Nonclinical Overview	
Drug Substance	Ticagrelor (AZD6140)
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2.4 Nonclinical Overview

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2.4. NONCLINICAL OVERVIEW

2.4.1 Overview of the Nonclinical Testing Strategy

A comprehensive nonclinical package has been developed with ticagrelor, in order to understand its pharmacology, safety pharmacology, pharmacokinetics, toxicokinetics, metabolism, distribution, excretion, and safety.

The pharmacological properties of ticagrelor were investigated relative to its ability to block platelet aggregation both *in vitro*, in human and rat washed platelets, human platelet rich plasma and human whole blood, and *in vivo* in dog models using platelet aggregation and thrombosis as endpoints. Ticagrelor was also examined for interaction with a comprehensive set of enzymes and receptors for potential for activity unrelated to its inhibition of platelet aggregation through the $P2Y_{12}$ receptor. It was also evaluated in a standard battery of safety pharmacology studies in rats and dogs, and in human receptor-expressing hamster cells, to test for unwanted effects on the gastrointestinal, cardiovascular, respiratory, renal and central nervous organ systems. These studies demonstrate that ticagrelor is a potent, selective, orally active, direct $P2Y_{12}$ receptor antagonist, which produces reversible, concentration (dose)-related inhibition of ADP-induced platelet aggregation.

A series of studies were completed to investigate the absorption, distribution, metabolism and excretion of ticagrelor in the same species, and in most cases the same strains, used to establish its nonclinical pharmacologic and toxicologic profiles (eg., mice, rats, dogs and marmosets). Sensitive and specific analytical methods based on reversed phase liquid-chromatography and single mass spectrometry (LC-MS) were developed to analyze for ticagrelor and the metabolites AR-C124910XX and AR-C133913XX in fluids from various species. Isotopically labelled internal standards for ticagrelor and tandem mass spectrometry (LC-MS-MS) were introduced. The different methods were precise and accurate, performed consistently and the assay characteristics were proven adequate for all applications, including the GLP toxicokinetics analyses.

The toxicology of ticagrelor was well characterized through a program of *in vivo* and *in vitro* non-clinical studies conducted to support the chronic administration of ticagrelor to humans, which included single and/or repeat-dose toxicology studies in mice, rats, non-pregnant rabbits and/or marmosets, and several of the studies included recovery groups. Ticagrelor has been tested for genotoxicity in the Ames test, the mouse lymphoma assay, and in the rat micronucleus test. The active metabolite, AR-C124910XX, has been tested in the Ames test and in the mouse lymphoma assay. Ticagrelor has been tested for carcinogenic potential in lifetime studies in mice and rats. A series of *in vivo* and *in vitro* studies were conducted to investigate the mechanism of the increased incidence of uterine tumours seen in rats.

Pivotal reproduction toxicity studies have been conducted with ticagrelor, consisting of male and female rat fertility studies where male fertility was assessed as part of the 6 month toxicity

study, embryofetal development studies in rats and rabbits plus a follow up embryofetal development study in the rabbit and a pre-postnatal study in rats. The embryofetal development studies and pre-postnatal studies were supported by preceding dose range-finding studies in which exposure was assessed.

A series of genetic toxicity tests were conducted using key intermediates and potential impurities in the synthetic pathway for ticagrelor to establish their safety and provide the foundation for setting of specifications for the final drug product. Because of the short half life and absence of accumulation in skin and eye, no further phototoxicity testing was done.

The pivotal toxicology studies were appropriately designed and conducted in a manner consistent with ICH guidelines, as were the safety pharmacology studies and the supporting toxicokinetics studies. The studies were conducted under GLP where appropriate, as described in the individual pharmacology, toxicokinetic and toxicology sections of this submission, the tabular summaries and in the individual reports. The doses used were selected based on information gathered as the program developed. Exposures were determined through toxicokinetic evaluations, to ensure that adequate high doses were evaluated to establish the toxicity profile.

2.4.2 Pharmacology

2.4.2.1 Pharmacology Related to the Proposed Indication

Platelets play a central role in the thrombosis occurring after atherosclerotic plaque rupture, which is the pathological aetiology of most acute coronary syndromes (ACS). Vascular injury results in the activation of platelets, causing aggregation, the release of their granular contents, expression of negatively charged phospholipids and the activation of the coagulation cascade. Platelets express three types of purinergic P2 receptors on their surface, the $P2X_1$ receptor, activated by ATP, and the $P2Y_1$ and $P2Y_{12}$ receptors, activated by ADP. ADP-induced platelet aggregation is initiated by the $P2Y_1$ receptor and amplified and sustained in a synergistic manner by the $P2Y_{12}$ receptor. Co-activation is required for a full aggregation response. Activation of the $P2Y_{12}$ receptor also sustains aggregation and amplifies responses to other platelet activators, and blockade of this receptor also leads to increased reversibility of aggregation. As a result of this, antiplatelet therapy has become the mainstay for initial management of patients with ACS or undergoing percutaneous coronary intervention (PCI), with the $P2Y_{12}$ being a preferred target for development of antagonists.

Ticagrelor was developed as a potent, selective, orally active, direct $P2Y_{12}$ receptor antagonist. The studies presented in the pharmacology section of this document support these claims, and demonstrate that it produces a reversible, concentration (dose)-related inhibition of ADP-induced platelet aggregation. The activity *in vitro* and the rapid onset of effect observed in the dog *in vivo,* together with demonstrated reversibility of effect, distinguish ticagrelor from thienopyridine agents, which serve as pro-drugs that inhibit the $P2Y_{12}$ receptor via its active metabolites for the life-span of the platelet.

2.4.2.2 *In vitro* Pharmacology

Ticagrelor potently inhibits the binding of a specific $P2Y_{12}$-receptor radioligand ([^{125}I]AR-C98597XX) to human washed platelets (K_i value, 2.0 nM). Ticagrelor is a potent inhibitor of ADP-induced platelet aggregation in suspensions of human and rat washed platelets, in human platelet rich plasma, and in marmoset and human whole blood (Table 1).

In addition to the effects seen in platelets, ticagrelor has been shown to inhibit the $P2Y_{12}$ – mediated vasoconstriction of mouse vascular smooth muscle cells induced by 2MeS-ADP.

Table 1 IC_{50} values for the inhibition of platelet aggregation by ticagrelor in a variety of *in vitro* systems

Compound	System	IC_{50} (nM)
Ticagrelor	Human washed platelets	13
Ticagrelor	Rat washed platelets	20
Ticagrelor	Human platelet rich plasma	398
AR-C124910XX	Human platelet rich plasma	126
Ticagrelor	Marmoset whole blood	35
Ticagrelor	Human whole blood	58

2.4.2.3 *In vivo* Pharmacology

Oral administration of a single dose of ticagrelor in the conscious male Beagle produced >90% inhibition of ADP-induced platelet aggregation measured *ex vivo*. Ticagrelor, administered by 30-minute stepped iv infusion, abolished all cases of platelet-mediated thrombosis visualized as cyclic blood flow reduction (CFR) in the damaged, stenosed femoral artery of the anaesthetized dog.

In a beagle combined thrombosis/bleeding model, there was a non-statistically significant increase in the separation of the anti-thrombotic and anti-haemostatic effects for ticagrelor compared to clopidogrel. However, both agents demonstrated a significantly better separation than observed with the active metabolite of the GPIIb/IIIa antagonist orbofiban. A subsequent study to profile ticagrelor against the two thienopyridines AR-H076866XX and clopidogrel in the model confirmed the increased separation between anti-thrombotic- and bleeding- effect for ticagrelor compared to both thienopyridines.

2.4.2.4 Pharmacology of Metabolites

The O-deethylated metabolite, AR-C124910XX, has also been shown to be a potent inhibitor of specific radioligand binding to human washed platelets (K_i value, 2.0 nM) and of ADP-induced platelet aggregation in human platelet rich plasma (IC_{50} = 126 nM, Table 1).

