表 2.6.5.5-2　有色ラット単回経口投与後の組織中濃度（続）

組織／血漿中濃度比

	Tissue:Plasma Concentration Ratio											
	Sacrifice Time (Hours)											
Tissue	1	SD	3	SD	8	SD	24	SD	72	SD	168	SD
Adrenal gland(s)	7.95	2.22	16.5	2.18	24.7	4.25	36.7	7.65	41.6	1.15	NA	NA
Arterial wall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Blood (QWBA analysis)	1.93	0.0881	2.05	0.152	2.65	0.308	NA	NA	NA	NA	NA	NA
Bone	NA	NA	0.324	0.109	0.598	0.252	NA	NA	NA	NA	NA	NA
Bone marrow	2.09	0.315	5.66	0.664	15.5	2.10	15.4	0.957	19.9	0.913	NA	NA
Brain cerebellum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Brain cerebrum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Brain choroid plexus	2.61	0.624	3.59	0.354	8.10	1.63	14.3	2.36	28.8	8.45	NA	NA
Brain medulla	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Brain olfactory lobe	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bulbo-urethral gland	2.75	0.261	6.38	0.450	13.1	1.59	19.2	2.86	13.4	0.876	NA	NA
Cecum	1.55[a]	NA	3.71	0.716	13.0	5.01	9.90	0.560	15.1[b]	NA	NA	NA
Diaphragm	2.58	0.575	4.34	0.362	7.45	0.290	6.69	0.600	NA	NA	NA	NA
Epididymis	NA	NA	0.402	0.0383	1.50	0.138	7.63	2.45	16.7[a]	NA	NA	NA
Esophagus	1.43	0.0902	3.21	0.572	5.98	0.717	6.36	0.684	NA	NA	NA	NA
Exorbital lacrimal gland	1.22	0.269	4.32	0.727	14.5	2.09	47.0	8.87	100	2.55	NA	NA
Eye lens	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Eye uveal tract	1.92	0.358	6.31	1.12	32.3	4.90	234	45.7	1391	258	NA	NA
Eye(s)	0.340	0.0526	1.24	0.184	5.02	1.47	40.5	8.40	240	53.7	NA	NA
Fat (abdominal)	0.419[b]	NA	0.664	0.147	1.37	0.131	NA	NA	NA	NA	NA	NA
Fat (brown)	2.09	0.378	3.88	0.240	7.30	0.268	7.72	1.69	13.9[a]	NA	NA	NA
Harderian gland	0.692	0.159	2.92	0.722	15.7	1.24	104	11.9	259	28.4	NA	NA
Intra-orbital lacrimal gland	1.16	0.326	3.77	0.791	13.2	1.17	52.9	18.0	120	28.0	NA	NA
Kidney cortex	6.41	1.25	13.5	1.21	19.3	0.794	21.6	3.02	42.1	2.14	NA	NA
Kidney medulla	5.81	1.07	12.0	1.28	21.7	2.59	28.8	4.42	53.8	5.91	NA	NA
Kidney(s)	6.06	1.14	12.7	1.31	20.5	1.64	25.4	3.97	47.8	3.60	NA	NA

NA: Not applicable. SD: Standard deviation.

[a] Mean is the average of two values.

[b] Mean is based on one value.

表 2.6.5.5-2　有色ラット単回経口投与後の組織中濃度（続）

組織／血漿中濃度比

	Tissue:Plasma Concentration Ratio											
	Sacrifice Time (Hours)											
Tissue	1	SD	3	SD	8	SD	24	SD	72	SD	168	SD
Large intestine	1.19	0.170	3.16	0.215	5.52	0.733	15.9	8.93	13.8[a]	NA	NA	NA
Liver	15.5	1.30	25.7	1.99	29.9	3.38	32.5	4.37	56.9	9.25	NA	NA
Lung(s)	7.25	1.22	20.8	0.821	63.8	0.690	28.3	4.40	14.4	3.65	NA	NA
Lymph node(s)	1.41	0.288	4.15	0.504	14.8	2.86	17.1	3.00	29.1	3.66	NA	NA
Muscle	0.937	0.156	2.30	0.460	4.81	0.410	4.32	0.582	NA	NA	NA	NA
Myocardium	3.76	0.300	6.32	0.531	8.97	0.793	6.91	0.725	NA	NA	NA	NA
Nasal turbinates	0.460	0.0924	0.771	0.0511	2.55	0.773	7.52	3.21	13.0	3.41	NA	NA
Pancreas	3.78	0.728	8.22	0.827	15.5	0.954	17.4	1.88	22.9	4.47	NA	NA
Pituitary gland	3.70	1.10	13.2	2.21	44.7	13.6	56.9	30.1	122	24.1	NA	NA
Preputial gland	0.868	0.213	1.73	0.345	5.72	3.00	41.2	12.6	157	21.6	NA	NA
Prostate gland	0.762	0.132	1.80	0.267	5.88	1.69	6.66	0.532	NA	NA	NA	NA
Salivary gland(s)	2.76	0.529	7.22	0.599	17.2	0.506	17.0	3.85	16.5	0.837	NA	NA
Salivary gland(s), parotid	2.08	0.379	5.51	0.531	11.6	0.403	15.2	3.71	17.3	2.29	NA	NA
Seminal vesicle(s)	0.297[b]	NA	0.688	0.111	1.64	0.555	2.17	1.89	NA	NA	NA	NA
Skin (nonpigmented)	0.382	0.0360	0.891	0.161	2.62	0.149	5.42	0.368	NA	NA	NA	NA
Skin (pigmented)	0.410	0.0393	1.00	0.0804	3.03	0.497	9.67	2.63	29.8	3.33	NA	NA
Small intestine	4.45	4.46	5.14	0.978	9.96	0.746	10.2	4.76	3.58	6.21	NA	NA
Spinal cord	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Spleen	6.12	0.883	11.2	1.01	23.4	3.77	24.4	3.48	45.4	2.85	NA	NA
Stomach	1.45	0.363	2.38	0.153	4.66	0.160	7.22	0.569	3.60	6.24	NA	NA
Stomach mucosa	1.81	0.420	3.39	0.229	4.99	0.705	8.72	2.07	13.2	1.90	NA	NA
Stomach wall	1.25	0.281	2.00	0.254	5.74	0.357	4.91	0.290	NA	NA	NA	NA
Testis(es)	NA	NA	0.191	0.0127	0.732	0.106	4.23	0.292	NA	NA	NA	NA
Thymus	0.796	0.0876	2.65	0.182	8.90	0.667	12.4	3.23	14.8	3.72	NA	NA
Thyroid	5.41	1.11	10.2	0.999	18.2	3.81	26.6	4.22	39.7	3.93	NA	NA
Urinary bladder	0.676	0.252	3.43[a]	NA	4.52	0.478	10.9[a]	NA	NA	NA	NA	NA

NA: Not applicable. SD: Standard deviation.

[a] Mean is the average of two values.

[b] Mean is based on one value.

表 2.6.5.5-3　ラット単回経口投与後の血漿・血液濃度比

									Test Article: [^{14}C]entrectinib				
									Location in CTD: 4.2.2.3-5				
									Study No. SR-[redacted]-003 (1087284)				
Species: Rat (Sprague Dawley and Long Evans)													
Gender (M/F)/Number of Animals: 3 M per group per sampling time													
Feeding Condition: Fed													
Vehicle/Formulation: 0.5% methylcellulose and 1% Tween® 80 in reverse osmosis water													
Method of Administration: PO													
Dose (mg/kg): 20													
Radionuclide: ^{14}C													
Specific Activity: 5.49 µCi/mg in dosing formulation													
Analyte/Assay: Radioactivity/Liquid scintillation counting (LSC)													
Sampling Time: l, 3, 8, 24, 72, and 168 h postdose													
	Blood:Plasma Concentration Ratio												
Species	1 h		3 h		8 h		24h		72h		168 h		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Sprague Dawley	1.71	0.137	2.52	0.219	2.53	0.195	1.43	0.0974	1.28	NA	NA	NA	
Long Evans	2.11	0.205	2.34	0.136	2.70	0.198	1.51	0.134	1.20	0.330	NA	NA	
Additional Information: With means ranging from 1.20 to 2.70, blood:plasma concentration ratios were greater than 1 through 72 hours postdose. The blood-to-plasma ratio data suggest that [^{14}C]entrectinib-derived radioactivity partitioned into the cellular components of blood, regardless of rat species.													

2.6.5.6　薬物動態試験：蛋白結合

表 2.6.5.6-1　*In vitro* 血漿蛋白結合（entrectinib）

			Test Article: Entrectinib	
			Location in CTD: 4.2.2.3-1	
			Study No. 0218-[redacted] (1087282)	
Study System: *In vitro*				
Test System and Method: Plasma from mouse, rat, dog, monkey and human, Equilibrium Dialysis (37°C, 6 h), n=3				
Analyte: Entrectinib				
Assay: LC-MS/MS				
Species	Conc. Tested	%Bound (mean ± SD)	Conc. Tested	%Bound (mean ± SD)
Mouse	5 µmol/L	97.43 ± 1.26	50 µmol/L	98.35 ± 0.79
Rat	5 µmol/L	99.37 ± 0.12	50 µmol/L	99.32 ± 0.01
Dog	5 µmol/L	98.80 ± 0.73	50 µmol/L	>99.98
Monkey	5 µmol/L	98.39 ± 1.35	50 µmol/L	99.17 ± 0.00
Human	5 µmol/L	99.51 ± 0.14	50 µmol/L	99.39 ± 0.41

表 2.6.5.6-2 *In vitro*血漿蛋白結合（M5）

					Test Article: M5	
					Location in CTD: 4.2.2.3-2	
					Study No. SR-[redacted]-005 (1087285)	
Study System: *In vitro*						
Test System and Method: Plasma from mouse, rat, dog, monkey and human, Equilibrium Dialysis (37°C, 5 h), n=3						
Analyte: M5						
Assay: LC-MS/MS						
Species	Conc. Tested	%Bound (mean ± SD)	Conc. Tested	%Bound (mean ± SD)	Conc. Tested	%Bound (mean ± SD)
Mouse	0.5 µmol/L	99.7 ± 0.0228	2.5 µmol/L	99.8 ± 0.0227	10 µmol/L	99.8 ± 0.0233
Rat	0.5 µmol/L	100 [a,b]	2.5 µmol/L	99.8 ± 0.0286	10 µmol/L	99.8 ± 0.0103
Dog	0.5 µmol/L	99.8 ± 0.0661	2.5 µmol/L	99.7 ± 0.0135	10 µmol/L	99.8 ± 0.0122
Monkey	0.5 µmol/L	99.6 ± 0.0645	2.5 µmol/L	99.7 ± 0.0433	10 µmol/L	99.7 ± 0.00262
Human	0.5 µmol/L	100 ± 0.00241[b]	2.5 µmol/L	99.9 ± 0.0104	10 µmol/L	99.9 ± 0.0146
Additional Information: [a] n = 2 (measurable concentration in the buffer side for one rat sample at 0.5 µmol/L was below 0.200 nmol/L) [b] Round to 100						

