485 (31.5);5	2371 (37.7);6	5238 (31.6);3	4721 (39.1);6	2076 (NC);1
AUC_{last} (ng·h/mL)	2469 (32.4);5	2378 (38.4);6	5204 (30.8);3	4724 (39.1);6	2076 (NC);1
C_{max} (ng/mL)	634 (24.1);5	539 (44.6);6	1483 (71.1);3	1132 (36.7);6	856 (NC);2
T_{max}[b] (h)	2.00 (0.50; 4.02);5	5.00 (0.50; 6.08);6	1.00 (0.50; 2.00);3	2.00 (1.07, 3.00);6	3.00 (2.00; 4.00);2
t½ (h);n	5.59 (49.0);3	2.64 (NC);1	3.32 (13.9);3	2.57 (33.0);3	6.22 (NC);1

NC = Not Calculated;
[a]Geometric Mean (GeoCV%), n;
[b]Median (Min, Max)
[c]Subject 005-0055-003 did not have samples collected on Cycle 1, Days 14 and 21, PK parameters were not calculated for this subject.
[d]Subject 005-0005-037 had only a pre-dose sample collected in Cycle 1, Days 14 and 21, PK parameters were not reported for this subject.
[e]Subject 005-0003-022 and Subject 005-0003-051 did not have samples collected on Cycle 1, Day 14, PK parameters were not calculated for these subjects.
Source: ONT-380-005 PK Report, Table 5-2

Table 5. Geometric mean (Geo Mean CV%) PK parameters for plasma ONT-993 following multiple dose administrations of tucatinib in combination with capecitabine alone or capecitabine + trastuzumab

Cohort	1A		1B	3A	3E
Subject Population	mBC		mBC	mBC	CNSM
Tucatinib	300 mg BID		350 mg BID	300 mg BID	300 mg BID
Capecitabine	1000 mg/m^2 BID		1000 mg/m^2 BID	1000 mg/m^2 BID	1000 mg/m^2 BID
Trastuzumab	-		-	8 mg/kg then 6 mg/kg q21	8 mg/kg then 6 mg/kg q21
Plasma PK Parameters[a]	Cycle 1, Day 14	Cycle 1, Day 21	Cycle 1, Day 14	Cycle 1, Day 14	Cycle 1, Day 14
N	6[c]	6[c]	3[d]	9[e]	2
AUC_{0-6} (ng·h/mL)	291 (37.3);4	259 (80.6);6	372 (NC);2	477 (35.6);5	151 (NC);1
AUC_{last} (ng·h/mL)	253 (46.6);5	260 (82.0);6	420 (22.1);3	421 (45.4);6	275 (NC);2
C_{max} (ng/mL)	62.7 (47.6);5	60.7 (101.5);6	118 (60.0);3	107 (33.7);6	85.2 (NC);2
T_{max}[b] (h)	2.00 (1.00; 4.02);5	2.00 (1.00; 6.08);6	2.00 (1.00; 2.00);3	1.53 (1.00; 3.00);6	3.00 (2.00; 4.00);2
t½ (h); n	5.78 (NC);1	3.65 (NC);1	3.90 (NC);1	2.95 (21.8);3	6.99 (NC);1

BID = twice daily; CNSM = central nervous system metastases; mBC = metastatic breast cancer; NC = not calculated; q21 = every 21 days
[a]Geometric Mean (GeoCV%), n;
[b]Median (Min, Max)
[c]Subject 005-0055-003 did not have samples collected on Cycle 1, Days 14 and 21, PK parameters were not calculated for this subject.
[d]Subject 005-005-037 had only a pre-dose sample collected in Cycle 1, Days 14 and 21, PK parameters were not reported for this subject.
[e]Subject 005-0003-022 and Subject 005-0003-051 did not have samples collected on Cycle 1, Day 14, PK parameters were not calculated for these subjects.
Source: ONT-380-005 PK Report, Table 5-3

2.1.2.2. HER2 陽性 mBC 患者にツカチニブとカペシタビン及びトラスツズマブを併用投与した第 2 相比較試験：ONT-380-206（HER2CLIMB）試験

本治験は、前治療歴を有する HER2 陽性 mBC 患者にツカチニブとトラスツズマブ及びカペシタビンを併用したときの無作為化、国際多施設共同、二重盲検試験である。

全例を対象に、ツカチニブ 300 mg BID 投与時のツカチニブ及び ONT-993 の定常状態での PK を評価し、Cycle 2～6 Day 1 の投与前に C_{trough}、Cycle 3 Day 1 の投与後 1～4 時間以内に投与後の濃度を評価した。休薬又は投与中断にかかわらず、スケジュール通りに PK 評価用検体を採取した。初回評価時（Cycle 2 Day 1）では、評価可能な 373 例中 333 例で C_{trough} が測定可能であった（ONT-380-206 CSR Table 14.2.9.1）。Cycle 2～6 では 333～257 例で PK 評価が可能であった（ONT-380-206 CSR Table 14.2.9.1）。

- ツカチニブ及び ONT-993 の血漿中濃度

全投与サイクルを通じたツカチニブの C_{trough} の中央値は 183.5～202 ng/mL、幾何平均値は 150.3～182.5 ng/mL であり、変動が大きかった（%CV 範囲：91%～111%）。前夜のツカチニブ投与時刻が記録されなかったため、前夜のツカチニブ投与から次のサイクルの来院までの時間が治験参加者によってばらついた可能性があり、C_{trough} の変動要因の 1 つと考えられた。Cycle 3 Day 1 のツカチニブ投与後 1～4 時間における血漿中ツカチニブ濃度の中央値及び幾何平均値（%CV）は、それぞれ 458.0 ng/mL 及び 361.0 ng/mL（70%）であった。

全投与サイクルを通じた ONT-993 の C_{trough} の中央値は 16.8～20.5 ng/mL で、血漿中ツカチニブ濃度の約 1/10 であった。ONT-993 の C_{trough} の幾何平均値は 17.8～21.5 ng/mL で、変動が大きかった（%CV 範囲：86%～125%）。Cycle 3 Day 1 のツカチニブ投与後 1～4 時間の血漿中 ONT-993 濃度の中央値及び幾何平均値（%CV）はそれぞれ 38.6 ng/mL 及び 35.0 ng/mL（99%）であった。

血漿中ツカチニブ濃度の治験参加者別一覧を ONT-380-206 CSR Listing 16.2.6.7 に示す。血漿中ツカチニブ濃度の規定採血時点毎の箱ひげ図を ONT-380-206 CSR Figure 14.2.9.2 に示す。血漿中 ONT-993 濃度の治験参加者別一覧を ONT-380-206 CSR Listing 16.2.6.8 に示す。血漿中 ONT-993 濃度の規定採血時点毎の箱ひげ図を ONT-380-206 CSR Figure 14.2.9.3 に示す。

- 曝露反応解析

血漿中ツカチニブ濃度と有効性及び安全性との関連性に関する曝露反応解析の結果は 3.3 に示す。

2.1.2.3. HER2 陽性 mBC 患者にツカチニブとカペシタビン及びトラスツズマブを併用投与した第 2 相試験：MK-7119-001（C4251013）試験

本治験は、前治療歴を有する HER2 陽性 mBC 患者にツカチニブとトラスツズマブ及びカペシタビンを併用したときの非盲検、単群、多施設、第 2 相試験である。本治験は日本、韓国及び台湾で実施し、各国で組み入れた治験参加者をそれぞれ日本人、韓国人及び台湾人集団と定義した。本治験では主試験に患者を登録する前に日本人患者 4 例を対象として、トラスツズマブ及びカペシタビンとの併用下でのツカチニブの安全性、忍容性及び PK を評価するため、safety

run-in を実施した。主試験では全例を対象に、ツカチニブ 300 mg BID 投与時（各サイクル 21 日間）のツカチニブ及び ONT-993 の定常状態での PK を評価し、Cycle 2～6 Day 1 の投与前に C_{trough}、Cycle 3 Day 1 の投与後 1～4 時間以内に投与後の濃度を評価した。

- ツカチニブ及び ONT-993 の PK

Safety run-in において日本人患者 4 例に Cycle 1 Day 1 でツカチニブ（300 mg）をトラスツズマブ及びカペシタビンとの併用で投与したときのツカチニブの血漿中濃度-時間推移を Figure 9 に示し、ツカチニブ及び ONT-993 の PK パラメータを Table 6 に要約する。Safety run-in のみで評価した Cycle 1～2 Day 8 及び Day 15 のツカチニブ及び ONT-993 の C_{trough} を Table 7 に要約する。

主試験における全集団（PK 解析対象 65 例）での Cycle 2～6 Day 1 のツカチニブ及び ONT-993 の C_{trough}、及び Cycle 3 Day 1 の投与後 1～4 時間以内のツカチニブ及び ONT-993 の血漿中濃度の箱ひげ図を Figure 10 に示し、日本人集団（PK 解析対象 52 例）の結果を Figure 11 に示す。

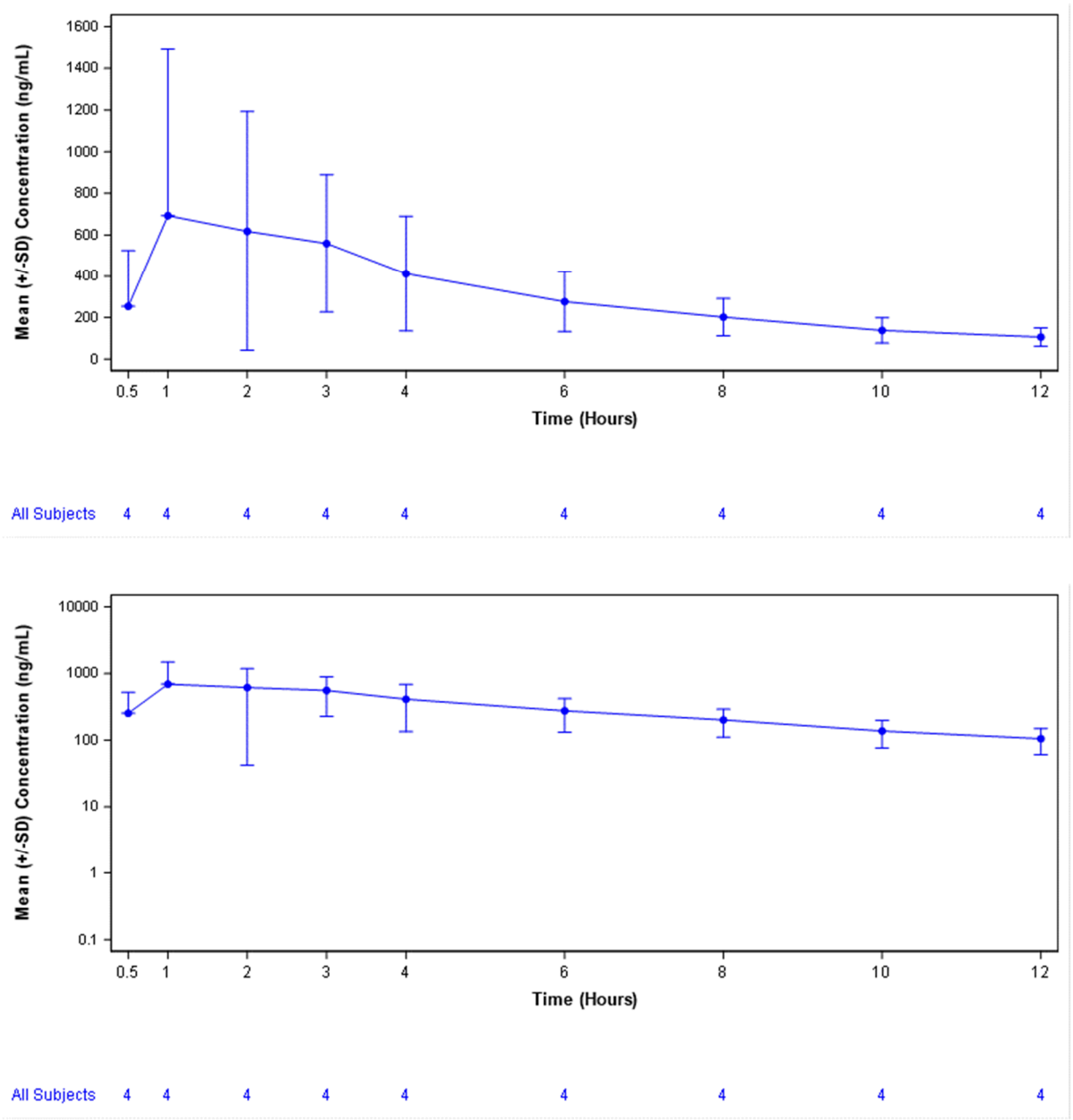
Safety run-in における Cycle 1 Day 1 のツカチニブの T_{max}（中央値）は 2.9 時間、C_{max} 及び AUC_{last}（幾何平均値）は、それぞれ 561.2 ng/mL 及び 2953.4 ng•h/mL、$t_{½}$（算術平均値）は 3.7 時間であった。ONT-993 は、それぞれ 2.9 時間、66.9 ng/mL、365.2 ng•h/mL 及び 5.4 時間であった。Cycle 1～2 Day 8 及び Day 15 の定常状態におけるツカチニブ及び ONT-993 の C_{trough}（幾何平均値）は、それぞれ 244.5～355.9 ng/mL 及び 43.3～49.1 ng/mL であった。

主試験の全集団における Cycle 2～6 Day 1 のツカチニブ及び ONT 993 の C_{trough}（幾何平均値）は、それぞれ 350.9～421.5 ng/mL 及び 23.3～30.2 ng/mL であった。Cycle 3 Day 1 の投与後 1～4 時間以内のツカチニブ及び ONT 993 の血漿中濃度（幾何平均値）は、それぞれ 711.2 ng/mL 及び 51.7 ng/mL であった。日本人集団では、Cycle 2～6 Day 1 のツカチニブ及び ONT 993 の C_{trough}（幾何平均値）は、それぞれ 307.1～421.7 ng/mL 及び 23.3～31.3 ng/mL、Cycle 3 Day 1 の投与後 1～4 時間以内では、それぞれ 689.3 ng/mL 及び 53.7 ng/mL であった。全集団と日本人集団では、ツカチニブ及び ONT 993 の C_{trough} 及び投与後の血漿中濃度は同様であった。

- 曝露反応解析

血漿中ツカチニブ濃度と有効性及び安全性との関連性に関する曝露反応解析の結果は 3.3 に示す。

Figure 9　Mean ± SD plasma tucatinib concentration vs time plot by cycle overlaid by dose for subjects without dose change (Upper: linear, Bottom: log-linear scales)



The vertical lines extend to mean +/- SD. The lower part of the vertical line is not presented if the mean minus SD is a negative value.
Source: MK-7119-001 CSR, Figure 14.8.3.1, Figure 14.8.3.2

Table 6. Summary of tucatinib and ONT-993 pharmacokinetic parameters, safety run-in set

Analyte	Statistic	C_{max} (ng/mL)	T_{max} (h)	AUC_{last} (ng•h/mL)	AUC_{inf} (ng•h/mL)	$t_{½}$ (h)
Tucatinib	N	4	4	4	3	3
	Mean	826.3	3.2	3648.8	5123.4	3.7
	SD	741.0	2.1	2406.9	2351.7	0.4
	Geometric Mean	561.2	2.7	2953.4	4698.0	3.7
	Geometric CV%	156.0	85.9	95.1	57.7	9.7
	Min	132	1	1068	2575	3
	Median	661.5	2.9	3563.3	5586.3	3.9
	Max	1850	6	6400	7209	4
ONT-993	N	4	4	4	3	3
	Mean	86.4	3.9	423.3	651.8	5.4
	SD	61.0	2.8	228.5	228.6	1.7
	Geometric Mean	66.9	3.4	365.2	627.7	5.2
	Geometric CV%	114.5	69.1	77.2	33.7	31.2
	Min	19	2	139	503	4
	Median	79.9	2.9	428.4	537.4	4.7
	Max	167	8	697	915	7

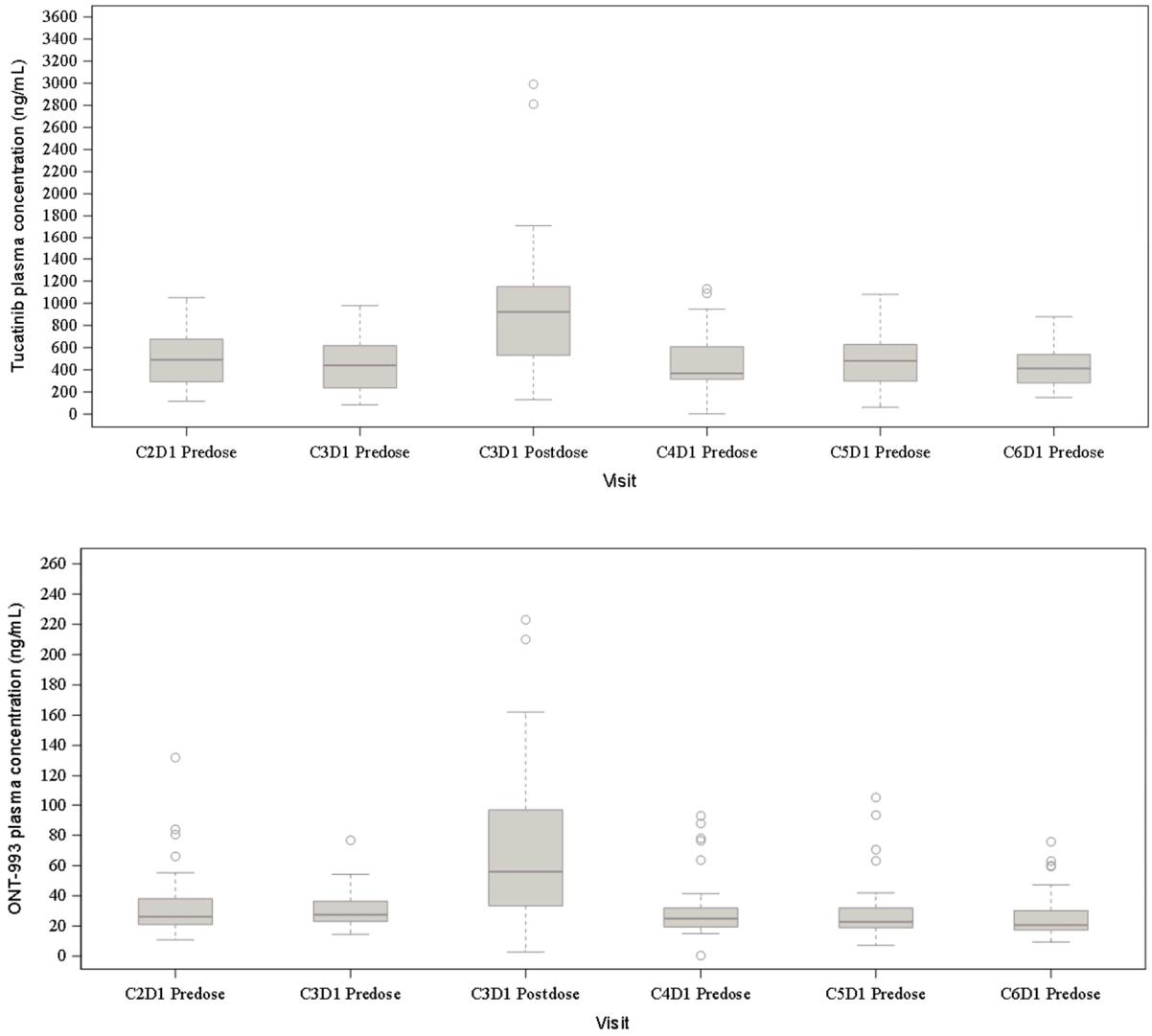
AUC_{inf}: area under the plasma concentration time curve from time zero extrapolated to infinity. AUC_{last}: area under the plasma concentration time curve from time zero until the last quantifiable concentration. C_{max}: maximum observed plasma concentration. T_{max}: time of C_{max}.
Source: MK-7119-001 CSR, Table 30, Table 14.8.2.1, Table 14.8.2.2

Table 7. Summary of tucatinib and ONT-993 C_{trough} on Day 8 and 15 in Cycle 1-2, safety run-in set

Analyte	Statistic	Cycle 1 Day 8	Cycle 1 Day 15	Cycle 2 Day 8	Cycle 2 Day 15
Tucatinib	N	4	3	4	1
	Mean	334.5	262.3	372.0	NC
	SD	151.2	126.0	124.9	NC
	Geometric Mean	297.8	244.5	355.9	NC
	Geometric CV%	68.1	47.0	35.8	NC
	Min	120	177	242	NC
	Median	373.0	203.0	368.5	269
	Max	472	407	509	NC
ONT-993	N	4	3	4	1
	Mean	46.7	49.0	50.5	NC
	SD	19.1	23.0	13.1	NC
	Geometric Mean	43.3	45.8	49.1	NC
	Geometric CV%	49.6	46.5	28.0	NC
	Min	23	32	34	NC
	Median	50.1	39.6	51.0	37.3
	Max	63	75	66	NC

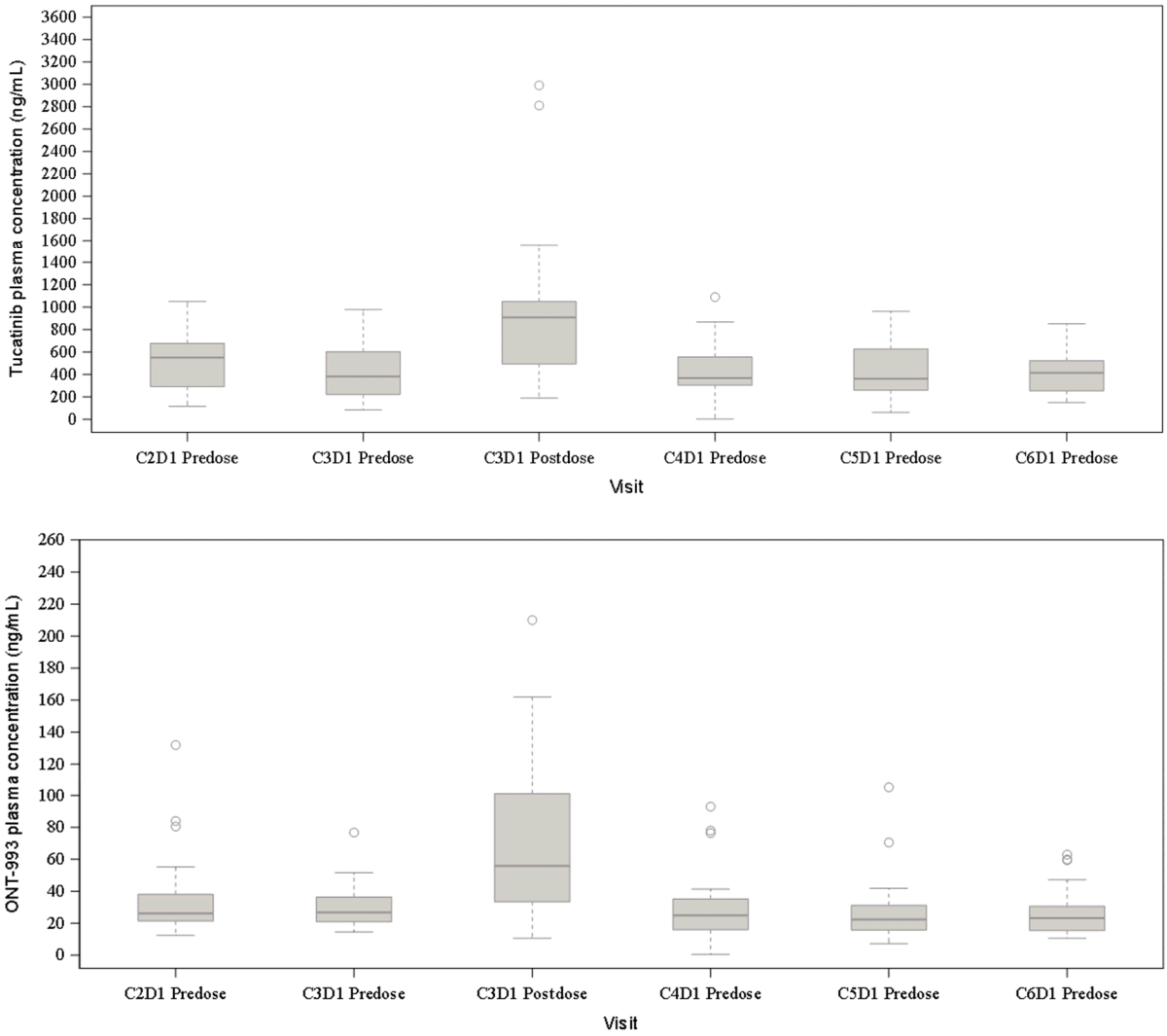
For n < 3, STD and geometric mean of coefficient of variation are not calculated and set to missing.
The lower limit of qualification (LLOQ) is 1 ng/mL. The values < LLOQ were analyzed as LLOQ/2. Timepoints were not summarized if more than 50% of results were < LLOQ. Pre-dose samples taken outside the 8-16 hours window post the previous dosing, and post-dose samples taken outside of protocol defined time frame were excluded.
Source: MK-7119-001 CSR, Table 14.8.1.1, Table 14.8.1.2

Figure 10　Boxplot of tucatinib and ONT-993 concentration by nominal time point (Upper: Tucatinib, Bottom: ONT-993)



The length of the box represents the interquartile range (the distance between the 25th and 75th percentiles). The horizontal line in the box interior represents the group median. The whiskers extend to 1.5 times the interquartile range.
The lower limit of qualification (LLOQ) is 1 ng/mL. The values < LLOQ were analyzed as LLOQ/2. Timepoints were not summarized if more than 50% of results were < LLOQ. Pre-dose samples taken outside the 8-16 hours window post the previous dosing, and post-dose samples taken outside 1-4 hours window were excluded.
Source: MK-7119-001 CSR, Figure 14.8.1.1, Figure 14.8.1.3

Figure 11　Boxplot of tucatinib and ONT-993 concentration by nominal time point in Japanese population (Upper: Tucatinib, Bottom: ONT-993)



The length of the box represents the interquartile range (the distance between the 25th and 75th percentiles). The horizontal line in the box interior represents the group median. The whiskers extend to 1.5 times the interquartile range.
The lower limit of qualification (LLOQ) is 1 ng/mL. The values < LLOQ were analyzed as LLOQ/2. Timepoints were not summarized if more than 50% of results were < LLOQ. Pre-dose samples taken outside the 8-16 hours window post the previous dosing, and post-dose samples taken outside 1-4 hours window were excluded.
Source: MK-7119-001 CSR, Figure 14.8.1.2, Figure 14.8.1.4

2.1.2.4. HER2 陽性 mBC 患者にツカチニブと T-DM1 を併用投与した第 1b 相試験：ONT-380-004 試験

本治験は、HER2 陽性 mBC 患者にツカチニブを T-DM1 と併用したときの安全性、忍容性及び MTD/RP2D を評価する非盲検試験である。本治験では、3 + 3 用量漸増デザインを用いて最大 4 用量のツカチニブを T-DM1 と併用投与したときの MTD/RP2D を決定した。Cycle 1 Day 1 はツカチニブのみを BID 投与し、Cycle 1 Day 2 に T-DM1 を投与した。その後のすべてのサイクルでは Day 1 に T-DM1 を投与した。本治験に組み入れられてツカチニブの投与を 1 回以上受け、定量可能なツカチニブ又は ONT-993 濃度が 1 回以上得られた 39 例を PK 解析対象集団とした。Cycle 1 で 32 例がツカチニブ 300 mg BID に、7 例がツカチニブ 350 mg BID に割り付けられた。Cycle 2 では、150 mg BID（300 mg から減量）投与を受けた 2 例、200 mg BID（300 mg から減量）投与を受けた 1 例、250 mg BID（300 mg から減量）投与を受けた 1 例、300 mg BID 投与を受けた 27 例（用量変更なし 26 例、350 mg から減量 1 例）及び 350 mg BID 投与を受けた 5 例（用量変更なし）の計 36 例を解析対象とした。

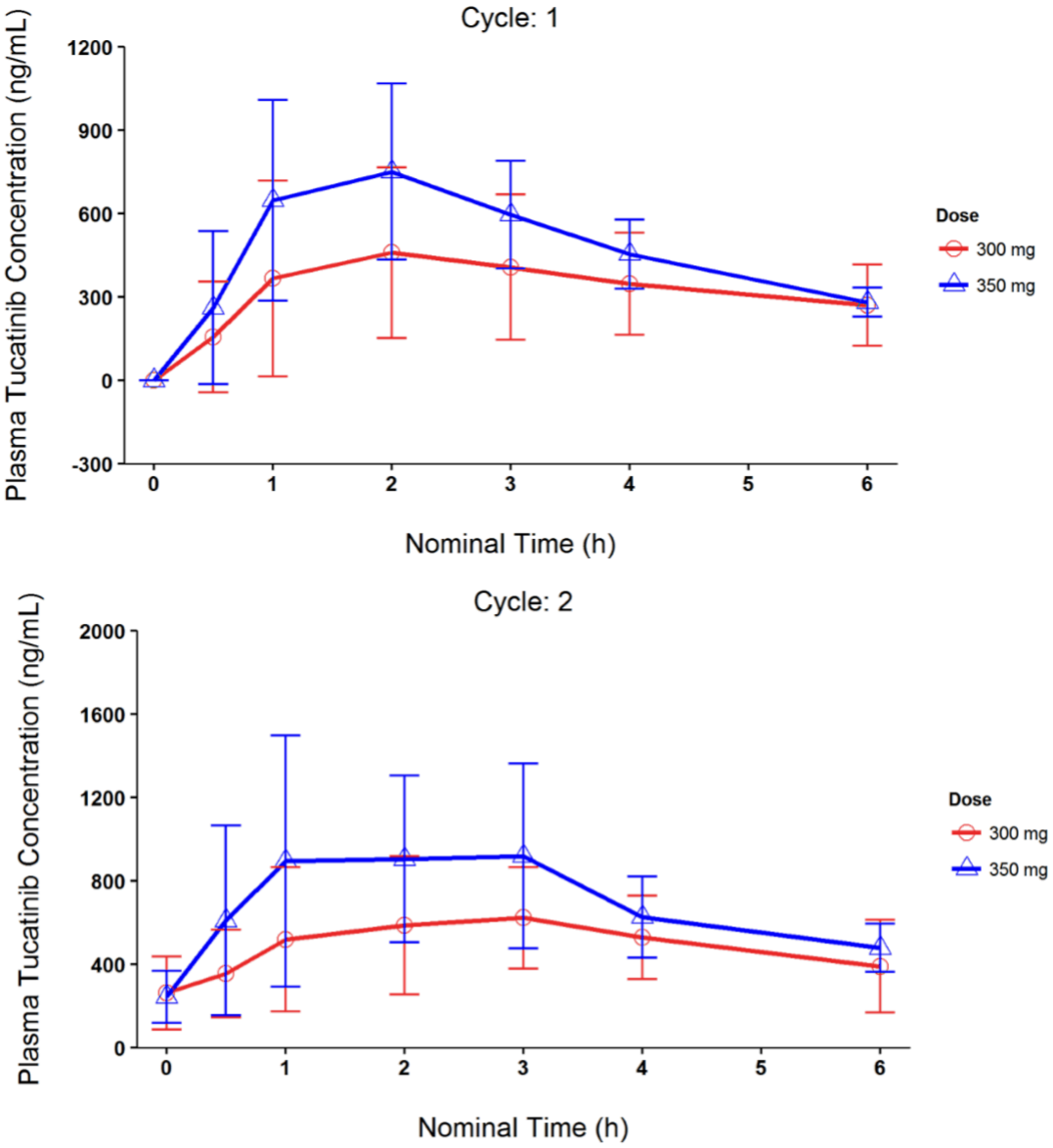
- ツカチニブ及び ONT-993 の血漿中濃度

ツカチニブの血漿中濃度-時間推移（Cycle 1 Day 1 及び Cycle 2 Day 1）及び各サイクルの C_{trough}（Cycle 2～4）をそれぞれ Figure 12 及び Figure 13 に示す。ONT-993 の血漿中濃度-時間推移（Cycle 1 Day 1 及び Cycle 2 Day 1）を Figure 14 に示す。血漿中濃度-時間推移は、ツカチニブ投与後 6 時間まで評価し、患者間で変動が認められたものの、ツカチニブの用量に応じた推移が示された。ツカチニブの C_{trough} は Cycle 2～4 で一貫していた。

- ツカチニブ及び ONT-993 の PK パラメータ

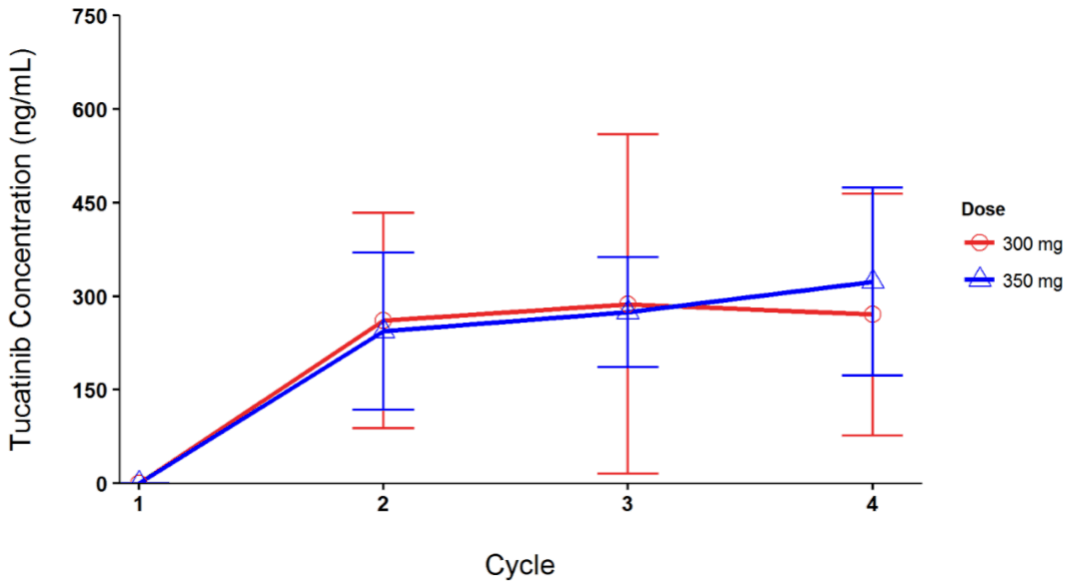
Cycle 1 及び Cycle 2 で用量を変更しなかった患者における 300 mg 及び 350 mg 投与時のツカチニブ及び ONT-993 の PK パラメータを Table 8 及び Table 9 に要約する。ツカチニブの T_{max}（中央値）は、Cycle 1 ではツカチニブ 300 mg 及び 350 mg で約 2 時間、Cycle 2 では 300 mg で約 2 時間、350 mg で約 1 時間であった。ONT-993 の T_{max}（中央値）は各サイクルでいずれの投与量においても約 2 時間であり、ツカチニブと同様であった。消失相における PK 用採血時点は限定的であったものの、ツカチニブの $t_{½}$（幾何平均値）は、約 3～4 時間であった。反復投与時のツカチニブの C_{max} 及び AUC_6 の AR は、それぞれ 300 mg では 1.54 及び 1.70、350 mg では 1.44 及び 1.54 であった。ONT-993 の PK 結果より、代謝物/親化合物比は 11%～13%であり、C_{max} 及び AUC_6 の AR はツカチニブと同程度であった。

Figure 12　Mean ± SD plasma tucatinib concentration vs time plot by cycle overlaid by dose for subjects without dose change



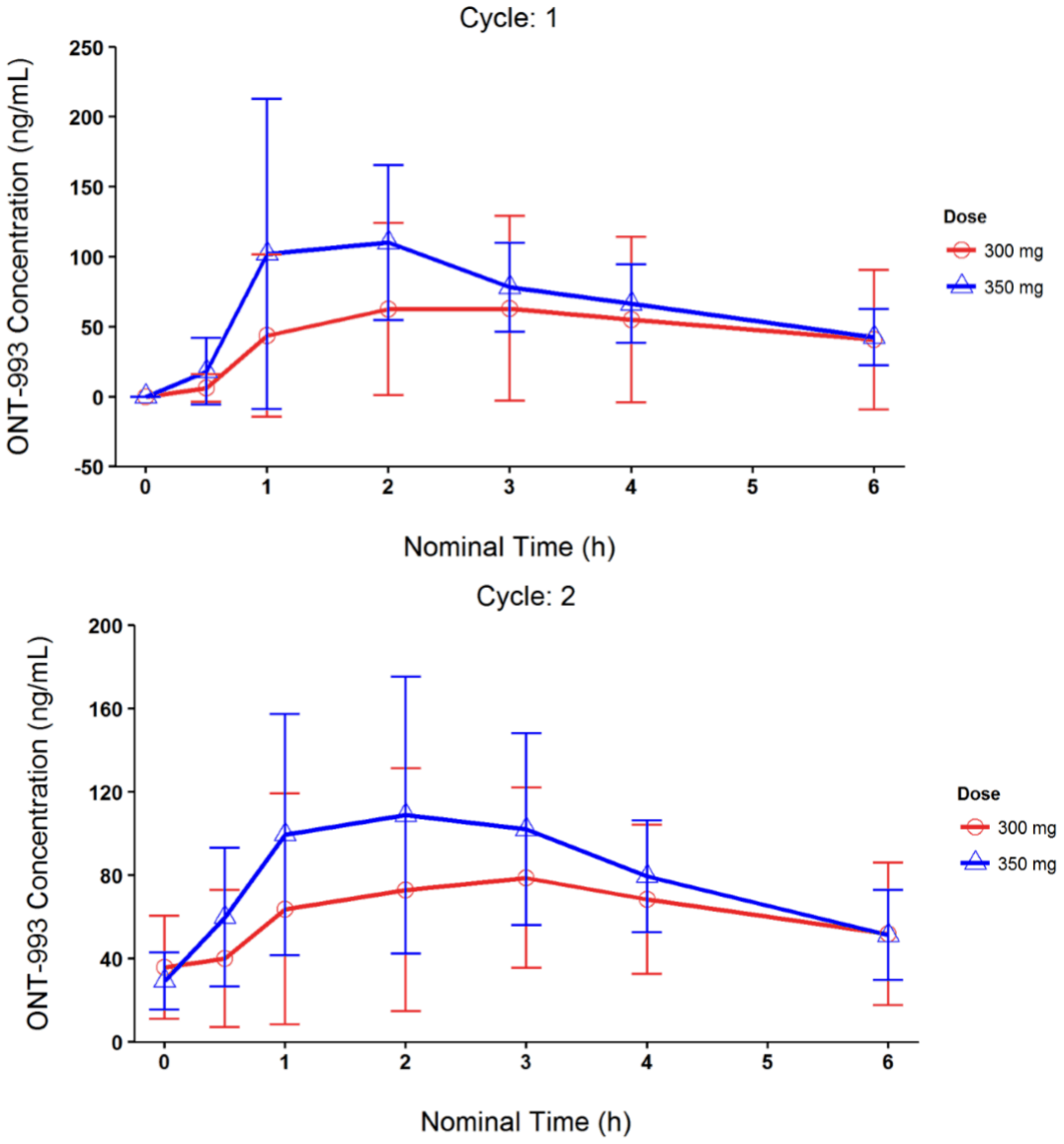
Source: ONT-380-004 PK Report, Figure 4-1