2.4.2.5 Reversibility of $P2Y_{12}$-receptor binding

In vitro receptor protection protocol studies indicated that binding of ticagrelor to the $P2Y_{12}$-receptor is reversible but suggested a relatively slow dissociation rate. Further studies indicated a low K_{on} for ticagrelor of $0.11x10^{-3}$ $nM^{-1}*s^{-1}$ and K_{off} of $0.87x10^{-3}$ s^{-1}.

2.4.2.6 Secondary Pharmacology

The selectivity of ticagrelor for the $P2Y_{12}$ receptor was established through *in vitro* screening against a variety of receptors, enzymes and transporters. The only inhibitory effects seen with IC_{50} values less than 10 μM were phospholipase C, adenosine transporters, adenosine A_3 receptor, platelet activating factor and phosphodiesterases (PDE1-5). Ticagrelor also inhibited the human ether-a-go-go-related (hERG) gene in hERG-expressing Chinese Hamster Ovary (CHO) cells, with an IC_{50} of 1.72 μM. Ticagrelor demonstrated potent inhibition of GPR17, a G-protein coupled receptor activated by both uracil nucleotides and cysteinyl-leukotrienes, and presumed to moderate secondary inflammatory responses as shown in a rat stroke model.

Ticagrelor showed potent inhibition of adenosine uptake by human red blood cells, with an IC_{50} of 100 nM. Sodium-independent adenosine uptake was inhibited in a concentration dependent manner in dog MDCK, human MCF7 and rat H4IIE cells (IC_{50} values 34.3, 60.6, 104.2 nM respectively). Studies in the dog MDCK kidney cell line and sodium free conditions indicated that this is likely via inhibition of sodium independent nucleoside transporter ENT-1.

In a range of functional assays, ticagrelor did not show significant activity against any other P2 receptor types. Studies demonstrated that ticagrelor has a low affinity for adenosine receptors, with a weak agonistic activity at the A3 receptor as the most potent activity (K_i=0.104 μM). Ticagrelor did not significantly inhibit ADP-independent platelet aggregation in human washed platelets at a concentration of 10 μM. No inhibition of aromatase or adenosine deaminase was observed at ticagrelor concentrations (or its major metabolites) up to 10 and 20 μM, respectively, nor did it bind to the human or bovine oestrogen receptor.

2.4.2.7 Safety Pharmacology

Safety pharmacology studies were conducted in conscious adult male rats and in anesthetized adult male Beagle dogs to detect any acute undesirable pharmacodynamic effects on the central, peripheral and autonomic nervous systems and on cardiovascular, respiratory, gastrointestinal and renal systems.

Ticagrelor produced no significant adverse effects on the cardiovascular system in anesthetized dogs at single oral doses up to 100 mg/kg, or on the peripheral or central nervous systems, respiratory system or gastrointestinal system in the rat at single oral doses up to 100 mg/kg. Changes in respiratory (increased rate, decreased expiration time and renal functions (increased excretion of sodium and chloride, increased pH) at doses of 10 and 100 mg/kg were not considered to be dose limiting with regard to doses proposed for humans. There were no effects on the gastrointestinal system at 1 and 10 mg/kg, but a reduction in gastrointestinal transit time was noted at the high dose of 100 mg/kg.

2.4.2.7.1 Dyspnoea

An increased incidence of dyspnoea was observed in the clinical trials DISPERSE and DISPERSE2. Evidence of an effect on the respiratory system described above was seen in the original rat safety pharmacology study (990632P (SR-99346-01)). Therefore, the rat was used to investigate the hypothesis that the increase in dyspnoea was secondary to adenosine release from damaged cardiac tissue and activating adenosine receptors in the lung. However, dosing with ticagrelor in rats in subsequent studies did not consistently reproduce the original observations(1116SR, 1266SR, 1267SR, 1764SR, 1864SR). Administration of DPSPX, an inhibitor of adenosine receptors, showed stimulatory effects in rats, although the effects following co-administration of DPSPX and ticagrelor were inconsistent and not dose-dependent, (20040510SPC (0789SR)). Definitive conclusions related to the hypothesis could not be drawn based on the results of these studies.

Ticagrelor inhibits adenosine uptake in human mouse, rat, dog, and human erythrocytes or cell lines (1929KV; 1878KV, SC-103269). In the beagle dog (26709 (3428-01)), coronary blood flow measurements after local ischaemia by temporary left anterior descending artery (LAD) occlusion showed that both ticagrelor and the reference compound dipyridamole dose-dependently and significantly augment the adenosine-induced increase in coronary blood flow. This effect is observed both when adenosine is endogenously induced via temporary occlusion of the left anterior descending coronary artery or administered locally via a direct adenosine infusion. These dog data suggest there may be a clinical relevance for the *in vitro* inhibition of adenosine uptake by red blood cells seen in the secondary pharmacology studies.

2.4.3 Pharmacokinetics

2.4.3.1 Absorption

Ticagrelor was found to be moderately absorbed in a dose-dependent manner at lower doses in the species used for nonclinical safety testing, and in more than a dose-dependent manner at higher doses used in the toxicology studies. The *in vivo* oral bioavailability was determined in the rat and marmoset to be about 90 and 40%, respectively. No gender differences were observed in any species except in the rat, where females generally had greater exposure to ticagrelor than male rats. Ticagrelor and its active metabolite AR-C124910XX were both Pgp-substrates as well as weak inhibitors.

2.4.3.2 Distribution

Volumes of distribution at steady state were calculated to be approximately 4.8 and 3.7 L/kg in the rat and marmoset, respectively.

2.4.3.2.1 Plasma Protein Binding

Plasma protein binding of ticagrelor was high in all species tested, resulting in an unbound fraction ranging between 0.7% in mouse plasma to 1.0% in dog plasma. The unbound fraction of the metabolite AR-C124910XX ranged from 0.32% in rabbit to 2.0% in mouse plasma. These compare to the unbound fractions of ticagrelor and the metabolite AR-C124910XX in human plasma of 0.6% and 0.14%, respectively.

2.4.3.2.2 Tissue Distribution

Following oral administration of radiolabelled ticagrelor to the rat, radioactivity was widely distributed, with the highest concentrations of total radioactivity observed in the liver and kidney and in the adrenal, pituitary and thyroid glands. Very low levels of radioactivity were observed in the brain. Elimination of radiolabelled components from all tissues was relatively rapid ($t_{1/2}$ 3-10h) with no retention in any tissue including pigmented skin and eye. Following dosing with [^{14}C]-ticagrelor, radioactivity distributed to the placenta, but did not significantly transfer from the placenta to the fetus.