表 2.6.5.6-3　*In vitro* 血漿蛋白結合（entrectinib 及び M5）

			Test Article: Entrectinib, M5	
			Location in CTD: 4.2.2.3-10	
			Study No. 1088992	
Study System: *In vitro*				
Test System and Method: Plasma from mouse, rat, dog and human, Equilibrium Dialysis (37°C, 5 h)				
Analyte: Entrectinib, M5				
Assay: LC-MS/MS				
	Entrectinib		M5	
Species (Strain) or protein	Conc. Tested	% fraction unbound (Geometric mean ± SD)	Conc. Tested	% fraction unbound (Geometric mean ± SD)
Human, HV	1 µmol/L	0.22 ± 0.05　(n=15)	1 µmol/L	0.31 ± 0.05　(n=13)
Mouse (C57bl/6j)	1 µmol/L	0.34 ± 0.03　(n=12)	1 µmol/L	0.53 ± 0.08　(n=12)
Rat (Wistar)	1 µmol/L	0.32 ± 0.09　(n=13)	1 µmol/L	0.46 ± 0.08　(n=9)
Dog (Beagle)	1 µmol/L	0.36 ± 0.10　(n=12)	1 µmol/L	0.46 ± 0.12　(n=11)
HSA 45 g/L	1 µmol/L	1.3 ± 0.1　(n=9)		
AGP 0.7 g/L	1 µmol/L	1.8 ± 0.6　(n=9)		
HSA 45 g/L / AGP 0.7 g/L	1 µmol/L	0.3 ± 0.03　(n=8)		

HV: healthy volunteer, HSA: human serum albumin, AGP: α1-acid glycoprotein

2.6.5.7　薬物動態試験：妊娠又は授乳動物における試験

該当なし

2.6.5.8　薬物動態試験：その他の分布試験

表 2.6.5.8-1　*In vitro* 血球移行並びにヒト肝細胞及びヒト肝ミクロソーム試料への結合性

			Test Article: Entrectinib, M5	
			Location in CTD: 4.2.2.3-10	
			Study No. 1088992	
Study System: *In vitro*				
Test System and Method: Blood from mouse, rat and human, (37°C, 30 min), n=3 Human hepatocyte medium, Human microsome, Equilibrium Dialysis (37°C, 5 h), n=9				
Analyte: Entrectinib, M5				
Assay: LC-MS/MS				
	Entrectinib		M5	
Species (Strain)	Conc. Tested	Blood /Plasma ratio (Geometric mean ± SD)	Conc. Tested	Blood/Plasma ratio (Geometric mean ± SD)
Human, HV	3 µmol/L	1.3 ± 0.09	3 µmol/L	1.0 ± 0.12
Rat (Wistar)	3 µmol/L	2.0 ± 0.08	3 µmol/L	1.5 ± 0.11
Mouse (C57bl/6j)	3 µmol/L	1.8 ± 0.03	3 µmol/L	1.2 ± 0.04

Matrix	Conc. Tested	% fraction unbound (Geometric mean ± SD)
Human hepatocyte medium	1 µmol/L	20.6 ± 1.6
Human microsome　0.05 mg/mL	1 µmol/L	8.8 ± 2.0
0.1 mg/mL	1 µmol/L	5.6 ± 0.7
0.25 mg/mL	1 µmol/L	3.0 ± 0.1
0.5 mg/mL	1 µmol/L	1.3 ± 0.1
1 mg/mL	1 µmol/L	0.6 ± 0.1

HV: healthy volunteer

表 2.6.5.8-2 *In vivo* 血球移行

					Test Article: [^{14}C]entrectinib	
Location in CTD:	4.2.2.2-3				4.2.2.2-5	
Study No.	SR-[redacted]-004 (1087302)				SR-[redacted]-013 (1087301)	
Species:	Rat (Sprague Dawley)				Dog (Beagle)	
Radionuclide:	^{14}C				^{14}C	
Specific Activity:	2.83 (PO) and 26.8 (IV) µCi/mg in formulation				4.24 (PO) and 41.1 (IV) µCi/mg in formulation	
Method of Administration :	PO		IV		PO	IV
Dose (mg/kg):	20		2		10	1
Analyte:	Radioactivity		Radioactivity		Radioactivity	Radioactivity
Assay:	LSC		LSC		LSC	LSC
Time Points (hours)	Blood/Plasma Concentration Ratio				Blood/Plasma Concentration Ratio	
	M	F	M	F	M	M
0.083	NA	NA	2.53	2.37	NA	3.84
0.25	2.03	2.69	3.44	3.38	7.70	4.71
0.50	2.14	2.39	3.61	3.47	5.93	5.17
1	1.78	1.80	3.57	4.17	4.50	5.13
2	1.91	2.73	4.36	4.44	4.07	5.11
4	2.52	2.63	3.80	4.14	4.11	5.06
6	2.59	2.73	2.78	3.69	4.21	4.76
8	2.15	2.59	3.22	4.13	4.18	4.12
12	2.77	3.14	2.59	3.53	3.77	3.47
24	1.76	3.11	1.55	1.98	2.21	1.66
48	1.59	1.61	1.67	1.99	1.58	1.31

表 2.6.5.8-2 *in vivo* 血球移行（続）

					Test Article: [^{14}C]entrectinib	
Study No.	SR-[redacted]-004 (1087302)				SR-[redacted]-013 (1087301)	
Method of Administration:	PO		IV		PO	IV
Dose (mg/kg):	20		2		10	1
	Blood/Plasma Concentration Ratio				Blood/Plasma Concentration Ratio	
Time Points (hours)	M	F	M	F	M	M
72	NA	NA	NA	NA	1.50	1.36
96	NA	NA	NA	NA	1.44	1.30
120	NA	NA	NA	NA	1.37	1.30
AUC_{inf}	2.25	2.74	2.99	3.47	2.71	2.51
Additional Information: Preferential distribution of [^{14}C]entrectinib-derived radioactivity in red blood cell component was observed.						

NA: Not applicable; LSC: Liquid scintillation counting.

表 2.6.5.8-3　脳への移行性（ラット経口投与）

					Test Article: Entrectinib	
					Location in CTD: 4.2.2.3-6	
					Study No. SR-[redacted]-001 (1087283)	
Species	Rat (Sprague Dawley)					
Gender (M/F)/Number of Animals:	3 M/time point/dose (Total 24 M)					
Feeding Condition:	Fasted					
Vehicle/Formulation:	0.5% methylcellulose water					
Method of Administration:	PO					
Dose (mg/kg):	20					
Sample Type:	Plasma, brain, cerebrospinal fluid (CSF)					
Analyte:	Entrectinib, M5					
Assay:	LC-MS/MS (not-validated)					
	Entrectinib		M5		M5:Entrectinib Ratio	
PK parameters	Plasma	Brain	Plasma	Brain	Plasma	Brain
T_{max} (h)	3.00	6.00	3.00	12.0	NA	NA
C_{max} (nmol/L)	820	151	63.8	8.43	0.0778	0.0557
AUC_{inf} (nmol/L·h)	6375	1395	473	149 [1]	0.0743	0.107 [1]
Brain:Plasma C_{max} ratio	NA	0.185	NA	0.132	NA	NA
Brain:Plasma AUC_{inf} ratio	NA	0.219	NA	0.315[1]	NA	NA
Additional Information: There was no measurable concentration of entrectinib and M5 in CSF samples.						

[1] AUC_{last} used for analysis, NA: Not applicable.

表 2.6.5.8-4　脳への移行性（ラット点滴静脈内投与）

					Test Article: Entrectinib	
					Location in CTD: 4.2.2.3-11	
					Study No. 1090366	
Species	Rat (Sprague Dawley)					
Gender (M/F)/Number of Animals:	4 M/time point/dose (Total 8 M)					
Feeding Condition:	Not specified					
Vehicle/Formulation:	10 mmol/L lactic acid and 5% glucose at pH 5.0					
Method of Administration:	A single IV bolus followed by an IV infusion					
Dose (mg/kg):	IV bolus 6 mg/kg, IV infusion 0.032 mg/min/kg for 5 h or 6 h					
Sample Type:	Plasma, brain, cerebrospinal fluid (CSF)					
Analyte:	Entrectinib, M5					
Assay:	LC-MS/MS (not-validated)					
	Entrectinib		M5		M5/Entrectinib Ratio (%)	
Time from start of infusion (h)	5	6	5	6	5	6
Concentration (mean ± SD)						
Plasma (nmol/L)	1260 ± 430	1400 ± 109	71.3 ± 32.3	84.8 ± 13.1	5.8 ± 1.9	6.0 ± 0.1
Brain (nmol/L)	567 ± 71.2	843 ± 186	22.8 ± 4.16	37.3 ± 10.6	4.1 ± 1.1	4.5 ± 0.1
CSF (nmol/L)	0.99 ± 0.2	0.81 ± 0.2	BLQ	BLQ		
Brain/Plasma ratio	0.49 ± 015	0.60 ± 0.1	0.35 ± 0.08	0.44 ± 0.07		
CSF/Free plasma ratio	0.23 ± 0.10	0.18 ± 0.03	NA	NA		

NA: Not applicable,　BLQ: below the lower limit of quantitation (<0.0178 nmol/L for M5 in CSF)

表 2.6.5.8-5 LIMBAによる脳組織への結合性

		Test Article: Entrectinib	
		Location in CTD: 4.2.2.3-12	
		Study No. 1090266	
Study System: *In vitro*			
Test System and Method: Lipid membrane binding assay (LIMBA), microemulsion of porcine brain polar lipids			
Analyte: Entrectinib			
Assay: LC-UV/MS			
45 min incubation at 37°C		18 h incubation at 21°C	
Conc. Tested (µmol/L)	% fraction unbound	Conc. Tested (µmol/L)	% fraction unbound
35	> 44	8	1.2
		16	1.2
		19	0.8
		25	0.7
		26	0.8
		27	0.8
		34	0.8
		35	0.8
Additional Information: Entrectinib showed a very strong time dependent effect on binding to brain polar lipids in the LIMBA assay. At 45 min the fraction unbound is >44% whereas the fraction unbound after 18 h incubation is only 0.8%. This strong time dependent effect needs to be further investigated. The fraction unbound after 18 h corrected for lysosomal trapping is estimated to be 0.41% (by taking into account the basic pKa value of 7.54 the fraction unbound needs to be reduced by a factor of 2.13 leading to a fu,b (corrected for lysosomal trapping)).			

