Nonclinical Overview	
Drug Substance	Brilique
Version ID.	001811475
Date	16 January 2014

2.4 Addendum to Nonclinical Overview

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2.4.1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

A comprehensive nonclinical package, including pharmacology, safety pharmacology, pharmacokinetics, and toxicology studies, supported the original Marketing Authorization Application (MAA) of ticagrelor.
This addendum includes data from internal AstraZeneca studies conducted with ticagrelor during the past 5 years since submission of the original MAA (22-October-2009 to 8-November-2014). A review of the published literature has also been conducted (Embase, Medline, Knowledge Discovery Platform).

2.4.2. PHARMACOLOGY

In the following sections, 19 nonclinical pharmacology studies conducted with ticagrelor by the Sponsor are summarized along with information from the literature. These information include but are not limited to reports and published papers 1) validating equilibrative nucleoside transporter (ENT) 1 inhibition as an additional clinically relevant target of ticagrelor, 2) exploring potential mechanistic explanations regarding an interaction between ticagrelor and aspirin, 3) exploring different strategies to reverse the anti-platelet effect of ticagrelor, and 4) exploring the effects of ticagrelor in animal models of atherosclerosis, restenosis, stroke, cancer and sepsis.

2.4.2.1 Primary Pharmacology

The original MAA included studies that defined the primary pharmacologic mechanism of action of ticagrelor, $P2Y_{12}$ antagonism. A limited number of additional primary pharmacology studies have been conducted and some data has been published in the past five years. The relative potency of ticagrelor, its major circulating metabolite AR-C124910XX and the major metabolite excreted in urine, AR-C133913XX have been explored in *in vitro* $P2Y_{12}$ receptor binding and signaling assays (AZMR10095) as well as in *in vitro* whole blood ADP-induced aggregation assays using blood from healthy human subjects, and the two toxicology species, rats and marmosets (AZMR10112). In these studies, performed by the Sponsor, ticagrelor and AR-C124910XX showed similar potency whereas AR-C133913XX had low or no potency, see Table 1.

Table 1 Potency of ticagrelor and its metabolites, in vitro

Compound	IC_{50} (mean±SD) Receptor binding [125I]-AZ11931285 binding (nM)	Receptor signalling [35S]-GTPgS (nM)	ADP-induced aggregation (µM) Human	Rat	Marmoset
Ticagrelor	11 ±8 (n=63)	66 ±28 (n=98)	0.24 (n=4)	0.13 (n=5)	0.12 (n=3)
AR-C124910XX	6.5 (n=2)	50 (n=2)	0.17 (n=4)	0.06 (n=5)	0.06 (n=3)
AR-C133913XX	2500	10000 (n=1)	>10 (n=4)	>10 (n=4)	1.91 (n=4)

Co-crystals of the $P2Y_{12}$ receptor in complex with both a $P2Y_{12}$ antagonist, AZD1283, and a $P2Y_{12}$ agonist, 2-methylthioadenosine diphosphate, have been published (Zhang et al 2014a, Zhang et al 2014b). These new co-crystal data are compatible with the previously published two binding site model where ticagrelor has been proposed to bind to one site and ADP to another site on the receptor (van Giezen et al 2009). A published *in vitro* study using different functional assays concluded that ticagrelor has a competitive mode of antagonism vs $P2Y_{12}$-receptors activated by ADP (Hoffman et al 2014). However, these functional data do not contradict the non-competitive binding mode of action previously reported for ticagrelor vs. ADP (van Giezen et al 2009).

Two Sponsor conducted studies relevant to the potential use of ticagrelor in paediatric populations have been performed. First, the anti-platelet *in vitro* potency of ticagrelor was approximately three times greater in whole blood from neonatal rats than from adult rats (AZMR10213). However, this apparent increased *in vitro* potency in blood from neonatal rats did not translate into human as a later study comparing the *in vitro* potency of ticagrelor in blood from children (0 to12 years) with blood from adults found no evidence of the *in vitro* potency of ticagrelor being different in children than in adults (BS000096-01).