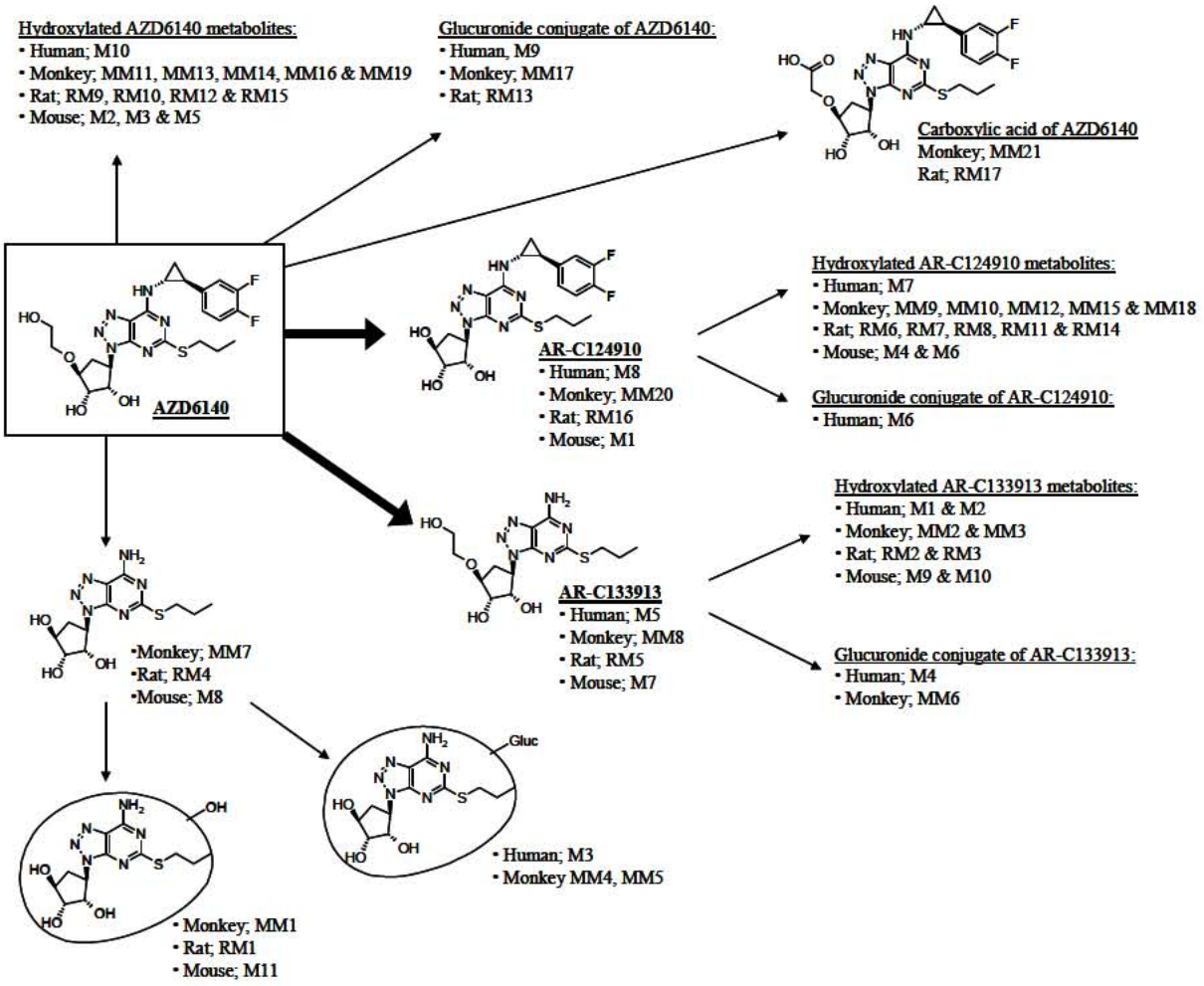
2.4.3.3 Metabolism

2.4.3.3.1 *In vivo* Metabolism

The major metabolic pathways were qualitatively similar among the species used for toxicity testing, and comparable to those seen in clinical studies. An ascending dose range-finding study was conducted in dogs, but production of the active metabolite AR-C124910XX in this species was found to be sufficiently different quantitatively from humans such that the dog was not pursued as a suitable non-rodent species for toxicity testing.

Metabolic pathways involve loss of hydroxyethyl side chain to form the active metabolite AR-C124910XX and loss of difluorophenyl-cyclopropyl group to form AR-C133913XX. Other minor metabolites detected included further oxidized metabolites or glucuronide conjugates of ticagrelor, AR-C124910XX and AR-C133913XX and in addition loss of both the hydroxyethyl side chain and the difluorophenyl-cyclopropyl group followed by hydroxylation or conjugation. A carboxylic acid of ticagrelor was also detected. The proposed *in vivo* metabolic pathways of ticagrelor are summarised in Figure 1.

Figure 1 Proposed *in vivo* metabolic pathways of ticagrelor



2.4.3.3.2 Identification of CYP enzymes involved in ticagrelor metabolism

The major CYP enzymes involved in the metabolism of ticagrelor, *in vitro,* were CYP3A4 and CYP3A5. CYP3A4 was the major enzyme responsible for the formation of AR-C124910XX and contributed as well to further metabolic elimination of AR-C124910XX. The contribution of other CYP enzymes to the metabolism of ticagrelor was less significant.

2.4.3.3.3 Enzyme induction and inhibition

In rats, ticagrelor caused induction of hepatic CYP enzymes. After 1 month treatment with 20, 80 or 300 mg/kg ticagrelor to male and female rats, increases in activities associated with hepatic CYP1A2, 2B, 2C, 2E1, 2A, 3A and 4A were observed. This enzyme induction had no observable consequence upon systemic exposure to parent and/or metabolite.

The enzyme inducing potential of ticagrelor was confirmed in a 1 month investigative study where a dose of 180 mg/kg/day produced increased levels of CYP1A1/2 and CYP4A1 in female rat livers. The induction was detected after 1 week of dosing as well as at the end of

the study. This induction property was extended in a 3 month investigative study in which a dose of 180 mg/kg/day produced increased expression of hepatic CYP1A1/2, together with increased expression of uterine CYP1A1 (protein and mRNA). No increases in levels of hepatic or uterine CYP1B1 (mRNA) were detected.

In contrast, in human hepatocytes, ticagrelor or AR-C124910XX did not produce induction of CYP1A1, 1A2 or 3A4 enzymes. Modest effects on activities associated with CYP2B6 and CYP2C9 were produced.

In human microsomes *in vitro,* ticagrelor and/or its major metabolites were moderate inhibitors of CYP2C9 and CYP2D6 activities. CYP3A5 was also moderately inhibited. CYP3A4 was activated or moderately inhibited dependent on substrate used in the assay. Ticagrelor showed no propensity to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C19 and CYP2E1.

In vitro studies with liver microsomes from female rats demonstrated that intrinsic clearance of testosterone was inhibited by ticagrelor with an IC_{50} of 12 μM. Similar studies with human liver microsomes showed testosterone intrinsic clearance was inhibited by ticagrelor with an IC_{50} of 23 μM. In the rat 6 month toxicology study, plasma concentrations in the high dose group met or exceeded the IC_{50} for inhibition hepatic microsomal intrinsic clearance. In contrast, at the human therapeutic dose, maximal plasma concentrations were markedly lower than the IC_{50} for inhibition human hepatic microsomal intrinsic clearance of testosterone.

2.4.3.3.4 Excretion

In the nonclinical species tested, the major route of elimination was via faeces, independent of the route of administration of radiolabelled ticagrelor (oral or intravenous), most likely the result of biliary excretion. Experiments using bile duct-cannulated rats showed that >70% of total radioactivity was excreted in the bile within 24h of dosing. Faecal excretion was >90% and >80% in the mouse and rat, respectively, and approximately 60% in the marmoset. Urinary excretion was approximately 2, 5 and 10% of the radioactivity in these respective species.

Ticagrelor and the metabolite AR-C124910XX were generally the major components found in faeces in all species, including humans. AR-C133913XX was the major component found in urine, except for the mouse, where parent compound was the largest fraction.

Ticagrelor, AR-C124910XX and AR-C133913XX were found to inhibit organic anion transporter 3 (OAT-3)-mediated uptake of [^{14}C]-urate *in vitro* in oocytes. AR-C124910XX also inhibited organic anion transporter 1 (OAT-1)-mediated uptake *in vitro.* All three compounds weakly inhibited urate transporter 1 (URAT-1)-mediated [^{14}C]-urate uptake in URAT1-transfected HEK293 cells. OAT1 and OAT3 mediate urate secretion in the human kidney, and URAT1 is a transporter for urate reabsorption in the human kidney. An inhibition of net secretion of urate was seen following addition of the compounds in bi-directional transport studies across monolayers of human proximal tubule cells, and significantly inhibited net secretion of urate when added to the basolateral compartment.