2.6.5.9 薬物動態試験：*In Vivo* における代謝

表 2.6.5.9-1 ラット血漿中及び糞中代謝物プロファイル

					Test Article: [^{14}C]entrectinib				
					Location in CTD: 4.2.2.4-5				
					Study No.: SR-[redacted]-004-Met (1087296)				
Gender (M/F)/Number of Animals:				3 M, 3 F/ dose route for urine and feces collection group; 4 M, 4 F/dose route for blood metabolite ID group					
Feeding Condition:				Fed					
Vehicle/Formulation:				10% Tween®80, 2 molar equivalents hydrochloric acid (HCl) in sterile saline/IV 0.5% methylcellulose and I% Tween®80 in reverse osmosis (RO) water/PO					
Method of Administration:				PO, IV					
Dose (mg/kg)/Route:				20/PO, 2/IV					
Species	Sample	Dosing	Gender	Time (h)	% of Radioactivity (Radio-HPLC)				
					M5	M12	Others [1]	Entrectinib	Total
Rat	Plasma (pooled)	PO	M	1	ND	90.4	ND	9.57	100
				4	ND	5.99	ND	94.0	100
			F	1	ND	46.3	ND	53.7	100
				4	ND	ND	ND	100	100
		IV	M	0.5	ND	ND	ND	100	100
				4	ND	ND	ND	100	100
			F	0.5	ND	ND	ND	100	100
				4	ND	ND	ND	100	100
	Feces (pooled)	PO	M	0-24	25.6	ND	1.63	72.7	100
				24-48	19.4	ND	7.26	73.4	100
			F	0-24	20.4	ND	ND	79.6	100
				24-48	28.4	ND	ND	71.6	100
		IV	M	0-24	40.6	ND	9.78	49.6	100
				24-48	71.1	ND	10.2	18.7	100
			F	0-24	36.7	ND	12.6	50.6	100
				24-48	62.9	ND	10.0	27.1	100
Additional Information: Metabolic profiles of entrectinib in rat urine were not evaluated as overall urinary excretion was less than 1% of dose after single oral administration or less than 2% of dose after single IV administration. M1 and M2 were detected in feces samples by HPLC-MS/MS, though they were under detection limit in radioactive profiling.									

[1] Others include M6, M9, M10 and several unassigned minor peaks ND: Not detected.

表 2.6.5.9-2　イヌ血漿中及び糞中代謝物プロファイル

					Test Article: [^{14}C]entrectinib				
					Location in CTD: 4.2.2.4-6				
					Study No.: SR-[redacted]-013-Met (1087297)				
Gender (M/F)/Number of Animals:				3 M/dose					
Feeding Condition:				Fasted overnight through 4 hours postdose/PO,　Fed/IV					
Vehicle/Formulation:				0.15% lactic acid, 0.05% benzyl alcohol in 5% D-mannitol, in 5% dextrose water /IV 0.5% methylcellulose and 1% Tween®80 in reverse osmosis (RO) water/PO					
Method of Administration:				PO, IV					
Dose (mg/kg)/Route:				10/PO, 1/IV					
Species	Sample	Phase	Dosing	Time (h)	% of Radioactivity (Radio-HPLC)				
					M4	M5	M7	Entrectinib	Total
Dog	Plasma (pooled)	I	PO	2	ND	38.1	ND	61.9	100
				6	ND	69.9	ND	30.1	100
		II	IV	0.083 [l]	ND	ND	26.9	73.1	100
				2	ND	12.1	ND	87.9	100
				6	ND	46.5	ND	53.5	100
	Feces (pooled)	I	PO	6-12	ND	94.8	ND	5.16	100
				12-24	ND	93.7	ND	6.31	100
				24-48	ND	100	ND	ND	100
		II	IV	12-24	8.20	91.8	ND	ND	100
				24-48	ND	100	ND	ND	100
				48-72	ND	100	ND	ND	100
Additional Information: Metabolic profiles of entrectinib in dog urine were not evaluated as overall urinary excretion was less than 1% of dose after single oral or IV administration. The presence of M1, M2, M3, and M12 in feces was confirmed by HPLC-MS/MS analysis.									

[l] Sample from PK study

ND: Not detected.

表 2.6.5.9-3　ヒト血漿中及び尿糞中代謝物プロファイル

						Test Article: [^{14}C]entrectinib			
						Location in CTD: 5.3.3.1-2			
						Study No.: RXDX-101-05			
Gender (M/F)/Number of Animals:	6 male healthy volunteers								
Feeding Condition:	Fasted overnight through 4 hours postdose								
Vehicle/Formulation:	200 mg capsules								
Method of Administration:	PO								
Dose (mg)/Route:	600								
Specific Activity:	200 µCi/body								
Plasma	% of Radioactivity Profile (Radio-HPLC)								
	M1	M2	M3[a]	M4	M5	M7	M8	M11[a]	Entrectinib
2 h	ND	ND	ND	ND	ND	ND	ND	ND	100
4 h	ND	ND	0.215	ND	14.1	2.89	ND	1.60	81.2
8 h	ND	ND	0.126	ND	9.99	ND	ND	12.5	77.4
0-24 h (pooled)	ND	ND	1.31	ND	11.5	ND	ND	18.6	68.6
Matrix	% of Dose (Radio-HPLC)								
	M1	M2	M3[a]	M4	M5	M7	M8	M11[a]	Entrectinib
Urine	0.482	0.782	0.0312	ND	0.171	0.279	0.488	0.165	0.663
Feces	14.2	9.45	1.22	0.185	22.1	ND	ND	0.0583	35.7
Total	14.7	10.2	1.25	0.185	22.2	0.279	0.488	0.224	36.4
Additional Information: [a] Relative percent was based on the response of selective ion monitoring in mass spectra, as M3 and M11 were overlapped in the radioactive chromatogram.									

ND: Not detected.

2.6.5.10　薬物動態試験：*In Vitro* における代謝

表 2.6.5.10-1　肝細胞を用いた *in vitro* 代謝試験

		Test Article: Entrectinib
		Location in CTD: 4.2.2.4-1
		Study No. T0627-R (1087704)
Study System: Hepatocytes from male CD-1 mice, male SD rats, male beagle dogs, male cynomolgus monkeys, and pooled humans were incubated for up to 1 hours with l μmol/L of entrectinib for intrinsic clearance or 10 μmol/L of entrectinib for metabolic profiling.		
Results for intrinsic clearance:		
Species	$t_{1/2}$ (min)	*In vitro* CL_{int} (mL/min/kg)
Mouse	130	47
Rat	39	85
Dog	83	64
Monkey	34	110
Human	76	22

表 2.6.5.10-1　肝細胞を用いた *in vitro* 代謝試験（続）

Test Article: Entrectinib					
Results for metabolic profiling in hepatocytes:					
Compound ID	% of Drug Related Material				
	Mouse	Rat	Dog	Monkey	Human
Entrectinib	70	44	46	45	68
M2	6	4	<1	11	2
M15	<1	4	<1	<1	<1
M14	6	19	7	3	10
M7	8	12	36	14	12
M5	7	11	8	16	3
M13		<1	<1	3	<1
M6		3	<1	<1	
M1	<1	1		3	<1
M3	<1	<1	<1	1	<1
M16	2	<1	1	1	1
M11				<1	3
Additional Information: No marked differences were observed between species both from a quantitative and a qualitative point; no human specific metabolite was observed in hepatocytes incubations.					

表 2.6.5.10-2　ヒト肝細胞を用いた *in vitro* 代謝試験　（固有クリアランス）

		Test Article: Entrectinib, M5
		Location in CTD: 4.2.2.4-7
		Study No. 1090334
Study System: Entrectinib and M5 was incubated using a long-term hepatocyte co-culture system HepatoPac®, which is a 96-well plate containing human liver cells and fibroblast control cells. Incubations (5% CO_2 atmosphere and 37°C) of entrectinib and M5 at different concentrations (0.1, 1 and 10 µmol/L) were performed for 96 hours. The concentrations of entrectinib and M5 in the cell medium (sampling time points: 2, 18, 26, 48, 72 and 96 h) were analyzed by LC-MS/MS, then calculated the intrinsic clearance.		
Results		
	CL_{int} (µL/min/mg protein) [a]	
Initial conc. (µmol/L)	Entrectinib	M5
0.1	20.3	9.3
1	17	8.7
10	5.8	3.9
Additional Information: The intrinsic clearance of M5 was ~2-fold lower than that of entrectinib at clinically relevant concentrations. A concentration-dependent decrease of the *in vitro* CL_{int} was observed for both entrectinib and M5.		

[a] CL_{int} (µL/min/mg protein) = HepatoPac® CL_{int} (µL/min/mg protein) – (0.75 × Fibroblasts (stromal cells) CL_{int} (µL/min/mg protein))

表 2.6.5.10-3　ヒト肝細胞を用いた *in vitro* 代謝試験（120分間インキュベーション）

		Test Article: [^{14}C]entrectinib
		Location in CTD: 4.2.2.4-8
		Study No. 1090424
Radionuclide: ^{14}C		
Specific Activity: 102.2 μCi/mg (radiochemical purity: >99%)		
Analyte/Assay: Radiochromatography and LC-MS/MS		
Study System: *in vitro* metabolism entrectinib was investigated in human hepatocytes using [^{14}C] labeled entrectinib at 10 μmol/L.		
Results:		
	Presence in Sample after 120 min	Relative Contribution to Metabolism
	% drug related material in peaks	%
Entrectinib	71	—
M1	1.8	6.2
M2	5.5	19
M3	0.5	1.7
M5	12	41
M7	3.4	12
M11	5.5	19
Unknown	0.7	2.4
Additional Information: Direct N-glucuronidation of entrectinib yielded a quaternary glucuronide M11. Structure elucidation of M11 by NMR spectroscopy demonstrated that the glucuronidation to M11 took place at the N-methylpiperazine.		

表 2.6.5.10-4　肝ミクロソームを用いた *in vitro* 代謝試験

	Test Article: [^{14}C]entrectinib
	Location in CTD: 4.2.2.4-2
	Study No. SR-[redacted]-021 (1087479)

Study System: Liver microsomes from mice, rats, dogs, cynomolgus monkeys, and pooled humans were incubated for 1 hour with 2 µmol/L of [^{14}C]entrectinib.

Compound ID	% of Radioactivity				
	Mouse	Rat	Dog	Monkey	Human
Entrectinib	79.3	70.9	33.6	32.6	46.5
M1	ND	ND	ND	5.05	7.48
M2	ND	ND	ND	ND	8.05
M3 [1]	ND	ND	ND	5.40	1.97
M5	14.1	17.8	29.9	40.4	28.4
M7	6.53	8.96	36.5	3.77	1.69
M13[1]	ND	ND	ND	10.6	5.74
Others [2]	ND	2.30	ND	2.20	0.108
Total	100	100	100	100	100

Additional Information: No human specific metabolite observed in liver microsomes incubations.