Figure 13　Mean ± SD tucatinib trough (Day 1 predose) concentration vs time plot for Cycles 1-4 overlaid by dose for subjects without dose change



Source: ONT-380-004 PK Report, Figure 4-2

Figure 14　Mean ± SD ONT-993 concentration vs time plot by cycle overlaid by dose for subjects without dose change



Source: ONT-380-004 PK Report, Figure 4-3

Table 8. Tucatinib plasma pharmacokinetic parameters by cycle and dose for subjects without dose change

Cycle	Dose (mg)	Statistic	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-6} (h*ng/mL)	$t_{½}$ (h)	ARC_{max}	$ARAUC_{0-6}$
1	300	N	32	32	30	29	0	0
		Mean	601	NC	2050	5.83	NC	NC
		SD	318	NC	1080	9.30	NC	NC
		CV%	52.8	NC	52.7	159	NC	NC
		Geometric Mean	514	NC	1780	3.93	NC	NC
		Geometric CV%	66.4	NC	60.8	81.5	NC	NC
		Min	144	0.92	581	1.91	NC	NC
		Median	542	2.05	1890	3.18	NC	NC
		Max	1310	6.00	5330	51.9	NC	NC
	350	N	7	7	7	7	0	0
		Mean	796	NC	2840	2.99	NC	NC
		SD	283	NC	727	0.878	NC	NC
		CV%	35.5	NC	25.6	29.4	NC	NC
		Geometric Mean	743	NC	2750	2.88	NC	NC
		Geometric CV%	44.1	NC	28.8	30.5	NC	NC
		Min	366	0.95	1700	1.95	NC	NC
		Median	935	1.97	2970	2.77	NC	NC
		Max	1070	3.00	3750	4.16	NC	NC
2	300	N	26	26	22	22	26	20
		Mean	791	NC	2920	4.83	1.76	1.90
		SD	335	NC	1100	3.09	1.02	1.01
		CV%	42.4	NC	37.6	64.0	58.2	53.4
		Geometric Mean	725	NC	2720	4.14	1.54	1.70
		Geometric CV%	45.1	NC	41.5	58.2	53.1	49.8
		Min	311	0.00	1130	2.05	0.634	0.877
		Median	728	2.12	2850	3.74	1.49	1.65
		Max	1590	5.50	5620	13.7	4.71	4.87
	350	N	5	5	5	5	5	5
		Mean	1120	NC	4220	3.37	1.46	1.56
		SD	525	NC	1670	1.24	0.238	0.303
		CV%	46.8	NC	39.5	36.6	16.3	19.4
		Geometric Mean	1010	NC	3910	3.21	1.44	1.54
		Geometric CV%	57.0	NC	47.2	34.6	16.0	20.2
		Min	541	1.00	2390	2.37	1.23	1.16
		Median	1250	1.02	5280	2.73	1.48	1.58
		Max	1700	3.03	5650	5.35	1.82	1.95

AR = accumulation ratio, ARC_{max} = maximum accumulation ratio; N = sample size, Mean = arithmetic mean; SD = standard deviation; CV% = arithmetic percent coefficient of variation; Geo CV% = geometric percent coefficient of variation; Geo mean = geometric mean; Min = minimum; Max = maximum; NC = not calculated
Source: ONT-380-004 PK Report, Table 4-2

Table 9. ONT-993 plasma pharmacokinetic parameters by cycle and dose for subjects without dose change

Cycle	Dose (mg)	Statistic	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-6} (h*ng/mL)	$t_{½}$ (h)	ARC_{max}	$ARAUC_{0-6}$
1	300	N	31	31	27	26	0	0
		Mean	87.9	NC	308	3.56	NC	NC
		SD	80.3	NC	281	2.40	NC	NC
		CV%	91.3	NC	91.3	67.4	NC	NC
		Geometric Mean	59.9	NC	212	3.09	NC	NC
		Geometric CV%	118	NC	115	53.4	NC	NC
		Min	5.81	1.00	23.6	1.40	NC	NC
		Median	63.5	2.03	207	3.02	NC	NC
		Max	338	5.62	1180	11.9	NC	NC
	350	N	7	7	7	7	0	0
		Mean	126	NC	405	5.26	NC	NC
		SD	92.6	NC	226	5.40	NC	NC
		CV%	73.4	NC	55.8	103	NC	NC
		Geometric Mean	100	NC	350	3.84	NC	NC
		Geometric CV%	86.2	NC	65.8	91.0	NC	NC
		Min	33.0	0.97	154	1.99	NC	NC
		Median	94.5	1.97	347	2.82	NC	NC
		Max	05	2.00	742	16.9	NC	NC
2	300	N	26	26	21	20	25	18
		Mean	104	NC	374	5.49	2.04	2.16
		SD	60.6	NC	216	5.19	1.41	1.64
		CV%	58.3	NC	57.9	94.5	68.9	75.8
		Geometric Mean	85.0	NC	307	4.29	1.66	1.74
		Geometric CV%	81.7	NC	79.1	71.3	72.1	73.3
		Min	12.3	0.00	59.6	1.83	0.662	0.730
		Median	108	2.11	352	3.52	1.61	1.55
		Max	254	6.00	863	23.6	5.68	6.64
	350	N	5	5	5	5	5	5
		Mean	127	NC	499	70.6	1.71	1.83
		SD	42.4	NC	209	149	1.80	1.84
		CV%	33.5	NC	41.9	211	105	101
		Geometric Mean	119	NC	458	9.57	1.22	1.32
		Geometric CV%	46.3	NC	52.3	735	104	102
		Min	55.1	1.00	209	3.25	0.511	0.574
		Median	131	2.92	492	3.99	0.984	1.01
		Max	161	3.03	776	337	4.88	5.03

AR = accumulation ratio, AR_{max} = maximum accumulation ratio; N = sample size, Mean = arithmetic mean; SD = standard deviation; CV% = arithmetic percent coefficient of variation; Geo CV% = geometric percent coefficient of variation; Geo mean = geometric mean; Min = minimum; Max = maximum; NC = not calculated

AUC_{0-6} extrapolated greater than 20% for subject 004-0005-043, Cycle 1 Day 1 and for subject 004-0212-029, Cycle 2 Day 1.

Source: ONT-380-004 PK Report, Table 4-3

2.2. 健康成人及び特定の背景を有する患者を対象とした臨床試験

2.2.1. 健康成人に ^{14}C 標識ツカチニブを投与したときの吸収、代謝及び排泄を評価するマスバランス試験：ONT-380-008 試験

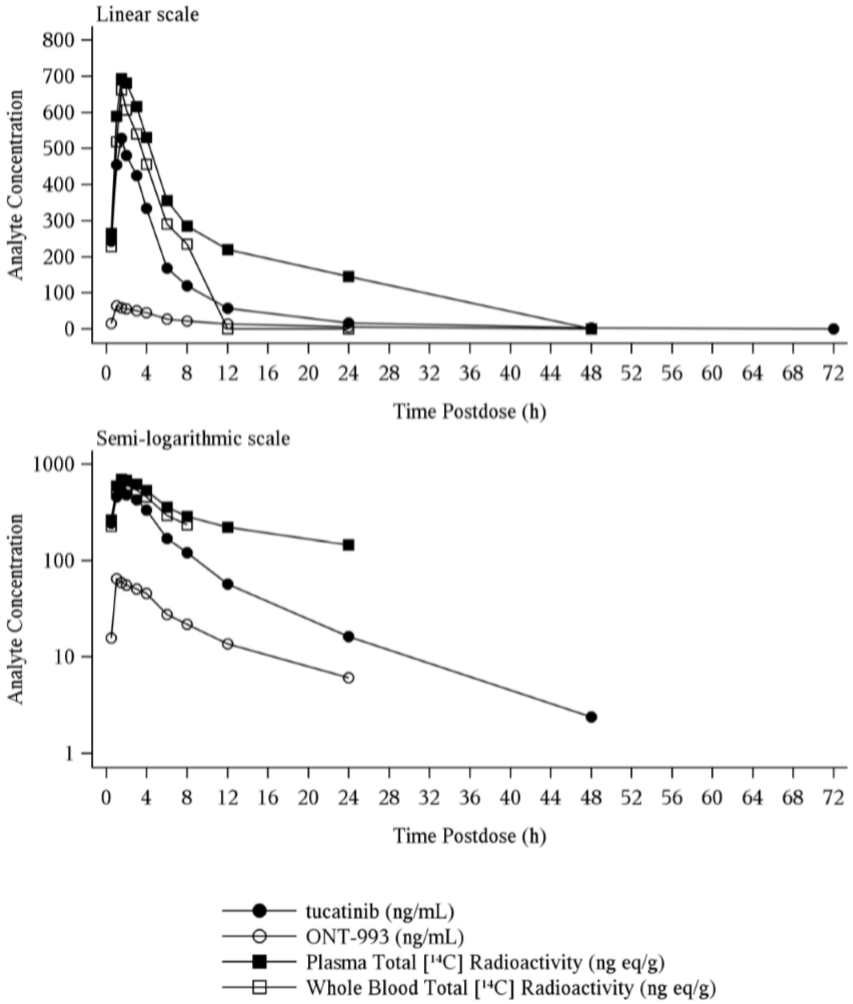
本治験は、健康成人を対象に、^{14}C 標識ツカチニブを単回経口投与後のツカチニブの吸収、代謝及び排泄を評価し、血漿中、尿中及び糞便中に存在する代謝物の特性を検討する非盲検試験である。本治験に 8 例（男性 4 例、女性 4 例）を組み入れ、^{14}C 標識ツカチニブ約 150 μCi を含むツカチニブ 317 mg の単回経口投与後、全例を対象に PK 解析を実施した。

2.2.1.1. 血漿中及び全血中の総放射能の PK

ツカチニブ及び ONT-993 の血漿中及び全血中濃度-時間推移を Figure 15 に示し、PK パラメータを Table 10 に要約する。

ツカチニブ及び ONT-993 の血漿中及びは速やかな吸収を示し、T_{max}（中央値）はそれぞれ 1.5 時間及び 1 時間であった。血漿中ツカチニブ及び ONT-993 の $t_{½}$（幾何平均値）は、それぞれ 7.13 時間及び 9.98 時間であった。血漿中ツカチニブ/総放射能 AUC 比の幾何平均値から、血漿中の未変化体は総放射能の約 33%（AUC_{inf}）～43%（AUC_t）であった。血液/血漿中総放射能 AUC 比の幾何平均値は 0.557（AUC_{inf}）及び 0.508（AUC_t）であり、放射能の赤血球への移行率は低いことが示された。

Figure 15　Arithmetic mean concentration profiles of tucatinib and ONT-993 in plasma (ng/mL), total [^{14}C] radioactivity in plasma and whole blood



Source: ONT-380-008 CSR, Figure 2

Table 10. Summary of pharmacokinetic parameters for tucatinib and ONT-993 in plasma and for total radioactivity in plasma and whole blood

	[^{14}C]-tucatinib 300 mg			
	Tucatinib in Plasma	ONT-993 in Plasma	Plasma Total [^{14}C] Radioactivity	Blood Total [^{14}C] Radioactivity
AUC_{0-t} (h*ng eq/g)	---	---	7410 (37.7)	3770 (39.4)
AUC_{0-t} (h*ng/mL)	3200 (34.9)	541 (28.1)	---	---
$AUC_{0-\infty}$ (h*ng eq/g)	---	---	10600 (46.2)	5790 (46.2)
$AUC_{0-\infty}$ (h*ng/mL)	3220 (34.7)	582 (24.2)	---	---
$\%AUC_{extrap}$ (%)	NA	NA	NA	NA
C_{max} (ng eq/g)	---	---	679 (27.0)	648 (24.3)
C_{max} (ng/mL)	515 (30.2)	63.8 (21.3)	---	---
t_{max}[a] (h)	1.50 (1.00-2.00)	1.00 (1.00-2.00)	1.50 (1.50-2.00)	1.50 (1.50-2.00)
$t_{1/2}$ (h)	7.13 (26.8)	9.98 (23.8)	18.5 (39.0)	6.58 (54.5)
CL/F (L/h)	98.4 (34.7)	---	---	---
V_z/F (L)	1010 (26.3)	---	---	---
MR $AUC_{0-\infty}$	---	0.181 (30.8)	---	---
MR C_{max}	---	0.124 (22.3)	---	---
Plasma tucatinib/Total [^{14}C] Radioactivity AUC_{0-t} Ratio	0.432 (18.6)	---	---	---
Plasma tucatinib/Total [^{14}C] Radioactivity $AUC_{0-\infty}$ Ratio	0.331 (23.3)	---	---	---
Blood/Plasma Total [^{14}C] Radioactivity/AUC_{0-t} Ratio	---	---	---	0.508 (13.6)
Blood/Plasma Total [^{14}C] Radioactivity/$AUC_{0-\infty}$ Ratio	---	---	---	0.557 (6.65)

Geometric mean (CV%) data are presented
Abbreviations: "---" = not determined; AUC_{0-t} = area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration; $AUC_{0-\infty}$ = area under the plasma concentration time curve from time zero to infinity; $\%AUC_{extrap}$ = percentage of AUC that is due to extrapolation from the last measurable concentration to infinity; CL/F = apparent total plasma clearance; C_{max} = maximum observed plasma concentration; CV = coefficient of variation; NA = not applicable;
$t_{1/2}$ = apparent plasma terminal elimination half life; t_{max} = time of the maximum observed plasma concentration; V_z/F = apparent volume of distribution during the terminal phase; MR $AUC_{0-\infty}$ = metabolic ratio based on $AUC_{0-\infty}$; MR C_{max}= metabolic ratio based on C_{max}.
[a] Median (min-max)
The actual dose of [^{14}C]-tucatinib administered was 317 mg.
Program Location: /cvn/projects/prj/ecb/programs/000000158854/dev/tables/t_pp_itt.sas
Program Run: 17DEC18 cvn_jwilliam7 Program Status: FINAL

Source: ONT-380-008 CSR, Table 7

2.2.1.2. 尿中及び糞便中への排泄

ツカチニブ及び ONT-993 の尿中排泄に関する PK パラメータを Table 11 に要約する。^{14}C 標識ツカチニブ約 150 μCi を含むツカチニブ 317 mg の経口投与後、尿中から回収された未変化体はツカチニブ投与量の 1%未満であった。ツカチニブの CL_R は 0.854 L/h、ONT-993 の CL_R は 9.46 L/h であった。

尿及び糞便中の 312 時間までの放射能の総平均回収率は 89.9%であり、個々の治験参加者における回収率は 62.5%～97.1%であった。投与した放射能は、糞便中及び尿中にそれぞれ 85.8%及び 4.09%が排泄された。放射能の大部分（83.4%）は投与後 120 時間以内に回収された。

Table 11. Summary of urine pharmacokinetic parameters for tucatinib and ONT-993

	[^{14}C]-tucatinib 300 mg	
	Tucatinib in Urine	ONT-993 in Urine
Cum A_{eu} (mg)	2.75 (33.1)	5.52 (23.8)
Cum f_{eu} (%)	0.867 (33.1)	---
CL_R (L/h)	0.854 (31.3)	9.46 (19.3)

Geometric mean (CV%) data are presented.
Abbreviations: Cum A_{eu} = cumulative amount excreted in urine; Cum f_{eu} = cumulative percentage excreted in urine; CL_R = renal clearance; CV = coefficient of variation
The actual dose of [^{14}C]-tucatinib administered was 317 mg.
Source: ONT-380-008 CSR, Table 8

2.2.1.3. 代謝物プロファイル

- 血漿中の代謝物

本治験の治験参加者においてツカチニブは広範囲に代謝され、11 種類の代謝物が同定された。全治験参加者の血漿中に最も豊富に存在した放射性成分はツカチニブ及び ONT-993 で、平均血漿中曝露量（AUC_{24}）はそれぞれ血漿中総放射能の 75.6%及び 9.16%であった（Table 12）。M19、M22、M25、M31 並びに未同定代謝物 M17、M32、M33 及び M34 を含むその他の血漿中代謝物はいずれも微量～少量であった。

- 尿中及び糞便中の代謝物

ツカチニブは、糞便中の主成分の 1 つ（投与量の 15.9%、Table 13）、及び尿中の微量成分（投与量の 0.714%、Table 14）の 1 つとして検出された。一方、排泄物中の代謝物は尿中では投与量の約 3%、糞便中では投与量の約 59%を占めた。最も多く排泄された放射性成分は ONT-993（M1）であり、尿中では平均して投与量の 1.47%、糞便中では投与量の 36.8%を占めた。糞便中で検出された少量の代謝物は、ONT-380 のジオキシ体 M18、ジオキシデヒドロ体 M19 及び M28 並びにオキシデヒドロ体 M25 で、それぞれ平均で投与量の 6.07%、5.29%、3.86%及び 1.54%を占めた。代謝物 M20 及び M3 は平均で投与量の 2.65%を、代謝物 M22 及び M23 は平均で投与量の 2.01%を占めた。尿中及び糞便中のそれ以外の放射性標識代謝物は微量であった。

代謝には主にジメチルオキサゾール部分の酸化が介在し、最も多い循環中代謝物及び最も多い排泄成分として ONT-993 が生じる。尿中で少量の代謝物が複数検出されたことから、定量限界未満の代謝物が血漿中に多数存在すると推定される。循環血中に最も多く存在した成分はツカチニブであった。糞便中にはツカチニブの酸化代謝物が多く存在し、尿中及び糞便中のツカチニブは比較的少なかったことから、ツカチニブは十分吸収され、実質的に肝代謝を受けること、及び主にジメチルジヒドロオキサゾール部分の酸化を介した代謝クリアランスがヒトにおける主要な消失経路であることが示唆された。

Table 12. Summary of metabolites in plasma

Component	Concentration (ng eq/g)				AUC (ng eq h/g)	Percent of Total	Percent of AUC
Designation	2 h	12 h	24 h	AUC Pool[a]	AUC Pool[b]	AUC Pool	Radioactivity[c]
M1 (ONT-993)	60.3	ND	ND	26.3	631	9.65	9.16
M17*	3.89	ND	ND	ND	ND	NA	NA
Tucatinib (ONT-380)	440	55.5	118	217	5210	79.7	75.6
M19	20.4	ND	ND	3.22	77.3	1.18	1.12
M22	4.89	ND	ND	ND	ND	NA	NA
M25	ND	ND	ND	3.61	86.6	1.32	1.26
M31	18.5	ND	ND	11.6	278	4.25	4.03
M32*	ND	ND	ND	0.163	3.91	0.0598	0.0568
M33*	1.94	ND	ND	1.71	41.0	0.627	0.595
M34*	ND	ND	ND	8.80	211	3.23	3.06

AUC Area under the concentration time curve (ng equivalents h/g).
h Hours.
NA Not applicable.
ND Peak not detected or below the established limit of quantitation (1% of run and 10 cpm peak height).
Notes: The AUC_{0-24} hour for total plasma radioactivity was 6890 ng eq h/g.
The total AUC pool exposure was 6540 ng eq h/g.
a Intersubject mean time-weighted plasma pool from 1 through 24 hours.
b Calculated AUC = AUC Pool x pooling interval.
c AUC for ONT-380 or metabolite / AUC total radioactivity through 24 hours x 100.
* Unknown metabolite.

Source: ONT-380-008 CSR Addendum

Table 13. Summary of metabolites in feces

Component Designation	Mean Percent of Dose
M1 (ONT-993)	36.8
M18	6.07
tucatinib (ONT-380)	15.9
M19	5.29
M20 (Array M9/M10)/M3	2.65
M21*	0.528
M22/M23 (Array M5)	2.01
M24*	0.394
M25	1.54
M28	3.86
M30	0.348
Total % Dose Quantitated:	75.3
Total % Dose Known:	74.3
Total % Dose Unknown:	0.922
Total % Dose Excreted:	82.0

* Unknown metabolite.

Source: ONT-380-008 CSR Addendum

Table 14. Summary of metabolites in urine

Component Designation	Mean Percent of Dose
M14*	0.00695
M15*	0.00448
M16*	0.00873
M1 (ONT-993)	1.47
M17*	0.0188
M18	0.0839
tucatinib (ONT-380)	0.714
M19	0.322
M20 (Array M9/M10)/M3	0.0549
M21*	0.0508
M22/M23 (Array M5)	0.321
M24*	0.0278
M25	0.0284
M26*	0.0446
M27*	0.0227
M29*	0.161
M30	0.0143
Total % Dose Quantitated:	3.35
Total % Dose Known:	3.00
Total % Dose Unknown:	0.346
Total % Dose Excreted:	3.93

* Unknown metabolite.

Source: ONT-380-008 CSR Addendum

2.2.2. 肝機能障害を有する治験参加者及び健康成人にツカチニブを投与した第 1 相試験：ONT-380-009 試験

本治験は、肝機能障害を有する患者及び健康成人に絶食下でツカチニブ錠 300 mg を単回経口投与したときの PK を比較評価する非盲検、並行群間比較試験である。Child-Pugh 分類を用いて肝機能障害を軽度、中等度又は重度に分類した。投与前から投与後 48 時間にかけて、ツカチニブ及び ONT-993 の血漿中濃度を分析するための PK 評価用血液検体を逐次採取した。本治験に計 37 例を組み入れ、全例を対象に PK 解析を実施した。

- ツカチニブ及び ONT-993 の PK

ツカチニブ及び ONT-993 の血漿中濃度-時間推移をそれぞれ Figure 16 及び Figure 17 に示す。ツカチニブと遊離型ツカチニブ及び ONT-993 の PK パラメータを Table 15 及び Table 16 に要約する。ツカチニブ、遊離型ツカチニブ及び ONT-993 の C_{max} 及び AUC の統計解析をそれぞれ Table 17、Table 18 及び Table 19 に示す。以下に T_{max} 以外は幾何平均値の結果を示す。

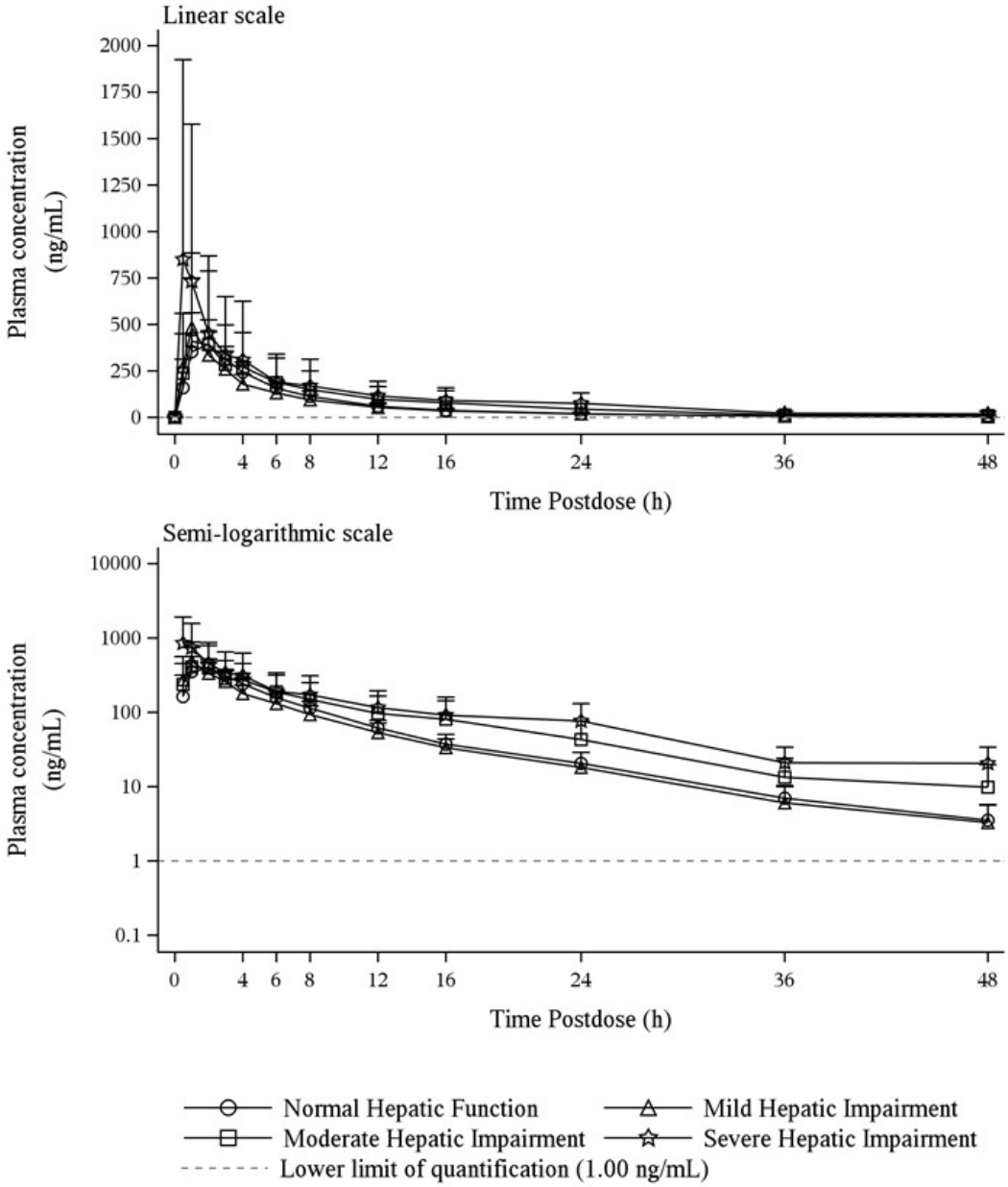
ツカチニブ 300 mg 単回投与後のツカチニブ及び ONT-993 の血漿中濃度-時間推移は、軽度、中等度又は重度の肝機能障害（軽度 8 例、中等度 8 例、重度 6 例）及び正常な肝機能（15 例）を有するすべての治験参加者で速やかな吸収を示し、T_{max}（中央値）は 1～2 時間であり、いずれの集団でも同程度であった。

ツカチニブの曝露量（C_{max}、AUC_{last} 及び AUC_{inf}）を人口統計学的特性で 1:1 マッチングさせた肝機能障害群と正常肝機能群について統計解析した結果、正常肝機能群と比べ、軽度肝機能障害群では変化はなく、中等度及び重度肝機能障害群では全般的に増加した。C_{max}、AUC_{last} 及び AUC_{inf} それぞれの対照群（正常肝機能群）に対する試験群（肝機能障害群）の GMR（90% CI）は、軽度肝機能障害群で 103.9%（61.6, 175.3）、98.0%（74.3, 129.3）及び 99.0%（76.3, 128.4）、中等度肝機能障害群で 88.5%（42.1, 186.1）、113.4%（65.2, 197.2）及び 114.8%（65.3, 201.8）、重度肝機能障害群で 117.4%（36.6, 376.5）、143.0%（70.6, 289.9）及び 161.0%（67.3, 385.3）であった。中等度肝機能障害群の AUC_{last} 及び AUC_{inf}、重度肝機能障害群の C_{max}、AUC_{last} 及び AUC_{inf} は個体間変動（CV%）が大きく、90% CI は 100%を跨ぎ、統計的に有意ではなかった。

非結合形ツカチニブの曝露量（C_{max}、AUC_{last} 及び AUC_{inf}）は、統計解析の結果、正常肝機能群と比べ、軽度肝機能障害群では変化はなく、中等度肝機能障害群では増加し、重度肝機能障害群では若干増加がみられたものの、個体間変動（CV%）は大きかった。

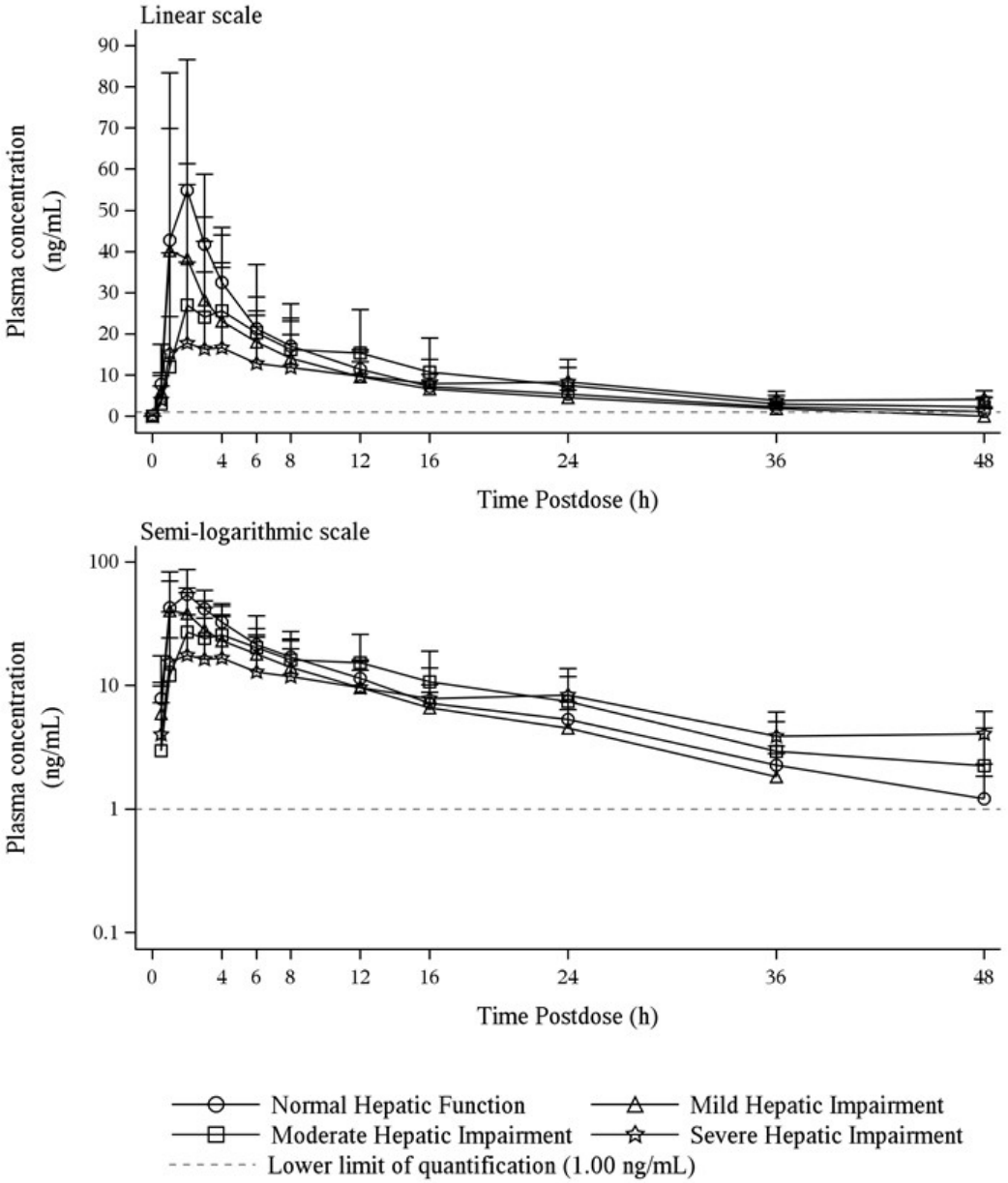
ONT-993 に関する統計解析の結果、C_{max} は肝機能障害の重症度が高いほど減少することが示され、AUC_{last} 及び AUC_{inf} はいずれの肝機能障害群でも正常肝機能群と比べて減少したものの、これらの影響は小さく、90% CI では統計的に有意ではなかった。

Figure 16　Arithmetic mean (+SD) plasma concentration profiles of tucatinib following administration of a single oral dose of tucatinib 300 mg to subjects with normal hepatic function and subjects with mild, moderate, or severe hepatic impairment



Source: ONT-380-009 CSR, Figure 2

Figure 17　Arithmetic mean (+SD) plasma concentration profiles of ONT-993 following administration of a single oral dose of tucatinib 300 mg to subjects with normal hepatic function and subjects with mild, moderate, or severe hepatic impairment



Source: ONT-380-009 CSR, Figure 3

Table 15. Summary of the pharmacokinetic parameters of tucatinib and unbound tucatinib following administration of a single oral dose of tucatinib 300 mg to subjects with normal hepatic function and subjects with mild, moderate, or severe hepatic impairment

Parameter	Normal Hepatic Function (N=15)	Mild Hepatic Impairment (N=8)	Moderate Hepatic Impairment (N=8)	Severe Hepatic Impairment (N=6)
AUC_{0-t} (h*ng/mL)	2710 (25.7)	2450 (29.0)	3040 (76.1)	3830 (103)
$AUC_{0-\infty}$ (h*ng/mL)	2760 (25.7)	2510 (26.9)	3140 (77.7)	4770 (102)[c]
C_{max} (ng/mL)	436 (36.3)	423 (78.2)	374 (104)	471 (240)
T_{max}[a] (h)	2.00 (1.00-3.00)	1.00 (1.00-3.00)	2.00 (1.00-4.00)	1.50 (0.50-4.00)
$t_{1/2}$[b] (h)	8.67 (1.35)	9.64 (3.62)	9.16 (1.48)	12.4 (1.85)[c]
CL/F (L/h)	109 (25.7)	120 (26.9)	95.5 (77.7)	62.9 (102)[c]
V_d/F (L)	1340 (32.2)	1570 (49.5)	1250 (67.9)	1120 (120)[c]
MRT (h)	9.09 (18.2)	8.89 (41.9)	11.8 (25.4)	15.2 (32.2)[c]
$AUC_{0-t, u}$ (h*ng/mL)	76.5 (55.9)	75.7 (52.5)	122 (88.5)	103 (112)
$AUC_{0-\infty, u}$ (h*ng/mL)	77.9 (56.0)	77.6 (52.7)	126 (90.6)	114 (130)[c]
$C_{max, u}$ (ng/mL)	12.3 (63.4)	13.1 (64.2)	15.0 (98.6)	12.7 (232)
CL/F_u (L/h)	3850 (56.0)	3860 (52.7)	2390 (90.6)	2640 (130)[c]
V_d/F_u (L)	47600 (56.4)	50700 (54.3)	31200 (80.9)	46700 (141)[c]

Abbreviations: AUC_{0-t} = area under the concentration-time curve from time 0 to the time of last measurable concentration; $AUC_{0-t, u}$ = unbound AUC_{0-t}; $AUC_{0-\infty}$ = area under the concentration time curve from time 0 to infinity; $AUC_{0-\infty, u}$ = unbound $AUC_{0-\infty}$; C_{max} = maximum concentration; $C_{max, u}$ = unbound C_{max}; CL/F = apparent total plasma clearance; CL/F_u = unbound CL/F; MRT = mean residence time; $t_{1/2}$ = terminal half-life; T_{max} = time to maximum concentration; V_d/F = apparent volume of distribution during the terminal elimination phase; V_d/F_u = unbound V_d/F.
Note: Geometric mean (CV%) data are presented.
[a] Median (Min-Max) data are provided for T_{max}.
[b] Arithmetic mean (SD) data are provided for $t_{1/2}$.
[c] N = 5.
Source: ONT-380 CSR, Table 9

Table 16. Summary of the pharmacokinetic parameters of ONT-993 following administration of a single oral dose of tucatinib 300 mg to subjects with normal hepatic function and subjects with mild, moderate, or severe hepatic impairment

Parameter	Normal Hepatic Function (N=15)	Mild Hepatic Impairment (N=8)	Moderate Hepatic Impairment (N=8)	Severe Hepatic Impairment (N=6)
AUC_{0-t} (h*ng/mL)	412 (39.4)	323 (56.6)	368 (55.9)	291 (88.8)
$AUC_{0-\infty}$ (h*ng/mL)	439 (38.7)	365 (44.2)	448 (57.7)[c]	NC
C_{max} (ng/mL)	54.5 (56.0)	34.1 (93.7)	26.1 (71.3)	13.0 (151)
T_{max}[a] (h)	2.00 (1.00-4.00)	1.50 (1.00-3.00)	3.00 (1.00-12.00)	3.00 (1.00-24.00)
$t_{1/2}$[b] (h)	11.6 (1.89)	13.5 (5.84)	14.5 (4.52)[c]	27.2 (9.68)[d]
MRT (h)	13.9 (16.4)	15.8 (45.5)	21.2 (37.2)[c]	NC
MR_{AUC}	0.159 (30.2)	0.146 (49.1)	0.129 (29.3)[c]	NC
MR_{Cmax}	0.125 (36.6)	0.0806 (61.1)	0.0698 (82.8)	0.0275 (46.4)

Abbreviations: AUC_{0-t} = area under the concentration-time curve from time 0 to the time of last measurable concentration; $AUC_{0-\infty}$ = area under the concentration time curve from time 0 to infinity; C_{max} = maximum concentration; MR_{AUC} = metabolic ratio based on $AUC_{0-\infty}$; MR_{Cmax} = metabolic ratio based on C_{max}; MRT = mean residence time; $t_{1/2}$ = terminal half-life; NC = not calculated; T_{max} = time to maximum concentration.
Note: Geometric mean (CV%) data are presented.
[a] Median (Min-Max) data are provided for T_{max}.
[b] Arithmetic mean (SD) data are provided for $t_{1/2}$.
[c] N = 7
[d] N = 4
Source: ONT-380-009 CSR, Table 10