Following administration of radiolabelled ticagrelor to lactating rats, milk samples displayed significantly higher levels of total radioactivity than plasma levels in the dam. The majority of the radioactivity found in the milk samples was unchanged ticagrelor, although significant levels of AR-C124910XX and AR-C133913XX were also detected.

2.4.4 Toxicology

A comprehensive program of *in vivo* and *in vitro* nonclinical studies has been conducted to evaluate the toxicological profile of ticagrelor in support of its administration to humans. Single and/or repeat-dose toxicology studies were conducted in mice, rats rabbits and marmosets and several of the repeat-dose studies included recovery groups. All nonclinical species used were considered pharmacologically relevant. The dog was not utilised as the non-rodent species for toxicity testing because the production of the active metabolite AR-C124910XX in dogs was found to be sufficiently different quantitatively from humans. Ticagrelor and the active metabolite, AR-C124910XX, as well as impurities in the final drug product, have been tested for genotoxicity in standard test batteries. Ticagrelor has been tested for carcinogenic potential in lifetime studies in mice and rats. A series of *in vivo* and *in vitro* studies were conducted to investigate the mechanism of the increased incidence of tumours seen in female rats. Reproduction toxicity studies were conducted with ticagrelor in rats and in rabbits.

2.4.4.1 Toxicokinetics

A summary of the C_{max} and AUC toxicokinetic parameters for ticagrelor and the metabolite AR-C124910 determined from the repeat-dose studies with ticagrelor are presented in Table 2 and Table 3. The exposure multiples in Table 2 and Table 3 are calculated using total exposure (bound + unbound). Protein binding is high in all species, highest in human. Therefore, calculating multiples based on a total exposure basis provides the more conservative estimate. Exposure was seen to increase with increasing dose in all species tested. After repeat dosing, the exposure to ticagrelor generally increased in a dose-proportional manner in rats and marmosets and increased in a less than dose-proportional manner in mice and rabbits. At the highest doses tested, where toxicity was evident, exposures generally deviated inconsistently from proportionality, possibly due to the gastrointestinal effects seen at those higher doses. There were no obvious differences in exposures based on gender except in rats, where female rats generally had greater ticagrelor, and lower AR-C124910XX, exposure than male rats. This difference was most evident at higher doses, as demonstrated with the toxicokinetics data from the 6-month study 456930 (0400PR), shown in Table 3.

Table 2 Selected toxicokinetic parameters for ticagrelor from toxicity studies

Species (strain)	Study	Dose (mg/kg)	Study Day	C_{max}[a] (µg/mL)	Exposure Multiple[b]	AUC[a] (µg-h/mL)	Exposure Multiple[c]
Mouse (CD-1)	456925 (0085PM	50	85	3.03	3.80	12.8	1.20
	456925 (0085PM)	250	85	14.1	17.7	165	15.4
	456925 (0085PM)	750	85	55.9	70.0	611	57.1
	456768 (0061AM	1000	14	47.8	59.9	282	26.4
	456925 (0085PM)	1250	1	74.2	93.0	716	66.9
	456768 (0061AM)	2000	1	62.5	78.3	401	37.5
Rat (Wistar)	456930 (0400PR)	10	172	0.67	0.84	3.41	0.32
(Sprague-Dawley)	TPR3143	20	90	2.00	2.51	19.0	1.78
(Wistar)	456930 (0400PR)	60	172	5.54	6.94	50.7	4.74
(Sprague-Dawley)	99302 (SR99302-01)	80	28	6.52	8.17	78.8	7.36
(Wistar)	456930 (0400PR)	180	172	16.7	20.9	228	21.3
(Sprague-Dawley)	93302 (SR99302-01)	300	28	38.1	47.7	633	59.2
Rabbit (pregnant)	0038RB	21	19	1.88	2.36	9.78	0.91
(NZ White)	0038RB	42	19	2.63	3.30	11.8	1.10
	0073KB	63	19	8.26	10.4	65.3	6.10
	0038RB	84	19	6.7	8.40	65.1	6.08
	0038RB	99	19	22.8	28.6	414	38.7
Marmoset	505453 (0008FT)	10	Week 52	1.08	1.35	7.37	0.69

Table 2 Selected toxicokinetic parameters for ticagrelor from toxicity studies

Species (strain)	Study	Dose (mg/kg)	Study Day	C_{max}[a] (µg/mL)	Exposure Multiple[b]	AUC[a] (µg-h/mL)	Exposure Multiple[c]
(Callithrix jacchus)	00019	20	56/57	3.16	3.96	23.9	2.23
	505453 (0008FT)	50	Week 52	6.54	8.20	52.8	4.93
	505453 (0008FT	100	Week 52	8.65	10.8	89.9	8.40
	99228 (SR99228-01)	200	28	10.2	12.8	158	14.8
	99228 (SR99228-01)	2000	28	26.7	33.4	554	51.8

[a] C_{max} and AUC are presented as the average for males and females, determined on the last sampling day for each study, using the last sampling from the study of longest duration (excluding the carcinogenicity studies). Conversion of µmol/L or µmol-h/L to µg/mL or µg-h/mL was accomplished using the molecular weight of ticagrelor (522.57 amu) (µmol-h/L x 522.57/1000 = µg-h/L).

[b] Exposure multiple calculated as the ratio of the animal exposures (C_{max}) listed in the table to the highest daily C_{max} measured in humans following the 100 mg bid dose used in Study D5130C0008 on Day 28, 0.798 µg/mL.

[c] Exposure multiple calculated as the ratio of the animal exposures (AUC) listed in the table to the highest total daily AUC measured in humans following the 100 mg bid dose used in Study D5130C0008 (Day 28, 2X the 12-hour AUC of 5.34 = 10.7 µg·h/mL total daily AUC).

Table 3 Selected toxicokinetic parameters for AR-C124910 from toxicity studies following dosing with ticagrelor

Species	Study	Dose (mg/kg)	Study Day	C_{max}[a] (µg/mL)	Exposure Multiple[b]	AUC[a] (µg-h/mL)	Exposure Multiple[c]
Mouse	456925 (0085PM)	50	85	2.62	10.9	17.5	4.60
	456925 (0085PM)	250	85	10.3	43.1	167	43.9
	456925 (0085PM)	750	85	49.2	206	870	229
	456768 (0061AM)	1000	14	40.2	154	225	54.2
	456925 (0085PM)	1250	1	24.6	103	478	126
	456768 (0061AM)	2000	1	19.7	75.3	108	26
Rat	456930 (0400PR)	10	172	0.215	0.74	1.50	0.39
	TPR3143	20	90	ND	-	ND	-
	456930 (0400PR)	60	172	1.88	7.87	26.4	6.95
	99302 (SR99302-01)	80	28	ND	-	ND	-
	456930 (0400PR)	180	172	6.69	28.0	109	28.7
	99302 (SR99302-01)	300	28	ND	-	ND	-
Rabbit	0038RB	21	19	0.70	2.93	5.03	1.32
	0038RB	42	19	0.81	3.39	6.86	1.80
	0073KB	63	19	2.99	12.5	34.3	9.03
	0038RB	84	19	2.93	12.2	34.7	9.13
	0038RB	99	19	9.23	38.6	181	47.6
Marmoset	505453 (0008FT)	10	Week 52	0.38	1.60	2.78	0.74
	00019	20	56/57	ND	-	ND	-

Table 3 Selected toxicokinetic parameters for AR-C124910 from toxicity studies following dosing with ticagrelor

Species	Study	Dose (mg/kg)	Study Day	C_{max}[a] (µg/mL)	Exposure Multiple[b]	AUC[a] (µg-h/mL)	Exposure Multiple[c]
Marmoset (Cont.)	505453 (0008FT)	50	Week 52	1.36	5.69	21.5	5.66
	505453 (0008FT)	100	Week 52	2.85	11.9	38.9	10.2
	99228 (SR99228-01)	200	28	ND	-	ND	-
	99228 (SR99228-01)	2000	28	ND	-	ND	-

ND Not determined.