[1] Overlapped in the radioactive chromatogram, relative percentage was based on the mass response from selective ion monitoring.
[2] Others refer to unassigned minor radioactive peaks

表 2.6.5.10-5　発現系ヒト CYP 及びヒト肝ミクロソームを用いた *in vitro* 代謝試験

		Test Article: Entrectinib	
		Location in CTD: 4.2.2.4-3	
		Study No. 0305-[redacted] (1087482)	
Study System: Entrectinib (10 µmol/L) was incubated for 60 min with human liver microsomes (HLM) and cDNA expressed human CYPs enzymes, including CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2Cl8, CYP2Cl9, CYP2D6, CYP2El, CYP3A4, and CYP3A5.			
Matrix	% of Remaining (N=2)	$t_{1/2}$ (min) (N=2)	CL_{int} (µL/min/pmol P450) (N=2)
CYP1A1	90.4	> 423.7	<0.08
CYP1A2	95.0	>480	<0.07
CYP2A6	96.1	>433.6	<0.08
CYP2B6	98.2	>480	<0.07
CYP2C8	86.1	217.8	0.18
CYP2C9	84.5	284.5	0.12
CYP2C18	102.4	>480	<0.07
CYP2C19	79.1	174.3	0.20
CYP2D6	98.0	>480	<0.07
CYP2E1	89.0	369.3	0.10
CYP3A4	55.5	74.8	0.47
CYP3A5	99.8	>480	<0.07
HLM	73.1	134.6	0.26 [1]
Additional Information: CYP2C18 and CYP2E1: 40 pmol/mL, Other CYPs: 20 pmol/mL			

[1] CL_{int} expressed as µL/min/mg protein

HLM: Human liver microsomes (0.8 mg/mL)

表 2.6.5.10-5　発現系ヒト CYP 及びヒト肝ミクロソームを用いた *in vitro* 代謝試験（続）

				Test Article: Entrectinib		
				Location in CTD: 4.2.2.4-3		
				Study No. 0305-[redacted] (1087482)		
	% contribution at 60 min (mean, n=2)					
	Metabolite					
CYP	M2	M7	M5	M4	M1	Total
CYP1A1	＜0.1	＜0.1	0.1	-		0.1
CYP1A2			0.2			0.2
CYP2A6						0.0
CYP2B6			0.1			0.1
CYP2C8		0.2	0.4			0.7
CYP2C9			0.1			0.1
CYP2C18						0.0
CYP2C19		＜0.1	0.5			0.6
CYP2E1	0.5	0.2	0.4			1.1
CYP2D6		＜0.1	0.1			0.1
CYP3A4	51.8	9.7	11.3	11.5	4.7	88.9
CYP3A5	2.3	2.0	3.8			8.1

表 2.6.5.10-6　発現系ヒト UGT 及びヒト肝ミクロソームを用いた *in vitro* 代謝試験（UGT Phenotyping）

	Test Article: [^{14}C]entrectinib
	Location in CTD: 4.2.2.4-4
	Study No. SR-[redacted]-016 (1087298)

Study System: [^{14}C]entrectinib (2 μmol/L) was incubated for 60 min with human liver microsomes (0.5 mg/mL), and recombinant human UGT isoforms (0.125 mg/mL), including UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, lA10, 2B4, 2B7, 2B10, 2Bl5, and 2Bl7.

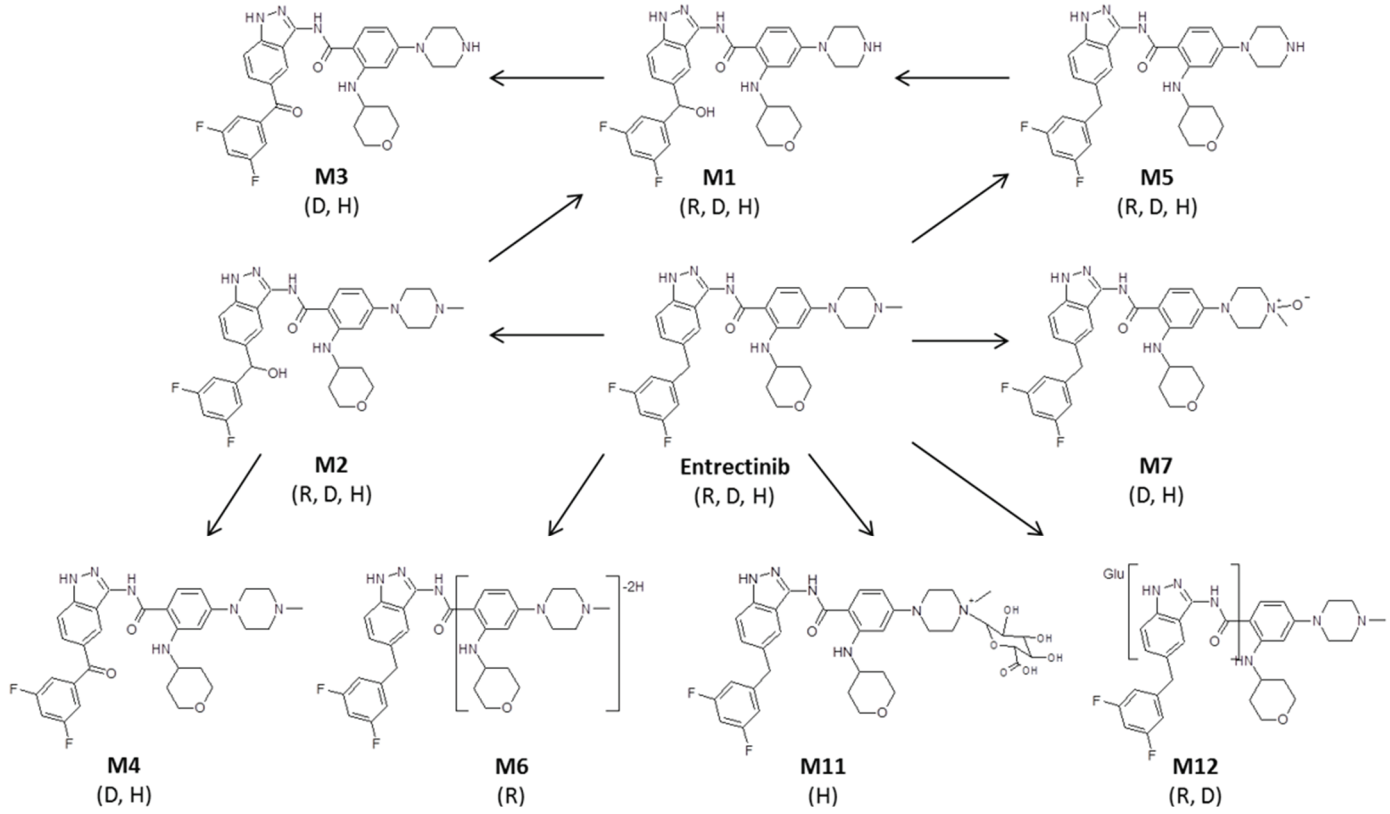
Matrix	% of Radioactivity		
	M11	Entrectinib	Total
UGT1A1	ND	100	100
UGT1A3	ND	100	100
UGT1A4	3.94	96.1	100
UGT1A6	ND	100	100
UGT1A7	ND	100	100
UGT1A8	ND	100	100
UGT1A9	ND	100	100
UGTlA10	ND	100	100
UGT2B4	ND	100	100
UGT2B7	ND	100	100
UGT2B10	ND	100	100
UGT2B15	ND	100	100
UGT2B17	ND	100	100
HLM	ND	100	100

Additional Information: Further evaluated by HPLC-MS/MS analyses, the formation of glucuronide conjugate of entrectinib (M11) was only observed in human liver micosomes and UGT1A4.

HLM: Human liver microsomes; ND: Not detected; UGT: human UDP-Glucuronosyl transferases.

2.6.5.11　薬物動態試験：推定代謝経路

	Test Article: Entrectinib
	Location in CTD: 4.2.2.4-1, 4.2.2.4-3, 4.2.2.4-5, 4.2.2.4-6, 4.2.2.4-8
	Study No. T0627-R (1087704), 0305-[redacted] (1087482), SR-[redacted]-004-Met (1087296), SR-[redacted]-013-Met (1087297), 1090424
Summary of entrectinib Metabolism: Biotransformation of entrectinib *in vivo* mainly includes N-demethylation, oxidation and phase II conjugation with glucuronide. Desmethyl metabolite M5 was a predominant component in dog feces and also a major metabolite in dog plasma and rat feces. M11 a glucuronide conjugate of entrectinib, was not detected in rat and dog plasma and fecal samples.	



R: Rat, D: Dog, H: Human

2.6.5.12　薬物動態試験：薬物代謝酵素の誘導／阻害

2.6.5.15 薬物相互作用に記載した。

2.6.5.13　薬物動態試験：排泄

表 2.6.5.13-1　ラットにおける尿糞中排泄

							Test Article: [^{14}C]entrectinib					
							Location in CTD: 4.2.2.2-3					
							Study No. SR-[redacted]-004 (1087302)					
Species:	Rat (Sprague Dawley)											
Gender (M/F)/No. of Animals:	3 M, 3 F /dosing route											
Feeding Condition:	Fed											
Vehicle/Formulation:	10% Tween®80, 2 molar equivalents hydrochloric acid (HCl) in sterile saline/IV 0.5% methylcellulose and 1% Tween®80 in reverse osmosis (RO) water/PO											
Analyte	% of ^{14}C recovery											
Assay	Liquid Scintillation Counting											
Method of Administration:	PO						IV					
Dose (mg/kg):	20						2					
Gender	M			F			M			F		
Excretion Route:	Urine	Feces	Cage Rinse	Urine	Feces	Cage Rinse	Urine	Feces	Cage Rinse	Urine	Feces	Cage Rinse
	% Total dose			% Total dose			% Total dose			% Total dose		
0-8 h	0.162	NA	NA	0.204	NA	NA	0.789	NA	NA	0.677	NA	NA
0-24 h	0.685	69.0	0.0137	0.545	50.8	0.0091	1.42	82.7	0.0369	1.09	72.7	0.0235
0-48 h	0.786	94.4	0.0184	0.629	93.6	0.0148	1.54	98.4	0.0588	1.19	95.9	0.0352
0-72 h	0.815	96.4	0.0184	0.654	96.6	0.0148	1.59	101	0.0669	1.22	98.1	0.0391
0-96 h	0.830	97.0	0.0184	0.669	97.2	0.0148	1.61	102	0.0669	1.24	98.8	0.0391
0-120 h	0.838	97.2	0.0184	0.677	97.5	0.0148	1.62	102	0.0707	1.26	99.1	0.0391
0-144 h	0.844	97.3	0.0184	0.681	97.6	0.0148	1.64	102	0.0738	1.27	99.3	0.0391
0-168 h	0.849	97.4	NA	0.686	97.7	NA	1.64	102	NA	1.28	99.5	NA
Additional Information: In rats, following an oral or IV dose of [^{14}C]entrectinib, fecal elimination was the predominant elimination pathway for drug-derived radioactivity.												