A number of publications have highlighted the role for $P2Y_{12}$ in thrombus formation and the anti-thrombotic effect of ticagrelor. $P2Y_{12}$ inhibition by ticagrelor inhibited thrombus formation (laser-injured cremasteric arterioles) to the same extent as seen in $P2Y_{12}$ deficient mice (Patil et al 2010). Ticagrelor also disrupted the stability of newly formed platelet aggregates *in vitro*, promoting disaggregation, and reversed thrombotic vascular occlusion *in vivo* in a mouse arterial thrombosis model ($FeCl_3$-injury) (Speich et al 2014). Acute plaque rupture in ApoE deficient mice by ultrasound treatment provoked rapid formation of non-occlusive thrombi, which were smaller in size and unstable in the presence of ticagrelor (Nergiz-Unal et al 2010). In the same study, when mouse or human blood was perfused over collagen or atherosclerotic plaque material *in vitro*, ticagrelor reduced the thrombus formation and increased platelet disaggregation at high shear rates. Finally, the recovery of platelet function after ticagrelor differs mechanistically from that after the thienopyridine clopidogrel. Thus as the recovery of platelet function of the direct acting and reversibly binding antagonist ticagrelor requires elimination of drug, whilst recovery of platelet function following cessation

of an irreversible antagonist such as clopidogrel require formation of new platelets. As the active metabolite of clopidogrel is only detectable for about 4h in the circulation, theoretically the newly formed platelets will not be exposed and should thus be un-inhibited whereas the remaining ticagrelor will redistribute, due to its reversibly binding mode of action, to inhibit the old and the newly formed platelets to a similar extent.
This difference is masked by conventional platelet aggregation methods, but is revealed by thrombus formation measurement under flow. Juvenile platelets formed at later time points after clopidogrel treatment promoted thrombus formation (Kuijpers et al 2011).

2.4.2.1.1 Non-platelet effects of $P2Y_{12}$ inhibition

The previously published ability of ticagrelor to inhibit ADP-induced vasoconstriction mediated by vascular smooth muscle cell expressing $P2Y_{12}$ (Högberg et al 2010) has been confirmed in two publications (Grzesk et al 2012, Grzesk et al 2013). The latter publication suggests that high dose aspirin impaired the anticontractile effect of ticagrelor following ADP stimulation in rat tail artery smooth muscle cells. The clinical relevance is unknown.

2.4.2.2 Secondary Pharmacology

2.4.2.2.1 Selectivity against other P2 receptor subtypes

In the original MAA application it was demonstrated that ticagrelor has activity at the $P2Y_{12}$ receptor but no relevant activity at the other P2Y and P2X receptors tested. Since then a functional assay for $P2Y_{13}$ has been established and in a Sponsor conducted study, ticagrelor and AR-C124910XX but not AR-C133913XX, concentration dependently inhibited 10 nM 2MeSADP induced $P2Y_{13}$ responses with IC_{50}-values of 0.46±0.04 µM (mean±SEM) and 0.20±0.05 µM (mean±SEM), respectively (AZMR10392). The potential clinical relevance is unclear.

2.4.2.2.2 Adenosine transporter (ENT1) inhibition

The additional secondary pharmacology work conducted since the original MAA has focused on elucidating and validating the additional mechanism of action of ticagrelor related to adenosine. Previously reported, now published, *in vitro* data on adenosine uptake inhibition indicated that the transporter involved likely was ENT1 (van Giezen et al 2012). Using human recombinant cell systems this has now been verified as ticagrelor, in a published Sponsor conducted study, was shown to inhibit the adenosine transporter ENT1 with an IC_{50} of 200 nM (Ki 41 nM) with no significant inhibition (IC_{50} >10 µM) of ENT2, concentrative nucleoside transporter (CNT)2 or CNT3 (2549KV, Armstrong et al 2014).