Table 17. Statistical analyses of plasma concentrations of tucatinib following administration of a single oral dose of tucatinib 300 mg to subjects with normal hepatic function and subjects with mild, moderate, or severe hepatic impairment

Analyte: Tucatinib

		Test (Mild, Moderate or Severe)		Reference (Normal)			
Parameter	Comparison (Test vs Reference)	n[a]	Geometric Mean[b]	n[a]	Geometric Mean[b]	Geometric Mean Ratio[c](%)	90% Confidence Interval[d](%)
C_{max} (ng/mL)	Mild vs. Normal	8	423	8	407	103.9	(61.6, 175.3)
	Moderate vs. Normal	8	374	8	423	88.5	(42.1, 186.1)
	Severe vs. Normal	6	471	6	401	117.4	(36.6, 376.5)
AUC_{0-t} (h*ng/mL)	Mild vs. Normal	8	2450	8	2500	98.0	(74.3, 129.3)
	Moderate vs. Normal	8	3040	8	2690	113.4	(65.2, 197.2)
	Severe vs. Normal	6	3830	6	2680	143.0	(70.6, 289.9)
$AUC_{0-\infty}$ (h*ng/mL)	Mild vs. Normal	8	2510	8	2540	99.0	(76.3, 128.4)
	Moderate vs. Normal	8	3140	8	2740	114.8	(65.3, 201.8)
	Severe vs. Normal	5	4770	5	2960	161.0	(67.3, 385.3)

[a] n was the number of subjects in each group used in the analysis.
[b] Geometric means calculated by transforming the natural-log means back to the linear scale.
[c] Geometric mean ratio for natural log transformed parameter (expressed as a percent), natural log transformed back to the linear scale.
[d] 90% confidence interval for geometric mean ratio of natural log transformed parameter (expressed as a percent), natural log transformed back to the linear scale.
Source: ONT-380-009 CSR, Table 11

Table 18. Statistical analyses of plasma concentrations of unbound tucatinib following administration of a single oral dose of tucatinib 300 mg to subjects with normal hepatic function and subjects with mild, moderate, or severe hepatic impairment

Analyte: Tucatinib

		Test (Mild, Moderate or Severe)		Reference (Normal)			
Parameter	Comparison (Test vs Reference)	n[a]	Geometric Mean[b]	n[a]	Geometric Mean[b]	Geometric Mean Ratio[c](%)	90% Confidence Interval[d](%)
$C_{max,u}$ (ng/mL)	Mild vs. Normal	8	13.1	8	12.7	103.4	(60.2, 177.4)
	Moderate vs. Normal	8	15.0	8	9.88	151.7	(69.1, 333.2)
	Severe vs. Normal	6	12.7	6	11.9	106.4	(35.6, 318.1)
$AUC_{0\text{-}t,u}$ (h*ng/mL)	Mild vs. Normal	8	75.7	8	77.7	97.5	(63.6, 149.4)
	Moderate vs. Normal	8	122	8	62.7	194.2	(96.7, 390.2)
	Severe vs. Normal	6	103	6	79.6	129.7	(58.4, 287.8)
$AUC_{0\text{-}\infty,u}$ (h*ng/mL)	Mild vs. Normal	8	77.6	8	78.9	98.4	(64.7, 149.9)
	Moderate vs. Normal	8	126	8	63.9	196.7	(96.6, 400.6)
	Severe vs. Normal	5	114	5	99.1	114.8	(46.6, 282.9)

[a] n was the number of subjects in each group used in the analysis.
[b] Geometric means calculated by transforming the natural-log means back to the linear scale.
[c] Geometric mean ratio for natural log transformed parameter (expressed as a percent), natural log transformed back to the linear scale.
[d] 90% confidence interval for geometric mean ratio of natural log transformed parameter (expressed as a percent), natural log transformed back to the linear scale.
Source: ONT-380-009 CSR, Table 12

Table 19. Statistical analyses of plasma concentrations of ONT-993 following administration of a single oral dose of tucatinib 300 mg to subjects with normal hepatic function and subjects with mild, moderate, or severe hepatic impairment

Analyte: ONT-993

		Test (Mild, Moderate or Severe)		Reference (Normal)			
Parameter	Comparison (Test vs Reference)	n[a]	Geometric Mean[b]	n[a]	Geometric Mean[b]	Geometric Mean Ratio[c](%)	90% Confidence Interval[d](%)
C_{max} (ng/mL)	Mild vs. Normal	8	34.1	8	46.2	73.8	(36.0, 151.1)
	Moderate vs. Normal	8	26.1	8	54.5	48.0	(25.9, 89.1)
	Severe vs. Normal	6	13.0	6	44.8	28.9	(11.3, 73.9)
AUC_{0-t} (h*ng/mL)	Mild vs. Normal	8	323	8	356	90.6	(60.4, 135.9)
	Moderate vs. Normal	8	368	8	426	86.5	(54.9, 136.4)
	Severe vs. Normal	6	291	6	353	82.3	(43.3, 156.5)
$AUC_{0-\infty}$ (h*ng/mL)	Mild vs. Normal	8	365	8	381	95.9	(67.7, 135.8)
	Moderate vs. Normal	7	448	7	490	91.5	(52.2, 160.2)

[a] n was the number of subjects in each group used in the analysis.
[b] Geometric means calculated by transforming the natural-log means back to the linear scale.
[c] Geometric mean ratio for natural log transformed parameter (expressed as a percent), natural log transformed back to the linear scale.
[d] 90% confidence interval for geometric mean ratio of natural log transformed parameter (expressed as a percent), natural log transformed back to the linear scale.
There were insufficient data to present the Severe vs Normal comparison for the $AUC_{0-\infty}$ parameter
Source: ONT-380-009 CSR, Table 13

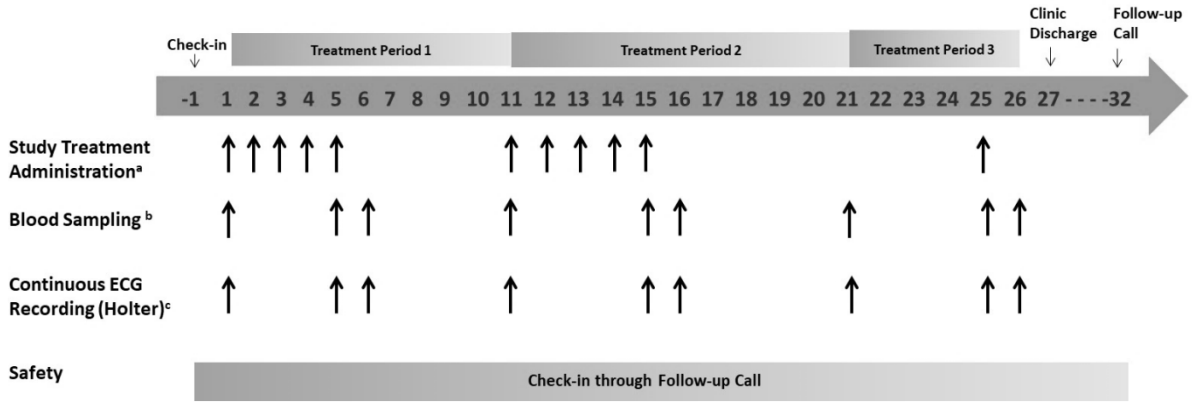
2.2.3. 健康成人にツカチニブを投与したときの心室再分極への影響を評価する第 1 相試験：ONT-380-011 試験

本治験は、健康成人を対象に、ツカチニブの臨床用量を反復投与し定常状態において、心室再分極に及ぼす影響を評価する部分的二重盲検（ツカチニブ及びプラセボは二重盲検、モキシフロキサシンは非盲検）、3 期クロスオーバー試験である。本治験に組み入れた 55 例中、ツカチニブの投与を受け、PK の評価が可能な 51 例を PK 解析に含めた。治験参加者を 6 投与順序のいずれかに無作為に割り付け、以下の 3 種類の各投与を実施した。

- 投与 A：ツカチニブ 300 mg を 4 日間 BID 経口投与し、5 日目の朝にツカチニブ 300 mg を単回経口投与
- 投与 B：ツカチニブに対応するプラセボを 4 日間経口 BID 投与し、5 日目の朝にツカチニブに対応するプラセボを単回経口投与
- 投与 C：モキシフロキサシン 400 mg を単回経口投与

本治験の概略図を Figure 18 に示す。本治験の分析感度を確認するためにモキシフロキサシンを陽性対照とした。予想されるモキシフロキサシンの QT に対する最大効果（ΔΔQTcF）は 10～15 msec である。

Figure 18　Study ONT-380-011 study schema



Abbreviation: BID = twice daily; ECG = electrocardiogram.
a Study treatment administration is presented for Treatment Sequence ABC. Subjects received study treatment in Treatment Periods 1, 2, and 3 according to the randomization schedule.
b Blood samples for determination of plasma tucatinib and ONT 993 concentrations or moxifloxacin concentrations were collected predose and through 36 hours postdose on Days 5 to 6, Days 15 to 16, and Days 25 to 26. A single predose blood sample was collected predose on Days 1, 11, and 21.
c Continuous ECG recording (Holter) were perfomed from approximately 1.5 hours predose until just prior to dosing on Days 1, 11, and 21 to establish baseline ECG readings. Continuous ECG recording (Holter) were perfomed from approximately 45 minutes predose through 36 hours postdose on Days 5 to 6, Days 15 to 16, and Days 25 to 26.

2.2.3.1. ツカチニブ及び ONT-993 の PK

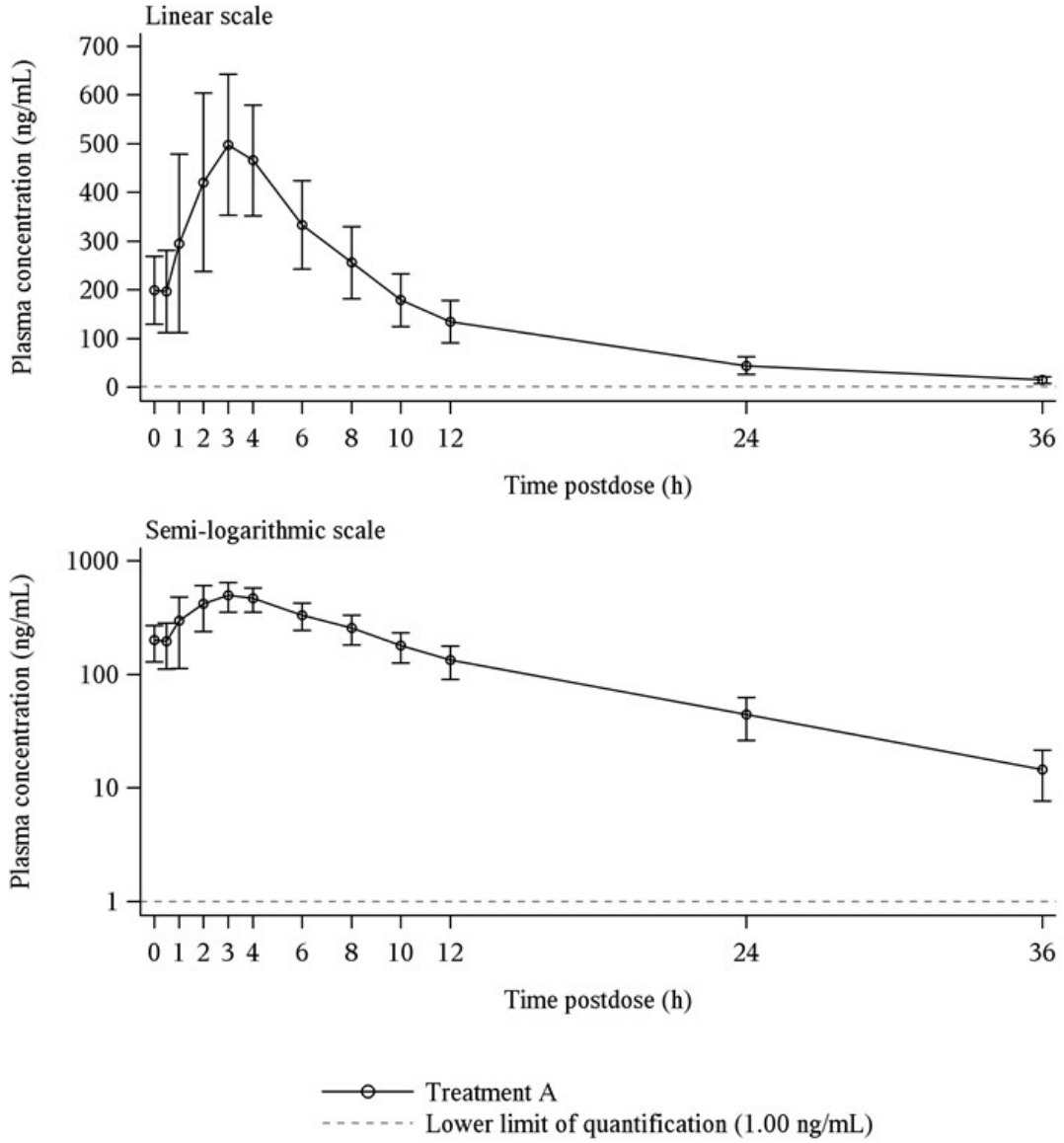
ツカチニブ及び ONT-993 の血漿中濃度-時間推移をそれぞれ Figure 19 及び Figure 20 に示す。ツカチニブ及び ONT-993 の PK パラメータを Table 20 に要約する。

ツカチニブ 300 mg 反復投与後のツカチニブの T_{max}（中央値）は 3 時間（範囲：1～4.1 時間）であった。血漿中ツカチニブ濃度は C_{max}（幾何平均値 519 ng/mL）に達した後に概ね二相性の低下を示し、$t_{½}$（算術平均値）は 7.43 時間であった。AUC_{tau}（幾何平均値）は 3520 h•ng/mL、CL/F（幾何平均値）は 85.3 L/h であった。

ONT-993 の T_{max}（中央値）は 3 時間（範囲：1～4.10 時間）でツカチニブと類似していた。血漿中 ONT-993 濃度は C_{max}（幾何平均値 65.0 ng/mL）に達した後に概ね二相性の低下を示し、$t_{½}$（算術平均値）は 11.4 時間であった。AUC_{tau}（幾何平均値）は 409 h•ng/mL、MR_{AUC}（幾何平均値）は 0.116 であった。

PK パラメータの治験参加者間変動（C_{max} 及び AUC_{tau} の幾何 CV%）は、ツカチニブで 26.0%～26.7%、ONT-993 で 36.5%～40.5%であった。

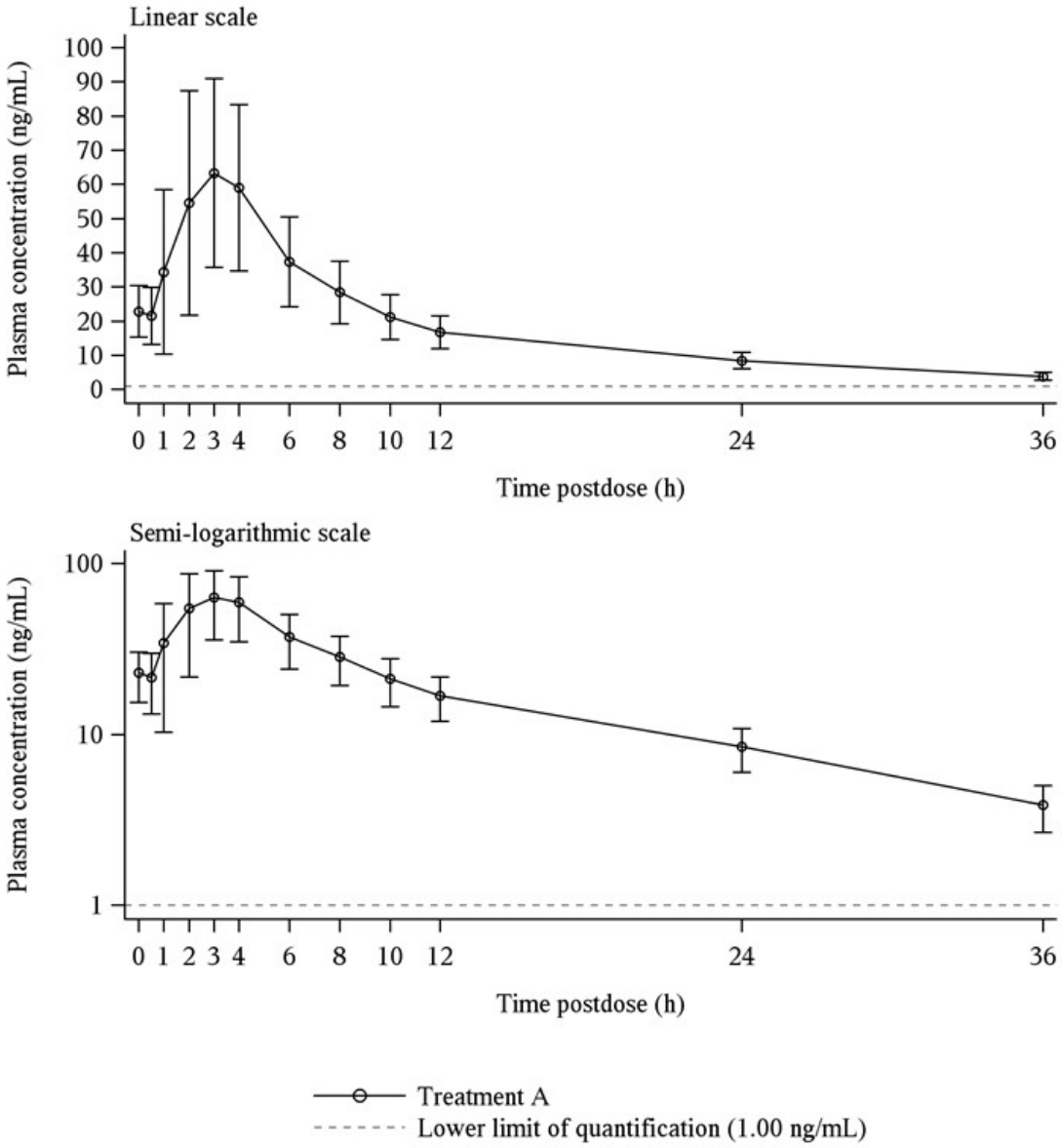
Figure 19　Arithmetic mean ± SD plasma concentrations of tucatinib after last dose administration following multiple oral doses (300 mg twice daily for 4 days and single dose on the fifth day)



Treatment: A = Tucatinib 300 mg BID for 4 days, single dose on the fifth day.
Timepoints are relative to the last dose of study treatment.
Reference: Table 14.2.1-2
Program Location: /cvn/projects/prj/ecb/programs/000000161764/dev/figures/f_pc.sas
Program Run: 10JUL2019 cvn_jwilliam7
Program Status: FINAL　Sponsor Reference: 000000161764

Source: Study ONT-380-011 CSR, Figure 2

Figure 20　Arithmetic mean ± SD plasma concentrations of ONT-993 after last dose administration following multiple oral doses of tucatinib (300 mg twice daily for 4 days and single dose on the fifth day)



Treatment: A = Tucatinib 300 mg BID for 4 days, single dose on the fifth day.
Timepoints are relative to the last dose of study treatment.
Reference: Table 14.2.1-2
Program Location: /cvn/projects/prj/ecb/programs/000000161764/dev/figures/f_pc.sas
Program Run: 10JUL2019 cvn_jwilliam7
Program Status: FINAL　Sponsor Reference: 000000161764

Source: Study ONT-380-011 CSR, Figure 3

Table 20. Summary of the pharmacokinetic parameters for tucatinib following multiple oral doses (300 mg twice daily for 4 days and single dose on the fifth day)

Parameter	Tucatinib (N = 50)	ONT-993 (N = 50)
$AUC_{0-\tau}$ (h*ng/mL)	3520 (26.0)	409 (36.5)
C_{max} (ng/mL)	519 (26.7)	65.0 (40.5)
t_{max} (h)[a]	3.00 (1.00, 4.10)	3.00 (1.00, 4.10)
$t_{1/2}$ (h)[b]	7.43 (1.10)	11.4 (1.51)
CL/F (L/h)	85.3 (26.0)	---
V_z/F (L)	905 (29.6)	---
MR_{AUC}	---	0.116 (35.4)

Source: Table 14.2.1-1
Abbreviations: $AUC_{0-\tau}$ = area under the plasma concentration time curve over dosing interval (τ = 12 hours); C_{max} = maximum observed concentration; CL/F = apparent total clearance; CV% = coefficient of variation; MR_{AUC} = metabolic ratio based on $AUC_{0-\tau}$; t_{max} = time of the maximum observed concentration; $t_{1/2}$ = apparent terminal elimination half life; V_z/F = apparent volume of distribution during the terminal phase.
Note: Geometric mean (CV%) data are presented.
[a] For t_{max}, median (minimum, maximum) are presented.
[b] For $t_{1/2}$, arithmetic mean (SD) are presented.
Program Location: /cvn/projects/prj/ecb/programs/000000161764/dev/tables/t_pp_itt.sas
Program Run: 10JUL2019 cvn_jwilliam7　Program Status: FINAL
Source: Study ONT-380-011 CSR, Table 9

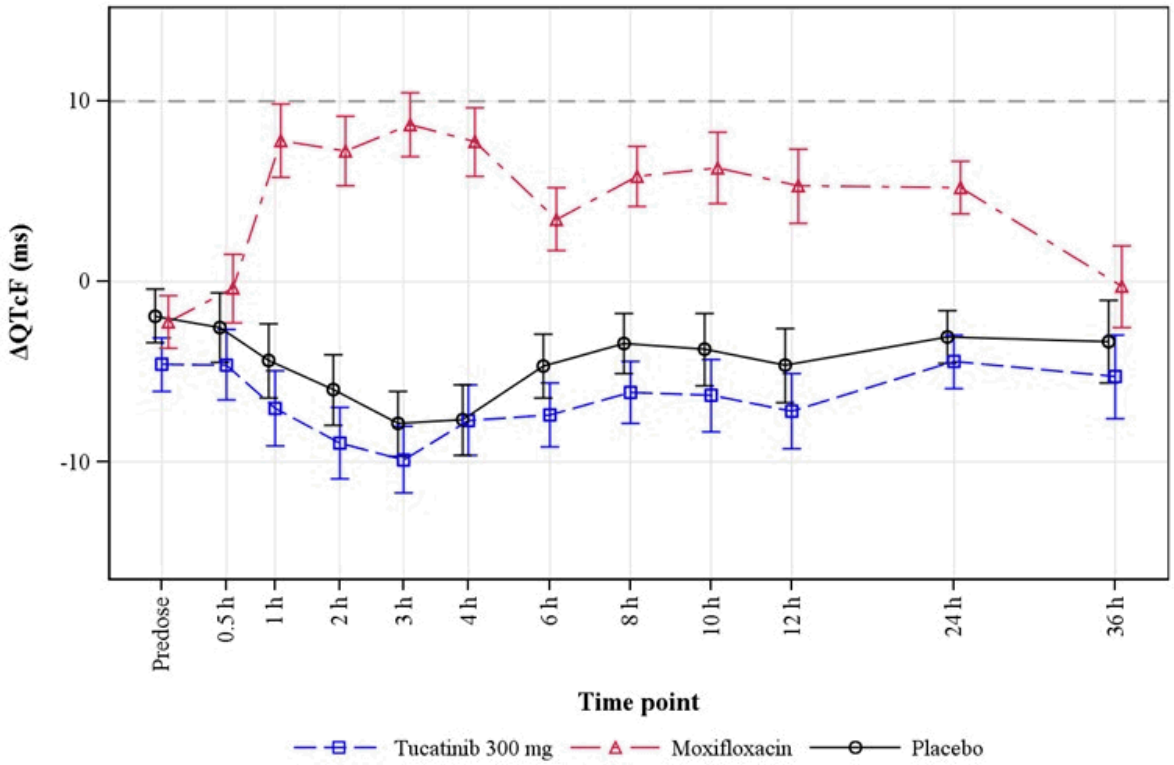
2.2.3.2. QTcF への影響

ツカチニブ、プラセボ及びモキシフロキサシンを投与したときの QTcF のベースラインからの平均変化量（ΔQTcF）の推移を Figure 21 に示す。

ツカチニブ投与時のΔQTcF は、ツカチニブ投与後のすべての QTcF 測定時点でプラセボ投与時のΔQTcF と同様であった。このため、プラセボで補正した平均ΔQTcF（ΔΔQTcF）は非常に小さく、-2.9 msec（投与後 2 時間）から 0.0 msec（投与後 4 時間）の範囲であった。ツカチニブ投与後に QTcF が 450 msec を超えた治験参加者は認められず、ΔQTcF が 30 msec を超えた治験参加者は認められなかった。ツカチニブ投与後のすべての時点でΔΔQTcF の 90% CI の上限は 5 msec 未満であった。したがって、ツカチニブはΔΔQTcF に対して臨床的に意味のある影響を及ぼさなかった。

モキシフロキサシン 400 mg 投与後には平均ΔΔQTcF の明らかな延長が認められ、最大値は投与後 3 時間の 16.6 msec（90% CI：14.03、19.08）であった。事前に規定したすべての時点（1、2 及び 3 時間）でΔΔQTcF の 90% CI の下限値が 5 msec 超であったことから、分析の感度が立証された。

Figure 21　Change from baseline QTcF (ΔQTcF) across timepoints (QT/QTc population)



LS mean and 90% CI based on a linear mixed-effects model.
Source: ONT-380-011 CSR, Figure 6, ERT Cardiac Safety Report, Figure 14.2.2.1.

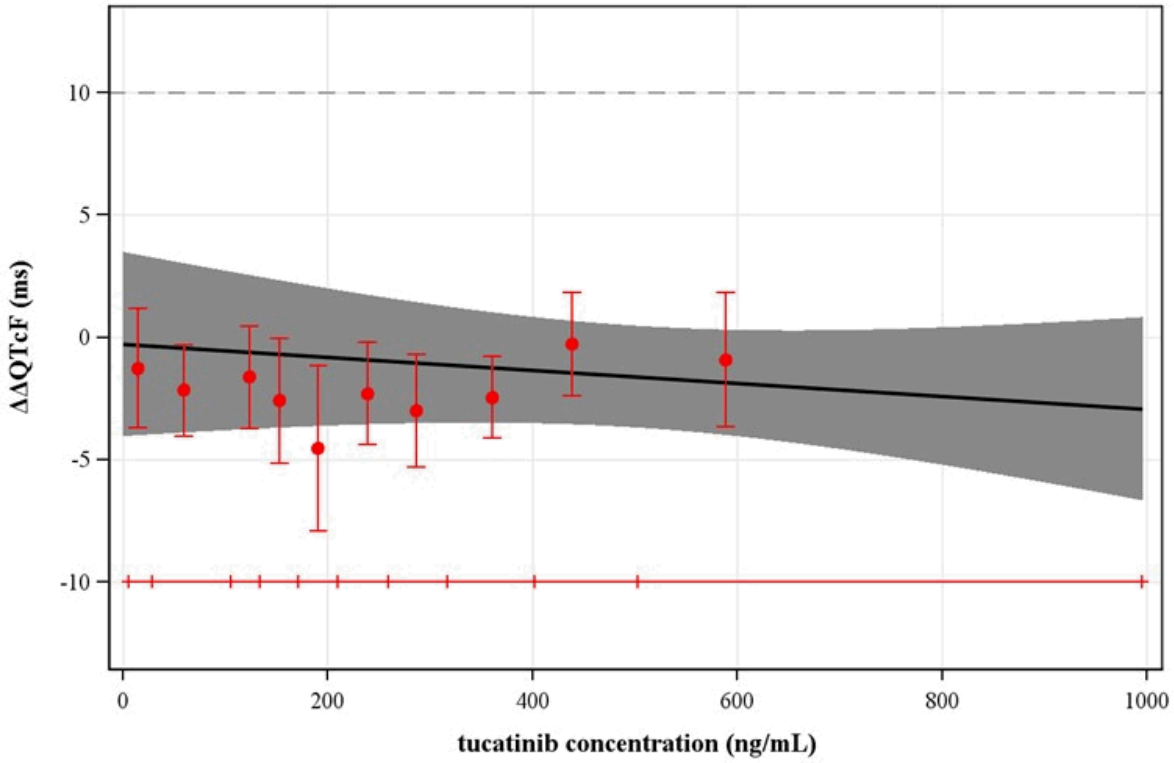
2.2.3.3. 曝露-反応解析（ΔΔQTcF）

ツカチニブ及び ONT-993 の血漿中濃度とΔΔQTcF の関連性について線形混合効果モデルを用いて解析した。ツカチニブ及び ONT-993 の血漿中濃度の各十分位範囲における平均ΔΔQTcF（90% CI）及びモデルで予測された平均ΔΔQTcF（90% CI）を Figure 22 及び Figure 23 に示す。

ΔΔQTcF の予測値は実測値に近く、モデルはツカチニブ及び ONT-993 の血漿中濃度とΔΔQTcF との関係性を示す妥当性が示された。血漿中濃度−QTc 関係の傾きの推定値は、ツカチニブで −0.003 msec/ng/mL（90% CI：−0.0090, 0.0036）、ONT-993 で 0.04 msec/ng/mL（90% CI：−0.009, 0.085）であり、ツカチニブ及び ONT-993 の傾きは統計的に有意ではなく、90% CI は 0 を跨っていた。

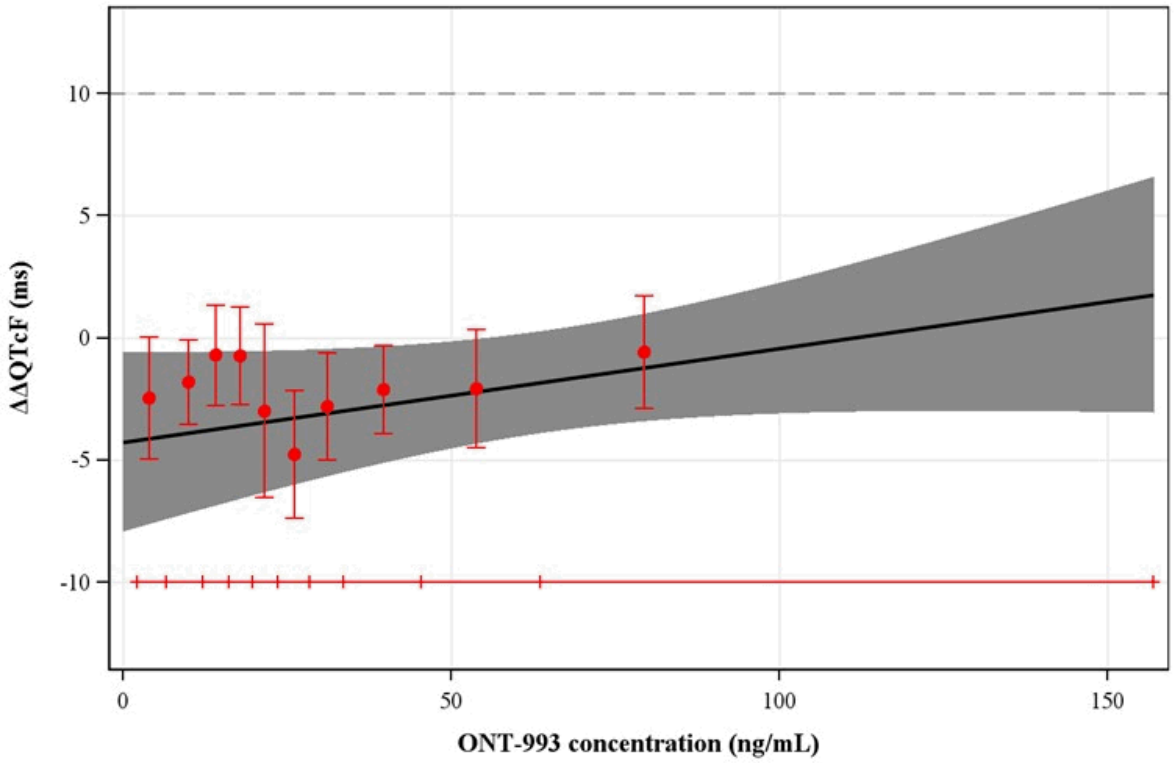
ツカチニブの臨床用量を反復投与した結果、評価した ECG パラメータに臨床的に意味のある影響を及ぼさなかった。よって本治験の結果、ICH-E14 ガイドライン[1,2]も踏まえ、ツカチニブが QT/QTc に及ぼす影響は明らかに陰性であることを確認した。

Figure 22　Model-predicted ΔΔQTcF (mean and 90% CI) and observed ΔΔQTcF (mean and 90% CI) across deciles of plasma concentrations for tucatinib from full model (PK/QTc population)



The red filled circles with vertical bars denote the mean ΔΔQTcF with 90% CI displayed at the median plasma concentration within each decile for tucatinib. The solid black line with gray shaded area denotes the model-predicted mean ΔΔQTcF with 90% CI based on the equation: ΔΔQTcF=-2.88 + -0.0027 *concentration of tucatinib + 0.04 * 67.67 where 67.67 is the arithmetic mean concentration of ONT-993 at Tmax of tucatinib. The horizontal red line with notches shows the range of concentrations divided into deciles for tucatinib. The distance between each decile represents the point at which 10% of the data are present; the first notch to second notch denotes the first 10% of the data, the second notch to third notch denotes the 10-20% of the data and so on.
Source: ONT-380-011 CSR, Figure 12, ERT Cardiac Safety Report, Figure 14.2.10.3.1.

Figure 23　Model-predicted ΔΔQTcF (mean and 90% CI) and observed ΔΔQTcF (mean and 90% CI) across deciles of plasma concentrations for ONT 993 from full model (PK/QTc population)



The red filled circles with vertical bars denote the mean ΔΔQTcF with 90% CI displayed at the median plasma concentration within each decile for ONT-993. The solid black line with gray shaded area denotes the model-predicted mean ΔΔQTcF with 90% CI based on the equation: ΔΔQTcF=-2.88 + -0.0027 * 521.80 + 0.04 * concentration of ONT-993 where 521.80 is the arithmetic mean concentration of tucatinib at Tmax of ONT-993. The horizontal red line with notches shows the range of concentrations divided into deciles for ONT-993. The distance between each decile represents the point at which 10% of the data are present; the first notch to second notch denotes the first 10% of the data, the second notch to third notch denotes the 10-20% of the data and so on.
Source: ONT-380-011 CSR, Figure 13, ERT Cardiac Safety Report, Figure 14.2.10.3.2.

2.2.4. 健康成人を対象としたツカチニブと複数の薬剤（CYP 及び P-gp）の DDI 試験：ONT-380-012 試験

本治験は、健康成人を対象とした非盲検、固定順序、クロスオーバー、5 つのパートからなるツカチニブと CYP3A4 及び CYP2C8 の阻害薬及び誘導薬、及び CYP2C8、CYP2C9、CYP3A4、P-gp の基質の DDI 試験である。本治験の 5 つの DDI パートはいずれも互いに独立しており、各パートではツカチニブ錠を用いて評価した。PK 結果の C_{max} 及び AUC_{inf} は幾何平均値の結果を示す。

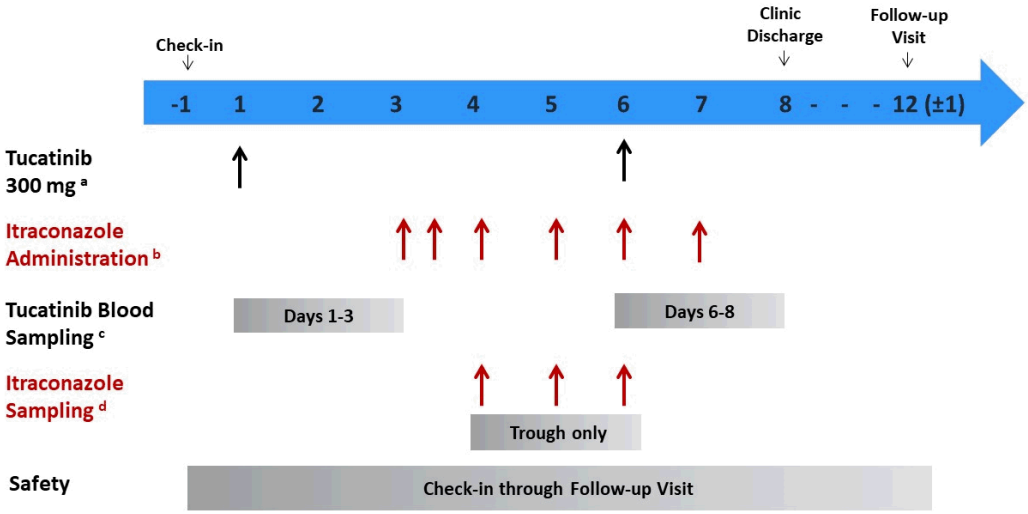
2.2.4.1. パート A：イトラコナゾールがツカチニブの PK に及ぼす影響

パート A の主要目的は、強い CYP3A4 阻害薬（イトラコナゾール）がツカチニブの PK に及ぼす影響を評価することであり、ONT-993 の PK に対する影響も評価した。パート A に計 28 例を組み入れ、全例を対象に PK 解析を実施した。治験参加者に以下の投与を実施した。

- 第 1 日：ツカチニブ 300 mg 単回経口投与
- 第 3 日：イトラコナゾール 200 mg BID 経口投与
- 第 4～7 日：イトラコナゾール 200 mg QD 経口投与
- 第 6 日：ツカチニブ 300 mg 単回経口投与

パート A の試験デザインの概略図を Figure 24 に示す。

Figure 24　Study schematic – Study ONT-380-012 part A



a A single oral dose of tucatinib 300 mg was administered 2 hours after completion of breakfast on Days 1 and 6 (approximately 2 hours after itraconazole on Day 6).
b Itraconazole 200 mg was administered twice daily on Day 3 (with doses administered immediately after breakfast and the evening meal) and once daily immediately after breakfast on Days 4 through 7.
c Blood samples for tucatinib plasma pharmacokinetics were collected predose and through 48 hours postdose on Days 1 through 3 and Days 6 through 8
d Single blood samples for trough plasma concentrations of itraconazole were collected predose on Days 4 through 6.