NA: Not applicable

表 2.6.5.13-2 イヌにおける尿糞中排泄

				Test Article: [^{14}C]entrectinib		
				Location in CTD: 4.2.2.2-5		
				Study No. SR-[redacted]-013 (1087301)		
Species:	Dog (Beagle)					
Gender (M/F)/No. of Animals:	3 M/dose					
Feeding Condition:	Fasted overnight through 4 hours postdose/PO, Fed/IV					
Vehicle/Formulation:	0.15% lactic acid, 0.05% benzyl alcohol in 5% D-mannitol, in 5% dextrose water /IV 0.5% methylcellulose and 1% Tween®80 in reverse osmosis (RO) water/PO					
Analyte	% of ^{14}C recovery					
Assay	Liquid Scintillation Counting					
Method of Administration:	PO			IV		
Dose (mg/kg):	10			1		
Excretion Route:	Urine	Feces	Cage Rinse	Urine	Feces	Cage Rinse
	% Total dose			% Total dose		
Predose	0.00	0.00	NA	0.000429	0.0295	NA
0-6 h	0.00	0.00	NA	0.0940	0.0295	NA
0-12 h	0.0968	10.4	NA	0.234	0.871	NA
0-24 h	0.247	40.4	0.0367	0.402	30.1	0.0601
0-48 h	0.336	76.2	0.139	0.515	59.4	0.0963
0-72 h	0.368	81.9	0.152	0.564	71.0	0.113
0-96 h	0.388	83.8	0.173	0.590	76.0	0.127
0-120 h	0.401	84.6	NA	0.612	78.0	NA
Additional Information: In male dogs, following an oral or IV dose of [^{14}C]entrectinib, fecal elimination was the predominant elimination pathway for drug-derived radioactivity.						

NA: Not applicable.

2.6.5.14　薬物動態試験：胆汁中排泄

該当なし

2.6.5.15　薬物動態試験：薬物相互作用

表 2.6.5.15-1　ヒト肝ミクロソームを用いたヒト CYP 代謝酵素に対する entrectinib の阻害作用

<table>
<tr><td colspan="5"></td><td colspan="3">Test Article: Entrectinib</td></tr>
<tr><td colspan="5"></td><td colspan="3">Location in CTD: 4.2.2.6-1</td></tr>
<tr><td colspan="5"></td><td colspan="3">Study No. 0205-[redacted] (1087287)</td></tr>
<tr><td colspan="8">Type of Study: To predict the potential for drug-drug interactions of entrectinib with co-administered drugs in vivo by evaluating the capacity of entrectinib to inhibit selected human cytochrome P450 (CYP) enzymes in vitro using CYP specific probe substrates.</td></tr>
<tr><td colspan="8">Method: The potential inhibitory effect of entrectinib (1, 10, 100 µmol/L) was investigated against human liver microsomes for CYP1A2, CYP2C8, CYP2C19, CYP2D6, and CYP3A4. Recombinant heterologously expressed P450 enzyme was used to study the inhibition of entrectinib on CYP2C9. The respective probe substrate used to determine potential inhibition were tacrine (CYP1A2), paclitaxel (CYP2C8), diclofenac (CYP2C9), S-mephenytoin (CYP2C19), bufuralol (CYP2D6), testosterone, and midazolam (CYP3A4).</td></tr>
<tr><td colspan="8">Liver microsome</td></tr>
<tr><td>Matrix:</td><td>CYP1A2</td><td>CYP2C8</td><td>CYP2C19</td><td>CYP2D6</td><td>CYP3A4 (Midazolam)</td><td>CYP3A4 (Testosterone)</td><td></td></tr>
<tr><td>IC_{50} (µmol/L):</td><td>91.9</td><td>31.1</td><td>> 100</td><td>5.6</td><td>6.3</td><td>2.8</td><td></td></tr>
<tr><td colspan="8">Recombinant heterologously expressed P450 enzyme</td></tr>
<tr><td>Matrix:</td><td>CYP2C9</td><td>CYP2D6</td><td>CYP3A4 (Testosterone)</td><td colspan="4" rowspan="2"></td></tr>
<tr><td>IC_{50} (µmol/L):</td><td>5.5</td><td>2.6</td><td>5.1</td></tr>
<tr><td colspan="8">Additional Information: The in vitro inhibition data suggests that entrectinib is able to inhibit CYP2C9, CYP2D6 and CYP3A4 isoforms at µmol/L concentrations, while the activities of other CYP isoforms were either slightly or not affected. Therefore, depending on the level of exposure reached in humans, entrectinib may give rise to clinical drug-drug interaction with drugs that are mainly metabolized by CYP2C9, CYP2D6 and CYP3A4.</td></tr>
</table>

表 2.6.5.15-2　ヒト肝ミクロソームを用いたヒト CYP 代謝酵素に対する entrectinib の時間依存的阻害作用

					Test Article: Entrectinib		
					Location in CTD: 4.2.2.6-2		
					Study No. 0370-[redacted] (1087295)		
Type of Study: The purpose of this study was to investigate *in vitro*, the potential Time-Dependent Inhibition (TDI) of entrectinib towards the major isoforms of human cytochrome P450 (CYP450): CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4.							
Method: At 50 µmol/L, the potential TDI effect of entrectinib was investigated in human liver microsomes (1 mg protein/mL) with 30 min preincubation. The respective probe substrate used to determine potential TDI were tacrine (CYP1A2), bupropion (CYP2B6), paclitaxel (CYP2C8), diclofenac (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6), and midazolam (CYP3A4).							
Matrix:	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
% of Activity Loss:	14.8	23.5	-3.3	2.7	-7.4	-1.2	10.9
Additional Information: At 50 µmol/L, entrectinib showed low potential risk to cause time dependent inhibition, with an activity loss value, close to the fixed threshold (25%) only for CYP2B6 (23.5%).							

表 2.6.5.15-3　ヒト肝ミクロソームを用いたヒト CYP2B6代謝酵素に対する entrectinib の阻害作用

				Test Article: Entrectinib	
				Location in CTD: 4.2.2.6-3	
				Study No. SR-[redacted]-018 (1087300)	
Type of Study: The purpose of this study was to investigate *in vitro*, the potential inhibition of entrectinib towards CYP2B6.					
Method: The potential inhibition effect of entrectinib (0.025-25 µmol/L) on CYP2B6 was investigated against human liver microsomes. The probe substrate used for CYP2B6 was efavirenz.					
Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition	
0-min preincubation		30-min preincubation without NADPH		30-min preincubation with NADPH	
IC_{50} (µmol/L)	Inhibition observed at 25 µmol/L (%) [2]	IC_{50} (µmol/L)	Inhibition observed at 25 µmol/L (%)	IC_{50} (µmol/L)	Inhibition observed at 25 µmol/L (%)
>25	NA	>25	NA	>25	27
Additional Information: Entrectinib appeared to cause little to no direct or time-dependent inhibition of CYP2B6; however, there was some evidence of potential metabolism-dependent inhibition. After a 30-min preincubation with pooled human liver microsomes in the presence of NADPH, CYP2B6 exhibited an increase in inhibition of approximately 27% by entrectinib at 25 µmol/L when compared to the 30-min preincubation in absence of NADPH, however, the IC_{50} value was greater than 25 µmol/L.					

NA: Not applicable;　NADPH: β-Nicotinamide adenine dinucleotide phosphate, reduced form.

表 2.6.5.15-4　ヒト肝ミクロソームを用いたヒト CYP 代謝酵素に対する entrectinib の阻害作用（追加）

	Test Article: Entrectinib
	Location in CTD: 4.2.2.6-11
	Study No. 1089383
Type of Study: The objective of this study was to evaluate the ability of entrectinib to reversibly inhibit the metabolism of isoform-selective probe substrates of CYPs. In addition, the ability of entrectinib to inhibit the CYPs in a time-dependent (NADPH-independent) manner or time- plus NADPH-dependent manner was also examined.	
Method: The potential inhibitory effect of entrectinib was investigated against human liver microsomes for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Seven concentrations of entrectinib (0.00729, 0.0243, 0.081, 0.27, 0.9, 3 and 10 µmol/L) and a no compound control were incubated in triplicate with HLM, a Km concentration of CYP probe substrate, and 1 mmol/L NADPH for the required incubation time in 100 mmol/L sodium phosphate buffer, pH 7.4. Incubations under the same experimental conditions were also performed with a 30 minute pre-incubation step in the absence or presence of NADPH to assess for possible time-dependent (NADPH-independent) inhibition or time- plus NADPH-dependent inhibition.	

表 2.6.5.15-4 ヒト肝ミクロソームを用いたヒト CYP 代謝酵素に対する entrectinib の阻害作用（追加）（続）

Enzyme (probe substrate)	Incubation Time /Protein Conc	Reversible Inhibition (μmol/L ± SE)	Reversible Inhibition: % Activity remaining at highest test concentration (mean ±SD)	Time-dependent (NADPH-independent) inhibition (μmol/L ± SE)	Signal[a]	Time- plus NADPH-dependent inhibition (μmol/L ± SE)	Signal[a]
CYP1A2 (phenacetin)	20 min 0.125 mg/mL	> 10	110.1 ± 2.5	> 10	No	> 10	No
CYP2B6 (efavirenz)	30 min 0.125 mg/mL	> 10	98.1 ± 5.0	> 10	No	> 10	No
CYP2C8 (amodiaquine)	5 min 0.125 mg/mL	> 10	44.2 ± 5.3	9.31 ± 1.41	No	> 10	No
CYP2C9 (diclofenac)	10 min 0.125 mg/mL	> 10	61.9 ± 0.6	> 10	No	> 10	No
CYP2C19 (S-mephenytoin)	30 min 0.25 mg/mL	> 10	85.8 ± 3.3	> 10	No	> 10	No
CYP2D6 (R-bufuralol)	15 min 0.1 mg/mL	> 10	49.6 ± 3.2	8.23 ± 1.18	No	> 10	No
CYP3A4 (midazolam)	2 min 0.1 mg/mL	> 10	51.5 ± 3.7	> 10	No	7.66 ± 0.69	No
CYP3A4 (testosterone)	10 min 0.05 mg/mL	2.04 ± 0.11	9.2 ± 1.0	2.58 ± 0.21	No	1.28 ± 0.23	Yes
Additional Information: Entrectinib inhibited the activity of CYP3A4, using testosterone as the probe substrate, with an IC_{50} value of 2.04 μmol/L, but >10 μmol/L for other CYPs. Entrectinib exhibited a signal for time- plus NADPH-dependent inhibition of CYP3A4, with testosterone as the probe substrate, based on a 2.01-fold decrease to a lower IC_{50}.							