2.4.2.2.3 General selectivity

The original secondary pharmacology profiling of ticagrelor, AR-C124910XX and AR-C133913XX have also been complemented and expanded using radioligand binding, enzyme and functional assays *in vitro* in 4 Sponsor conducted studies (1064SY, 0951SY, 0952SY, 1055SY). Using a binding affinity of $K_i \leq 200$ nM as a cut-off, corresponding to a100-fold selectivity vs. $P2Y_{12}$, (K_i 2.0 nM), yielded 3 significant activities. Mean affinities (K_i) and functional activities (IC_{50}) are summarized in Table 2.

Table 2 Non-P2Y12 activities of ticagrelor and AR-C124910XX in vitro

	Ticagrelor (µM)		AR-C124910XX (µM)	
	K_i	IC_{50}	K_i	IC_{50}
Adenosine transporter ENT1	0.041	0.2		
Dopamine transporter	0.2	0.1		
Adenosine A_3 receptor	0.2	6.4	0.2	5.6

A previous Sponsor supported external study reported activity vs GPR17 (AZM090128-04, Martini 2010). However, this could not be confirmed in a second Sponsor supported external study using an alternative investigator (AZMR10330). Thus there is no conclusive evidence that ticagrelor has activity vs. GPR17.

2.4.2.2.4 Pharmacological relevance of ENT1 inhibition

A number of Sponsor conducted studies have been carried out to understand and explore the physiological relevance of ticagrelor mediated ENT1 inhibition.

A previously reported study, now published, showed that ticagrelor could augment adenosine-induced coronary blood flow increases in dog model (van Giezen et al 2012). This observation has now been shown to be relevant to humans as ticagrelor, 180 mg, augmented adenosine-induced coronary blood flow increases in a randomized published Sponsor conducted study in healthy human subjects (Wittfeldt et al 2013).

A Sponsor conducted *in vitro* study in human blood showed that ticagrelor could attenuate the degradation of exogenously added adenosine and thereby augment adenosine-induced platelet inhibition (AZMR10120). The platelet function data was repeated and confirmed in an external laboratory that also performed parallel experiments in blood from $P2Y_{12}$ deficient patients (Nylander et al 2013). The potency by which ticagrelor attenuated the degradation of the added adenosine in these *in vitro* experiments predicted that the effect should be present in patients treated with ticagrelor and indeed the adenosine plasma concentrations have been shown to be increased in patients treated with ticagrelor relative patients treated with clopidogrel (Bonello et al 2013).

Another *in vitro* experiment, published as a meeting abstract, showed that ticagrelor can augment adenosine-induced stimulation of neutrophil chemotaxis in the presence of erythrocytes (Alsharif et al 2014).

A previous report, now published, showed that ticagrelor but not clopidogrel could reduce infarct size in a dog tPA-induced reperfusion model (Wang et al 2010). Both $P2Y_{12}$ antagonists were dosed to equal $P2Y_{12}$ inhibition as evidenced by complete inhibition of ADP-induced aggregation *ex vivo*, thus indicating a non-$P2Y_{12}$ mediated cardioprotective effect of ticagrelor. In a recent publication ticagrelor dose-dependently reduced infarct size in a rat model of reperfusion injury whereas again no effect was seen for clopidogrel despite dosed to

similar $P2Y_{12}$ inhibition (Nanhwan et al 2014). In this rat model the infarct size reduction induced by ticagrelor could be reversed by adenosine antagonism providing indirect evidence that in this model the mechanism responsible for the cardioprotective effect was adenosine mediated. Furthermore, the infarct size reduction could partly be reversed by high but not low dose aspirin providing a plausible mechanistic explanation for the apparent interaction with high dose aspirin in patients (see 2.4.2.4.1). The adenosine mediated effects of ticagrelor and their potential clinical relevance have recently been reviewed (Cattaneo et al 2014).