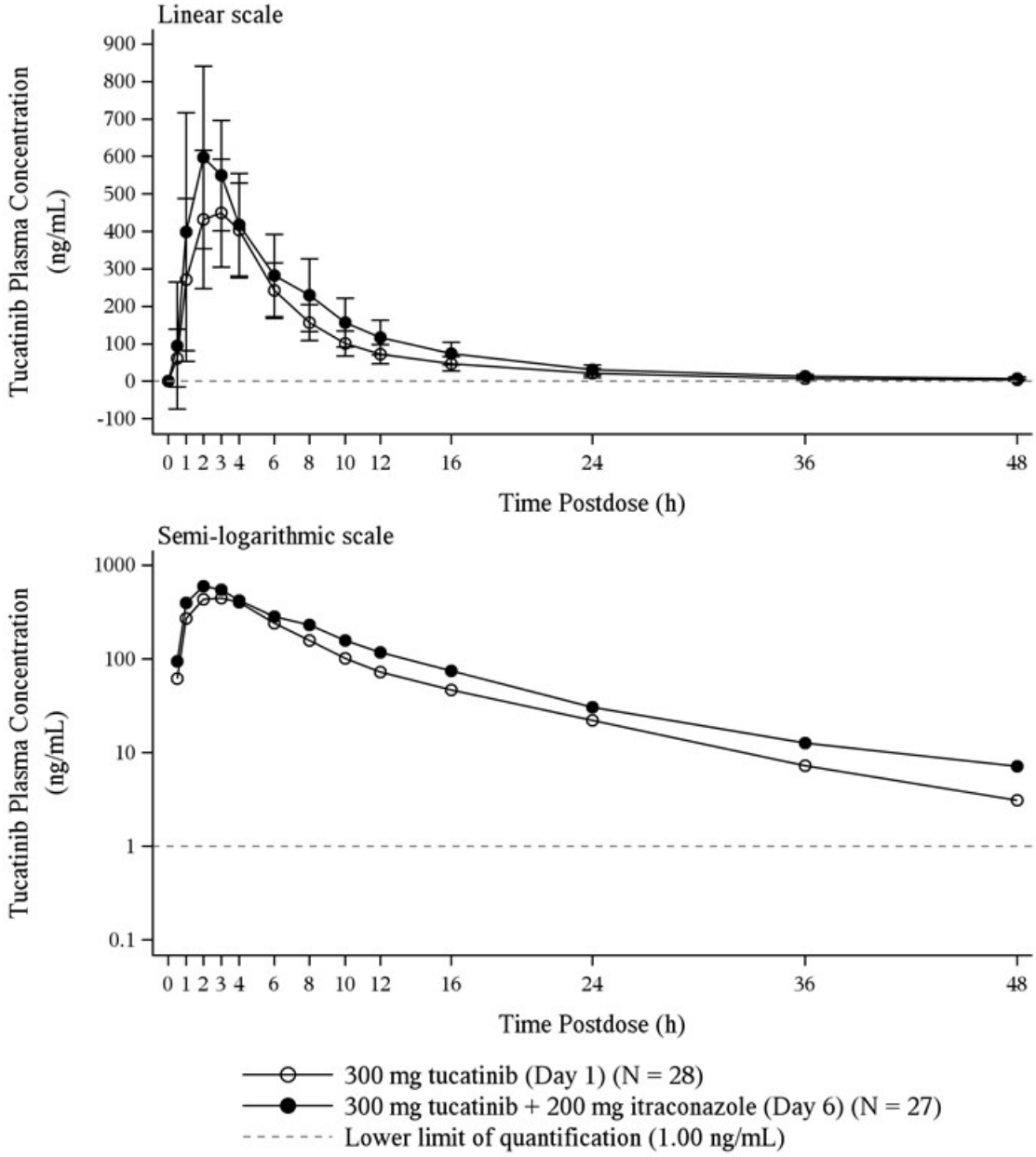
- ツカチニブ及び ONT-993 の PK

ツカチニブ単独又はイトラコナゾールと併用投与したときのツカチニブ及び ONT-993 の血漿中濃度-時間推移をそれぞれ Figure 25 及び Figure 26 に示す。ツカチニブ及び ONT-993 の PK パラメータの統計解析結果をそれぞれ Table 21 及び Table 22 に示す。

ツカチニブとイトラコナゾールを併用投与したときのツカチニブの C_{max} 及び AUC_{inf} は、ツカチニブ単独投与時に比べていずれも約 1.3 倍に増加した。同様に ONT-993 の C_{max} 及び AUC_{inf} はそれぞれ約 2 倍及び約 1.6 倍に増加した。

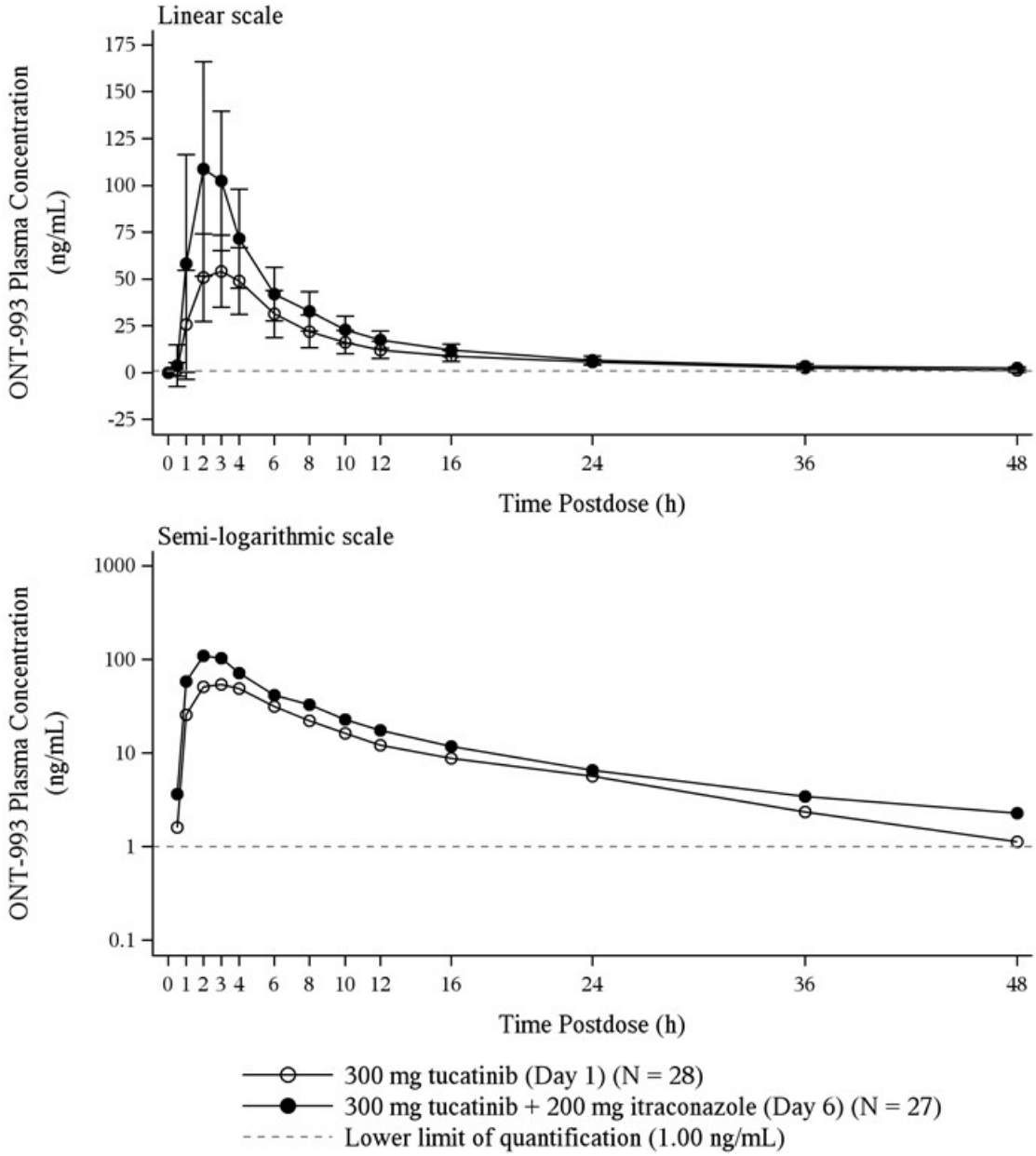
強い CYP3A4 阻害薬であるイトラコナゾールは、ツカチニブ及び ONT-993 の曝露量に対して全般的に弱い影響であり、ツカチニブに対する明らかな DDI の影響は認められなかった。

Figure 25　Arithmetic mean ± SD plasma concentrations of tucatinib following oral administration of tucatinib 300 mg alone (Day 1) and in combination with itraconazole (Day 6) – Part A



Source: Study ONT-380-012 CSR, Figure 6

Figure 26　Arithmetic mean ± SD plasma concentrations of ONT-993 following oral administration of tucatinib 300 mg alone (Day 1) and in combination with itraconazole (Day 6) – Part A



Source: Study ONT-380-012 CSR, Figure 6

Table 21. Statistical analysis to assess the effect of itraconazole on the pharmacokinetic data of tucatinib – Part A

Analyte: Tucatinib

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	300 mg tucatinib (Reference)	28	3320				
	300 mg tucatinib + 200 mg itraconazole (Test)	27	4400	1.33	1.25	1.41	13.7
$AUC_{0-\infty}$ (h*ng/mL)	300 mg tucatinib (Reference)	28	3350				
	300 mg tucatinib + 200 mg itraconazole (Test)	27	4500	1.34	1.26	1.43	13.7
C_{max} (ng/mL)	300 mg tucatinib (Reference)	28	488				
	300 mg tucatinib + 200 mg itraconazole (Test)	27	645	1.32	1.23	1.42	15.3

Abbreviations: AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 21

Table 22. Statistical analysis to assess the effect of itraconazole on the pharmacokinetic data of ONT-993 – Part A

Analyte: ONT-993

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	300 mg tucatinib (Reference)	28	482				
	300 mg tucatinib + 200 mg itraconazole (Test)	27	761	1.58	1.49	1.68	12.7
$AUC_{0-\infty}$ (h*ng/mL)	300 mg tucatinib (Reference)	28	512				
	300 mg tucatinib + 200 mg itraconazole (Test)	27	808	1.58	1.49	1.67	12.6
C_{max} (ng/mL)	300 mg tucatinib (Reference)	28	61.7				
	300 mg tucatinib + 200 mg itraconazole (Test)	27	125	2.03	1.89	2.18	15.8

Abbreviations: AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 22

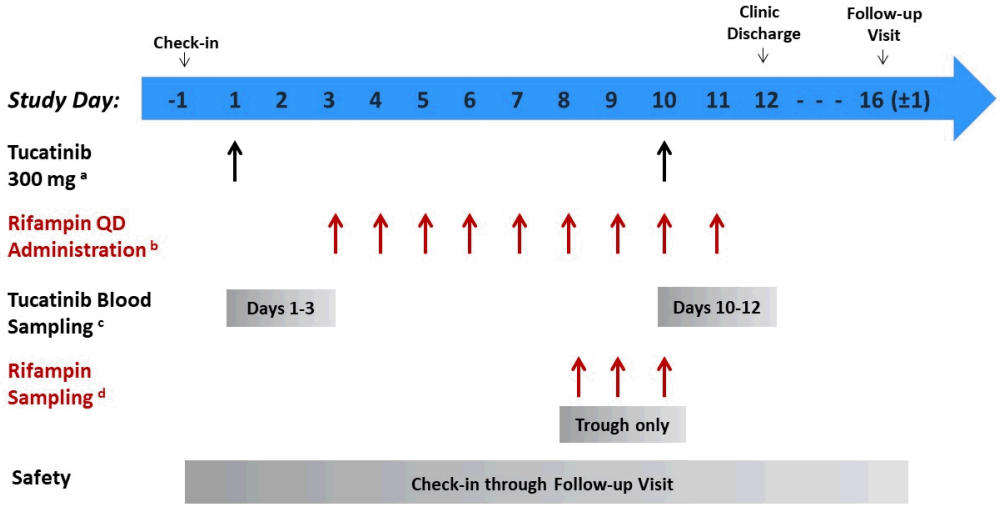
2.2.4.2. パート B：リファンピシンがツカチニブの PK に及ぼす影響

パート B の主要目的は、強い CYP3A4 誘導薬及び中程度の CYP2C8 誘導薬（リファンピシン）がツカチニブの PK に及ぼす影響を評価することであり、ONT-993 の PK に対する影響も評価した。パート B に計 28 例を組み入れ、全例を対象に PK 解析を実施した。治験参加者に以下の投与を実施した。

- 第 1 日：ツカチニブ 300 mg 単回経口投与
- 第 3～11 日：リファンピシン 600 mg QD 経口投与
- 第 10 日：ツカチニブ 300 mg 単回経口投与

パート B の試験デザインの概略図を Figure 27 に示す。

Figure 27　Study schematic – Study ONT-380-012 Part B



a A single oral dose of tucatinib 300 mg was administered in the morning on Days 1 and 10 following an at least 8 hour overnight fast.
b Rifampin 600 mg were administered once daily (QD) on Days 3 through 11 following at least an 8 hour overnight fast.
c Blood samples for tucatinib plasma pharmacokinetics were collected predose and through 48 hours postdose on Days 1 through 3 and Days 10 through 12.
d Single blood samples for trough plasma concentrations of rifampin were collected predose on Days 8 through 10.

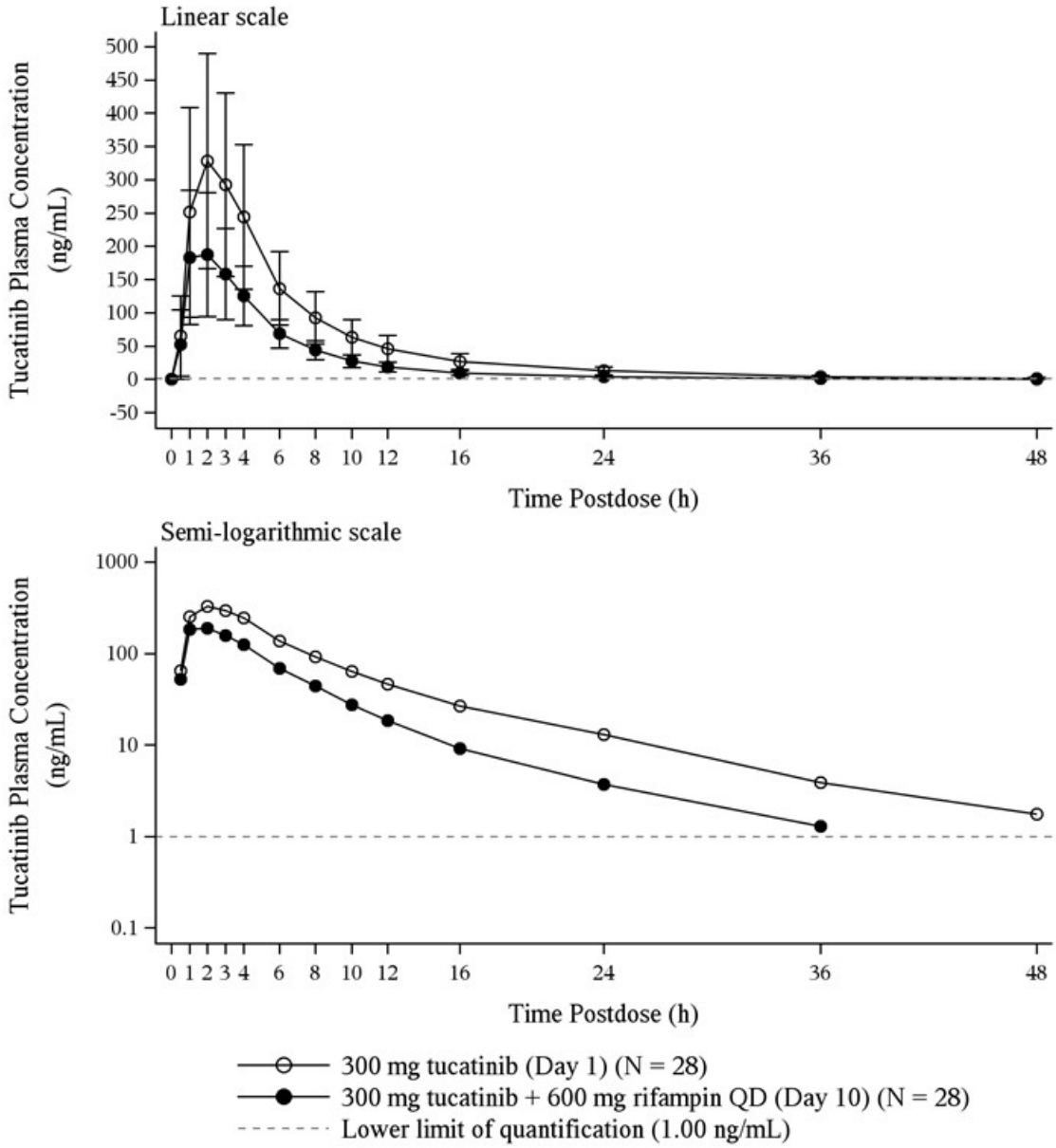
- ツカチニブ及び ONT-993 の PK

ツカチニブ単独又はリファンピシンと併用投与したときのツカチニブ及び ONT-993 の血漿中濃度-時間推移をそれぞれ Figure 28 及び Figure 29 に示す。ツカチニブ及び ONT-993 の PK パラメータの統計解析結果をそれぞれ Table 23 及び Table 24 に示す。

ツカチニブとリファンピシンを併用投与したときのツカチニブの C_{max} 及び AUC_{inf} は、ツカチニブ単独投与時に比べてそれぞれ約 37%及び約 48%減少した。ONT-993 の C_{max} 及び AUC_{inf} はそれぞれ約 2.1 倍増加及び約 25%減少した。ONT-993 の MR_{AUC} 及び MR_{Cmax} は、ツカチニブとリファンピシンの併用投与では、ツカチニブ単独投与よりもそれぞれ約 1.4 倍及び約 3.3 倍に増加した。

強い CYP3A4 誘導薬及び中程度の CYP2C8 誘導薬であるリファンピシンは、ツカチニブ及び ONT-993 の曝露量に対して影響を及ぼすことが示された。このため、ツカチニブとリファンピシンの併用投与は注意が必要と考えられる。

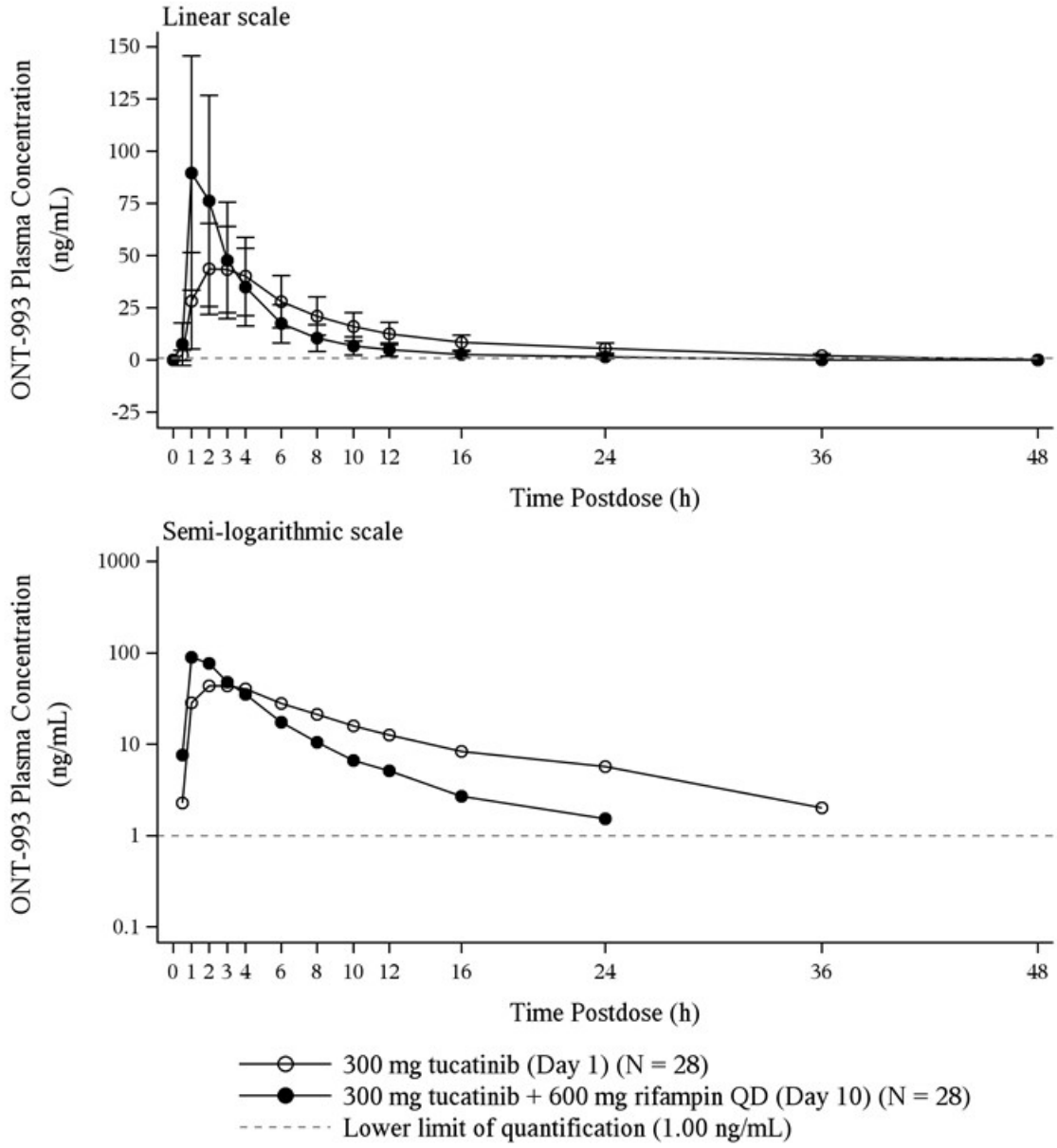
Figure 28　Arithmetic mean ± SD plasma concentrations of tucatinib following oral administration of tucatinib 300 mg alone (Day 1) and in combination with rifampin (Day 10) – Part B



QD = once daily

Source: Study ONT-380-012 CSR, Figure 9

Figure 29　Arithmetic mean ± SD plasma concentrations of ONT-993 following oral administration of tucatinib 300 mg alone (Day 1) and in combination with rifampin



QD = once daily

(Day 10) – Part B

Source: Study ONT-380-012 CSR, Figure 10

Table 23. Statistical analysis to assess the effect of rifampin on the pharmacokinetic data of tucatinib – Part B

Analyte: Tucatinib

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	90% CI for the ratio (Test : Reference) Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	300 mg tucatinib (Reference)	28	2000				
	300 mg tucatinib + 600 mg rifampin QD (Test)	28	1040	0.517	0.449	0.596	32.0
$AUC_{0-\infty}$ (h*ng/mL)	300 mg tucatinib (Reference)	28	2030				
	300 mg tucatinib + 600 mg rifampin QD (Test)	28	1050	0.520	0.452	0.597	31.3
C_{max} (ng/mL)	300 mg tucatinib (Reference)	28	302				
	300 mg tucatinib + 600 mg rifampin QD (Test)	28	191	0.632	0.531	0.753	39.8

AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum observed concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 25

Table 24. Statistical analysis to assess the effect of Rifampin on the pharmacokinetic data of ONT-993 – Part B

Analyte: ONT-993

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	90% CI for the ratio (Test : Reference) Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	300 mg tucatinib (Reference)	28	414				
	300 mg tucatinib + 600 mg rifampin QD (Test)	28	311	0.750	0.636	0.884	37.3
$AUC_{0-\infty}$ (h*ng/mL)	300 mg tucatinib (Reference)	28	439				
	300 mg tucatinib + 600 mg rifampin QD (Test)	28	328	0.748	0.640	0.873	35.1
C_{max} (ng/mL)	300 mg tucatinib (Reference)	28	42.6				
	300 mg tucatinib + 600 mg rifampin QD (Test)	28	88.5	2.08	1.70	2.55	46.9

AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 26

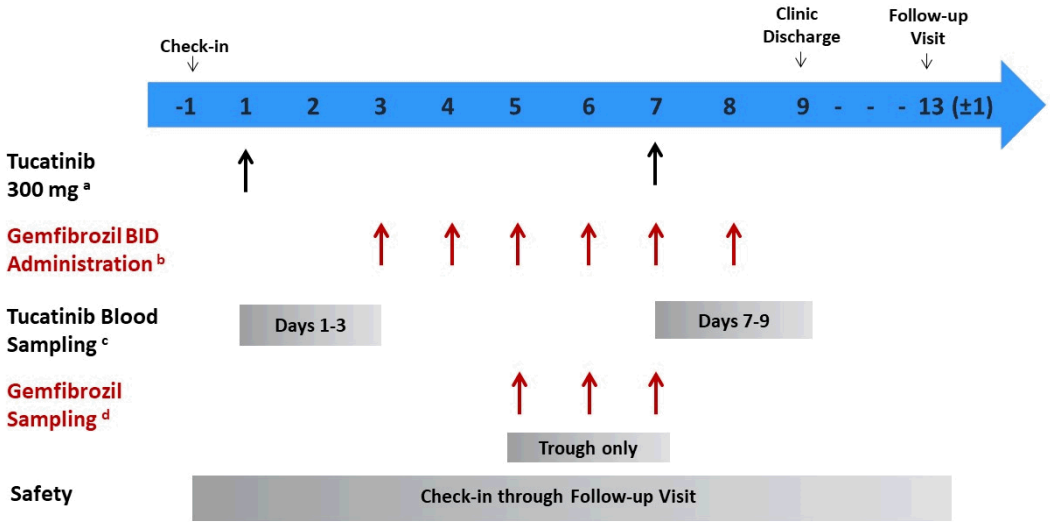
2.2.4.3. パート C：ゲムフィブロジルがツカチニブの PK に及ぼす影響

パート C の主要目的は、強い CYP2C8 阻害薬（ゲムフィブロジル）がツカチニブの PK に及ぼす影響を評価することであり、ONT-993 の PK に対する影響も評価した。パート C に計 28 例を組み入れ、全例を対象に PK 解析を実施した。治験参加者に以下の投与を実施した。

- 第 1 日：ツカチニブ 300 mg 単回経口投与
- 第 3～8 日：ゲムフィブロジル 600 mg BID 経口投与
- 第 7 日：ツカチニブ 300 mg 単回経口投与

パート C の試験デザインの概略図を Figure 30 に示す。

Figure 30　Study schematic – Study ONT-380-012 Part C



a A single oral dose of tucatinib 300 mg was administered in the morning on Days 1 and 7 following at least an 8 hour overnight fast.
b Gemfibrozil 600 mg was administered twice daily (BID) on Days 3 through 8, with the morning doses administered following at least an 8 hour overnight fast and the evening doses administered approximately 30 minutes prior to dinner.
c Blood samples for tucatinib plasma pharmacokinetics were collected predose and through 48 hours postdose on Days 1 through 3 and Days 7 through 9.
d Single blood samples for trough plasma concentrations of gemfibrozil were collected predose on Days 5 through 7.

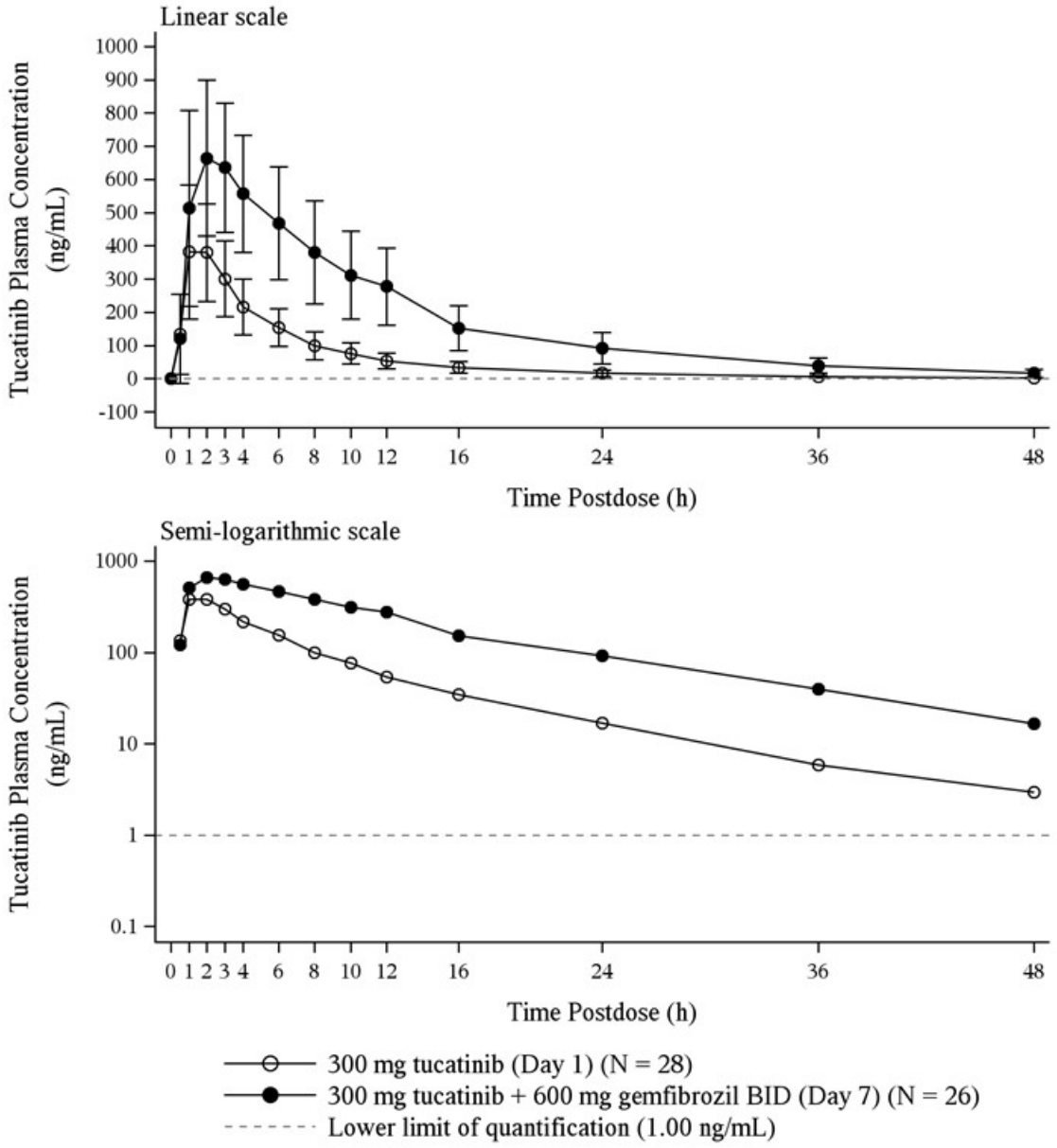
- ツカチニブ及び ONT-993 の PK

ツカチニブ単独又はゲムフィブロジルと併用投与したときのツカチニブ及び ONT-993 の血漿中濃度-時間推移をそれぞれ Figure 31 及び Figure 32 に示す。ツカチニブ及び ONT-993 の PK パラメータの統計解析結果をそれぞれ Table 25 及び Table 26 に示す。

ツカチニブとゲムフィブロジルを併用投与したときのツカチニブの C_{max} 及び AUC_{inf} は、ツカチニブ単独投与時に比べてそれぞれ約 1.6 倍及び約 3.0 倍に増加した。ONT-993 の C_{max} 及び AUC_{inf} はそれぞれ約 69%及び約 11%減少した。ONT-993 の MR_{AUC} 及び MR_{Cmax} は、ツカチニブとゲムフィブロジルの併用投与では、ツカチニブ単独投与よりもそれぞれ約 71%及び約 81%減少した。

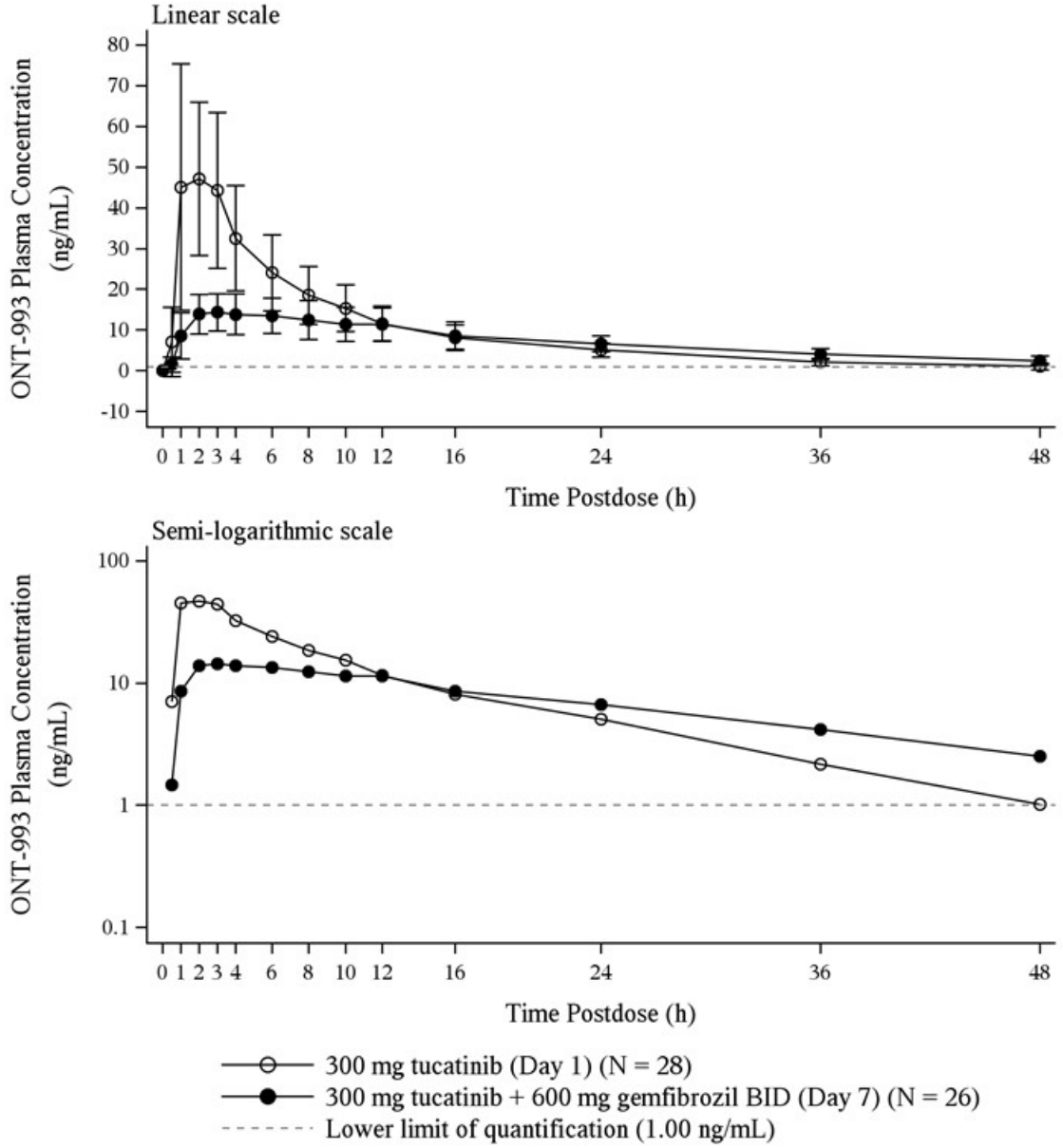
強い CYP2C8 阻害薬であるゲムフィブロジルは、ツカチニブ及び ONT-993 の曝露量に対して影響を及ぼすことが示された。このため、ツカチニブとゲムフィブロジルの併用投与は注意が必要と考えられる。

Figure 31　Arithmetic mean ± SD plasma concentrations of tucatinib following oral administration of tucatinib 300 mg alone (Day 1) and in combination with gemfibrozil (Day 7) – Part C



BID = twice daily
Source: Study ONT-380-012 CSR, Figure 12

Figure 32　Arithmetic mean ± SD plasma concentrations of ONT-993 following oral administration of tucatinib 300 mg alone (Day 1) and in combination with gemfibrozil (Day 7) – Part C



BID = twice daily
Source: Study ONT-380-012 CSR, Figure 13

Table 25. Statistical analysis to assess the effect of gemfibrozil on the pharmacokinetic data of tucatinib – Part C

Analyte: Tucatinib

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	90% CI for the ratio (Test : Reference) Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	300 mg tucatinib (Reference)	28	2440				
	300 mg tucatinib + 600 mg gemfibrozil BID (Test)	26	7280	2.99	2.62	3.41	28.5
$AUC_{0-\infty}$ (h*ng/mL)	300 mg tucatinib (Reference)	28	2480				
	300 mg tucatinib + 600 mg gemfibrozil BID (Test)	26	7520	3.04	2.66	3.46	28.4
C_{max} (ng/mL)	300 mg tucatinib (Reference)	28	410				
	300 mg tucatinib + 600 mg gemfibrozil BID (Test)	26	665	1.62	1.47	1.79	21.1

Abbreviations: AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum observed concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 29

Table 26. Statistical analysis to assess the effect of gemfibrozil on the pharmacokinetic data of ONT-993 – Part C

Analyte: ONT-993

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	90% CI for the ratio (Test : Reference) Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	300 mg tucatinib (Reference)	28	415				
	300 mg tucatinib + 600 mg gemfibrozil BID (Test)	26	318	0.767	0.686	0.858	24.0
$AUC_{0-\infty}$ (h*ng/mL)	300 mg tucatinib (Reference)	28	444				
	300 mg tucatinib + 600 mg gemfibrozil BID (Test)	25	394	0.887	0.801	0.982	21.4
C_{max} (ng/mL)	300 mg tucatinib (Reference)	28	48.7				
	300 mg tucatinib + 600 mg gemfibrozil BID (Test)	26	14.8	0.304	0.263	0.352	31.6