[a] A signal for either type of time-dependent inhibition was determined by a >50% decrease in IC_{50}. If IC_{50} values could not be determined, a signal was determined by a decrease in the % remaining activity to <75% of the % remaining activity in the comparator curve.

表 2.6.5.15-5　ヒト肝ミクロソームを用いたヒト CYP 代謝酵素に対する M5の阻害作用

				Test Article: M5		
				Location in CTD: 4.2.2.6-4		
				Study No. SR-[redacted]-023 (1087289)		
Type of Study: To predict the potential for drug-drug interactions of metabolite M5 with co-administered drugs *in vivo* by evaluating the capacity of M5 to inhibit selected human cytochrome P450 (CYP) enzymes *in vitro* using CYP specific probe substrates.						
Method: The potential inhibitory effect of M5 was investigated against human liver microsomes for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A. The respective probe substrate used to determine potential inhibition were phenacetin (CYP1A2), efavirenz (CYP2B6), amodiaquine (CYP2C8), diclofenac (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6), testosterone and midazolam (CYP3A4/5).						
Matrix	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition	
	0-min preincubation		30-min preincubation without NADPH		30-min preincubation with NADPH	
	IC_{50} (µmol/L) [1]	Inhibition observed at 25 µmol/L (%) [2]	IC_{50} (µmol/L) [1]	Inhibition observed at 25 µmol/L (%) [2]	IC_{50} (µmol/L) [1]	Inhibition observed at 25 µmol/L (%)[2]
CYP1A2	>25	7.9	>25	9.6	>25	39
CYP2B6	17	66	16	63	15	70
CYP2C8	4.9	100	3.9	100	3.7	100
CYP2C9	>25	35	>25	29	>25	36
CYP2C19	>25	34	>25	37	>25	42
CYP2D6	21	60	21	60	20	62
CYP3A4/5 (Midazolam)	15	68	19	66	20	58
CYP3A4/5 (Testosterone)	12	72	18	66	13	74
Additional Information: M5 directly inhibited CYP2B6, CYP2C8, CYP2D6 and CYP3A4/5 (as measured by midazolam 1'-hydroxylation and testosterone 6β-hydroxylation) with IC_{50} values of 17, 4.9, 21, 15 and 12 µM, respectively. M5 appeared to cause some potential metabolism-dependent inhibition of CYP1A2 with an increase in inhibition of approximately 29% after M5 (i.e., 25 µM) was preincubated with pooled human liver microsomes in the presence of NADPH for 30 min.						

[1] Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50}

[2] Inhibition observed(%) is calculated with the following formula (results are rounded to two significant figures): Inhibition observed (%) = 100% - Percent solvent control.

表 2.6.5.15-6　ヒト肝細胞を用いたヒト CYP 代謝酵素に対する entrectinib の誘導作用

		Test Article: Entrectinib	
		Location in CTD: 4.2.2.6-5	
		Study No. 0248-[redacted] (1087293)	
Type of Study: To predict the potential for drug-drug interactions of entrectinib with co-administered drugs *in vivo* by evaluating the capacity of entrectinib to induce selected human cytochrome P450 (CYP) enzymes *in vitro* using cryopreserved human hepatocytes.			
Method: Human hepatocytes (1 donor) were incubated for 24 hours with entrectinib at 3 different concentrations (1, 10, and 100 µmol/L）and CYP mRNA expression was compared to vehicle treated samples.			
Analysis		Entrectinib concentration (µmol/L)	
		1	10
CYP1A2	Fold change on mRNA expression level	No significant change	7
CYP2B6	Fold change on mRNA expression level	No significant change	No significant change
CYP3A4	Fold change on mRNA expression level	2.1	29
Additional Information: The investigation of the induction potency of entrectinib failed at 100 µmol/L, probably due to the toxicity of the compound at the highest concentration.			

表 2.6.5.15-7　ヒト肝細胞を用いたヒト CYP 代謝酵素に対する entrectinib の誘導作用

	Test Article: Entrectinib
	Location in CTD: 4.2.2.6-6
	Study No. SR-[redacted]-017 (1087299)

Type of Study: To predict the potential for drug-drug interactions of entrectinib with co-administered drugs *in vivo* by evaluating the capacity of entrectinib to induce selected human cytochrome P450 (CYP) enzymes *in vitro* using cryopreserved primary human hepatocytes.

Method: Three preparations of cultured human hepatocytes from 3 separate donors were treated once daily for 3 consecutive days with DMSO (0.1% v/v, vehicle control), flumazenil (25 µmol/L, negative control), one of six concentrations of entrectinib (1, 3, 10, 30, 60 or 100 µmol/L) or one of two known human CYP enzyme inducers: omeprazole (50 µmol/L) for CYP1A2, rifampin (20 µmol/L) for CYP2C8, CYP2C9, CYP2C19 and CYP3A4.

Analysis		Entrectinib concentration (µmol/L)		
		1	3	10
CYP1A2 Activity	Fold change	1.15± 0.07	1.02± 0.22	0.676 ± 0.243
	Percent positive control ± Standard deviation	0.313 ±0.107	0.00537 ± 0.39795	- 0.749 ± 0.634
CYP1A2 mRNA	Fold change	1.13± 0.12	1.34± 0.20	1.42± 0.47
	Percent positive control ± Standard deviation	0.227 ± 0.155	0.692 ± 0.434	0.886 ± 0.865
CYP2C8 mRNA	Fold change	1.43 ± 0.26	2.08 ± 0.62	4.24 ± 1.48
	Percent positive control ± Standard deviation	11.7± 5.9	30.8 ± 16.2	90.2±28.6
CYP2C9 mRNA	Fold change	1.28± 0.12	1.87 ± 0.29	3.46 ± 0.62
	Percent positive control ± Standard deviation	12.3 ± 3.0	38.3 ± 7.7	109 ± 9
CYP2C19 Activity	Fold change	1.09± 0.23	1.07± 0.50	0.614 (N =2)
	Percent positive control ± Standard deviation	- 0.166 ± 1.852	-2.62 ± 5.36	-11.2 (N = 2)
CYP3A4/5 Activity	Fold change	1.33± 0.09	1.57± 0.36	1.72 ± 0.59
	Percent positive control ± Standard deviation	3.87 ±0.31	6.34 ± 3.42	7.85 ±5.09
CYP3A4 mRNA	Fold change	1.57 ±0.38	2.63 ± 1.07	7.22± 3.61
	Percent positive control ± Standard deviation	4.60 ± 3.65	12.5 ± 11.0	47.9 ± 38.3

Additional Information: Due to observed cytotoxicity, the 30, 60 and 100 µmol/L treatment groups were excluded from the evaluation of CYP induction for both mRNA and enzyme activities.

Values in the table are the average of 3 individual cultures for the specified analysis endpoint unless otherwise specified (n = 2).

表 2.6.5.15-8　ヒト肝細胞を用いたヒト CYP 代謝酵素に対する M5の誘導作用

		Test Article: M5			
		Location in CTD: 4.2.2.6-7			
		Study No. SR-[redacted]-022 (1087288)			
Type of Study: To predict the potential for drug-drug interactions of M5 with co-administered drugs *in vivo* by evaluating the capacity of M5 to induce selected human cytochrome P450 (CYP) enzymes *in vitro* using cryopreserved primary human hepatocytes.					
Method: Three preparations of cultured human hepatocytes from 3 separate donors were treated once daily for 3 consecutive days with acidified methanol (45 mM HCl) with DMSO (0.1% v/v, vehicle control), flumazenil (25 µmol/L, negative control), one of six concentrations of M5 (0.1, 0.5, 2.5, 10, 25, or 50 µmol/L) or one of three known human CYP enzyme inducers: omeprazole (50 µmol/L) for CYP1A2, phenobarbital（750 µmol/L) for CYP2B6, and rifampin (20 µmol/L) for CYP2C19 and CYP3A4.					
Analysis		M5 concentration (µmol/L)			
		0.1	0.5	2.5	10
CYP1A2 Activity	Fold change	1.08 ± 0.16	1.19 ± 0.19	1.43 ± 0.39	1.17 ± 0.09
	Percent positive control ± Standard deviation	0.274 ± 0.496	0.575 ± 0.657	1.30 ± 1.40	0.445 ± 0.272
CYP1A2 mRNA	Fold change	1.02 ± 0.10	1.10 ± 0.09	1.59 ± 0.32	1.92 ± 0.24
	Percent positive control ± Standard deviation	0.0748 ± 0.2222	0.220 ± 0.231	1.22 ± 0.87	1.70 ± 0.15
CYP2B6 Activity	Fold change	1.11 ± 0.14	1.16 ± 0.19	1.54 ± 0.72	1.46 ± 0.44
	Percent positive control ± Standard deviation	2.11 ± 2.38	2.98 ± 2.91	9.02 ± 8.75	9.56 ± 8.52
CYP2B6 mRNA	Fold change	1.15 ± 0.17	1.22 ± 0.24	2.09 ± 0.63	2.50 ± 0.25
	Percent positive control ± Standard deviation	2.89 ± 3.03	4.22 ± 4.37	19.3 ± 11.4	26.5 ± 10.7
CYP2C19 Activity	Fold change	0.943 ± 0.067	0.945 ± 0.028	0.901 ± 0.199	0.639 (n = 2)
	Percent positive control ± Standard deviation	- 0.599 ± 1.274	- 1.21 ± 0.94	-3.22 ± 3.41	-9.87 (n = 2)
CYP3A4/5 Activity	Fold change	1.10 ± 0.09	1.12 ± 0.11	1.45 ± 0.32	1.53 ± 0.45
	Percent positive control ± Standard deviation	1.05 ± 0.91	1.25 ± 1.17	4.61 ± 3.06	5.42 ± 4.45
CYP3A4 mRNA	Fold change	1.14 ± 0.19	1.22 ± 0.19	2.14 ± 0.59	3.98 ± 0.89
	Percent positive control ± Standard deviation	0.618 ± 1.362	1.16 ± 1.47	5.90 ± 5.01	16.5 ± 11.2
Additional Information: Due to observed cytotoxicity, results from the 25 and 50 µmol/L treatment groups were excluded from the evaluation of CYP induction potential by M5.					