[a] C_{max} and AUC are presented as the average for males and females, determined on the last sampling day for each study, using the last sampling from the study of longest duration (excluding the carcinogenicity studies). Conversion of µmol/L or µmol-h/L to µg/mL or µg-h/mL was accomplished using the molecular weight of AR-C124910 (478 amu) (µmol-h/L x 478/1000 = µg-h/L).

[b] Exposure multiple calculated as the ratio of the animal exposures (C_{max}) listed in the table to the highest daily C_{max} measured in humans following the 100 mg bid dose used in Study D5130C0008 on Day 28, 0.239 µg/mL.

[c] Exposure multiple calculated as the ratio of the animal exposures (AUC) listed in the table to the highest total daily AUC measured in humans following the 100 mg bid dose used in Study D5130C00008 (Day 28, 2X the 12-hour AUC of 1.9 = 3.8 µg·h/mL total daily AUC).

Table 4 **Comparison of male and female C_{max} and AUC values for ticagrelor and AR-C124910 on Study Day 172 from the 6-month toxicity study in rats (456930 (0400PR))**

	Male		Female	
Dose (mg/kg/day)	C_{max} (µg/mL)	AUC (µg-h/mL)	C_{max} (µg/mL)	AUC (µg-h/mL)
		Ticagrelor		
10	0.621	3.39	0.731	3.43
60	6.22	49.7	4.84	51.8
120/180 (M/F)	13.6	168	19.7	289
		AR-C124910		
10	0.254	1.93	0.174	1.08
60	2.80	38.5	0.96	14.2
120/180 (M/F)	8.99	144	4.36	74.1

2.4.4.2 Single Dose Toxicity

The acute toxicity of ticagrelor is considered low. The results of single dose studies in CD-1 mice and Sprague-Dawley rats showed that ticagrelor was well tolerated when given orally by gavage. No deaths were observed following doses up to 2000 mg/kg (the highest dose tested) in both species. The primary observation was a transient body weight loss seen in rats at the higher doses tested.

2.4.4.3 Repeat Dose Toxicity

2.4.4.3.1 Mice

The repeat-dose toxicity of ticagrelor was investigated in CD-1 mice in 2 studies, a 14-day dose range-finding study and a 3-month dose range-finding study. Mortality was observed early in the dosing periods at 750 mg/kg/day and above, doses also associated with decreased body weights and food consumption. The primary clinical signs at that dose and above were related to the gastrointestinal tract, with swollen ventral abdomens and increased stomach weights seen at necropsy. There was no histological correlate seen in the gastrointestinal tract. Increased liver weights were seen at 250 mg/kg/day and above, and hepatic centrilobular hypertrophy was seen at 250 mg/kg/day and above in males and 750 mg/kg/day in females. Increases in ALP were also seen at 750 mg/kg/day.

Also at doses of 750 mg/kg/day and above, subclinical bleeding was occurring, as evidenced by increased spleen weights and splenic haematopoiesis, with associated clinical pathology changes such as increased reticulocytes. Increased water consumption was seen during the in-

life phase, with nephropathy seen in both sexes. An increase in adrenal weights in males was associated with adrenal cortical cell hypertrophy. Decreased ovarian weights were seen, with interstitial cell hypertrophy and absent corpora lutea seen in some females. Testicular seminiferous epithelial degeneration (minimal to moderate) observed at 250 mg/kg/day and above in some males was not reflected in any organ weight change. Doses of 250 mg/kg/day (15-fold safety margin to human therapeutic exposures) and lower were generally well tolerated with observations comparable to controls, although no NOEL could be established in the 3-month study due to changes in reticulocytes and red blood cell parameters.

2.4.4.3.2 Rats

The repeat-dose toxicity of ticagrelor was investigated in rats (Sprague-Dawley and Wistar) in an ascending dose range-finding/5-day repeat dose study and in 1-, 3- and 6-month toxicity studies. In the 1-month study, treatment-related mortality (killed in extremis) was seen at 300 mg/kg/day. Clinical signs, reduced food consumption and decreased body weights or body weight gain seen in all studies at 60 mg/kg/day and above. The gastrointestinal tract was a primary target organ, with increased stomach weights correlating with microscopic signs of irritancy including erosion, squamous hyperplasia, oedema and inflammation at doses of 60 mg/kg/day and above (5- to 10-fold safety margin to human therapeutic exposures). The effects were reversible upon withdrawal of treatment.

Liver weights were seen to increase inconsistently at 60 mg/kg/day and consistently at higher doses, with small increases of ALP and ALT and increases in cholesterol and triglycerides. Bilirubin increased at 300 mg/kg. There were no histological correlate other than centrilobular hypertrophy at doses above 60 mg/kg. These observations suggest adaptive changes in liver function were occurring possibly associated with enzyme induction, in response to the high doses of ticagrelor since there were no degenerative changes and since all the effects were seen to reverse upon removal of drug.

Adrenal weights were increased inconsistently at 60 mg/kg/day and consistently at higher doses. Microscopic examination revealed vacuolization or inflammatory cell foci at 80 mg/kg/day and above, and at 300 mg/kg/day, cortical hypertrophy. The effects were reversible upon withdrawal of treatment. These observations may have been related to the stress associated with higher doses of ticagrelor, although they may be the result of inhibition of steroid synthesis in the adrenals, since ticagrelor was shown to inhibit corticosterone synthesis in adrenal cell cultures *in vitro*. This is consistent with the increased water consumption and urinary output seen at these dose, suggesting that aldosterone levels may have been affected.

Increased pulmonary alveolar histiocytosis was seen following doses of 300 mg/kg/day for 1 month, and at 60 mg/kg/day and above following longer duration of dosing. It was not observed in studies of less than 1 month. Severity of the finding did not increase with duration of dosing, and was found to recover upon removal of treatment.

Changes in hematological parameters were seen, including decreases in hemoglobin, RBC counts, and/or hematocrit, and increases in reticulocytes and platelets. No frank hemorrhage

was seen at necropsy, but the observations of hemorrhage associated with stomach irritation and erythrophagocytosis in the mesenteric lymph nodes suggest subclinical bleeding, consistent with the pharmacological activity of inhibition of platelet aggregation.

2.4.4.3.3 Marmosets

The repeat-dose toxicity of ticagrelor was investigated in marmosets (Callithrix jacchus) in an ascending consecutive dose/5-day repeat dose range-finding study and in 1-, 3- and 12-month toxicity studies. The primary observation in marmosets was related to gastrointestinal effects normally associated with handling of the species. The effects were reversible in surviving animals upon withdrawal of treatment. Few other treatment-related effects were seen that were not considered secondary to the gastrointestinal effects. Single ascending doses up to 1600 mg/kg were well tolerated and no treatment-related effects were seen following 5 daily 1600 mg/kg/day doses. Mortality (found dead or killed in extremis) was observed on days 18 and 22 in the 1-month study at 2000 mg/kg/day, but was also seen within the first week of dosing at 500 mg/kg/day in the 3-month study. Clinically, mortality was preceded by subdued behaviour, lack of appetite, diarrhoea, and rapid body weight loss. Tolerability studies, conducted to investigate the reason for the disparity, demonstrated gastrointestinal lesions in control and treated animals that were reduced upon improvement of handling procedures and not necessarily refinement of vehicle. Even with the improved procedures, mortality related to gastrointestinal effects preceded by rapid body weight loss was seen late in the 12-month study in all dose groups, but mostly in the high-dose group. Reduced hemoglobin, RBC and haematocrit were seen in the decedent animals. Marmosets are characterized as being susceptible to enteritis as a result of the stress of handling and captivity, and these observations are consistent with that characterization, although the incidence and severity increased with dosing with ticagrelor. It is likely that the enteritis frequently observed in marmosets is potentiated by treatment with ticagrelor, at least in part related to its pharmacology of inhibition of platelet aggregation.