2.4.2.2.5 Other *in vitro* and *in vivo* pharmacodynamic observations

Human erythrocytes responded to ticagrelor *in vitro* by releasing ATP in a dose-dependent manner (IC_{50} 14 μM) (Öhman et al 2012). It is not known whether this effect occurs in humans. If present it may further enhance the local presence of adenosine.

A published mouse study showed that thienopyridines (clopidogrel and prasugrel) but not elinogrel (a direct reversible $P2Y_{12}$ antagonist) prolonged primary haemostasis in a tail vein blood loss model in $P2Y_{12}$ KO mice compared with untreated $P2Y_{12}$ KO mice (André et al 2011). This indicates that the thienopyridines but not elinogrel could further increase bleeding via an unknown non-$P2Y_{12}$ related effect. However a similar Sponsor conducted mouse study showed that ticagrelor, clopidogrel and elinogrel can all increase bleeding in the absence of $P2Y_{12}$, by which mechanism is unclear (AZMR10231).

2.4.2.3 General Pharmacology

2.4.2.3.1 Reversal of anti-platelet effect

A number of *in vitro* and *ex vivo* studies have been conducted to predict the effect of platelet transfusion in patients. A Sponsor conducted study, using human platelet rich plasma, concluded that the platelet inhibition achieved by ticagrelor can be reversed to different extents depending on the level of platelet inhibition induced by ticagrelor, the incubation time with un-inhibited platelets, and the amount of added un-inhibited platelets (AZMR10395). Similar independent studies have been published (Hobl et al 2013, Hansson et al 2014, O´Connor et al 2013, Ibrahim et al 2014, Martin et al 2014). A rat study indicate that a platelet transfusion may be less effective in the setting of ticagrelor, reversible inhibition, as compared with prasugrel, irreversible inhibition (Sugidachi et al 2013) which also was indicated in a few of the studies mentioned above.
Both NovoSeven and human recombinant FII were able to attenuate ticagrelor enhanced bleeding when evaluated in a mouse tail bleeding model (AZMR10333, AZMR10335).

2.4.2.3.2 Effect in animal models of atherosclerosis, restenosis, stroke, cancer and sepsis

A number of published studies have explored the effect of ticagrelor in different disease models.

Following 8 weeks of treatment, ticagrelor attenuated the initiation of atherosclerosis in hypercholesterolemic ApoE deficient mice (Schirmer et al 2012). However, a similar study using the same model but only 4 weeks treatment showed no effect of ticagrelor or clopidogrel treatment (West et al 2014). Ticagrelor induced a more stable plaque phenotype (thicker

fibrous cap and reduced necrotic core) with a trend for a reduction in plaque size after 25 week treatment of hypercholesterolemic ApoE deficient mice (Rusnak et al 2014).

Ticagrelor reduced neointima formation in a mouse carotid artery model ($FeCl_3$ injury) (Patil et al 2010) and reduced neointimal hyperplasia in a rabbit carotid anastomosis model (Sürer et al 2014).

Ticagrelor attenuated the progression of brain damage at 24 and 48 h after middle cerebral occlusion in rat model of stroke. The protective effect was accompanied by (or due to) a significant reduction of typical markers of brain inflammation, such as the number of blood-borne infiltrating macrophages and of locally activated microglial cells (AZMR10259, Gelosa et al 2014).

In mouse models of experimental metastasis, ticagrelor reduced metastases and improved survival (Gebremeskel et al 2014).

Ticagrelor reduced neutrophil recruitment and lung damage in a mouse sepsis model (cecal ligation and puncture) (Rahman et al 2013).