表 2.6.5.15-9 Caco-2単層膜透過性

	Test Article: Entrectinib
	Location in CTD: 4.2.2.2-1
	Study No. SR-[redacted]-012 (1087481)
Type of Study: To evaluate the Caco-2 cell monolayer tolerability, solubility in the permeability assay buffer, and bidirectional permeability of entrectinib.	
Method: Assays were performed with six replicates (n=6) in each direction. First, the Caco-2 monolayers were pre-incubated with buffer, with or without 10 µmol/L cyclosporine A (CsA) on both sides, for 30 min, followed by a further pre-incubation (with or without CsA on both sides) with test article on the donor side for10 min. Dosing solution for the permeability assessment contained entrectinib at 0.10 µmol/L, minoxidil at 10 µmol/L, and atenolol at 100 µmol/L, and samples were analyzed for entrectinib, minoxidil, and atenolol. The assay buffer was HBSSg, pH 7.4 on the apical and basolateral sides.	

Analyte	Conc. (µmol/L)	CsA (10 µmol/L)	A-to-B (mean ± SD, n=6)		B-to-A (mean ± SD, n=6)		Efflux Ratio[c]
			P_{app}[a] (10^{-6} cm/s)	Recovery (%)	P_{app}[b] (10^{-6} cm/s)	Recovery (%)	
Entrectinib	0.1	–[f]	0.838[d] ± 0.292	43.6 ± 5.38	3.54 ± 0.523	30.7 ± 0.973	4.22
		+	1.07[e] ± 0.108	32.3 ± 1.55	0.862 ± 0.175	50.5 ± 3.85	0.808
Minoxidil	10	–	5.67 ± 0.215	97.7 ± 7.30	NA	NA	NA
		+	5.25 ± 0.499	92.5 ± 1.48	NA	NA	NA
Atenolol	100	–	0.244 ± 0.0672	90.9 ± 7.29	NA	NA	NA
		+	0.191[g] ± 0.0603	106 ± 1.42	NA	NA	NA
Additional Information: Under the conditions of this study, entrectinib cannot be classified as highly permeable, as its mean A-to-B P_{app} was lower than that of the co-dosed high permeability reference compound minoxidil, both in the absence and presence of CsA, an inhibitor of drug transporters. The results suggest that entrectinib may be a substrate of an apical efflux transporter such as P-glycoprotein.							

[a] Minoxidil P_{app} / atenolol $P_{app} > 3$ for each replicate.
[b] PELY $P_{app} < 0.8 \times 10^{-6}$ cm/s for all 3 replicates.
[c] Efflux ratio calculated using the mean P_{app} values.
[d] P_{app} calculated at 45 min, including the receiver rinse concentration, is $2.57 \pm 0.463 \times 10^{-6}$ cm/s.
[e] P_{app} calculated at 45 min, including the receiver rinse concentration, is $2.95 \pm 1.07 \times 10^{-6}$ cm/s. P_{app} calculated at 45 min, including receiver rinse concentration and using donor zero concentration rather than nominal, is $3.72 \pm 1.36 \times 10^{-6}$ cm/s .
[f] P_{app}, recovery for entrectinib -CsA were calculated using the initial donor concentration rather than the default method of using the nominal concentration, because the mean measured dosing concentration and mean initial donor concentration were more than 20% lower than the nominal concentration.
[g] One replicate failed linearity ($r^2<0.9$ for cumulative receiver concentration vs. time) for atenolol, and P_{app} was calculated using the last two time points for that replicate.
NA: not applicable

表 2.6.5.15-10　MDR1あるいは Mdr1a 発現細胞での entrectinib 及び M5の能動輸送並びに透過性

	Test Article: Entrectinib, M5
	Location in CTD: 4.2.2.6-12
	Study No. 1088452

Type of Study: To characterize the permeability and human and mouse P-gp substrate potential of entrectinib and M5, metabolite of entrectinib, *in vitro*.

Method: Parent porcine kidney epithelial cells (LLC-PK1) transfected with human MDR1 (L-MDR1) or mouse (L-mdr1a) were used. The inhibitor (zosuquidar, 1 µmol/L) was added to both sides. The transport experiment was performed in both directions in triplicates. The plates containing the inserts were incubated at 37°C and 5% CO_2 with continuous shaking (100 rpm). Samples were taken from the donor and the opposite (acceptor) side after 3 hours incubation. Concentrations of substrate in both compartments were determined by scintillation counting for radioactive compounds (digoxin) or by LC-MS/MS for the test compound and the non-radio-labelled control (edoxaban).

Substrate	Conc. (µmol/L)	Zosuquidar (1 µmol/L)	P_{app} (nm/s) L-MDR1 (mean ± SD, n=3)			P_{app} (nm/s) L-Mdr1a (mean ± SD, n=3)		
			A-to-B	B-to-A	ER	A-to-B	B-to-A	ER
Digoxin	0.015	−	10.9 ± 0.7	121 ± 7.3	11.1 ± 1.0	12.2 ± 1.0	125 ± 2.8	10.2 ± 0.8
		+	42.0 ± 2.3	67.0 ± 0.9	1.60 ± 0.1	41.0 ± 3.8	88.0 ± 2.0	2.20 ± 0.2
Crizotinib	1	−	13.8 ± 0.3	388 ± 17.0	28.1 ± 1.4	12.0 ± 1.5	345 ± 16.8	28.7 ± 3.8
		+	47.9 ± 10.5	52.3 ± 1.0	1.09 ± 0.2	58.6 ± 15.6	91.9 ± 3.2	1.57 ± 0.4
Entrectinib	1	−	13.5 ± 3.4	197 ± 4.5	14.6 ± 3.7	9.90 ± 0.4	256 ± 19.4	25.8 ± 2.2
		+	15.2 ± 1.9	30.0 ± 2.1	1.98 ± 0.3	14.6 ± 0.9	29.0 ± 3.2	1.99 ± 0.3
Edoxaban	1	−	11.0 ± 3.6	407 ± 8.2	37.1 ± 12.1	11.4 ± 4.3	262 ± 17.6	23.0 ± 8.8
		+	98.2 ± 5.5	162 ± 21.0	1.65 ± 0.2	86.6 ± 4.7	159 ± 9.1	1.84 ± 0.1
M5	1	−	2.70 ± 0.3	146 ± 10.0	54.0 ± 6.7	2.60 ± 0.4	102 ± 2.0	39.4 ± 6.5
		+	13.5 ± 1.2	30.4 ± 7.2	2.25 ± 0.6	12.0 ± 0.7	30.7 ± 3.6	2.56 ± 0.3

表 2.6.5.15-10 MDR1あるいは Mdr1a 発現細胞での entrectinib 及び M5の能動輸送並びに透過性（続）

Summary of *in vitro* bidirectional permeability and P-gp efflux potential			
Substrates	Inhibitors	Results	Comment
Apparent permeability across LLCPK1-MDR1/Mdr1a cells in presence of inhibitor			
Entrectinib (1 μmol/L)	zosuquidar (1 μmol/L)	22 nm/s	Low permeability
M5 (1 μmol/L)		20 nm/s	Low permeability
Human P-gp substrate interaction			
Entrectinib (1 μmol/L)	- + zosuquidar (1 μmol/L)	ER = 14.6 ± 3.7 Partial sensitivity	Partial substrate interaction
M5 (1 μmol/L)	- + zosuquidar (1 μmol/L)	ER = 54.0 ± 6.7 Full sensitivity	Strong substrate
Mouse P-gp substrate interaction			
Entrectinib (1 μmol/L)	- + zosuquidar (1 μmol/L)	ER = 25.8 ± 2.2 Partial sensitivity	Partial substrate interaction
M5 (1 μmol/L)	- + zosuquidar (1 μmol/L)	ER = 39.4 ± 6.5 Full sensitivity	Strong substrate
Additional Information: Results revealed that entrectinib and M5 are low permeable compounds, with an average passive permeability across LLC-PK1 monolayers of ~20 nm/s. Entrectinib displays apparent substrate interaction with P-gp (ER = 14.6 and 25.8 with human and mouse P-gp, respectively: reduced ≤ 2 with inhibitor in both species); however, entrectinib did not display sensitivity to a P-gp inhibitor on P_{app} (A-to-B) values (i.e. similar P_{app} [A-to-B] values with or without the presence of a P-gp inhibitor), in contrast to the reference P-gp substrates digoxin and crizotinib that showed sensitivity in both P_{app} (A-to-B) and P_{app} (B-to-A), in human and mouse P-gp expressing cell lines. M5 displays strong substrate interaction with human and mouse P-gp (ER= 54.0 and 39.4, respectively; reduced to ≤ 2.5 in the presence of P-gp inhibitor). Furthermore, P_{app} (A-to-B) and P_{app} (B-to-A) both displayed sensitivity to P-gp inhibitor, unlike what was observed with entrectinib.			

表 2.6.5.15-11　ヒト薬物トランスポーターに対する entrectinib の作用

		Test Article: Entrectinib
		Location in CTD: 4.2.2.6-8
		Study No. XT148029 (1087291)
Type of Study: To predict the potential for drug-drug interactions of entrectinib with co-administered drugs *in vivo* by evaluating entrectinib as an inhibitor and a substrate of human transporters.		
Method: The ability of entrectinib (0.1, 0.3, 1, 3, 10 and 30 μmol/L) to inhibit P-gp and BCRP was evaluated by measuring the bidirectional permeability of a probe substrate (digoxin or prazosin) across a monolayer of Caco-2 and MDCKII-BCRP cells in the presence of entrectinib. The ability of entrectinib (0.03, 0.1, 0.3, 1, 3, 10 and 30 μM) to inhibit OATP1Bl, OATP1B3, OCT2, OAT1, and OAT3 was evaluated by measuring the accumulation of probe substrate, estradiol-17β-glucuronide (OATP1B1 and OATP1B3)], metformin (OCT2), p-aminohippurate (OAT1), or estrone-3-sulfate (OAT3) in transporter-expressing and control HEK293 cells in the presence of entrectinib. To determine whether entrectinib (0.3, 3 and 30 μmol/L) is a substrate of P-gp and BCRP, the bidirectional permeability of entrectinib across MDCKII-MDR1 and MDCKII-BCRP cells was measured. To determine whether entrectinib (1, 5 and 25 μmol/L) is a substrate of OATP1Bl and OATP1B3, the accumulation of entrectinib (1, 5 and 25 μmol/L) in transporter-expressing and control HEK293 cells was measured.		
Transporter	Substrate	Inhibition IC_{50} (μmol/L)
P-gp	Yes	1.33
BCRP	No	3.02
OATP1Bl	No	6.46
OATP1B3	No	>30
OAT1	Not examined	>30
OAT3	Not examined	>30
OCT2	Not examined	>30
Additional Information: In the presence of entrectinib, P-gp, BCRP and OATP1B1 were inhibited with IC_{50} values of 1.33, 3.02 and 6.46 μmol/L, respectively. OATP1B3, OAT1 and OAT3 were not inhibited more than 50% in the presence of up to 30 μmol/L entrectinib 1. The efflux ratio of entrectinib (3 μmol/L) across MDCKII-MDR1 cells was 13.8 and reduced to less than 2 in the presence of the P-gp inhibitor valspodar indicating entrectinib is a substrate of P-gp (MDR1). A follow up experiment in the presence of 0.1% BSA confirmed the results. The efflux ratio of entrectinib across MDCKII-BCRP cells was less than 2 at all concentrations tested (0.3, 3 and 30 μmol/L) and not affected by the BCRP inhibitor Ko143 suggesting entrectinib is not a substrate of BCRP. The accumulation of entrectinib (1, 5 and 25 μmol/L) was similar in OATP1B1 and control cells and in OATP1B3 and control cells and not affected by the OATP1B1 inhibitor rifampin suggesting entrectinib is not a substrate of OATP1B1 or OATP1B3.		