Abbreviations: AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 30

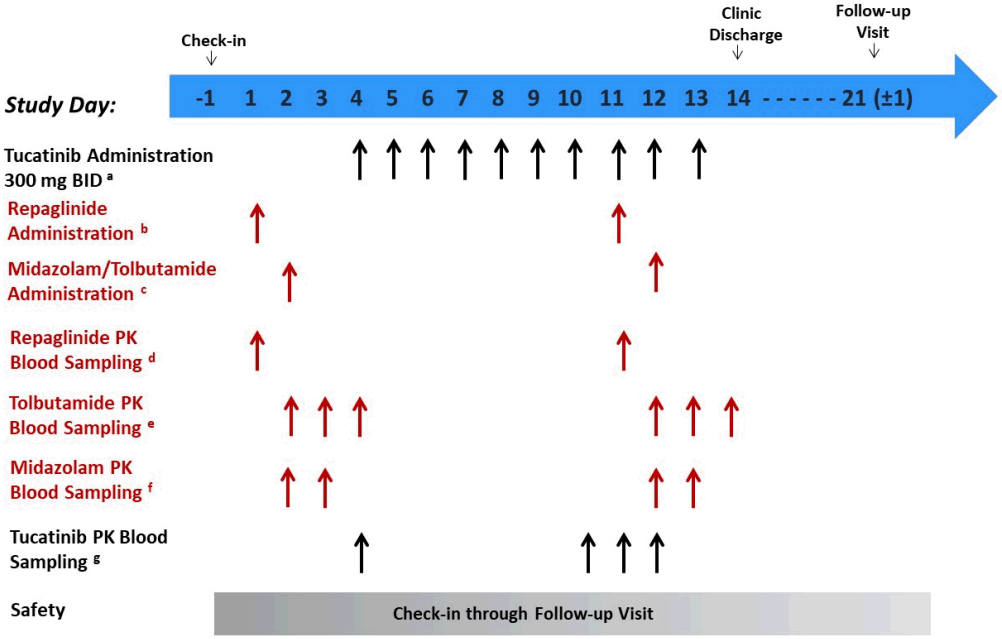
2.2.4.4. パート D：ツカチニブが CYP2C8、CYP2C9 及び CYP3A4 の基質の PK に及ぼす影響

パート D の主要目的は、ツカチニブが CYP2C8、CYP2C9 及び CYP3A4 のプローブ基質（それぞれレパグリニド、トルブタミド及びミダゾラム）の PK に及ぼす影響を評価することであり、トルブタミドの代謝物（4-ヒドロキシトルブタミド）及びミダゾラムの代謝物（1-ヒドロキシミダゾラム）の PK に対する影響も評価した。ミダゾラム及びトルブタミドは両薬剤間の DDI がないため同時投与した。パート D に計 17 例を組み入れ、全例を対象に PK 解析を実施した。治験参加者に以下の投与を実施した。

- 第 1 日：レパグリニド 0.5 mg 単回経口投与
- 第 2 日：ミダゾラム 2 mg 単回経口投与及びトルブタミド 500 mg 単回経口投与（同時）
- 第 4～13 日：ツカチニブ 300 mg BID 経口投与
- 第 11 日：レパグリニド 0.5 mg 単回経口投与
- 第 12 日：ミダゾラム 2 mg 単回経口投与及びトルブタミド 500 mg 単回経口投与（同時）

パート D の試験デザインの概略図を Figure 33 に示す。

Figure 33　Study schematic – Study ONT-380-012 Part D



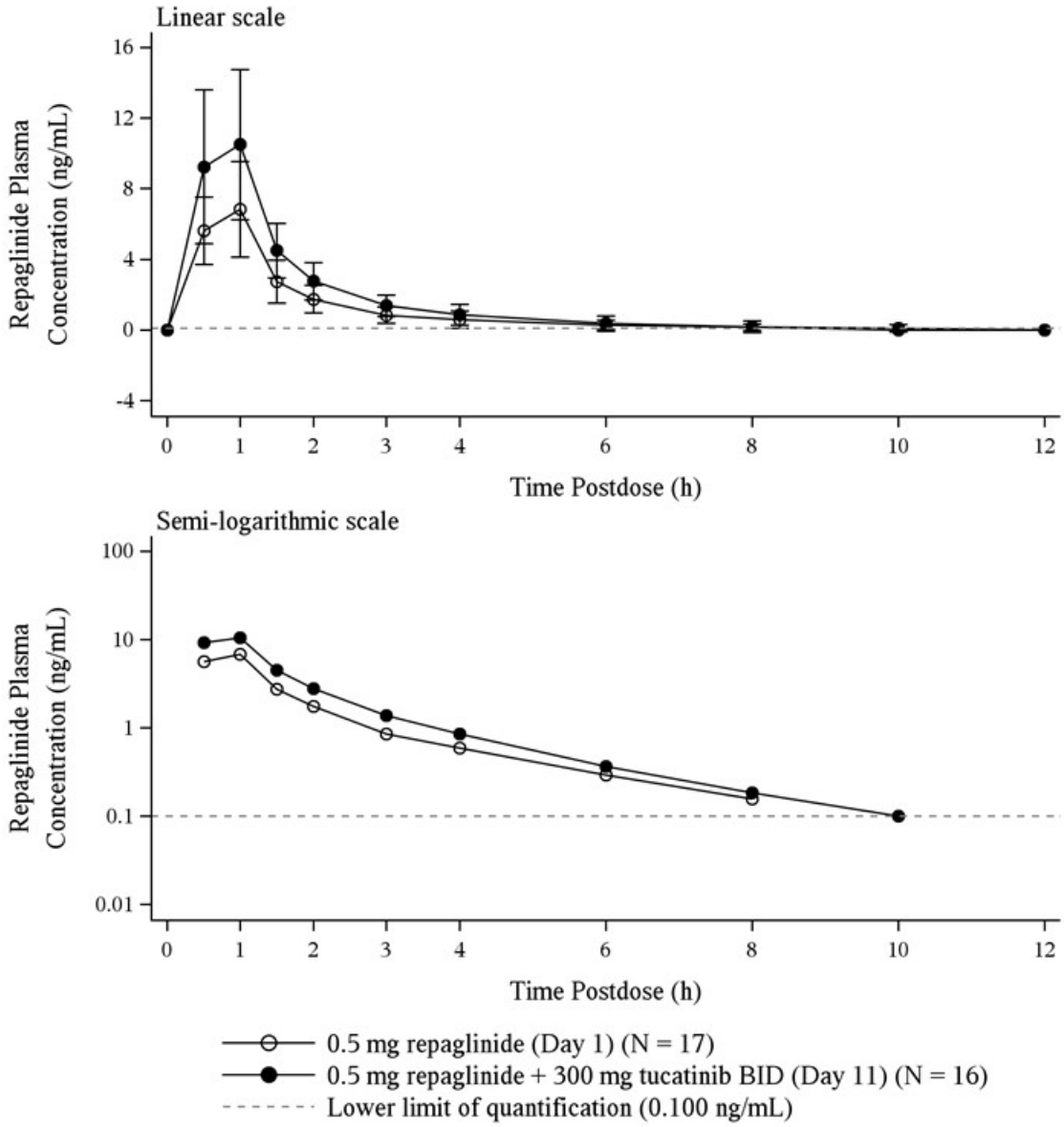
a Tucatinib 300 mg twice daily (BID; approximately 12 hours apart) was administered orally as 2 x 150-mg tablets on each dosing occasion on Days 4 through 13. On Days 4, 10, 11, and 12, the morning dose of tucatinib was administered after at least an 8-hour overnight fast. On Days 11 and 12, the morning dose of tucatinib was administered immediately after the probe drug.
b Repaglinide 0.5 mg was administered orally after an overnight fast on Days 1 and 11.
c Tolbutamide 500 mg and midazolam 2 mg were coadministered orally after an overnight fast on Days 2 and 12.
d Blood samples for plasma concentrations of repaglinide were collected predose and through 12 hours postdose on Days 1 and 11.
e Blood samples for plasma concentrations of tolbutamide and the 4-hydroxytolbutamide metabolite were collected predose and through 48 hours postdose on Days 2 to 4 and Days 12 to 14.
f Blood samples for plasma concentrations of midazolam and the 1-hydroxymidazolam metabolite were collected predose and through 24 hours postdose on Days 2 to 3 and Days 12 to 13.
g Blood samples for tucatinib plasma pharmacokinetics (PK) were collected predose and through 12 hours postdose on Days 4, 10, 11, and 12.

- レパグリニド（CYP2C8 の基質）の PK

レパグリニド単独及びツカチニブと併用投与したときのレパグリニドの血漿中濃度-時間推移を Figure 34、レパグリニドの PK パラメータの統計解析結果を Table 27 に示す。

ツカチニブとレパグリニドを併用投与したときのレパグリニドの C_{max} 及び AUC_{inf} は、レパグリニド単独投与時に比べていずれも約 1.7 倍に増加した。この結果、この結果、ツカチニブは CYP2C8 の基質であるレパグリニドの曝露量をやや上昇させたことから、ツカチニブは CYP2C8 の弱い阻害薬であることが示された。

Figure 34　Arithmetic mean ± SD plasma concentrations of repaglinide following oral administration of repaglinide 0.5 mg alone (Day 1) and in combination with tucatinib (Day 11) – Part D



BID = twice daily
Source: Study ONT-380-012 CSR, Figure 15

Table 27. Statistical analysis to assess the effect of tucatinib on the pharmacokinetic data of repaglinide – Part D

Analyte: Repaglinide

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	90% CI for the ratio (Test : Reference) Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	0.5 mg repaglinide (Reference)	17	10.5				
	0.5 mg repaglinide + 300 mg tucatinib BID (Test)	16	18.0	1.72	1.55	1.91	16.7
$AUC_{0-\infty}$ (h*ng/mL)	0.5 mg repaglinide (Reference)	16	10.9				
	0.5 mg repaglinide + 300 mg tucatinib BID (Test)	15	18.4	1.69	1.51	1.90	17.4
C_{max} (ng/mL)	0.5 mg repaglinide (Reference)	17	7.34				
	0.5 mg repaglinide + 300 mg tucatinib BID (Test)	16	12.4	1.69	1.37	2.10	35.7

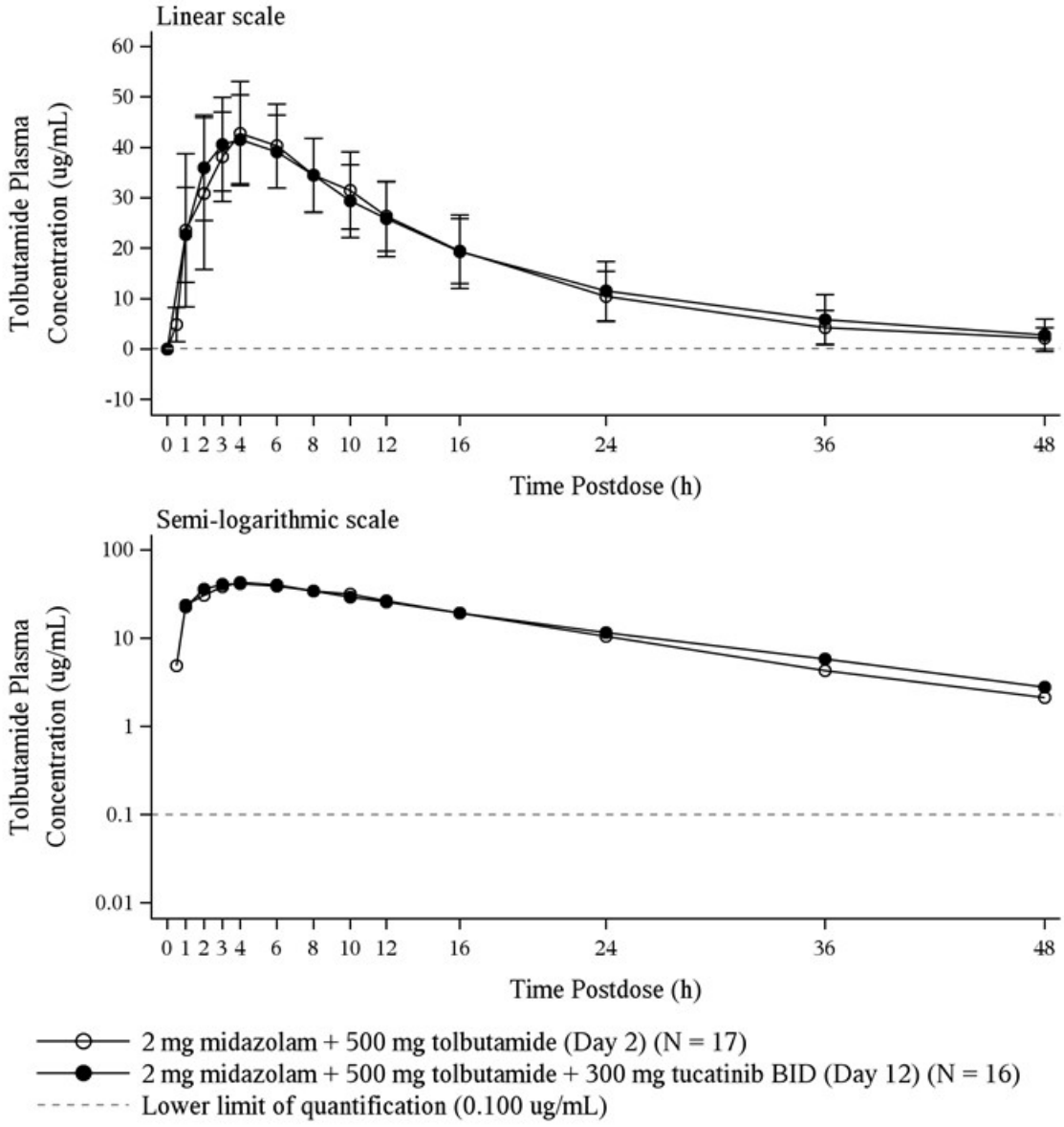
Abbreviations: AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 32

- トルブタミド（CYP2C9 の基質）の PK

トルブタミド（トルブタミドとミダゾラム同時投与）をツカチニブと非併用あるいは併用投与したときのトルブタミド及び 4-ヒドロキシトルブタミドの血漿中濃度-時間推移をそれぞれ Figure 35 及び Figure 36 に示す。トルブタミド及び 4-ヒドロキシトルブタミドの PK パラメータの統計解析結果を Table 28 及び Table 29 に示す。

ツカチニブとトルブタミドを併用投与したときのトルブタミド及び 4-ヒドロキシトルブタミドの C_{max} 及び AUC_{inf} は、ツカチニブ非併用投与時に比べて、トルブタミドでは約 5%、4-ヒドロキシトルブタミドでは約 10%程度の変化であり、大きな影響は認められなかった。この結果、ツカチニブは CYP2C9 の基質であるトルブタミドの曝露量には影響を及ぼさず、CYP2C9 を阻害あるいは誘導しないことが示された。

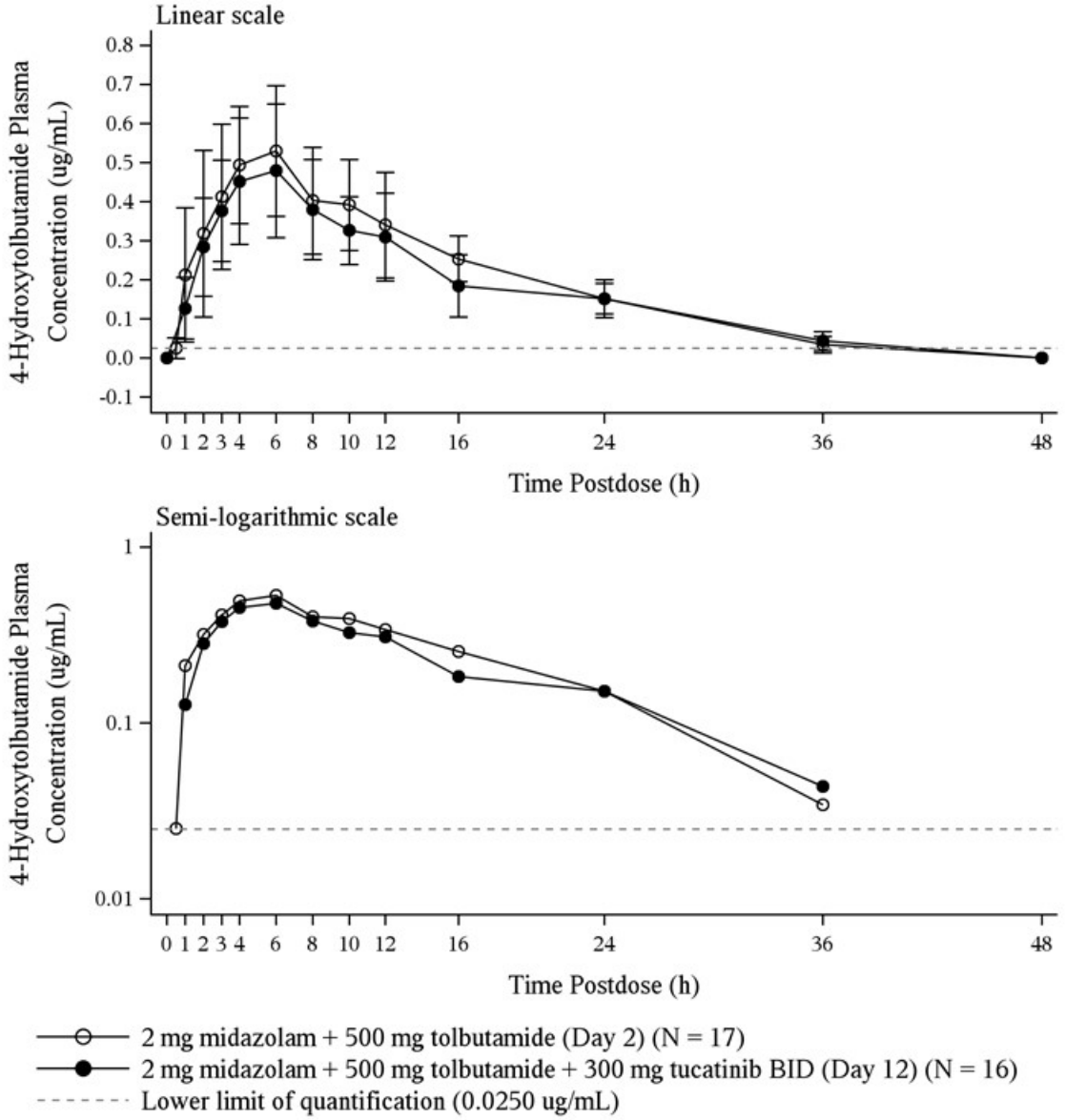
Figure 35　Arithmetic mean ± SD plasma concentrations of tolbutamide following oral administration of tolbutamide 500 mg and midazolam 2 mg alone (Day 2) and in combination with tucatinib (Day 12) – Part D



BID = twice daily

Source: Study ONT-380-012 CSR, Figure 17

Figure 36　Arithmetic mean ± SD plasma concentrations of 4-hydroxytolbutamide following oral administration of tolbutamide 500 mg and midazolam 2 mg alone (Day 2) and in combination with tucatinib (Day 12) – Part D



BID = twice daily

Source: Study ONT-380-012 CSR, Figure 18

Table 28. Statistical analysis to assess the effect of tucatinib on the pharmacokinetic data of tolbutamide – Part D

Analyte: Tolbutamide

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	Upper	Within-subject CV%
AUC_{last} (h*ug/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	693				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	715	1.03	1.01	1.06	4.3
$AUC_{0-\infty}$ (h*ug/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	720				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	755	1.05	1.01	1.09	5.8
C_{max} (ug/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	46.9				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	45.1	0.961	0.904	1.02	10.1

Abbreviations: AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 35

Table 29. Statistical analysis to assess the effect of tucatinib on the pharmacokinetic data of 4-hydroxytolbutamide – Part D

Analyte: 4-Hydroxytolbutamide

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	Upper	Within-subject CV%
AUC_{last} (h*ug/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	8.28				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	7.45	0.900	0.868	0.934	5.9
$AUC_{0-\infty}$ (h*ug/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	9.06				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	8.32	0.918	0.880	0.958	6.9
C_{max} (ug/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	0.546				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	0.481	0.881	0.831	0.934	9.5

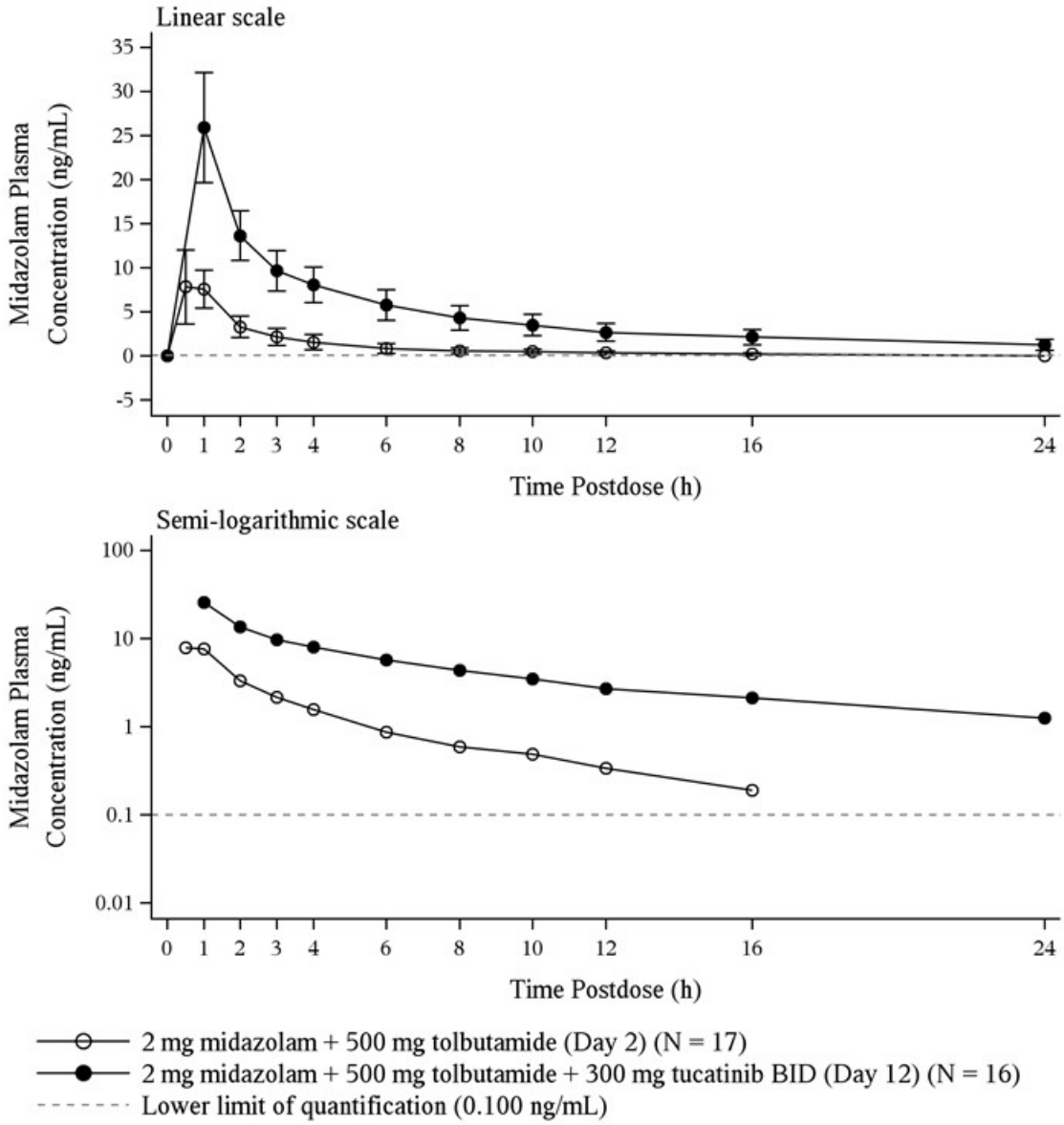
Abbreviations: AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 36

- ミダゾラム（CYP3A4 の基質）の PK

ミダゾラム（トルブタミドとミダゾラム同時投与）をツカチニブと非併用あるいは併用投与したときのミダゾラム及び 1-ヒドロキシミダゾラムの血漿中濃度-時間推移をそれぞれ Figure 37 及び Figure 38 に示す。ミダゾラム及び 1-ヒドロキシミダゾラムの PK パラメータの統計解析結果を Table 30 及び Table 31 に示す。

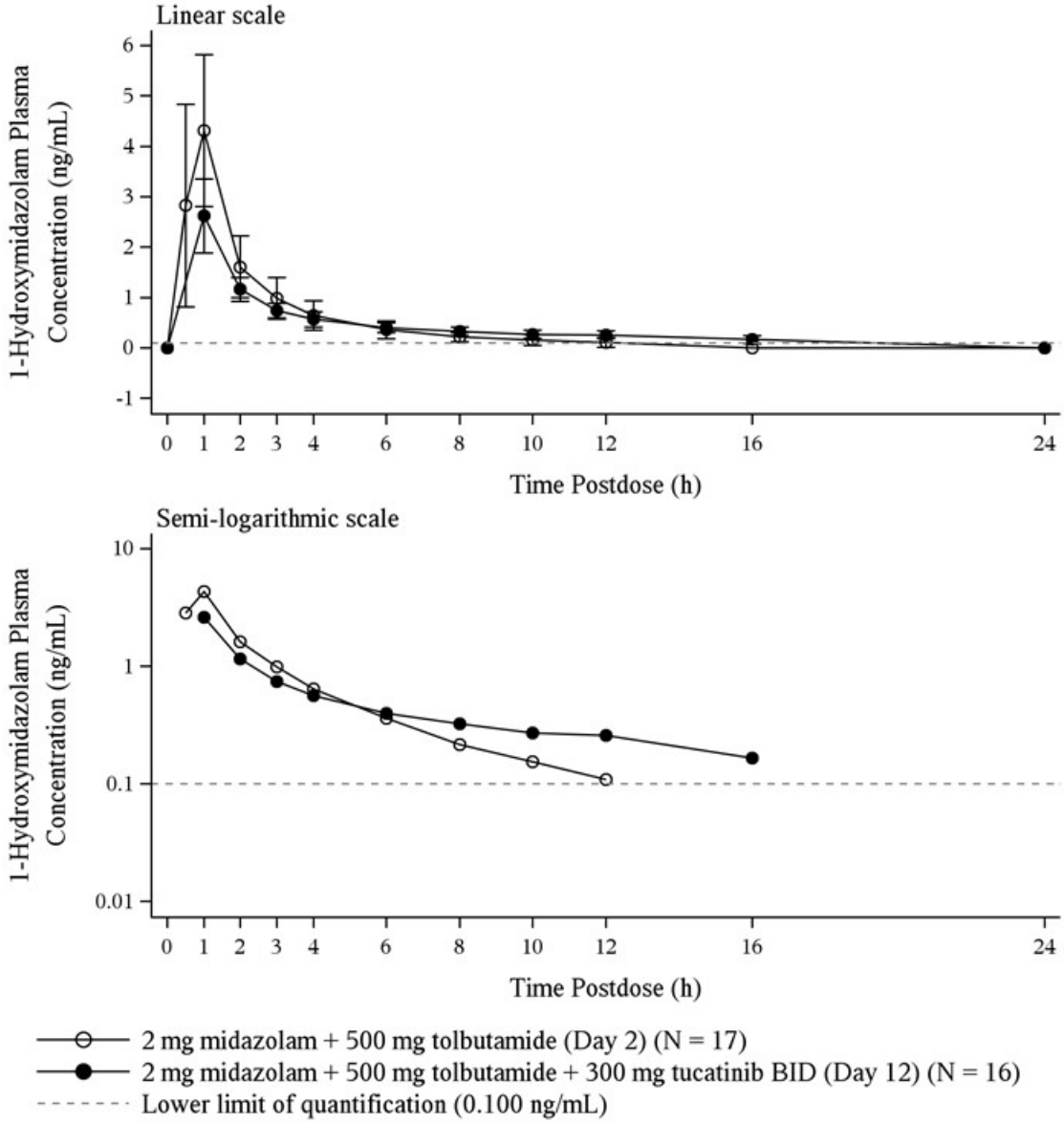
ツカチニブとミダゾラムを併用投与したときのミダゾラムの C_{max} 及び AUC_{inf} は、ツカチニブ非併用投与時に比べて、それぞれ約 3.0 倍及び約 5.3～5.7 倍に増加した。一方、1-ヒドロキシミダゾラムの C_{max} は約 40%減少し、AUC_{inf} はツカチニブ併用時と同程度であった。この結果、ツカチニブは CYP3A4 の基質であるミダゾラムの曝露量に大きな影響を及ぼしたことから、CYP3A4 の強い阻害薬であることが示された。このため、ツカチニブと CYP3A4 の基質の併用投与は注意が必要と考えられる。

Figure 37　Arithmetic mean ± SD plasma concentrations of midazolam following oral administration of tolbutamide 500 mg and midazolam 2 mg alone (Day 2) and in combination with tucatinib (Day 12) – Part D



BID = twice daily
Source: Study ONT-380-012 CSR, Figure 20

Figure 38　Arithmetic mean ± SD plasma concentrations of 1-hydroxymidazolam following oral administration of tolbutamide 500 mg and midazolam 2 mg alone (Day 2) and in combination with tucatinib (Day 12) – Part D



BID = twice daily

Source: Study ONT-380-012 CSR, Figure 21

Table 30. Statistical analysis to assess the effect of tucatinib on the pharmacokinetic data of midazolam – Part D

Analyte: Midazolam

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	21.4				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	113	5.30	4.65	6.04	21.5
$AUC_{0-\infty}$ (h*ng/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	22.7				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	130	5.74	5.05	6.53	21.1
C_{max} (ng/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	8.71				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	26.2	3.01	2.63	3.45	22.4

AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 39

Table 31. Statistical analysis to assess the effect of tucatinib on the pharmacokinetic data of 1-hydroxymidazolam – Part D

Analyte: 1-Hydroxymidazolam

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	9.55				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	9.03	0.945	0.848	1.05	17.8
$AUC_{0-\infty}$ (h*ng/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	10.5				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	14	10.7	1.02	0.903	1.16	18.9
C_{max} (ng/mL)	2 mg midazolam + 500 mg tolbutamide (Reference)	17	4.44				
	2 mg midazolam + 500 mg tolbutamide + 300 mg tucatinib BID (Test)	16	2.63	0.593	0.507	0.694	26.0

AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 40

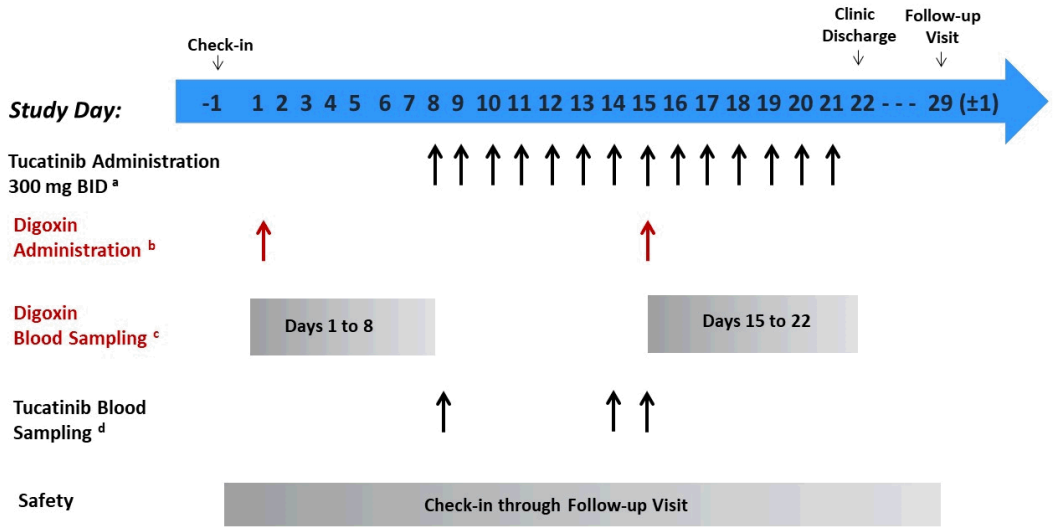
2.2.4.5. パート E：ツカチニブが P-gp 基質の PK に及ぼす影響

パート E の主要目的は、ツカチニブが P-gp のプローブ基質（ジゴキシン）の PK に及ぼす影響を評価することである。パート E に計 15 例を組み入れ、全例を対象に PK 解析を実施した。治験参加者に以下の投与を実施した。

- 第 1 日：ジゴキシン 0.5 mg 単回経口投与
- 第 8～21 日：ツカチニブ 300 mg BID 経口投与
- 第 15 日：ジゴキシン 0.5 mg 単回経口投与

パート E の試験デザインの概略図を Figure 39 に示す。

Figure 39　Study schematic – Study ONT-380-012 Part E



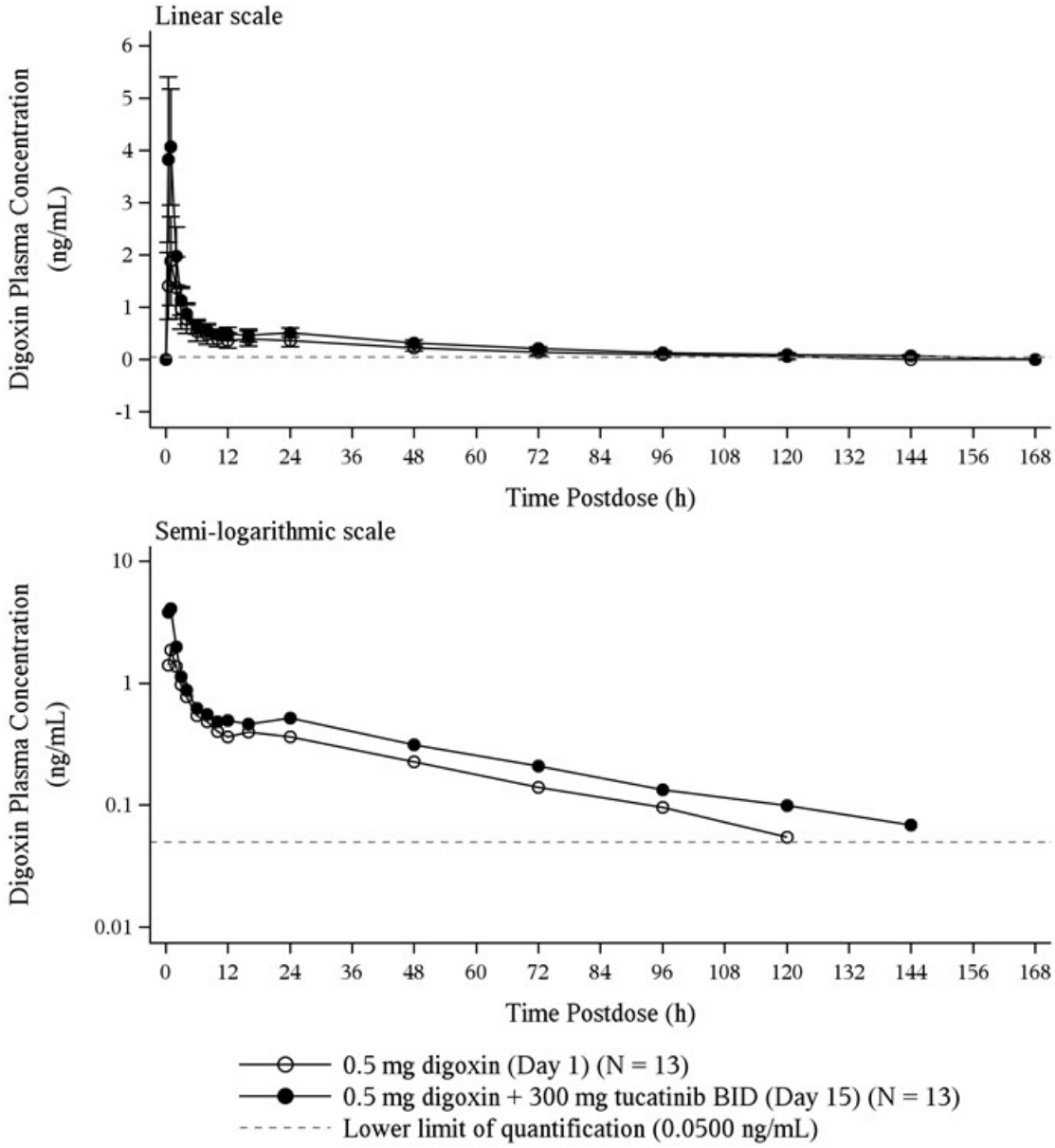
a Tucatinib 300 mg twice daily (BID; approximately 12 hours apart) was administered orally as 2 x 150-mg tablets on each dosing occasion on Days 8 through 21. On Days 8, 14, and 15, the morning dose of tucatinib was administered after at least an 8-hour overnight fast. On Day 15 the morning dose of tucatinib was administered immediately after digoxin.
b Single oral doses of digoxin 0.5 mg were administered orally as 2 x 0.25-mg tablets in the morning of Days 1 and 15 following an at least 8-hour overnight fast.
c Blood samples for determination of the plasma concentrations of digoxin were collected predose and through 7 days (168 hours) postdose on Days 1 through 8 and Days 15 through 22.
d Blood samples for tucatinib plasma pharmacokinetics (PK) were collected predose and through 12 hours postdose on Days 8, 14, and 15.