2.4.2.4 Pharmacodynamic drug interactions

2.4.2.4.1 Potential interaction with aspirin

A number of studies were conducted to explore the mechanism of the interaction with aspirin seen in the PLATO study where high maintenance dose of aspirin appeared to reduce the clinical benefit of ticagrelor relative to clopidogrel (Mahaffey et al 2011). In a Sponsor conducted *in vitro* study pre-incubation with aspirin (0.1 to 5.0 mM) had no effect on the inhibition of 2Me-S-ADP-induced $P2Y_{12}$ signalling by ticagrelor (AZMR10072). In human platelets *in vitro* (Warner 2010, Kirkby et al 2013) as well as in a Sponsor published *in vivo* study in dog (AZMR10239, Björkman et al 2013) it was shown that $P2Y_{12}$ inhibition resulted in inhibition of thromboxane-dependent pathways of platelet activation independently of aspirin. In the dog model, aspirin, when added to sub-maximal $P2Y_{12}$ inhibition, contributed additional anti-thrombotic and anti-platelet effect, but when added to maximal $P2Y_{12}$ inhibition, contributed no additional anti-thrombotic effect and a smaller anti-platelet effect compared to in combination with sub-maximal $P2Y_{12}$ inhibition. The differences in terms of anti-thrombotic and anti-platelet effects were related to the level of $P2Y_{12}$ inhibition but not the particular $P2Y_{12}$ antagonist used. High dose aspirin significantly decreased *in vivo* PGI_2 production and increases vascular resistance, effects that were independent of $P2Y_{12}$ antagonist at any dose tested (AZMR10239, Björkman et al 2013). Hence the balance between the added anti-platelet benefit of high-dose aspirin in combination with $P2Y_{12}$ antagonism and its potential harm may be different depending on the level of $P2Y_{12}$ blockade. This is further discussed in a review on the use of aspirin in regards to dual anti-platelet therapy (Warner et al 2011).

2.4.2.4.2 Potential interaction with clopidogrel and cangrelor

The potential pharmacodynamic drug-drug interactions between ticagrelor and clopidogrel and ticagrelor or clopidogrel and cangrelor were investigated in a Sponsor conducted dog study (27053, 27263, Ravnefjord et al 2012). The observed interactions between clopidogrel and cangrelor or ticagrelor appeared to be dependent on the level of receptor occupancy when clopidogrel is administered. Importantly, no significant pharmacodynamic interaction occurred between ticagrelor/clopidogrel when clopidogrel was given at clinical trough levels in terms of platelet inhibition with ticagrelor. No significant pharmacodynamic interaction occurred between cangrelor and ticagrelor.

2.4.2.4.3 Potential interaction with rivaroxaban

Using tissue factor (TF)-induced platelet aggregation and TF-induced thrombin generation, and a rat arteriovenous shunt model *in vivo,* concomitant administration of ticagrelor and rivaroxaban enhanced the antithrombotic effects of the latter (Perzborn et al 2011a, Perzborn et al 2011b, Perzborn et al 2012).

2.4.3. PHARMACOKINETICS

To support the 5-year renewal of the marketing authorization of ticagrelor, 7 studies which related to the nonclinical pharmacokinetics of ticagrelor were conducted by the Sponsor and are summarized in this section. A review of the literature from the past 5 years did not reveal any additional information pertaining to the nonclinical metabolism and pharmacokinetics of ticagrelor.

2.4.3.1 Absorption

Ticagrelor is a P-glycoprotein (Pgp) substrate and, to support the paediatric programme, an *in vitro* study has been performed by the Sponsor to quantify the amount of Pgp in liver homogenate from neonatal rats (7, 14 and 25 days old) and adult rats (AZM110520-04). The results indicate that the Pgp amount in the liver increased with age in young rats. However, in the adult rats there was significantly less Pgp compared to the amount in 25 days old rats.

2.4.3.2 Distribution

To support the paediatric programme, the *in vitro* plasma protein binding of ticagrelor and its metabolite AR-C124910XX was determined by the Sponsor by equilibrium dialysis at 0.37 and 37 µg/mL and 0.35 and 34 µg/mL for ticagrelor and AR-C124910XX, respectively, in plasma from neonatal Han Wistar rats (7, 14 and 25 days old) (YAT/265). Rat plasma for this study was obtained under a separate study protocol (VKS0827). The degree of protein binding was very high in all age groups for both ticagrelor and AR-C124910XX. For ticagrelor the mean unbound fraction ranged between 0.3% and 0.5% and for the metabolite between 0.3% and 0.4%. In conclusion, the plasma protein binding of ticagrelor and the metabolite AR-C124910XX was high and similar in the age range of 7 to 25 days old rats and the plasma protein binding in young rats was in the same range as for adult rats.