2.4.4.4 Genotoxicity

Ticagrelor and its metabolite AR-C124910XX have shown no genotoxic potential *in vitro* in the Ames and mouse lymphoma tests, and ticagrelor showed no genotoxic potential *in vivo* in the rat micronucleus test. Genetic toxicity testing of key starting materials, intermediates and impurities have identified a single potential genotoxic impurity (PGI), C3, which is controlled at less that the toxicological threshold of concern for PGI (calculated to be 8 ppm in the drug substance for a clinical dose of 90 mg b.i.d. ticagrelor).

2.4.4.5 Carcinogenicity

Carcinogenicity studies with ticagrelor via the oral (gavage) route of administration have been conducted in CD-1 mice utilising doses of 50, 100 and 250 mg/kg/day, and in Wistar rats utilising doses of 20, 60 and 120 mg/kg/day for males and 20, 60 and 180 mg/kg/day for females. Different top doses were used for the genders in the rat study in part because of the difference in tolerability seen in chronic toxicity studies (See Section 2.4.4.1., Table 4).

2.4.4.5.1 Mouse

There was no evidence that ticagrelor was carcinogenic in the mouse study at doses up to 250 mg/kg/day There were no statistically significant differences in mortality, which ranged across the combined control and ticagrelor treated groups from 40 to 56% for males and from 54 to 71% in females. Surviving fractions were adequate for evaluation of tumourigenic potential in the remaining populations. Toxicokinetic evaluation indicated that exposure to ticagrelor and the metabolite AR-C124910XX increased with dose and was consistent throughout lifetime dosing. Slight, non-statistically significant decreases in body weights were seen in ticagrelor-treated animals (4% in males and 8% in females in the high dose group).

It is noteworthy that adrenal cortical hypertrophy and renal tubular degeneration, seen to increase in the 3-month study at 750 mg/kg/day, showed a dose-dependent decrease in males and females, respectively, in this study. No other treatment-related toxicity was observed. Since the results of the 3-month study in mice indicated unacceptable rates of survival at 750 mg/kg/day (see Section 2.6.6.3.2), the upper dose utilized in this study is considered appropriate.

There was a slight but statistically significant increase in pulmonary bronchio-alveolar adenomas in females given 250 mg/kg/day, compared to controls. There was no significant linear trend and the incidence of this tumour was within 5-year background rates at the laboratory of conduct. Statistically significant increases were seen in other tumour types (*follicular centre cell lymphomas, granulocytic leukaemias)* that also showed no dose dependence or were within 5-year background rates at the laboratory of conduct. It was concluded that ticagrelor was not carcinogenic in the mouse.

2.4.4.5.2 Rat

There was no evidence that ticagrelor was carcinogenic in male rats. In female rats, ticagrelor at the high dose only, produced a change in the tumour incidence pattern consisting of increased incidence of uterine tumours (adenocarcinomas), increased incidence of hepatic adenomas, reduced incidence of mammary fibroadenomas and reduced incidence of pituitary adenomas and hyperplasia.

Based on survival rates, the rat study is considered sufficient for evaluation of the carcinogenic potential of ticagrelor. There were no statistically significant differences in mortality between the combined controls and any other groups for males and females receiving ticagrelor. Mortality ranged from 18 to 32% for males across the dose groups and from 30 to 44% for controls and the two lower dose groups in females, with a numerically higher fraction for the high dose group, at 62%.

Toxicokinetic evaluations indicated that exposure to ticagrelor and the metabolite AR-C124910XX increased with increasing dose and was consistent or increased throughout lifetime dosing. Decreases in body weights (8% less than controls in males and 19% less in females) and body weight gains (12% less than controls in males and 33% less in females)

were seen in the high dose groups. Non-neoplastic observations were consistent with those seen in the toxicity studies with ticagrelor (stomach changes, adrenal vacuolization). Based on these observations, the upper doses utilized in this study are considered appropriate.

There was a change in the tumour spectrum that included an increased incidence of uterine tumours (adenocarcinoma), reduced incidence of mammary adenomas and a slight increase in hepatic adenomas, at the high dose only (180 mg/kg/day). There was a statistically significant reduction in pituitary adenomas (anterior lobe) in decedent high-dose females. In all female animals (decedents and survivors) there was a reduction in the incidence of pituitary adenomas and hyperplasia combined. There was an increase in the combined incidence of sex cord stromal tumours in the ovary of high dose females, but no increase in the individual tumours.

The pattern of this and other observations such as no increase in uterine weights and increased ovarian interstitial cell vacuolization and cysts suggested there was a disturbance in endocrine homeostasis in the high dose group females. These observations lead to the hypothesis that the increased incidence of uterine tumours are the result of a prolonged pattern of hormonal imbalance following lifetime exposure to high doses of ticagrelor in the rat.

The incidence of hepatocellular adenoma was slightly increased above 5-year historical controls for Wistar rats at the laboratory of conduct, and the Peto analysis indicated a positive trend test for combined adenomas and carcinomas.

In toxicology studies with ticagrelor, the effects on the liver included increased liver weight, hepatic enzyme induction, small increases in ALT (in females after 1 and 3 months of dosing at 180 mg/kg/day an above and in males and females in the 6-month study at 60 mg/kg/day and above) and ALP (males and females at 180 mg/kg/day and above) and centrilobular hypertrophy. There was no histological evidence of hepatocellular injury that could explain the increases in ALT and ALP. Therefore, these effects are considered to be an adaptive enzyme induction response to the high liver load of compound which is often associated with increased adenomas and carcinomas in rodent liver. The increase in liver tumours in the rat carcinogenicity study is modest reflecting the modest enzyme induction response.

2.4.4.6 Reproductive Toxicity

Definitive reproduction toxicity studies conducted with ticagrelor included a female fertility study in female rats at doses up to 200 mg/kg/day, a male fertility study at doses up to 180 mg/kg/day performed as part of the 6-month general toxicity study in rats, embryofetal development studies in rats at doses up to 300 mg/kg/day and in rabbits at doses up to 63 mg/kg/day, and a pre-postnatal development study in rats at doses up to 180 mg/kg/day.

The studies showed ticagrelor did not adversely affect fertility or significantly effect fetal development at clinically relevant exposures. There were no effects on male fertility in male rats which had been dosed for 10 weeks (as part of the 6 month toxicity study) prior to mating. Irregular oestrous cycles were seen in rats in the female rat fertility study, with extended oestrus the most common observation, but female fertility or reproductive performance was

not affected. In the rat embryo-fetal development study, maternal toxicity was seen when 300 mg/kg/day was administered during gestation, with animals demonstrating gastric and duodenal erosions consistent with ticagrelor effects seen in the repeat-dose toxicity studies. Ticagrelor reduced fetal weights at the maternally toxic doses, but had no other adverse effects on embryonic or fetal survival.

Doses of 180 mg/kg/day ticagrelor during gestation and through lactation reduced maternal body weight gain in rats and also reduced birth weight, post-natal pup viability and growth rates of the pups. These effects were not seen at lower doses. All other parameters were normal in the F1 groups. Analysis of milk from dams determined that ticagrelor was excreted into milk, at concentrations exceeding that in maternal plasma. Necropsy examinations of the F0 dams and F1 pups at weaning did not reveal any treatment-related lesions, and there was no compound-related mortality of the selected F1 rats. Sexual maturation was not affected, and mating performance and fertility of the F1 generation were not obviously influenced by treatment of the parental females.

Severe retinal folding was seen in a few fetuses from the mid- and top-dose groups of the rabbit embryofetal development study. A follow-up study was performed which established that the retinal folding was a background event and not related to the administration of ticagrelor. Based on these observations, it was concluded that the retinal effects seen in the initial study in the rabbit were not treatment-related.