- ジゴキシン（P-gp の基質）の PK

ジゴキシン単独及びツカチニブと併用投与したときのジゴキシンの血漿中濃度-時間推移を Figure 40、ジゴキシンの PK パラメータの統計解析結果を Table 32 に示す。

ツカチニブとジゴキシンを併用投与したときのジゴキシンの C_{max} 及び AUC_{inf} は、ジゴキシン単独投与時に比べて、それぞれ約 2.3 倍及び約 1.5 倍に増加した。この結果、ツカチニブは P-gp の基質であるジゴキシンの曝露量にやや影響を及ぼしたことから、P-gp の弱い阻害薬であることが示された。このため、ジゴキシンのような治療域の狭い P-gp の基質をツカチニブと併用するときは注意が必要と考えられる。

Figure 40　Arithmetic mean ± SD plasma concentrations of digoxin following oral administration of digoxin 0.5 mg alone (Day 1) and in combination with tucatinib (Day 15) – Part E



BID = twice daily
Source: Study ONT-380-012 CSR, Figure 25

Table 32. Statistical analysis to assess the effect of tucatinib on the pharmacokinetic data of digoxin – Part E

Analyte: Digoxin

Parameter	Treatment	n	Geometric least squares mean	Ratio of geometric least squares mean (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	90% CI for the ratio (Test : Reference) Upper	Within-subject CV%
AUC_{last} (h*ng/mL)	0.5 mg digoxin (Reference)	13	28.2				
	0.5 mg digoxin + 300 mg tucatinib BID (Test)	13	43.2	1.53	1.35	1.74	18.7
$AUC_{0-\infty}$ (h*ng/mL)	0.5 mg digoxin (Reference)	13	32.6				
	0.5 mg digoxin + 300 mg tucatinib BID (Test)	13	47.7	1.46	1.29	1.66	18.3
C_{max} (ng/mL)	0.5 mg digoxin (Reference)	13	1.84				
	0.5 mg digoxin + 300 mg tucatinib BID (Test)	13	4.31	2.35	1.90	2.90	30.8

AUC_{last} = area under the concentration-time curve from time 0 to the time of last quantifiable concentration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 extrapolated to infinity; CI = confidence interval; C_{max} = maximum concentration; CV = coefficient of variation; n = number of observations.
Note: The ratio and corresponding confidence limits are back-transformed from the difference and confidence limits calculated on the log scale.
Source: Study ONT-380-012 CSR, Table 44

2.2.5. 健康成人を対象としたツカチニブとメトホルミンの DDI 試験：SGNTUC-020 試験

本治験は、健康成人を対象とし、ツカチニブがトランスポーターMATE1/MATE2-K の基質（メトホルミン）の PK に及ぼす影響を評価した非盲検、固定順序の DDI 試験である。本治験ではイオヘキソールを GFR の指標として腎機能に対するツカチニブの影響も評価した。本治験に 18 例を組み入れ、以下の投与を実施し、そのうち 17 例を対象に PK 及び PD 解析を実施した。

- Day 1 及び Day 8 の午前中にメトホルミン 850 mg を絶食下で経口投与
- Day 1 及び Day 8 のメトホルミン投与 10 時間後にイオヘキソール 5 mL（ヨード含有量 300 mg/mL）を静脈内投与（総投与量はヨード 1500 mg、イオヘキソール 3235 mg）
- Day 2～Day 8 までツカチニブ 300 mg BID を経口投与（ツカチニブは 150 mg 錠を 2 錠投与し、午前中の投与は絶食下、Day 8 ではメトホルミン投与直後に投与）

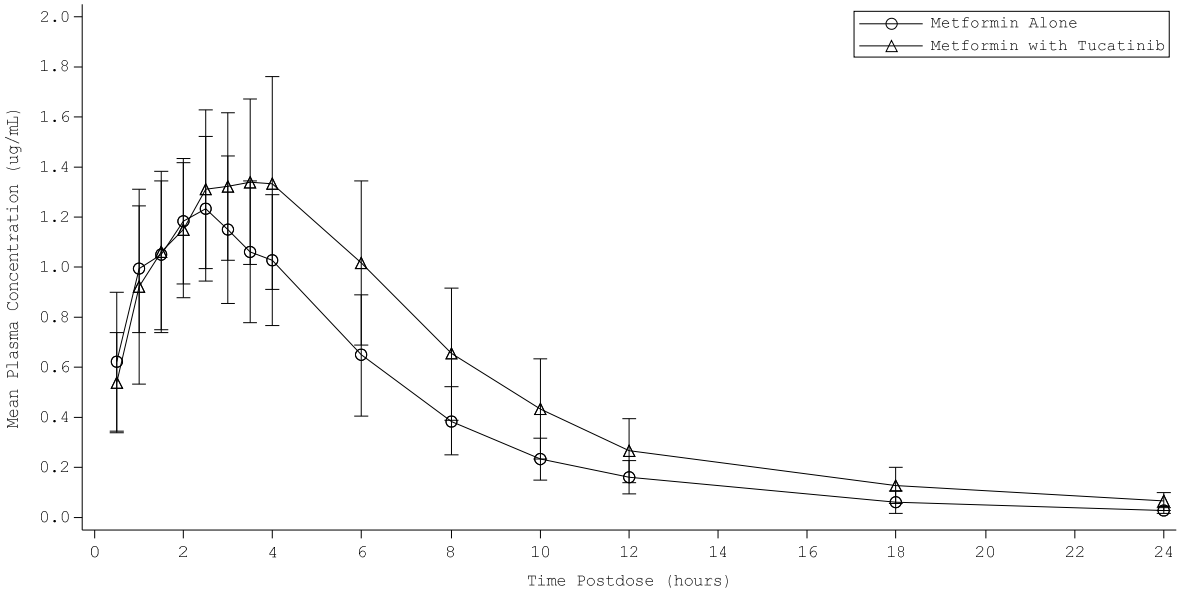
2.2.5.1. 血漿中及び尿中メトホルミンの PK

メトホルミン単独及びツカチニブと併用投与したときのメトホルミンの血漿中濃度-時間推移を Figure 41、メトホルミンの血漿中及び尿中 PK パラメータの統計解析結果をそれぞれ Table 33 及び Table 34 に示す。

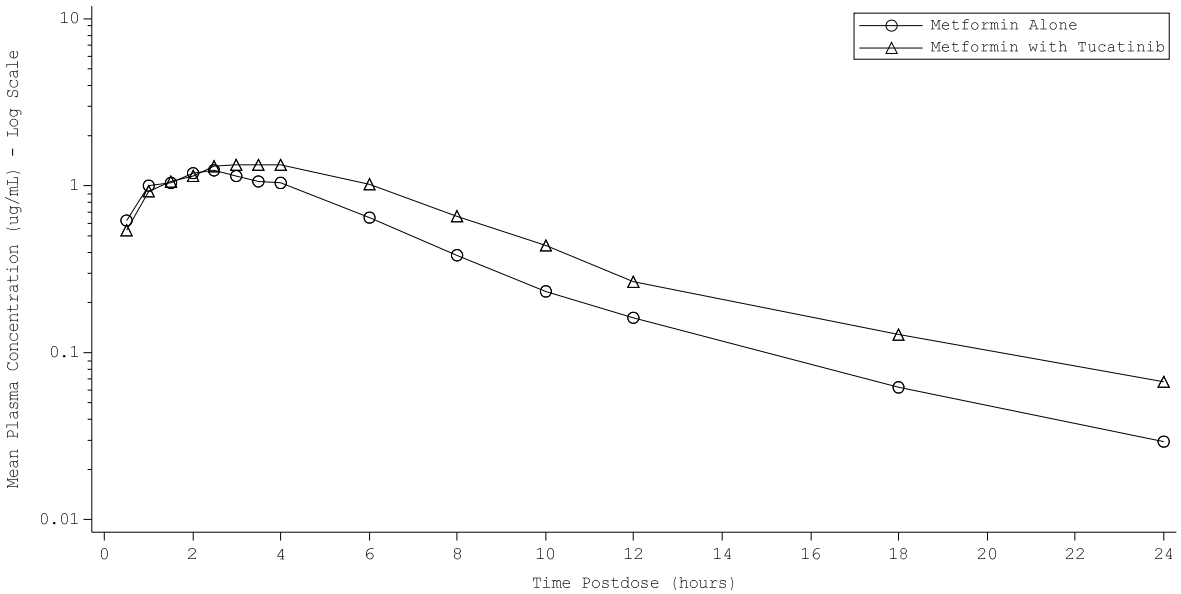
ツカチニブとメトホルミンを併用投与したとき、メトホルミン単独投与時に比べて血漿中メトホルミンの C_{max} 及び AUC_{inf} はそれぞれ約 1.1 倍及び約 1.4 倍に増加した。この結果、ツカチニブは MATE1/MATE2-K の基質であるメトホルミンの曝露量にやや影響を及ぼしたことから、MATE1/MATE2-K の弱い阻害薬であることが示された。尿中のメトホルミン PK 解析の結果、メトホルミンの Ae24、Fe24 及び CLr は、メトホルミン単独投与時に比べて、ツカチニブ併用投与時ではやや減少する傾向が認められた。

Figure 41　Mean ± SD plasma metformin concentrations versus time profiles

Linear



Semi-log



Source: Study SGNTUC-020 CSR, Figure 1

Table 33. Statistical analyses of the effect of tucatinib on metformin pharmacokinetic parameters

Parameter	Geometric Least Squares (LS) Mean				Geometric LS Mean Ratio (Test/Reference)	
	Metformin with Tucatinib (Test)		Metformin Alone (Reference)			
	n	Result	n	Result	Estimate	90% CI
AUC_{last} (h* µg/mL)	17	11.054	17	8.147	1.357	(1.220, 1.509)
AUC_{inf} (h* µg/mL)	17	11.558	17	8.333	1.387	(1.251, 1.539)
C_{max} (µg/mL)	17	1.418	17	1.314	1.079	(0.951, 1.225)

CI = confidence interval
Source: Study SGNTUC-020 CSR, Table 9

Table 34. Summary statistics for urine metformin pharmacokinetic parameters

Pharmacokinetic Parameters	Arithmetic Mean (CV%)	
	Metformin Alone (n=17)	Metformin + Tucatinib (n=17)
$A_{e0\text{-}24}$ (mg)	242.62 (26.5)	190.79 (31.7)
$F_{e0\text{-}24}$ (%)	28.54 (26.5)	22.44 (31.7)
CL_R (L/h)	29.99 (27.0)	17.64 (36.0)

CV = coefficient of variation
Source: Study SGNTUC-020 CSR, Table 8

2.2.5.2. 血漿中イオヘキソールの PK

イオヘキソールの PK パラメータは Jodal and Brochner-Mortensen 式に従って算出した。血漿中イオヘキソールの PK パラメータの要約統計量を Table 35 に示す。

イオヘキソールの PK パラメータは、ツカチニブとメトホルミンの併用投与後とメトホルミン単独投与後とで同程度であったため、ツカチニブは GFR に影響を及ぼさなかったことが示された。シスタチン C データを用いて投与別に推定した GFR は、ツカチニブの非併用投与時と併用投与時でそれぞれ 101.4 mL/min/1.73 m^2 及び 111.4 mL/min/1.73 m^2 であり、有意な影響は認められなかった。

Table 35. Summary statistics for plasma iohexol pharmacokinetic parameters

Pharmacokinetic Parameters	Arithmetic Mean (CV%)	
	Metformin Alone	Metformin + Tucatinib
AUC_{last} (h*ug/mL)	345.5 (21.2)	346.4 (21.7)
AUC_{inf} (h*ug/mL)	440.5 (27.9)	441.9 (27.2)
CL_{syst} (mL/min)	129.8 (22.7)	129.0 (22.0)
$t_{1/2}$ (h)	1.762 (18.9)	1.784 (17.5)
C_0 (ug/mL)	175.6 (18.0)	178.5 (21.5)
aGFR (mL/min/1.73 m^2)	94.99 (17.4)	94.56 (16.9)

CV = coefficient of variation; CLsyst = systemic clearance
Source: Study SGNTUC-020 CSR, Table 6

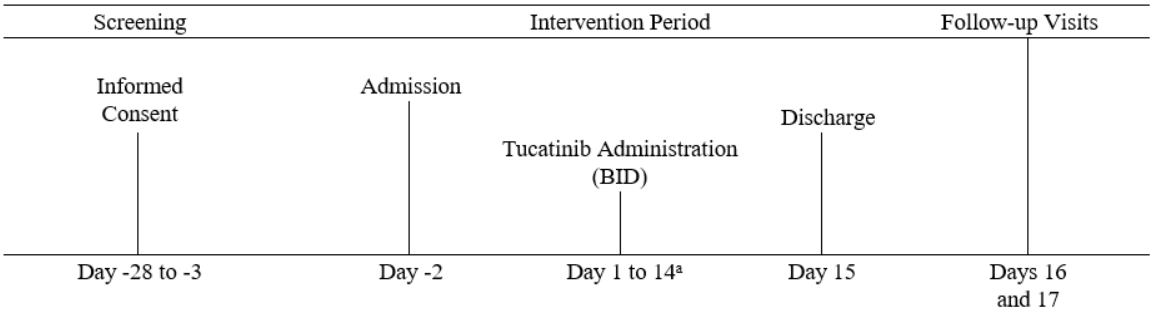
2.2.6. 日本人及び白人の健康成人を対象としたツカチニブの PK 試験：SGNTUC-015 試験

本治験は、日本人及び Caucasian（コーカサス系人種、本 M2.7.2 では白人と記す）の健康成人を対象にツカチニブの PK を評価し、両人種の PK を比較する第 1 相、非盲検試験である。3 用量の 3 つのコホートにおいて、14 日間（第 1～14 日）ツカチニブ錠を反復経口投与した。Day 1 の初回及び Day 14 では絶食下投与し、その他では食事は問わずに投与した。各投与コホートに日本人及び白人を各 6 例ずつ組み入れた。

- コホート 1：Day 1～13 に 50 mg BID、Day 14 に QD（日本人 6 例、白人 6 例）
- コホート 2：Day 1～13 に 150 mg BID、Day 14 に QD（日本人 6 例、白人 6 例）
- コホート 3：Day 1～13 に 300 mg BID、Day 14 に QD（日本人 6 例、白人 6 例）

SGNTUC-015 試験のフローチャートを Figure 42 に示す。

Figure 42　SGNTUC-015 study flow chart



BID = twice daily

a. On Day 14, only the morning dose will be administered in order to get a full PK and metabolite profile up to 72 hours.

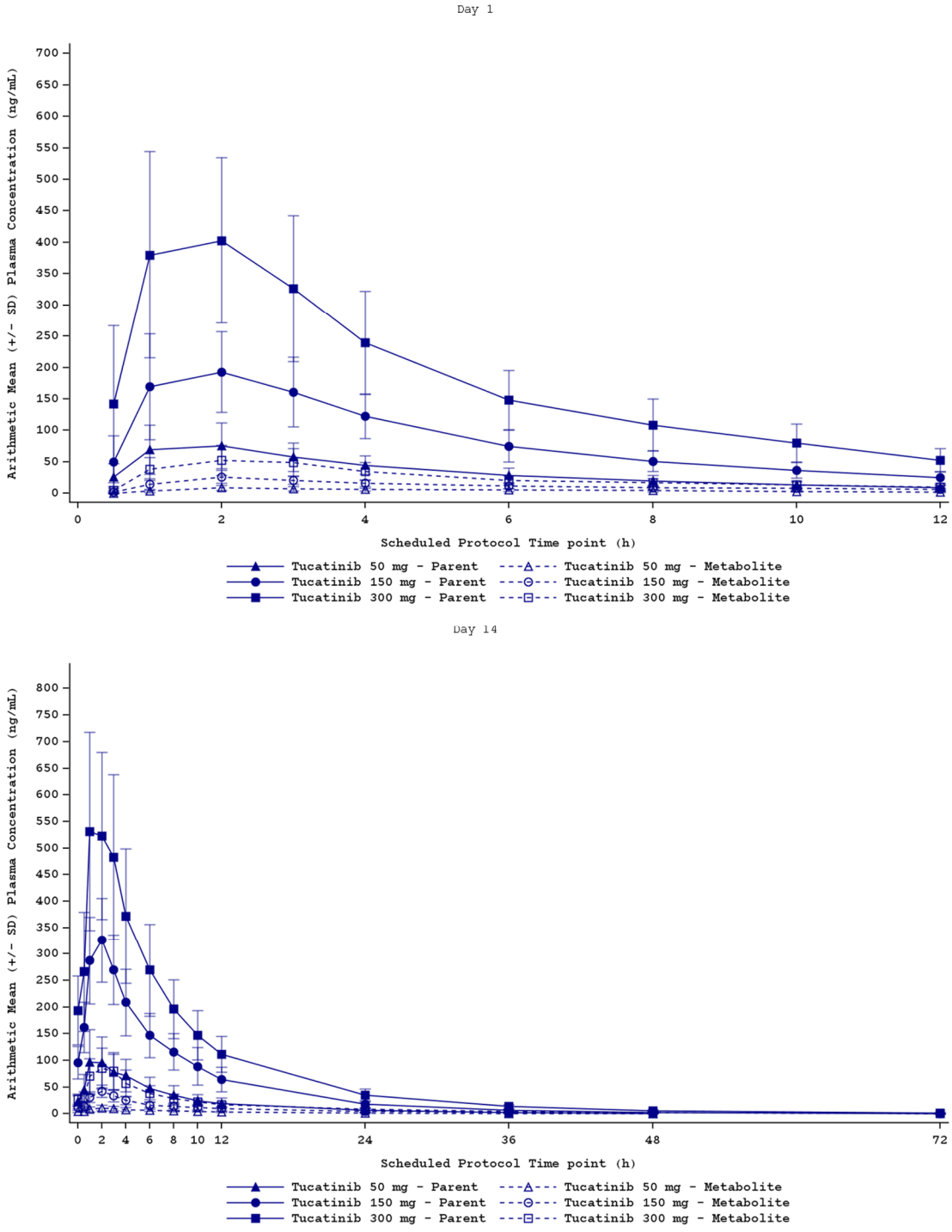
2.2.6.1. ツカチニブ及び ONT-993 の血漿中濃度

ツカチニブ（50、150 及び 300 mg）を投与後の Day 1 及び Day 14 におけるツカチニブ及び ONT-993 の血漿中濃度-時間推移を Figure 43（線形目盛）及び Figure 44（片対数目盛）に示す。日本人及び白人別におけるツカチニブの血漿中濃度-時間推移を Figure 45（線形目盛）及び Figure 46（片対数目盛）に示し、Day 2～14 の血漿中 C_{trough}-時間推移を Figure 47 に示す。

ツカチニブ及び ONT-993 の血漿中濃度は投与量に応じて、それぞれ吸収相から消失相まで同様の推移を示した。ツカチニブ 150 mg 及び 300 mg では Day 1 及び Day 14 で日本人と白人で概ね同様の血漿中濃度-時間推移であった。ツカチニブ 50 mg では白人で全体の用量の結果よりもやや低い傾向であったが、変動があり日本人の分布と概ね重なっていた。

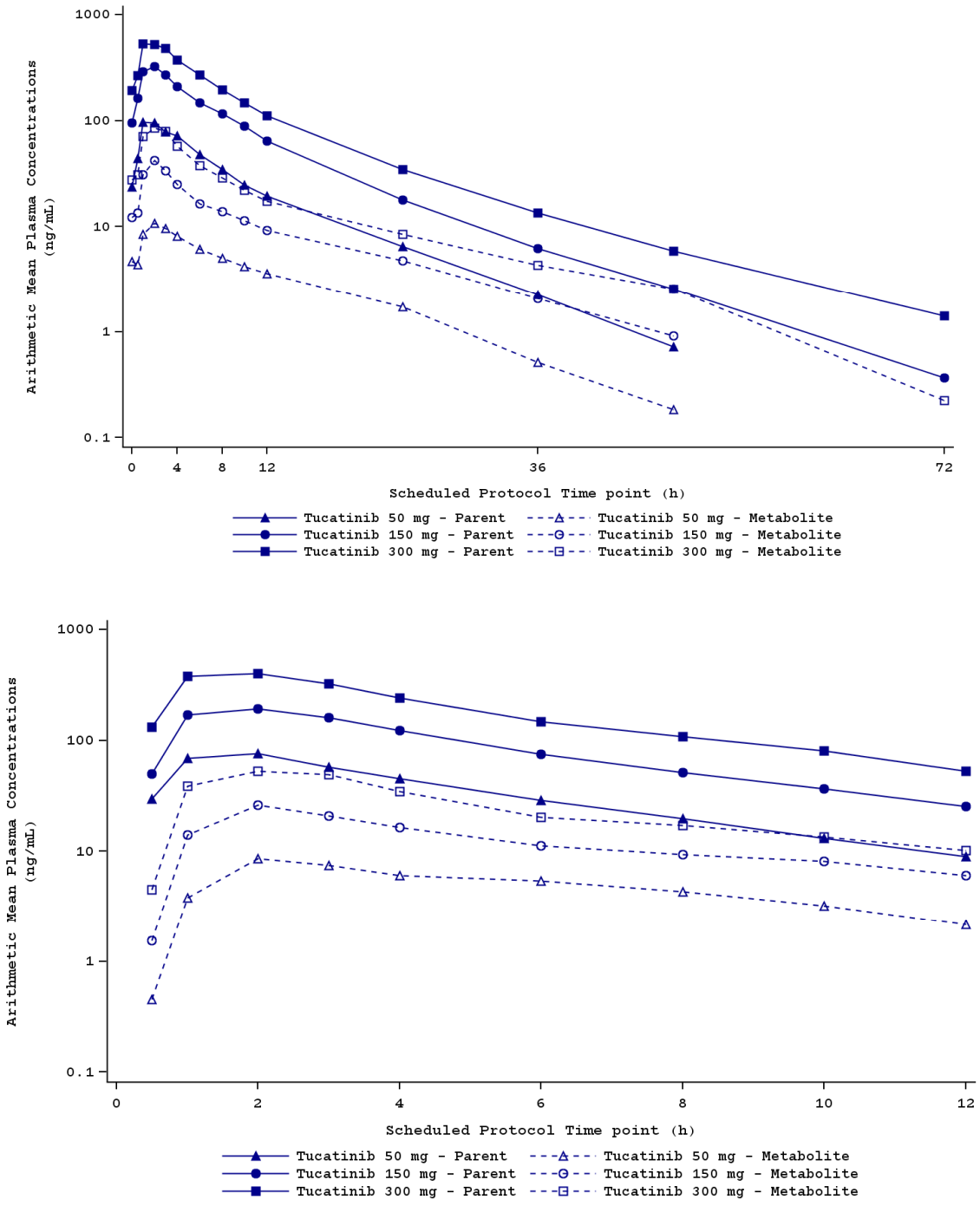
反復投与の結果、Day 5 までに定常状態に達し、血漿中 C_{trough} は検討したすべての用量でほぼ用量比例性を示した。日本人と白人の血漿中 C_{trough} はいずれの用量でも同様の推移であった。

Figure 43　Arithmetic mean ± SD tucatinib and ONT-993 plasma concentration time data (pharmacokinetic population) all subjects (linear scale) – Day 1 and Day 14



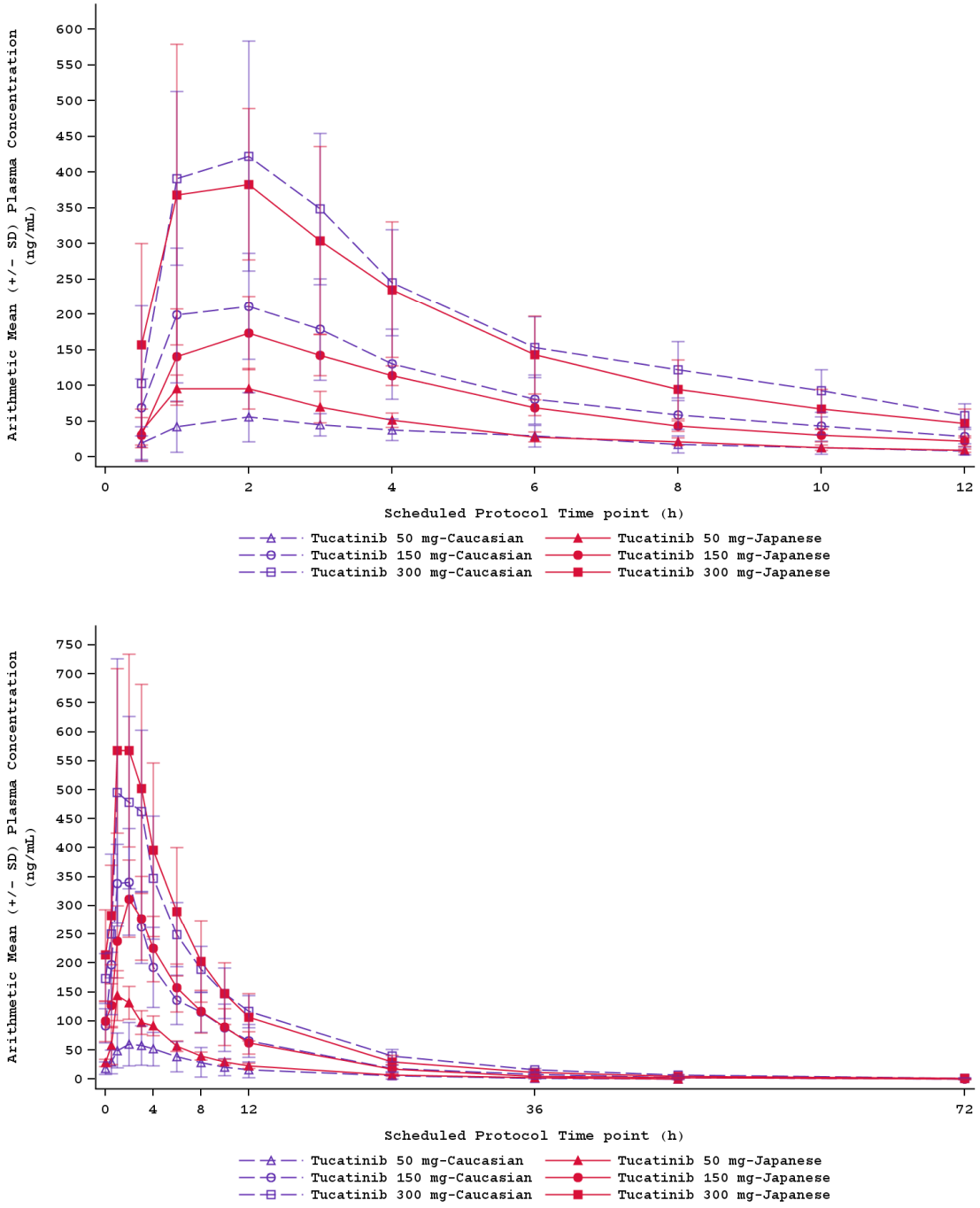
Note: When actual time of sample collection deviated greater than 30 minutes from planned collection time for predose time point (trough) or was greater than 20% from planned collection time for all other time points, the concentrations at those time points were excluded from the mean concentrations used for plotting.
Source: SGNTUC-015 CSR, Figure 11-4

Figure 44　Arithmetic mean ± SD tucatinib and ONT-993 plasma concentration time data (pharmacokinetic population) all subjects (semi-logarithmic scale) – Day 1 and Day 14



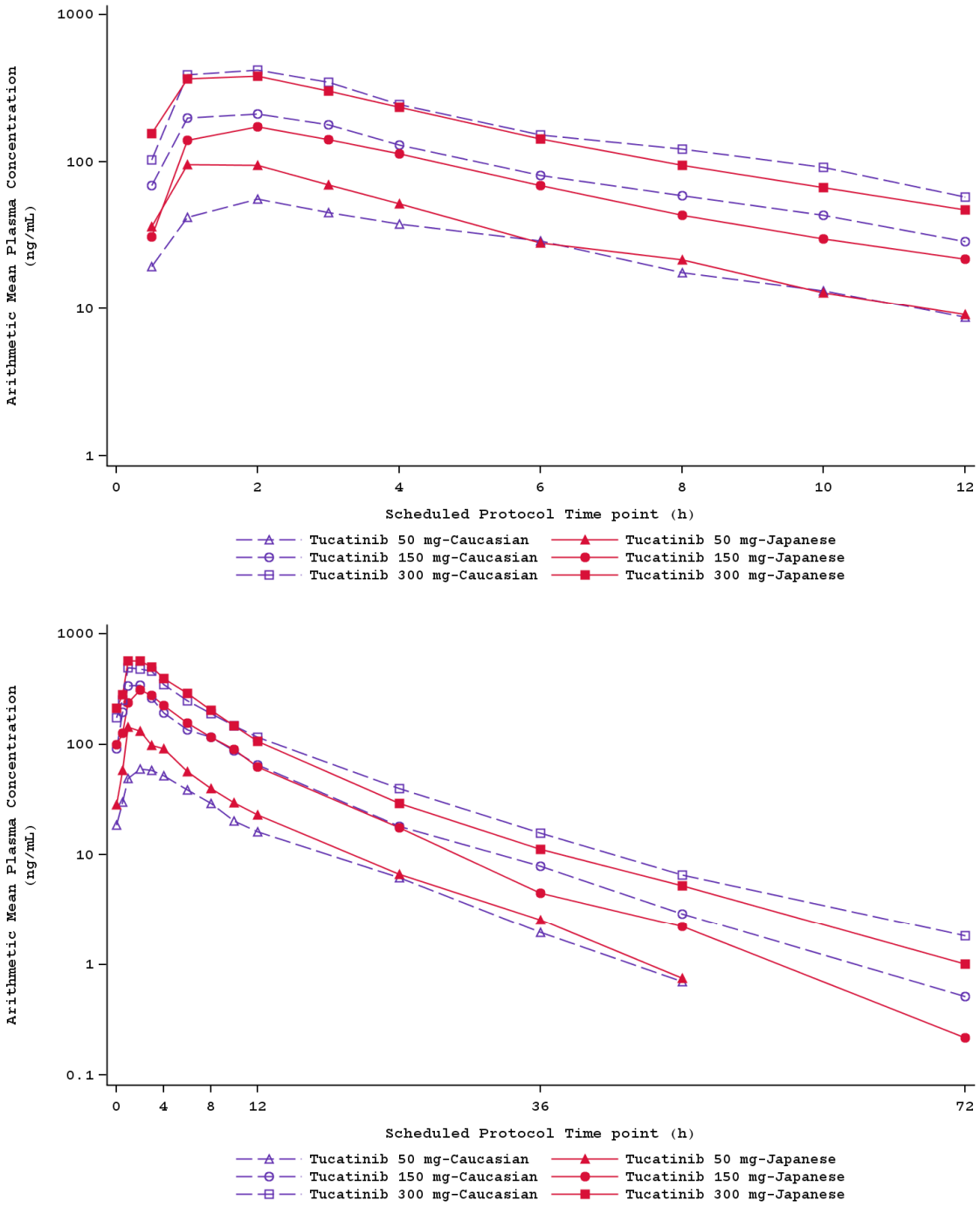
Note: When actual time of sample collection deviated greater than 30 minutes from planned collection time for predose time point (trough) or was greater than 20% from planned collection time for all other time points, the concentrations at those time points were excluded from the mean concentrations used for plotting.
Source: SGNTUC-015 CSR, Figure 11-5

Figure 45　Linear mean concentration-time profiles of tucatinib on Day 1 and Day 14 (top panel: Day 1, bottom panel: Day 14) (pharmacokinetic population)



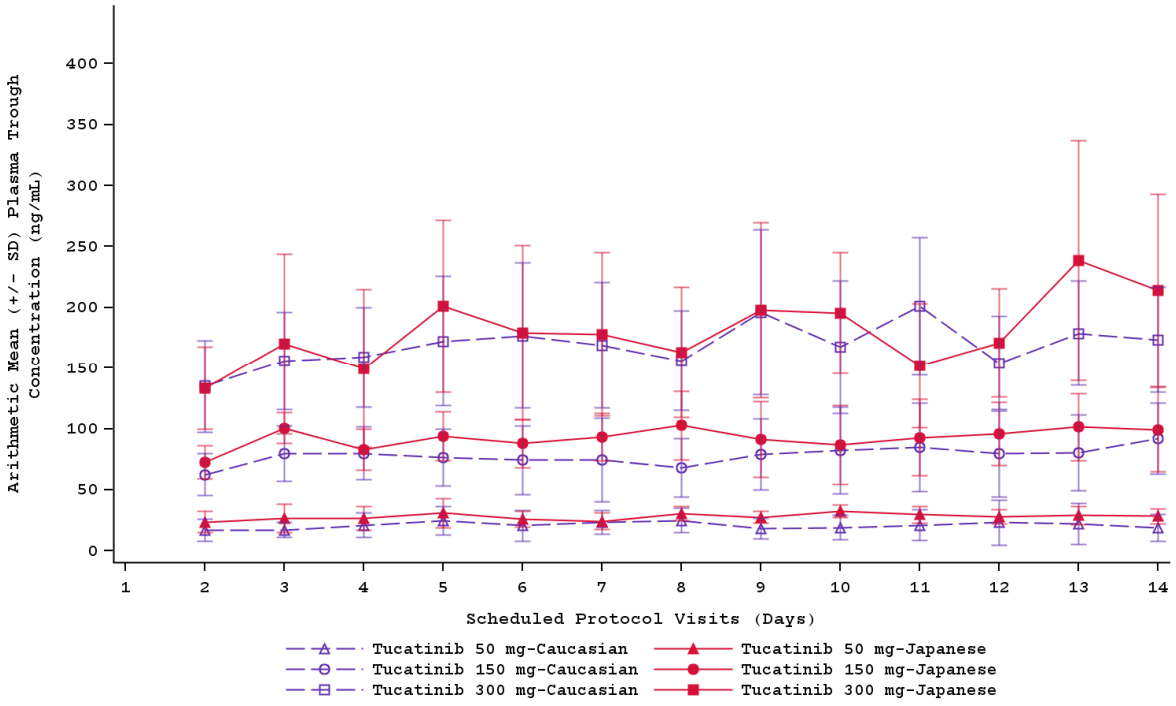
When actual time of sample collection deviated greater than 30 minutes from planned collection time for predose time point (trough) or was greater than 20% from planned collection time for all other time points, the concentrations at those time points were excluded from the mean concentrations used for plotting.
Data Source: SGNTUC-015, CSR, Figure 11-1.

Figure 46　Semi-logarithmic mean concentration-time profiles of tucatinib on Day 1 and Day 14 (top panel: Day 1, bottom panel: Day 14) (pharmacokinetic population)



When actual time of sample collection deviated greater than 30 minutes from planned collection time for predose time point (trough) or was greater than 20% from planned collection time for all other time points, the concentrations at those time points were excluded from the mean concentrations used for plotting.
Data Source: SGNTUC-015, CSR, Figure 11-2.