2.4.3.3 Metabolism

The metabolism of ticagrelor and AR-C124910XX was investigated by the Sponsor in liver microsomes from male and female Han Wistar rats of various ages (7, 14, 25 days and 13 weeks old) to support the paediatric programme (VKS0826). The livers used were from a separate study (VKS0827). The results indicated that there was little metabolism of ticagrelor by rat liver microsomes, at 0.5 or 2.5 mg/mL, under the experimental conditions used. Consequently these data were not considered suitable for the determination of accurate intrinsic values. There was evidence that some microsomal formation of AR-C124910XX was seen, which increased with age of both male and female rats. The data provided did not suggest any metabolism of AR-C124910XX by rat liver microsomes under the conditions used in this study.

2.4.3.4 Excretion

Not applicable

2.4.3.5 Drug Interactions

The involvement of the cytochrome P450 (CYP) enzymes 2E1 and 2C8 in the metabolism of ticagrelor have been investigated by the Sponsor (AZM100709-04). Recombinant human P450 enzymes 2E1 and 2C8 (rCYP2E1 and 2C8) were used in the study as well as CYP selective inhibitors to estimate the relative contribution of various CYPs in the metabolism of ticagrelor in human liver microsomes (HLM) and changes in formation rates of the metabolites AR-C124910XX and AR-C133913XX. Incubations of ticagrelor in rCYP2C8 and rCYP2E1 showed less than 3% and 0.05% substrate depletion, respectively and intrinsic clearance was not possible to calculate. Diethyldithiocarbamate (20 µM) and quercetin (5 µM), which are inhibitors of CYP2E1 and CYP2C8, respectively, reduced the formation of the active metabolite AR-C124910XX by approximately 20% and the formation of the inactive metabolite AR-C133913XX by 50% from ticagrelor in human liver microsomes. However, the inhibition of CYP3A-mediated hydroxylations of midazolam and testosterone by the same inhibitors in HLM were in the same order of magnitude, indicating a non-specific effect of the CYP inhibitors used. In conclusion, the complementary *in vitro* experimental results from this study indicate that CYP2E1 and CYP2C8 do not contribute substantially to the metabolism of ticagrelor.

The potential of acetylsalicylic acid (ASA) and salicylic acid (SA) to induce the human cytochrome P450 (CYP) enzymes CYP1A2, CYP2B6 and CYP3A4 has been evaluated by the Sponsor in the human hepatoma cell line HepaRG (AZM090914-04). HepaRG cell monolayers were exposed to ASA and SA at concentrations up to 1000 µM to investigate CYP enzyme activity and gene expression. Omeprazole (CYP1A2), phenobarbital (CYP2B6) and rifampicin (CYP3A4) were used as prototypical inducers. The CYP enzyme activities were measured by the following marker substrates: phenacetin (CYP1A2), bupropion (CYP2B6) and midazolam (CYP3A4). The induction of CYP enzyme activities and gene expressions by the test compounds were evaluated by calculating the concentrations leading to a 2-fold increase over baseline (F_2 values). In cases when full dose response curves were reached, EC_{50} values were calculated. The results from the enzyme activity measurements

showed that ASA and SA are not inducers of CYP1A2, CYP2B6 or CYP3A4 at the concentrations tested and gene expression analyses confirmed that ASA and SA are not inducers of CYP1A2, CYP2B6 or CYP3A4. In conclusion, the results of this study demonstrate that ASA and SA up to a concentration of 1000 µM does not induce CYP1A2, CYP2B6 or CYP3A4 enzyme activity or gene expression in the human hepatoma cell line HepaRG.