2.4.5 Integrated Overview and Conclusions

The results of nonclinical studies with ticagrelor support its use in the prevention of secondary cardiovascular events in patients with ACS, as well as the safety of prolonged use of ticagrelor in human populations.

2.4.5.1 Pharmacodynamics of ticagrelor

The nonclinical data collected with ticagrelor describe a potent, selective, orally active, direct $P2Y_{12}$ receptor antagonist in a variety of *in vitro* and *in vivo* nonclinical models, providing mechanistic support for its utility use in the prevention of secondary cardiovascular events in patients with ACS. The primary mechanism of action, inhibition of platelet $P2Y_{12}$ receptors resulting in the prevention of platelet aggregation, is supported by nonclinical pharmacodynamic studies, and may be supplemented by effects in other systems such as effects on GP17 and smooth muscle.

Ticagrelor is a potent inhibitor of radioligand binding to human washed platelets and of ADP-induced platelet aggregation in suspensions of human and rat washed platelets, in human platelet rich plasma, and in marmoset and human whole blood. It inhibits ADP-induced platelet aggregation measured *ex vivo* in conscious male Beagle dogs and eliminates platelet-mediated thrombosis in the damaged, stenosed femoral artery of the anaesthetized dog. Its reversible mechanism of action distinguishes it from thienopyridine agents, which are pro-drugs requiring metabolic activation forming active metabolites which covalently bind to the $P2Y_{12}$ receptor, inhibiting aggregation for the life-span of the platelet. In addition to the effects seen in platelets, ticagrelor has been shown to inhibit the $P2Y_{12}$ – mediated

vasoconstriction of mouse vascular smooth muscle cells, and demonstrates nano-molar affinity for GPR17 *in vitro*, displaying antagonistic or partial agonistic properties against this receptor, activities which may also contribute to its clinical utility in ACS.

Its major circulating metabolite, O-deethylated AR-C124910XX, shows pharmacological activity comparable to that of the parent molecule in those tests where it has been examined. Since ticagrelor is eliminated from the body primarily as AR-C124910XX and parent, these observations indicate that the primary metabolic pathway does not alter its activity against platelet aggregation while in circulation.

Investigations into potential for secondary pharmacological effects demonstrate low potential for unwanted pharmacological activities, consistent with the safety profile seen in toxicity studies with the compound. Ticagrelor increases intestinal transit time, which may be related to the primary dose-limiting effects on intestinal tissues including irritation, inflammation and damage described below. The *in vitro* IC_{50} for inhibition of hERG of 1.72 μM did not translate to any potential for effects on the action potential *in vitro* or on the ECG *in vivo*.

2.4.5.2 Dyspnoea

Effects on respiratory parameters seen in safety pharmacology studies in the rat could not be consistently repeated in the laboratory, but may be related to the dyspnoea seen clinically. Data showed that ticagrelor itself has low activity at adenosine receptors, but inhibits adenosine uptake in red blood cells, does not inhibit adenosine deamination (deactivation), does not interfere with the hypoxanthine salvage pathway of mouse lymphoma L5178Y TK+/-3.7.2C cells, inhibits adenosine-induced depolarization in isolated rat and guinea pig vagal nerve fibres through adenosine A_1, A_{2B} or A_3 receptors and enhances adenosine-mediated effects on blood flow in the canine heart. Adenosine causes dyspnoea in humans when given intravenously, and changes in respiratory parameters are observed when adenosine is given intravenously to rats, observations which are mediated via adenosine receptor activation. These observations are consistent with the hypothesis that dyspnoea in some ACS patients is secondary to adenosine released from damaged cardiac tissue and activating adenosine receptors in the lung. The adenosine is more likely to reach the lung as a result of inhibition of red blood cell adenosine uptake. It is not thought to be related to the increase in alveolar histiocytosis seen in rat lung, since this effect was only seen in rats given high doses for at least a month, while the respiratory effects in rats or the dyspnoea seen clinically were observed following single or only a few multiple doses. Because the increase in alveolar histiocytosis occurred at high multiple doses, was minimal to moderate in severity, did not increase in severity with duration of dosing, was reversible and was not seen in any other species, it is not considered to represent a risk to humans.

2.4.5.3 Toxicology of ticagrelor

Toxicity testing was sufficient to characterize the potential hazards of high-dose ticagrelor, since exposures in the species used exceeded those required for clinical therapeutic use. Toxicokinetic data collected with ticagrelor indicate increased exposure with dose, allowing for adequate testing for toxicity as the dose was increased. Exposure to the active metabolite AR-C124910XX in nonclinical species was high; fractional conversion in species used in

toxicity testing was as high or higher than that seen in humans, allowing for adequate testing of the safety of the metabolite as well as parent. No unique human metabolites have been identified.

The primary target organ of toxicity seen across species was the gastrointestinal tract, although the location and type of effect seen varied across the species. Gastrointestinal effects were reversible as long as the effects were not so severe as to cause mortality. In mice, abdominal distension was observed with no histological correlates detected. In rats, increased stomach weights, and effects generally in the upper gastrointestinal tract, including mucosal erosion, squamous hyperplasia, oedema, and inflammation were observed. Marmosets inconsistently demonstrated effects in the small and/or large intestine consistent with enteritis frequently seen in this species. The observations were seen in treated animals and in controls in most of the studies, increasing in incidence and severity with increasing ticagrelor dose and duration of dosing. Thus, it is likely that the enteritis often observed in marmosets in captivity is potentiated by treatment with ticagrelor. Although it is not known whether the gastrointestinal effects are a local, irritative effect or due to a systemic pharmacological effect of increased bleeding due to inhibition of platelet aggregation, hemorrhagic necrosis was seen only on occasion in marmosets, and not in rodents. No other target organs of toxicity were observed resulting in high safety margins in marmosets excluding gastrointestinal effects. Extrapolating to the clinic, a potential for gastrointestinal effects can be monitored readily, although no routinely dose-limiting clinical correlates have been observed in clinical trials with ticagrelor.

Effects were seen in the liver in rodents, but not in marmosets. In mice and rats, increased alkaline phosphatase (ALP) and/or alanine aminotransferase (ALT), increased liver weights, and centrilobular hypertrophy, were seen. These observations are consistent with mild enzyme induction in the rodent, and CYP protein and activities (CYP4A and CYP1A1) were shown to be marginally increased in the rat. The lifetime carcinogenicity study in rats showed a slight increase in hepatic adenomas (4/50 females at 180 mg/kg/day), and there was also a single adenocarcinoma in the high dose female group. The incidence of hepatocellular adenoma was slightly increased above 5-year historical controls for Wistar rats at the laboratory of conduct, and the Peto analysis indicated a positive trend test for combined adenomas and carcinomas. The increased liver weights (20 to 25%) seen consistently in studies in rats of 1 month in duration or greater, with slight induction of CYP1A1 and 4A and no consistent related histological changes other than hypertrophy, may be related to these observations. This is consistent with the liver in rats adapting to the metabolic load of high doses of ticagrelor producing a slightly increased incidence of liver adenomas. The observation was considered not important for humans because the incidence was low at the high dose, with large safety margins, and the tumours were benign, and considered secondary to the liver response to the metabolic load placed on the liver from the high doses of ticagrelor.

Evidence was seen for subclinical bleeding at high doses in all species tested, as evidenced by increased spleen weights and splenic haematopoiesis, clinical pathology changes including decreases in haemoglobin, RBC counts, and/or haematocrit, and increases in reticulocytes and

platelets. No frank haemorrhage was seen at necropsy, but the observations of haemorrhage associated with stomach irritation and erythrophagocytosis in the mesenteric lymph nodes are also suggestive of subclinical bleeding. These observations are consistent with ticagrelor's pharmacological activity of inhibition of platelet aggregation.