Figure 47　Arithmetic mean ± SD tucatinib plasma trough concentration time data profiles (pharmacokinetic population) all treatment groups and ethnicity



When actual time of sample collection deviated greater than 30 minutes from planned collection time for predose time point (trough) or was greater than 20% from planned collection time for all other time points, the concentrations at those time points were excluded from the mean concentrations used for plotting.
Source: SGNTUC-015 CSR, Figure 11-3

2.2.6.2. ツカチニブ及び ONT-993 の PK パラメータ

ツカチニブ（50、150 及び 300 mg）を投与したときの全体集団、日本人、白人の Day 1 及び Day 14 のツカチニブの主な PK パラメータは Table 36 及び Table 37 に要約し、C_{max} 及び AUC の箱ひげ図を Figure 48 に示す。同様に Day 1 及び Day 14 の ONT-993 の主な PK パラメータはそれぞれ Table 38 及び Table 39 に要約する。

全体集団において、Day 1 のツカチニブの C_{max} 及び AUC_{inf}（幾何平均値）は、50 mg 群で 73.8 ng/mL 及び 370 h•ng/mL、150 mg 群で 199 ng/mL 及び 1126 h•ng/mL、300 mg 群で 436 ng/mL 及び 2203 h•ng/mL であった。Day 14 のツカチニブの C_{max} 及び AUC_{12}（幾何平均値）は、50 mg 群で 88.8 ng/mL 及び 531 h•ng/mL、150 mg 群で 333 ng/mL 及び 1937 h•ng/mL、300 mg 群で 571 ng/mL 及び 3332 h•ng/mL であった。Day 1 及び Day 14 の T_{max}（中央値）はすべての用量群で約 2 時間（範囲：0.98～4.02 時間）であった。Day 14 の AUC_{inf}（50、150 及び 300 mg のそれぞれの幾何平均値 810.3、2563.5 及び 4599.9 h•ng/mL、SGNTUC-015 CSR Table 14.2.2.2）は、Day 1 の約 2 倍であった。

全体集団の結果、ツカチニブ 50 mg、150 mg 及び 300 mg を投与したときの Day 1 及び Day 14 のツカチニブの C_{max} 及び AUC は、用量に概ね比例して増加することが示された。ツカチニブの

C_{trough}（幾何平均値）は 50 mg 群で 21.4 ng/mL、150 mg 群で 91.4 ng/mL、300 mg 群で 184 ng/mL であり、C_{trough} も用量に概ね比例して増加した。

全体集団での ONT-993 の代謝物/親化合物比（幾何平均比）は、Day 1 の AUC_{inf} では各用量群で同程度（0.1456～0.1735）であり、Day 14 の AUC_{12} においても各用量群で同程度（0.1150～0.1428）であった。

日本人と白人では、ツカチニブ 150 mg 及び 300 mg では Day 1 及び Day 14 において C_{max} 及び AUC は両集団で同様であった。ツカチニブ 50 mg では白人の C_{max} 及び AUC は全体の用量の結果よりもやや低い傾向であったが、変動があり日本人の分布と概ね重なっていた。

日本人と白人の C_{max} 及び AUC の人種の影響を比較した統計解析の結果（SGNTUC-015 CSR Section 11.4.1.2）、ANCOVA 調整後の幾何平均比は、50 mg 群では Day 1 及び Day 14 で変動（0.6～2.63）がみられたものの、150 mg 群及び 300 mg 群では、いずれも 0.86～1.33 の範囲であり、人種間で有意な差がないことが示された。

Table 36. Summary of key tucatinib pharmacokinetic parameters Day 1 (pharmacokinetic population)

Parameter	Statistic	Tucatinib 50 mg			Tucatinib 150 mg			Tucatinib 300 mg		
		Caucasian (N = 6)	Japanese (N = 6)	Overall (N = 12)	Caucasian (N = 6)	Japanese (N = 6)	Overall (N = 12)	Caucasian (N = 6)	Japanese (N = 6)	Overall (N = 12)
C_{max} (ng/mL)	n	6	6	12	6	6	12	6	6	12
	GM	54.8149	99.3334	73.7899	216.2259	182.3432	198.5632	429.8723	441.6023	435.6978
	gCV%	61.1	22.8	54.9	52.8	32.0	42.2	32.3	37.6	33.4
T_{max} (h)	n	6	6	12	6	6	12	6	6	12
	Median	2.000	1.500	2.000	2.000	2.000	2.000	2.000	1.017	1.492
	Minimum	1.00	0.98	0.98	0.98	1.00	0.98	1.00	0.98	0.98
	Maximum	3.07	2.02	3.07	3.00	3.00	3.00	2.03	3.00	3.00
AUC_{inf} (h*ng/mL)	n	5	5	10	5	4	9	5	3	8
	GM	290.0307	471.3061	369.7205	1222.8293	1016.3972	1126.3565	2338.5473	1994.8471	2203.2179
	gCV%	40.4	23.4	41.2	42.0	6.8	31.1	29.6	17.8	25.6
$\%AUC_{extrap}$ (%)	n	5	5	10	5	4	9	5	3	8
	GM	10.5266	7.8050	9.0642	11.3391	9.7042	10.5809	13.3927	7.5440	10.7993
	gCV%	40.4	34.1	38.9	36.6	30.4	32.9	28.1	24.2	39.9

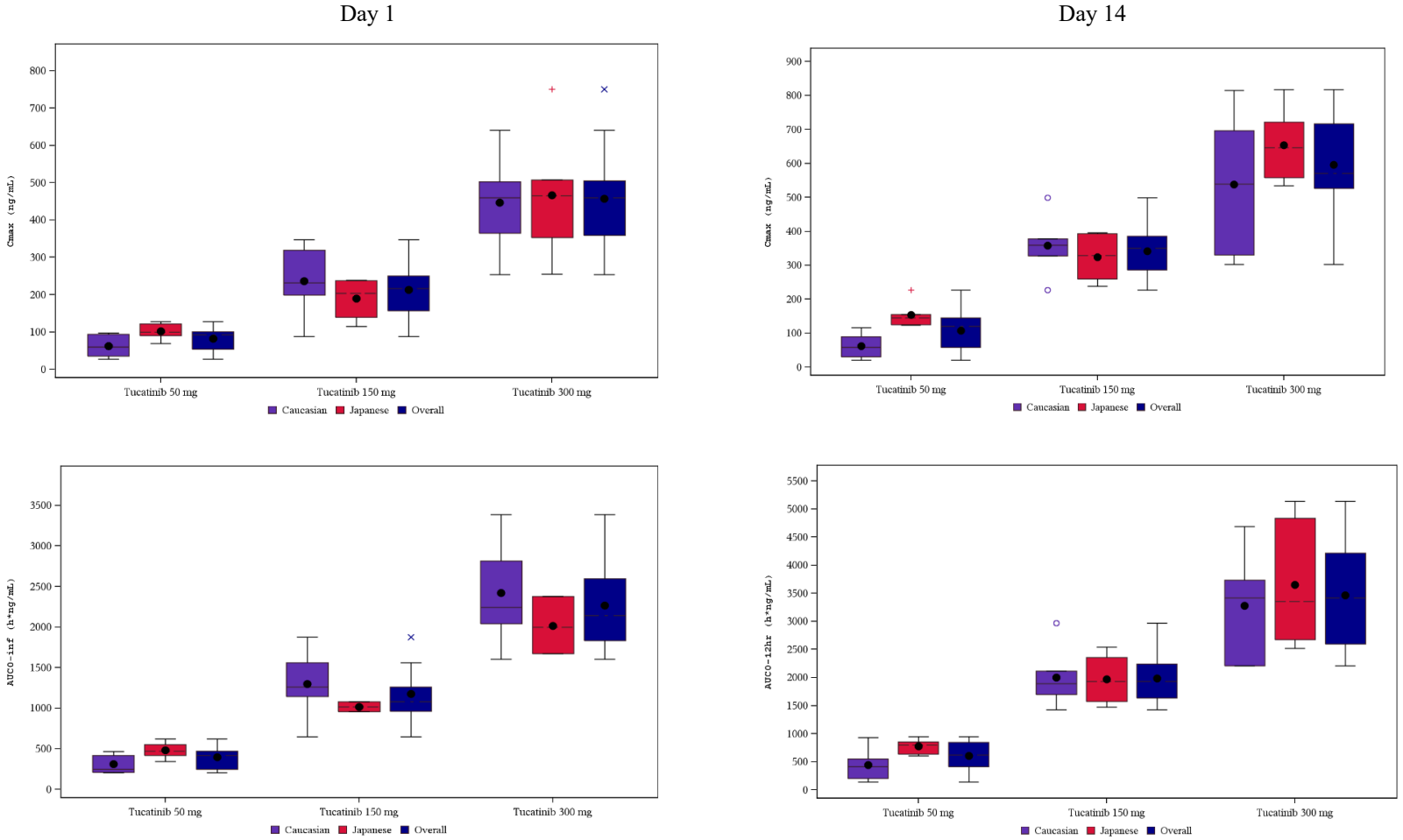
$\%AUC_{extrap}$ = percentage of $AUC_{0\text{-}inf}$ due to extrapolation; $AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 extrapolated to infinity; C_{max} = maximum observed concentration; gCV = geometric coefficient of variation; GM = geometric mean; N = number of subjects used in the analysis; n = number of subjects in the specific category; NC = not calculated; t_{max} = time of the maximum observed concentration
Source: Study SGNTUC-015 CSR, Table 11-4

Table 37. Summary of key tucatinib pharmacokinetic parameters Day 14 (pharmacokinetic population)

Parameter	Statistic	Tucatinib 50 mg			Tucatinib 150 mg			Tucatinib 300 mg		
		Caucasian (N = 6)	Japanese (N = 6)	Overall (N = 12)	Caucasian (N = 6)	Japanese (N = 6)	Overall (N = 12)	Caucasian (N = 6)	Japanese (N = 6)	Overall (N = 12)
C_{max} (ng/mL)	n	6	6	12	6	6	12	6	6	12
	GM	52.5035	150.2715	88.8244	349.0903	318.2815	333.3302	504.6152	646.0824	570.9842
	gCV%	73.6	22.6	82.7	25.9	21.5	23.2	41.3	17.2	32.7
T_{max} (h)	n	6	6	12	6	6	12	6	6	12
	Median	2.000	1.117	1.975	1.008	2.000	2.000	2.000	2.000	2.000
	Minimum	1.07	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Maximum	4.02	2.00	4.02	2.02	3.02	3.02	3.02	3.03	3.03
AUC_{0-12} (h*ng/mL)	n	6	6	12	6	6	12	6	6	12
	GM	367.6276	766.0874	530.6928	1943.7357	1930.0122	1936.8618	3160.5687	3512.9461	3332.1026
	gCV%	77.2	17.6	67.3	25.6	21.9	22.7	30.8	30.4	29.7
CL/F (L/h)	n	5	6	11	6	6	12	6	6	12
	GM	112.3755	65.2667	83.5517	77.1710	77.7197	77.4449	94.9196	85.3984	90.0332
	gCV%	60.4	17.6	49.7	25.6	21.9	22.7	30.8	30.4	29.7
Vz/F (L)	n	5	6	11	6	6	12	6	6	12
	GM	1319.0627	752.7668	971.3819	966.4458	885.3368	925.0028	1507.9399	1262.0180	1379.5098
	gCV%	58.5	16.3	49.2	50.6	30.5	39.7	29.8	37.0	33.3
C_{trough} (ng/mL)	n	6	6	12	6	6	12	6	6	12
	GM	16.5115	27.7499	21.4054	88.3353	94.6724	91.4490	168.3890	200.8988	183.9270
	gCV%	58.4	21.8	50.6	30.6	34.4	31.2	27.6	41.2	34.6

AUC_{0-12hr} = area under the concentration-time curve from time 0 to 12 hours postdose; CL/F = apparent total clearance; C_{max} = maximum observed concentration; gCV = geometric coefficient of variation; GM = geometric mean; N = number of subjects used in the analysis; n = number of subjects in the specific category; t_{max} = time of the maximum observed concentration; Vz/F = apparent volume of distribution during the terminal phase
Source: Study SGNTUC-015 CSR, Table 11-5

Figure 48　Box plots of key pharmacokinetic exposure parameters (pharmacokinetic population)



AUC = area under the concentration-time curve; $AUC_{0\text{-}inf}$ = AUC from time 0 extrapolated to infinity; AUC_{last} = AUC from time 0 to the time of last quantifiable concentration; $AUC_{0\text{-}12hr}$ = area under the concentration-time curve from time 0 to 12 hours postdose; C_{max} = maximum observed concentration
The X, circle and plus marks represent the outliers. Filled circles and dashed or solid lines inside the box plot represent the mean and median, respectively.
The boxes represent the 25 and 75 percentile and the whiskers represent the 5 and 95 percentile.
Source: SGNTUC-015 CSR, Figure 11-3

Table 38. Summary of key ONT-993 pharmacokinetic parameters Day 1 (pharmacokinetic population)

Parameter	Statistic	Tucatinib 50 mg			Tucatinib 150 mg			Tucatinib 300 mg		
		Caucasian (N=6)	Japanese (N=6)	Overall (N=12)	Caucasian (N=6)	Japanese (N=6)	Overall (N=12)	Caucasian (N=6)	Japanese (N=6)	Overall (N=12)
C_{max} (ng/mL)	n	6	6	12	6	6	12	6	6	12
	GM	6.4372	10.2740	8.1324	22.1555	26.1395	24.0652	50.4572	59.9515	54.9998
	gCV%	57.6	29.1	50.5	61.9	49.0	53.6	43.5	46.4	43.8
T_{max} (h)	n	6	6	12	6	6	12	6	6	12
	Median	2.550	2.000	2.017	2.000	2.000	2.000	2.017	1.017	1.983
	Minimum	2.00	2.00	2.00	2.00	2.00	2.00	1.02	0.98	0.98
	Maximum	6.00	2.02	6.00	2.02	3.98	3.98	3.00	3.00	3.00
AUC_{inf} (h*ng/mL)	n	1	2	3	2	2	4	2	2	4
	GM	71.2770*	84.3487	79.7448	184.7766	166.2977	175.2939	387.5946	310.8965	347.1337
	gCV%	NC	65.2	45.3	25.9	23.6	21.1	18.0	11.1	17.7
$\%AUC_{extrap}$ (%)	n	1	2	3	2	2	4	2	2	4
	GM	18.6653*	21.7889	20.6936	19.5739	21.9773	20.7408	19.2438	14.5614	16.7397
	gCV%	NC	17.9	15.5	32.8	15.2	21.7	25.9	26.5	27.0
MR_{AUCinf}	n	1	2	3	2	2	4	2	2	4
	GM	0.1657*	0.1776	0.1735	0.1489	0.2001	0.1726	0.1427	0.1485	0.1456
	gCV%	NC	18.8	13.8	18.9	1.7	20.4	57.9	11.4	32.7

$\%AUC_{extrap}$=percentage of $AUC_{0\text{-}inf}$ due to extrapolation; $AUC_{0\text{-}inf}$=area under the concentration-time curve from time 0 extrapolated to infinity; C_{max}=maximum observed concentration; gCV=geometric coefficient of variation; GM=geometric mean; MR_{AUCinf}=metabolite-to-parent molar ratio based on $AUC_{0\text{-}inf}$; N=number of subjects used in the analysis; n=number of subjects in the specific category; NC=not calculated; $t_{½}$=terminal elimination half-life; t_{max}=time of the maximum observed concentration; λ_z=terminal elimination rate constant.

* Derived value presented for a single subject.

Several subjects were excluded from summary statistics and analyses for λ_z dependent parameters as estimates were considered unreliable for at least one of the following reasons:

λ_z interval was <1.5 × the estimated $t_{½}$

R^2 adjusted was <0.80

$\%AUC_{extrap}$ >20%

Data Source: SGNTUC-015, CSR, Table 11-6.

Table 39. Summary of key ONT-993 pharmacokinetic parameters Day 14 (pharmacokinetic population)

Parameter	Statistic	Tucatinib 50 mg			Tucatinib 150 mg			Tucatinib 300 mg		
		Caucasian (N=6)	Japanese (N=6)	Overall (N=12)	Caucasian (N=6)	Japanese (N=6)	Overall (N=12)	Caucasian (N=6)	Japanese (N=6)	Overall (N=12)
C_{max} (ng/mL)	n	6	6	12	6	6	12	6	6	12
	GM	6.4687	15.5084	10.0160	40.9535	41.3200	41.1364	65.5402	109.6632	84.7782
	gCV%	39.9	15.7	57.6	29.8	30.8	28.9	25.7	32.9	39.9
T_{max} (h)	n	6	6	12	6	6	12	6	6	12
	Median	2.500	2.000	2.000	2.000	2.000	2.000	2.508	2.000	2.008
	Minimum	1.97	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.00
	Maximum	8.02	2.00	8.02	2.10	2.00	2.10	3.02	3.03	3.03
AUC_{0-12} (h*ng/mL)	n	6	6	12	6	6	12	6	6	12
	GM	51.2443	97.7289	70.7675	225.5752	234.8334	230.1578	405.0273	597.0457	491.7518
	gCV%	37.2	11.4	44.2	28.6	27.2	26.7	18.9	31.6	32.5
$MR_{AUC0-12}$	n	6	6	12	6	6	12	6	6	12
	GM	0.1349	0.1235	0.1291	0.1123	0.1178	0.1150	0.1240	0.1645	0.1428
	gCV%	41.1	14.0	29.2	9.3	31.1	21.8	28.8	22.3	28.8

AUC_{0-12hr}=area under the concentration-time curve from time 0 to 12 hours postdose; C_{max}=maximum observed concentration; gCV=geometric coefficient of variation; GM=geometric mean; N=number of subjects used in the analysis; n=number of subjects in the specific category; t_{max}=time of the maximum observed concentration; $MR_{AUC0-12}$=metabolite-to-parent molar ratio based on AUC_{0-12}.
Data Source: SGNTUC-015, CSR, Table 11-7.

3. 全試験を通しての結果の比較と解析

ツカチニブの臨床薬理は、臨床試験においてツカチニブを投与したときの PK 及び PD の結果に基づき評価した。臨床薬理に関する全体的な結論は 1.3 に示す。ツカチニブの生物薬剤学に関する詳細は M2.7.1 に示す。

3.1. ツカチニブ投与時の PK 及び PD

がん患者、健康成人及び肝機能障害を有する治験参加者において、ツカチニブを投与したときの PK 及び PD を評価した。ツカチニブ錠を用いた各臨床試験で評価したツカチニブの PK パラメータの要約一覧を Appendix 1、Appendix 2 及び Appendix 3 に示す。各臨床試験で得られた臨床薬理に関するツカチニブ及び ONT-993 の PK の全体的な結論は 1.3 に示す。

ツカチニブを 800 mg BID までの用量で経口投与したとき、ツカチニブの C_{max} 及び AUC は用量に概ね比例して増加し、T_{max}（中央値）は約 2 時間であった。ツカチニブ錠 300 mg 投与時の $t_{½}$（算術平均値）は、約 4～9 時間であり、BID 反復投与後、5 日目までには概ね定常状態に達し、AR_{AUC} は約 2 であった。ツカチニブの代謝に主に関与する酵素は CYP2C8 であり、複数の代謝物が生成され、ONT-993 が主な循環血中代謝物であった。放射性標識体を用いたマスバランス試験（ONT-380-008 試験）の結果、ONT-993 が占める割合は血漿中総放射能の 10%未満であり、ツカチニブ投与後の曝露量に対する大きな寄与は示されなかった。

ツカチニブ 300 mg BID を反復投与したとき、ツカチニブ及び ONT-993 の血漿中濃度と QT 間隔には関連性は認められず、ツカチニブは QT 間隔を延長しなかった。

3.2. 母集団 PK 解析

ツカチニブの臨床試験における治験参加者の背景等及びツカチニブの PK の結果を用いて、ツカチニブ PK に対する共変量を評価するために母集団 PK 解析を実施した。

3.2.1. 方法

ツカチニブの臨床試験 7 試験（健康成人対象の 4 試験 ARRAY-380-103、ONT-380-012、SGNTUC-015、SGNTUC-020、及び患者対象の 3 試験 ONT-380-004、ONT-380-005、SGNTUC-017[a]）における 283 例から得られた 3942 点の定量可能な PK データを解析に用いた。SGNTUC-015 試験には日本人健康成人、ONT-380-004 試験及び ONT-380-005 試験は HER2 陽性 mBC 患者のデータも含めて解析した。基本モデルは、一次吸収及び吸収ラグタイムを含む 2-コンパートメントモデルで構築した。

共変量の探索には、Vc/F、CL/F 及び ka と Frel に対して臨床的に意義があると考えられる以下の項目を含めた。

- Vc/F：体重、性別、人種、対象集団（健康成人、mBC 患者、CRC 患者）、年齢、アルブミン

[a] HER2 陽性 CRC 患者を対象にツカチニブとトラスツズマブを併用投与した海外第 2 相試験（M5.3.3.5.1）

- CL/F：体重、性別、人種、対象集団（健康成人、mBC 患者、CRC 患者）、クレアチニンクリアランス、年齢、肝機能、アルブミン、ECOG

- ka と Frel：性別、Frel に対して対象集団（健康成人、mBC 患者、CRC 患者）

肝機能障害を有する患者における PK への影響は、ONT-380-009 試験で評価した（2.2.2）。人種も共変量に含め、日本人及び白人におけるツカチニブの PK の直接比較は SGNTUC-015 試験で評価した（2.2.6）。ONT-380-206 試験で得られた C_{trough} は直前の投与時刻の特定ができなかったため（2.1.2.2）、母集団 PK 解析対象には含めなかった。MK-7119-001 試験は東アジア人患者対象の試験であり、内因性民族的要因の影響について評価する際に PK の結果を用いた（3.4.2）。各臨床試験の PK より、カペシタビン及びトラスツズマブと併用したときのツカチニブへの DDI の影響は認められなかったため（3.5.1）、共変量にこれらの併用薬は含めなかった。

母集団 PK モデルは非線形混合効果モデルを用いて構築し、母集団 PK 解析のガイドライン[3,4]を踏まえて解析した。

3.2.2. 結果

最終母集団 PK モデルは、ラグタイムを伴う一次吸収及び線形消失の 2 コンパートメントモデルであり、共変量は対象集団（mBC 患者、CRC 患者）が CL/F 及び Frel に含むモデルであった（Table 40）。体重、性別、人種、年齢及びアルブミンは、Vc/F 及び CL/F の共変量に特定されず、クレアチニンクリアランス、肝機能及び ECOG も CL/F の共変量に特定されなかった。

モデルの診断及び VPC により、血漿中ツカチニブ濃度の測定結果とモデルで算出した結果がよく適合することが確認された。ブートストラップ法で評価（1000 回）した結果、ブートストラップで得られたパラメータは最終モデルと類似しており、最終母集団 PK モデルは安定していることが示された。

最終母集団 PK モデルを用いて、HER2 陽性 mBC 患者にツカチニブを 300 mg BID 反復投与したときの血漿中ツカチニブ濃度の推移をシミュレーションした（Figure 49）。ツカチニブ投与後、血漿中ツカチニブ濃度の $t_{½}$（幾何平均値）は 16.7 時間であり、5 日目までには概ね定常状態に達することが示された。

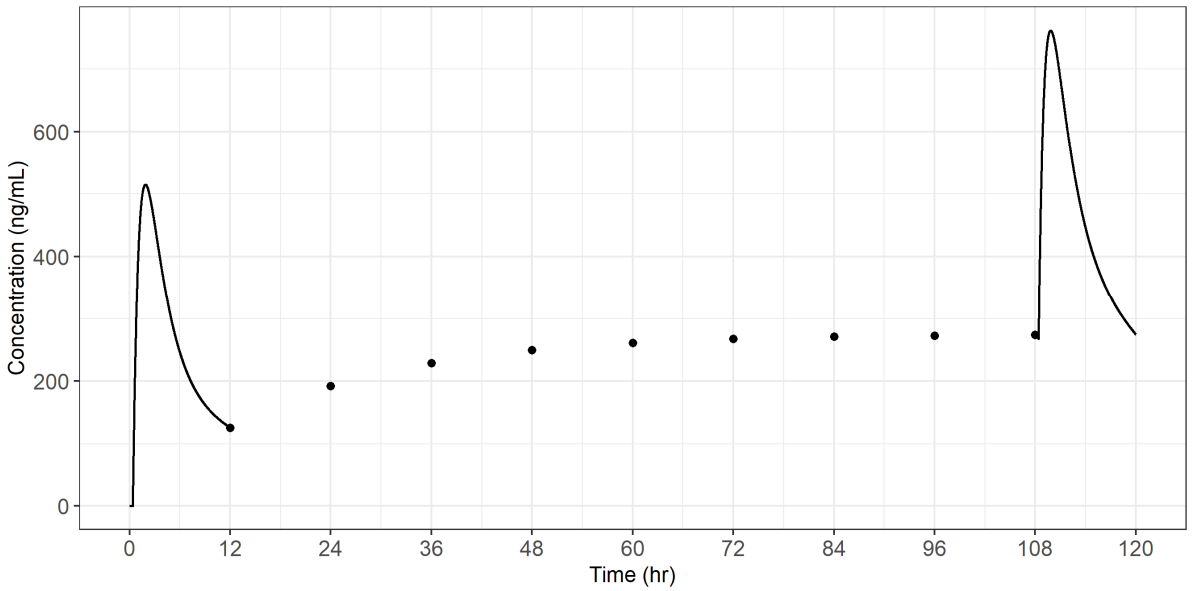
母集団 PK 解析結果の詳細は M5.3.3.5.1 に示した。

Table 40. Final model parameter estimates for tucatinib population PK

Parameter	Fixed Effects		BSV CV%		
	Estimate	RSE	Estimate	RSE	Shrinkage
CL/F (L/h), healthy subject	112	4.3%	45%	4.6%	4.8%
Vc/F Volume (L)	125	11.3%	135%	5.7%	10.8%
Q/F (L/h)	89.9	5.4%	43.9%	7.6%	31.3%
Vp/F (L)	635	4.0%	42.2%	7.4%	32.1%
ka (1/h)	0.424	5.3%	0 FIXED	-	-
Tlag (h)	0.392	0.56%			
Additive res. Error SD (ng/mL)	1.07	2.14%			7.5%
Proportional res. error CV	0.329	0.73%			
Covariate Effects					
CL ~ Subject with HER2+ mBC	-0.519	6.3%			
CL ~ Subject with HER2+ mCRC	-0.705	3.6%			
Frel ~ Subject with HER2+ mCRC	-0.637	3.8%			
BSV correlations					
Corr (CL/F, Vc/F)	0.470	6.8%			

Source: run2005_fda_cov_03.lst
Notes: BSV CV% is calculated as sqrt(exp(variance) – 1) × 100%. The reference subject is a healthy subject.
Abbreviations: BSV=between subject's variability; CL= clearance; CL/F= apparent clearance; Corr(X,Y)=X, Y correlation; CV=coefficient of variation; Frel=relative bioavailability; HER2+= human epidermal growth factor 2 positive; ka=first-order absorption rate constant; mBC=metastatic breast cancer; mCRC =metastatic colorectal cancer; PK=pharmacokinetic; Q/F=apparent intercompartmental clearance; Res.=residual; RSE=relative standard error; SD=standard deviation; Tlag=lag time; Vc/F=apparent central volume of distribution; Vp/F=apparent peripheral volume of distribution.

Figure 49 Simulation of a typical concentration-time PK profile following 300 mg BID tucatinib in subjects with HER2+ mBC (Studies ONT-380-004 and ONT-380-005)



Abbreviations: BID = twice daily; HER2+= human epidermal growth factor 2 positive; mBC = metastatic breast cancer; PK = pharmacokinetic.
Source: M5.3.3.5.1 Population PK report, Figure 19

3.3. 曝露-反応解析

3.3.1. ツカチニブの曝露量と有効性の関連性

ONT-380-206 試験及び MK-7119-001 試験でツカチニブの C_{trough} と有効性の関連性を評価した。

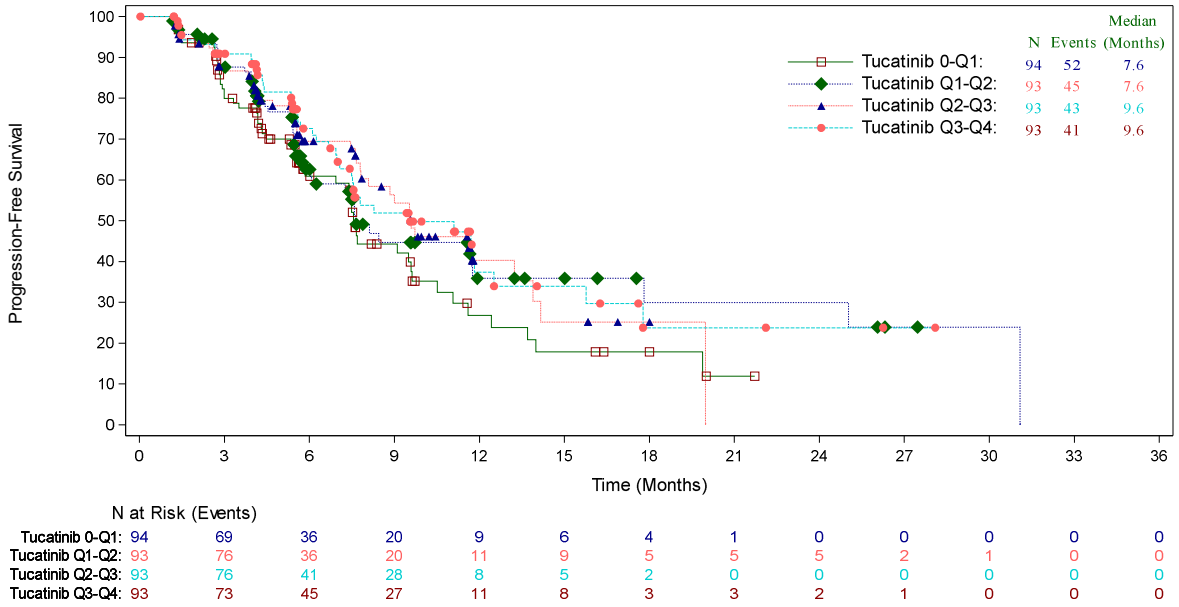
- ONT-380-206 試験

ツカチニブの投与時点も確認した Cycle 3 Day 1 の C_{trough} を曝露量の指標として曝露-有効性の解析を実施した。C_{trough} の上昇に伴って PFS が延長する傾向が認められたが、すべての四分位範囲で CI が重なり、すべての四分位範囲の患者に対照群と比較して意味のある治療効果が認められた。この結果には、Cycle 3 Day 1 の C_{trough} のばらつきが大きかったこと（CV：93%、ONT-380-206 CSR, Table 14.2.9.1）、及び Cycle 3 Day 1 より前に進行した又は治療を中止した患者を除外したことが交絡している可能性がある。

Cycle 3 Day 1 の C_{trough} を四分位範囲別にして曝露量のばらつきの影響を最小化することにより、PFS との関連性を評価する追加の解析を実施した（Figure 50 及び Table 41）。PFS の Kaplan-Meier 解析には、全サイクルの曝露量の幾何平均値の四分位範囲を用いた。これらの結果、Cycle 3 Day 1 までの PFS 解析と同様の傾向を示した。CI はすべての四分位範囲で重なったが、PFS に C_{trough} の増加に伴う傾向が認められ、すべての四分位範囲の患者における PFS の中央値が対照群（5.6 ヵ月、95% CI: 4.2, 7.1）に比べて高かった。

ツカチニブの C_{trough} の上昇に伴って PFS が延長する傾向が示されたが、すべての四分位範囲で CI が重なり、すべての四分位範囲の治験参加者に治療効果が認められた。

Figure 50　Kaplan-Meier plots for PFS per BICR by quartiles of average tucatinib trough concentrations (pharmacokinetics analysis population)



Subjects in Tuc+Cap+Tra arm were categorized into four groups based on the geometric means of their tucatinib predose concentration values on C2D1, C3D1, C4D1, C5D1, and C6D1, using the overall quartiles of the geometric means in Tuc+Cap+Tra arm.
The intervals used for categorization are inclusive all the upper bounds and exclusive of the lower bound, with the exception of 0 - Q1 (inclusive on both sides). Missing concentration values are not included. BLOQ values are imputed as 1/2 BLOQ (0.5 ng/mL) for the calculation of quartiles.
The Q1, Q2 and Q3 values of the geometric means of tucatinib trough concentration in Tuc+Cap+Tra are 53.3 ng/mL, 142.1 ng/mL, and 268.8 ng/mL, respectively.
Source: ONT-380-206 CSR, Figure 11

Table 41.　PFS per BICR by quartiles of average tucatinib trough concentrations (PK analysis set)

	Tuc+Cap+Tra (N=385)			
	Tucatinib 0-Q1 (N=94)	Tucatinib Q1-Q2 (N=93)	Tucatinib Q2-Q3 (N=93)	Tucatinib Q3-Q4 (N=93)
Median PFS (months) (95% CI)	7.6 (5.8, 9.6)	7.6 (6.0, 11.8)	9.6 (7.8, 13.2)	9.6 (7.5, 12.5)
Number of Events (%)	52 (55.3)	45 (48.4)	43 (46.2)	41 (44.1)
Number Censored (%)	42 (44.7)	48 (51.6)	50 (53.8)	52 (55.9)

Subjects in Tuc+Cap+Tra arm were categorized into four groups based on the geometric means of their tucatinib predose concentration values on C2D1, C3D1, C4D1, C5D1, and C6D1, using the overall quartiles of the geometric means in Tuc+Cap+Tra arm.
The intervals used for categorization are inclusive all the upper bounds and exclusive of the lower bound, with the exception of 0 - Q1 (inclusive on both sides). Missing concentration values are not included. BLOQ values are imputed as 1/2 BLOQ (0.5 ng/mL) for the calculation of quartiles.
The Q1, Q2 and Q3 values of the geometric means of tucatinib trough concentration in Tuc+Cap+Tra are 53.3 ng/mL, 142.1 ng/mL, and 268.8 ng/mL respectively.
Source: ONT-380-206 CSR, Table 23

- MK-7119-001 試験

MK-7119-001 試験の主試験で評価した Cycle 2～6 の C_{trough} を四分位範囲別にして、ツカチニブの曝露量と BOR 及び確定 ORR との関連性を評価した。全集団及び日本人集団での解析結果をそれぞれ Table 42 及び Table 43 に示す。

全集団における各四分位の確定 ORR（90% CI）は、第 1 四分位（0～Q1）28.6%（10.4～54.0%）、第 1～2 四分位（Q1～Q2）30.8%（11.3～57.3%）、第 2～3 四分位（Q2～Q3）76.9%（50.5～93.4%）、第 3～4 四分位（Q3～Q4）15.4%（2.8～41.0%）であった。

日本人集団における各四分位の確定 ORR（90% CI）は、第 1 四分位（0～Q1）27.3%（7.9～56.4%）、第 1～2 四分位（Q1～Q2）20.0%（3.7～50.7%）、第 2～3 四分位（Q2～Q3）63.6%（35.0～86.5%）、第 3～4 四分位（Q3～Q4）10.0%（0.5～39.4%）であった。

各四分位の例数は限られていたものの、全集団及び日本人集団ともに第 2～3 四分位（Q2～Q3）では、他の四分位範囲よりも確定 ORR はやや高めの傾向であり、第 1 四分位（0～Q1）及び第 1～2 四分位（Q1～Q2）の確定 ORR は同様の傾向であった。全集団と日本人集団で各四分位の確定 ORR の傾向に明らかな違いは認められなかった。

Table 42. Summary of confirmed objective response per ICR by quartiles of tucatinib trough (average trough from Cycle 2 through Cycle 6) concentration, PK analysis set in response evaluable set per ICR in all participants

	Total (N=53)			
	Tucatinib 0-Q1 (N=14)	Tucatinib Q1-Q2 (N=13)	Tucatinib Q2-Q3 (N=13)	Tucatinib Q3-Q4 (N=13)
Best Overall Response[a], n (%)				
Complete Response (CR)	1 (7.1)	0	1 (7.7)	0
Partial Response (PR)	3 (21.4)	4 (30.8)	9 (69.2)	2 (15.4)
Stable Disease (SD)[b]	8 (57.1)	9 (69.2)	2 (15.4)	10 (76.9)
Progressive Disease (PD)	2 (14.3)	0	1 (7.7)	1 (7.7)
Not Available (NA)[c]	0	0	0	0
Confirmed ORR, n (%)	4 (28.6)	4 (30.8)	10 (76.9)	2 (15.4)
90% CI[d]	(10.4, 54.0)	(11.3, 57.3)	(50.5, 93.4)	(2.8, 41.0)

Subjects were categorized into four groups based on the geometric means of their tucatinib predose concentration values on C2D1, C3D1, C4D1, C5D1, and C6D1, using the overall quartiles of the geometric means. The intervals used for categorization are inclusive of the upper bounds and exclusive of the lower bound, with the exception of 0 - Q1 (inclusive on both sides). Missing concentration values are not included. The values below the lower limit of qualification (LLOQ) were imputed as LLOQ/2 for the calculation of quantiles. Geometric mean of tucatinib trough for a subject is not calculated if more than 50% of the results were < LLOQ. Pre-dose samples taken outside the 8-16 hours window post the previous dosing were excluded.

The minimum, Q1, Q2, Q3, maximum values of the geometric means from Tucatinib pre-dose concentration (from Cycle 2 through Cycle 6) are 75.5 ng/mL, 249.0 ng/mL, 411.1 ng/mL, 583.0 ng/mL, and 975.6 ng/mL, respectively.

In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 5 weeks.

a. Confirmed best overall response assessed per RECIST 1.1.

b. Include stable disease and non-CR/non-PD.

c. Include subjects with no post-baseline response assessment and subjects whose disease assessment are not evaluable.

d. Two-sided 90% exact confidence interval, computed using the Clopper-Pearson method.

Source: MK-7119-001 CSR, Table 32

Table 43. Summary of confirmed objective response per ICR by quartiles of tucatinib trough (average trough from Cycle 2 through Cycle 6) concentration, PK analysis set in response evaluable set per ICR in Japanese participants

	Total (N=42)			
	Tucatinib 0-Q1 (N=11)	Tucatinib Q1-Q2 (N=10)	Tucatinib Q2-Q3 (N=11)	Tucatinib Q3-Q4 (N=10)
Best Overall Response[a], n (%)				
Complete Response (CR)	1 (9.1)	0	0	0
Partial Response (PR)	2 (18.2)	2 (20.0)	7 (63.6)	1 (10.0)
Stable Disease (SD)[b]	7 (63.6)	7 (70.0)	3 (27.3)	8 (80.0)
Progressive Disease (PD)	1 (9.1)	1 (10.0)	1 (9.1)	1 (10.0)
Not Available (NA)[c]	0	0	0	0
Confirmed ORR, n (%)	3 (27.3)	2 (20.0)	7 (63.6)	1 (10.0)
90% CI[d]	(7.9, 56.4)	(3.7, 50.7)	(35.0, 86.5)	(0.5, 39.4)

Subjects were categorized into four groups based on the geometric means of their tucatinib predose concentration values on C2D1, C3D1, C4D1, C5D1, and C6D1, using the overall quartiles of the geometric means. The intervals used for categorization are inclusive of the upper bounds and exclusive of the lower bound, with the exception of 0 - Q1 (inclusive on both sides). Missing concentration values are not included. The values below the lower limit of qualification (LLOQ) were imputed as LLOQ/2 for the calculation of quantiles. Geometric mean of tucatinib trough for a subject is not calculated if more than 50% of the results were < LLOQ. Pre-dose samples taken outside the 8-16 hours window post the previous dosing were excluded.

The minimum, Q1, Q2, Q3, maximum values of the geometric means from Tucatinib pre-dose concentration (from Cycle 2 through Cycle 6) are 75.5 ng/mL, 233.0 ng/mL, 404.6 ng/mL, 583.0 ng/mL, and 907.7 ng/mL, respectively.

In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 5 weeks.

a. Confirmed best overall response assessed per RECIST 1.1.

b. Include stable disease and non-CR/non-PD.

c. Include subjects with no post-baseline response assessment and subjects whose disease assessment are not evaluable.

d. Two-sided 90% exact confidence interval, computed using the Clopper-Pearson method.