A pharmacokinetic *in vivo* interaction study of ASA and SA after oral administration of ASA alone or in combination with oral dosing of ticagrelor or intravenous dosing of clopidigrel was conducted by the Sponsor in the dog (AZM090928-01). In summary, there were no major differences in the pharmacokinetics of ASA and SA, respectively, after co-administration of ASA with ticagrelor or clopidogrel compared to when ASA was given alone.

2.4.4. TOXICOLOGY

A substantial nonclinical toxicology package supported the original Marketing Authorization Application (MAA) of ticagrelor. To support the paediatric program and to investigate the effects of ticagrelor on steroidogenesis the Sponsor conducted 7 nonclinical toxicology studies during the past 5 years. These studies are listed (see Table 3) and summarised below. In addition, a review of the literature covering the past 5 years was conducted and did not reveal any additional noteworthy information pertaining to the nonclinical safety risk assessment of ticagrelor.

Table 3 Nonclinical Toxicology Studies with ticagrelor

Study type and duration	Route of administration	Species	Study number	GLP compliant
Juvenile Toxicity:				
DRF – Neonatal Rats (19 days)	po	Rat	2835LR	Yes
Neonatal Rats (19 days)	po	Rat	2836LR	Yes
Weanling Rats (5 weeks)	po	Rat	2885LR	Yes
Respiratory effects (single dose)	po	Rat	3233SR	Yes
Investigative Toxicity:				
Effects on Steroidogenesis in Ovarian Cell Culture	in vitro	Rat	2189KV	No
Effects on Steroidogenesis in Adrenal Cell Culture	in vitro	Rat	2190KV	No
Prolactin Release in Ovariectomized Rats (4 days)	po	Rat	3100KR	No

2.4.4.1 Juvenile toxicity

Four nonclinical toxicology studies were conducted to support administration of ticagrelor to paediatric patients.

A dose range- finding study (2835LR) was conducted by the Sponsor in neonatal rats. The data suggested that oral administration of ticagrelor in young rats for 19 days, starting at 7 days of age, at dose levels of 10, 60 or 180 mg/kg/day was well tolerated and only resulted in a minimally transient lower body weight gain in animals given the high dose (180 mg/kg/day).

In the subsequent main neonatal toxicity study in the rat (2836LR) administration of ticagrelor to neonatal Wistar rats for 19 days, starting at 7 days of age, at doses of 0, 10, 60 and 180 mg/kg/day, resulted in mortality of a number of main test and satellite animals at the high dose level of 180 mg/kg/day. An involvement of ticagrelor in the observed mortality in the high dose group was considered likely, even though no cause of death could be established and intubation trauma was suspected in one of the high dose animal deaths. Microscopic examination revealed a higher incidence and severity of congestion and haemorrhage in the mesenteric lymph node in the high dose group. This was considered to be due to an exaggeration of an agonal effect based on the pharmacology of ticagrelor. The dose level of 60 mg/kg/day was considered to be a No Observed Adverse Effect Levels (NOAEL) which was equivalent to at least 7 fold the human adult therapeutic exposures at 90 mg bid (based on AUC total).

The nonclinical toxicology profile of ticagrelor was also investigated by the Sponsor in weanling rats (2885LR). In this study oral administration of ticagrelor at dosages of 0, 10, 60 or 180 mg/kg/day to weanling rats for five weeks caused transient reductions in body weight gain and food consumption together with increased liver transaminases and organ weight but without associated histopathological changes in the liver at a dose level of 180 mg/kg/day. These effects were reversible and were not noted at the end of the 4-week recovery period. The death of a single high dose female on Day 3 of the study may have been attributable to the test compound, however, cause of death could not be established at histopathological examination. The NOAEL for this study was 60 mg/kg/day which was equivalent to at least 10 fold the human adult therapeutic exposures at 90 mg bid (based on AUC total).