The effects seen in the adrenals in rodents following dosing with ticagrelor at doses high enough to also produce clinical signs and mortality may be stress-related, but may also be related to the inhibition of steroid hormone (corticosterone) synthesis by ticagrelor seen *in vitro*. In mice and rats, ticagrelor caused a reversible increase in adrenal weight, which was sometimes associated with either cortical vacuolization or focal hypertrophy of the adrenal cortex (reticularis, fasciculata). These observations suggest that the adrenal is responding to feedback pressure to regain homeostasis of steroid hormone precursor synthesis in rodents, since it was also shown that endogenous levels of ACTH can overcome the inhibitory effect *in vitro*. Pituitary weights were not affected. This is also consistent with the increased water consumption seen in rodents, since aldosterone synthesis is downstream from corticosterone. Similar effects have not been seen in non-human primates, even at dose levels that produce clinical signs and mortality, and clinical indications for adrenal hypo- or hyperactivity have not been evident in clinical trials with ticagrelor.

2.4.5.4 Genotoxicity studies

Ticagrelor and its metabolite AR-C124910XX have shown no genotoxic potential *in vitro* in the Ames and mouse lymphoma tests, and ticagrelor showed no genotoxic potential *in vivo* in the rat micronucleus test.

2.4.5.5 Carcinogenicity studies

There was no evidence that ticagrelor was carcinogenic in the mouse study. There was no evidence that ticagrelor was carcinogenic in male rats. In female rats, ticagrelor at the high dose only, produced a change in the tumour incidence pattern consisting of increased incidence of uterine tumours (adenocarcinomas), increased incidence of hepatic adenomas, reduced incidence of mammary fibroadenomas and reduced incidence of pituitary adenomas and hyperplasia. The mid-dose did not show any effect and represents a 8-fold margin of safety.

2.4.5.5.1 Liver tumours

In high dose females a small increase in the incidence of liver tumours was observed. The increased incidence is thought to be related to the increase in CYP induction observed (centrilobular hypertrophy and liver weight increases). In rats, ticagrelor caused induction of hepatic CYP enzymes. After 1 month treatment with 20, 80 or 300 mg/kg/day ticagrelor to male and female rats, increases in activities associated with hepatic CYP1A2, 2B, 2C, 2E1, 2A, 3A and 4A were observed. This enzyme induction had no observable consequence upon systemic exposure to parent and/or metabolite.

The enzyme inducing potential of ticagrelor was confirmed in a 1-month investigative study where doses of 180 mg/kg/day produced increased levels of CYP1A1/2 and CYP4A1 in

female rats. The induction was detected after 1 week of dosing as well as at the end of the study. This induction property was further evaluated in a 3 month investigative study in which a dose of 180 mg/kg/day produced increased expression of hepatic CYP1A1/2, together with increased expression of uterine CYP1A1 (protein and mRNA). No increases in levels of hepatic or uterine CYP1B1 (mRNA) were detected. The increase in liver tumours in the rat carcinogenicity study is modest reflecting the modest enzyme induction response.

2.4.5.5.2 Uterine adenocarcinomas

Observations in the repeat-dose toxicity and carcinogenicity studies, including adrenal cortical and ovarian interstitial cell hypertrophy and vacuolisation, increase in ovarian cysts, cycle changes, as well as the pattern of tumour incidence, resulted in the hypothesis that a sustained endocrine imbalance in high dose female rats occurred. Mechanistic studies conducted to test that hypothesis showed that high dose ticagrelor produced several simultaneous effects consistent with extensive cross talk in the endocrine system. The mechanistic studies showed effects on testosterone and testosterone clearance, but did not identify any direct effect on oestrogen receptors nor any effect on aromatase activity.

2.4.5.5.3 Effects on testosterone

The time course studies identified an increase of testosterone during proestrus. This finding is possibly linked to the inhibition of testosterone clearance seen *in vitro*. *In vitro* studies with liver microsomes from female rats demonstrated that intrinsic clearance of testosterone was inhibited by ticagrelor with an IC_{50} of 12 µM. Similar studies with human liver microsomes showed testosterone intrinsic clearance was inhibited by ticagrelor with an IC_{50} of 23 µM. In the rat 6 month toxicology study, plasma concentrations in the high dose group substantially exceeded the IC_{50} for inhibition of hepatic microsomal intrinsic clearance. In contrast, at the human therapeutic dose, maximal plasma concentrations were markedly lower than the IC_{50} for inhibition of human hepatic microsomal intrinsic clearance of testosterone. Hence this effect is only likely to be relevant at the high doses used in the toxicology and carcinogenicity studies.

According to the literature, the androgen receptor is expressed in the uterus (Pelletier 2000, Pelletier et al 2000), and testosterone can be trophic for both the endometrium and stroma in ovariectomised rats (Nantermet et al 2005). The investigative three month study (1800KR) failed to show a trophic effect at the top dose of 180 mg/kg/day in non-ovariectomised rats. Parallel studies were carried out to determine whether there was evidence of an androgenic drive in the uterus, and whether pre-neoplastic changes to the uterus could be detected concomitantly with the occurrence of ticagrelor-associated endocrine imbalance. The data did not provide evidence of an androgenic drive in the uterus, although changes to gene expression within the whole uterus in the non-ovarectomised animal may be a relatively insensitive measure. This study, limited in duration, also failed to demonstrate convincing evidence of a pro-proliferative effect in the uterus.

2.4.5.5.4 Adrenal and ovarian interstitial cell hypertrophy

The adrenal and ovarian interstitial cell hypertrophy observed in the repeat-dose and carcinogenicity studies are considered to be adaptive in nature and possibly caused by effects seen on steroidgenenesis *in vitro*.

2.4.5.5.5 Changes in tumour pattern

The increase in the incidence of uterine carcinomas was associated with a reduction of mammary fibroadenomas and a reduction in the number of pituitary adenomas and hyperplasia. This shift in tumour pattern mirrors that seen with food restriction in the rat and studies with Bromocriptine, which is thought to be mediated by a reduced prolactin drive (Roe et al 1995; Griffith 1977; Keenan et al 1994; Keenan et al 1995a; Keenan et al 1995b). In food restriction studies, the incidence of uterine tumours is inversely related to body weight (Roe et al 1995). In the 2-year ticagrelor rat carcinogenicity study, a marked loss of bodyweight gain(33%) only occurred with the high dose, and only in the females. Most hyperplasias and adenomas in the rat pituitary are derived from prolactin-producing cells and the reduced incidence of pituitary adenomas and hyperplasias, and mammary fibroadenoma seen in the rat carcinogenicity study is consistent with reduced prolactin drive. Prolactin in the rat has a direct effect on ovarian steroidogenesis (luteotropic effect), an effect not seen in primate. Therefore the tumour effects seen in the rat would not be expected in primates, including humans.

2.4.5.5.6 Carcinogenicity studies: Conclusions

The weight of evidence and mechanistic data support the hypothesis that the increased incidence of uterine carcinomas is caused by a sustained change of hormonal balance in the high dose female rats only. Several of the mechanisms identified associated with these effects are seen in high dose female rats only and not relevant for man at the therapeutic doses. The increase in liver tumours is likely to be related to the enzyme induction observed. Ticagrelor is non-mutagenic and the mid-dose in the carcinogenicity study is the NOEL with a safety margin of approximate 8-fold. No carcinogenic effects were seen in the mouse or in the male rats. In total, these data lead to the conclusion that ticagrelor does not represent a carcinogenic risk to man.

2.4.5.6 Reproductive toxicology

The results from the reproductive toxicity studies do not indicate reproductive risk to the fetus, suckling neonate or to adults at tolerated exposures. Based on this, it is considered that ticagrelor is unlikely to affect reproduction at therapeutic exposures.

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