Source: MK-7119-001 CSR, Table 31

3.3.2. ツカチニブの曝露量と安全性の関連性

ONT-380-206 試験及び MK-7119-001 試験で評価したツカチニブの C_{trough} と安全性の関連性を評価した。

- ONT-380-206 試験

ツカチニブの投与時点も確認した Cycle 3 Day 1 の C_{trough} と AESI（潜在的な薬物性肝障害、脳浮腫及び用量変更又は投与中止に至る左室収縮機能障害）との関連性の解析結果を Table 44 に示す。C_{trough} の四分位範囲別に、AESI の発現割合を評価した。AESI の全体的な発現割合は低く、C_{trough} の各四分位範囲における発現割合はわずか 1.5%～3.1%であった。各四分位範囲における AESI の発現頻度は低かったものの、Cycle 3 Day 1 のツカチニブの C_{trough} との関係に明らかな傾向は認められなかった。

Table 44. Summary of adverse events of interest by quartiles of tucatinib trough (Cycle 3 Day 1) concentration (PK analysis set)

	Tuc+Cap+Tra (N=385)			
	Tucatinib 0-Q1 (N=66) n (%)	Tucatinib Q1-Q2 (N=65) n (%)	Tucatinib Q2-Q3 (N=66) n (%)	Tucatinib Q3-Q4 (N=65) n (%)
Potential drug-induced liver injury	1 (1.5)	2 (3.1)	0	2 (3.1)
Left ventricular systolic dysfunction leading to a change/discontinuation of study treatment	0	2 (3.1)	2 (3.0)	1 (1.5)
Cerebral Edema	0	0	0	0

Source: ONT-380-206 CSR, Table 14.3.1.6

- MK-7119-001 試験

MK-7119-001 試験の主試験で評価した Cycle 2～6 の C_{trough} を四分位範囲別にして、ツカチニブの曝露量と AESI として肝毒性（全グレード）発現率との関連性を評価した。全集団及び日本人集団での解析結果をそれぞれ Table 45 及び Table 46 に示す。

全集団における各四分位の肝毒性発現率は、第 1 四分位（0～Q1）53.3%、第 1～2 四分位（Q1～Q2）57.1%、第 2～3 四分位（Q2～Q3）53.3%、第 3～4 四分位（Q3～Q4）85.7%であった。日本人集団では、それぞれ 58.3%、54.5%、58.3%及び 90.9%であった。

各四分位の例数は限られていたものの、第 3～4 四分位では肝毒性発現率は高めの傾向であったが、観察及びツカチニブの用量調節により管理可能であった（M2.7.4.2.1.5.2）。

Table 45. Summary of adverse events of special interest prevalence by quartiles of tucatinib trough (average trough from Cycle 2 through Cycle 6) concentration, PK analysis set in all participants

	Total (N=58)			
	Tucatinib 0-Q1 (N=15)	Tucatinib Q1-Q2 (N=14)	Tucatinib Q2-Q3 (N=15)	Tucatinib Q3-Q4 (N=14)
Hepatotoxicity[a], n (%)	8 (53.3)	9 (64.3)	7 (46.7)	12 (85.7)

Subjects were categorized into four groups based on the geometric means of their tucatinib predose concentration values on C2D1, C3D1, C4D1, C5D1, and C6D1, using the overall quartiles of the geometric means. The intervals used for categorization are inclusive of the upper bounds and exclusive of the lower bound, with the exception of 0 - Q1 (inclusive on both sides). Missing concentration values are not included. The values below the lower limit of qualification (LLOQ) were imputed as LLOQ/2 for the calculation of quantiles. Geometric mean of tucatinib trough for a subject is not calculated if more than 50% of the results were < LLOQ. Pre-dose samples taken outside the 8-16 hours window post the previous dosing were excluded.
The minimum, Q1, Q2, Q3, maximum values of the geometric means from Tucatinib pre-dose concentration (from Cycle 2 through Cycle 6) are75.5 ng/mL, 233.0 ng/mL, 404.6 ng/mL, 581.7 ng/mL, and 975.6 ng/mL, respectively.
a. Hepatotoxicity includes TEAEs with preferred terms from the drug related hepatic disorders - comprehensive search SMQ (narrow).
Source: MK-7119-001 CSR, Table 34

Table 46. Summary of adverse events of special interest prevalence by quartiles of tucatinib trough (average trough from Cycle 2 through Cycle 6) concentration, PK analysis set in Japanese participants

	Total (N=46)			
	Tucatinib 0-Q1 (N=12)	Tucatinib Q1-Q2 (N=11)	Tucatinib Q2-Q3 (N=12)	Tucatinib Q3-Q4 (N=11)
Hepatotoxicity[a], n (%)	7 (58.3)	6 (54.5)	7 (58.3)	10 (90.9)

Subjects were categorized into four groups based on the geometric means of their tucatinib predose concentration values on C2D1, C3D1, C4D1, C5D1, and C6D1, using the overall quartiles of the geometric means. The intervals used for categorization are inclusive of the upper bounds and exclusive of the lower bound, with the exception of 0 - Q1 (inclusive on both sides). Missing concentration values are not included. The values below the lower limit of qualification (LLOQ) were imputed as LLOQ/2 for the calculation of quantiles. Geometric mean of tucatinib trough for a subject is not calculated if more than 50% of the results were < LLOQ. Pre-dose samples taken outside the 8-16 hours window post the previous dosing were excluded.
The minimum, Q1, Q2, Q3, maximum values of the geometric means from Tucatinib pre-dose concentration (from Cycle 2 through Cycle 6) are 75.5 ng/mL, 222.0 ng/mL, 375.5 ng/mL, 560.2 ng/mL, and 907.7 ng/mL, respectively.
a. Hepatotoxicity includes TEAEs with preferred terms from the drug related hepatic disorders - comprehensive search SMQ (narrow).
Source: MK-7119-001 CSR, Table 33

3.4. 内因性要因の影響

3.4.1. 年齢、性別、体重の影響

母集団 PK 解析の結果、年齢、性別、体重は、ツカチニブの Vc/F 及び CL/F の共変量と特定されなかったため（3.2）、これらの患者背景は、ツカチニブの PK に大きな影響を及ぼさないことが示された。

3.4.2. 人種による民族的要因の影響

ツカチニブは主に CYP2C8（主要な代謝酵素）及び CYP3A（副次的な代謝酵素）によって代謝され、これらの CYP に対する民族的遺伝子多型の影響は想定されない。ツカチニブを経口投与したとき、ツカチニブの C_{max} 及び AUC は用量に比例して増加し、食事の影響は大きくなく、制酸薬と併用投与したときの本薬の BA に及ぼす影響は軽微であった。これらの結果より、ツカチニブの PK は内因性民族的要因の影響を受けにくいと考えられる。

日本人及び白人の健康成人にツカチニブ単剤を経口投与したときの PK に人種による民族差は認められなかった（2.2.6）。母集団 PK 解析の結果、人種はツカチニブの Vc/F 及び CL/F の共変量と特定されなかった（3.2）。日本人及び外国人の mBC 患者にツカチニブ 300 mg BID とカペシタビン及びトラスツズマブを併用したときのツカチニブの定常状態時の C_{trough}（算術平均値）は、それぞれ 262.3 ng/mL（Cycle 1 Day 15）及び 310.0 ng/mL（Cycle 1 Day 14）であり、大きな違いは認められなかった（2.1.2.3、2.1.2.1）。以上の結果より、人種による民族的要因はツカチニブの PK に影響を及ぼさないことが示された。

3.4.3. 肝機能障害の影響

肝機能障害を有する患者（軽度、中等度及び重度）にツカチニブを投与したときの PK を解析した結果、正常肝機能群と比べ、軽度肝機能障害群では変化はなく、中等度肝機能障害群では AUC の増加は 15%以下であり、重度肝機能障害群の AUC_{last} 及び AUC_{inf} はそれぞれ 143.0%及び 161.0%の増加が認められた（2.2.2）。このため、重度肝機能障害を有する患者では、ツカチニブの開始用量を減量する必要があると考えられる。重度肝機能障害を有する患者でのツカチニブの PK、mBC 患者での肝機能に対する安全性（M2.7.4.2.1.5.2）及びツカチニブ錠の市販用含量規格（50 mg 及び 150 mg 錠）も踏まえ、重度肝機能障害群を有する患者ではツカチニブ 200 mg BID を開始用量とすることが妥当と考える。

3.4.4. 腎機能障害の影響

腎機能障害を有する患者を対象にしたツカチニブの臨床試験は実施してないものの、ONT-380-008 試験の結果、尿中に排泄される総放射能は投与量の 5%未満であることが示され、ツカチニブは主に代謝及び胆汁中排泄を介して消失するため、腎機能障害はツカチニブの PK にほとんど影響しないと考えられる。母集団 PK 解析の結果、クレアチニンクリアランスは CL/F の共変量に特定されなかった。このため、腎機能障害に対するツカチニブの用量調節は必要ないと考えられる。

ただし、「重篤な腎障害のある患者」に対しては、カペシタビンの投与が禁忌であるため、カペシタビンとの併用において、本剤の投与も推奨されない。

3.5. 外因性要因の影響

ツカチニブとカペシタビン及びトラスツズマブ併用の影響、ツカチニブの DDI 試験（ONT-380-012 試験及び SGNTUC-020 試験）の結果及び PBPK で評価した結果概要を示す。食事の影響及び制酸薬の影響の概要は M2.7.1 に示す。

3.5.1. カペシタビン及びトラスツズマブ併用の影響

ツカチニブとカペシタビンあるいはトラスツズマブとの DDI 試験は実施していないもの、ツカチニブ単剤投与時と、ツカチニブとカペシタビン及びトラスツズマブ併用投与時のツカチニブの PK 結果より、カペシタビン及びトラスツズマブ併用がツカチニブの PK に及ぼす影響を評価した。

カペシタビンはツカチニブの主な代謝酵素である CYP2C8 への阻害あるいは誘導作用は報告されてなく、トラスツズマブは抗体であり、CYP2C8 への阻害あるいは誘導作用は報告されていない。このため、各薬剤の代謝経路及び PK 特性からも 3 剤併用時における DDI は想定されない。

進行がん患者にツカチニブ単剤（PIC 製剤）を反復投与した結果、定常状態のツカチニブの AUC_{tau} は、Cycle 1 Day 1 の AUC_{tau} と比較して AR は約 2 であり、T_{max}（中央値）はいずれも約 2 時間、MR_{AUC} は約 0.1 であった（ARRAY-380-101 試験）。mBC 患者にツカチニブ錠 300 mg BID とカペシタビン及びトラスツズマブ併用を反復投与したときの定常状態のツカチニブの AUC_6 は、ツカチニブ錠単剤 300 mg を Cycle 1 Day 1 に投与したときの AUC_6 と比較して AR は約 1.2～2.7 であり、併用時の T_{max}（中央値）は 2 時間、MR_{AUC} は約 0.1 であった（ONT-380-004 試験及び ONT-380-005 試験）。このため、カペシタビン及びトラスツズマブ併用時にツカチニブを反復投与したときのツカチニブの PK は、ツカチニブ単剤投与時と比較して、AR では変動はみられたものの明らかな一定の傾向はなく、T_{max} 及び MR_{AUC} は同様であった。

以上のことから、カペシタビン及びトラスツズマブ併用はツカチニブの PK に大きな影響を及ぼさないと考えられる。

3.5.2. CYP3A4、CYP2C8、CYP2C9、P-gp 及び MATE1/MATE2-K に関する DDI

ツカチニブと CYP3A4 及び CYP2C8 の阻害薬及び誘導薬、及び CYP2C8、CYP2C9、CYP3A4、P-gp の基質との DDI 試験（ONT-380-012 試験）、及びトランスポーターMATE1/MATE2-K の基質との DDI 試験（SGNTUC-020 試験）の結果の概略を以下に示す。ツカチニブと CYP3A4 及び CYP2C8 の阻害薬及び誘導薬との DDI 結果概要の一覧を Table 47 に示し、CYP2C8、CYP3A、P-gp 及び MATE1/MATE2-K の基質との DDI 結果概要の一覧を Table 48 に示す。

- ツカチニブとイトラコナゾールを併用投与したときのツカチニブの C_{max} 及び AUC_{inf} は、ツカチニブ単独投与時に比べていずれも約 1.3 倍に増加した。同様に ONT-993 の C_{max} 及び AUC_{inf} はそれぞれ約 2 倍及び約 1.6 倍に増加した。強い CYP3A4 阻害薬であるイトラコナゾールは、ツカチニブ及び ONT-993 の曝露量に対して全般的に弱い影響であり、ツカチニブに対する明らかな DDI の影響は認められなかった。

- ツカチニブとリファンピシンを併用投与したときのツカチニブの C_{max} 及び AUC_{inf} は、ツカチニブ単独投与時に比べてそれぞれ約 37%及び約 48%減少した。ONT-993 の C_{max} 及び

AUC_{inf}はそれぞれ約 2.1 倍増加及び約 25%減少した。強い CYP3A 誘導薬及び中程度の CYP2C8 誘導薬であるリファンピシンは、ツカチニブ及び ONT-993 の曝露量に対して影響を及ぼすことが示された。このため、ツカチニブとリファンピシンの併用投与は注意が必要と考えられる。

- ツカチニブとゲムフィブロジルを併用投与したときのツカチニブの C_{max} 及び AUC_{inf} は、ツカチニブ単独投与時に比べてそれぞれ約 1.6 倍及び約 3.0 倍に増加した。ONT-993 の C_{max} 及び AUC_{inf} はそれぞれ約 69%及び約 11%減少した。強い CYP2C8 阻害薬であるゲムフィブロジルは、ツカチニブ及び ONT-993 の曝露量に対して影響を及ぼすことが示された。このため、ツカチニブとゲムフィブロジルの併用投与は注意が必要と考えられる。

- ツカチニブとレパグリニドを併用投与したときのレパグリニドの C_{max} 及び AUC_{inf} は、レパグリニド単独投与時に比べていずれも約 1.7 倍に増加した。この結果、ツカチニブは CYP2C8 の基質であるレパグリニドの曝露量をやや上昇させたことから、ツカチニブは CYP2C8 の弱い阻害薬であることが示された。

- ツカチニブとトルブタミドを併用投与したときのトルブタミド及び 4-ヒドロキシトルブタミドの C_{max} 及び AUC_{inf} は、ツカチニブ非併用投与時に比べて、トルブタミドでは約 5%、4-ヒドロキシトルブタミドでは約 10%程度の変化であり、大きな影響は認められなかった。この結果、ツカチニブは CYP2C9 の基質であるトルブタミドの曝露量には影響を及ぼさず、CYP2C9 を阻害あるいは誘導しないことが示された。

- ツカチニブとミダゾラムを併用投与したときのミダゾラムの C_{max} 及び AUC_{inf} は、ツカチニブ非併用投与時に比べて、それぞれ約 3.0 倍及び約 5.3～5.7 倍に増加した。一方、1-ヒドロキシミダゾラムの C_{max} は約 40%減少し、AUC_{inf} はツカチニブ併用時と同程度であった。この結果、ツカチニブは CYP3A4 の基質であるミダゾラムの曝露量に大きな影響を及ぼしたことから、CYP3A4 の強い阻害薬であることが示された。このため、ツカチニブと CYP3A4 の基質の併用投与は注意が必要と考えられる。

- ツカチニブとジゴキシンを併用投与したときのジゴキシンの C_{max} 及び AUC_{inf} は、ジゴキシン単独投与時に比べて、それぞれ約 2.3 倍及び約 1.5 倍に増加した。この結果、ツカチニブは P-gp の基質であるジゴキシンの曝露量にやや影響を及ぼしたことから、P-gp の弱い阻害薬であることが示された。このため、ジゴキシンのような治療域の狭い P-gp の基質をツカチニブと併用するときは注意が必要と考えられる。

- ツカチニブとメトホルミンを併用投与したとき、メトホルミン単独投与時に比べて血漿中メトホルミンの C_{max} 及び AUC_{inf} はそれぞれ約 1.1 倍及び約 1.4 倍に増加した。この結果、ツカチニブは MATE1/MATE2-K の基質であるメトホルミンの曝露量にやや影響を及ぼしたことから、MATE1/MATE2-K の弱い阻害薬であることが示された。

- イオヘキソールの PK パラメータは、ツカチニブとメトホルミンの併用投与後とメトホルミン単独投与後とで同程度であったため、ツカチニブは GFR に影響を及ぼさなかったことが示された。シスタチン C データを用いて投与別に推定した GFR は、ツカチニブの非併用投与時と併用投与時でそれぞれ 101.4 mL/min/1.73 m^2 及び 111.4 mL/min/1.73 m^2 であり、有意な影響は認められなかった。

Table 47. Summary of observed tucatinib victim drug-drug interactions

Concomitant Drug (Dose)	Tucatinib Dose	Geometric Mean Ratio (90% CI) of Exposure Measures of Tucatinib Combination/No Combination	
		C_{max}	AUC
CYP3A4 Inhibition Itraconazole (200 mg BID)	300 mg single dose	1.32 (1.23, 1.42)	1.34 (1.26, 1.43)
CYP3A4/2C8 Induction Rifampin (600 mg once daily)		0.632 (0.531, 0.753)	0.520 (0.452, 0.597)
CYP2C8 Inhibition Gemfibrozil (600 mg BID)		1.62 (1.47, 1.79)	3.04 (2.66, 3.46)

BID = twice daily; C_{max} = maximum concentration; AUC = area under the curve

Table 48. Summary of observed tucatinib perpetrator drug-drug interactions

Concomitant Drug (Dose)	Tucatinib Dose	Geometric Mean Ratio (90% CI) of Exposure Measures of Tucatinib Combination/No Combination	
		C_{max}	AUC
Repaglinide (CYP2C8) (0.5 mg single dose)	300 mg twice daily	1.69 (1.37, 2.10)	1.69 (1.51, 1.90)
Midazolam (CYP3A4) (2 mg single dose)		3.01 (2.63, 3.45)	5.74 (5.05, 6.53)
Digoxin (P-gp) (0.5 mg single dose)		2.35 (1.90, 2.90)	1.46 (1.29, 1.66)
Metformin (MATE1/2-K)[1] (850 mg single dose)		1.08 (0.95, 1.23)	1.39 (1.25, 1.54)

AUC = area under the curve; C_{max} = maximum serum concentration; CYP3A4=cytochrome P450 3A4; CYP2C8=cytochrome P450 2C8; MATE=multidrug and toxin extrusion; P-gp = P-glycoprotein

3.5.3. UGT1A1 及び OATP1B1/3 に関する DDI

ツカチニブの in vitro 及び臨床 PK データを用いて、ツカチニブ及び ONT-993 の PBPK モデルを構築した（M5.3.3.4.3）。PBPK モデルを用いて、ツカチニブと UGT1A1 及び OATP1B1/3 の基質との DDI（ツカチニブが基質に及ぼす影響）について予測した。

バリデートした最終 PBPK モデルを用いて、UGT1A1 の基質であるラルテグラビルとツカチニブを併用したときの DDI（ツカチニブがラルテグラビルに及ぼす影響）の程度を予測した。ツカチニブと併用したときのラルテグラビルの C_{max} 及び AUC_{inf} の幾何平均比及び 90% CI は、それぞれ 1.5 倍以下であった（Table 49）。このため、ツカチニブによる UGT1A1 を阻害する影響は低いことが示された。

OATP1B1/3 の基質であるロスバスタチンとツカチニブを併用したときの DDI（ツカチニブがロスバスタチンに及ぼす影響）の程度を予測した。ツカチニブと併用したときのロスバスタチン

の C_{max} 及び AUC_{inf} の幾何平均比及び 90% CI は、それぞれ約 1 倍であった（Table 50）。このため、ツカチニブによる OATP1B1/3 を阻害する影響はないことが示された。

Table 49. Simulated tucatinib inhibition of raltegravir clearance

Substrate	UGT1A1 K_i (μM)	C_{max} ratio	AUC_{inf} ratio
Raltegravir	1.81	1.13 (1.12, 1.15)	1.11 (1.10, 1.13)
Raltegravir	0.181	1.40 (1.36, 1.43)	1.36 (1.33, 1.39)

Geometric mean ratio (90% CI): C_{max}; AUC_{inf}
Simulated data source model-app-73.xlsx; model-app-74.xlsx
Source: M5.3.3.4.3 PBPK report, Table 4.3-1

Table 50. Simulated tucatinib inhibition of rosuvastatin clearance

Substrate	OATP1B1 Ki (μM)	OATP1B3 Ki (μM)	C_{max} ratio	AUC_{inf} ratio
Rosuvastatin	5	4	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Rosuvastatin	0.5	0.4	1.04 (1.03, 1.04)	1.03 (1.02, 1.03)

Geometric mean ratio (90% CI): C_{max}; AUC_{inf}
Simulated data source model-app-71.xlsx; model-app-72.xlsx
Source: M5.3.3.4.3 PBPK report, Table 4.3-2

4. 用量設定の根拠

4.1. RP2D の設定根拠

ONT-380-206 試験におけるツカチニブの用量は、進行固形がんを有する患者を対象にしたツカチニブ単剤の第 1 相用量漸増試験（ARRAY-380-101 試験）の結果並びにトラスツズマブ及びカペシタビンと併用したツカチニブの第 1b 相試験（ONT-380-005 試験）の結果に基づいて決定した。ツカチニブ錠を単回及び反復投与した ARRAY-380-103 試験及び ONT-380-004 試験における 300 mg BID 経口投与後の曝露量（M2.7.1.2.3、2.1.2.4、Appendix 1、Appendix 2）は、ARRAY-380-101 試験における MTD（PIC 製剤として 600 mg を BID 経口投与）の曝露量（Table 3、Figure 5、Figure 6）と同程度であった。このため、ONT-380-005 試験における第 1b 相用量漸増計画では、ツカチニブの 300、350 及び 400 mg BID の用量を予定した（ONT-380-005 試験 CSR、9.4.4）。また、ツカチニブの用量調節はそれぞれ 50 mg ずつの減量とし、減量用に 50 mg 錠も導入した。ONT-380-005 試験の用量漸増期に 300 mg BID が MTD/RP2D と決定し、この用量についてさらなる評価を行った。

ONT-380-206 試験で評価した用量は、ONT-380-005 試験で得られたトラスツズマブ及びカペシタビンとの併用下でツカチニブ 300 mg BID 連続経口投与の MTD/RP2D に基づき決定し、食事は問わずとした。MK-7119-001 試験でのツカチニブの用量も ONT-380-206 試験と同じく、トラスツズマブ及びカペシタビンとの併用下でツカチニブ 300 mg BID で実施した。ARRAY-380-101 試験及び ARRAY-380-103 試験の結果、ツカチニブの曝露量に対する食事の影響は大きくなかったことから（M2.7.1.3.2）、ONT-380-206 試験及び MK-7119-001 試験において、ツカチニブの用法は食事に関係なく BID 経口投与に設定した。

4.2. 申請用法・用量の設定根拠

ONT-380-206 試験で得られた主要評価項目及び主な副次評価項目の結果から、トラスツズマブ及びカペシタビンと併用したツカチニブ 300 mg BID 投与に伴う疾患進行及び死亡のリスクの臨床的に意味のある低下が示された（M2.7.3）。ツカチニブ 300 mg BID 投与時の定常状態におけるツカチニブの曝露量は、BT-474 細胞を用いた HER2 リン酸化の阻害能の IC_{50}（9.88 nmol/L、M2.6.2.21.2）から算出した約 160 ng/mL[a]を上回ることが示唆された。有効性についての曝露-反応解析でツカチニブの C_{trough} の上昇に伴って PFS が延長する傾向が示されたものの、すべての四分位範囲で CI が重なり、対照群の患者に対する治療効果が認められた。AESI の発現割合は低く、Cycle 3 Day 1 のツカチニブ C_{trough} との関係に明らかな傾向は認められなかった。ツカチニブ投与後の AE は、治験実施計画書に規定された用量変更によって適切に管理された（M2.7.4）。

MK-7119-001 試験の結果、日本人集団の PK は外国人患者と大きな違いは認められず、有効性は同様であり、日本人集団に新たな安全性上の懸念は認められなかった。

以上の臨床試験の結果に基づき、ツカチニブの申請用法・用量は、食事に関係なくツカチニブ 300 mg を BID 経口投与（21 日間サイクルで 14 日間のカペシタビン 1000 mg/m^2 BID 経口投与及び 21 日ごとのトラスツズマブ 6 mg/kg 静脈内投与との併用）に設定することは妥当と考える。

5. 特殊集団における用量調節

肝機能障害を有する患者を対象とした臨床試験（ONT-380-009 試験）の結果より、重度の肝機能障害を有する患者では、ツカチニブの用量は減量する必要があると考える（3.4.3）。ツカチニブの PK に臨床的に意味のある影響を及ぼす内因性要因は認められないことから、年齢、体重、人種及び腎機能が異なる集団において、ツカチニブの開始用量調節は不要と考える。

6. その他の試験

6.1. 髄液中の PK 評価

IST-380-004 試験（TBCRC049、NCT03501979）は髄膜播種を伴う HER2 陽性 mBC 患者を対象として、ツカチニブとトラスツズマブ及びカペシタビンを併用投与する医師主導の海外第 2 相試験である。CSF 中のツカチニブ及び ONT-993 の PK を評価することは、脳転移患者におけるツカチニブの有効性の機序を説明できる可能性がある。この目的のために 15 例の患者を対象に CSF 中のツカチニブ及び ONT-993 の PK 解析を実施した。IST-380-004 試験は、CSF 中のツカチニブ及び ONT-993 濃度を評価した最初の試験である。詳細は PK 報告書（M5.3.5.4.1）に記載した。

ツカチニブ 300 mg BID 投与し、Cycle 1 及び Cycle 2（定常状態）の Day 1 に血漿及び CSF 検体を採取し、血漿中及び CSF 中のツカチニブ及び ONT-993 濃度を定量した。CSF 中タンパク濃度は血中タンパク濃度よりも微量であるため、遊離型（タンパク非結合型）血漿中濃度の算出

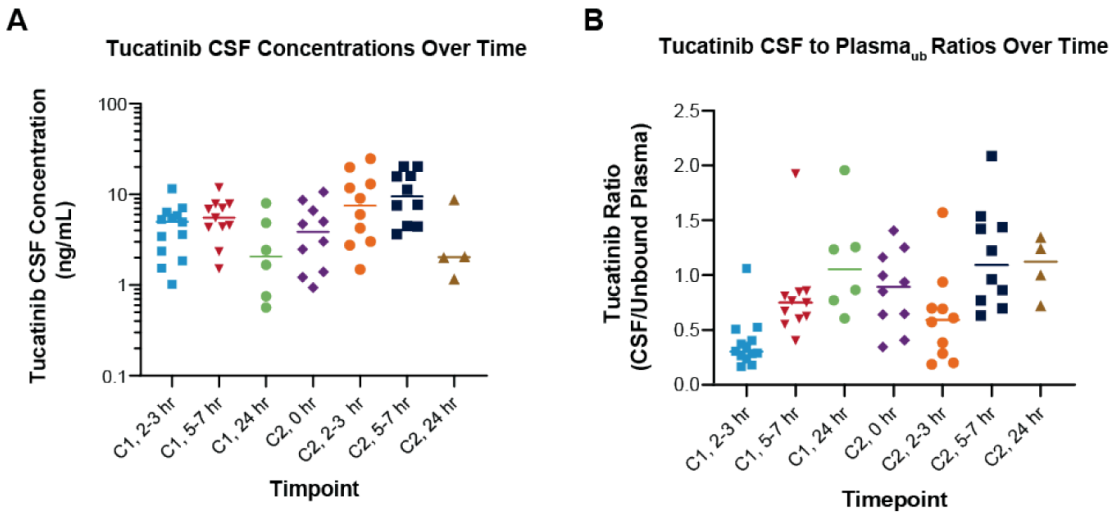
[a] ツカチニブ（$C_{26}H_{24}N_8O_2$）の分子量 480.52 及びヒト血漿蛋白結合率 97.1%（M2.6.4.4.1）を用いて、9.88 nmol/L*480.52/ (0.029*1000)で算出

は、過去の試験で報告された血漿中の平均遊離型分率 0.029 を用いた。CSF 中濃度は全て CSF 中の総ツカチニブ濃度を用いた。

CSF 中のツカチニブ濃度はツカチニブ投与後 2 時間以内に検出された（Figure 51、A）。各時点におけるツカチニブの CSF/遊離型血漿中濃度の比（中央値）は、Cycle 1 では 0.3～1.0、Cycle 2 では 0.59～1.1 であった（Figure 51、B）。ツカチニブと同様に ONT-993 もツカチニブ投与後 2 時間以内に CSF から検出された。ONT-993 の各時点の CSF/遊離型血漿中濃度の比（中央値）は、Cycle 1 では 0.32～2.4、Cycle 2 では 0.81～2.1 であった。

本治験より、髄膜播種を伴う HER2 陽性 mBC 患者にツカチニブとカペシタビン及びトラスツズマブを併用投与したとき、ツカチニブ及び ONT-993 はツカチニブ初回投与後から CSF 中に検出され、定常状態では、CSF と遊離型血漿中濃度は同程度であることが示された。このため、ツカチニブの CSF への移行性により、脳転移患者でツカチニブの有効性が示されたことが示唆された。

Figure 51　Tucatinib CSF concentrations and CSF to unbound plasma ratios over time



(A) Tucatinib concentrations in the CSF at 0, 2, 5, and 24 hours after tucatinib administration (300 mg BID) on Day 1 of Cycles 1 and 2. (B) Tucatinib CSF-to- plasma unbound ratios at 0, 2-3, 5-7, and 24 hours after tucatinib administration (300 mg BID) on Day 1 of Cycles 1 and 2.
Source: IST-380-004 TBCRC049

7. 付録

Appendix 1. Summary of the pharmacokinetic parameters of tucatinib from relevant studies (Tablet, Day 1 and Steady-state)

Tucatinib, Day 1								
Study	Subjects or HV	Dose (mg)	n	Part/Arm	Co-medication(s)	T_{last} (h)	AUC_{last} (hr*ng/mL)[a]	AUC_{inf} (hr*ng/mL)[a]
ARRAY-380-103	HV	300	12	B	None	24	2330 (36.2)	2410 (36.2)
ONT-380-009	HV	300	15	Normal	None	48	2710 (25.7)	2760 (25.7)
ONT-380-012	HV	300	28	A	None	48	3320 (30.9)	3350 (31.1)
ONT-380-012	HV	300	28	B	None	48	2000 (56.1)	2030 (55.3)
ONT-380-012	HV	300	28	C	None	48	2440 (38.7)	2480 (38.5)
ONT-380-012	HV	300	17	D	None	12	1870 (37.6)	2170 (36.7)
ONT-380-012	HV	300	13	E	None	12	2190 (34.3)	2450 (37.1)
SGNTUC-015	HV	50	12	Day 1	None	12	362 (44.9)	370 (41.2)
SGNTUC-015	HV	150	12	Day 1	None	12	982 (32.3)	1126 (31.1)
SGNTUC-015	HV	300	12	Day 1	None	12	2061 (29.1)	2203 (25.36)
ONT-380-004	Subjects, mBC	300	32	Cycle 1	None	6	1780 (60.8)	n/a
ONT-380-004	Subjects, mBC	350	7	Cycle 1	None	6	2750 (28.8)	n/a
MK-7119-001	Subjects, mBC	300	4	Cycle 1	Capecitabine + Trastuzumab	12	2953.4 (95.1)	4698.0 (57.7)

Tucatinib (BID), Steady-State								
Study	Subjects or HV	Dose (mg)	n	Part/Arm	Co-medication(s)	T_{last} (h)	AUC_{last} (h*ng/mL) [a]	AUC_{tau} (hr*ng/mL) [a]
ONT-380-011	HV	300	50	n/a	None	36	NC	3520 (26.0)
ONT-380-012	HV	300	17	D	None	12	3460 (31.6)	3470 (31.6)
ONT-380-012	HV	300	13	E	None	12	3300 (40.9)	3300 (40.9)
SGNTUC-015	HV	50	12	Day 14	None	72	696	531 (67.3)
SGNTUC-015	HV	150	12	Day 14	None	72	2541	1937 (22.7)
SGNTUC-015	HV	300	12	Day 14	None	72	4529	3332 (29.7)
ONT-380-004	Subjects, mBC	300	26	Cycle 2	T-DM1	6	2720 (41.5)	NC
ONT-380-004	Subjects, mBC	350	5	Cycle 2	T-DM1	6	3910 (47.2)	NC

Tucatinib (BID), Steady-State								
Study	Subjects or HV	Dose (mg)	n	Part/Arm	Co-medication(s)	T_{last} (h)	AUC_{last} (h*ng/mL) [a]	AUC_{tau} (hr*ng/mL) [a]
ONT-380-005	Subjects, mBC	300	6	1A, D14	Capecitabine	6	2469 (32.4)	NC
ONT-380-005	Subjects, mBC	300	6	1A, D21	Capecitabine	6	2378 (38.4)	NC
ONT-380-005	Subjects, mBC	350	3	1B	Capecitabine	6	5204 (30.8)	NC
ONT-380-005	Subjects, mBC	300	9	3A	Capecitabine + Trastuzumab	6	4724 (39.1)	NC
ONT-380-005	Subjects, CNSM	300	2	3E	Capecitabine + Trastuzumab	6	2076 (NC)	NC

AUC_{last}=area under the plasma-concentration time curve from time 0 to the time of the last quantifiable concentration; CNSM=central nervous system metastases; HV=healthy volunteer; mBC=metastatic breast cancer; n/a=not available; NC=not calculated; T-DM1= trastuzumab emtansine
a geometric mean (gCV%)

Appendix 2. Summary of the pharmacokinetic parameters of tucatinib from relevant studies (Tablet, Day 1 and Steady-State)

Tucatinib, Day 1							
Study	Subjects or HV	Dose (mg)	n	Part/Arm	Co-medication(s)	C_{max} (ng/mL) (CV%) [a]	T_{max} (h) Median (min-max)
ARRAY-380-103	HV	300	12	B	None	429 (47.8)	1.5 (1.5-2.0)
ONT-380-009	HV	300	15	Normal	None	436 (36.3)	2.0 (1.0-3.0)
ONT-380-012	HV	300	28	A	None	488 (31.5)	3.0 (1.0-6.0)
ONT-380-012	HV	300	28	B	None	302 (69.7)	2.0 (1.0-6.0)
ONT-380-012	HV	300	28	C	None	410 (42.3)	1.0 (0.97 -3.0)
ONT-380-012	HV	300	17	D	None	378 (43.5)	2.0 (1.0-4.0)
ONT-380-012	HV	300	13	E	None	437 (37.5)	2.0 (1.0-2.1)
SGNTUC-015	HV	50	12	Day 1	None	73.8 (54.9)	2.0 (0.98-3.0)
SGNTUC-015	HV	150	12	Day 1	None	199 (42.2)	2.0 (0.98-3.0)
SGNTUC-015	HV	300	12	Day 1	None	436 (33.4)	1.5 (0.98-3.0)
ONT-380-004	Subjects, mBC	300	32	Cycle 1	None	514 (66.4)	2.1 (0.92-6.0)
ONT-380-004	Subjects, mBC	350	7	Cycle 1	None	743 (44.1)	2.0 (0.95-3.0)
MK-7119-001	Subjects, mBC	300	4	Cycle 1	Capecitabine + Trastuzumab	561.2 (156.0)	2.9 (1-6)

Tucatinib (BID), Steady-State							
Study	Subjects or HV	Dose (mg)	n	Part/Arm	Co-medication(s)	C_{max} (ng/mL) (CV%)[a]	T_{max} (h) Median (min-max)
ONT-380-011	HV	300	50	n/a	None	519 (26.7)	3.0 (1.0-4.1)
ONT-380-012	HV	300	17	D	None	667 (29.9)	1.0 (0.98-2.0)
ONT-380-012	HV	300	13	E	None	598 (40.8)	2.0 (1.0-3.0)
SGNTUC-015	HV	50	12	Day 14	None	88.8 (82.7)	2.0 (1.0-4.0)
SGNTUC-015	HV	150	12	Day 14	None	333 (23.2)	2.0 (1.0-3.0)
SGNTUC-015	HV	300	12	Day 14	None	571 (32.7)	2.0 (1.0-3.0)
ONT-380-004	Subjects, mBC	300	26	Cycle 2	T-DM1	725 (45.1)	2.1 (0.0-5.5)
ONT-380-004	Subjects, mBC	350	5	Cycle 2	T-DM1	1010 (57.0)	1.0 (1.0-3.3)
ONT-380-005	Subjects, mBC	300	6	1A, D14	Capecitabine	634 (24.1)	2.0 (0.5-4.0)
ONT-380-005	Subjects, mBC	300	6	1A, D21	Capecitabine	539 (44.6)	5.0 (0.5-6.1)
ONT-380-005	Subjects	350	3	1B	Capecitabine	1483 (71.1)	1.00 (0.5-2.00)

Tucatinib (BID), Steady-State							
Study	Subjects or HV	Dose (mg)	n	Part/Arm	Co-medication(s)	C_{max} (ng/mL) (CV%)[a]	T_{max} (h) Median (min-max)
ONT-380-005	Subjects, mBC	300	9	3A	Capecitabine + Trastuzumab	1132 (36.7)	2.0 (1.1-3.0)
ONT-380-005	Subjects, CNSM	300	2	3E	Capecitabine + Trastuzumab	856 (NC)	3.0 (2.0-4.0)

C_{max}=maximum concentration; CNSM=central nervous system metastases; HV=healthy volunteer; mBC=metastatic breast cancer; NC=not calculated; T-DM1= trastuzumab emtansine; t_{max}=time to maximum concentration
a geometric mean (CV%)

Appendix 3. CL/F, CL_{ss}/F and half-life values in healthy subjects from relevant studies (Tablet, D1 and SS)

Tucatinib, Day 1						
Study	Dose (mg)	n	Part/Arm	T_{last} (h)	CL/F (L/h)[a]	Half-Life (h)[b]
ONT-380-012	300	28	A	48	89.5 (31.1)	7.87 (0.995)
ONT-380-012	300	28	B	48	148 (55.3)	7.98 (1.61)
ONT-380-012	300	28	C	48	121 (38.5)	8.70 (1.75)
ONT-380-012	300	17	D	12	138 (36.7)	4.14 (0.676)
ONT-380-012	300	13	E	12	122 (37.1)	3.88 (0.532)
ONT-380-009	300	15	Normal	48	109 (25.7)	8.67 (1.35)
Tucatinib (BID), Steady-State						
Study	Dose (mg)	n	Part/Arm	T_{last} (h)	CL_{ss}/F (L/h)	Half-Life (h)
ONT-380-012	300	17	D	12	86.3 (31.6)	4.70 (1.07)
ONT-380-012	300	13	E	12	90.9 (40.9)	4.10 (0.630)
ONT-380-011	300	50	n/a	36	85.3 (26.0)	7.43 (1.10)
SGNTUC-015	50	12	Day 14	72	83.6 (49.7)	NC
SGNTUC-015	150	12	Day 14	72	77.4 (22.7)	NC
SGNTUC-015	300	12	Day 14	72	90.0 (29.7)	NC

BID=twice daily; n/a=not available; NC=not calculated
a Geometric mean (%CV)
b Arithmetic mean (SD)

8. 参考文献

[1] 「非抗不整脈薬における QT/QTc 間隔の延長と催不整脈作用の潜在的可能性に関する臨床的評価について」（平成 21 年 10 月 23 日、薬食審査発 1023 第 1 号）

[2] 「非抗不整脈薬における QT/QTc 間隔の延長と催不整脈作用の潜在的可能性に関する臨床的評価」に関するＱ＆Ａについて（平成 29 年 5 月 23 日、事務連絡）

[3] US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Population pharmacokinetics guidance for industry; Feb 2022.

[4] 「母集団薬物動態／薬力学解析ガイドライン」について（令和元年 5 月 15 日薬生薬審発 0515 第 1 号）