A single dose study in the suckling rat was conducted by the Sponsor to assess the effects of ticagrelor on the respiratory system, using whole body plethysmography (3233SR). In this study, which was designed to better evaluate the risk of potential respiratory effects occurring in the very young paediatric population, ie, preterm/term newborn and infants, ticagrelor (180 mg/kg) had no compound-related effect on respiratory rate, tidal volume, minute volume, inspiration time and expiration times and peak inspiratory and expiratory flows at 120 min post-dose. The NOEL for effects on the respiratory system of suckling rats was at least 180 mg/kg ticagrelor which was equivalent to at least 40 fold the human adult therapeutic exposures at 90 mg bid (based on Cmax total).

2.4.4.2 Investigative Toxicity Studies

Three nonclinical toxicology studies were conducted by the Sponsor to investigate the effects of ticagrelor on steroidogenesis.

As already reported and discussed in the original MAA, ticagrelor was found to increase the incidence of uterine adenocarcinomas in a 2-year rat carcinogenicity study (0508CR). Effects on circulating testosterone and oestradiol concentrations were demonstrated in a subsequent 3-month investigative study (1800KR) and it had been demonstrated *in vitro* that ticagrelor can inhibit steroidogenesis in ovarian cell cultures isolated from AP Han Wistar rats (1778KV). To better understand the underlying mechanism of this decrease in steroidogenesis an additional study (2189KV) was conducted by the Sponsor. Since the strain of rat used in (1778KV), ie, AP Han Wistar rats, was no longer available, this study was conducted using ovarian cells derived from Harlan rats. Unfortunately, the effects of ticagrelor on steroidogenesis, seen previously could not be reproduced in this study and therefore no conclusion could be drawn. An exception to this was the assessment of the effects of ticagrelor on 3ß-HSD activity where ticagrelor was shown not to inhibit 3ß-HSD activity in Harlan-derived ovarian cell cultures.

Another investigative toxicology study was conducted to investigate the mechanism whereby ticagrelor can inhibit steroidogenesis in rat adrenal cell cultures (2190KV). It was shown that ticagrelor can effectively inhibit the unstimulated synthesis and release of corticosterone and progesterone by cultured rat adrenal cells, but its effect on maximally ACTH-stimulated cells is minimal. This inhibitory effect of ticagrelor is unlikely to be related to its primary pharmacology since the P2Y12 inhibitor cangrelor, unlike ticagrelor, did not inhibit adrenal

steroidogenesis. In addition, ticagrelor appeared not to competitively inhibit steroidogenic enzymes in rat adrenal cell cultures. The observed effects of ticagrelor on adrenal cells preincubated with ACTH were consistent with it inhibiting ACTH-mediated stimulation of steroidogenesis.

Secondary pharmacology studies of AZD6140 reveal an ability to bind to the dopamine transporter (DAT), see Table 2. Since dopamine negatively regulates lactotroph function and inhibitors of DAT have been shown to block estradiol-induced prolactin release in ovariectomized rats, the effect of 4 daily doses of ticagrelor on estradiol-induced prolactin release was studied by the Sponsor in an ovariectomized rat investigative toxicity study (3100KR). Whilst a surge in prolactin release was noted in vehicle-treated rats, oral administration of ticagrelor (180 mg/kg/day) almost completely blocked estradiol-induced prolactin release in ovariectomized rats.

2.4.5. INTEGRATED OVERVIEW AND CONCLUSIONS

The data from internal AstraZeneca studies conducted with ticagrelor as well as information published during the past 5 years since submission of the original MAA (22-October-2009 to 8-November-2014) add to the knowledge of pharmacological, pharmacokinetic and toxicological effects of ticagrelor.
However, none of the new information listed above is considered to significantly change the already established and previously reported pharmacological, pharmacokinetic and toxicological properties of ticagrelor as already discussed in the original MAA and thus none of the new data created over the past five years alter the favourable benefit/risk profile of ticagrelor for continued use in the treatment of patients with acute coronary syndrome.

2.4.6. LIST OF LITERATURE CITATIONS

Alsharif et al 2014
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