BCRP: Breast cancer resistance protein; Caco-2 cell: Continuous cell of heterogeneous human epithelial colorectal adenocarcinoma cells;
MDCKII: Madin- Darby canine kidney II; MDR1: Multi drug resistance 1; OAT: Orgamc anion transporter;
OATP: Organic anion-transportingpolypeptide; OCT: Organic cationtrasporter; P-gp: P-glycoprotein

表 2.6.5.15-12　ヒト OAPT1B1に対する entrectinib の阻害作用

	Test Article: Entrectinib
	Location in CTD: 4.2.2.6-13
	Study No. 1090527
Type of Study: To determine the *in vitro* inhibitory profile of entrectinib against OATP1B1 using probe substrates pitavastatin and rosuvastatin.	
Method: The ability of entrectinib (0.04, 0.12, 0.37, 1.10, 3.30, 10, 30 µmol/L) to inhibit OATP1B1 was evaluated by using HEK-OATP1B1 and HEK-vector control TransportoCells™ cells. For inhibition experiments the net uptake for each condition was calculated by subtracting the mean response (triplicate wells) in HEK-empty vector control cells from the mean response (triplicate wells) in HEK-OATP1B1 cells.	

Probe substrate	Inhibition IC_{50} (µmol/L)
Rosuvastatin (1.0 µmol/L)	3.89
Pitavastatin (0.2 µmol/L)	4.22

表 2.6.5.15-13　ヒト薬物トランスポーターに対する entrectinib 及び M5の作用

	Test Article: Entrectinib, M5
	Location in CTD: 4.2.2.6-10
	Study No. SR-[redacted]-023 (1087486)
Type of Study: To predict the potential for drug-drug interactions of entrectinib and/or M5 via inhibition of human transporters.	
Method: Inhibition potential of M5 (0.1, 0.3, 1, 3, 10 and 30 μmol/L) was evaluated for the following transporters (substrates): MATE1, MATE2-K, OCT2 (metformin), OAT1, OAT3 (p-aminohippurate), OCT1 (MPP+), OATP1Bl (estradiol-17β-D-glucuronide), OATP1B3 (CCK-8), P-gp (quinidine), BCRP (prazosin), and BSEP(taurocholate). MATE1, MATE2-K, OCT1, uptake test system was a polarized monolayer of MDCK-II cells treated to express the transporter or a control vector. For BCRP, the experimental system was a polarized monolayer of MDCK-II cells treated to express BCRP. The test system for P-gp transport was a polarized monolayer of MDCK-MDR1 cells. For BSEP, the experimental system was comprised of Sf9 membrane vesicles containing human BSEP. Inhibition potential of entrectinib across the same concentration range was evaluated for OCT1, BSEP, MATE1, and MATE2-K using the same methodology.	

Transporter	Entrectinib Inhibition IC_{50} (μmol/L)	M5 Inhibition IC_{50} (μmol/L)
BCRP	Not examined	8.35
P-gp	Not examined	10.1
OAT1	Not examined	>30
OAT3	Not examined	>30
OCT2	Not examined	>30
OATP1B1	Not examined	>30
OATP1B3	Not examined	>30
OCT1	> 30	>30
BSEP	13.3	>30
MATE1	1.10	0.642
MATE2-K	19.4	3.14

BCRP: Breast cancer resistance protein; MDCKII: Madin-Darby canine kidney II; MDR1: Multi drug resistance l; OAT: Organic anion transporter;
OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter; P-gp: P-glycoprotein; BSEP: Bile salt export pump;
MATE: Multi-drug and toxic compound extrusion transporter

表 2.6.5.15-14　ヒト薬物トランスポーターに対する M5の作用

			Test Article: M5		
			Location in CTD: 4.2.2.6-9		
			Study No. SR-[redacted]-016 (1087292)		
Type of Study: To predict the potential for drug-drug interactions of entrectinib with co-administered drugs *in vivo* by evaluating M5 as a substrate of human transporters.					
Method: The uptake test system for the transporters represented by the SLC family, OCT1, OATP1B1 and OATP1B3 was a polarized monolayer of MDCK-II treated to express the transporter of interest or treated with a control vector. For BCRP, the experimental system was a polarized monolayer of MDCK-II cells treated to express BCRP, no control cells were used for BCRP. The test system for P-gp transport was a polarized monolayer of MDCK-MDR1 cells. The transport of M5 (0.15, 1.5 and 15 μmol/L) was determined by LC-MS/MS.					
Transporter	P-gp	BCRP	OATP1B1	OATP1B3	OCT1
M5 determined to be a substrate	Yes	Yes	No	No	No

BCRP: Breast cancer resistance protein; MDCKII: din-Darby canine kidney II; MDR1 Multi drug resistance 1;
OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter; P-gp: P-glycoprotein.

2.6.5.16　薬物動態試験：その他

表 2.6.5.16-1　結晶形 Form Ⅰ及び Form Ⅱ の PK 比較

	Test Article: Entrectinib			
	Location in CTD: 4.2.2.7-1			
	Study No. 0275-[redacted] (1087279)			
Species	Dog (Beagle)			
Gender (M/F)/Number of Animals:	4 M			
Feeding Condition:	Not specified			
Vehicle/Formulation:	0.5% Methylcellulose 400 cP solution			
Method of Administration:	PO			
Sample (eg, whole blood, plasma, serum):	Plasma			
Analyte:	Entrectinib			
Assay:	LC-MS/MS			
Dose (mg/kg)	60			
Polymorphic Form	I		II	
PK parameters	Mean	Standard Deviation	Mean	Standard Deviation
T_{max} (h)	2.75	1.50	3.00	1.15
C_{max} (μmol/L)	2.27	0.49	2.65	1.15
AUC_{inf} (μmol/L·h)	40.3	7.94	39.4	20.9
$t_{1/2}$ (h)	8.58	2.30	6.85	1.66
Additional Information: Overall, no differences in C_{max} and AUC as well as in T_{max} and $t_{1/2}$ were evident between the two polymorphs.				

表 2.6.5.16-2　剤型及び食餌の影響

	Test Article: Entrectinib		
	Location in CTD: 4.2.2.7-2		
	Study No. 0303-[redacted] (1087280)		
Species	Dog (Beagle)		
Gender (M/F)/Number of Animals:	6 F/test period		
Feeding Condition:	Fasted (overnight through 4 hours postdose) & Fed		
Vehicle/Formulation:	0.5% Methylcellulose 400 cP solution		
Method of Administration:	PO		
Sample (eg, whole blood, plasma, serum):	Plasma		
Analyte:	Entrectinib		
Assay:	LC-MS/MS		
Dose (mg/kg)	60 (60 for suspension, 52–72 for capsule, 48–70 for tablet)		
Feeding Condition:	Fasted		
Formulation:	Suspension	Capsule	Tablet
PK parameters	Mean ± SD	Mean ± SD	Mean ± SD
T_{max} (h)	8.83 ±11.8	5.33 ± 9.16	12.7 ± 12.4
C_{max}/dose (µmol/L/mg/kg)	20.6 ± 16.9	23.5 ± 14.5	15.2 ± 8.68
AUC_{last}/dose (µmol/L·h/mg/kg)	295 ± 187	298 ± 162	261 ± 130
$t_{1/2}$ (h)	7.16 ± 1.57 [1]	6.94 ± 0.798[1]	9.53 ± 2.47
AUC_{inf}/dose (µmol/L·h/mg/kg)	330 ± 186[1]	353 ± 102[1]	263 ± 128
Feeding Condition:	Fed		
Formulation:	Capsule	Tablet	
PK parameters			
T_{max} (h)	3.67 ± 0.816	4.17 ± 3.13	
C_{max}/dose (µmol/L/mg/kg)	47.4 ± 12.6	45.9 ± 4.56	
AUC_{last}/dose (µmol/L·h/mg/kg)	603 ± 173	706 ± 141	
$t_{1/2}$ (h)	9.89 ± 2.90	9.61 ± 1.84	
AUC_{inf}/dose (µmol/L·h/mg/kg)	604 ± 173	708 ± 141	
Additional Information: Overall, the pharmacokinetic data indicated that, either with capsules or tablet, the presence of food increased the systemic exposure to entrectinib and markedly reduced the variability of the systemic exposure to the compound.			

[1] N = 5,　%CV: Coefficient of variation of the mean as percent; SD: Standard deviation.

表 2.6.5.16-3　食餌の影響

	Test Article: Entrectinib	
	Location in CTD: 4.2.2.7-3	
	Study No. [redacted]-0054 (1087281)	
Species	Dog (Beagle)	
Gender (M/F)/Number of Animals:	6 F/ feeding condition	
Feeding Condition:	Fasted (overnight through 4 hours post-dose) & Fed	
Vehicle/Formulation:	Capsule	
Method of Administration:	PO	
Sample (eg, whole blood, plasma, serum):	Plasma	
Analyte:	Entrectinib	
Assay:	LC-MS/MS	
Dose (mg/kg)	60	
Feeding Condition:	Fasted	Fed
PK Parameters	Mean ± SD	Mean ± SD
T_{max} (h)	5.83 ± 8.95	4.83 ± 2.71
C_{max}/dose (μmol/L/mg/kg)	44.1 ± 19.6	55.1 ± 18.9
AUC_{last} /dose (μmol/L·h/mg/kg)	857 ± 309	831 ± 304
$t_{1/2}$ (h)	7.84 ± 3.37	6.02 ± 1.24
AUC_{inf} /dose (μmol/L·h/mg/kg)	860 ± 308	831 ± 304
Additional Information: Fed/fasting ratios of normalized C_{max} and AUC_{1ast} were 1.40 and 1.17, respectively; suggesting a similar systemic exposure in dogs under fasted and fed conditions.		